



<i>Title:</i> AOS Protocol and Procedure: SDG- Surface Water Dissolved Gas Sampling		<i>Date:</i> 01/02/2025
<i>NEON Doc. #:</i> NEON.DOC.001199	<i>Author:</i> K. Goodman	<i>Revision:</i> Q

AOS PROTOCOL AND PROCEDURE: SDG – SURFACE WATER DISSOLVED GAS SAMPLING

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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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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.
M	12/17/2018	ECO-05959	<ul style="list-style-type: none"> • 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'
N	10/09/2019	ECO-06247	<ul style="list-style-type: none"> • Added information regarding replicates



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			<ul style="list-style-type: none"> • Updated to new template.
O	10/11/2021	ECO-06677	<ul style="list-style-type: none"> • Removed human readable labels • Updated processing instructions and added additional temperature and pressure measurements at final sample collection • Simplified rescheduling guidelines • Guidance on transporting vials
P	12/23/2021	ECO-06747	<ul style="list-style-type: none"> • Updated to template NEON.DOC.050006 Rev K
Q	01/02/2025	ECO-07111	<ul style="list-style-type: none"> • Updated short barcode and placement information • Added clarity regarding water in vials after sampling • Updated to template NEON.DOC.050006 Rev M



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1 OVERVIEW

1.1 Background

The following protocol outlines field sampling of surface water dissolved gas chemistry (i.e., CO₂, CH₄, and N₂O) 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.

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 in controlled environments) have spurred the interest in measuring dissolved gas in natural aquatic environments (Danley et al. 2005). Currently, little is known about how increased CO₂ in natural freshwater systems impacts primary and secondary production. Stream dissolved chemistry can 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 pCO₂ saturation over time, annual net CO₂ balances may be inferred for the system and provide insight on how changes in the surrounding landscape may influence aquatic system 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.



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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, rivers, 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).



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2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.002384	Datasheets for AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
RD[06]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[07]	NEON.DOC.001646	NEON General AQU Field Metadata Sheet
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy document
RD[09]	NEON.DOC.004257	Standard Operating Procedure: Decontamination of Sensors, Field Equipment and Field Vehicles
RD[10]	NEON.DOC.002905	AOS Protocol and Procedure: SWC – Water Chemistry Sampling in Surface Waters and Groundwater
RD[11]	NEON.DOC.001886	AOS Protocol and Procedure: ASI – Stable Isotope Sampling in Surface and Ground Waters
RD[12]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[13]	NEON.DOC.003044	AOS Protocol and Procedure: AMC – Aquatic Microbial Sampling
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples, Sensors and Equipment
RD[15]	NEON.DOC.000693	AOS Protocol and Procedure: REA – Reaeration in Streams

2.3 Acronyms

Acronym	Definition
CH ₄	Methane
cm	centimeter
CO ₂	Carbon Dioxide



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m	meter
mL	milliliter
N ₂ O	Nitrous oxide
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

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

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.

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

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.

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

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

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3 METHOD

The following protocol describes the collection, processing, and storage of dissolved gas samples from lakes, streams, and non-wadeable streams (i.e., rivers). In streams, samples are collected in the thalweg, immediately downstream of the sensor set and in conjunction with water chemistry sample collection. In lakes and rivers, sample collection depends on the system depth and stratification (**Figure 1b**). For all lakes and rivers, one sample is taken at 0.5 m below the surface of the water. If the lake or river is stratified at the time of sampling, an additional sample(s) will be collected from the hypolimnion at the buoy location. If the hypolimnion is very shallow/thin (i.e., hypolimnion thickness < 2 m), no hypolimnion sample is collected. 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 hypolimnion sample is collected (**Figure 1b**). If true inflow and outflow streams are present at lake sites, additional samples are collected at inflow infrastructure and at the lake-outflow interface (just inside the lake boundary). For these locations, samples are collected following the wadeable stream sampling design.

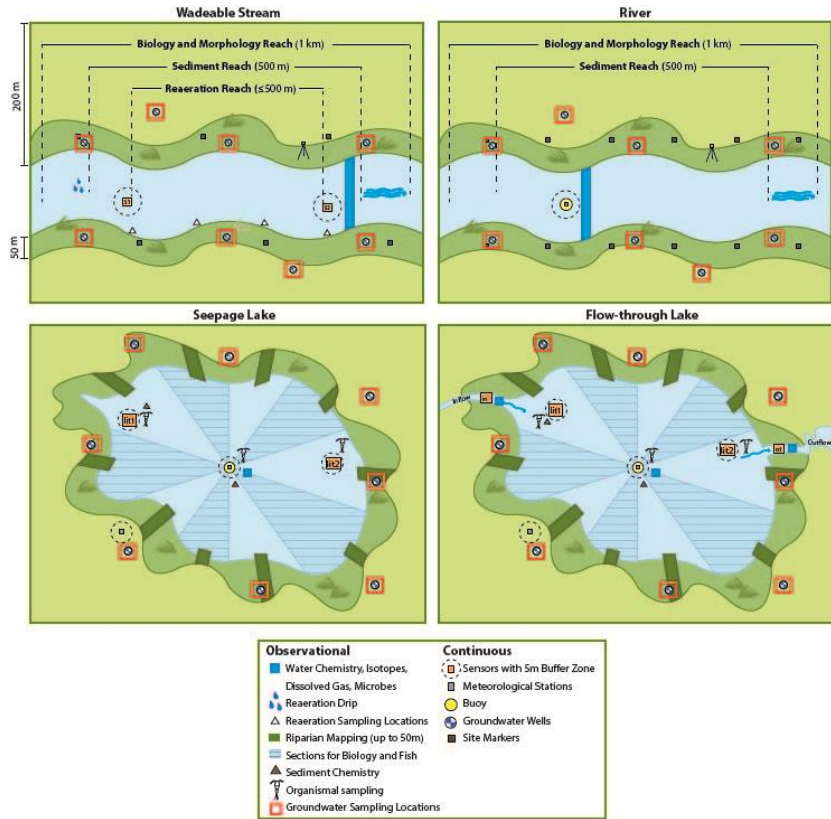
Once all samples are collected at a site, samples are processed as soon as possible by equilibrating with air. Reference air samples are also collected. Gas samples are transferred to evacuated gas vials to be analyzed at an external facility.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field ecologists **must** follow the protocol and associated SOPs and 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. If local conditions create uncertainty about carrying out these steps, it is critical that field ecologists document the problem and enter it in NEON’s problem tracking system.

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

a)



b)

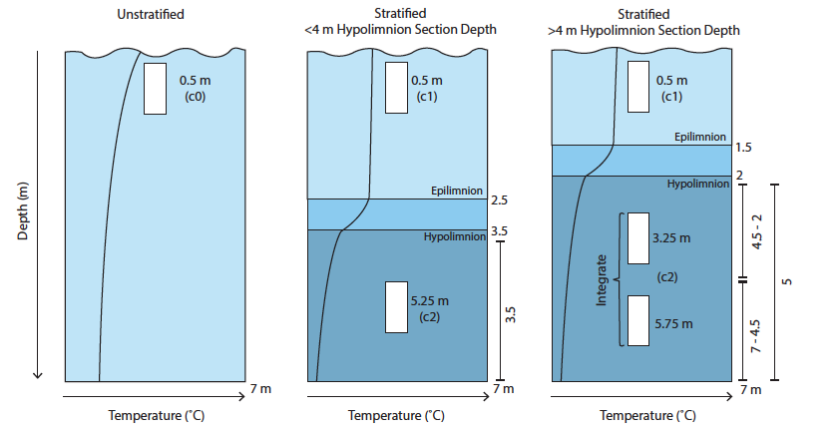


Figure 1. a) Generic aquatic site layouts with dissolved gas sampling locations and b) Lake center sampling depths dependent on stratification and hypolimnion depth. Note, hypolimnion sampling is determined by the hypolimnion section depth (i.e., thickness 2-4 m, collect at midpoint of hypolimnion. If hypolimnion thickness is >4 m, collect an integrated sample.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Dissolved gas chemistry sampling occurs in conjunction with surface water chemistry and aquatic stable isotopes (RD[09] and RD[10]) sampling up to 26 times per year in wadeable streams and rivers and 12 times per year in lakes. In general, stream and river samples are collected monthly with an additional 14 flow-weighted samples. Lakes are sampled monthly, with lakes that freeze modifying sampling in spring and fall to capture ice-on and ice-off events. Sample timing should follow site-specific timing guidelines found in the Domain Specific Sampling Design documents (**Table 6**). Dissolved gas samples should attempt to be collected at the same time of day relative to sunrise or solar noon (i.e., 2 hours following sunrise +/- 1hr) throughout the year to minimize errors incurred by natural diel variations in dissolved gases.

Table 1. Sampling frequency for Surface Water Dissolved Gas procedures on a per SOP per site type basis.

SOP	Site Type	Bout Duration	Bouts Per Year	Remarks
SOP A-D	Stream/ River	1 day	26	Sampling dates are synchronized with the Surface Water Chemistry protocol. See Domain Specific Sampling Design documents for site-specific sampling dates (Table 6).
	Lake	1 day	12	Sampling dates are synchronized with the Surface Water Chemistry protocol. See Domain Specific Sampling Design documents for site-specific sampling dates (Table 6).

Scheduling Considerations

1. Coordinating with water chemistry sampling suite: Surface water dissolved gas samples are collocated with surface water chemistry and aquatic stable isotope samples on each sampling event. Ensure there is enough time to process all samples within the appropriate time frame.
2. **Replicates:** Once per year, NEON will quantify measurement uncertainty and environmental variability by collecting two additional surface water and air samples for external analysis.
 - a. Field Science is not required to collect dissolved gas replicates on the same day as water chemistry replicate collection if there are timing/logistical constraints.
 - b. In stratified lakes, replicate sampling should occur during periods when the lake is NOT stratified.

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 solid during the winter are sampled more intensively during periods of higher flow. Systems that have a snowmelt dominated or storm dominated flow regime are sampled more intensively during elevated flow. Site specific sampling dates can be found in Aquatic Site Sampling Design Documents (**Table 6**).

4.3 Timing for Laboratory Processing and Analysis

Samples should be processed (i.e., head space equilibration completed and transferred to appropriate containers) as soon as possible and no longer than 4 hours after the end of sample collection.

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

Sample type	Activity	Maximum Holding Time
Unprocessed syringe samples	Shake to equilibrate	4 hours before headspace equilibration (complete immediately if possible)
Gas (headspace) samples	Store in evacuated vials in water and ship to external lab	One month after headspace sampling

4.4 Sampling Timing Contingencies

Dissolved gas sampling should always occur as part of the water chemistry sampling suite. If sampling must be rescheduled, reschedule as soon as possible. Schedule dates no greater than plus or minus (+/-) 14 days of original schedule date.

Table 3. Contingency decisions for Surface Water Dissolved Gas Sampling.

Delay/Situation	Action	Outcome for Data Products
Hours	If water chemistry sampling location is too shallow to obtain a sample: <ol style="list-style-type: none"> 1. Sample in another location more conducive to sampling. 2. Record the coordinates for the new location in the app. 3. In app, record stationID of WAT sample as 're'. Air sample stationID will remain as 'ss' or 'c0'. 	No adverse outcome
	If the stream is entirely dry or frozen solid (i.e., no flowing water under the ice): <ol style="list-style-type: none"> 1. Complete sampling impractical record. 2. Reschedule for a time when conditions are expected to be more conducive to sampling. 	Potential reduced data availability.
	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

Delay/ Situation	Action	Outcome for Data Products
	If the lake surface is frozen: <ol style="list-style-type: none"> 1. Test the thickness of the ice 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]). <ol style="list-style-type: none"> a. Minimum of 15 cm thickness for walking and 20 cm thickness for use of UTV/snowmobiles, etc. b. If the ice is greater than the minimal thickness and safe for the transportation method being used, travel to sampling location, make a hole in the ice, and proceed with sampling. c. If the ice is not thick enough to safely travel on, return to the Domain Support Facility and sample at another time. 	No adverse outcome
	During lake sampling, if you are unable to use the tubing to collect a sample because the water is freezing in the tube: <ol style="list-style-type: none"> 1. You may collect out of the top of the Kemmerer while minimizing the time the water sample is exposed to air. 2. 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, 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/river becomes too windy (>20 mph), has unsafe wave heights (e.g., >1 m), and you are unable to hold the boat stationary at sampling location: <ol style="list-style-type: none"> 1. Return to shore and wait in a safe location for 30 minutes. 2. If wind subsides, resume sampling. 3. If not, return to the Domain Support Facility and sample at another time. Be sure to complete a sampling impractical record. 	No adverse outcome
Days-Weeks	If sampling must be rescheduled and can be rescheduled within 14 days of the originally scheduled sampling dates, reschedule event. No additional action is needed.	No adverse outcome
Weeks	If sampling must be rescheduled but cannot be rescheduled within 14 days of the originally scheduled sampling dates, submit an IS/OS Schedule Change Request.	Potentially reduced data availability
Cancelled	If sampling is cancelled completely, submit an incident ticket.	Reduced data availability

4.5 Missed or Incomplete Sampling

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

- Logistics – e.g., insufficient staff or equipment

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- Environment – e.g., deep snow, flooding, inclement weather, or
- Management activities – e.g., controlled burns, pesticide application

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

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates:** Bout-specific sampling dates (Appendix C, **Table 6**).
- **Scheduled Sampling Dates:** Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling:** Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical:** The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled:** Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

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

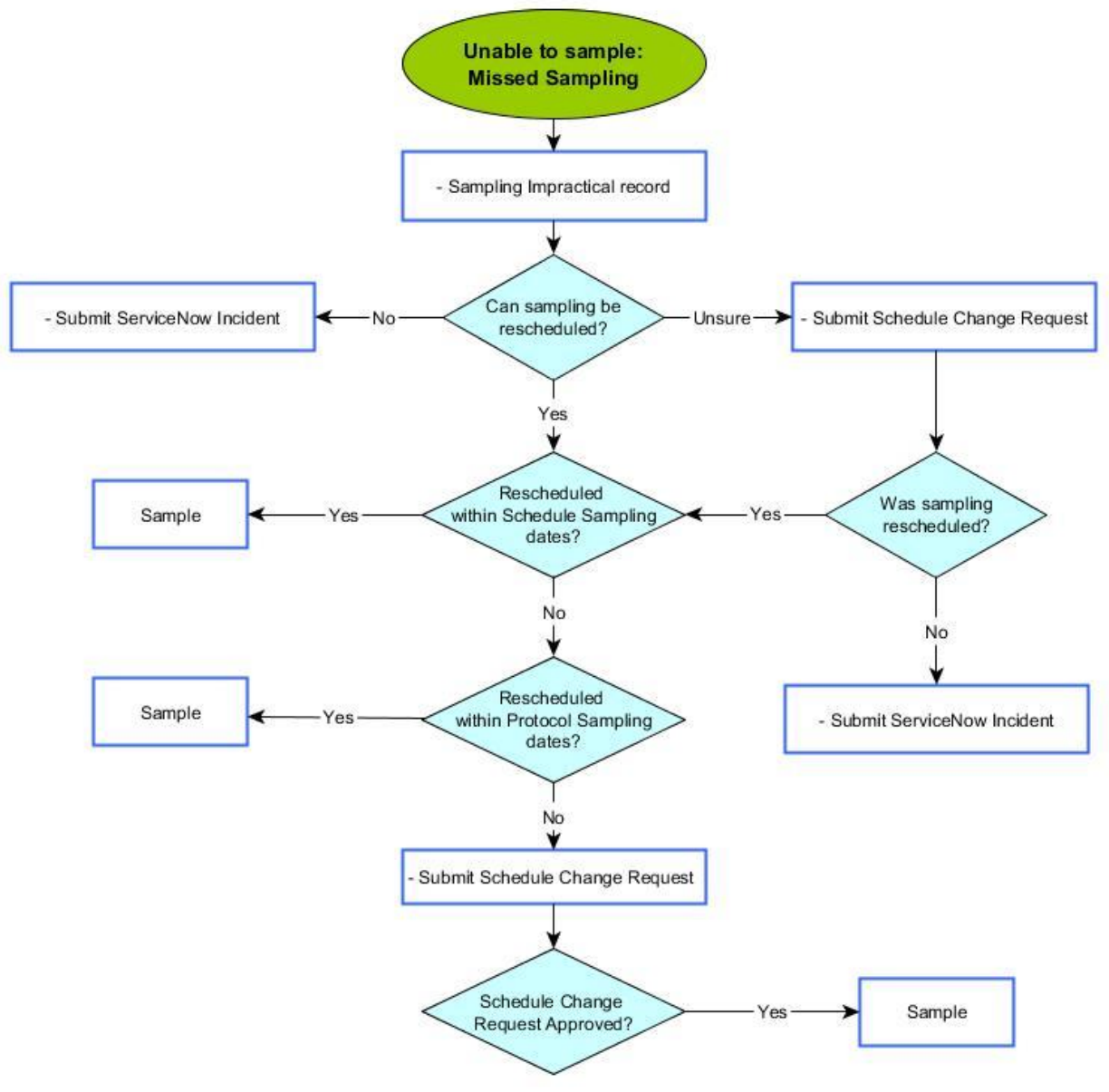


Figure 2. The documentation to account for a Missed Sampling event. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management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

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling must be communicated to Science by a Service Now Incident if sampling cannot be rescheduled within 14 days of the originally scheduled sampling dates (**Figure 2**).
 - a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 2**).
 - b. Consult **Figure 2** to determine required actions if scheduled activities are delayed or canceled. This protocol is the ultimate source of information should any discrepancy exist between this document and other summary materials – e.g., the ‘Scheduled Field Activities – Delays and Cancellations’ spreadsheet linked via the SSL.
2. Create a Fulcrum record in the parent Water Chemistry application for each Missed Sampling event in the field.
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the parent Water Chemistry mobile collection device (**Table 4**).

Table 4. Protocol-specific Sampling Impractical reasons entered in the parent Water Chemistry Fulcrum application. If more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
High water velocity	High water velocity
Location dry	Location dry
Location frozen	Location frozen
Location snow covered	Location snow covered
Other	Sampling location inaccessible due to other ecological reason described in the remarks

Surface water dissolved gas sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and Appendix C. 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 (gradient sites). However, sampling may be shifted from one location to another when a sampling location is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced. If a sampling location is only temporarily impacted, follow the sampling contingency steps (**Table 3**).

4.6 Estimated Time

The time required to implement a protocol will vary depending on several factors, such as skill level, system diversity, environmental conditions, and distance between sampling locations. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing

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progress. If a task is taking significantly longer than the estimated time, an incident ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Table 5. Estimated staff and labor hours required for implementation of surface water dissolved gas sampling. More time may be required at sites with multiple sampling stations, such as a stratified lake or a lake with inflows and outflows.

SOP	Estimated time	Suggested staff	Total person hours
SOP A.1: Preparing for sampling	0.2 h	1	0.2 h
SOP A.2: Labels and Identifiers	0.1 h	1	0.1 h
SOP B.2-B.3: Collecting samples	0.1 h per station	1	0.1 h per station
SOP B.4: Processing and transporting samples	0.2 h	1	0.2 h
SOP D: Sample shipment	0.5 h	1	0.5 h



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5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the EHS Safety Policy and Program Manual (AD[01]) and Operations Field Safety and Security Plan (AD[02]). Additional safety issues associated with this field procedure are outlined below. If an employee witnesses any unsafe conditions or uncontrolled hazards that present an imminent danger, they should immediately take action to stop work and report such conditions to their manager. Employees must also report all workplace injuries, illnesses, incidents, or releases to the environment as soon as possible, regardless of the severity.

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 if depth of ice is less than 6" (+15 cm) or operate UTV or snowmobile on frozen lake if depth of ice is less than 8" (20 cm). 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. Use caution and good judgment to carefully evaluate site conditions including ice strength. Local guidelines from natural resource officials, property owners or site 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, field ecologists may perform GPS positioning around the lake, and measurements for inflow and outflow, without dismounting from the vessel. In addition, field ecologists 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. Field ecologists should be aware of any site-specific water hazards of that particular location (e.g., flooding, high water velocity, freezing, 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.



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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

6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

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.

6.2 Specialized Skills

Where applicable, personnel will be licensed to operate a boat and able to safely handle a motor and drive a boat.



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7 STANDARD OPERATING PROCEDURES

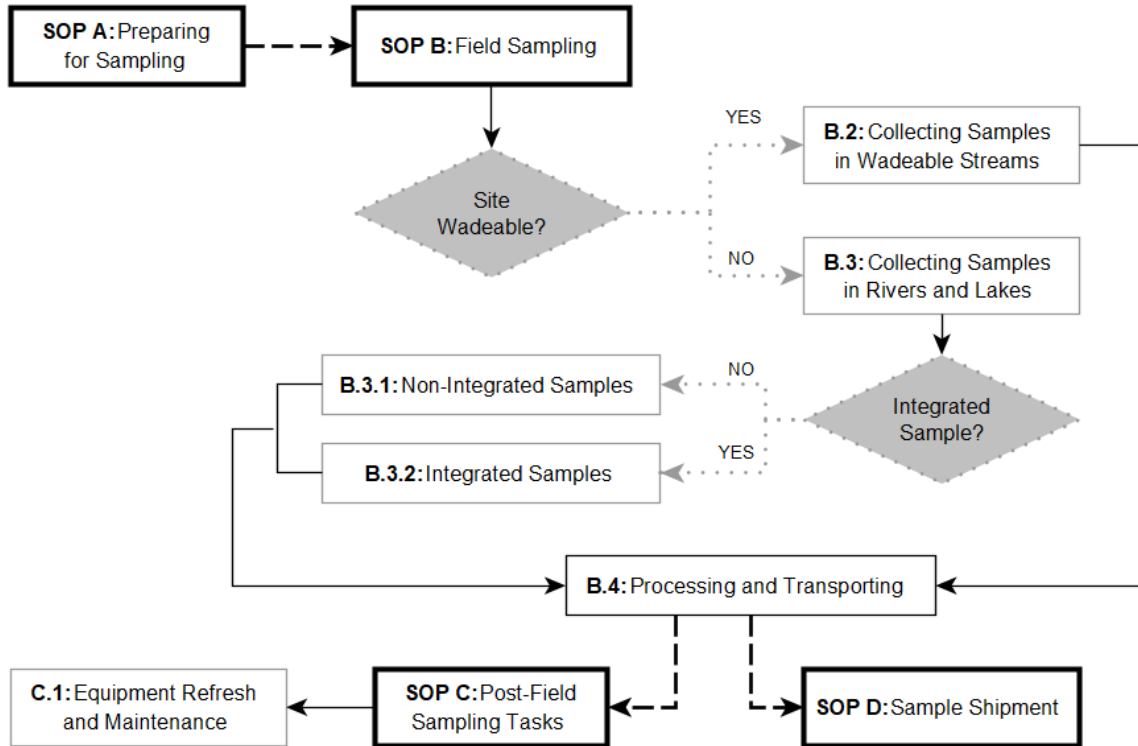


Figure 3. High level workflow diagram of how the included SOPs are sequentially connected. Bold boxes represent SOPs and gray boxes represent parts of SOPs, with dashed lines between SOP sections. Diamonds represent a decision that must be addressed.

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged 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 Labels and Identifiers

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



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



Figure 4. NEON Example of Type I barcode.

1. Prepare sample vials with labels before going in the field.
2. Remove submerged gas vials from storage containers and dry before applying labels. Make sure vials are fully dry before applying the barcode.
3. Attach a short (1 * 0.63 in label) Type 1 barcode label (**Figure 4**). NOTE that the barcodes used for vials are ~ 0.57 inches shorter than the typical NEON Type 1.
 - a. Add adhesive barcode labels to the sample containers along the length of the vial (**Figure 5**). Barcode must be placed at the top of the vial. Align the end of the label with the top lip of the glass, right before it narrows.

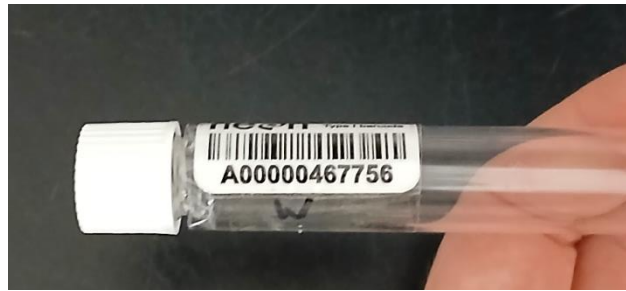


Figure 5. Example of appropriate attachment of barcode label.

- b. Allow the adhesive to cure for at least 30 minutes. It is OK for them to sit out longer, as long as no major changes in temperature or pressure occur.
4. Write the ID (W or A, or W2, W3, A2, A3 if collecting replicates) on the top of the vial cap (**Figure 6**) to help you quickly locate the correct sample during processing.

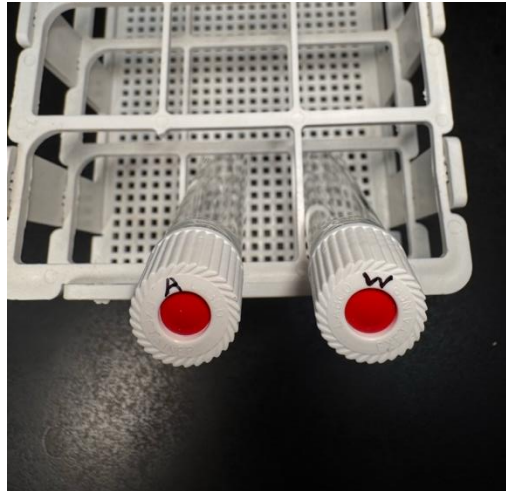


Figure 6. Gas vial with labeled septum lid.

- a. Sites with multiple locations (i.e., stratified lakes and lakes with permanent inflow and outflow sampling locations) will need to clearly identify which sampling location each sample is from.
- b. Workflow tip (not required): writing on vial in an area that will be covered by clear tape (Step 5; to keep sharpie from rubbing off) can help identify the correct vial during processing. Do not write on the barcode.
5. Cover the barcode with tape:
 - a. Wrap the tape completely around the vial, perpendicular to the label.
 - i. Do not place tape on cap, so as not to impact vial vacuum integrity by accidentally unscrewing the cap.



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- ii. Start with tape on the glass portion of the vial.
- iii. Press tape as you roll it around vial to avoid ripples or bubbles that may make it difficult to scan the barcode.
- b. Ensure the ends of the tape overlap slightly to keep the tape from coming unstuck.
- c. Use as LITTLE tape as possible (i.e., do not make multiple wraps around vial. If possible, cut tape to just slightly wider than barcode prior to wrapping).
- d. DO NOT use heavy duty clear packing tape.

SOP B Field Sampling



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

B.1 Spatially and Temporally Linked Protocols

Synchronized protocols and SOPs include:

- AOS Protocol and Procedure: SWC – Water Chemistry Sampling in Surface Waters and Groundwater (RD[10])
- AOS Protocol and Procedure: ASI – Stable Isotope Sampling in Surface and Ground Waters (RD[11])
- AOS Protocol and Procedure: AMC – Aquatic Microbial Sampling (RD[13]), occurs at a subset of water chemistry sampling events.

Surface water dissolved gas samples are collected at the same time and place as surface water chemistry and aquatic stable isotope samples. Aquatic microbes are collected in coordination with the water chemistry suite during monthly sampling events in streams and every other month in lakes and rivers.

B.2 Collecting Samples in Wadeable Streams

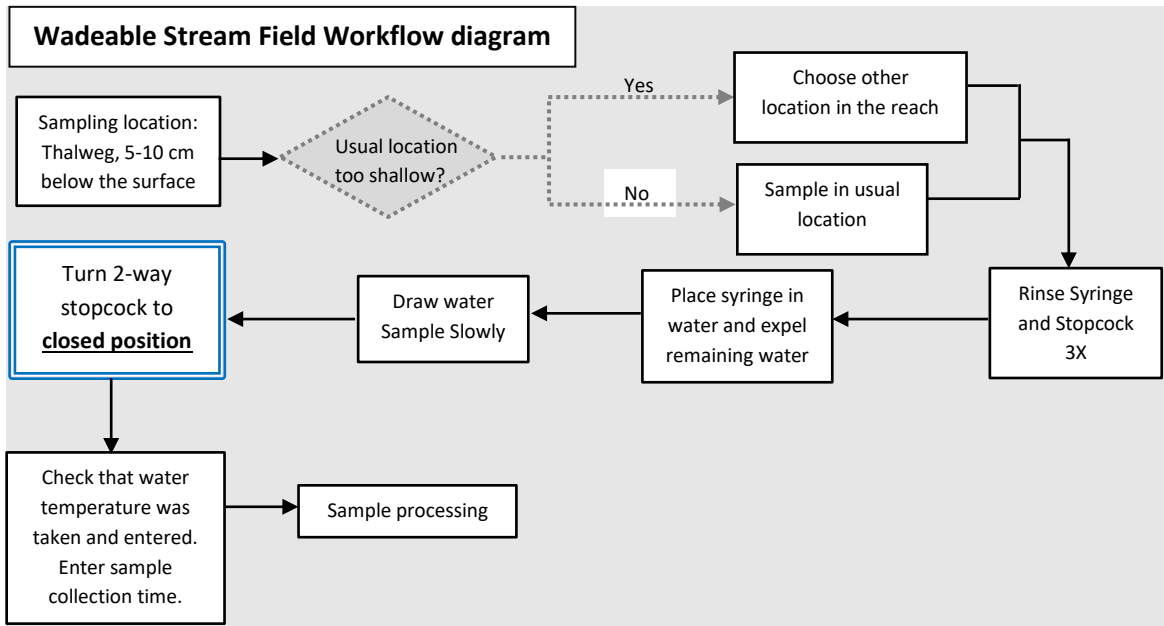


Figure 7. An expanded diagram of the workflow for SOP B.2 Collecting Samples in Wadeable Streams.

1. When traveling to and from the field site, vials should be protected from extreme temperatures.
 - a. Vials should not be allowed to overheat, as this changes the pressure in the vial and impacts their integrity. Do not leave in a hot vehicle. Keep vials in a cooler with ice packs to keep them from overheating.
 - b. If below freezing, make sure centrifuge tubes are not completely filled with water, this way if water freezes and expands, you will not break the glass vial.
2. Ensure the General AQU Field Metadata information is completed (RD[06]).
3. Record the Date (YYYYMMDD), time of day (use military time and record the local time (Ex. 13:46), and water temperature, and barometric pressure at the time of sample collection in the Water Chemistry App.
4. Ensure syringe graduations are covered with clear packing tape so they don't rub off.
5. Dissolved gas samples are collected in the same location and at the same depth as surface water chemistry and aquatic stable isotope samples (RD[10] and RD[11]), away from of any disturbance and in a flowing area, if possible.
 - a. ALWAYS sample in the thalweg and 5-10 cm 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 and be sure to take samples upstream from the standing location.
6. 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 8**) into the stream so that the water is sampled ~10 cm under the surface. Stopcock should always remain on the syringe.

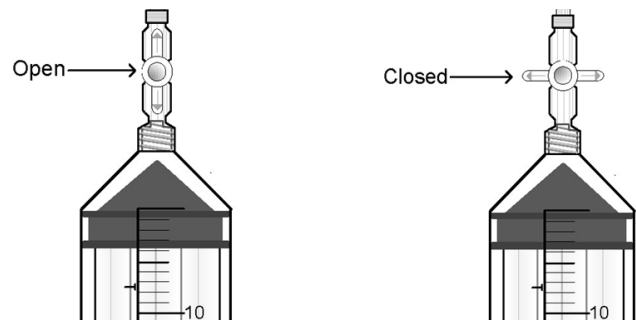


Figure 8. Syringe with a 2-way Luer lock stopcock in the “Open” and “Closed” positions.

- 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 one more time.



- e. On the third rinse:
 - i. 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.
 - ii. Roll the air slowly around the syringe and plunger tip to collect air bubbles.
 - iii. 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.
 - iv. Holding the syringe upright, expel the air and all but 3-5 mL of the water sample.
7. 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.
8. **SLOWLY** pull the plunger to draw a water sample until the syringe is completely full, and the plunger is at the 60-mL mark (**Figure 9**). 60 mLs 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, remove bubbles, or resample.

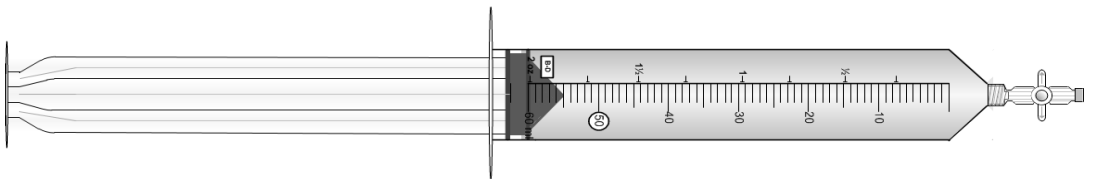


Figure 9. A full 60 mL syringe with a closed 2-way stopcock.

9. **BEFORE** removing the syringe from the stream, immediately turn the 2-way stopcock to the closed position (**Figure 8**).
10. Ensure water temperature at the time of collection is recorded in the water chemistry app.
11. If collecting replicates be sure to bring additional syringes in the field:
 - a. Replicate samples should be collected as close to the same time and location as the primary sample as possible, no more than 5 minutes in between samples.
 - b. Only complete one dissolved gas Fulcrum record. All of the WAT samples (1, 2, and 3) will be children of the rep 1 AIR sample. AIR rep 2 and 3 will not have any child WAT samples associated with them (**Figure 10**).
12. Proceed to B.4 for sample processing.

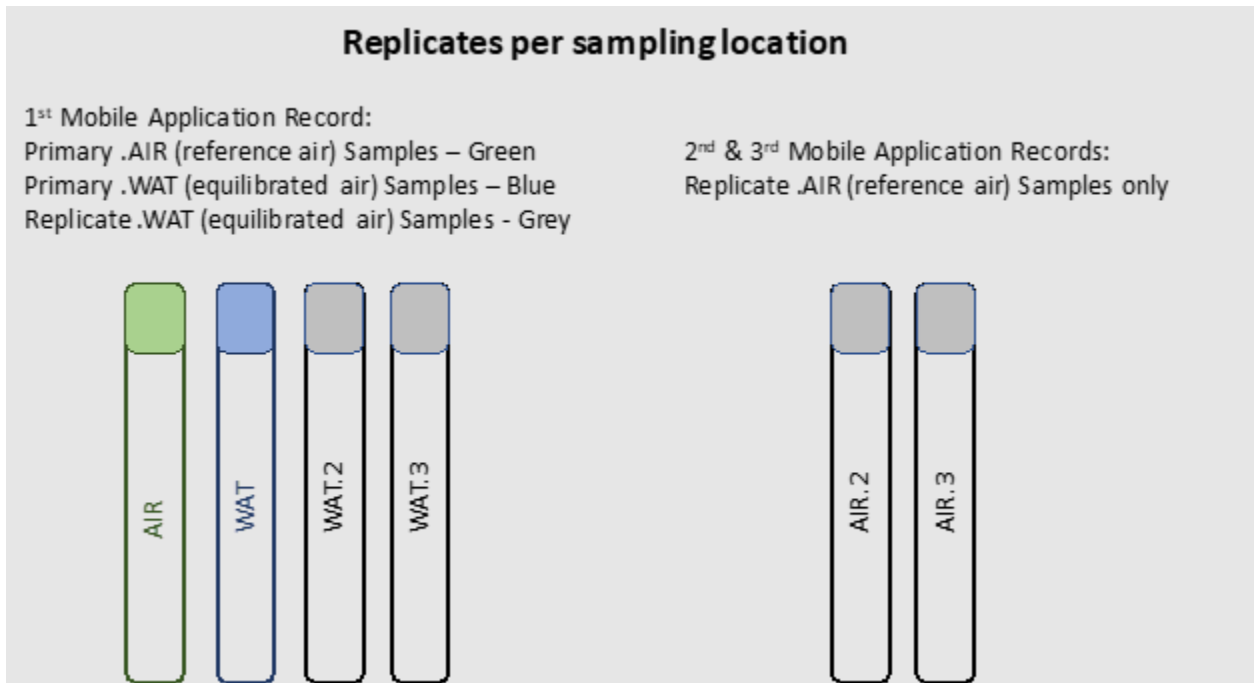


Figure 10. Dissolved gas replicate sample collection workflow.

B.3 Collecting Samples in Rivers and Lakes

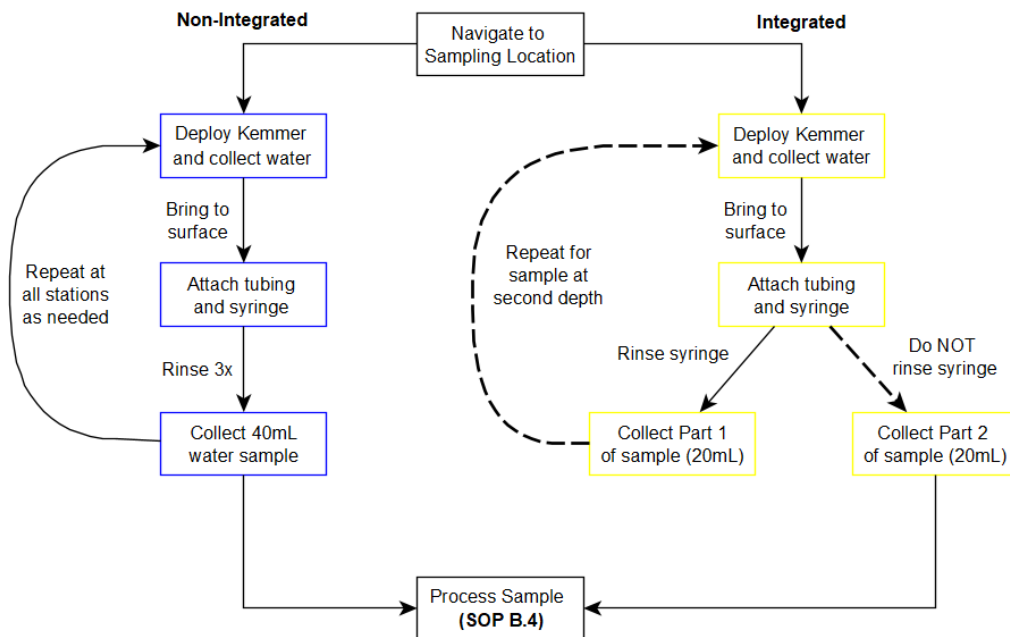


Figure 11. An expanded diagram of the workflow for SOP B.3 Collecting Samples in Rivers and Lakes. You will always collect one non-integrated sample at 0.5 m depth. Additional samples are collected if the lake is thermally stratified.

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1. When traveling to and from the field site, vials should be protected from extreme temperatures.
 - a. Vials should not be allowed to overheat, as this changes the pressure in the vial and impacts their integrity. Do not leave in a hot vehicle. Keep vials in a cooler with ice packs to keep them from overheating.
 - b. If below freezing, make sure centrifuge tubes are not completely filled with water, this way if water freezes and expands, you will not break the glass vial.
2. Determine if you need to collect a non-integrated or integrated
 - a. Take one sample at a 0.5-meter depth at buoy station (Kemmerer should be placed with top at 0.25 m and bottom at 0.75 m) below the surface of the water. For more details on sample collection steps, see section B.3.1 For Non-integrated Samples.
 - 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:
 - i. If NO, do not take any more samples.
 - ii. 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 1b**). For more detail, see Section B.3.2 For Integrated Samples
 - 1) If hypolimnion section depth (i.e., hypolimnion thickness) is <2 m, do not take any more samples.
 - 2) If hypolimnion depth/thickness ≥ 2 m but ≤ 4 m, then collect a sample from the midsection of the hypolimnion depth.
 - 3) 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.
 - iii. Is there a true (i.e., flow-through) inflow and outflow to the lake (**Figure 1a**)?
 - 1) If No, do not take any more samples
 - 2) If Yes, collect samples sampled just downstream of the inflow infrastructure and at the lake-outflow interface (just inside the lake boundary), following the wadeable stream protocol (RD[10]).
3. Ensure the General AQU Field Metadata information is completed (RD[06]).



4. Record the Date (YYYYMMDD), time of day (use military time and record the local time (Ex. 13:46), and water temperature, and barometric pressure at the time of sample collection in the Water Chemistry App.
5. Ensure syringe graduations are covered with clear packing tape so they don't rub off.

B.3.1 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.
 - a. 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 from the buoy location (**Figure 1b**).
 - b. And additional non-integrated sample is collected if the hypolimnion thickness is 2-4 m thick (**Figure 1b**). Note, if hypolimnion thickness is <2 m, no hypolimnion sample is collected.
3. Give the rope a slight tug to ensure the sampler has shut properly.
4. Gently bring the Kemmerer back to the surface and ensure the sampler is tightly closed. If the sampler is not be properly closed, discard the sample, and collect another sample.
5. Attach the tubing to the spigot of the Kemmerer (**Figure 12**).
6. Open the spigot valve and allow some water to pour out to flush the tubing.
7. Attach the open end of the tubing to the Luer-lock stopcock and 60 mL syringe (**Figure 12**).
8. Rotate the stopcock valve so that the sample syringe is in the 'open' position.
9. Rinse the syringe by filling the syringe with 20-30 mL of water. Turn Kemmerer spigot to the 'off' position. 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.
 - a. TIP: Keep the syringe and stopcock attached to the Kemmerer the entire time by drawing in air, shaking, and then discarding rinse water through the side-port of the 3-way stopcock. This avoids extra air in the Kemmerer tubing and makes sampling without air bubbles easier.
10. Repeat, rinsing 1 more time.
11. After the second rinse (NOTE: the following steps are 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.
12. SLOWLY pull the plunger to draw a water sample until the plunger is at the 60-mL mark (**Figure 9**). 60 mLs 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.
 13. Collect all water samples at all stations before processing.
 14. If collecting replicates, samples should be collected as close to the same time and location as the primary sample and from different Kemmerer grabs to capture environmental variability (**Figure 10**).
 - a. Every sample location will have three WAT samples associated with it.
 - b. Only complete one dissolved gas Fulcrum record. All of the WAT samples (1, 2, and 3) will be children of the rep 1 AIR sample. AIR rep 2 and 3 will not have any child WAT samples associated with them.
 - c. Lakes – If the lake has true inflow/outflow, collect two additional samples from each of these locations and follow the wadeable stream protocol for sample collection.
 15. Proceed to B.4 once all samples are collected for sample processing.

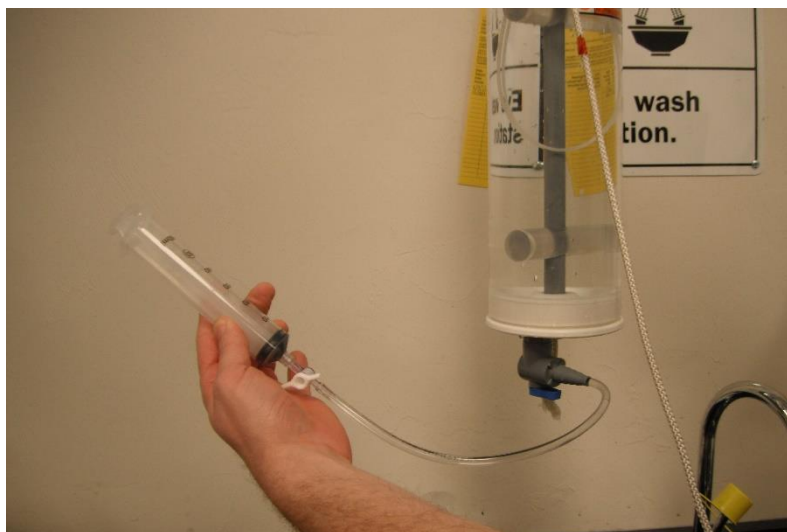


Figure 12. Syringe and 2-way stopcock attached to Kemmerer spigot.



B.3.2 For Integrated Samples

1. Sample 1 of 2

- a. Open and secure the Kemmerer bottle.
- b. Gently lower the Kemmerer 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. If sampler is not be properly closed, discard the sample and resample. Since we are attempting to collect a sample at a specific depth, the Kemmerer should be sealed completely to ensure no contamination from other depths when retrieving.
- d. Attach the tubing to the spigot of the Kemmerer (**Figure 13**).
- e. Attach the other end of the tubing to the 3-way stopcock and the syringe.
- f. Turn 3-way stopcock so the 'off' position is towards the syringe (**Figure 13**).
- g. Open the spigot valve and allow some water to pour out of the 3-way stopcock side port 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. This 20-mL sample is the first half of your integrated sample and is what you will use when collecting 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.

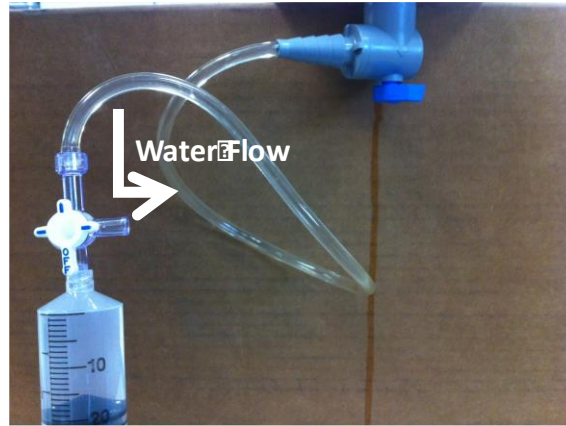


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- c. Turn stopcock so that the 'off' position is towards the syringe. Flush tubing through the side port of 3-way stopcock.
 - d. DO NOT RINSE syringe with second sample.
 - e. Rotate the stopcock so the 'off' position is at the side port.
 - f. Draw in 20 mL of sample water into the syringe. (Plunger should be at 40 mL mark).
 - g. Rotate stopcock so the 'off' position is towards the syringe and remove stopcock from tubing.
3. If collecting replicates (**Figure 10**), samples should be collected as close to the same time and location as the primary sample and from DIFFERENT Kemmerer grabs to capture environmental variability.
- a. Every sample location will have three WAT samples associated with it.
 - b. Only complete one dissolved gas Fulcrum record. All of the WAT samples (1, 2, and 3) will be children of the rep 1 AIR sample. AIR rep 2 and 3 will not have any child WAT samples associated with them (**Figure 10**).
 - c. **Rivers** – If the river is stratified, only collect replicates at the 0.5 m depth.
 - d. **Lakes** – Collect 2 WAT replicates per station/lake layer (i.e., if stratified: two samples from c1 and 2 sample from c2). If lake has true inflow/outflow, collect two additional samples from each of these locations. Every lake sample location has three WAT samples associated with it. Collect a total of 3 AIR samples from the processing location (AIR, AIR.2, and AIR.3). Lake inflow and outflow samples should be treated as stream samples.
4. Your sample is complete.
 5. Place the syringe in a small cooler or pitcher of sample water at ambient water temperature to help maintain temperature prior to processing and to avoid degassing.
 6. Record water temperature at the sampling location.
 7. Collect water samples at all stations before processing.
 8. Proceed to B.4 for sample processing.

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a)



b)

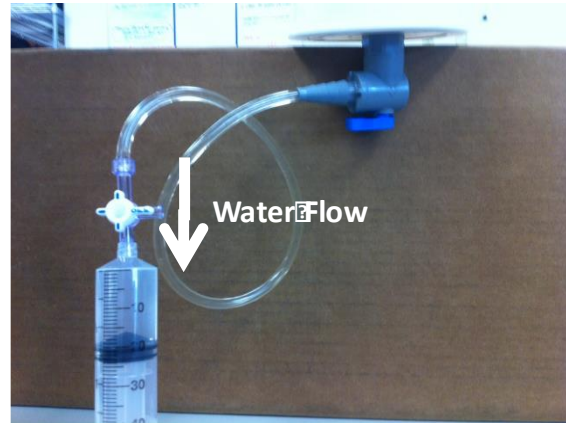


Figure 13. a) 3-way Luer-lock to Kemmerer spigot via tubing. Syringe is in the 'off' position allowing water to flow out the side port and b) 3-way Luer-lock to Kemmerer spigot via tubing. Side port is in the 'off' position, allowing water to flow into syringe.

B.4 Processing and Transporting Samples

NOTE: Samples should be processed immediately, if possible, and no longer than 4 hours after sample collection. If you are not able to process the samples immediately (within a few minutes), place the syringe in a storage container of ambient water (with stopcock down so water in vial is immersed in storage container water) in a storage container (i.e., small cooler, pitcher, or bucket) of ambient water to help maintain temperature and to decrease degassing prior to processing. If you unable to process samples immediately or store in water, please contact Science. Samples should be processed away from the stream/lake/river. When adding air to syringe collect the air at least 5 m from water's edge to avoid contamination by gas evasion from surface water. All water and air samples must be processed at the same location and time, so that the AIR sample provides a consistent atmospheric gas reference across all samples. All air samples, including the reference air and replicate air samples, should be pulled into the syringes as close to the same time as possible (i.e., one right after the other) and no more than 5 minutes apart.

Properly evacuated vials will often suck gas from the syringe into the vial; however, this is not always the case and should not be used as an indicator of a properly evacuated vial. If liquid water is observed in the evacuated gas vial prior to sampling or you are unable to push the typical volume of gas into the vial (even after attaching a new needle), the vial evacuation is likely compromised and should not be used. If a gas vial is not fully evacuated at time of processing, resample and use another evacuated gas vial. Label all improperly evacuated vials as such and return to the external lab. While properly evacuated gas vials will often suck in some volume of the sample, the collector must push on the syringe plunger to finish collecting the sample and ensure the samples are over pressurized. Always attempt to transfer the same amount of gas/sample to each vial (~18 mL) for all samples.

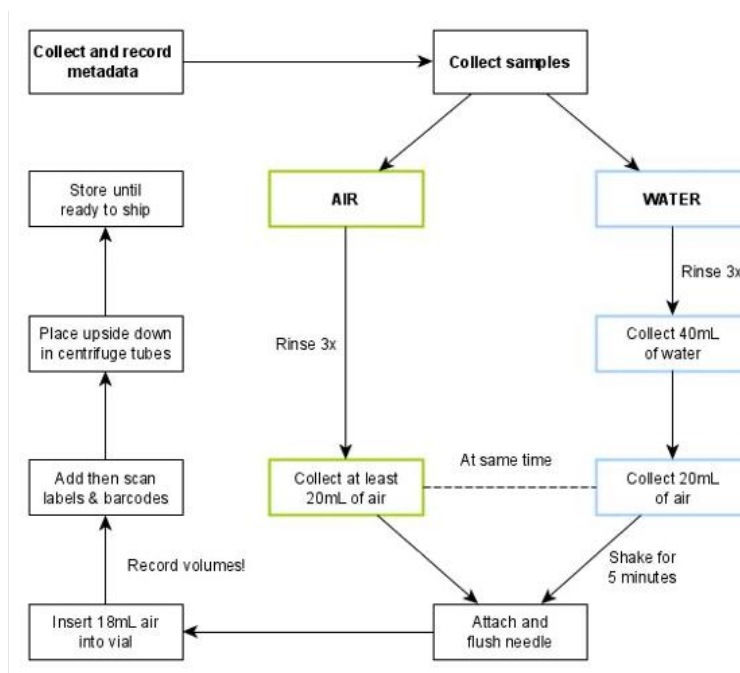


Figure 14. An expanded workflow for SOP B.4 Processing and Transportation Samples.

1. Always, avoid holding the syringe where the water sample is located, as the heat from your hand can warm up the sample and alter the dissolved gas concentration.
2. Move to an appropriate location to collect the ambient air for the WAT and AIR samples:
 - a. At least 5 m from the water's edge at stream and river sites
 - b. At the same height within a collection (i.e., collect both samples 1 or 2 m above the ground. Stay consistent.). This is because gas concentrations change as you move further away from vegetation.
 - c. Do not sample near exhaust from a running truck.
 - d. It is OK to process on a boat, but sample away/upwind from boat exhaust.



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 15a**). You will not need to do this step for integrated samples, since you only sampled 40 mL.

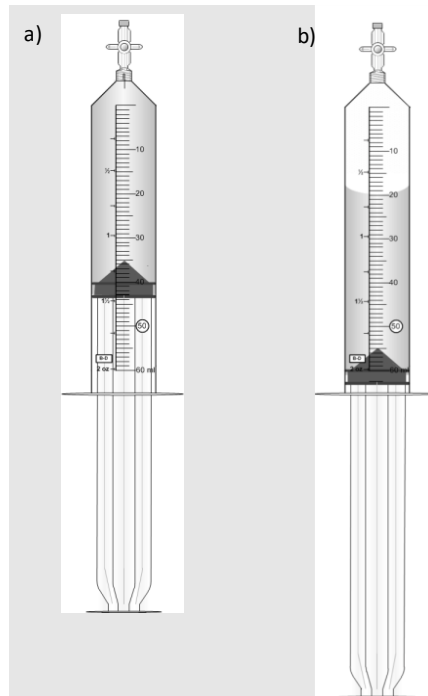


Figure 15. A closed syringe with a) 40 mL of sample water and b) 40 mL of water and 20 mL of ambient air.

4. Add 20 mL of ambient air to the sample syringe:
 - a. Hold syringe at 1 - 2 m above the ground and at a full arm's length from the body. Point syringe into the wind and away from contamination (e.g., human breath). **Be consistent on the height at which the air is sampled across all syringes.**
 - b. Pull the syringe plunger to 60 to draw in 20 mL of ambient air (**Figure 15b**). Rotate stopcock to close syringe (**Figure 8**).
 - i. **NOTE:** Use the plunger as your measurement mark, rather than the air/water line.
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 15**). 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 that will be used to correct the dissolved gas concentrations:
 - a. Collect the air sample at same height as the WAT sample air was collected.
 - b. Hold syringe into the wind and away from contamination (e.g., human breath).



- c. Draw a full syringes worth of air into the syringe and expel, rinsing the syringe with air. Rinse with ambient air 3 times.
 - d. Draw in at least 20 mL of ambient air.
 - e. Rotate stopcock to close syringe (**Figure 8**).
 - f. In lakes, the air sample will be considered station “c0” no matter where the sample was collected (i.e., boat, shore, lab, etc.).
7. Proceed to equilibration location.
- a. Shaking for 5 minutes can influence sample temperature. Thus, it is highly preferable to shake in ambient water, if possible.
 - b. If shaking in ambient water is not possible, record additional fields at time of collection (detailed below).
8. Shake the sample syringe for 5 minutes so that the dissolved gases in the sample water come to equilibrium with the ambient air in the headspace.
- a. **NOTE:** It is important to be consistent and shake the syringe(s) 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 where the sample water is contained, as this will increase the sample water temperature.
 - c. Avoid holding the syringe in direct sunlight.
8. Sample processing location (reference air collection):
- Field over water (Lake/River sites only)
 - Field over land, or
 - Indoor laboratory
9. Record sample equilibration location (shaking):
- In water at sampling location
 - In water at other location (e.g., at the boat ramp), or
 - In air
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 or after gas transfer**. Be sure the barcode labels on the gas vials match the sample type on the mobile application.
11. **Check gas vials to ensure no water is in vial prior to sampling.** Water in vials is an indication that the vacuum has been lost.

12. Attach needle to stopcock
 - a. Push and slightly turn base of needle to improve connection.
 - b. Needles can be reused within a Site and Collect Date, as long as needles are purged first to remove any non-sample gas.
13. Holding the syringe upright, open the stopcock and push a small amount of air (~0.5 mLs) through the needle to purge the needle.
14. Immediately after purging, transfer dissolved gas from the water (WAT) to an evacuated gas vial (**Figure 16**).
 - a. Do not turn stopcock off after purging needle or you will draw air back into needle.
 - b. Make note of the plunger position to determine amount of air added to vial.



Figure 16. Transfer of dissolved gases to an evacuated gas vial.

15. Push ~18 mL of gas into vial.
 - 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.
 - i. Tip: If possible, hold needle at the base where it connects to the stopcock when removing needle.
 - 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, first try a new needle as the needle may be clogged from the septa. If you can still not push gas in after changing needles, **DO NOT use the vial**. Use one of the provided spares.

- i. 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.
 - ii. Return unusable vials to the external lab.
16. **Avoid pushing sample water into the vial.** A small amount of sample water in the vial is acceptable but should be minimized or avoided, if possible.
- a. To reduce pushing water into the vial, stop transferring gas when the water meniscus is at the 0 mark.
 - b. If you accidentally get too much water in the vial, please resample.
 - c. A few tiny drops/mist are OK, but lots of drops or a pooled drop at the bottom is too much (**Figure 17**). If you are having trouble filling the vials without getting water in them, please reach out for help (certified trainers, Science).

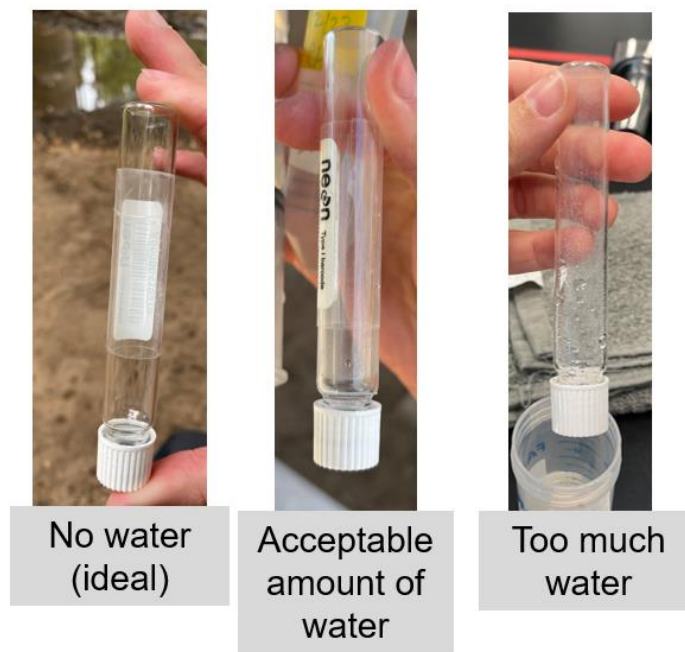


Figure 17. Example of acceptable dissolved gas samples with differing water levels in sample.

17. Repeat steps as needed for any additional dissolved gas water samples collected in stratified systems. You may use the same needle if the needle is **purged** with sample first before inserting into the vial.
18. If collecting replicates (**Figure 10**):
 - a. Replicate AIR samples should be collected as close as possible to the same time and location as the primary sample.

- b. Only complete one dissolved gas Fulcrum record. When creating the sample record, all the WAT samples (1, 2, and 3) will be children of the rep 1 AIR sample. AIR rep 2 and 3 will not have any child WAT samples associated with them.
19. Transfer AIR sample(s) 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 16**), 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).
20. If sample equilibration location was 'In air' or 'in water at other location', record the syringe water temperature and barometric pressure immediately after injecting gas samples into the vials. To collect those additional measurements for WAT syringes:
 - a. **Remove** the metal guard of the YSI handheld meter.
 - b. Ensure stopcock is closed.
 - c. Invert syringe and remove plunger.
 - d. Place the YSI handheld meter (WITHOUT guard) into the syringe (**Figure 18**).
 - e. Visually verify that the temperature probe is covered with water.
 - f. Record sample temperature and barometric pressure.

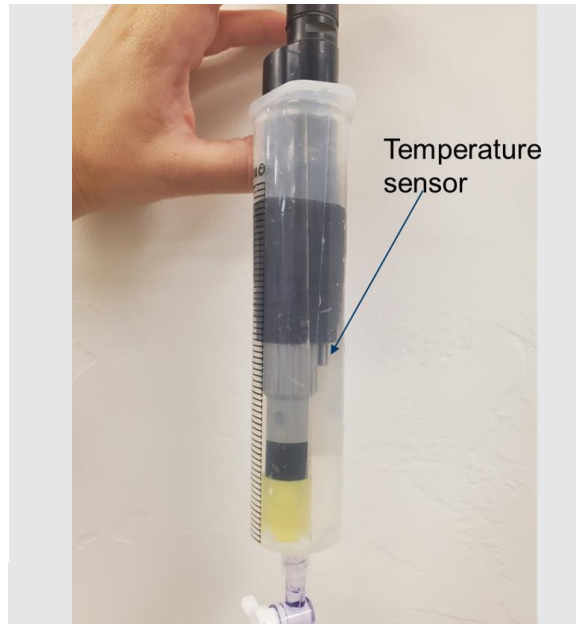


Figure 18. Handheld temperature probe without metal guard inserted into syringe after sample collection, with temperature sensor submerged in water.

21. Place needle(s) in a Sharps container.
22. 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]). **NOTE:** Read the change in plunger position to determine amount of air added to vial.
23. **Record data in the mobile app.**
 - a. Scan the barcode label with the tablet (**Figure 19**). Barcode labels should be located on the actual dissolved gas vial, not the centrifuge tube.



Figure 19. Barcode label scanning.

24. Store gas vials by submersing upside down in water filled 50-mL centrifuge tubes (**Figure 20**). You may re-use the water that was previously in the centrifuge tubes. If additional water is needed, top off with DI water.
25. Return to the Domain Support Facility for sample storage and/or shipping.



Figure 20. Dissolved gas samples stored upside-down in centrifuge tubes.



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SOP C Post-Field Sampling Tasks

C.1 Equipment Refresh and Maintenance

1. Refreshing the sampling kit
 - a. Restock the sampling kit with new evacuated dissolved gas sampling vials, 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. Be sure to cover new syringes with clear packing tape over the graduations so that they don't wear off.
2. Equipment maintenance, cleaning, and storage
 - a. Ensure all equipment is properly decontaminated (see RD[08]) and dry prior to storage.
3. Inventory Samples and perform barcode QC. It is best practice to use the domain inventory app for tracking samples that are held for longer than a couple of days.
4. If for some reason you must discard samples, remove barcode from the vial(s) and place into a separate bag from the samples. Label the bag "Do not analyze"
 - a. Update Fulcrum record to reflect that no SDG samples were collected.

C.2 Document Incomplete Sampling Within a Site

This section is not applicable to the Surface Dissolved Gas SOP, since only one location is sampled.



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SOP D 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. Data collected on paper data sheets must be transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data are accurate and complete. Certain QA checks can be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before the sampling window closes. Invalid metadata (e.g. collection dates, plotIDs) are difficult to correct when field crews are no longer at a sampling location.

Office QA procedures are meant to ensure that sampling activities are consistent across bouts, that sampling has been carried out to completion, and that activities are occurring in a timely manner. The Office QA will also assess inadvertently duplicated data and transcription errors to maintain data validity and integrity. See the Data Management Protocol (RD[04]) for more discussion of QA measures.

Before samples ship to external facilities and/or their digital records load to the NEON database, the data must undergo thorough quality checks. The steps needed to accomplish this are outlined in the SDG QC Checklist, which is available on the [NEON SSL](#).

Sample Identifiers & Barcodes

By default, each (sub)sample produced by this protocol receives a sample identifier, which contains information about the location, date, and sample type. Each (sub)sample will also be associated with a scannable barcode, which will not contain information about sample provenance, but will improve sample tracking and reduce transcription errors introduced by writing sample identifiers by hand.

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, thus it is encouraged to apply these in



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advance. Use the appropriate barcode label type with each container (i.e., cryogenic Type II barcode labels only used for samples that are stored at -80°C, etc). Note that a barcode label is applied in addition to a sample identifier (hand-written or printed).

Barcodes are scanned into the data entry application when a sample is placed into a container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data.



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SOP E Sample Shipment

1. Follow sample shipping timelines in Section 4 to maintain appropriate sample hold times and storage conditions.
 - a. Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.
2. Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment in order to ship samples to external laboratories or the biorepository (RD[14]).

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8 REFERENCES

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

APPENDIX A QUICK REFERENCES

Summary of Surface Water Dissolved Gas Sampling Procedure

Step 1. Sampling Preparation

- A. Data Capture Preparation
- B. Prepare Labels and Identifiers

Step 2. Field Sampling

- A. Sample Collection Location – record date, time, water temperature and barometric pressure at time of collection
- B. Collect Sample Using Appropriate Technique for the Site

Step 3. Process Samples

- A. Record related information (i.e., date time, barometric pressure)
- B. Collect surface water samples
- C. Remove Air Bubbles
- D. Add 20mL of Ambient Air to Sample Syringe
- E. Collect Ambient Air Sample
- F. Shake WAT sample for 5 min, preferably in water at collection site
- G. Record sample processing location and all relevant information
- H. Record Sample Type
- I. Check Vials for Correct Labeling and Void of Water
- J. Transfer equilibrated air sample to the vial(s)
- K. Transfer AIR Sample to vial
- L. Record Data in Mobile App -record sample temperature and barometric pressure, as needed

Step 4. Transport Samples

- A. Store the Gas Vials Upside Down in D.I. Water Filled 50mL Centrifuge Tubes
- B. Return to Domain Office and Ship or Store Samples

Surface Water Dissolved Gas Sampling Reference Tables

- SOP B.2 Wadeable stream field workflow diagram
- SOP B.3 River and Lake field workflow diagram
- SOP B.4 Processing and transporting samples workflow diagram

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APPENDIX B REMINDERS

Sampling Locations: Make sure to collect samples:

- Wadeable streams and Rivers: Just downstream of the primary sensor set and in the main flow at the Water Chemistry sampling location.
- Lakes:
 - For non-stratified lakes: from 0.5 m below the surface of the water.
 - For stratified lakes with a very shallow hypolimnion (hypolimnion thickness of <2 m), only one sample is collected at 0.5 m below the surface of the water, as in a non-stratified lake.
 - For stratified lakes with shallow hypolimnion (hypolimnion thickness of 2-4 m), 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 stratified lakes with a hypolimnion thickness >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 water sample in the syringe, remove large air bubbles from water sample syringe.
- Process immediately, if possible, or store water sample syringe(s) in a bucket of ambient water if transporting back to the lab to process. Process within 4 hours of collection.
- 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. Collect all air samples (for WAT and AIR samples) at least 5 meters from stream/river edge and at same height above ground.
- Shake syringe sideways for **5 minutes**, while holding syringe at base to decrease temperature changes. Shake syringe in sample water when possible.
- Carefully record all metadata, measurements, and observations.

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Each domain has site specific guidelines for timing of sample collection and can be found in Domain Specific Sampling Designs (**Table 6**). The dates in the Sampling Design documents are estimated from historical hydrologic data. Dates presented are only a guide and are derived according to the logic presented in Section 4.2. Because individual years may vary widely from the average dates provided below, it is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling per environmental conditions, as described in Section 4 of this protocol.

Table 6. Aquatic Site Sampling Design documents.

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



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APPENDIX D SITE-SPECIFIC INFORMATION

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

D.1 D18/19 Vial Evacuation Procedures are detailed in the Reaeration Protocol Appendix G (RD[15]).

APPENDIX E 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 7. Equipment list – Sample collection for surface water dissolved gas in wadeable streams, rivers, and lakes.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Mobile data entry tablet, fully charged and synced before field work	Field data entry	1
	N	60 mL syringe, polyethylene	Sample collection	2*
	N	1-way male Luer-lock stopcock, polycarbonate	Sample collection, attach and close syringe	2*
	N	3-way male Luer-lock stopcock, polycarbonate	Sample collection for integrated samples	1
	N	Sharps container	Needle disposal	1
YSI 6052030	Y	Handheld Conductivity/Temperature Meter; YSI Pro2030	Temperature and Conductivity Meter – must order DO Probe addition separately	1
YSI 605202	Y	DO probe for conductivity/temperature handheld meter – Galvanic for YSI Pro2030	DO Galvanic Probe and replacement tips – must order separately	1
YSI 605913	Y	Replacement DO sensor tips for Galvanic probes on YSI Pro2030	Replacement part for DO sensor tips – order when appropriate.	As needed
	N	Storage container (pitcher)	Field storage and transport	1
Consumable items				
Ordered from lab by HQ based on sampling schedule	Y	12 mL evacuated gas vial(s) with rubber septa; evacuated <50 mTorr	Prepared sample container shipped direct to Domain from external lab	2*

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Adhesive barcode labels (Type I) – short, 1 * 0.63 inches	Labeling sample bottles with barcode-readable	1 sheet
	N	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	Needles, 27 G ½ inch	Sample transfer from syringe/stopcock to gas vial	2*
	N	Clear Packing tape (Not heavy-duty clear tape)	Covering syringe graduations, and securing labels to vials prior to shipping, if necessary	

*per station (Lakes may have up to 4 stations). Always take extra to the field. When collecting replicates, additional items per station will be required.

Table 8. Equipment list – Additional equipment and consumables for dissolved gas sampling in lakes and rivers.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Boat and oars		1
	N	Safety/First aid kit		1
Cole-Parmer 05485-10	Y	Kemmerer water sampler (or Van Dorn sampler were approved)	Water collection	1
	N	Tubing (1/8" ID)	Sample transfer from Kemmerer to syringe	1
	N	Tubing (3/8" ID)	Sample transfer from Kemmerer to syringe	1
	N	Flow control hose clamp	Sample transfer from Kemmerer to syringe	1
	N	3-way stopcock	Sample transfer from Kemmerer to syringe	1
	N	3/8" to 1/8" tubing adaptor	Sample transfer from Kemmerer to syringe	1



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Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	GPS (Accuracy < 4m)		1
	N	Waders or boots		2
	N	Personal Flotation Devices		1 per person