

<i>Title:</i> AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling		<i>Date:</i> 11/07/2014
<i>NEON Doc. #:</i> NEON.DOC.001199	<i>Author:</i> K. Goodman	<i>Revision:</i> D

AOS PROTOCOL AND PROCEDURE: SURFACE WATER DISSOLVED GAS SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	05/30/2014	ECO-01838	Initial release
B	08/29/2014	ECO-02233	Minor updates based on feedback from the field
C	10/21/2014	ECO-02366	Migration to new protocol template
D	11/07/2014	ECO-02441	Minor updates following D04/D08 training, and shipment meetings with CLA and FOPS.

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1 OVERVIEW

1.1 Background

The following protocol outlines field sampling of surface water dissolved gas chemistry in aquatic environments. The movement of dissolved gases in water (e.g., diffusing, dissolving and reacting) is governed by the partial pressure of the gas, not the concentration. Gases move from zones of high to low pressure until equilibrium is reached. The dissolution of gases in water is a function of the solubility of the gas, which is dependent on the temperature and salinity of the medium. The distribution and partial pressure of dissolved gases in water at any point in time are dependent on physical (e.g., evaporation, evasion and advection), chemical (e.g., binding, pH), and biological (e.g., heterotrophic and autotrophic metabolism, methanogenesis, denitrification) processes and reactions occurring within the water body and across the sediment-water and water-atmosphere interfaces. The rates of these processes and reactions will govern if a system is undersaturated or supersaturated in dissolved gases relative to the atmosphere, which can fluctuate both daily and seasonally. In lakes, stratification can limit the exchange and movement of dissolved gases between the hypolimnion and epilimnion, further influencing the chemical speciation of gases (i.e., CO₂ and CH₄).

Although less attention is given to dissolved gases than dissolved nutrients, environmental changes such as the release of greenhouse gases and ozone degradation have led to increased measurements of dissolved gases in aquatic environments. Additionally, concerns over the effect of increased CO₂ on fish populations (i.e., elevated CO₂ can decrease metabolic efficiencies) have spurred the interest in measuring dissolved gas in aquatic environments (Danley et al. 2005). Stream dissolved chemistry may provide scientists, managers and decision makers with valuable information to consider potential water quality responses to natural and anthropogenic changes. Supersaturation and undersaturation of pCO₂ in freshwaters may result from nutrient loading, point and non-point pollution sources, and groundwater inputs. By assessing the degree of saturation of pCO₂ over time, annual net CO₂ balances may be inferred for the system and may provide insight about how changes in the surrounding landscape may influence stream function and structure. For example, how do increased nitrogen inputs influence stream denitrification? How are increases in CO₂ affecting the biological communities?

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.

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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 collecting surface water dissolved gas samples in small, wadeable streams, non-wadeable streams and lakes broadly follows the requirements set forth by Lotic Intersite Nitrogen eXperiment (LINX), the Environmental Protection Agency, the laboratories of Dr. Stephen Hamilton, Michigan State University, and the USGS Lake Monitoring Field Manual by Nevers & Whitman (2007).

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.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.014051	Field Audit Plan
AD[07]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002383	Datasheets for AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
RD[06]	NEON.DOC.001646	NEON General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy document
RD[08]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[09]	NEON.DOC.000694	AOS Protocol and Procedure: Surface Water Chemistry Sampling in Wadeable Streams
RD[10]	NEON.DOC.00 1190	AOS Protocol and Procedure: Lake and Non-Wadeable Stream Water Chemistry

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2.3 Acronyms

Acronym	Definition
mL	milliliter
P&P	Procedure and Protocol
USGS	United States Geological Survey

2.4 Definitions

Ambient: The conditions of the surrounding environment, such as the temperature of a stream where a sample was collected.

Headspace: A gaseous space above a closed liquid sample.

Supersaturation: A solution that contains a vapor of a compound that has a higher (partial) pressure than the vapor pressure of that compound.

Stratification: The thermal stratification of lakes refers to a change in the temperature at different depths in the lake that results from the change in water's density with temperature

Thalweg: Deepest part of the stream channel.

3 METHOD

The following protocol describes the collection, processing, storage and shipping of dissolved gas samples from aquatic environments, both for lakes and streams. In streams, samples are collected in the deepest part of the channel (A.K.A. thalweg, the line of least resistance to water flow, Figure 1), where it is assumed that the stream channel is well mixed. The sampling location is located away from, or upstream of, any major local disturbances and other areas where NEON sampling activities commonly occur. In streams with a shallow water column, technicians must be cautious not to disturb the benthic sediments when sampling. In lakes, samples are collected along a depth profile at the deepest point of the lake near the buoy (Figure 1). The vertical location of samples to be taken in lakes depends upon lake depth and stratification. For shallow (<5m) non-stratified lakes, one sample is taken from 0.5 m below the surface of the water. For shallow stratified lakes, two samples are taken: one from 0.5 m below the surface of the water and one 1 m above the sediment-water interface. For deep stratified lakes (>5 m), up to three samples are taken: one from 0.5 m below the surface of the water, one below the base of the thermocline and one 1 m from the sediment- water interface.

Disruption of the sediments by walking or by sampling too close to the stream and lake bottom can contaminate samples. Thus, always sample upstream from wading activity and minimize the suspension of sediments when sampling. If sediments are disrupted, wait until the area has cleared before sampling. Due to the diel variability in partial pressure of dissolved gases, samples should be consistently taken at the same time of day to allow for researchers to compare across sites and seasons. This is more important in lake environments than in streams.

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To ensure sample quality, dissolved gas samples should be collected upstream of any solute injection work occurring on the same day as sampling, as well as upstream and upwind of any fieldwork disrupting the stream or lake bottom (e.g., morphology mapping, invertebrate collection, macrophyte collection, etc.). When sampling ambient air, collectors must take care not to contaminate samples. Thus, the collector should sample by holding the syringe into the wind and away from humans or any other sources of carbon or nitrogen gas.

Properly evacuated vials will suck gas from the syringe into the vial. If a gas vial is improperly evacuated, resample and use another evacuated gas vial. Label all improperly evacuated vials as such and return to the gas lab with samples. While properly evacuated gas vials will suck in some sample, the collector must push as much gas into the gas vial as possible by pushing on the syringe plunger. Always hold the syringe upright when transferring gas from the syringe to the gas vial to avoid transferring liquid to the gas vial.

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

If the water chemistry sampling location is too shallow to obtain a sample, sample upstream in a flowing section of the stream (always sample in the thalweg, if possible) and note this change in field sheet. If no flow exists, sample in a nearby pool where the water is deep enough to obtain a clean, sediment free sample, and note this change in the field sheet. For lakes, also record new GPS position and total depth of the water column sampled from (RD[05]). Be sure to note this on the field sheet as a “non-flowing sample.” If the stream is entirely dry or frozen solid, note that on the field sheet. If the stream is ice-covered, but is still flowing, the ice should be broken so the stream can be sampled (following an few minute period to allow the water to clear). Always make note of any weather or stream conditions that could influence chemistry, including but not limited to wind, activities in the surrounding watershed, prior flood or rain events, ice, and changes in sampling locations (RD[05]).

If the lake is frozen only surficially and safe to walk on (minimum of 15 cm thickness) make a hole in the ice and proceed with sampling. The thickness of the ice shall be tested on the nearshore environment prior walking on the lake, by drilling a hole in the ice and measuring the ice thickness and consistency.

Samples should be processed as soon as possible. If necessary, stream water may be collected in a 60 mL syringe, submerged in a container of streamwater to reduce gas loss, and processed within 4 hours at a base camp or Domain Lab (i.e., if weather dictates that need to get out of the field immediately and stream discharge is increasing). Sample collection time, processing station and processing time must be recorded on the Dissolved Gas Sampling Data Sheet (RD[05]).

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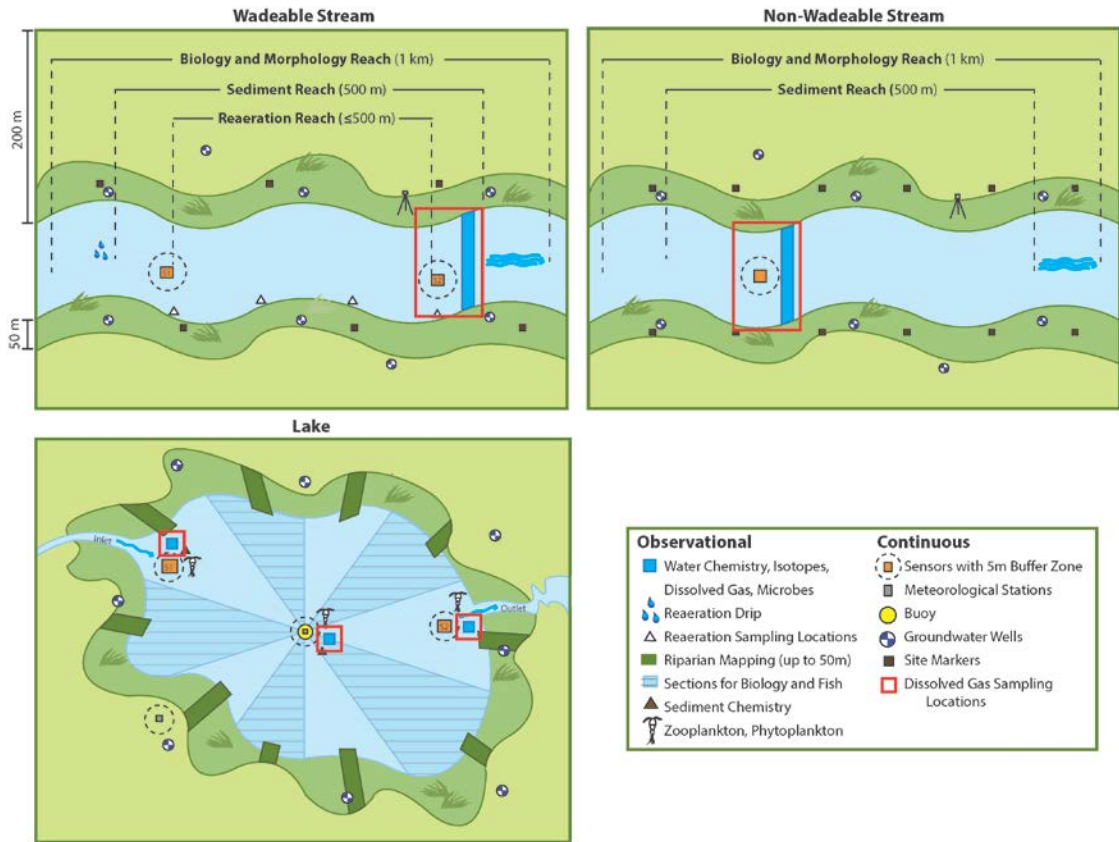


Figure 1. Generic aquatic site layouts with Dissolved Gas sampling locations

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

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

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[06]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[07]).

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4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Dissolved gas chemistry sampling occurs in conjunction with water chemistry (RD[09] and RD[10]) sampling 26 times per year in wadeable and non-wadeable streams/ rivers (approximately every other week) and 12 times per year in lakes. Samples should always be taken at the same time of day (i.e., 2 hours following sunrise +/- 1hr) in both lakes and streams in order to minimize errors incurred by natural diel variations in dissolved gases.

4.2 Criteria for Determining Onset and Cessation of Sampling

The timing of sampling allows researchers to assess aquatic biogeochemical cycles, and therefore timing depends on the dominant driver(s) of nutrient flux and cycling within each system. Timing of sampling is site-specific and determined by rules developed using historical discharge for streams and environmental data for both streams and lakes (RD[07]). For example, streams with little or no flow during the summer dry-season or streams that are frozen during the winter are sampled more intensively during other periods that have more flow. Systems that have a snowmelt-dominated or storm-dominated flow regime are sampled more intensively during elevated flows (i.e., time periods when the majority of the nutrients are moving through the system), and sampled sporadically during times of baseflow (RD[07]). Stream systems that are heavily influenced by winter rains are more heavily sampled in the winter. Samples in lakes will be taken approximately monthly, with several samples being taken to capture major events such as ice-off, major storm, turnover and stratification.

4.3 Timing for Laboratory Processing and Analysis

Stream and Lake Dissolved Gas Samples should be processed within 3-4 hours of sample collection. It is preferable for samples to be processed in the field immediately after collection; however, samples may be processed in the lab if conditions prevent this from happening. For storage and shipping timelines see SOP D.

4.4 Sampling Timing Contingencies

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Hours	If sampling stirred up sediments or added chemical constituents to the stream/lake (i.e., gas additions) within the past hour, allow the water to clear and disturbance to pass; sample upstream/upwind of the disturbance	No adverse outcome.

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5 SAFETY

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

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

Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is $\geq 10 \text{ ft}^2/\text{s}$ ($0.93 \text{ m}^2/\text{s}$) (AD[01]). When working around ice, use caution and good judgment to carefully evaluate site conditions including ice strength. Do not continue if the risk is too great.

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6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 2. Equipment list – Sample collection for surface water dissolved gas in wadeable streams, non-wadeable streams, and lakes

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	60 mL syringe, polyethylene	Sample collection	2*	N
	R	1-way male Luer-lock stopcock, polycarbonate	Sample collection, attach and close syringe	2*	N
	R	Sharps container	Needle disposal	1	N
	R	Handheld Conductivity/Temperature Meter	Temperature	1	N
	R	Storage container (pitcher)	Field storage and transport	1	N
Consumable items					
	R	12 mL evacuated gas vial(s) with rubber septa; evacuated <50 mTorr	Sample container	2*	N

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Dissolved Gas Labels (Waterproof Labels 1 * 2 ⁵ / ₈)	Labeling sample	2*	N
	R	50 mL polypropelene centrifuge tubes	Sample storage and shipping	2*	N
	R	Needles, 27 G ½ inch	Sample transfer from syringe/stopcock to gas vial	2*	Y

R/S=Required/Suggested

*1 per station (Lakes may have up to 4 stations) plus one background sample. Always take extra to the field.

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Table 3. Equipment list – Additional equipment and consumables for dissolved gas sampling in lakes and non-wadeable streams

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat and oars		1	N
	R	Safety kit		1	N
	R	Kemmerer sampler	Water collection	1	N
	R	Tubing with flow control hose clamp	Sample transfer from Kemmerer to bottles	1	N
	R	GPS		1	N
	R	First Aid Kit		1	N
	R	Waders or boots		2	N
	R	Personal Flotation Devices		2	N
Consumable items					
(None)					

R/S=Required/Suggested

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6.2 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians 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 wading stream dissolved gas measurements and safe working practices for stream fieldwork.

6.3 Specialized Skills

None.

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

Sampling and shipping will require 1-2 technicians for 1-2 hours each sampling day plus travel to and from the site.

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7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Prepare Labels

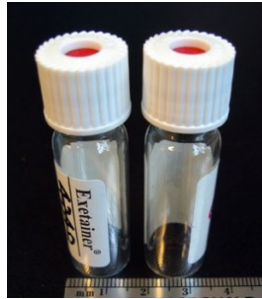


Figure 2. Gas vial with septum lid.
Image from <http://isotopes.forestry.ubc.ca>



NOTE: Labels are waterproof but should be filled out before getting wet to ensure ink will stick to the labels.

1. Attach pre-printed labels (1 * 2 ⁵/₈)(Figure 3) to gas vials (Figure 2). Use a **Sharpie or computer** to fill out labels (Figure 3) before going into the field. Site ID is the 4 letter site code. StationID is '1' for wadeable and non-wadeable streams, and for lakes "in", "out", "center". If "center" is stratified, "center1" or "center2", as necessary, with "center1" being the top layer. Sample Type should be labeled as W (sample water) or A (Air).

SampleID: _____
(siteID.stationID.YYYYMMDD.sampleType)
Gas Sample Type: W (Water) A (Air)
Project: NEON

Figure 3. NEON dissolved gas chemistry labels

2. Determine the depths for sampling lakes according to the data downloaded from the real-time data on the website or using the latest profiling data available acquired by the buoy. Look at the temperature profile and determine if and where stratification occurs and how many samples will be taken.
 - a. For shallow (<5 m) non-stratified lakes, one sample will be taken from 0.5 m below the surface of the water.
 - b. For shallow stratified lakes, two samples will be taken: one from 0.5 m below the surface of the water and one at the mid-point of the hypolimnion.
 - c. For deep stratified lakes (>5 m) where a deep hypolimnion is present, up to three samples will be taken: one from 0.5 m below the surface of the water, and one sample each at 30 and 60% depth of the hypolimnion.
 - d. Dissolved gas sampling in streams will be completed in same location as surface water chemistry (RD[09]), upstream of any stream disruption and in a flowing area of the stream, if possible.

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SOP B Field Sampling

B.1 Before Sampling



NOTE: Be cautious when sampling. Items can easily fall into the water while bending to sample.

1. Ensure the General AQU Field Metadata Sheet (RD[06]) is completed.
2. Rinse the collection syringe and stopcock twice with the stream/lake water.
 - a. Place the syringe tip (with 2-way stopcock attached and turned to open; Figure 4) into the stream or lake so that the water is sampled ~10 cm under the surface.

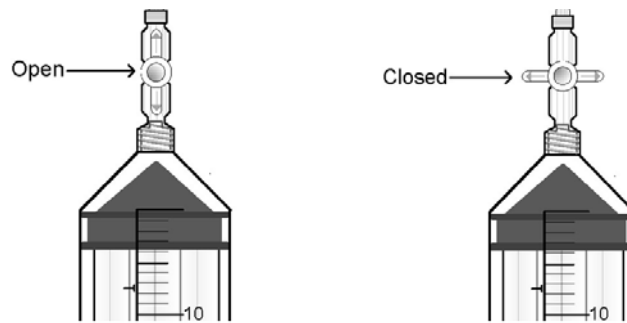


Figure 4. Syringe with a 2-way Luer lock stopcock in the “Open” and “Closed” positions

- b. Pull in ~20-30mL of water and remove syringe from stream or lake.
- c. Roll the water around the syringe and plunger tip to collect air bubbles.
- d. 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.
- e. Holding the syringe upright, expel the air.
- f. Repeat steps i-iv if large (>2 mm) air bubbles persist.

B.2 Collecting Samples in Wadeable Streams

1. ALWAYS sample in the THALWEG (the deepest location in the stream cross-section) and 5-10 centimeters below the surface (to avoid sampling floating material or surface film). If the usual location is too shallow select another location within the stream reach that is deep enough, preferably in the thalweg. Personnel can step into the stream, but must approach the sampling location from downstream. Be sure to take samples upstream from the standing location of the personnel.
2. 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, leaving approximately 1 mL in the syringe, which will help reduce the collection of air bubbles when sampling.

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- SLOWLY** pull the plunger to draw a water sample until the syringe is completely full with the plunger at the 60 mL mark (Figure 5). 60 mL is more than needed, but collecting more will make the processing step easier.



NOTE: Be careful not to entrain any air bubbles. If large air bubbles are captured in the syringe, resample.

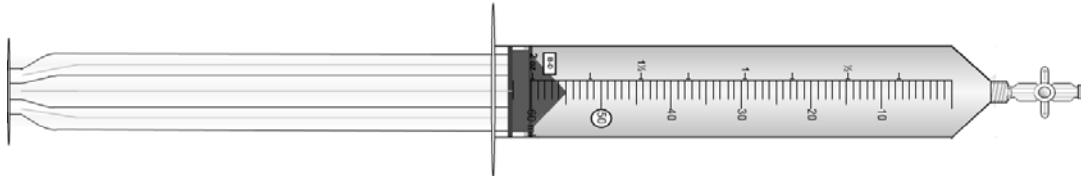


Figure 5. A full 60 mL syringe with a closed 2-way stopcock

- BEFORE** removing the syringe from the stream, immediately turn the 2-way stopcock to the closed position (Figure 4).
- Place the syringe in a storage container (i.e., small cooler, pitcher or bucket) of streamwater at ambient stream temperature to help maintain temperature prior to processing and to avoid degassing.
- Record water temperature at the time of collection on the Dissolved Gas Datasheet (RD[05])
- Proceed to B.4, Sample Processing.

B.3 Collecting Samples in Non-Wadeable Streams and Lakes

- Move to the sampling location.
- Note the GPS position on the field sheet.
- Open and secure the Kemmerer bottle.
- Gently lower the bottle to the desired depth (based on the center of the sampler) and release the messenger. Give the rope a slight tug to ensure the sampler has shut properly.
- Gently bring the Kemmerer back to the surface and ensure the sampler is tightly closed. Should the sampler not be properly closed, discard the sample and take another.
- Attach the tubing with the flow control hose clamp to the spigot of the Kemmerer (Figure 6).
- Open the spigot valve and then the hose clamp on the tubing and allow some water to pour out in order to flush the tubing.
- Attach the other end of the tubing with the female Luer-lock fitting to the Luer-lock stopcock (Figure 6).
- Rotate the stopcock valve so that the sample syringe is “Open.”
- Rinse the syringe by slowly filling the syringe to 30 mL with water and then 30 mL with air, shake vigorously for 30 seconds and discard the sample.
- SLOWLY** pull the plunger to draw a water sample until the syringe is completely full, the plunger at the 60 mL mark (Figure 5). 60 mL is more than needed, but collecting more will make the

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processing step easier. Be careful not to entrain any air bubbles. If large air bubbles are captured in the syringe, resample.

12. Place the syringe in a small cooler, pitcher or bucket of lake water at ambient water temperature to help maintain temperature prior to processing and to avoid degassing.
13. Record water temperature at the sampling location on the Dissolved Gas Datasheet (RD[05])
14. Proceed to B.4, Sample Processing.

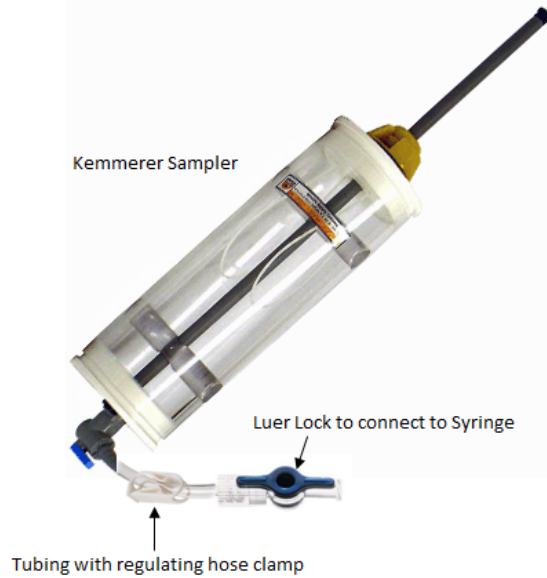


Figure 6. Tubing attachment with hose clamp and Luer-Lock to Kemmerer spigot

B.4 Processing and Transporting Samples



NOTE: Process stream and lake dissolved gas samples within 3-4 hours of sample collection.

1. Record the Date (YYYYMMDD) and the time of day (use military time and record the time zone, indicating standard or daylight (Ex. 13:46 MST)) that each sample was collected from the stream or lake on the bottle labels (Figure 3), the General Field Sampling Data Sheet and the Dissolved Gas Data Sheet (RD[05]). You DO NOT need to complete a second General Field Sampling Datasheet unless dissolved gas samples are collected on a different date than the water chemistry samples.
2. Point the sample syringe UPWARD and tap syringe to move any small air bubbles to the top of the syringe. With syringe still pointing up, push the plunger to the 40 mL mark to expel entrapped gas and excess sample (Figure 7).

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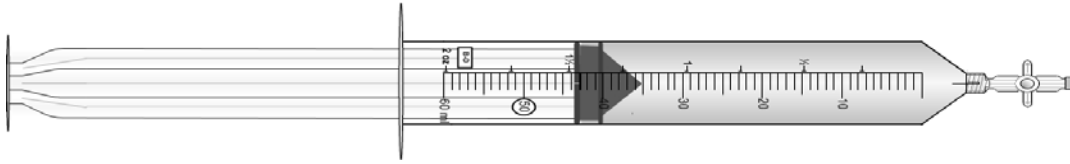


Figure 7. A syringe with 40 mL of sample water

3. Add 20 mLs of ambient air to the sample syringe:
 - a. Hold syringe at ~1.5 m above ground and at a full arms length from the body. Point syringe into the wind and away from contamination (e.g., human breath).
 - b. Draw in 20 mLs of ambient air. Rotate stopcock to close syringe (Figure 4).
4. The sample syringe should be filled to the 60 mL mark with 40 mL of sample water and 20 mL of ambient air headspace (Figure 8). Ensure stopcock is closed.

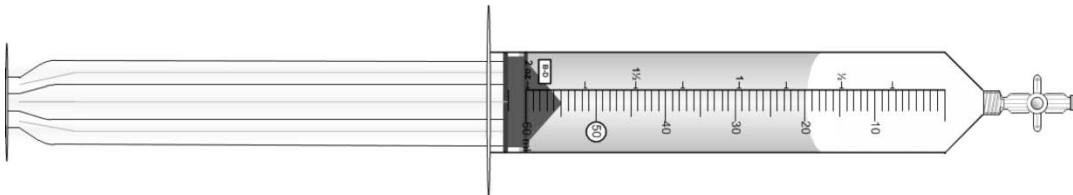


Figure 8. A syringe with 40 mL of water and 20 mL of ambient air

5. **Immediately** after adding 20 mLs of air to sample syringe, collect an ambient air sample using an empty syringe. This will be used to determine atmospheric gas concentrations, which will be used to correct the dissolved gas concentrations via subtraction:
 - a. Hold syringe into the wind and away from contamination (e.g., human breath).
 - b. Draw in 60 mLs of air and expel to flush the syringe and ensure no previous gas build-up.
 - c. Draw in at least 20 mLs of ambient air.
 - d. Rotate stopcock to close syringe (Figure 4).
6. Shake the sample syringe for the full 5 minutes so that the dissolved gases in the sample water come to equilibrium with the ambient air in the headspace.
 - a. **NOTE:** It is important to be consistent and shake the syringe for a minimum of 5 minutes since this affects the amount of dissolved gas released into the headspace.
 - b. Hold the syringe where the plunger enters the syringe. Avoid holding the syringe at the sample water, as this will increase the sample water temperature. Avoid holding the syringe in direct sunlight.
7. **Record** the temperature of the sample storage water (the water in the bucket) for each sample ID.
8. **Record** the sample type (i.e., sample water (S) or ambient air (A), volume of sample water and gas in the syringe on the Dissolved Gas Data Sheet (RD[05]). This should be done **immediately before gas transfer**. Be sure the labels on the gas vials match the sample ID's and type on the data sheet.
9. Transfer dissolved gases to an evacuated gas vial (Figure 9).



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- a. Attach the needle to the 2-way stopcock on the syringes.
 - b. With stopcock 'open' and syringe held upright, expel a few milliliters of the syringe headspace gas to flush the needle.
 - c. Insert the needle through the rubber septum of the gas vial (Figure 9) and push gas into the vial. Properly evacuated vials will automatically suck in some gas. Be sure to push as much gas into the vial as possible until gas vial is over-pressurized (i.e., inject more gas than the volume of the gas vial). If vial is not properly evacuated, re-do the sampling and processing (B.1) using the extra, evacuated gas vials.
 - d. Continue to push gas into the vial while removing the needle from the vial septum as quickly as possible. Do this before turning off the stopcock because turning the stopcock to the closed position can cause some air to be drawn back into the syringe.
 - e. Turn stopcock off.
 - f. Ensure sample labels are completed, as appropriate.
 - g. **Record** the volume of gas injected into the gas vial (**read the syringe to determine volume of gas injected**) on the Dissolved Gas Data Sheet (RD[05]).
 - h. Repeat step 9 for all Sample ID's.
10. Discard needles into a sharps container with a lid.



Figure 9. Transfer of dissolved gases to an evacuated gas vial.

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11. Store gas vial by submersing upside down in water filled 50-mL centrifuge tubes (Figure 10).



Figure 10. Dissolved gas samples stored in centrifuge tubes.

12. Return to the Domain Support Facility for sample storage and/or shipping.

B.5 Ending the Sampling Day

1. Refreshing the sampling kit
 - a. Restock the sampling kit with new evacuated dissolved gas sampling vial (with new labels attached), centrifuge tubes, needles, etc. Refer to equipment list, Section 10.3.1
 - b. Syringes will eventually begin to wear as the rubber of the plunger wears. Replace syringes when they become noticeably harder to draw in water.
2. Equipment maintenance, cleaning and storage
 - a. Ensure all equipment is properly decontaminated (see RD[08]) and dry prior to storage.

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SOP C Data Entry and Verification

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

Information from the Surface Water Dissolved Gas Datasheets (RD[05]) should be entered into excel file 'Dissolved Gas data entry'. Files should be uploaded as sdg_YYYYMMDD_in_DXX

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SOP D Sample Shipment

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

Shipments are to have a hardcopy of a shipping inventory sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking # in the email.

D.1 Handling Hazardous Materials

N/A

D.2 Supplies/Containers

1. Ensure gas vials are placed upside-down in 50-mL centrifuge tubes. Centrifuge tubes should be ~ 2/3 full with water. This will allow for water expansion if freezing occurs, without breaking the gas vial.
2. Place the 50-mL centrifuge tubes (with upside down gas vials) in resealable plastic bag inside the same cooler that the centrifuge tubes and evacuated vials were delivered in.
 - a. Centrifuge tubes should stand upright to ensure water surrounds the gas vial lid to reduce the risk of gas leakage.
3. Pack absorbent material around tubes and fill additional space with regular packaging materials.
4. Place shipping inventory in a resealable plastic bag and tape to the inside top of cooler

D.3 Timeline and Conditions

Samples can be stored at room temperature (20°C +/- 5°C) for a couple weeks. Store samples upside down in water filled centrifuge tubes. Ship gas samples Ground to the gas analysis lab bi-monthly.

D.4 Grouping/Splitting Samples

Organize by Site ID, if applicable.

D.5 Return of Materials or Containers

There is no return of materials or containers associated with this protocol. However, new evacuated vials will need to be ordered and shipped directly from the lab before each sampling bout.

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D.6 Shipping Inventory

Fill out the AOS Sample Shipping Inventory found on the CLA SharePoint page. Each box sent should have a shipping inventory of its contents. The electronic shipping inventory that includes IDs of all tubes shipped should be emailed to the appropriate contact at the receiving analytical laboratory as well as the NEON CLA contact on the day that samples ship. Include shipping IDs and estimated arrival date(s)/time(s) in the email as well.

D.7 Laboratory Contact Information and Shipping/Receipt Days

See the [CLA shipping document](#) on [CLA's NEON intranet site](#).

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8 REFERENCES

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- Hamilton, S.K., and N.E. Ostrom. 2007. Measurement of the stable isotope ratio of dissolved N₂ in ¹⁵N tracer experiments. *Limnology and Oceanography: Methods* 5: pp. 233-240.
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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 4. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.002383	Datasheets for AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
NEON.DOC.001646	NEON General AQU Field Metadata Sheet

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

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APPENDIX B QUICK REFERENCES

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

Step 2 – Prepare labels (1 * 2 ⁵/₈”).

SampleID : _____
(siteID.stationID.YYYYMMDD.sampleType)
Gas Sample Type: **W** (Water) **A** (Air)
Project: NEON

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

Step 4 – Navigate to the specified sampling location and rinse the collection syringe twice with stream/lake water.

Step 5 – Collect 60 mL of sample water. Close stopcock.

Step 6 – Point the sample syringe UPWARD, open stopcock, and push the plunger to the 40 mL mark to expel entrapped gas and excess sample.

Step 7 – Add 20 mLs of ambient air to the sample syringe. Close stopcock.

Step 8 – **Immediately** after adding 20 mLs of air to sample syringe, collect an ambient air sample using an empty syringe.

Step 9 – Shake the water sample syringe for **5 minutes**.

Step 10 – Record the temperature of the sample storage water, the sample type (i.e., sample water (S) or ambient air (A)), volume of sample water and gas in the syringe on the Dissolved Gas Data Sheet (RD[05]).

Step 11 – Open stopcock and transfer gas samples to an evacuated gas vial. Be sure to hold the syringe with water and gas upright so gas goes into gas vial. Continue to push gas into the vial while removing the needle from the vial septum as quickly as possible. Do not close stopcock before removing needle from vial. Discard needles into a sharps container with a lid.

Step 12 – Store gas vials by submersing upside down in water filled 50-mL centrifuge tubes.

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APPENDIX C REMINDERS

Sampling Locations: Make sure to collect samples...

- Wadeable streams: in the thalweg at the Water Chemistry sampling location near S2.
- Non-Wadeable streams: in the thalweg at the Water Chemistry sampling location near the sensor set.
- Lakes:
 - For shallow (<5 m) non-stratified lakes: from 0.5 m below the surface of the water.
 - For shallow stratified lakes, two samples will be taken: one from 0.5 m below the surface of the water and one at the mid-point of the hypolimnion.
 - For deep stratified lakes (>5 m) where a deep hypolimnion is present, up to three samples will be taken: one from 0.5 m below the surface of the water, and one sample each at 30 and 60% depth of the hypolimnion.

Collecting Samples: Make sure to...

- Rinse the sample syringe twice with stream/lake water.
- Remove large air bubbles from water sample syringe.
- Store water sample syringe in a bucket of water if transporting back to the lab to process.
- Use stopcock to ensure no sample is lost during storage or shaking.
- Collect ambient air sample **immediately** after adding 20 mLs of ambient air to sample water syringe.
- Shake for the full **5 minutes**.
- Carefully record all metadata, measurements, and observations on data sheet.