NEON Field Procedure and Protocol:
Reaeration
Measuring diffusion of O_2 across the water-air interface

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1 INTRODUCTION

The intent of this document is to provide a change controlled version of a field procedure and protocol. This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.1 Purpose

The purpose of this document is to provide:

- A format version for external review of procedures and protocols;
- Change control for the content of field procedures and protocols (e.g. methodologies);
- Content for training materials for NEON staff and contractors; and,
- Content for field documents for NEON staff and contractors.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- General safety practices (i.e. how to drive a boat).
- Site-specific safety practices (i.e. how to safely walk in a stream).
- Related laboratory processing practices.
- General maintenance (i.e. fill the car with gas).

1.3 Acknowledgements

This protocol is based closely on the work by the Lotic Intersite Nitrogen eXperiment (LINX), the laboratories of Dr. Bob Hall, University of Wyoming, and Dr. Michelle Baker, Utah State University.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference Documents

RD[01]	NEON.DOC.000008 NEON Glossary of Abbreviations
RD[02}	EHS Safety Policy and Program Manual
RD]03…]	<primary science design docs explaining/justifying this protocol/these procedures>
RD[04]	NEON Sampling Design Document
RD[05]	Training Plan
	QA/PA Plan
	DOORS requirements
	ATBD

3 BACKGROUND AND OBJECTIVES

3.1 Background

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

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

3.1.1 Assumptions

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

3.2 Science Requirements

This protocol fulfills the following Observatory science requirements:

NEON.AQU. 3.028	Stream reaeration-rating curves shall be verified every 1-2 years or after extreme hydrologic events.
NEON.AQU.3.029	Stream reaeration rating curves shall be quantified using a propane evasion method during a three-day period, where appropriate.
NEON.AQU.3.096	Stream reaeration rating curves shall be developed during site characterization.
NEON.AQU.3.102	During site characterization, stream reaeration rating curves shall be quantified using a propane evasion method during a three-day period per sample date, where appropriate.
NEON.AQU.3.034	System shall provide surface and groundwater chemistry data to enable ecohydrologic research.

NEON.AQU.3.103 During site characterization, field measurements to develop stream reaeration and discharge rating curves shall be conducted at up to ten discrete sample dates throughout the year.

3.3 Data Products

Data Product	Measurement
AQU.0.0101	Stream reaeration rating curve

4 PROTOCOL

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

This protocol outlines the procedures required to measure gas exchange (i.e., reaeration rate coefficient, K_2) from the atmosphere to the water by use of an inert (i.e., will not interact with any biogeochemical processes) gas tracer (e.g., Sulfur Hexafluoride (SF_6), as well as a conservative tracer (e.g., NaCl) to account for groundwater inputs to the system. Stream reaeration is often measured by injecting an inert gas (e.g., Propane or Sulfur Hexafluoride (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 are used to calculate an O_2 reaeration (or deaeration) rate coefficients (K_2). To account for dilution due to surface or groundwater inputs, a conservative solute tracer such as chloride (Cl^-) or bromide (Br^-) is added to the stream in addition to the inert gas.

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

5 QUALITY ASSURANCE AND QUALITY CONTROL

TBD

6 DECISION TREE

1. Are the weather conditions safe for sampling (i.e. no lightning, hail or flooding)?
    a. If YES, go to 2.
    b. If NO, stop sampling.
2. Has there recently been any stream sampling that occurred within the reaeration reach?
    a. If YES, go to 3.
    b. If NO, continue sampling.

3. Did any of the previous work stir up sediments or add chemical constituents to the stream (i.e. salt additions)?
    a. If Yes, allow stream to clear and disturbance to pass.
    b. If NO, continue sampling.
4. Is the sampling location dry?
    a. If Yes, move to a nearby location where water can be sampled. Record sampling location on field data sheet.
    b. If NO, continue sampling.

7 SAFETY

Personnel working at a NEON site should be familiar with and practice safe field work as outlined in the EHS Safety Policy and Program Manual. Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Samplers should make sure gas cylinder is secure 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 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.

8 PERSONNEL REQUIREMENTS

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

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

9 TRAINING REQUIREMENTS

Personnel are to be trained in stream reaeration measurements and safe working practices for stream fieldwork. Refer to NEON Training Plan (NEON.DOC.##).

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling Frequency and Timing

Reaeration measurements will be completed 10 - 12 times during site characterization at each NEON wadeable stream location. Timing of sampling is site specific and determined by rules developed using historical (flow regime) and environmental data. For example, streams with little or no flow during the summer dry-season are sampled more intensively during wet periods. Streams with snowmelt-

dominated hydrographs are sampled more intensively during spring/summer-elevated flows than during winter snow-covered months.

Following NEON site characterization, a relationship will be developed between reaeration and discharge. This relationship will be checked each year during Operations to ensure the curve has not shifted over time. To establish and maintain the reaeration-discharge rating curve, simultaneous measurements of reaeration and discharge must be made over a range of discharges. Therefore, the production of a continuous record of reaeration requires periodic manual reaeration measurement checks during Operations. NEON's target is to obtain 5 - 6 manual reaeration measurements per year during Operations. In general, the reaeration curve will be checked for a high, low and average discharge event. Rating curve checks may also be completed during times selected to help fill in gaps in the measured data for the reaeration-discharge rating curve (i.e., when discharge is in a range with minimal or no reaeration measurements) in order to refine parts of the curve with little data. In the event that the curve check does not produce results similar to the original reaeration-rating curve, such as after a major flooding or scouring event, a new curve will need to be established.

10.2 Contingent decisions

Reaeration measurements should not be conducted during or immediately after any fieldwork disrupting the stream bottom (i.e., morphology mapping, invertebrate collection, macrophytes collection, etc.). In streams with a shallow water column, samplers must be cautious not to stir up the benthic sediments when sampling the stream water. Disruption of the sediments by walking or by sampling too close to the stream bottom can contaminate your sample. Thus, always sample upstream from wading activity and minimize suspension of sediments when sampling. If sediments are disrupted, wait until the area has cleared before sampling.

The weather should be checked the day prior to the scheduled field sampling and adjusted to avoid any major storms. Should it begin raining during the reaeration injection enough to change the flow of the stream, stop the injection and restart at a later date. Reaeration measurements should not be made when the water level and discharge are changing rapidly.

If the stream is disconnected such that it is a series of pools not connected by surface water or the stream is dry, do not conduct an injection. Make a note of any weather or stream conditions that could influence reaeration, including but not limited to wind, channel alterations, activities in the surrounding watershed, prior flood or rain events.

10.3 Field Procedure

10.3.1 First Trip

1. Locate Reaeration Reach, which is representative of the stream and has an injection site (i.e. the location at the top of the reach where we will add the SF_6 gas and NaCl solution) with a good mixing zone and minimizing the inclusion of large pools or dead zones (which increase travel time and water storage).

a. There will be 4 sampling stations within the reach, located downstream of the injection site (Figure 3).
   i. The 1st sampling location (Station 1) should be just downstream of the distance it takes the conservative tracer to completely mix with the streamwater (25 - 100 m; higher flow streams often need more length to completely mix). During higher flows, mixing lengths may increase and injection sites may need to be moved upstream.
   ii. The last sampling location (Station 4) will be located at the bottom of the reaeration reach.

2. Determine Reach Length:
   a. The top of the reach must start with a good mixing zone to completely mix tracer with stream water. Best mixing zones are upstream of shallow pools with converging and diverging flows (Figure 4). This should allow for complete mixing before the first sampling location (Station 1), often occurring over a distance of 25 – 100 m. Wide, slow moving streams may have difficulties mixing. To ensure complete mixing at Station 1, during the continuous NaCl injection measure conductivity across main flowing section of the stream after steady state has been reached (i.e., the conductivity is no longer increasing). Depending on flows this could take 20 minutes to several hours to attain. If the site is well mixed, conductivity should be similar across this main flow.

   b. Measure travel time between Station 1 and Station 4 - The best reach lengths are those that take ~40-45 minutes, during baseflow, for water to travel from Station 1 to Station 4. A simple way to estimate water travel time is to place an orange in the stream at Station 1 and follow the orange as it moves downstream for ~40-45 minutes to Station 4. Salt-pulse additions may be necessary to estimate travel time in small streams (<25-30 L/s), where travel time between Stations 1 and 4 is the difference in the timing of the salt profile half-height as it passes station 1 and 4. Adjust Station 4 upstream or downstream to obtain the appropriate travel time.

10.3.2 Equipment and Materials

- Initial trip:

Equipment	Quantity per sampling event
5 gallon bucket/carboy (may need to be larger, depending on size of stream)	2
Conservative tracer: Chloride (Cl^-) or Bromide (Br^-) – Site Specific	1
Conductivity probes, handheld, calibrated	2
Stopwatch	1
Neutrally buoyant object (i.e., orange)	1
Meter tape, 50 – 100 m metric	1

- Gas Injection:

Equipment	Quantity per

	sampling event
Gas Tank (e.g., Sulfur Hexafluoride, SF_6 or propane – site specific) 5-10 lbs (10 lb tank contains sufficient gas for about ~30 reaeration measurements)	1
Gas Tank Regulator	1
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
Gas-impermeable tubing: ¼ inch ID (inner diameter), (e.g., Tygon)	1
Teflon tape	1
1-1/8 inch wrench to connect regulator to SF_6 tank	1
11/16 inch wrench tubing to gas flow regulator	1
Fine-pore diffuser	2
Small squirt bottle of SNOOP (or soapy water) to test for gas leaks	1

- Conservative Tracer Injection:

Equipment	Quantity per sampling event
5 gallon bucket/carboy (may need to be larger, depending on size of stream)	1
Extra bucket for filling conservative tracer reservoir	1
Conservative tracer: Chloride (Cl^-) or Bromide (Br^-) – Site Specific	1
Pump set-up: • Fluid Metering (FMI) Pump – QB metering pump, Stainless Steel pump head with ceramic piston and hose barb adapter (¼" Barb * ¼" MIP)	1
Gel cell batteries (12 Volt)	2
¼ inch I.D. tubing – 10 and 1 foot long	2
Plastic 200 mL graduated cylinder	1
Stopwatch	1
Battery charger	1
Grease (high grade machine oil)	1

- Sampling:

Equipment	Quantity per sampling event
5 gallon bucket/carboy (may need to be larger, depending on size of stream)	1
Extra bucket for filling conservative tracer reservoir	1
Conservative tracer: Chloride (Cl^-) or Bromide (Br^-) – Site	1

Specific	
Pump set-up: • Fluid Metering (FMI) Pump – QB metering pump, Stainless Steel pump head with ceramic piston and hose barb adapter (¼" Barb * ¼" MIP)	1
Gel cell batteries (12 Volt)	2
¼ inch I.D. tubing – 10 and 1 foot long	2
Binder Clips	2
Plastic 200 mL graduated cylinder	1
Stopwatch	1
Battery charger	1
Grease (high grade machine oil)	1
Flagging, roll	1
Field thermometer and conductivity meter, handheld - calibrated	1
Logging Conductivity probes - calibrated	2
60-mL HDPE sample bottles (e.g., Nalgene), pre-labeled. Plus extras	25
60-mL syringes, with luer-lok tip, 1mL graduations (individually #ed and covered with clear packing tape to protect syringe labeling)	24
2-way stopcocks, one per syringe	24
12 mL Exetainer gas vials with Double Wadded White Caps, pre-evacuated and pre-labeled	20
Gas vial rack	1
Labels for gas vials: 1 inch * 4 inches (e.g., Avery 5661)	20
Silicone sealant, tube	1
Needles, 25 gauge, disposable 1 – 1.5 inches in length	20
25 mm/ 0.45 µm pore size syringe filters	25
"Sharps" container for needle disposal	1
Meter-tape, 50- metric field tape	1
Discharge Equipment, see Measuring Discharge in Wadeable Streams Protocol	

- Site Specific Supplies:

Equipment	Quantity per sampling event
Infrastructure, such as stakes, rebar, or ring stand, for tracer injections	As needed
rope	As needed
Zip ties/Cable ties	As needed
Bridge/plank	As needed

- Shipping Equipment:

Equipment	Quantity per

	sampling event
Lock and Lock box, ~ 2qt	1
Shipping cooler and boxes	1
Frozen ice packs for gas sample shipping	1
Field sheets, laboratory data sheets, pencils and sharpies	1

10.3.3 Outline of Major Steps on All Reaeration Days

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

2. Measure discharge using the velocity-transect or salt-dilution method. See Wadeable Stream Discharge Protocol.

3. Continuous Injection of Gas (SF_6) and Salt (NaCl) – At the same time and location, add inert gas (to account for diffusion) and conservative tracer (to account for groundwater inputs) to the stream. Rate of addition depends on stream flow.
    a. SF_6 Addition:
        - We use about 100 mL/min for a 50-200 L/s stream.
        - In a big stream (>1000 L/s) we increase to 200-300 ml/min.

    b. Salt Addition:
        - 5-15-mg Cl^-/L NaCl above background (10-30 µS/cm)

4. Collect Plateau Samples – While injection continues, take samples after the furthest downstream location (Station 4) reaches plateau of conservative tracer (NaCl).
    a. Collect five 45-mL water samples at each of the 4 sampling stations into pre-labeled syringes – each syringe will provide a gas and water sample.
    b. Record stream temperature, conductivity and time when samples are taken at each of the 4 sampling stations.

5. Process samples at a base camp away from stream and upstream of the injection site to limit potential SF_6 contamination.

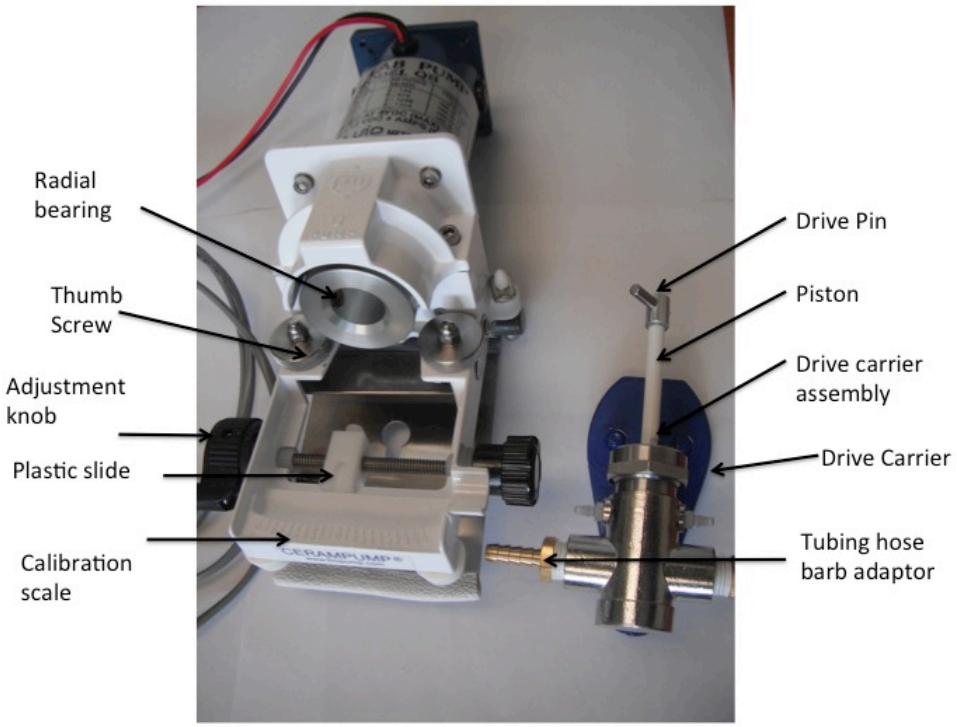
6. Store samples appropriately.
    a. Water samples → Tightly sealed in labeled 60-mL HDPE bottle.
    b. Gas samples → Sealed in Gas Vials to be processed within 1 - 2 months.

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

10.3.4 Preparation

1. The Day Before:
    a. Pump Assembly- Assembled and tested before going to field.
        i. Insert ceramic piston into drive carrier cylinder just enough so that it will not fall out (~1 inch, Figure 1a).
        ii. Add a small drop of grease (high grade machine oil) to the drive pin just before it is inserted into the radial bearing. Insert ceramic piston drive pin into the radial bearing in the spindle assembly (Figure 1b).
        iii. At the same time as you insert the drive pin into the bearing, slide the drive carrier into the pump base assembly (Figure 1b), 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 1a) at the same time.
        iv. 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 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.
            2. 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.
        v. Tighten thumbscrew (Figure 1a) to hold drive carrier in place.
        vi. 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)

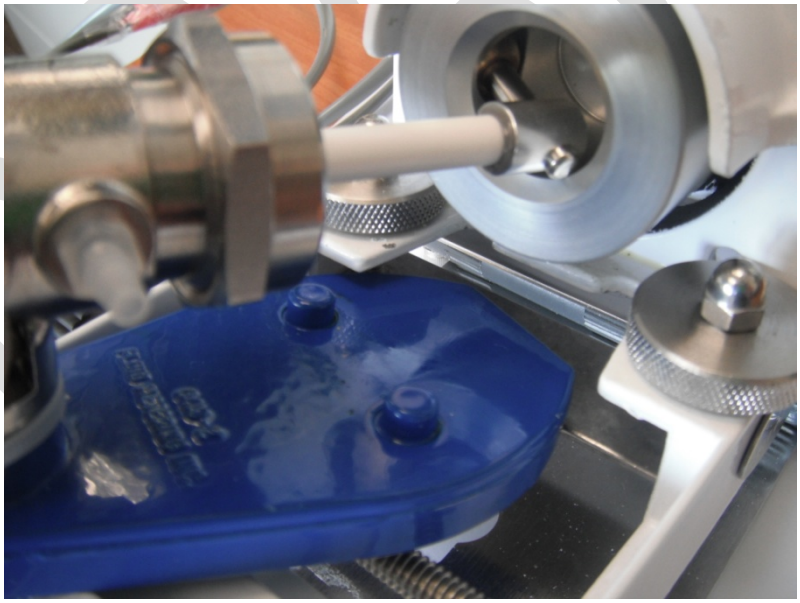


Figure 1. Image of fluid metering pump a) components and b) assembly.

a. Test Pump: Test pump by placing it on the rim of a 5-gallon bucket with inlet and outlet tubing ends placed in the bucket containing tap water. Connect the pump to a battery

and run the pump to make sure everything is connected and working properly. Look for leaks.

b. Use the injection calculation spreadsheet (Figure 2) to calculate the quantity of the conservative tracer (NaCl) you will need to add to the bucket/carboy. Tracer addition needs to be large enough to detect the tracer at the most downstream sampling station and will vary by location and time as discharge and background conductivity values change. Aim for a 5-15 mg/L increase in Cl^-, with <50% saturation of the conservative tracer. The calculation spreadsheet (Figure 2) will be provided by NEON.

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

Figure 2. Example of injection calculation worksheet.

c. The amount of salt required is a product of the target enrichment, the estimated stream flow and the injection rate.

d. Weigh out the amount calculated and dissolve the salt in tap water in a small container, as needed (i.e., a liter bottle or a 4-L jug). Ensure the tracer is completely dissolved.

e. **Record** the amount of salt added to the Injection Field Data Sheet (See Appendix A, Field Data Sheets).

f. Confirm that you have newly evacuated gas vials from a gas laboratory.

g. Charge two gel-cell batteries OVERNIGHT.

h. Calibrate the conductivity meter and probes and check their battery life. Set probes to begin logging on the reaeration field day. See user manual for calibration and usage instructions.

i. Label all gas vials and conservative tracer bottles (60 mL HDPE).
   i. Gas: Label the gas vials with Domain number (DXX), stream 4-letter identifier code, date (YYYYMMDD), and syringe number (1-20).
   ii. Conservative Tracer: Label 25 sixty (60) mL plastic bottles. Mark StationID, syringe (aka sample) number, date and time on the bottle label. Mark bottle with lab code **FU**. StationID is the Domain number (D##)_Site 4-letter Code_ Sample ID (Ex. Domain 15, Red Butte Creek, Sample 16 would be D15_REDB_16.). Sample IDs are: BS1-S4 – Background Station 1 – Station 4, 0-Injectate, 1-5 indicates the five samples taken at Station 1, 6-10 indicates the five samples taken at Station 2, 11-15 samples at Station 3, 16-20 samples at Station 4 (Table 1). The bottle sample IDs correspond to the field syringe IDs.

Table 1. List of SampleID numbers for the conservative tracer. Note the injectate sample bottle is SampleID #0. The SampleID number corresponds to the syringe number.

Sample station	SampleID
Background	B Station # (S1 – S4, where S1 is Station 1).
Injectate	0
Station 1	1-5
Station 2	6-10
Station 3	11-15
Station 4	16-20

10.3.5 Sample Collection in the Field

1. Background Sampling
   a. First, visit each of the four sampling stations (Figure 3), and at each station record four conductivity measurements from the stream thalweg (i.e. the main flow of the stream), for a total of 16 conductivity measurements (See Appendix A, Field Data Sheets). Make sure the hand-held conductivity meter is set on the temperature-corrected setting (the '°C' should be flashing). If the setting is not correct, press the mode button until you enter the temperature-corrected mode. Wait for the meter to adjust to the stream temperature for more accurate conductivity measurements.

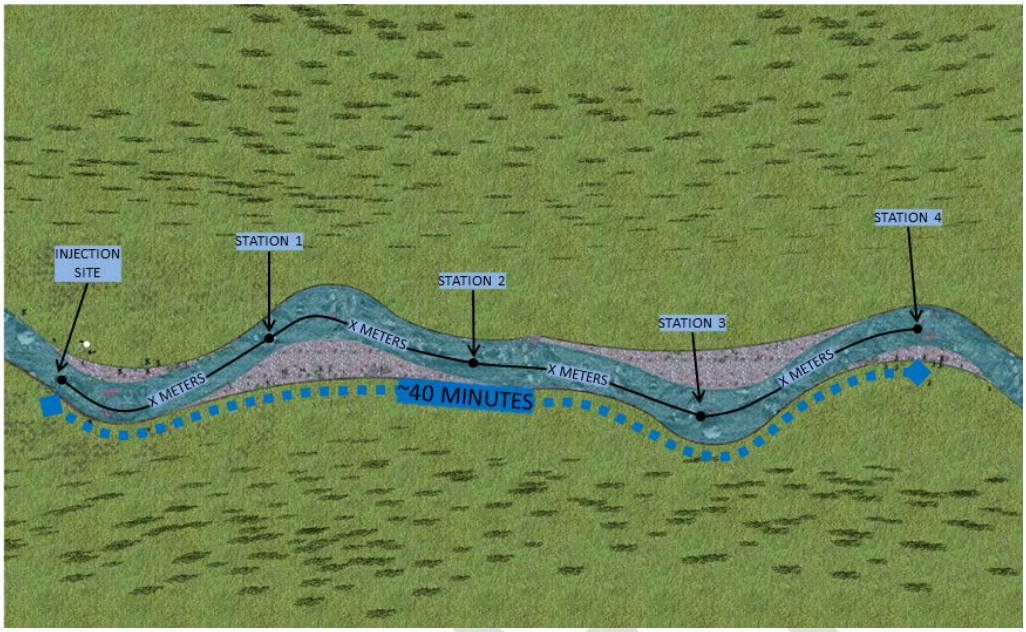


Figure 3. Schematic of stream reach with injection site and four downstream sampling stations. Travel time between Station 1 and Station 4 should be ~40 minutes.

b. At each location, collect 60 mLs of stream water from the thalweg using the corresponding background syringes. Using a syringe filter, rinse and filter the sample into a 60 mL bottle for a background Cl^- sample. Label as DXX (domain number), Stream ID, B (background), Station # (S1 – S4, where S1 is Station 1).
c. Mark and label each location with flagging tape, if necessary, to help find the sampling locations during the injection
d. Place logging conductivity probes in the thalweg at Station 1 and Station 2. Probes should already be logging.

2. In Field (plan 3-4 hours)
   a. Fill a 5-gallon bucket/carboy with stream water and stir in the concentrated salt solution from the day before. This will allow some time for the water to warm while you get the rest of the set-up ready and will allow any undissolved salt to dissolve more easily. You may need to increase the size of your bucket if the stream is large or flows are very high. **Record** the volume of the bucket/carboy on the injection data sheet (Appendix A).
   b. Set up the bucket/carboy, tubing, battery, and pump on a level surface at the injection site (Figure 4). If a level surface on the bank is not adequate, place pump set-up on a bridge or plank laid across the stream.

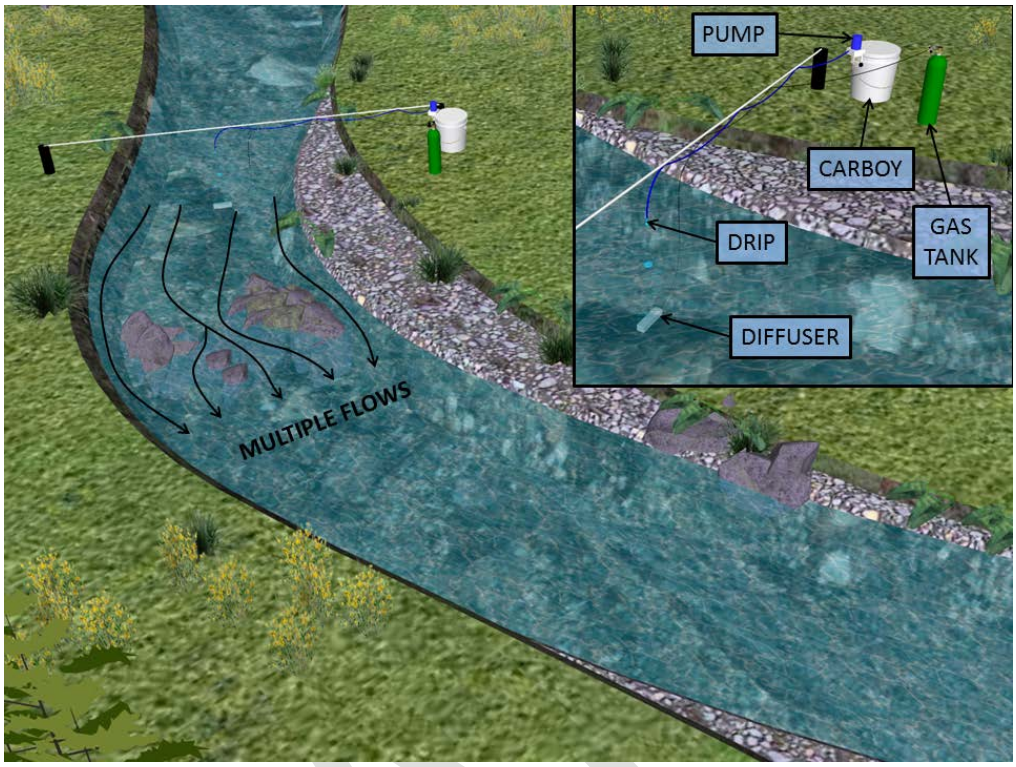


Figure 4. Field setup of the injection site with conservative tracer and inert gas.

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

i.

Figure 5. Mounting of the pump on the conservative tracer bucket.

d. 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 2). To ensure a more accurate calibration, make sure to test the injection at the same height that the tubing will be placed during the injection.
    i. NOTE: This step may take several minutes, but it is extremely important to get the correct pump rate.
e. Every time you test the rate, pour the injectate back into the bucket/carboy and return the outlet tubing to the pump. DO NOT dispose of injectate solution.
f. Record the actual (what was measured in the cylinder) pump rate on the data sheet as "Start Pump Rate" (See Appendix A, Field Data Sheets).
g. Collect a 60 mL bottle of conservative tracer injection solution into the bottle labeled with 'SampleID 0'. This is an **EXTREMELY IMPORTANT** step so that we know the exact concentration of the solution we added to the stream.
h. Inject (i.e., drip) the salt solution into the stream thalweg. The end of the tubing should be placed a few inches above the water surface. **DO NOT** put the tubing in the stream because it will change the pump rate. Attach the tubing to something stable, such as a piece of rebar pounded into the streambed or a tree above the stream. Make sure to INJECT INTO THE THALWEG so the solution will mix as quickly as possible with the stream water (Figure 4).
i. At the injection site, set-up the SF_6 gas tank, regulator and flowmeter (Figure 6).
    a. Attach the regulator to the gas tank using the appropriate size wrench (1-1/8 inch wrench).

b. 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.
c. Use gas-impermeable (e.g., Tygon) tubing to connect the TOP of the flowmeter to the diffuser (air stone). The tank can lie on the ground.
d. Make sure diffuser remains underwater and is at the same location in the stream as the conservative tracer injection (Figure 4).
e. Ensure the flowmeter is vertical. The flowmeter must remain upright for the best performance.

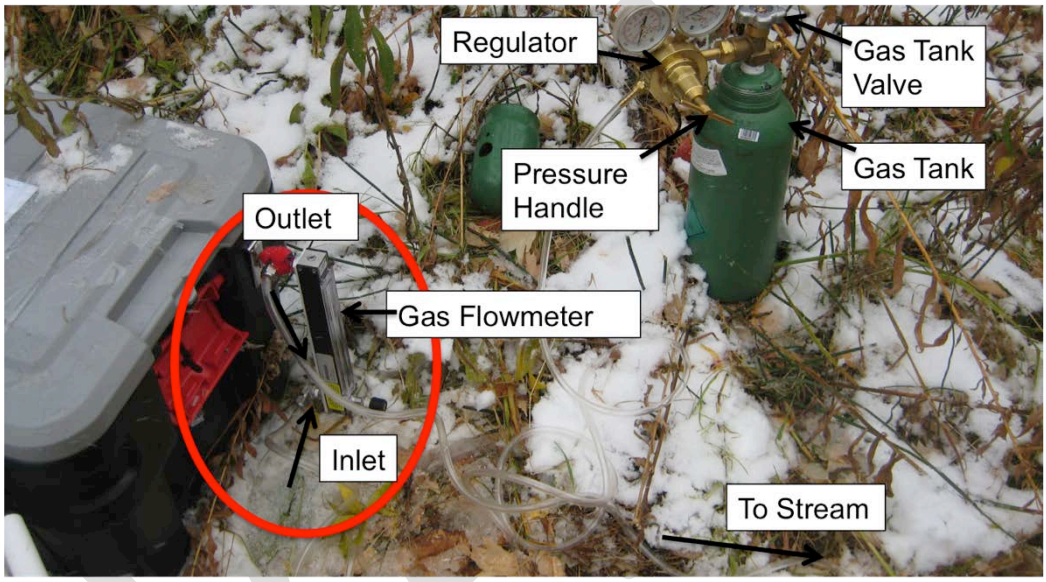


Figure 6. Gas tank, regulator, and flowmeter field set-up.

j. Connect all parts of the gas set-up before opening the main value on the tank.
   a. Turn regulator pressure handle completely to the right on the regulator. This will close the regulator valve and keep your gas from blowing through before you are ready.
   b. Open the gas tank valve.
   c. Slowly turn the regulator pressure handle to the left to allow gas to flow to the flowmeter.
k. Set the regulator at 12 psi.
l. On the flowmeter, turn the valve so that it reads approximately 35 psi. If stream flows are low, you will need to increase the gas pressure to ensure enough gas makes it to the downstream sampling stations. Make sure you see little bubbles coming out of the diffuser (air stone).
m. Start the pump for the salt injection at the **same time** as the gas injection.
n. Note the start time on the reaeration spreadsheet.
o. Spend a few more minutes at the injection site making sure the salt and gas injections are working properly.
p. Next, walk to the MOST DOWNSTREAM location (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.

q. Once at the most downstream station, place the handheld conductivity meter in the stream. Make sure the probe is fully submerged in the main flow. Do not put the probe in a side pool.

r. Continue to observe conductivity until the stream has reached plateau (when salt concentration/conductivity measurement is no longer rising). Sampling should not begin until the stream has reached plateau at the MOST DOWNSTREAM location, usually 45 minutes to an hour during low flows and will be faster during high flows.

s. Once the most DOWNSTREAM location has reached plateau, start sampling at the most UPSTREAM location (i.e., Station 1, the station closest to the injection site) and work downstream (Figure 3). The idea is that you are following a parcel of water as it moves downstream.

t. At Station 1, record 5 temperature-corrected conductivity measurements across the main flowing section of the stream. You only need to do this step at Station 1 (See Appendix A, Field Data Sheets).

u. Take 5 syringe samples in the thalweg at each sampling stations. You may need to get into the stream to do this. Only step into the stream at designated sampling locations and do your best to not disturb the sediment too much as you walk. If you do kick up benthic sediments, wait for the area to clear before sampling. ALWAYS take samples upstream of where you are standing.

v. RINSE: Place the syringe tip (with 2-way stopper attached and turned to open; Figure 7) into the stream so that you are sampling the water ~10 cm under the surface of the water. Pull in ~20 mL water and remove syringe from stream. Rinse the syringe by pulling the stopper all the way back (without removing it) and shake. Expel the rinse water downstream or onto the bank. Repeat.

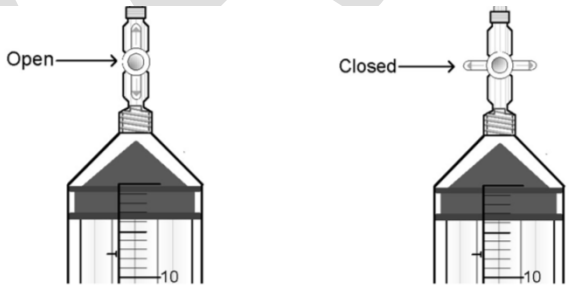


Figure 7. Example of a syringe and stopcock in the "Open" and "Closed" positions.

w. SAMPLE: Put the syringe tip back into the water, below the surface, and pull the plunger until the syringe is completely full. Remove the syringe and tap the sides of the syringe firmly with your hand/fingers to remove the air bubbles. EXPEL water, leaving approximately 1 mL in the syringe, which will help reduce air intake on the next sampling. Put the syringe back in the water, expel the final 1 mL of aerated water below the stream surface, and slowly fill to the 45 mL mark, being careful not to entrain any air bubbles (Figure 8). Immediately turn the 2-way stopper to the closed position before removing the syringe from the stream (Figure 7). Take 5 separate syringe samples all within the main flow of water. Place the syringe in a small cooler to help maintain temperature.

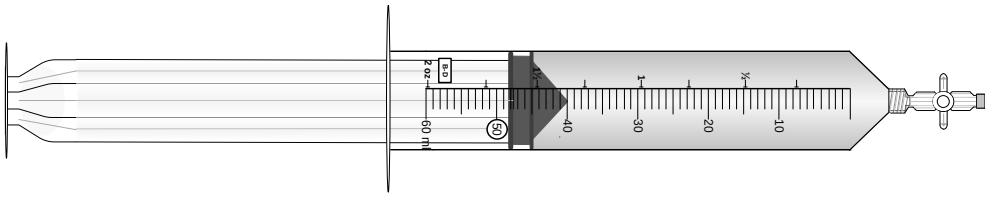


Figure 8. A syringe with a 45 mL water sample.

x. At each station, note the time, 5 syringe IDs, water temperature, and conductivity on the reaeration spreadsheet (See Appendix A, Field Data Sheets).
y. REPEAT (steps u-x) for each sampling station, always working from upstream to downstream. When walking between stations, do not walk in the stream.
z. Return to the Injection Site. Test and Record the 'End Pump Rate' and time on the field data collection sheet (See Appendix A, Field Data Sheets).
aa. END INJECTION: Turn off Gas and Pump.
bb. If you did not already, SAVE some conservative tracer solution in a 60 mL bottle, labeled with station number (DXX), stream name, Sample ID 0, and date (YYYYMMDD).
cc. RINSE Cl^- INJECTION EQUIPMENT:
    i. Return any remaining conservative tracer solution to 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.
    ii. Fill the bucket/carboy with fresh stream water and run the pump for 30 minutes to flush the NaCl from the pump equipment.
dd. COLLECT logging conductivity meters.
ee. BREAK DOWN the gas and NaCl injection set-ups.

3. Sample Processing
    a. Away from stream, where there will be no contamination in the air from the injection, such as upstream and upwind, open stopcock and draw in 15 mL of air so the syringe is filled to the 60 mL mark with air and water (Figure 8). Close stopcock. VERY IMPORTANT: To be consistent, be sure to have the bottom of the meniscus at the 15 mL mark (Figure 9).

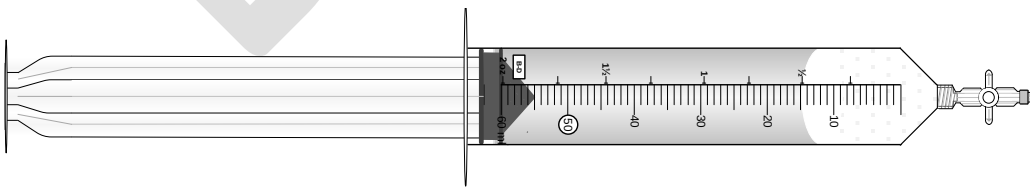


Figure 9. A syringe with 45 mL of water and 15 mL of air.

b. SHAKE: Once samples have been collected from ALL locations, shake each syringe for 5 minutes to equilibrate the air and SF_6. To save time, shake multiple syringes at once. You may need to do this in shifts if you have too many to hold during the first shaking.
c. SAMPLE GAS: Label the gas vials with station number (DXX), stream ID, date (YYYYMMDD), and syringe number.
d. After shaking, place needle with needle cover still attached on 2-way stopcock. Once needle is attached, remove the plastic covering. Holding the syringe upright, open the stopcock (Figure 9) and push a small (~0.5 mLs) through the needle to purge the air in needle.

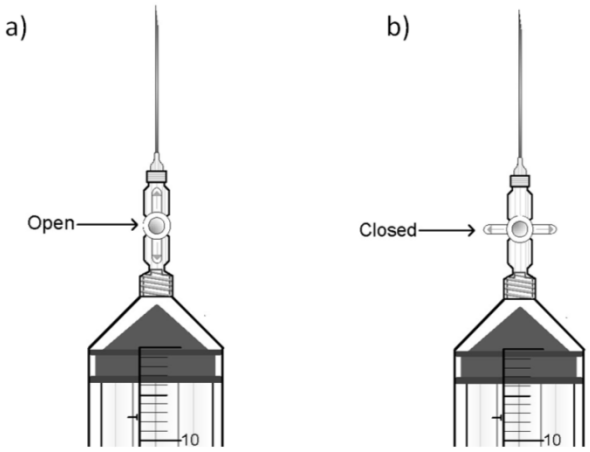


Figure 10. Example of a syringe with a stopcock with attached needles in the a) "Open" and b) "Closed" positions.

e. With stopcock still 'open' and syringe held upright, insert the needle through the rubber septum of the gas vial (Figure 10 and 11) and push gas into the vial. Properly evacuated vials will automatically suck in the gas. Push as much gas in as possible.
f. Make sure to OVER-PRESSURIZE THE VIALS to prevent gas from leaking into the vials (you can normally get ~13 mLs of air into the septum). Pull the syringe needle out quickly without closing the 2-way stopper. This makes loss of gas less likely.
g. Discard needle in a Sharps container with a lid.

Figure 11. Insertion of the needle into the gas vial through the rubber septum.

h. SAMPLE CONSERVATIVE TRACER: Remove the 2-way stopcock. Hold syringe upright and expel remaining air from the syringe (Pushing air through the filter can cause the filter to rupture). Attach a 25 mm/ 0.45 µm filter capsule onto the syringe. Rinse the 60 mL bottle with 5 mLs of filtered sample twice. Filter the remaining syringe sample (~35 mLs) into the bottle. Be sure the bottle is labeled with station number (DXX), stream ID, date (YYYYMMDD), and syringe number. Repeat for all syringes.

i. DISCARD filter and replace stopcock on each syringe.

j. Additional data to collect: 30 stream wetted width measurements evenly spaced throughout the reach, between Station 1 and Station 4 (e.g. 10 wetted width measurements between station 1 and 2, 10 measurements between station 2 and 3 and 10 measurements between station 3 and 4) completed at the end of each injection.

4. Troubleshooting
   a. Diffuser: If no bubbles are coming out of diffuser plate, then check 1) tank is turned on and regulator is open and 2) all tubing connections. Apply SNOOP (or soapy water) to the tube connections and watch for bubbles, which indicate a gas leak.
   b. Pump: If the pump is not working or is not working correctly:
      i. Check battery charge and try another battery. It is always a good precaution to bring an extra battery with you to the field with you even if it is not fully charged. If changing the battery changes the pump rate, it is a battery problem. If changing the battery doesn't affect the rate, check to make sure all wires are well connected.
      ii. Ensure pump thumbscrews are securely tightened.
      iii. Refer to Pump User's Manual.

10.3.6 Sample Preservation

1. Gas Samples:
   Cover the needle holes on the gas vials with silicon. This helps to keep gas from leaking out during storage. **VERY IMPORTANT:** Gas samples should be kept cool and away from a light and heat source.

2. Chloride Samples:
   There are no preservation requirements for chloride samples.

10.3.7 Sample Shipping

1. Gas Samples
   a. Ground ship samples immediately to laboratory for analysis. Changes in temperature and elevation can alter the gas pressure in the gas vials. Samples should be shipped in a watertight container and placed in a cooler with icepacks.

2. Conservative Tracer Samples
   b. Ship the injectate sample and the 20 plateau conservative tracer sample bottles for (Cl^-) analysis. Cl^- samples do not need to be frozen.
      i. Fill out the Analysis Service Request (ASR) forms for the lab (Appendix B). Each Cl sample requires its own form. You will have a total of 21 forms, including the injectate sample, sample ID 0.
      ii. Make sure to note what the expected concentration of the injectate is in the "comments to NWQL" section; they will need to dilute the sample before analysis. The expected concentration can be taken from the injection spreadsheet used to calculate the concentration and injection rate of the conservative tracer (Figure 2).

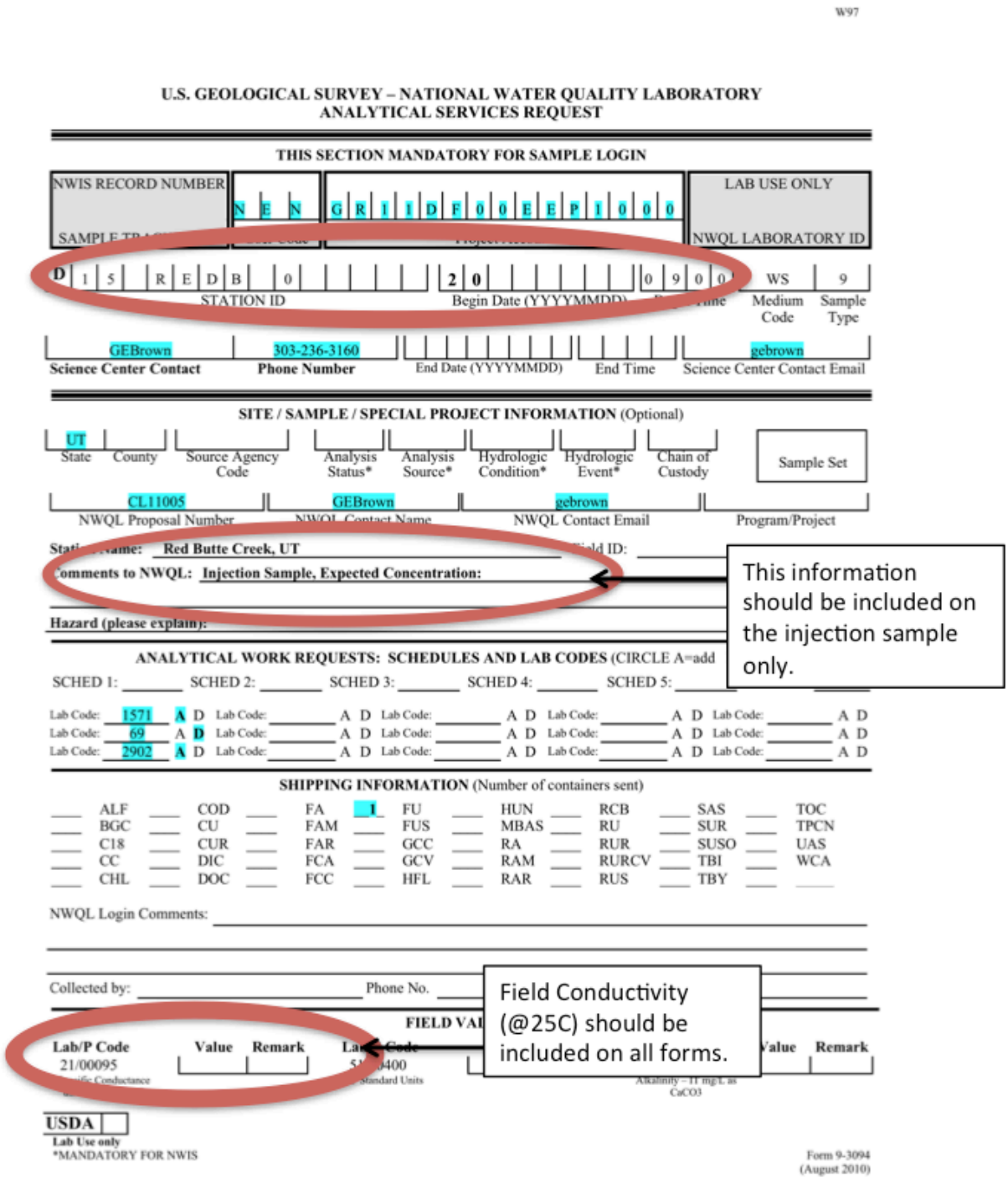


Figure 11. Example of ASR form to be filled out for each chloride sample. Red circles indicate areas of the form that must be completed

10.3.8 Data Handling

1. Calculations
    a. Discharge: Discharge can be calculated from the conductivity measurements at Station 1 and Station 4.
        i. Background-correct conductivity measurements at Station 1 and Station 4.
        ii. Calculate Discharge as: (a) $Q=(C_{salt} * Q_{salt})/ C_{station}$, Where C_{salt} is the conservative tracer concentration (Cl^-) in the injection solution, Q_{salt} is the injection rate (in L/s) and $C_{station}$ is the background-corrected conservative tracer concentration at the Station 4.
        iii. Station concentration can be calculated from conductivity measurements where 1 µS/cm = 0.5 ppm Cl^- (mg Cl^-/L).
    b. Reach Velocity: Calculate Average Velocity of the Reach: V=X/Travel Time, Where X is the distance between Station 1 and Station 4, and Travel Time is the time it takes from to reach ½ half height at Station 4 minus the time to reach ½ height at Station 1.
    c. Reaeration Coefficient
        i. Plot the natural log (LN) of the RATIO of the tracer gas concentration (or GC peak area) to the background-corrected Cl concentration (Y-axis) by Stream Distance (X-axis).
        ii. The slope of the line is the SF_6 loss rate (m^{-1}).
        iii. Calculate Reaeration Coefficient for SF_6: Multiply SF_6 loss rate by the reach velocity (m min^{-1}) to get reaeration rate coefficient for SF_6 (K_{SF6}; min^{-1}). Reach velocity is the distance between station 1 and 4 divided by the difference in timing of the maximum slope of the conservative tracer ascending limb at station 1 and 4.
        iv. Convert Reaeration Coefficient for SF_6 to O_2 ($K_{O2} = K_{SF6} * 1.34$).
        v. Convert K_2 values from ambient stream Temp to standard temperature (T=20C) $K_2(T=20C) = K_2(T) * (1.0241^{(20-(Upstream(T)+Downstream(T)/2))})$
        vi. Develop a relationship between discharge and K_{O2}.
2. Enter data into excel file named "Reaeration Field Data." Save file in format:
    Reaeration Field Data_DXX_StationID_YYYYMMDD.xls
    Station ID should be the 4-letter Station ID
3. Download conductivity probes. Save file as:
    Reaeration Conductivity Data_DXX_StationID_YYYYMMDD
    Station ID should be the 4-letter Station ID

10.3.9 Refreshing the Field Sampling Kit

1. Restock the sampling kit (shipping coolers) with newly evacuated gas vials, new chloride sampling bottles (with new labels), syringe filters, needles, etc.

10.3.10 Equipment Maintenance, Cleaning, and Storage

Pump:

- Run clean water (this can be stream water) through the pump and tubing for 20-30 minutes to rinse salt water from equipment. You can do this while you complete sample processing.
- Rinse of external parts of the pump with freshwater to remove any salt solution.
- Upon returning to Domain support facility remove drive carrier and piston from pump. Remove piston from drive carrier cylinder and allow all parts to air dry before storing pump in a protected case.
- Empty all water from tubing before storage.
- 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 1b).

Charge batteries.

11 DEFINITIONS

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

Deaeration – loss of oxygen molecules from a liquid (or gas).

Diffusion – the movement of particles from an area of higher concentration to an area of lower concentration.

Discharge – the volume of water flowing past a point on a stream during a specified unit of time.

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

Gas exchange rate – see reaeration rate.

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

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

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

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

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

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

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

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APPENDIX A Field Data Sheets

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

BACKGROUND CONDUCTIVITY VALUES

Domain #: **Site Name:**

Date: **Attendees:**

Be sure to take conductivity readings across main flow of water.

Background (Pre-Injection) Conductivity (uS/cm @25C)

Station #	Measurement 1	Measurement 2	Measurement 3	Measurement 4	Notes
1					
2					
3					
4					

Domain #: **Site Name:**

Date: **Attendees:**

Station ID: Site #

Time	Conductivity (uS/cm @25C)	Time	Conductivity (uS/cm @25C)

Date:	**Domain #:**	**Site Name:**
Amt. Salt Added (g):	**Carboy Total Volume (units):**	
Drip Start Time:	**Drip End Time:**	**Site Attendees:**
Drip Rate Start:	**Drip Rate End:**	

Station #	Distance from Injection (m)	Time	Water Temp (°C)	Conductivity (uS/cm @25C)	Syringe ID	Vacutainer ID	Bottle ID	Notes
Injectate								
1								
1								
1								
1								
1								
2								
2								
2								
2								
2								
3								
3								
3								
3								
3								
4								
4								
4								
4								
4								

Be sure to take conductivity readings in main flow of water.
Sample at Plateau: 45 mL water equilibrated with 15 mL air
Grey cells do not require info filled in.

Domain #:		**Site Name:**		
Date:		**Attendees:**		

Take 30 stream width measurements between reaeration site 1 and site 2.

Replicate #	Stream Wetted Width (m)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	

Take stream widths after the injection is complete.

APPENDIX B Lab Data Sheets

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

W97

U.S. GEOLOGICAL SURVEY – NATIONAL WATER QUALITY LABORATORY
ANALYTICAL SERVICES REQUEST

THIS SECTION MANDATORY FOR SAMPLE LOGIN

NWIS RECORD NUMBER / SAMPLE TRACKING ID	User Code	Project Account	LAB USE ONLY / NWQL LABORATORY ID
	NEN	GR11DF00EEP1000	

STATION ID	Begin Date (YYYYMMDD)	Begin Time	Medium Code	Sample Type
D	20	0900	WS	9

Science Center Contact	Phone Number	End Date (YYYYMMDD)	End Time	Science Center Contact Email
GEBrown	303-236-3160			gebrown

SITE / SAMPLE / SPECIAL PROJECT INFORMATION (Optional)

State	County	Source Agency Code	Analysis Status*	Analysis Source*	Hydrologic Condition*	Hydrologic Event*	Chain of Custody	Sample Set
TN								

NWQL Proposal Number	NWQL Contact Name	NWQL Contact Email	Program/Project
CL11005	GEBrown	gebrown	

Station Name: Walker Branch STREON Reach, Oak Ridge, TN Field ID: _____

Comments to NWQL: Injection Sample, Expected Concentration: _____

_____

Hazard (please explain): _____

ANALYTICAL WORK REQUESTS: SCHEDULES AND LAB CODES (CIRCLE A=add D=delete)

SCHED 1: _____ SCHED 2: _____ SCHED 3: _____ SCHED 4: _____ SCHED 5: _____ SCHED 6: _____

Lab Code: 1571 **A** D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D
Lab Code: 69 A **D**	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D
Lab Code: 2902 **A** D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D	Lab Code: _____ A D

SHIPPING INFORMATION (Number of containers sent)

	ALF		COD		FA	1	FU		HUN		RCB		SAS		TOC
	BGC		CU		FAM		FUS		MBAS		RU		SUR		TPCN
	C18		CUR		FAR		GCC		RA		RUR		SUSO		UAS
	CC		DIC		FCA		GCV		RAM		RURCV		TBI		WCA
	CHL		DOC		FCC		HFL		RAR		RUS		TBY		

NWQL Login Comments: _____

_____

_____

Collected by: _____ Phone No. _____ Date Shipped: _____

FIELD VALUES

Lab/P Code	Value	Remark	Lab/P Code	Value	Remark	Lab/P Code	Value	Remark
21/00095 Specific Conductance uS/cm @ 25 deg C			51/00400 pH Standard Units			2/39086 Alkalinity – IT mg/L as CaCO3		

USDA ☐
Lab Use only
*MANDATORY FOR NWIS

Form 9-3094
(August 2010)

APPENDIX C Considerations for implementation

Exposing gas vials to heat should be avoided as increases in temperature can influence gas vial storage and increase the risk of gas lost. Changes in pressure (e.g. elevation changes) can increase the risk of gas lost and samples should be shipped ground.

It is extremely important that good travel time estimates be calculated. This should be easy to do during NEON Operations, as there will be sensors installed at Station 1 and Station 2. However, during Construction, thorough conductivity measurements should be recorded manually to estimate travel time.

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, if not multiple times during the injection.

Extremely saturated salt solution can clog the filter in the filter housing and influence the pump rate. Try to keep salt solutions to <50% NaCl.

Samples must be processed away from stream to avoid contamination. Common errors include:

1) 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.
2) Not sampling in thalweg
3) Forgetting to shake syringes prior to gas sampling.