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| <i>Title:</i> AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling | | <i>Date:</i> 12/17/2018 |
| <i>NEON Doc. #:</i> NEON.DOC.001199 | <i>Author:</i> K. Goodman | <i>Revision:</i> M |

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. |
| L | 01/10/2018 | ECO-05285 | Updated wording on lake inlet and outlet sampling to indicate only lakes with permanently flowing inflows and outflows will be sampled as inlet and outlet locations. Lakes without permanent inflows and outflows will only be sampled at buoy location. |

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| | | | |
|---|------------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| M | 12/17/2018 | ECO-05959 | Added clarity about tape on vials, with barcode placed on top of tape. Updated barcode language, updated shipping manifest language to include STORK app. Clarity on stationID for AIR is always 'ss' |
|---|------------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

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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., 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 when evaluating potential water quality responses to natural and anthropogenic changes. Supersaturation and undersaturation of pCO₂ (partial pressure of Carbon Dioxide) 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 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.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.004257 | All Systems Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles |
| RD[09] | NEON.DOC.000694 | AOS Protocol and Procedure: Surface Water Chemistry Sampling in Wadeable Streams |
| RD[10] | NEON.DOC.001190 | AOS Protocol and Procedure: Lake and Non-Wadeable Stream Water |

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| | | |
|--------|-----------------|----------------------------------------------|
| | | 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.

Epilimnion: Top layer of water of a stratified lake, denoted by highest temperatures and least dense water in the summer

Headspace: A gaseous space above a closed liquid sample.

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline. This layer is denoted by cooler summer temperatures and slightly warmer winter temperatures relative to the Epilimnion.

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: The line that connects deepest part of the active channel.

3 METHOD

The following protocol describes the collection, processing, storage, and shipping of dissolved gas samples from aquatic environments, for lakes, streams, and rivers. In streams, samples are collected in the thalweg, where it is assumed that the stream channel is well mixed. The sampling location is located immediately downstream of 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 and non-wadeable streams (i.e. rivers), sample collection depends on lake or river depth and stratification, as detailed below (Figure 1b). For all lakes and rivers, 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

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from the buoy location. If the lake or river is stratified at the time of sampling, an additional sample will be collected from the hypolimnion at the buoy location. In lakes or rivers with a **very** shallow/thin hypolimnion (hypolimnion thickness < 2 m) do not collect a hypolimnion sample. If the hypolimnion is shallow (hypolimnion thickness 2-4 m), the sample is collected from the mid-point of the hypolimnion. In systems with a deep/thick hypolimnion (hypolimnion thickness >4 m) an integrated sample is collected as described in the water chemistry protocol (RD[10], Figure 1b). Note that if inlet and outlet streams are present, samples are collected just downstream of the inlet and outlet infrastructure, following the wadeable stream sampling design.

Disruption of the sediments by walking or by sampling too close to the system bottom or setting an anchor can contaminate samples. Thus, always sample upstream and away from disruptions 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 system 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 often suck gas from the syringe into the vial, however, this is not always the case and should not be the only indicator of a properly evacuated vial. However, if you are unable to push the typical volume of gas into the vial, it is not properly evacuated and should not be used. 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 on the syringe plunger to finish collecting the sample. Always attempt to transfer the same amount of gas/sample to each vial (~18 mL). 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 or too frozen to obtain a sample, sample in the nearest location where it is possible to obtain a sample and document the new GPS coordinates in the mobile app. If no flow exists in the **stream or river**, 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

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or river is ice-covered, but is still flowing, the ice should be broken so the sample can be collected (following a few minutes to allow the water to clear). If the **lake** is frozen only surficially and safe to walk on (minimum of 15 cm thickness for walking and 20 cm for using a UTV or snowmobile) 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 water 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]).

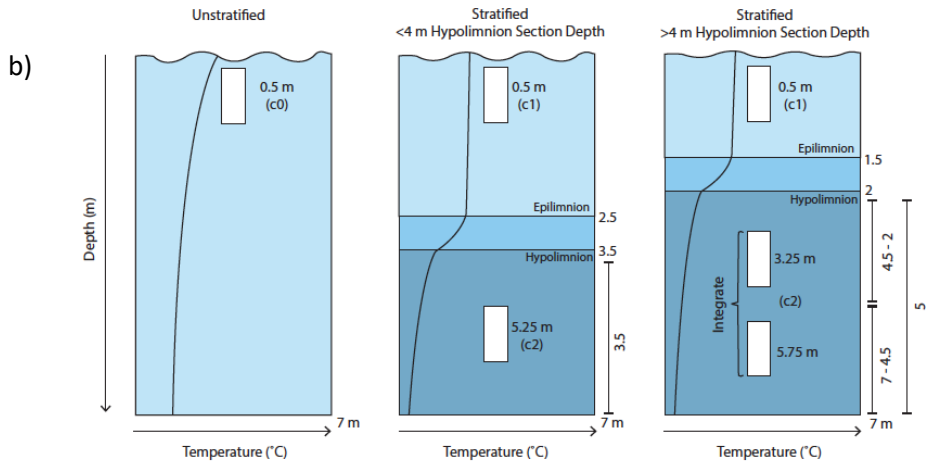
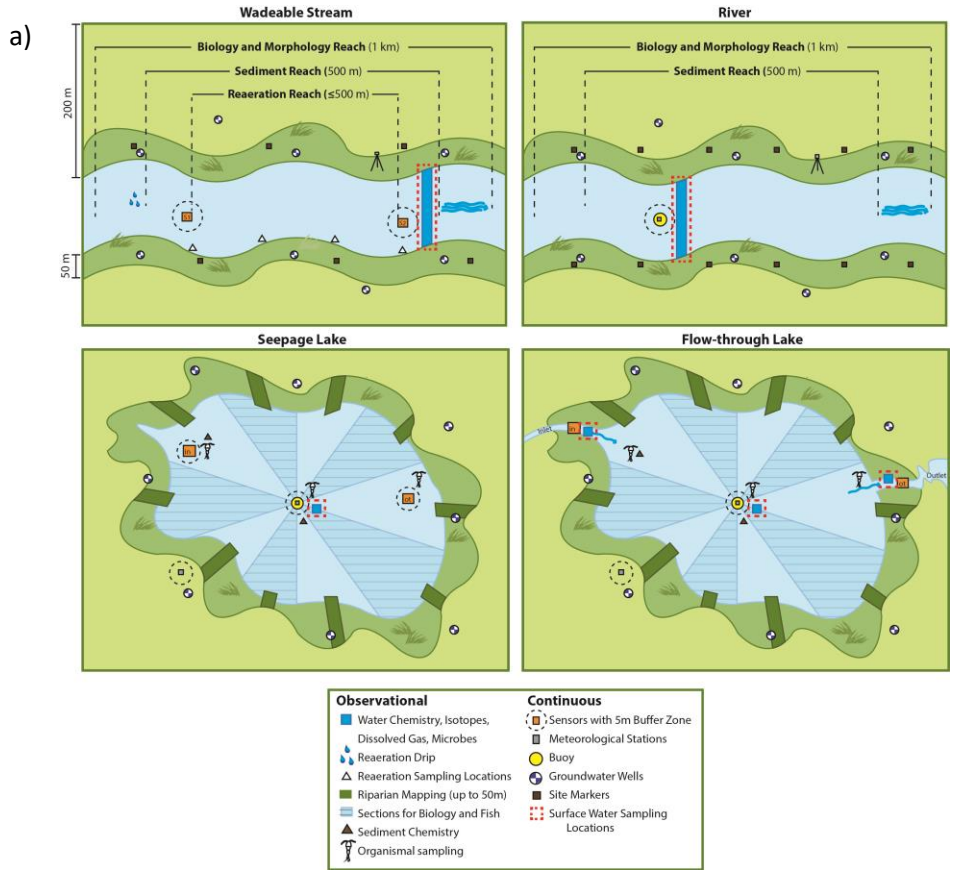
Samples should be processed as soon as possible. If necessary, water may be collected in a 60-mL syringe, submerged in a container of sample water to reduce gas loss, and processed within 4 hours at a base camp or Domain Lab (i.e., if weather dictates that need to get out of the field immediately and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

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]).

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4 FIGURE 1. A) GENERIC AQUATIC SITE LAYOUTS WITH DISSOLVED GAS SAMPLING LOCATIONS AND B) LAKE CENTER SAMPLING DEPTHS DEPENDENT ON STRATIFICATION AND HYPOLIMNION DEPTH. NOTE, HYPOLIMNION SAMPLING IS DETERMINED BY THE HYPOLIMNION SECTION DEPTH (I.E. THICKNESS). IF HYPOLIMNION THICKNESS IS < 4M, COLLECT ONE SAMPLE AT MIDPOINT OF HYPOLIMNION. IF HYPOLIMNION THICKNESS IS > 4 M COLLECT AN INTEGRATED SAMPLE.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 same time of day (i.e., 2 hours following sunrise +/- 1hr) 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 and rivers 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]). 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

Dissolved gas samples should be processed within 4 hours of sample collection. It is preferable for samples to be processed in the field immediately after collection because syringes are not air tight; however, samples may be processed in the lab if conditions prevent this from happening. For storage and shipping timelines see SOP D.

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4.4 Sampling Timing Contingencies

Table 1. Contingent decisions

| Delay/Situation | Action | Outcome for Data Products |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| Hours | If the water chemistry sampling location is too shallow to obtain a sample, sample upstream in a flowing section (always sample in the thalweg, if possible) and note this change on field sheet. | No adverse outcome. |
| | If no flow 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 or river is ice-covered, but is still flowing, the ice should be broken so the stream can be sampled (following a few minutes 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. |

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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 $\geq 10 \text{ ft}^2/\text{s}$ ($0.93 \text{ m}^2/\text{s}$) (AD[02]). When working around ice, refer to (AD[02], Section 9.3 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:

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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, minimum of 20 m, 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.

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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. | Supplier | Supplier ID | R/S | Description | Purpose | Quantity | Special Handling |
|----------------------|-------------------------|-------------|-----|----------------------------------------------------------------------|---------------------------------------------|----------|------------------|
| Durable items | | | | | | | |
| MX111388 | CDW-G | 4452963 | R | Mobile data entry tablet, fully charged and synced before field work | Field data entry | 1 | N |
| MX100554 | Thomas Scientific, Inc. | 8939D81 | R | 60 mL syringe, polyethylene | Sample collection | 2* | N |
| MX101261 | Cole-Parmer | EW3060001 | R | 1-way male Luer-lock stopcock, polycarbonate | Sample collection, attach and close syringe | 2* | N |
| MX110214 | Cole-Parmer | EW3060002 | R | 3-way male Luer-lock stopcock, polycarbonate | Sample collection for integrated samples | 1 | N |
| MX103240 | Grainger, W.W. | 2TUW7 | R | Sharps container | Needle disposal | 1 | N |

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| Item No. | Supplier | Supplier ID | R/S | Description | Purpose | Quantity | Special Handling |
|-------------------------|-----------------------------------------------------------------------|---------------------|-----|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|--------------|------------------|
| MX100514 | Thomas Scientific, Inc. Fisher Scientific Company, LLC | 1185K52 15177622 | R | Handheld Conductivity/Temperature Meter | Temperature and Conductivity Meter – must order DO Probe addition separately (MX110375) | 1 | N |
| MX110375 | Fondriest Environmental, Inc. Fisher Scientific Company, LLC | 605202 15177458 | R | DO probe for conductivity/temperature handheld meter (MX100514) | DO Galvanic Probe and replacement tips – must order separately | 1 | N |
| MX107554 | Fondriest Environmental, Inc. Fisher Scientific Company, LLC | 605913 15176211 | | Replacement DO sensor tips for MX110375 | Replacement part for DO sensor tips – order when appropriate. | As needed | N |
| MX106201 | Grainger, W.W. | 4UEF5 | R | Storage container (pitcher) | Field storage and transport | 1 | N |
| Consumable items | | | | | | | |

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|----------|-----------------------------------------------------------------|-----------------------------|-----|----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------|------------------|
| | | | 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 | Grainger, W.W. Amazon Capital Services Inc. Arrow SameDay | 5NHH1 B00006IBUV 5520 | R | Dissolved Gas Labels (Waterproof Labels 1 * 2 ⁵ / ₈) | Labeling sample, human readable | 2* | N |
| | | | S | Adhesive barcode labels (Type I) | Labeling sample bottles with barcode-readable | 1 sheet | 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 | Thomas Scientific, Inc. Fisher Scientific Company, LLC | 8956A70 1482648 | R | Needles, 27 G ½ inch | Sample transfer from syringe/stopcock to gas vial | 2* | Y |

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|----------|----------------|------------------|-----|--------------------|------------------------------------------|----------|------------------|
| MX105587 | Grainger, W.W. | 15F814 31HJ31 | 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. | Supplier | Supplier ID | R/S | Description | Purpose | Quantity | Special Handling |
|----------------------|------------------------------|-------------|-----|---------------|---------|----------|------------------|
| Durable items | | | | | | | |
| | | | R | Boat and oars | | 1 | N |
| MX100435 | Amazon Capital Services Inc. | B003QKQ4V0 | R | Safety kit | | 1 | N |

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| Item No. | Supplier | Supplier ID | R/S | Description | Purpose | Quantity | Special Handling |
|----------|--------------------------------------------------------------------------|----------------------------------|-----|-----------------------------|------------------------------------------|----------|------------------|
| MX100393 | Fisher Scientific Company, LLC | EW0548610 | R | Kemmerer sampler | Water collection | 1 | N |
| MX100364 | Thomas Scientific, Inc. Cole-Parmer Fisher Scientific Company, LLC | 1207W05 EW0640776 14171212 | R | Tubing (1/8" ID) | Sample transfer from Kemmerer to syringe | 1 | N |
| MX100662 | Fisher Scientific Company, LLC | 141697G | R | Tubing (3/8" ID) | Sample transfer from Kemmerer to syringe | 1 | N |
| | | | S | Flow control hose clamp | Sample transfer from Kemmerer to syringe | 1 | N |
| MX110214 | Cole-Parmer | EW3060002 | R | 3-way stopcock | Sample transfer from Kemmerer to syringe | | |
| MX100662 | Fisher Scientific Company, LLC | 141697G | R | 3/8" to 1/8" tubing adaptor | Sample transfer from Kemmerer to syringe | 1 | N |

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| Item No. | Supplier | Supplier ID | R/S | Description | Purpose | Quantity | Special Handling |
|-------------------------|--------------------------------------------------------------------------|------------------------------|-----|----------------------------|---------|--------------|------------------|
| MX110075 MX102739 | Forestry Suppliers, Inc. Cabela's Inc. Recreational Equipment Inc. | 39481 IK-270217 895022 | R | GPS (Accuracy < 4m) | | 1 | N |
| MX102603 | Grainger, W.W. | 11C657 | R | First Aid Kit | | 1 | N |
| | | | R | Waders or boots | | 2 | N |
| | | | R | Personal Flotation Devices | | 1 per person | N |
| Consumable items | | | | | | | |
| (None) | | | | | | | |

R/S=Required/Suggested

| | | |
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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 the Field Operations Job Instruction Training Plan (AD[04]). Personnel are to be trained in 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).

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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. Always sync your tablet before leaving for the field to capture any updates that may have been incorporated since the last sampling.

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 2 months of evacuation.

A.3 Prepare Labels

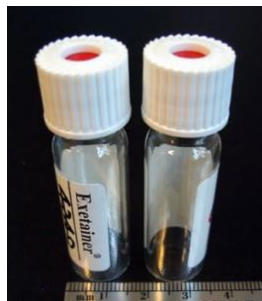


Figure 2. Gas vial with septum lid.
Image from <http://isotopes.forestry.ubc.ca>

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

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. Human readable labels should be attached along the horizontal axis of the tube (**Error! Reference source not found.**), with clear tape wrapped completely around the vial, perpendicular to the label.



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- a. Use as LITTLE tape as possible, but the ends of the tape should overlap slightly to keep the tape from coming unstuck. You may need two rows of tape.
 - b. DO NOT use thick clear tape.
3. 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 non-stratified lakes. 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).
 - a. For lakes, only one air sample should be collected per site and date.
 - b. For streams, if WAT sample is collected at a different sample location such that the stationID is 're', the AIR sample stationID will still be 'ss'. The AIR sample stationID is ALWAYS 'ss'.
 - c. 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

4. Add adhesive barcode labels to the sample containers and scan using the mobile app (**Error! Reference source not found.**). Add adhesive labels to dissolved gas vial along the length of the vial.
 - a. Do NOT cover the barcode tape. Place barcode on top of tape.



Figure 4. Example of appropriate attachment of dissolved gas vial labels. Do not tap over barcode

- b. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially

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associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

- c. Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.
- 5. In streams and rivers, 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, if possible.
- 6. In lakes:
 - a. Take one sample at a 0.5-meter depth at buoy station.
 - b. Is the lake thermally stratified at buoy station? Use the Secchi Depth App to determine sampling depths and stratification. If the secchi depth app is not available, use the decision tree:
 - 1) If NO, do not take any more samples.
 - 2) If YES, evaluate the **hypolimnion** section depth (i.e. hypolimnion thickness) at the buoy, calculated using the secchi disk app. If you are not using the secchi depth application, ensure you are calculating the hypolimnion section depth (i.e. hypolimnion thickness), NOT the maximum lake depth and NOT the depth that the hypolimnion starts (Figure 1 b).
 - a) If hypolimnion section depth (i.e. hypolimnion thickness) is <2 m, do not take any more samples.
 - b) If hypolimnion depth/thickness ≥ 2 m but ≤ 4 m, then collect a sample from the midsection of the hypolimnion depth.
 - c) If hypolimnion depth/thickness > 4 m, then divide the hypolimnion depth by 2 and collect a sample in the midsection of both those layers. Integrate the samples from the hypolimnion into 1 sample.
 - c. Is there a true (i.e. flow-through) inlet and outlet to the lake (Figure 1a)?
 - 1) If No, do not take any more samples
 - 2) If Yes, collect samples sampled just downstream of the inlet and outlet infrastructure, following the wadeable stream protocol.

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 information is completed in mobile app or data sheet (RD[06]).

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B.2 Collecting Samples in Wadeable Streams

1. ALWAYS sample in the thalweg 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 5)) into the stream so that the water is sampled ~10 cm under the surface. Stopcock should remain on the syringe at ALL times.

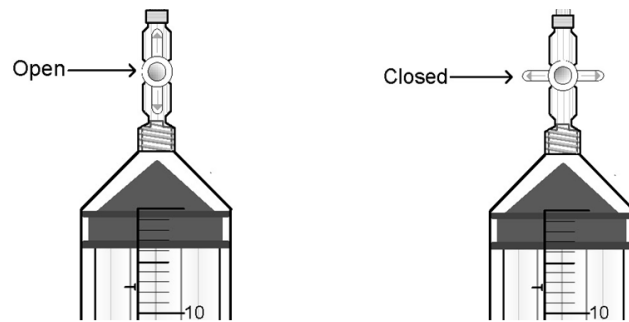


Figure 5 Syringe with a 2-way Luer lock stopcock in the “Open” and “Closed” positions

- 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. On the third 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 6). 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.

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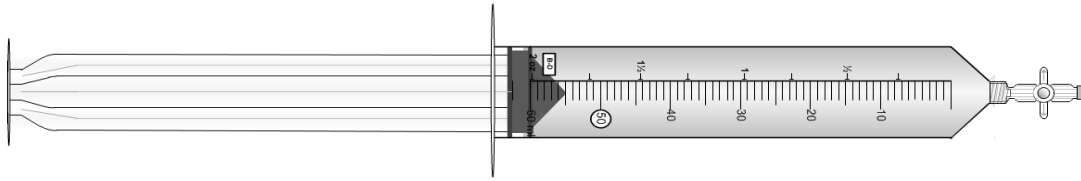


Figure 6. A full 60 mL syringe with a closed 2-way stopcock

5. **BEFORE** removing the syringe from the stream, immediately turn the 2-way stopcock to the closed position (Figure 5).
6. Ensure water temperature at the time of collection is recorded in the water chemistry app.
7. Proceed to B.4, Sample Processing
 - a. Samples should be processed immediately after collection, if possible.
 - b. If you are not able to process the samples immediately, 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 decrease degassing prior to processing.

B.3 Collecting Samples in Non-Wadeable Streams (i.e. Rivers) 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.
 4. Attach the tubing to the spigot of the Kemmerer (Figure 7).
 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 7).
 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 valve 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

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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 6). 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.

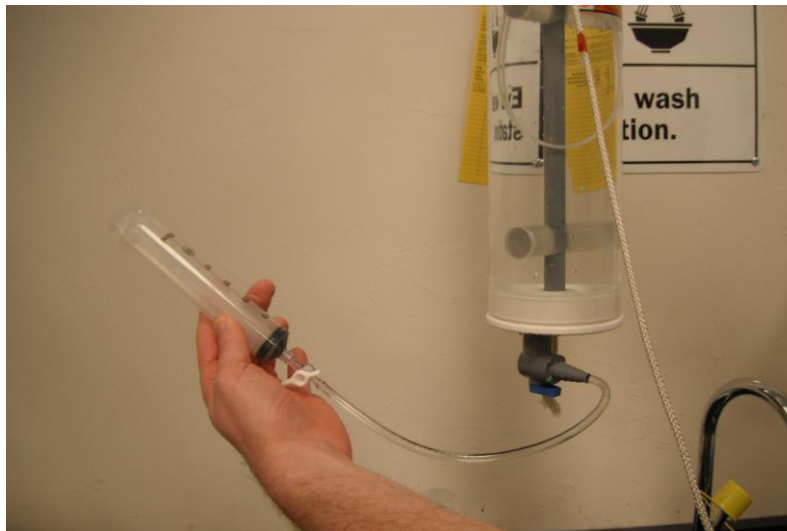


Figure 7. Syringe and 2-way stopcock attached to Kemmerer spigot

- b. For integrated samples:

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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 8).
- e) Attach the other end of the tubing to the 3-way stopcock and the syringe (**Figure 8**)
- f) Turn 3-way stopcock so the 'off' position is towards the syringe (**Figure 8**).
- 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)

2) **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.

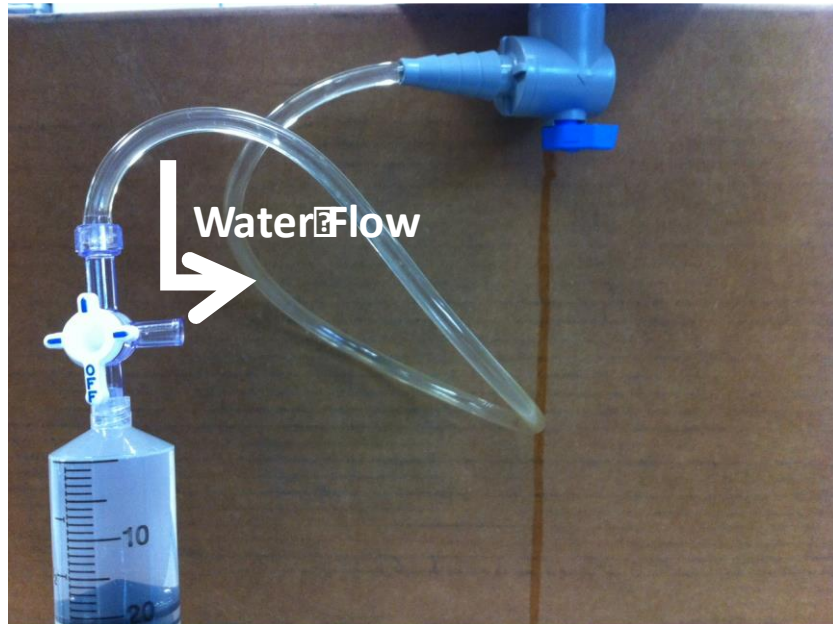
3) Your sample is complete.

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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
6. Collect all water samples at all stations before processing.
7. Proceed to B.4, Sample Processing.

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a)



b)

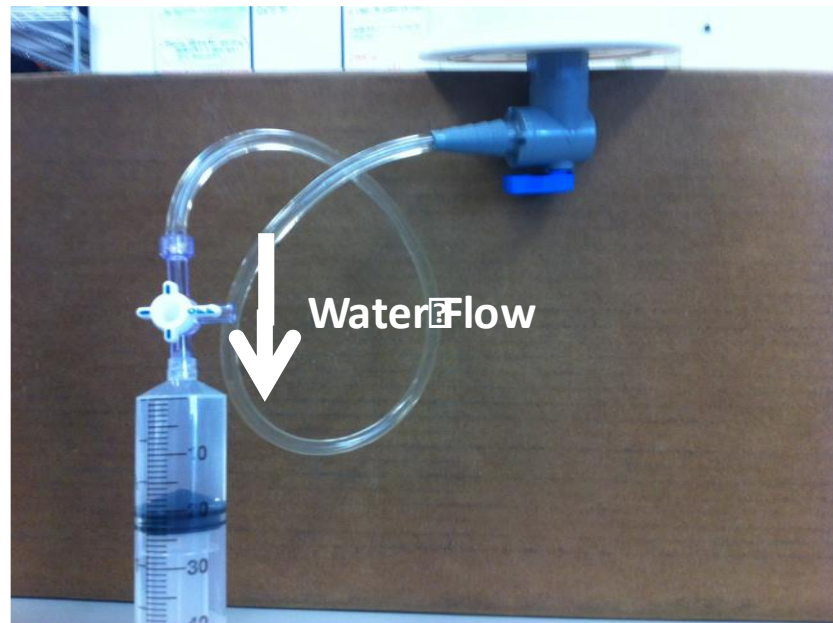


Figure 8 a) 3-way Luer-lock to Kemmerer spigot via tubing. Syringe is in the 'off' position allowing water to flow out the side port and b) 3-way Luer-lock to Kemmerer spigot via tubing. Side port is in the 'off' position, allowing water to flow into syringe.

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B.4 Processing and Transporting Samples



NOTE: Process dissolved gas samples within 4 hours of sample collection. 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, must 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 mobile app or 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 9). You will not need to do this step for integrated samples, since you only sampled 40 mL.

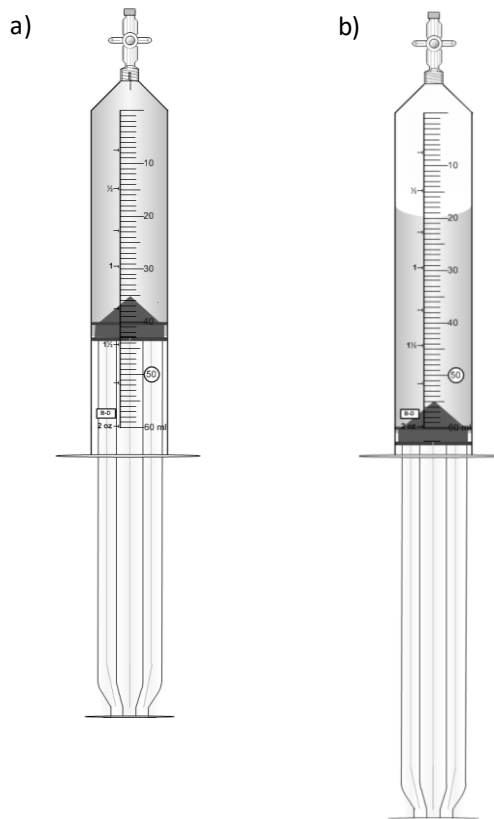


Figure 9. A closed syringe with a) 40 mL of sample water and b) 40 mL of water and 20 mL of ambient air.

4. Add 20 mL of ambient air to the sample syringe:

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- a. Hold syringe at ~1.5 m above ground and at a full arm's 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 5).
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.
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 at least 20 mL of ambient air.
 - d. Rotate stopcock to close syringe (Figure 5).
 - e. 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 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.
9. 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.
10. **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 mobile application or 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 mobile application.
11. **Check gas vials to ensure no water is in vial.** Water in vials is an indication that the vacuum has been lost. Transfer dissolved gas from the water (WAT) to an evacuated gas vial (**Figure 11**), as you did with the air sample.
 - a. You may re-use the needle from the AIR sample. Needles can be reused within a Site and Collect Date, as long as needles are purged first to remove any non-sample gas.
12. Push ~ 18 mL of gas into vial.



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- a. 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.
 - b. **Properly evacuated vials may automatically suck in some gas, but may not always do this even if properly evacuated. If you cannot push gas into the gas vial, DO NOT use the vial.** Look for crooked caps/bad seals and report these issues via NEON's problem tracking software. You only need to report issues if several of the vials were impacted in one shipment or if this is a recurring issue.
13. Repeat steps as needed depending for any additional dissolved gas from water samples collected in stratified systems. You may use the same needle as long as needle is purged with sample first before inserting into the vial.
14. Transfer AIR sampling into the evacuated gas vial.
 - 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. Note the air volume in the syringe after purging but before injecting the sample into the vial.
 - d. Insert the needle through the rubber septum of the gas vial (**Figure 11**), and push gas into the vial.
 - e. Push the same volume of air into the vial as you did for your water sample (this should be ~ 18 mL). DO NOT push as much air in as possible. We want the air sample to have approximately the same volume as the water sample.
 - f. Note volume of air remaining in the syringe after the sample has been transferred to the vial. Subtract that from air volume after purging (step c above) and record the difference as your sample volume (i.e. if you had 19 mL of air after purging needle and you have 1 mL remaining after injecting sample into vial, your sample volume is 18 mL).
15. Remove needle and when finished transferring samples to evacuated vials, and put needle(s) in a Sharps container.
16. Ensure sample labels are completed, as appropriate.
17. For all samples, **record** the volume of gas injected into the gas vial (**this is the difference in the volume of gas in the syringe after purging the needle and after sample is collected to determine volume of gas injected into the vial**) in the Dissolved Gas mobile application or Data Sheet (RD[05]).
18. **Record data in the mobile app.**
 - a. If available, scan the barcode label with the tablet (**Figure 10**). Barcode labels should be located on the actual dissolved gas vial, not the centrifuge tube.
 - b. Ensure that the human-readable sampleID matches the sampleID generated by the mobile app.

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Figure 10. Barcode label scanning..



Figure 11. Transfer of dissolved gases to an evacuated gas vial.

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19. Store gas vial by submersing upside down in water filled 50-mL centrifuge tubes (Figure 12). 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 12. Dissolved gas samples stored upside-down in centrifuge tubes.

20. 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

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. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). 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.

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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 Information to External Facilities’ document on CLA’s NEON intranet site.

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. Do not use thick tape.
2. Ensure human-readable labels are secured to vials with clear packing tape prior to shipping. Tape should be wrapped perpendicular to the label.
 - a. Ensure the barcode label is placed on top of tape.
3. 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.
4. 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.
5. Pack absorbent material around tubes and fill additional space with regular packaging materials.

D.3 Timeline and Conditions

Samples can be stored at room temperature (20°C +/- 5°C) and shipped monthly or as directed by the CLA shipping guidelines. Store samples upside down in water filled centrifuge tubes. Secure human-readable labels with clear packing tape and barcodes placed on top of tape.

D.4 Grouping/Splitting Samples

Organize by Site ID, if applicable.

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D.5 Return of Materials or Containers

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

D.6 Shipping Inventory

Whenever samples are shipped, they must be accompanied by a hard-copy Shipping Manifest enclosed within the shipping container. In addition, a corresponding electronic version of the Shipping Manifest (csv file) must be emailed to the taxonomic ID facility and NEON’s CLA contact using the Stork Shipment Verification Tool as soon as possible after the samples have been shipped. For locations to which to ship samples, and CLA contract information, please reference CLA’s NEON intranet site, available through the sampling support library.

1. Navigate to the “Shipping Information for External Facilities” document on [CLA’s NEON intranet site](#).
2. Prepare a shipping inventory detailing the contents of the shipment, using the Shipment Creation and Shipment Review applications. Include a printed copy of the inventory in the shipment box (downloaded from the Stork Shipment Verification Tool).

Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA “Shipping Information for External Facilities” document.

3. Email a digital copy of the shipping manifest emailed to the appropriate contact at the receiving analytical laboratory as well as the NEON CLA contact on the day that samples ship by submitting the shipment in the Stork Shipment Verification Tool.

D.7 Laboratory Contact Information and Shipping/Receipt Days

See CLA’s NEON intranet site, available through the sampling support library.

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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 | Mobile application |
|--------------------|---------------------------------------------------------------------------------|----------------------------------------------|
| NEON.DOC.002383 | Datasheets for AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling | AOS (SWC) PROD |
| NEON.DOC.001646 | NEON General AQU Field Metadata Sheet | (AOS) Field Metadata and Gauge Height [PROD] |

These datasheets can be found in Agile or the NEON Document Warehouse, user guides for mobile applications may be found in NEON’s internal sampling support library.

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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: WAT (Water) AIR (Air)
Project: NEON

Step 3 – Ensure the General AQU Field Metadata mobile application or, if necessary, data 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 mL of air into the collection vial.

Step 13 – 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/Rivers: 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 surface 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 **5 minutes**.
- Carefully record all metadata, measurements, and observations on data sheet.