AOS PROTOCOL AND PROCEDURE: SURFACE WATER DISSOLVED GAS SAMPLING

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See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	10/04/2013	ECO-01152	Initial release
B	05/30/2014	ECO-01838	Minor updates based on feedback from the field
C	08/29/2014	ECO-02233	Migration to new protocol template
D	10/21/2014	ECO-02366	Minor updates based on feedback from the field following D04/D08 training, and shipment meetings with CLA and FOPS.
E	11/07/2014	ECO-02441	Minor updates based on feedback from the field
F	03/26/2015	ECO-02646	Minor updates based on feedback from the field and changes to lake sampling design
G	06/25/2015	ECO-02985	Changes to lake and non-wadeable sampling depths and sample integration, and lake and non-wadeable equipment setup including the addition of a 3-way luer-lock stopcock
H	01/21/2016	ECO-03423	Updates following FOPs review
J	12/12/2016	ECO-04367	2016 updates following FOPS training and reviews; updated template; clarity added on sample volumes; River stationID changed to ‘c0’, no longer ‘rs’; Updated shipping info and data entry.
K	04/04/2017	ECO-04582	Pressure to be recorded at sample processing, not collection. Clarity added to lake sampling in winter.

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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, or 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., CO_2 and CH_4).

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 CO_2 on fish populations (i.e., elevated CO_2 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 pCO_2 in freshwaters may result from nutrient loading, point and non-point pollution sources, and groundwater inputs. By assessing the degree of saturation of pCO_2 over time, annual net CO_2 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 are increases in CO_2 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.

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.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC. 004104	NEON Science Performance QA/QC Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.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
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

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), where it is assumed that the stream channel is well mixed. The sampling location is located near the sensor set where water chemistry samples are collected and 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, sample collection depends on lake depth and stratification, as detailed below (Figure 1b). For all lakes, one sample is taken at 0.5 m (Kemmerer should be placed with top at 0.25 m and bottom at 0.75 m) below the surface of the water from the inlet, outlet and buoy locations (3 water dissolved gas samples). If the lake is stratified at the time of sampling, an additional sample will be collected from the hypolimnion at the buoy location (4 total dissolved gas samples). In lakes with **very** shallow hypolimnions (< 2 m) do not collect a hypolimnion sample. In lakes with shallow hypolimnions (2-4 m), the sample is collected from the mid-point of the hypolimnion. In lakes with deep hypolimnions (>4 m) an integrated sample is collected as described in the water chemistry protocol (RD[10], Figure 1b)

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.

To ensure sample quality, dissolved gas and associate air samples should not be collected on the same day as reaeration gas injections. Samples should be collected 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 **stream or lake** water chemistry sampling location is too shallow to obtain a sample, sample in the nearest location where it is possible to obtain a sample and note the new GPS coordinates in field sheet. If no flow exists in the **streams**, 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 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 a few minute period to allow the water to clear). If the **lake** is frozen only surficially and safe to walk on (minimum of 15 cm thickness for waling and 20 cm for using a UTV) make a hole in the ice and proceed with sampling. The thickness of the ice shall be tested on the nearshore environment prior to walking on the lake by drilling a hole in the ice and measuring the ice thickness and consistency. Always make note of any weather or stream/lake 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]).

Samples should be processed as soon as possible. If necessary, water may be collected in a 60 mL syringe, submerged in a container of sample water to reduce gas loss, and processed within 3 hours streams or 6 hours for lakes 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]).

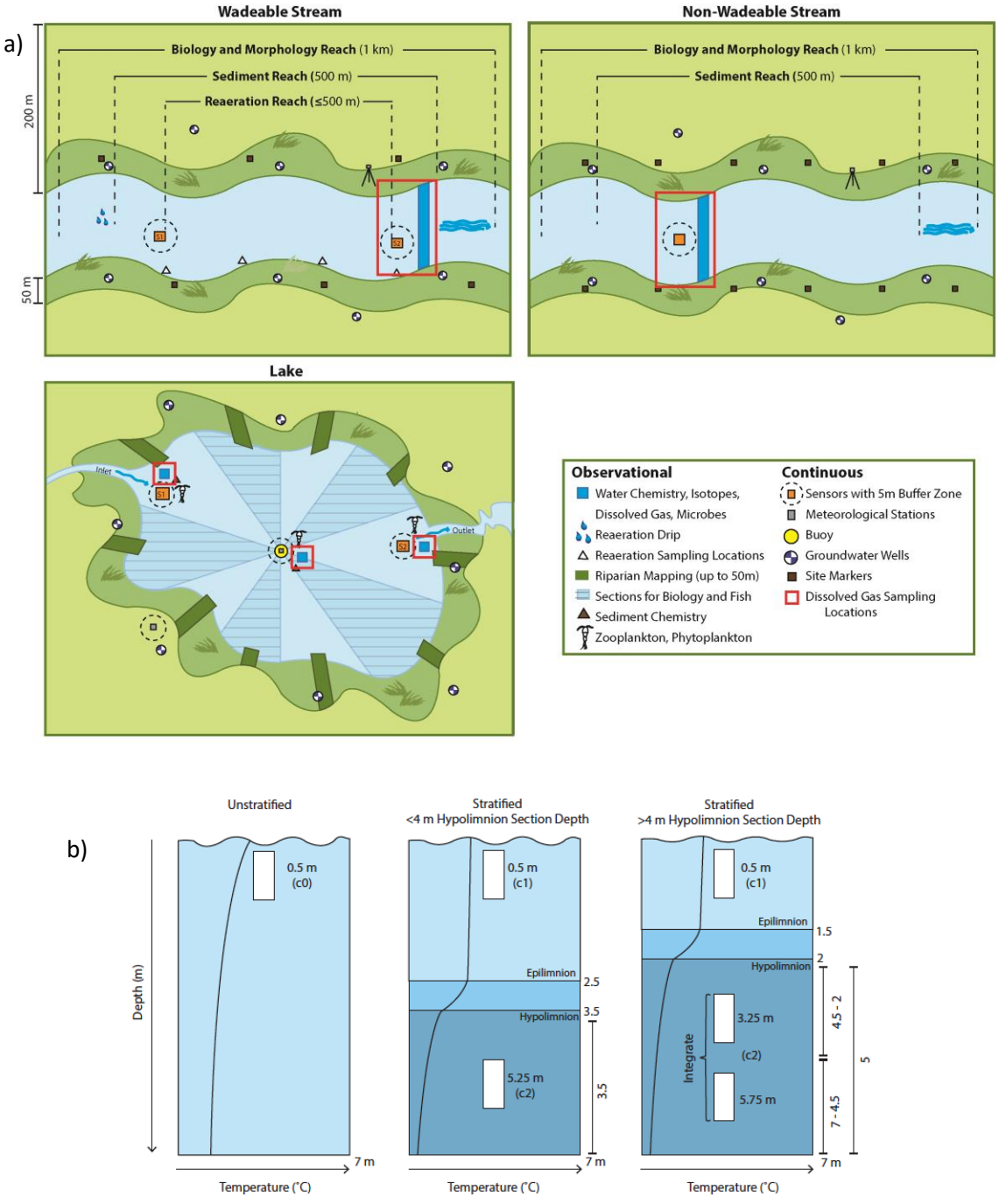


Figure 1. a) Generic aquatic site layouts with Dissolved Gas sampling locations and b) lake center sampling depths dependent on stratification and hypolimnion depth.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and

associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

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

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

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 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 the stream 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 on field sheet.	No adverse outcome.
	If no streamflow exists, sample in a nearby pool where the water is deep enough to obtain a clean, sediment free sample, and be sure to note this change in the field sheet as a "non-flowing sample."	No adverse outcome.
	If the stream is entirely dry or frozen solid, note that on the field sheet.	No adverse outcome.
	If the stream is ice-covered, but is still flowing, the ice should be broken so the stream can be sampled (following a few minute period to allow the water to clear).	No adverse outcome.
	If the lake surface is frozen and safe to walk on (minimum of 15 cm thickness for walking and 20 cm thickness for use of UTV/snowmobiles, etc.) make a hole in the ice and proceed with sampling. The thickness of the ice shall be tested on the nearshore environment prior to walking on the lake, by drilling a hole in the ice and measuring the ice thickness and consistency (RD[08]).	No adverse outcome.
	During lake sampling in freezing conditions, if you are unable to use the tubing to collect a sample because the water in the tube is freezing, you may collect out of the top of the Kemmerer while minimizing the time the water sample is exposed to air. Do not transfer the water to a 4L jug before collection, as that will increase air contamination.	No adverse outcome.
	If sampling stirred up sediments or added chemical constituents to the water within the past hour, allow the water to clear and disturbance to pass or sample in a different location.	No adverse outcome.

	If weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>20 mph) and has unsafe wave heights (>1 m) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	No adverse outcome.

4.5 Criteria for Permanent Reallocation of Sampling Within a Site

Dissolved Gas sampling will occur on the schedule described above at 1 (streams/rivers) and 3-4 (lakes) locations per site. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given station becomes compromised, a problem ticket should be submitted by Field Operations to Science.

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

5 SAFETY

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

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

Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s) (AD[02]). When working around ice, refer to (AD[02], Section 10.3 Winter Water Safety. Do not attempt to walk on frozen lake is depth of ice is less than 6" (+15cm) or operate UTV or snowmobile on frozen lake if depth of ice is less than 8" (20cm). Use

caution and good judgment to carefully evaluate site conditions including ice strength. Local guidelines from natural resource officials, property owners or hosts, and domain managers should be consulted regarding work on ice, prior to deploying employees and equipment. Do not continue if the risk is too great.

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

1. Due to site-specific hazards that may be encountered, technicians may perform GPS positioning around the lake, and measurements for inflow and outflow, without dismounting from the vessel. In addition, technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All personnel must be wearing a personal flotation device prior to entering the boat, and in wadeable streams when conditions are approaching the allowed wading limit (AD[02]).
3. All employees shall have access to a form of communication with other team members such as a two-way radio.
4. Technicians should be aware of any site-specific water hazards of that particular location (i.e., current status, tidal charts, etc.).
5. If personnel or loads will be on ice while performing their task for greater than 2 hours, all loads should be multiplied by 2 to determine safe ice thickness.
6. Access to Safety Data Sheet shall be available for work with chemicals (including Dry Ice) associated with this protocol.

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					
MX100554	R	60 mL syringe, polyethylene	Sample collection	2*	N
MX101261	R	2-way male Luer-lock stopcock, polycarbonate	Sample collection, attach and close syringe	2*	N
MX110214	R	3-way male Luer-lock stopcock, polycarbonate	Sample collection for integrated samples	1	N
	R	Sharps container	Needle disposal	1	N
MX100514	R	Handheld Conductivity/Temperature Meter	Temperature and Conductivity Meter – must order DO Probe addition separately (MX110375)	1	N
MX110375	R	DO probe for conductivity/temperature handheld meter (MX100514)	DO Galvanic Probe and replacement tips – must order separately	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX107554		Replacement DO sensor tips for MX110375	Replacement part for DO sensor tips – order when appropriate.	As needed	N
MX106201	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 -ordered from HQ based on sampling schedule	Prepared sample container shipped direct to Domain from external lab	2*	N
MX106268	R	Dissolved Gas Labels (Waterproof Labels 1 * 2 $^{5}/_{8}$)	Labeling sample	2*	N
	R	50 mL polypropylene centrifuge tubes – ordered from external lab with gas vials.	Sample storage and shipping - shipped direct to Domain from external lab	2*	N
MX106200	R	Needles, 27 G ½ inch	Sample transfer from syringe/stopcock to gas vial	2*	Y
	R	Clear Packing tape	Secure labels to vials prior to shipping		N

R/S=Required/Suggested

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

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
MX100393	R	Kemmerer sampler	Water collection	1	N
MX100364	R	Tubing (1/8” ID)	Sample transfer from Kemmerer to syringe	1	N
	R	Tubing (3/8” ID)	Sample transfer from Kemmerer to syringe	1	N
	R	Flow control hose clamp	Sample transfer from Kemmerer to syringe	1	N
MX110214	R	3-way stopcock	Sample transfer from Kemmerer to syringe		
MX100662	R	3/8” to 1/8” tubing adaptor	Sample transfer from Kemmerer to syringe	1	N
	R	GPS (Acuuracy < 4m)		1	N
	R	First Aid Kit		1	N
	R	Waders or boots		2	N
	R	Personal Flotation Devices		2	N

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

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete required safety training. Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]). Personnel are to be trained in wading stream dissolved gas measurements and safe working practices for stream fieldwork.

All personnel required to operate a boat shall be trained through a NEON Safety approved program (AD[02]). All others shall be aware of boating safety procedures.

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. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Sampling and shipping will require 1-2 technicians for 1-2 hours each sampling day plus travel to and from the site. More time may be required in lakes given the multiple sampling stations (~ 1 hour per station).

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

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

A.2 Check gas vials

Vials should be stored in the water they were shipped in prior to use. Check vial integrity before using. Vials should have no water in them prior to use. If there is water in the vials, DO NOT USE. Water in vials is an indication that the vacuum has been lost and the vials are no longer evacuated. The general recommendation is vials should be used within 1-2 months.

A.3 Prepare Labels

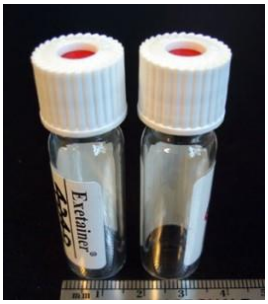


Figure 2. Gas vial with septum lid.
Image from http://isotopes.forestry.ubc.ca

NOTE: Labels are waterproof but should be filled out before getting wet to ensure ink will stick to the labels. Labels and vials must be wrapped with clear packing tape prior to shipping to ensure label adheres to vial.

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.
2. Use SampleID: SITE.StationID.YYYYMMDD.SampleType. SITE is the 4-letter site code. StationID is 'ss' for wadeable streams, 'c0' for non-wadeable streams/rivers, and for lakes 'in', 'ot', and 'c0' at lake center. If the center is stratified, use 'c1', 'c2', or 'c3' as necessary, with "c1" being the top layer. Sample Type should be WAT (sample water) or AIR (Air). For lakes, only one air

sample should be collected per site and date. The stationID for this lake 'AIR' sample should be c0.

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

Figure 3. NEON dissolved gas chemistry labels

3. Dissolved gas sampling will be completed in the same location and at the same depth as surface water chemistry samples (RD[09] and RD[10]), upstream of any stream disruption and in a flowing area of the stream, if possible.
4. 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 (Figure 1b).
    a. For non-stratified lakes, one sample will be taken from 0.5 m below the surface of the water at the inlet, outlet and buoy location.
    b. For stratified lakes where there hypolimnion section depth is <2 m, sample as if the lake was non-stratified and only collect one sample at 0.5 m below the surface.
    c. For shallow stratified lakes where the hypolimnion section depth 2-4 m, at the buoy one sample will be collected from 0.5 m below the surface of the water and one at the mid-point of the hypolimnion. Inlet and outlet locations sampled at 0.5 m depth.
    d. For deep stratified lakes where the hypolimnion segment depth >4 m: at the buoy one sample will be collected from 0.5 m below the surface of the water, and for the second sample, take samples at 25% and 75% depth of hypolimnion (divide the hypolimnion depth by 2 and take a sample in the midsection of both those layers). Integrate the samples from the hypolimnion into 1 sample. Inlet and outlet locations sampled at 0.5 m depth.

SOP B Field Sampling

B.1 Before Sampling

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

Ensure the General AQU Field Metadata Sheet (RD[06]) is completed.

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.
2. Rinse the collection syringe and stopcock three times with the sample water.
    a. Place the syringe tip (with 2-way stopcock attached and turned to open (Figure 4)) into the stream so that the water is sampled ~10 cm under the surface. Stopcock should remain on the syringe at ALL times.

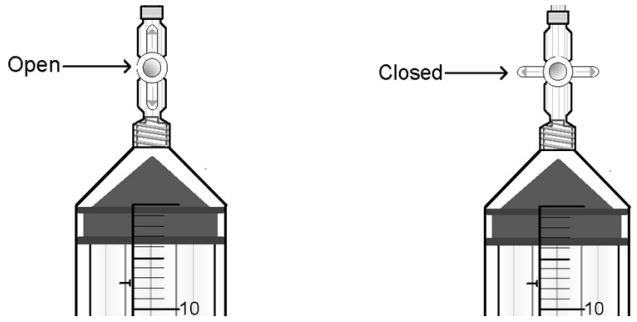


Figure 4 Syringe with a 2-way Luer lock stopcock in the "Open" and "Closed" positions

    b. Pull in ~20-30 mL of water and remove syringe from stream.
    c. Draw in air, close stopcock, and shake syringe vigorously for ~5-10 seconds.
    d. Expel air and water and repeat rinsing steps 1 more time.
    e. After the second rinse:
        1) Place the syringe under the water and draw in ~40 mL of water and 20 mL of air. These volumes do not have to be exact.
        2) Roll the air slowly around the syringe and plunger tip to collect air bubbles.
        3) Turn syringe tip-upward and tap the side of the syringe to release any trapped air bubbles. Large air bubbles affect volume readings so they should be removed. Tap hard to remove air bubbles. Removing small air bubbles may not be possible.
        4) Holding the syringe upright, expel the air and all but 3-5 mL of the water sample.

3. Place the syringe tip back into the water so that it is ~10 cm below the surface, and expel the remaining water into the stream, which will help reduce the collection of air bubbles when sampling.
4. **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.

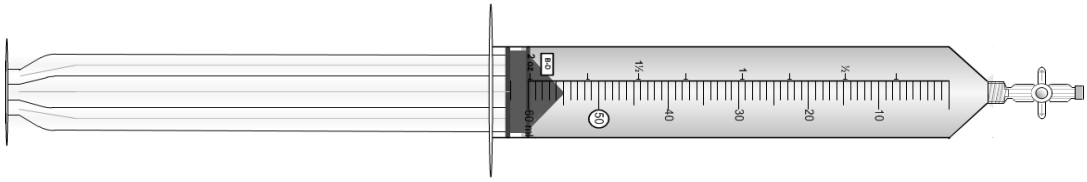


Figure 5. A full 60 mL syringe with a closed 2-way stopcock

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

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

1. Move to the sampling location.
2. Note the stationID on the field sheet.
3. Collect Samples:
   a. For non-integrated samples:
      1. Open and secure the Kemmerer bottle.
      2. 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.
      3. 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. Should the sampler not be properly closed, discard the sample and take another.
      4. Attach the tubing to the spigot of the Kemmerer (Figure 6).
      5. Open the spigot valve and allow some water to pour out in order to flush the tubing.

6. Attach the open end of the tubing to the Luer-lock stopcock and 60 mL syringe (Figure 6).
7. Rotate the stopcock valve so that the sample syringe is "Open."
8. Rinse the syringe by filling the syringe with 20-30 mL of water. Turn Kemmerer spigot to 'off'. Remove the syringe and stop-cock from tubing and rotate stopcock value to closed. Draw in ~30 mL of air, either by removing the syringe from the Kemmerer tubing or drawing in air through the side port of the 3-way stopcock., Shake vigorously for ~5-10 seconds and discard the sample. NOTE: I suggest you keep the syringe and stopcock attached to the Kemmerer this entire time by drawing in air, shaking, and then discarding rinse water through the side-port of the syringe. This removes extra air in the Kemmerer tubing and makes sampling without air bubbles easier.
9. Repeat, rinsing 1 more time.
10. After the second rinse (Note this step is considered your 3rd rinse);
    a) Draw in ~40 mL of water and 20 mL of air. These volumes do not have to be exact.
    b) Roll the air slowly around the syringe and plunger tip to collect air bubbles.
    c) 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.
    d) Holding the syringe upright, expel the air and all but ~1 mL of the water sample.
    e) If you removed the syringe from the Kemmerer tubing, re-attach the stopcock to Kemmerer tubing. Be sure tubing is full of water before attaching or you will get air bubbles in your sample. You can use a 3-way stopcock as described, in detail, in the integrated sample section below, as this can make obtaining an air-free sample easier.
11. 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 processing step easier. Be careful not to entrain any air bubbles. If large air bubbles are captured in the syringe, resample.
12. Collect all water samples at all stations before processing.
13. Proceed to B.4, Sample Processing, once all samples are collected.

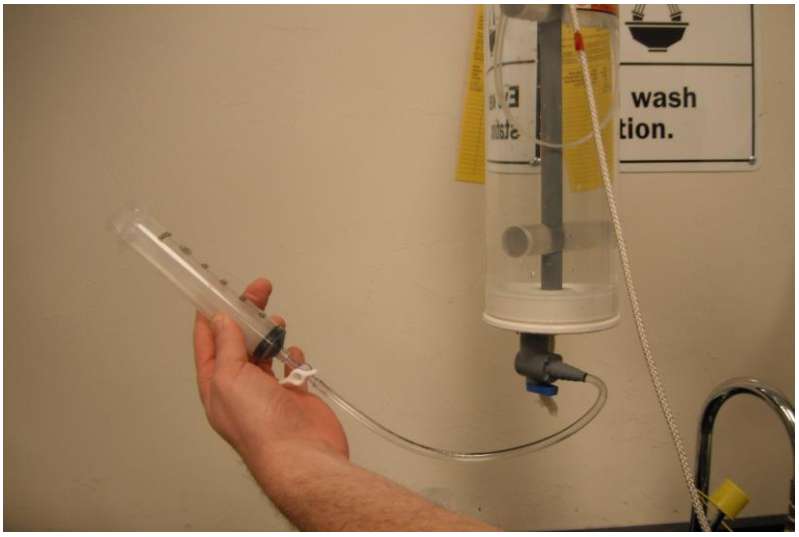


Figure 6. Syringe and 2-way stopcock attached to Kemmerer spigot

a. For integrated samples:

1) Sample 1 of 2

a) Open and secure the Kemmerer bottle.

b) Gently lower the bottle to the first 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.

c) 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.

d) Attach the tubing to the spigot of the Kemmerer (Figure 7).

e) Attach the other end of the tubing to the 3-way stopcock and the syringe (**Figure 7**)

f) Turn 3-way stopcock so the 'off' position is towards the syringe (**Figure 7**).

g) Open the spigot valve and allow some water to pour out of the 3-way stopcock side port in order to flush the tubing.

h) Rotate the 3-way stopcock valve so that the sample syringe is 'open' and the side-port is 'off'

i) Rinse the syringe three times by slowly filling the syringe with ~ 20- 30 mL of water and then ~30 mL with air, shake vigorously for 5-10 seconds, reattach the syringe to the 3-way stopcock, rotate stopcock so the Kemmerer is in the 'off' position, and discard the sample through the side port on the 3-way stopcock.

j) On the 3rd rinse, expel all be ~1 ml of sample water in the syringe through the side-port, rotate the stopcock valve so the 'off' position is at the side port.

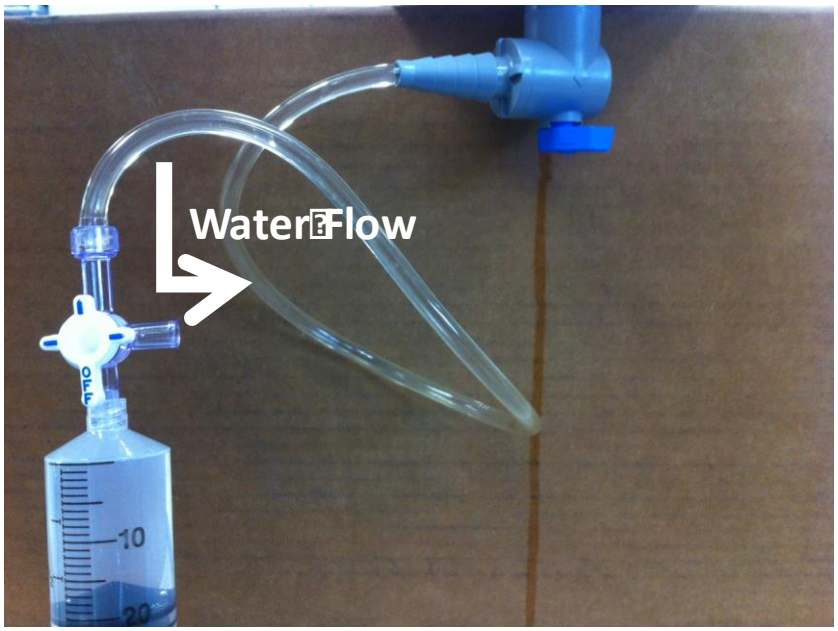
k) SLOWLY pull the plunger to draw a water sample until the plunger is at the **20 mL mark**. Be careful not to entrain any air bubbles. If large air bubbles are captured in the syringe, resample.

l) Use this 20 mL sample when collected sample 2 of 2 (step 2 below)

1) **Sample 2 of 2**
   a) Empty the water in the Kemmerer, and place it back in the water to sample at the second depth of the integrated sample.
   b) Pull the Kemmerer back up and attach tubing to the Kemmerer and the 3-way stopcock with the 20 mL sample you just collected above.
   c) Turn stopcock so that the 'off' position is towards the syringe. Flush tubing through the side port of 3-way stopcock.
   d) DO NOT RINSE syringe with second sample.
   e) Rotate the stopcock so the 'off' position is at the side port. Draw in 20 mL of sample water into the syringe. (Syringe plunger should be at 40 mL mark).
   f) Rotate stopcock so the 'off' position is towards the syringe and remove stopcock from tubing.
2) Your sample is complete.

4. Place the syringe in a small cooler, pitcher or bucket of sample water at ambient water temperature to help maintain temperature prior to processing and to avoid degassing.
5. Record water temperature at the sampling location on the Dissolved Gas Datasheet (RD[05])
6. Collect all water samples at all stations before processing.
7. Proceed to B.4, Sample Processing.

a)



b)

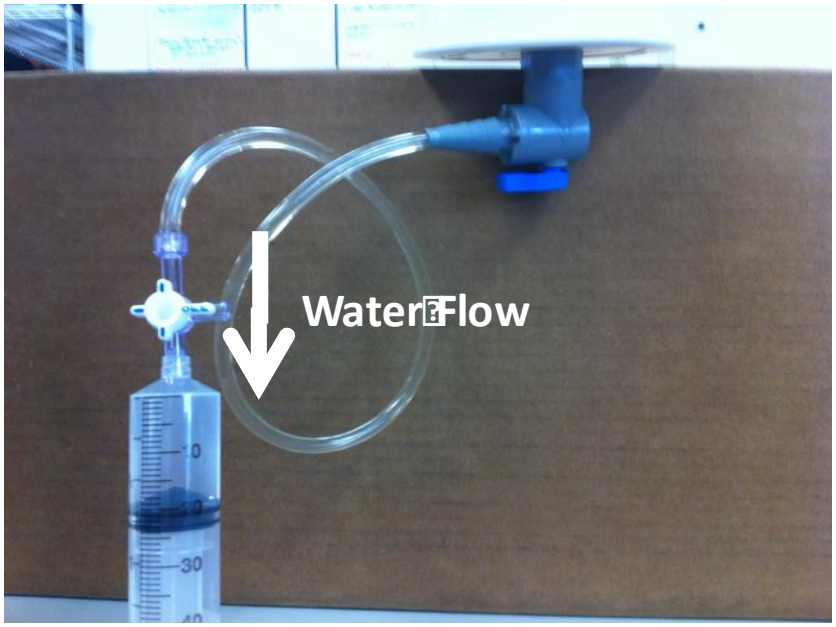


Figure 7 a) 3-way Luer-lock to Kemmerer spigot via tubing. Syringe is in the 'off' position allowing water to flow out the side port and b) 3-way Luer-lock to Kemmerer spigot via tubing. Side port is in the 'off' position, allowing water to flow into syringe.

B.4 Processing and Transporting Samples

NOTE: Process dissolved gas samples within 3 hours of sample collection (6 hours maximum allowable for lakes). All water and air samples must be processed at the same time, so that the AIR sample provides a consistent atmospheric gas reference. Air should be pulled into the syringes as close to the same time as possible (i.e. one right after the other). All air, including the air sample, should be collected within 5 minutes of each other.

1. Record the Date (YYYYMMDD) and the time of day (use military time and record the local time (Ex. 13:46) that each sample was collected on the Dissolved Gas Data Sheet (RD[05]). You DO NOT need to complete a second General Field Metadata sheet unless dissolved gas samples are collected on a different date than the water chemistry samples.
2. Record the barometric pressure at time of sample processing.
3. 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 8). You will not need to do this step for integrated samples, since you only sampled 40 mL.

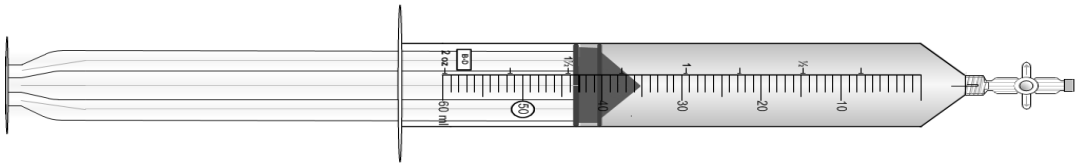


Figure 8. A syringe with 40 mL of sample water

4. Add 20 mL 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. Pull the syringe plunger to 60 to draw in 20 mL of ambient air. Rotate stopcock to close syringe (Figure 4).
5. 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 9). Ensure stopcock is closed.

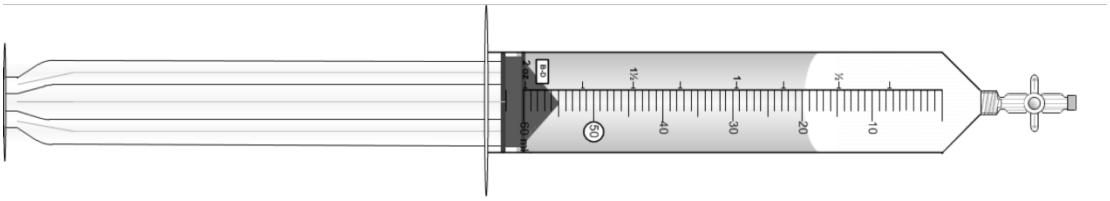


Figure 9. A syringe with 40 mL of water and 20 mL of ambient air

6. **Immediately** after adding 20 mL 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 a full syringes worth of air into the syringe and expel, rinsing the syringe with air. Rinse with ambient air 3 times.
c. Draw in 60 mL of air and expel to flush the syringe and ensure no previous gas build-up.
d. Draw in at least 20 mL of ambient air.
e. Rotate stopcock to close syringe (Figure 4).
f. In lakes, label the air sample as station "c0" no matter where the sample was collected (i.e., boat, shore, lab, etc).

7. Shake the sample syringe for the full 5 minutes so that the dissolved gases in the sample water comes to equilibrium with the ambient air in the headspace.
   a. **NOTE:** It is important to be consistent and shake the syringe for 5 minutes since this affects the amount of dissolved gas released into the headspace. Shaking the syringes sideways (laterally, with syringes turned horizontally) is preferred because it increases the surface area contact between water and gas, helping to ensure equilibration.
   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.
8. If samples were not processed immediately, **record** the temperature of the sample storage water (the water in the bucket at the time of processing) for each sample ID. This should be the same for all samples being processed, since all samples are processed at the same time.
9. **Record** the sample type (i.e., sample water (WAT) or ambient air (AIR), 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.
10. **Check gas vials to ensure no water is in vial.** Water in vials is an indication that the vacuum has been lost.
11. Transfer dissolved gas to an evacuated gas vial (Figure 10) within 5-10 minutes after equilibration.
    a. Attach the needle to the 2-way stopcock on the syringes.
    b. With stopcock 'open' and syringe held upright, expel ~ 1 mL of the syringe headspace gas to flush the needle.
    c. Insert the needle through the rubber septum of the gas vial (Figure 10) and push gas into the vial. **Properly evacuated vials will automatically suck in some gas. If the gas vial does not suck in gas from your sample, DO NOT use the vial. Look for crocked caps/bad seals and report these issues via JIRA.**
    d. Push ~ 18 mL of gas sample into the vial. Push the same volume of the air sample into the gas vial as you do from the water samples (~ 18 mLs).

e. 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.
f. Turn stopcock off.
g. Ensure sample labels are completed, as appropriate.
h. **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]).
i. Repeat step 9 for all Sample ID's.

11. Needles can be reused within a Site and Collect Date, as long as needles are purged first to remove any non-sample gas.
12. When finished sampling a site or when needles begin to bend, discard needles into a sharps container with a lid.

Figure 10. Transfer of dissolved gases to an evacuated gas vial.

13. Store gas vial by submersing upside down in water filled 50-mL centrifuge tubes (Figure 11). You may re-use the water that was previously in the centrifuge tubes. If additional water is needed, you may top it off with DI water.

Figure 11. Dissolved gas samples stored upside-down in centrifuge tubes.

14. Return to the Domain Support Facility for sample storage and/or shipping. Labels will need to be secured to vials with clear packing tape prior to shipping. Labels should be attached along the horizontal axis of the tube (Figure 11), with clear tape wrapped completely around the vial, perpendicular to the label. The ends of the tape should overlap to keep the tape from coming unstuck. You may need two rows of tape.

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.

SOP C Data Entry and Verification

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

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 the "per Sample" tab of the shipping inventory (RD[11]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. ShipmentID must be included in the electronic version of the shipping inventory, but is not necessary for the hard copy. Also include the shipment tracking # in the email.

D.1 Handling Hazardous Materials

N/A

D.2 Supplies/Containers

1. Use clear packing tape to secure labels to vials prior to shipping. Wipe excess water off vial prior to adding packing tape, and fully wrap tape around vial and label. Wrap tape completely around the vial so the tape does not come unstuck.
2. 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.
3. 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.
4. Pack absorbent material around tubes and fill additional space with regular packaging materials.
5. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site. Include a printed copy of the inventory in the shipment box. Place 'per sample' tab of AOS shipping inventory (RD[11]) in a resealable plastic bag and tape to the inside top of cooler.
6. Save the inventory with the following naming convention: "**DXX_MOD_ShippingInventory_YYYYMMDD**"
7. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.
8. Email an electronic copy of the shipping manifest and tracking number to the email addresses listed in the CLA "Shipping Information for External Facilities" document.
    a. Include the shipment tracking # (Shipment ID) in the email body, as well as the electronic copy of shipping manifest.

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. Secure labels with clear packing tape prior to shipping. Ship gas samples Ground to the gas analysis lab once or twice per month, depending on sample volume. Please coordinate with HQ.

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.

D.6 Shipping Inventory

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

D.7 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

Butman, D. and P.A. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nature Geoscience 4: pp. 839-842. Doi:10.1038/ngeo1294

Danley, M.L. *et al*. 2005. Effects of Carbon Dioxide Exposure on Intensively Cultured Rainbow Trout Oncorhynchus mykiss: Physiological Responses and Fillet Attributes. Journal of the World Aquaculture Society 36(3): pp. 249-261. DOI: 10.1111/j.1749-7345.2005.tb00329.x.

Hamilton, S.K., and N.E. Ostrom. 2007. Measurement of the stable isotope ratio of dissolved N_2 in ^{15}N tracer experiments. Limnology and Oceanography: Methods 5: pp. 233-240.

Nevers, M.B., and R.L. Whitman. 2007. Lake Monitoring Field Manual. USGS Lake Michigan Ecological Research Station.

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.

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: WAT (Water) **AIR** (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 three times with sample water.

Step 5 – Collect 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 mL of ambient air to the sample syringe. Close stopcock.

Step 8 – **Immediately** after adding 20 mL 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 water and, if applicable, the sample storage water at the time of processing. Record the sample type (i.e., sample water (WAT) or ambient air (AIR)), volume of sample water and gas in the syringe, and volume of gas injected into gas vial on the Dissolved Gas Data Sheet (RD[05]).

Step 11 – Open stopcock and transfer ~ 18 mL of gas sample 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 – Collect an equal volume of Air for the AIR sample as you did for the WAT sample(s). If you pushed 18 mL of equilibrated gas into the vial, push 18 mLs of air into the collection vial.

Step 13 – Store gas vials by submersing upside down in water filled 50-mL centrifuge tubes.

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 non-stratified lakes: from 0.5 m below the surface of the water.
    - For very shallow (hypolimnion depth of <2 m) stratified lakes, only one sample is collected at 0.5 m below the surface of the water, as in a non-stratified lake.
    - For shallow (hypolimnion depth of 2-4 m) 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 (hypolimnion depth >4 m), two samples will be taken: one from 0.5 m below the surface of the water, and one sample each at 25 and 75% depth of the hypolimnion (divide the hypolimnion depth by 2 and take a sample in the midsection of both those layers). Integrate these samples from the hypolimnion into 1 sample.

Collecting Samples: Make sure to…

- ☑ Rinse the sample syringe three times with stream/lake water. The third rinse is when you roll the air around the syringe to pick up any large air bubbles, before collecting the final sample.
- ☑ After collecting the sample, 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 mL of ambient air to sample water syringe.
- ☑ Shake syringe sideways for the full **5 minutes**.
- ☑ Carefully record all metadata, measurements, and observations on data sheet.