



Title: AOS Protocol and Procedure: ASI - Stable Isotope Sampling in Surface and Ground Waters		Date: 12/15/2021
NEON Doc. #: NEON.DOC.001886	Author: Z. Nickerson	Revision: H

AOS PROTOCOL AND PROCEDURE: ASI - STABLE ISOTOPE SAMPLING IN SURFACE AND GROUND WATERS

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F	01/10/2018	ECO-05285	<ul style="list-style-type: none"> Clarified replicate wording to match updated SWC 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. Updated shipping of H₂O samples to every 2 months per CLA schedule Added barcode language
G	12/17/2018	ECO-05959	<ul style="list-style-type: none"> Fill H₂O vial 80%, reduced from 90% to account for freezing Removed sample type from labels Added clarity on drying filters Updated barcode language Updated shipping manifest language to include STORK app.
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1 OVERVIEW

1.1 Background

The following protocol outlines field sampling of stable isotope chemistry in aquatic environments (e.g., stream, rivers, lakes, and groundwater). Naturally occurring stable isotopes are useful ecological research tools. Isotopes are forms of the same element, differing in the number of neutrons in the nucleus. These different forms have similar chemical reactivity, but the different number of neutrons affects the weight of the element. While these elements have similar chemical reactions, the extra weight of the neutron can result in slower reactions than observed in the lighter isotope, resulting in a changing ratio of heavy to light isotope. This is known as fractionation.

Isotopes are measured as the fractional amount of heavy isotope (^HF) over the light (^LF) isotope, relative to a standard.

$$\delta = [(R_{\text{sample}}/R_{\text{standard}} - 1)] * 1000,$$

where $R = {}^H\text{F}/{}^L\text{F}$.

Common stable isotopes of ecological interest include nitrogen, carbon, oxygen, hydrogen and sulfur, which are useful in studying elemental cycling, food web dynamics, nutrient transfer and hydrological modeling (Fry 2008).

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).



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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.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	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: Data Management
RD[05]	NEON.DOC.002211	Datasheets for AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.002905	AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
RD[09]	NEON.DOC.001199	AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
RD[10]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling
RD[11]	NEON.DOC.002494	Datasheets for AOS Shipping Manifest
RD[12]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[13]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples and Equipment

2.3 Acronyms

Acronym	Definition
Ah	Amp Hours
°C	Degrees Celsius
¹³ C/ ¹² C	Carbon stable isotope ratio
cm	centimeter

CLA	Collections and Laboratory Analysis
DI	De-ionized
ft ²	Square foot
GF/F	Glass fiber filter
GPS	Global positioning system
h	Hour
² H/ ¹ H	Hydrogen stable isotope ratio
H ₂ O	Water
Hg	Mercury
L	Liter
m	meter
m ²	Square meter
m ³	Cubic meter
mph	Miles per hour
mL	milliliter
mm	millimeter
μm	micrometer
NEON	National Ecological Observatory Network
¹⁵ N/ ¹⁴ N	Nitrogen stable isotope ratio
¹⁸ O/ ¹⁶ O	Oxygen stable isotope ratio
POM	Particulate organic matter
s	Second
SOP	Standard operating procedure

2.4 Definitions

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.

Hydrograph: A diagram depicting the change in discharge (m³/s) over a given time(s).

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.

Isotope: An atom with the same number of electrons and protons, but different numbers of neutrons.

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

Stable isotope: Isotopes (i.e., atomic species) that do not undergo spontaneous radioactive decay.

Thalweg: The deepest part of a stream channel.



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Thermocline: The vertical section of the lake where the rate of decrease in temperature with increasing depth is greatest. The denser and cooler layer below the thermocline is defined by the hypolimnion. The warmer upper layer is termed the epilimnion.



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3 METHOD

The following protocol describes the collection, processing, storage, and shipping of the stable isotopes $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$ of Particulate Organic Matter (POM) and $^2\text{H}/^1\text{H}$, $^{18}\text{O}/^{16}\text{O}$ of water samples from aquatic environments, including streams, rivers, and lakes. $^2\text{H}/^1\text{H}$, $^{18}\text{O}/^{16}\text{O}$ of water will also be sampled from groundwater.

In streams, disruption of the sediment by walking or by sampling too close to the stream bottom can contaminate samples. Thus, always sample upstream from wading activity and minimize the suspension of sediments when sampling. If sediments are disrupted, wait until the area has cleared before sampling.

In lakes and rivers, care must be taken to avoid contaminating the sample with re-suspended bed sediment. Such contamination may be minimized by anchoring the boat upwind (or upstream) of the sampling site and using an anchor line 2-3 times as long as the depth of the lake or stream. Ensure that when anchoring in rivers, the anchor and boat are within the permitted reach. If sediments are disrupted, wait until the area has cleared before sampling (normally this only takes a few minutes).

In **wadeable and non-wadeable streams**, samples are collected in the deepest part of the channel (A.K.A. thalweg, the line of least resistance to water flow), where it is assumed that the stream channel is well mixed. The stream sampling location is located away from, or upstream of, any major local disturbances and other areas where NEON sampling activities commonly occur. Stable Isotope samples should be collected with water chemistry samples immediately downstream of Sensor Set 2 in **wadeable streams** (Figure 1a) and just downstream of the only sensor set (Sensor Set 1) in **rivers** (Figure 1b). In streams with a shallow water column, technicians must be cautious not to disturb the benthic sediment when sampling.

In **lakes** (Figure 1b,c), sample collection depends on lake depth and stratification, as detailed in Figure 4. For all lakes, one sample is taken at 0.5 m (Kemmerer sampler 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. If the lake is stratified at the time of sampling, an additional sample will be collected from the hypolimnion at the buoy location (4 total samples). In lakes with **very** shallow hypolimnions (hypolimnion thickness <2 m) do not collect a hypolimnion sample. In lakes with shallow hypolimnions (hypolimnion thickness 2-4 m), the sample is collected from the mid-point of the hypolimnion. In lakes with deep (i.e., thick) hypolimnions (hypolimnion thickness >4 m) an integrated sample is collected as described in the water chemistry protocol (RD[08], Figure 4). Note that if inflow and outflow streams are present, samples are collected just downstream of the inflow and outflow infrastructure, following the wadeable stream sampling design (Figure 1c).

Groundwater well samples should be collected to align in space and time with the groundwater water chemistry samples (RD[08]). Groundwater samples are only sampled for H_2O isotopes, no POM isotopes are collected from groundwater wells.



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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 is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).

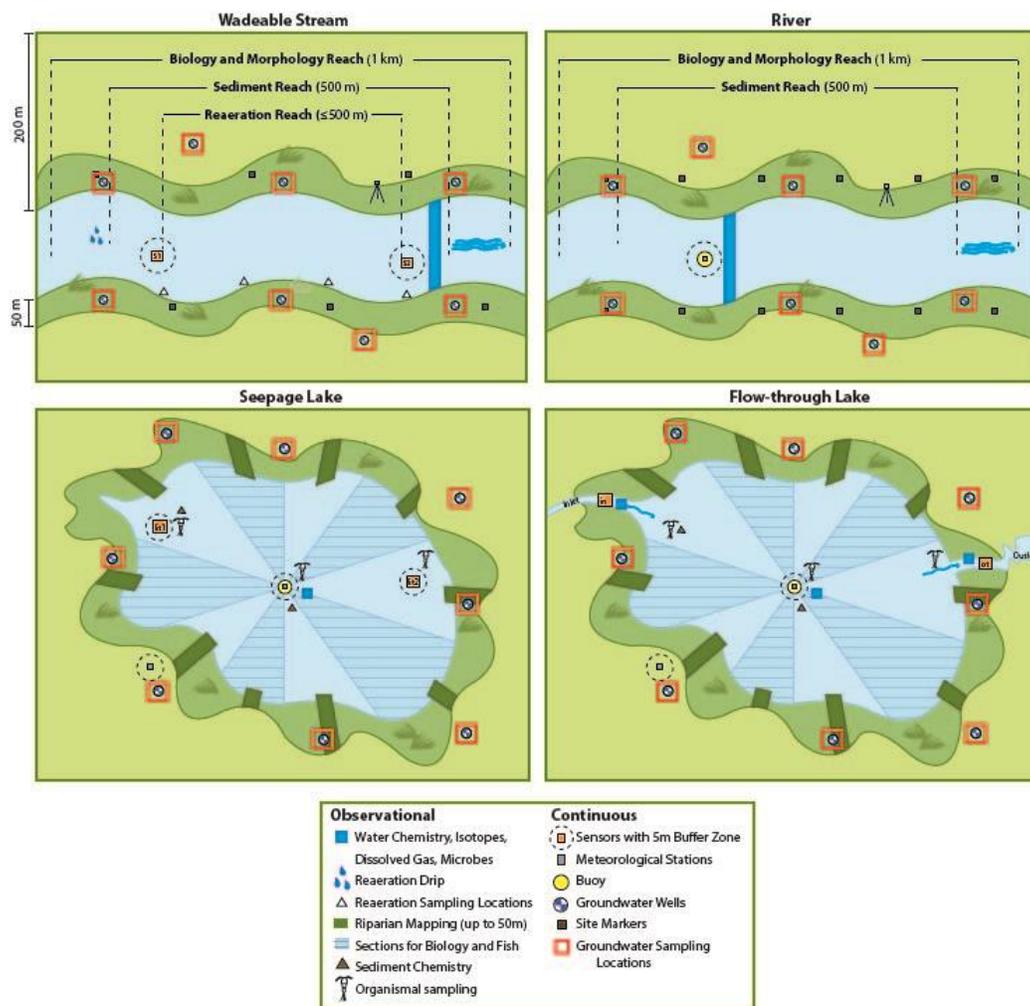


Figure 1. Generic site layouts for wadeable streams, rivers (AKA non-wadeable streams) and lakes surface water and groundwater sampling locations. Seepage lakes are lakes with no true inflow and outflow, while flow-through lakes have a true inflow and outflow.



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4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Stable Isotope samples are collected according to the schedule in **Table 1**, and implementation of this protocol is scheduled as part of the Water Chemistry Suite which also includes the protocols listed below. Science Operations will provide sample timing annually.

- AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwaters (RD[08])
- AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling (RD[09])
- AOS Protocol and Procedure: Aquatic Microbial Sampling (RD[10])

Table 1. Sampling frequency for Stable Isotope procedures on a per SOP per site type basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Remarks
SOP B.2	Wadeable and Non-wadeable Streams	Buoy, S2	1 day	26	Sampling is synchronized with Water Chemistry protocols during monthly and discharge related windows.
	Lakes	Buoy, Inflow, Outflow	1 day	12	Sampling is synchronized with Water Chemistry protocols monthly.
	Groundwater Wells	3-4 of 8 wells	1 day	2	Sampling is synchronized with Water Chemistry protocols (± 1 day) in early spring and late fall.

Scheduling Considerations

Wadeable and Non-wadeable Streams

- In wadeable and non-wadeable streams and rivers, aquatic stable isotope sampling occurs up to 26 times per year (**Table 1**). Standard recurrent stream and river sampling should take place one Tuesday per month (e.g., 12 times per year) in coordination with ‘co-located’ NEON atmospheric wet deposition chemistry sampling. The remaining 14 sampling dates are site specific and based on the cumulative discharge of the stream representing the increasing and decreasing periods of annual peak flow. Science Operations will provide sample-timing windows in domain specific sample strategy documents.

Lakes

- In lakes, aquatic stable isotope sampling shall occur up to 12 times per year (**Table 1**). Other than event based sampling, chemistry samples should be collected on Tuesday to coincide with other national chemistry sampling efforts (RD[07]). For lakes, the number of samples will vary with season, and depends on the timing of thermal stratification.



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Groundwater Wells

- c. For groundwater wells, a total of 6-8 samples per year per site will be sampled. Due to the limited number of samples available it is anticipated that a subset of wells will be sampled at multiple times per year (e.g., 3-4 wells sampled 2 times per year; **Table 1**). This will allow for evaluation of seasonal responses in groundwater constituent concentrations (RD[08]). Sample timing will be site-specific, but in general, samples are targeted for the early spring and late fall (See **Section 4.2** for detailed explanation on sampling criteria).

4.2 Criteria for Determining Onset and Cessation of Sampling

Stable isotope chemistry sampling occurs in conjunction with water chemistry sampling in streams, lakes and groundwater environment (RD[08]). The timing of sampling allows researchers to assess aquatic biogeochemical cycles, and therefore timing depends on the dominant driver(s) of nutrient flux and cycling within each system. Timing of sampling is site-specific and determined by rules developed using historical discharge for streams and environmental data for both streams and lakes (RD[07]). For example, streams with little or no flow during the summer dry-season or streams that are frozen during the winter are sampled more intensively during other periods that have more flow. Systems that have a snowmelt-dominated or storm-dominated flow regime are sampled more intensively during elevated flows, on both the rising and falling limb of the hydrograph (i.e., time periods when the majority of the nutrients are moving through the system), and sampled sporadically during times of baseflow (RD[07]). Stream systems that are heavily influenced by winter rains are sampled more frequently during 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.

Groundwater sampling dates are guided by the hydrologic cycle of the system that the site is located in. Samples are targeted for the early spring when the dominate precipitation events begin to increase the groundwater flow rate towards the aquatic system (stream, river, or lake); and for late fall when the production of surface constituents have had sufficient time to infiltrate into the groundwater. The timing of these seasons is site dependent, and the quantification mechanism is unique to the aquatic system. Sample timing will be defined by the cumulative discharge curve for streams and rivers, with the first sample occurring when the discharge is between 20-30%, and the second sample when the discharge is between 70-80% of the predicted annual cumulative discharge based on historic discharge data, when available. Sample timing for lakes will be similar to that of streams and rivers, but due to the lack of a discharge curve, the groundwater around the lakes will be sampled based on the lake level. The historic trends in annual lake level fluctuations, when available, will be used to define when the samples shall be collected. The first sample shall be obtained when the lake is 20-30% from its seasonal high level, and the second set of samples shall be collected when the lake level has reduced between 70-80% from its high level towards its seasonal low water level. When historical lake level data is not available, sample timing shall occur near the first and third biology sampling bouts and will be coupled with the surface water chemistry sampling bouts. The timeframe for collecting samples will range from a 2-week to a 2-month window of time within which to align a groundwater chemistry sampling event with a

surface water sampling event. This range is dependent on the discharge characteristics unique to each aquatic system.

4.3 Timing for Laboratory Processing and Analysis

Samples should be stored on ice (4 °C) immediately after collection. Process (filter and store appropriately) surface and subsurface isotope samples as soon as possible, and within 4 hours of sample collection. For storage and shipping timelines see the NEON Protocol and Procedure: Shipping Ecological Samples and Equipment Protocol (RD[14]).

Table 2. Timing for laboratory processing and handling of Stable Isotope samples

Sample type	Activity	Holding Time
Oven-dried (65°C) POM Samples	Filter, Dry, Store in desiccator, and ship to external lab	Shipped with Algal Chemistry or every 2 months
Room temperature H ₂ O samples	Filter, Cap, Parafilm cap, Store, and ship to external lab	Shipped every 2 months on CLA-defined schedule

4.4 Sampling Timing Contingencies

Table 3. Contingency decisions for Stable Isotope protocol in streams and lakes.

Delay/Situation	Action	Outcome for Data Products
Hours	If the stream water chemistry sampling location is too shallow to obtain a sample, sample upstream in a flowing section of the stream (always sample in the thalweg, if possible) and note this change in the mobile app or field sheet.	No adverse outcome.
	If no streamflow exists, sample in a nearby pool where the water is deep enough to obtain a clean, sediment free sample, and be sure to note this change in the field sheet as a “non-flowing sample.”	No adverse outcome.
	If the stream is entirely dry or frozen solid, note that on the field sheet.	No adverse outcome.
	If the stream is ice-covered, but is still flowing, the ice should be broken so the stream can be sampled (following a few minute period to allow the water to clear).	No adverse outcome.
	If the lake is frozen only on the surface 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.
	If lake surface is frozen, and the lake has in-lake inflow and outflow sampling locations, only sample at buoy location. Lakes with inflow and outflow streams should follow stream ice recommendations for inflow and outflow locations.	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.

Table 4. Contingency decisions for Stable Isotope protocol in groundwater.

Delay/ Situation	Action	Outcome for Data Products
Hours	If temperatures are below freezing and water in the pump discharge line is freezing, stop sampling and reinitialize the sampling effort when ambient temperatures are above freezing. Since groundwater well sampling occurs at most twice a year, the events should be timed with above freezing weather conditions.	No adverse outcome.
	Should time become limited during chemistry sampling, collect water samples in 4-L jugs and return the samples on ice to the designated sample processing location to filter.	No adverse outcome.
	If water samples cannot be processed in situ, the filtration and preservation must be completed within 4 hours of sample collection. Samples must be kept cold (~4 °C) to reduce nutrient transformation. Water jugs must be shaken before filtration to re-suspend particulates and homogenize water.	No adverse outcome.
	If when sampling a well following the low flow method, the well goes dry, turn off the pump and wait for groundwater to return to the well, then restart the pumping and collect groundwater for sampling.	No adverse outcome.
	Though wells are generally sited for slightly elevated locations, times will occur when standing water surrounds the base of the well. In this condition postpone sampling until the ground near the base of the well is free of standing water.	No adverse outcome.
Days – Months	If site conditions dictate that stream sampling is not possible due to the stream being dry, then postpone the groundwater sampling event until flow returns in the stream.	No adverse outcome.
	If a well goes dry, postpone the sampling event until the selected well has water again. If the well goes dry for an extended period of time, and the stream maintains flowing water, submit a trouble ticket so we can evaluate switching the selected well to a different well.	No adverse outcome.
	In some locations, the groundwater level will drop below the bottom of the well either seasonally (e.g., Taiga and Tundra sites) or in periods of drought, which are likely to occur at some point during the life of the Observatory. Under these conditions sampling of groundwater is not possible.	No adverse outcome.



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	For sites that have the “generation” of groundwater resulting from seasonal thawing of permafrost, sampling is targeted for times when the permafrost is sufficiently thawed to allow for collection of groundwater samples.	No adverse outcome.
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A groundwater chemistry sampling event must occur within a 2-week to a 2-month window of time to align with a stream sampling event. This range is dependent on the discharge characteristics unique to each stream. Groundwater well sampling can be time consuming; therefore, extending the groundwater chemistry sampling event over two days is acceptable. However, efforts should be taken to complete the sampling in one day. If site conditions dictate that stream sampling is not possible due to the stream being dry, then postpone the groundwater sampling event until flow returns in the stream. Additional information on Groundwater Sampling contingency planning can be found in the Groundwater Chemistry Sampling From Observation Wells (RD[08]).

Samples should be processed (filtered) as soon as possible. If necessary, surface water may be collected in a large container, kept on ice at 4 °C, and filtered and/or preserved within 4 hours at a base camp or DSF (i.e., if weather or other safety considerations dictate the need to get out of the field immediately and/or stream discharge is increasing quickly). Sample collection time and processing time must be recorded in the mobile app or on the Aquatic Isotope Sampling Data Sheet (RD[05]).

Table 5. Sampling specific contingent decisions.

Delay/ Situation	Action	Outcome for Data Products
If a groundwater well is damaged (i.e., casing is broken internally) or bent	Do not try to sample this well. It is likely that the pump may get stuck inside the well due to the limited tolerance between the pump and well casing. If this condition is observed submit a trouble ticket for a new well to be selected for sampling.	No adverse outcome.

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 plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- 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 plot suitability, and informing end users of NEON data availability. Some types of missed sampling 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]).



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Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates:** Bout-specific sampling dates.
- **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 according to one of the scenarios documented in **Figure 2**, 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**).



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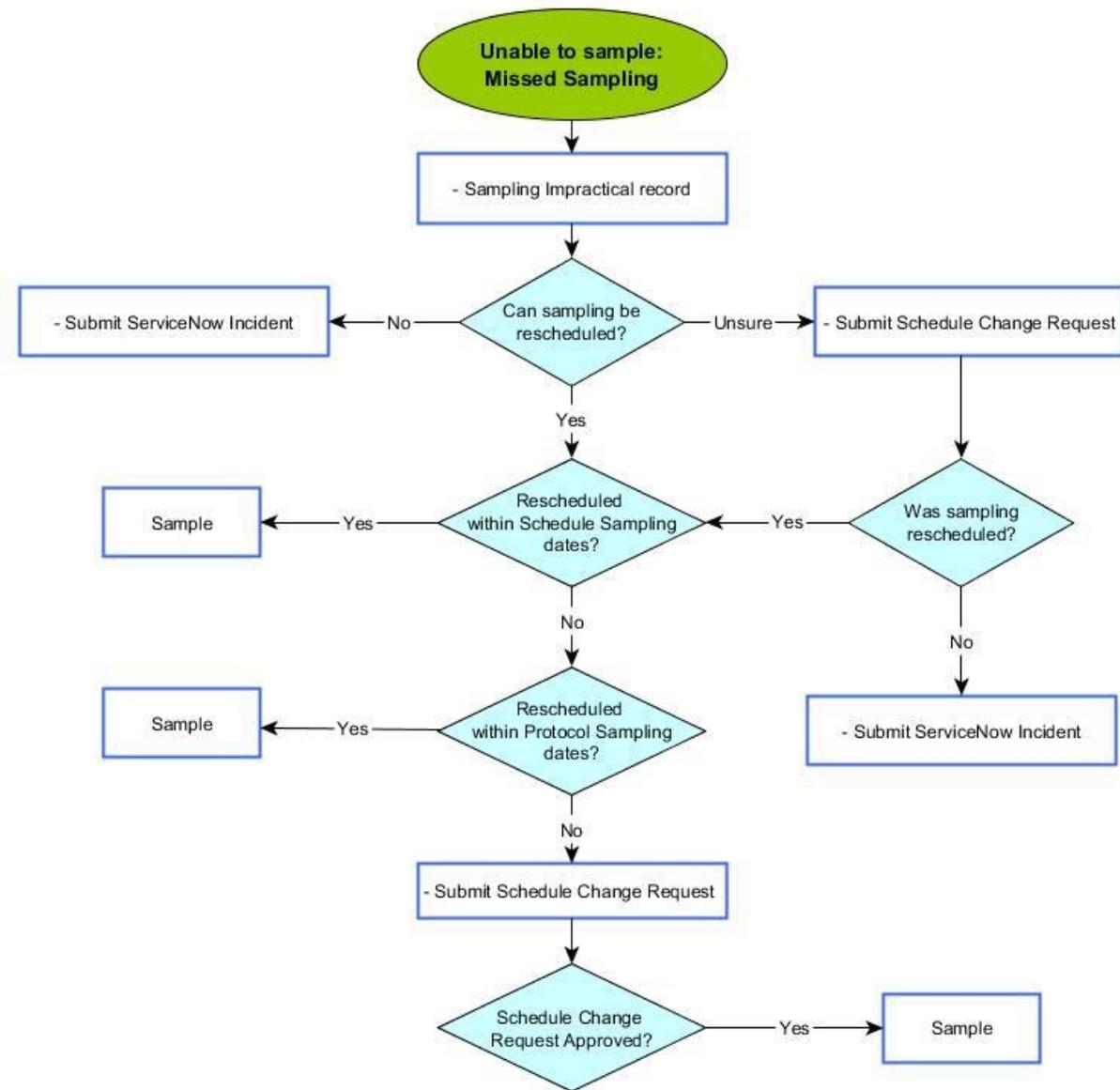


Figure 2. The documentation to account for a MissedSampling event. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNowincident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management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

To Report Missed or Incomplete Sampling:

2. Missed or Incomplete Sampling that cannot be rescheduled within the Schedule sampling dates must be communicated to Science by a ServiceNow Incident.
 - a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 2**).



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- b. Consult **Table 6** below to determine required actions if scheduled activities are delayed or canceled. Guidance for this and other NEON protocols is summarized for ease of use in a table posted to a Field Science Sharepoint library. However, this protocol is the ultimate source of information should any discrepancy exist.
3. Create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled. That is, if data are recorded in the field at the plot level, a record must be made for each plot missed.
 - a. For stable isotopes sampling, create a **Sampling Impractical** record at the water chemistry sampling metadata-level in accordance with RD[08].
 - b. Missing data in downstream applications (e.g., Lab apps) are not recorded. For example, the stable isotope-specific field data and external laboratory data (H₂O and POM) will not be returned if there is a **Sampling Impractical** record entered at the metadata label.
4. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 7**).
5. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Flag must also be recorded (**Figure 2**).

Table 6. Guidance for responding to delays and cancellations encountered during implementation of the Stable Isotope Sampling protocol.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Stable Isotopes – H ₂ O Shipping	If not shipped during scheduled week	Notify ^List-CLA	Notify ^List-CLA

Table 7. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that 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
Low yield groundwater	Low yield groundwater well
Other	Sampling location inaccessible due to other ecological reason described in the remarks
Shallow groundwater	Shallow groundwater; depth of groundwater column less than 0.5 m
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)



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4.6 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.

Table 8. Estimated staff and labor hours required for implementation of the Stable Isotope Sampling protocol.

SOP	Estimated time	Suggested staff	Total person hours
B.2: Field Sampling in Surface Water	1-2 h	1-2	1-4 h
B.2: Field Sampling in Groundwater	4-16 h	1-2	4-32 h
B.3: Sample Collection and Processing	1-2 h	1	1-2 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 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. Anyone can stop work, and you are expected to use that authority when there is uncertainty about the safe conduct of work.

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

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

1. Due to site-specific hazards that may be encountered, technicians may perform GPS positioning around the lake, and measurements for inflow and outflow, without dismounting from the vessel. In addition, technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance, 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

6.1 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

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 safely.



7 STANDARD OPERATING PROCEDURES

SOP Overview

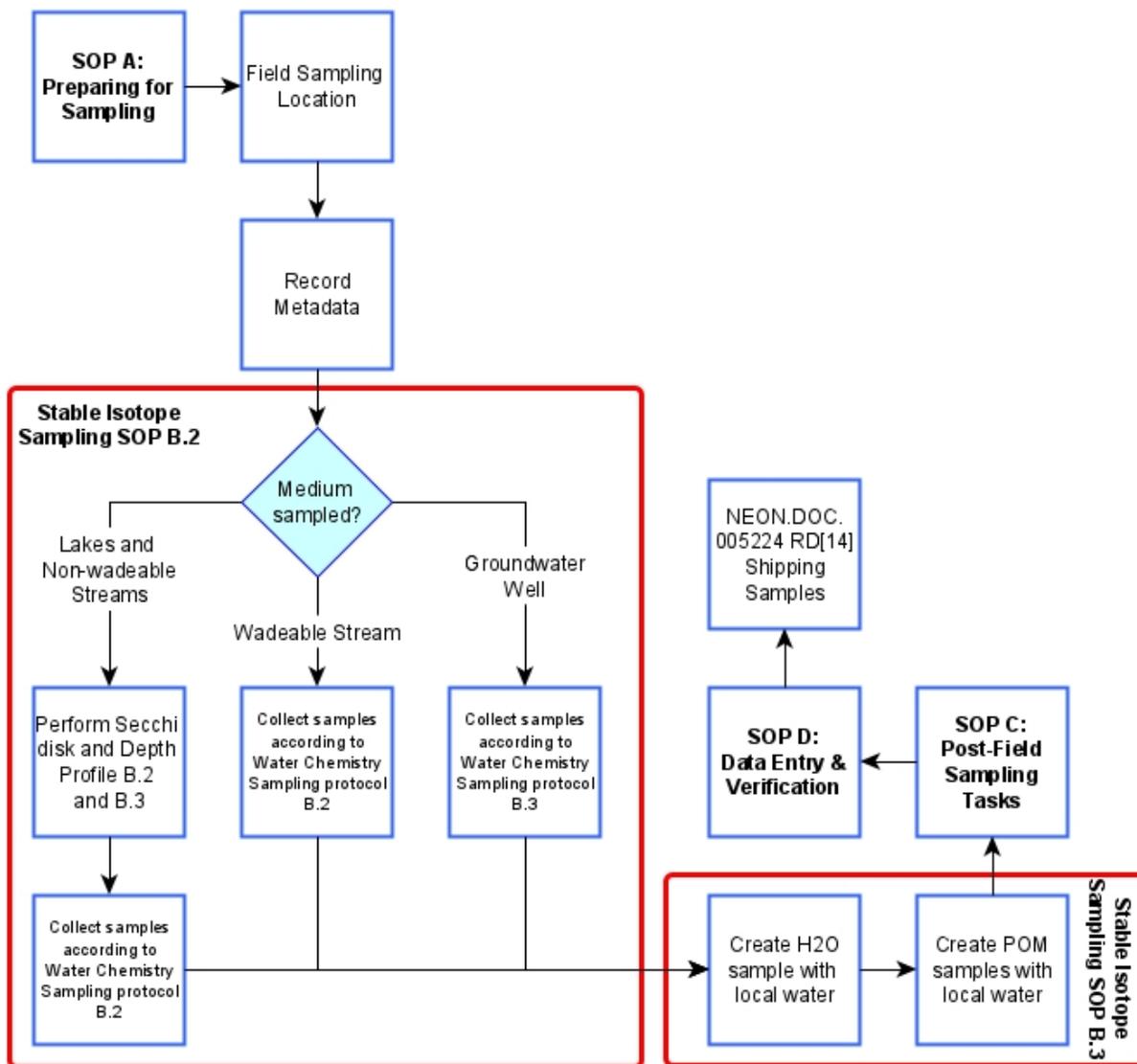


Figure 3. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.



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SOP A Preparing for Sampling

A.1 Selecting Sampling Depth in Lakes and Rivers

6. Take one sample at a 0.5 m depth at buoy station.
7. 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:
 - a. If NO, do not take any more samples.
 - b. 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 4**).
 - 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 into 2 sections and collect a sample in the midsection of both those layers. Integrate the samples from the hypolimnion into 1 sample.
8. Is there a true inflow and/or outflow to the lake (**Figure 1**)?
 - a. If No, do not take any more samples. If Yes, collect samples just downstream of the inflow and/or outflow infrastructure, following the wadeable stream protocol.

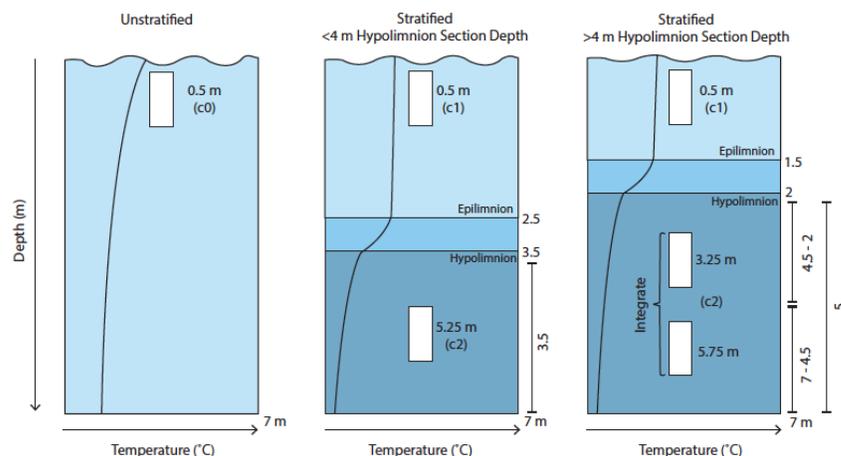


Figure 4. Examples of unstratified and stratified lake water column sampling depths with placement of thermocline. Note, hypolimnion sampling is determined by the hypolimnion section depth (i.e. thickness). If hypolimnion thickness is < 4 m, collect one sample



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A.2 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. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). 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.3 Preparing for Field Sampling

9. Pre-ash GF/F filters:

- a. Place layers of 25 mm GF/F filters on aluminum foil. Use multiple layers of foil if needed, filters can be touching and placed on top of one another but should not be stacked more than 3 filters deep.
- b. Place in muffle furnace (500 °C) for at least 6 hours.
- c. After the furnace has cooled, remove from the furnace and stack filters using filter forceps, and place in original box.
- d. Label box with permanent marker to read "ASHED, Your Name, Date".
- e. Place box in in sealed zip-top bag.
- f. Ashed filter may be stored indefinitely, as long as they remain in the box and stay dry.

10. Check the aquatic isotope field sampling kit to make sure all supplies are packed.

A.4 Labels and Identifiers

Many protocols use a mix of human intelligible labels and barcodes in their workflow. 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.** In addition, barcodes should be used for lab procedures where it makes sense.

Rules of thumb for using barcodes in the lab:

- In general, apply a barcode if a sample is generated and returned to for processing over a period of > 5 business days.
- Barcodes are optional for temporary samples that are created and consumed in < 1 day. For example, pooled samples that are then immediately sorted, split, or ground may not need a barcode.





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All barcodes need to be applied to dry containers for 30 minutes 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 5**).

1. Prepare final sample containers by affixing one Type I adhesive barcode label to each container used to contain each sample. Adhesive barcode labels should be applied at the DSF 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).
 - a. For H₂O isotope sample vials, barcode labels should be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode lengthwise along a vial, not horizontally wrapping around a vial.
 - b. For POM isotope foil packs, put on a pair of nitrile gloves and apply the label to a new piece of foil layed flat on an ethanol-cleaned surface (e.g., lab bench, tray). The side of the foil laid against the ethanol-cleaned surface will become the inside of the foil pack. Apply the labels to the upward-facing side of the foil.
 - i. DO NOT touch the area that will become the inside of the foil pack with ungloved hands
 - c. 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 associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.
2. Attach pre-printed labels (**Figure 6**).
 - a. SampleID: **site.stationID.YYYYMMDD.sampleType.Rep.siteID** is the 4-digit side code. *StationID* is the 2-digit station code where sample was taken (i.e., Station ID for streams = "ss", non-wadeable streams/rivers = 'c0'; in Lakes, inlet="in", outlet="ot", center="c0", if center is stratified: "c1", "c2", "c3" with "c1" being the top layer; "w1"-"w8" for groundwater wells). *sampleType* is the 3-letter sample code (H₂O or POM), do NOT include isotope numbers (i.e. 2H,18O or 15N13C) to sampleID. *Rep* is replicate #.
 - a. For regular H₂O collections without replicates, rep is always ".1" See example:
 - i. Ex.) **PRIN.ss.20151013.H2O.1**
 - b. Use a priner and/or sharpie to fill out labels before going into the field

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Figure 5. An example of a Type I barcode. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.

SampleID: _____
 (siteID.stationID.YYYYMMDD.sampleType.Rep)
NEON

Figure 6. NEON aquatics stable isotope chemistry labels example.

About Barcode Uses and Placement

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required.

Table 9 provides a quick reference to the types of sample this protocol generates that require barcodes.

The final disposition of all vial/filtered samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository or an external laboratory.

Table 9. Barcode requirements for sample types generated by the Stable Isotope Sampling protocol.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required	Barcode Qty
POM.1 POM.2	Particulate Organic Matter Isotopes	CARI.ss.20200429.POM.1 CARI.ss.20200429.POM.2	(AOS) Water Chemistry	Foil Packet	Type I	Always Required	1 per filter
H2O	Water Isotopes	CARI.ss.20200429.H2O.1	(AOS) Water Chemistry	15 mL vial	Type I	Always Required	1 per vial



SOP B Field Sampling

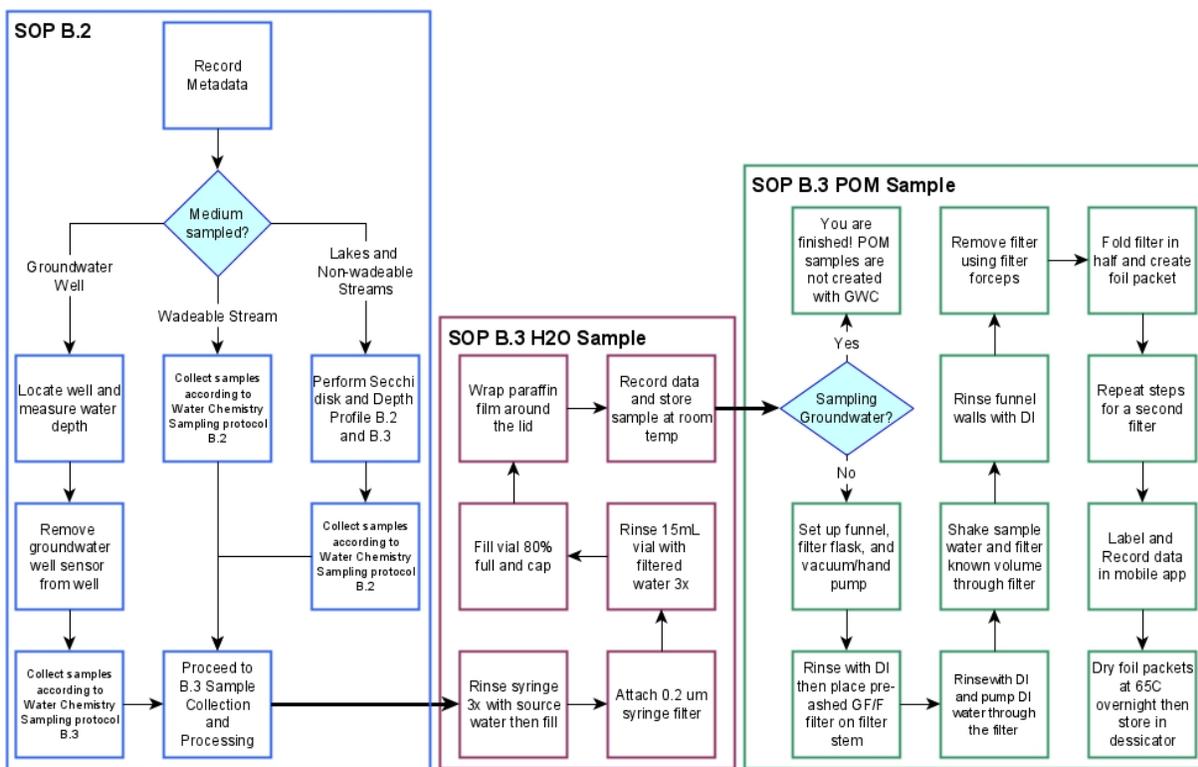


Figure 7. An expanded diagram of the workflow for Field Sampling.

B.1 Spatially and Temporally Linked Protocols

Water Chemistry Sampling in Surface Waters and Groundwater

- Isotope sampling in wadeable streams will be completed in the same location as surface water chemistry, immediately downstream of Sensor Set 2 (RD[08]) and upstream of any stream disruption and in a flowing area of the stream, if possible.
- Isotope sampling in lakes and rivers will follow the Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]).
- Groundwater wells are only sampled for H₂O, no POM samples are collected. Groundwater isotope H₂O sampling will be completed in the same location/assigned wells as water chemistry sampling occurs.

Dissolved Gas Sampling

- Isotope sampling in surface water will be completed in the same location as surface water dissolved gas sampling (RD[09]) in wadeable streams, lakes, and rivers.



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Aquatic Microbial Sampling

- Isotope sampling in surface water will be completed in the same location as surface water aquatic microbe sampling (RD[10]) in wadeable streams, lakes, and rivers.

B.2 Field Sampling

1. In the field, complete the General AQU Field Metadata and the Water Chemistry Application on the mobile data recorder. You only need to fill out one General Field Metadata Application per site per day.
 - a. Always make note of any weather or stream conditions that could influence chemistry, including but not limited to wind, activities in the surrounding watershed, prior flood or rain events, ice, and changes in sampling locations (RD[06]). For lakes, also record new Global Positioning System (GPS) coordinates (accuracy is <4 m) and total depth of the water column sampled with the water chemistry protocol (RD[08]).
2. Collect samples according to water chemistry sampling protocol (RD[08]) in streams, rivers and lakes, or groundwater wells, as appropriate. Samples should be collected at the same depth as the water chemistry samples are collected. Samples for isotopes should be collected out of the same sampler and collection jug as water chemistry, if possible.
3. Station ID is the 2-digit station code where sample was taken (i.e., Station ID for streams ="ss", non-wadeable streams/rivers ='c0'; in Lakes, inflow="in", outflow="ot", center="c0", if center is stratified: "c1", "c2", "c3" with "c1" being the top layer; "w1"-"w8" for groundwater wells).
4. Wadeable Streams
 - a. Collect stream isotope samples and metadata following the Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]).
 - b. ALWAYS sample in the thalweg (the deepest location in the stream cross-section) 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 be sure to take samples upstream from the standing location.
 - c. Proceed to SOP B.3, Sample Collection and Processing.
5. Lakes and Rivers (Non-wadeable streams)
 - a. Collect river and lake water samples and metadata following the Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]).
 - 1) Move to the sampling station and note the station ID in the mobile recorder (on the field sheet, if necessary) (RD[08]; SOP B.2).
 - 2) Determine the depths for sampling according to Secchi Application.
 - 3) Sample using the Kemmerer bottle, as described in the water chemistry protocol (**Figure 8**, RD[08]; SOP B.2).
 - b. Proceed to SOP B.3, Sample Collection and Processing.



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Figure 8. Image of a Kemmerer Sampler.

6. Groundwater Wells

- a. Collect groundwater sample water and associated metadata from selected wells following the Groundwater Chemistry Sampling Protocol (RD[08], SOP B.3).
 - 1) Locate well and measure water depth.
 - 2) Remove groundwater well sensor from the well.
 - 3) Extract groundwater from well, using appropriate pump method.
 - 4) Collect water samples for H₂O. No POM samples are collected for GW.
- b. Proceed to B.3, Sample Collection and Processing.

B.3 Sample Collection and Processing

¹⁸O/¹⁶O and ²H/¹H of H₂O samples from Surface and Ground Water

1. Rinse a 5 mL or 60 mL syringe 3 times with source water. Discard rinse water downstream or on the bank.
 - a. Some may prefer to use a 5 mL syringe because it is easier to press the water through the filter. However, you will need to fill the 5 mL syringe several times to collect the appropriate sample volume.
2. Fill the syringe with sample water.
3. Attach a 0.2 μm syringe filter.
4. Rinse collection bottle (15 mL glass vial with rubber closure) with filtered sample water 3 times. To rinse: filter at least 1-3 mL of sample water into vial, recap (you may just hold cap on with your hand instead of rescrewing cap each rinse), and shake to ensure water touches all parts of the bottle and cap.



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5. Filter the remaining water in the syringe into a 15 mL glass bottle (**Figure 9**). Fill the vial with **12 mL** of filtered water. This volume will minimize evaporation and will leave enough headspace to ensure bottle will not break if freezing occurs.
6. Securely attach lid and **wrap plastic paraffin film** around the lid and vial to secure the lid and to reduce air movement.
7. Record data in the mobile app.
 - a. If available, scan the barcode label with the tablet (**Figure 9**).
 - b. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.



Figure 9. Barcode label scanning.

8. No additional preservation or special shipping is required. Store samples at room temperature until shipment

$^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ of POM from Surface Waters

9. Put on a pair of nitrile gloves.
10. Collect water using a 4 L jug to be filtered within 4 hours. In shallow streams, you may need to use the peristaltic pump to get the water out of the stream and into the 4 L jug.
 - a. When transporting water back to the DSF, to reduce biological activity, keep jug on ice and in the dark until you are able to process the sample.
 - b. When processing samples in the field, keep the 4 L jug in a shaded and cool place until you are able to process the sample.



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11. Set up filter funnel, filter flask, and vacuum pump (**Figure 10**).
 - a. Connect flexible tubing from vacuum pump to hose connection on filter flask.
 - b. Make sure filter stem is inserted into the hole in the rubber stopper.
 - c. Insert rubber stopper into the top of the filter flask. Push in tightly.
 - d. Attach top of filter funnel to filter stem. This may be a magnetic connection or a screw-in connection.

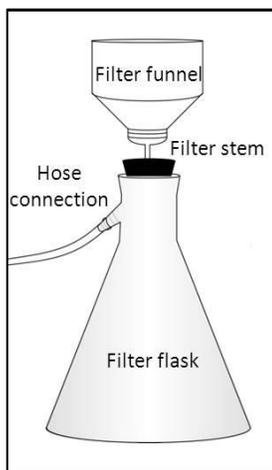


Figure 10. Filter apparatus setup.

12. Rinse the filter funnel and stem with de-ionized (DI) water, using a squirt bottle and wipe with kimwipes if needed.
13. Remove the top of funnel.
14. Place pre-ashed GF/F filter on top of the filter stem (**Figure 10**).
15. Re-attach the top of funnel.
16. Rinse the filter with DI water. Use the vacuum pump to create suction in the flask and draw the DI water through the filter.
17. Shake sample bottle vigorously for ~15 seconds to mix sample.
 - a. Filter known volume of sample.
 - i. Measure using a graduated cylinder.
 - ii. Triple rinse the graduated cylinder with sample water before measuring out the sample
 - iii. Pour sample slowly in ≤ 200 mL increments into filter funnel.



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- b. Do not pour more sample into funnel than will pass through the filter. All water in the filter funnel must pass through the filter, if filter becomes clogged while there is still sample water in the funnel, discard sample and filter and start again.



18. Keep track of the volume of sample filtered on the Mobile Data Recorder

19. Draw suction on filter apparatus using the hand vacuum pump (or vacuum manifold, if available). Do not exceed 15 inches of mercury (in Hg) vacuum on the pump dial. High pressure ruptures cells and causes chlorophyll and other compounds to dissolve and pass through the filter.



- a. If you have added too much sample and the filter appears to be clogged, you may discard the contents of the filter funnel and the filter and start over. **If you decant water from the filter funnel, you must discard the filter and start over.**

20. Check the filter, if it still appears white, filter more sample. If the filter appears green or yellow tinged, proceed to next step.

- a. **Record the volume of sample filtered for each Replicate on the mobile recorder.**

- b. Filter as much as possible (should be >500 mL, if possible). If unable to filter the minimum amount, filter as much sample as possible and record volume.

21. Rinse inside walls of funnel using wash bottle of DI.

- a. Do not include DI rinse water in the volume of sample filtered.

22. Continue to draw suction on the filter until there is no water left in the funnel and there is no excess water on top of the filter.

23. Remove top of filter funnel, release suction by turning the valve on the manifold. If using the hand pump, you may need to remove stopper.

- a. You may wish to remove the stopper entirely from the flask or manifold to reduce pressure on filter. This will make removing the filter easier.

24. Carefully remove the filter from the stem using filter forceps (forceps with flat ends). Take care not to touch the filter with your fingers.

25. Place the wet filter in the middle of the pre-labelled foil pack (**Figure 11**).

26. Create a crease in the filter by partially folding within the pre-labelled foil pack (**Figure 12**). Do not fold filter completely or let the two sides of the filter touch/smear sample.

- a. The wet filter will stick to the aluminum foil, creasing with relative ease as the foil is folded in on itself (**Figure 12**). If the filter is not folding easily within the foil pack, use a pair of blunt-ended filter forceps to hold the filter in place, then fold the foil.

27. Leave the filter unfolded, but creased, in the foil square. It will partially unfold on its own if it was not folded completely.



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28. Enclose the sample in the pre-labelled foil pack by folding one edge of the foil pack in then folding the top down (**Figure 11**). One end should be left open to allow drying in the drying oven (**Figure 12**).

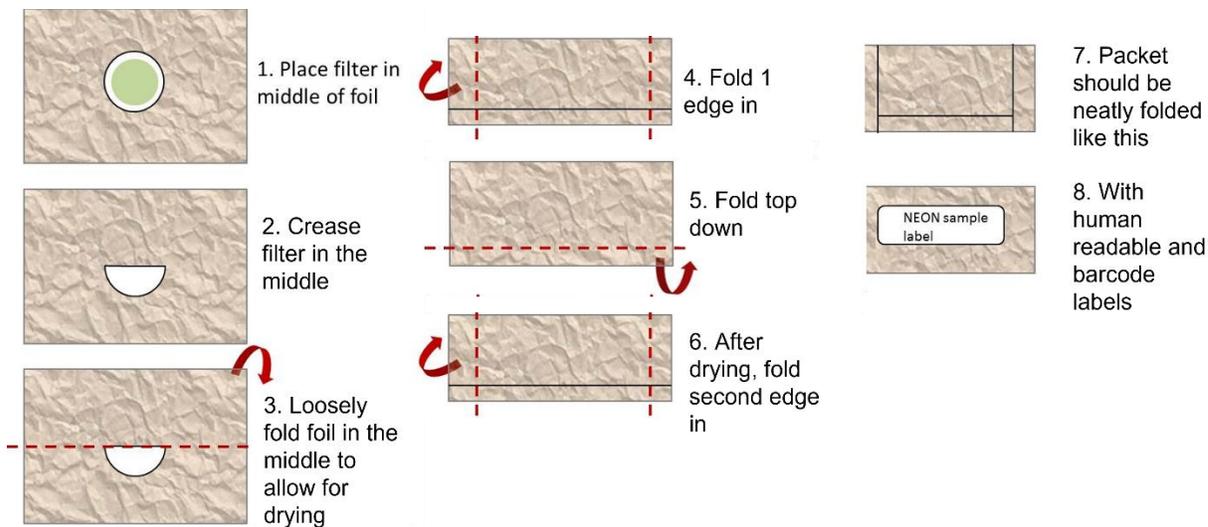


Figure 11. Correct filter packet folding procedure. Make sure packet can be easily opened at the external lab without destroying the NEON sample label or barcode label. Do not use excess foil.

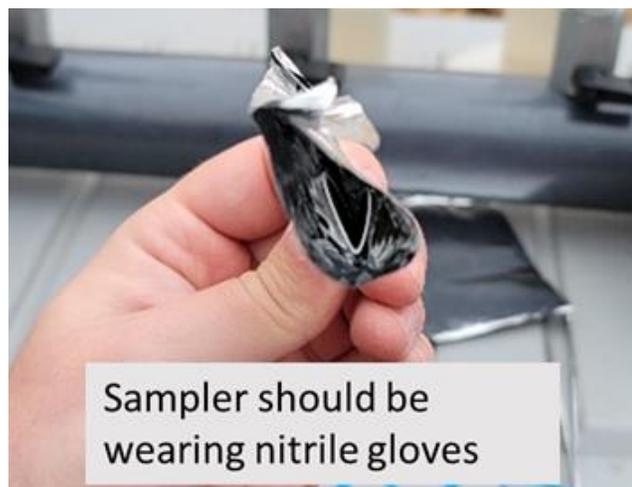


Figure 12. Correctly folded foil pack ready to be placed in the drying oven.

- 29. If transporting from the field, pack in a rigid container with light packing materials so that the foil pockets/samples are not crushed during transport. Keep foil containers right-side-up. Place filters in the cooler with the water sample(s).
- 30. At the DSF, place the pre-creased, but partially unfolded, sample in drying oven with foil pack opened on one end to allow air flow. Dry overnight at 65 °C.



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31. After filter is dried, remove the foil pack from the drying over and fold the remaining end closed. Ensure the label and barcode are clearly visible/scannable for shipping to the external lab (**Figure 11**).
32. Rinse funnel with DI and wipe with kimwipes if needed, and wipe filter forceps with kimwipes.
33. Repeat above steps until you have 2 POM replicate filters from the same sample.
34. Record data in the mobile app.
35. If available, scan the barcode label with the tablet (**Figure 9**).
36. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
37. Place all foil packs from one site inside a resealable bag. Using a permanent marker, label the outside of the resealable bag with Domain, Site, Date, and the "lab type" ($^{13}\text{C}/^{15}\text{N}$).
38. Place all dried filters in a desiccator until shipping.



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SOP C Post-Field Sampling Tasks

C.1 Ending the Processing Day

Refreshing the sampling kit

1. Restock the sampling kit (shipping cooler) with new isotope chemistry sampling bottles with new labels attached, filters, resealable plastic bags, etc. Refer to **Appendix E** for tables detailing equipment and materials.

Equipment maintenance, cleaning and storage

2. Generally, decontaminate and store all sampling equipment in accordance with RD[08] SOP D.5.
3. Peristaltic Pump:
 - a. Run clean water through the peristaltic pump to rinse tubing. Make sure to pump all water out of tubing before storage.
 - b. Charge batteries.
4. Rinse Filter Funnel apparatus with DI and wipe using kimwipes.

C.2 Document Incomplete Sampling Within a Site

Aquatic stable isotope 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 sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a terrestrial sampling plot is compromised after road-building activities, or a stream moves after a flood and the location is no longer within the stream channel. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

If sampling is not possible during a given bout, a problem ticket must be submitted by Field Science staff.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'AOS/TOS Sampling Incomplete: MOD – [Root Cause Description]'
 - a. E.g., 'AOS Sampling Incomplete: ASI – Could not access due to permanently closed road'



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3. Staff Scientists review tickets periodically to determine if a sampling location is compromised.



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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. 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 E Sample Shipment

For detailed shipping information see Shipping Ecological Samples and Equipment (RD[14]).



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8 REFERENCES

Fry, B. (2008). *Stable Isotope Ecology*. Springer, New York, New York.



APPENDIX A QUICK REFERENCES

A.1 Flowchart of Sample Collection and Filtration

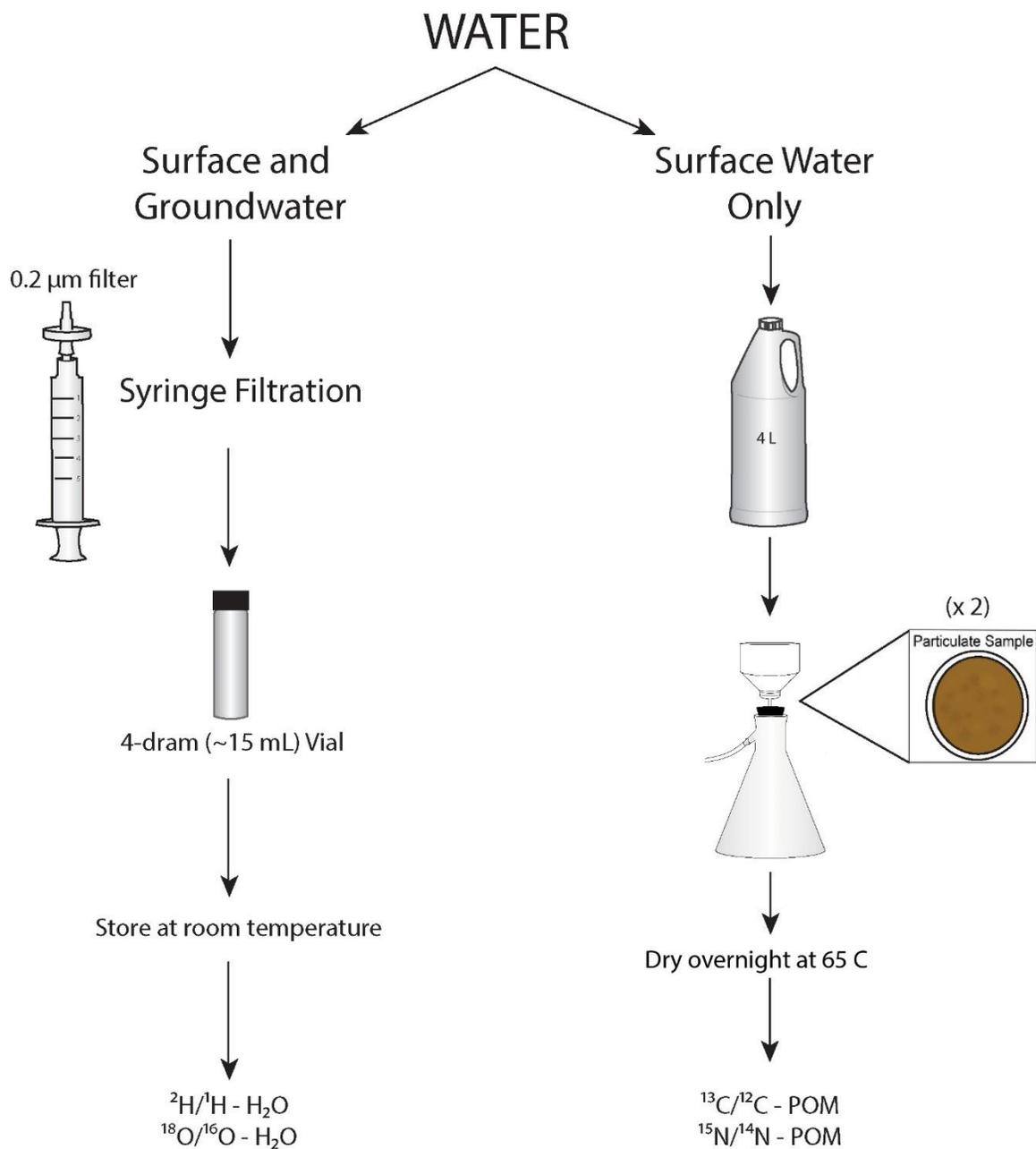


Figure 13. Flowchart of Sample Collection and Filtration



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A.2 Steps for Sample Collection and Filtration

Step 1 – Pre-ash filters.

Step 2 – Check the isotope field sampling kit to make sure all supplies are packed.

Step 3 – Prepare labels (1 x 2 5/8")

SampleID: _____

(siteID.stationID.YYYYMMDD.sampleType.Rep)

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Step 4 – Ensure the General AQU Field Metadata Sheet (RD[06]) is completed per field site visit.

Step 5 – Wadeable streams: Collect isotope samples in the thalweg following the Water Chemistry Sampling in Surface Waters and Groundwater protocol (RD[08]).

Step 6 – Lakes and non-wadeable streams: Collect isotope samples following the Water Chemistry Sampling in Surface Waters and Groundwater protocol (RD[08]). Select sampling depth and collect samples using the Kemmerer.

Step 7 – Groundwater wells: Collect isotope samples from selected wells following the Water Chemistry Sampling in Surface Waters and Groundwater protocol (RD[08]).

1. Locate well and measure water depth.
2. Remove groundwater well sensor from the well.
3. Extract groundwater from well, using appropriate pump method.
4. Collect water samples.

Step 8 – Process (filter and store appropriately) surface and subsurface isotope samples within 4 hours of sample collection:

1. $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ of H_2O samples from Surface and Ground Water:
 - a. Filter using a 0.2 μm filter with syringe into sample vials
2. $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ of POM from Surface Waters (2 filters):
 - a. Filter with a vacuum pump and ashed GF/F filters

Step 9 – Ship samples:

1. $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ of H_2O samples: Ship samples Ground every 2 months per CLA schedule.
2. Filters ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ of POM): Ship filters every 2 months or at the same time as the biology bout shipment. Filters shall be dried at 65 °C. Allow to dry at least overnight and store in a desiccator until shipped.



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APPENDIX B REMINDERS

Before heading into the field: Make sure you...

Collect and prepare all equipment including labels.

Pre-print labels on waterproof adhesive labels.

Fill out the labels before they get wet.

Sample collection: Be sure to...

Not sample anywhere you or other field technicians have walked, or locations that appear recently disturbed. Wait for disturbance to pass.

Avoid contaminating the sample with re-suspended bed sediment.

Use caution when sampling as items can easily fall into water while sampling.

ALWAYS sample in the THALWEG in wadeable and non-wadeable streams.

Sample filtering: Be sure to...

Keep track of the volume of sample filtered on the mobile data recorder or paper Data Sheet (RD[05]).

If you decant water from the filter funnel, you must discard the filter and start over.

DO NOT add more water into the filter tower than you can filter.



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APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.



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APPENDIX D SITE-SPECIFIC INFORMATION

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

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 10. Equipment list – Surface and subsurface (groundwater) water isotope sampling.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
CDW-G/4452963	Y	Mobile data entry tablet, fully charged and synced before field work	Field data entry	1
Thomas Scientific, Inc./8939D81	Y	60 mL syringe (numbers covered with clear packingtape)	$^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$ of H_2O Collection	1
Thomas Scientific, Inc./1227H00 Fisher Scientific Company, LLC/14823220	Y	5 mL syringe (numbers covered with clear packingtape)	$^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$ of H_2O Collection	6
GB08760000	Y	Vacuum-pump filter manifold assembly, pre-made	Filtering samples	1
Part of GB08760000	Y	Filter Unit and 25 mm, 200 mL funnel (Part #: 0341440000)	Filtering samples – part of filter manifold assembly	1
Part of GB08760000	Y	1 L Polypropylene vacuum flask (Part #: 0341520000)	Filtering samples – part of filter manifold assembly	1
Part of GB08760000	Y	2 L Polypropylene vacuum flask (Part #: 0342980000)	Filtering samples – part of filter manifold assembly	1
Amazon Capital Services, Inc. /MV8510	Y	Vacuum hand pump – back up to filtering manifold, metal handle	$^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$ of POM Filtering –	1
Amazon Capital Services, Inc./MVM8900	Y	Vacuum Hand Pump replacement parts	$^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$ of POM Filtering	1
Fisher Scientific Company, LLC/975350	N	Filter forceps (flat ends)	$^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$ of POM Filtering	1



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Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Fisher Scientific Company, LLC/300742	N	Graduated cylinder, 250mL	¹⁵ N/ ¹⁴ N, ¹³ C/ ¹² C of POM Filtering	1
Cole-Parmer/EW0608953 Fisher Scientific Company, LLC/0343814B Thomas Scientific, Inc./1212W94	N	Collection jug, 4L	¹⁵ N/ ¹⁴ N, ¹³ C/ ¹² C of POM Filtering	1 per station
Consumable items				
Fisher Scientific Company, LLC/033433G Thomas Scientific, Inc./1231B94	Y	Vial, 16 mL, 21 Dia. x 73 mm H., Black Phenolic Cap, 14B Rubber Liner Material, Clear, Glass; VWR or Equivalent	¹⁸ O/ ¹⁶ O, ² H/ ¹ H of H ₂ O Collection	1
Fisher Scientific Company, LLC/9719006	Y	Syringe Filter, non-sterile, nylon, 0.2 µm pore size, 33 mm diameter	¹⁸ O/ ¹⁶ O, ² H/ ¹ H of H ₂ O Collection	1* per station
Fisher Scientific Company, LLC/1337412	N	Plastic Paraffin film (e.g., Parafilm)	¹⁸ O/ ¹⁶ O, ² H/ ¹ H of H ₂ O Collection	As needed
Fisher Scientific Company, LLC/987464	Y	GF/F filters (25 mm diameter, pre-ashed)	¹⁵ N/ ¹⁴ N, ¹³ C/ ¹² C of POM Filtering	2 per station
Grainger, W.W./6CHG5	N	Aluminum foil squares (~4" X 4")	¹⁵ N/ ¹⁴ N, ¹³ C/ ¹² C of POM Filtering	2
Grainger, W.W./5NHH1 Amazon Capital Services Inc./B00006IBUV Arrow SameDay/5520	Y	Isotope waterproof labels (1" x 2 5/8"), pre-printed adhesive labels	Labeling samples, human readable	As needed
	Y	Adhesive barcode labels (Type I)	Labeling sample bottles with barcode-readable	1 sheet



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Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Amazon Capital Services Inc./ B00006IF13	Y	Permanent markers	Labeling samples	1

* May require more than 1 per sample in turbid waters

Table 11. Equipment list – Additional equipment and consumables for surface water isotope sampling in lakes and rivers.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
Fisher Scientific Company, LLC/EW0548610	Y	Kemmerer sampler	Collecting samples	1
Thomas Scientific, Inc./1207W05 Cole-Parmer/EW0640776 Fisher Scientific Company, LLC/14171212	Y	Tubing (1/8" ID)	Sample transfer from Kemmerer to syringe for H ₂ O sample	1
	N	Ice auger	Drilling a hole in the ice	1
	N	Boat		1
Amazon Capital Services Inc./B003ZZG5EM	N	Anchor with rope		2
	N	Oars		2
West Marine Products, Inc./13487178	N	Trolling Electric Motor		1
Grainger, W.W./2UKJ5	N	Battery (12 volt)		1
Amazon Capital Services Inc./B003QKQ4V0	N	Safety kit for boat (e.g., flares, bailer, float with rope)		1



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Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Personal Flotation Devices (PFDs)		1 per person
	N	Waders or boots		1 pair per person

Table 12. Equipment list – Additional equipment and consumables for sampling isotopes in groundwater.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
In-Situ, Inc./73310	Y	Water level tape	Measuring water level	1
Uline/S-7914	N	Clean 5 Gallon Bucket	Pumping sample water	1
Cole-Parmer/EW0608953 Fisher Scientific Company, LLC/0343814B Thomas Scientific, Inc./1212W94	N	4-L jug	Collecting sample water	1 per well sampled
QED Environmental Systems/MPSP4P	Y	QED Sample Pro Pump	Pumping sample water	1
QED Environmental Systems/MP50	Y	QED MP-50 Compressor / Controller	Pumping sample water	1
	N	Key	Unlocking well	1
Grainger, W.W./2UKJ5	N	Battery (12V, minimum of 3.6 Ah)	Pumping sample water	2*
QED Environmental Systems/DTTP4B	Y	Bucket of ¼" x ¼" dual bonded tubing (250 feet of tubing in each bucket). Tubing will be dedicated per each well for the duration of the sampling events.	Pumping sample water	1 per site, required for 1 st sampling event.



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Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
QED Environmental Systems/DTTP4B	Y	Dedicated tubing for wells (in 1-gallon bags). Make sure to get the tubing identified for the well that will be sampled. The sealable bags shall be labeled with the Well ID.	Pumping sample water	1 dedicated piece of tubing per well sampled (after 1 st sampling event)

*Always take Extra batteries into field

Table 13. Equipment list – Sample field storage, lab processing, and shipping.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	Cooler	Transporting sampling back to the lab for processing	1
	N	Squirt bottle	Rinsing filtration unit with DI	1
Thomas Scientific, Inc./1229N43	Y	Drying oven	Drying samples	1
Consumable items				
	N	Cardboard box	Shipping H ₂ O Glass Vials and POM samples	As needed
	N	Packing material	Filling up extra space and adding absorbent material	As needed
Grainger, W.W./5LH30	N	Resealable plastic bags (gallon and quart size)	Separately enclosing the shipping labels, ice packs and samples	1
Fisher Scientific Company, LLC./3532100	N	Ice or ice packs	Keeping the samples cool in field	As needed
Grainger, W.W./15F814	N	Clear Packing tape, roll	Labeling shipment	1
	N	Shipping labels	Labeling shipment and cooler return	2



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Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
	N	DI water	rinsing during filtration of POM samples	1 lb.