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

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See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	11/21/2014	ECO-02430	Initial release
B	01/22/2015	ECO-02632	Migration to new protocol template
C	09/03/2015	ECO-03164	Aligning lake sampling with water chemistry protocol

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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, ponds, 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 (^{H}F) over the light (^{L}F) isotope, relative to a standard.

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

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

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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC. 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.002494	Datasheets for AOS Shipping Inventory

2.3 Acronyms

Acronym	Definition
^{o}C	Degrees Celsius
cm	centimeter
DI	De-ionized
GF/F	Glass fiber filter
POM	Particulate Organic Matter
L	Liter
m	meter
mph	Miles per hour
mL	milliliter
mm	millimeter

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µm	micrometer
NEON	National Ecological Observatory Network

2.4 Definitions

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

Headspace: A gaseous space above a closed liquid sample.

Hydrograph: A diagram depicting the change in discharge (m^3) 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.

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

Thalweg: The deepest part of a stream channel.

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.

3 METHOD

The following protocol describes the collection, processing, storage and shipping of stable isotope ($^{15}N/^{14}N$, $^{13}C/^{12}C$ of Particulate Organic Matter (POM), and $^{2}H/^{1}H$, $^{18}O/^{16}O$ of water (H_2O)) samples from aquatic environments, including streams, rivers, and lakes. $^{2}H/^{1}H$, $^{18}O/^{16}O$ of water will also be sampled from groundwater.

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** and just downstream of the only sensor set (Sensor Set 1) in **non-wadeable streams** (Figure 1). In streams with a shallow water column, technicians must be cautious not to disturb the benthic sediments when sampling.

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Three locations per **lake** will be sampled, notably the deepest part of the lake and a location near the most prominent inlet (5 meters from the inlet mouth) and one near the outlet (5 meters upstream of the outlet). These locations are collocated with the lake buoy, inlet and outlet infrastructure, respectively (Figure 1). Care should be taken to not disturb the infrastructure and, where possible, samples should be collected downwind or downstream of the infrastructure. NEON will collect one sample each at the inlet, outlet, and buoy locations in unstratified lakes at 0.5 m depth to maintain comparability with water chemistry measurements (**Figure 3**, RD[08]). If the system is stratified, NEON will collect samples at 0.5 m depth at the inlet, outlet, and buoy locations to align with the water chemistry and dissolved gas sampling, as well as an integrated sample of the hypolimnion at the buoy (**Figure 3**). Note that at these inlet and outlet locations the samples are always taken at the surface (0.5 m depth).

Groundwater well samples should be collected to align in space and time with the groundwater water chemistry samples (RD[08]).

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

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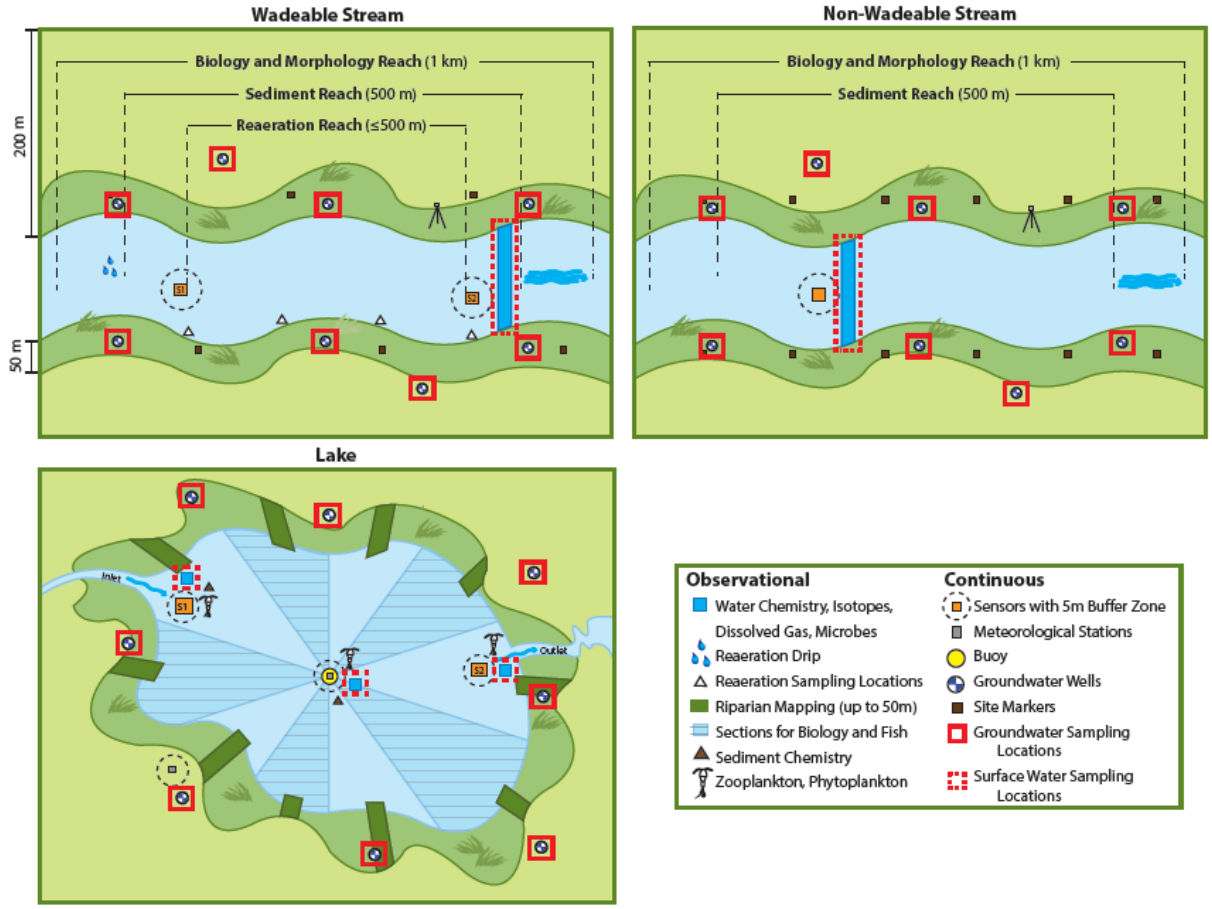

Figure 1. Generic site layouts for wadeable streams, non-wadeable streams and lakes with stable isotope sampling locations

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

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

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[05]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[06]).

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4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Isotope samples should be collected in conjunction with water chemistry samples. Science Operations will provide sample timing annually.

4.1.1 Wadeable and Non-wadeable Streams

In wadeable and non-wadeable streams and rivers, aquatic stable isotope sampling occurs up to 26 times per year. Standard recurrent stream and river sampling should take place one Tuesday per month (e.g., 12 times per year) in coordination with atmospheric 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.

4.1.2 Lakes

In lakes, aquatic stable isotope sampling shall occur up to 12 times per year. 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.

4.1.3 Groundwater Wells

For groundwater well samples, sample budgets are based on an average of one sample per well per year at each site, or a total of 8 samples per year per site. Due to the limited number of samples available it is anticipated that a subset of wells will be sampled at multiple times per year (likely 4 wells at 2 times per year). 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

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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

Process (filter and store appropriately) surface and subsurface isotope samples within 3 hours of sample collection (maximum of 6 hours allowable for lake sites). For storage and shipping timelines see SOP F.

4.4 Sampling Timing Contingencies

Table 1. Contingent decisions for 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 on field sheet.	No adverse outcome.
	If no streamflow exists, sample in a nearby pool	No adverse outcome.

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	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.”	
	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 surficially and safe to walk on (minimum of 15 cm thickness) make a hole in the ice and proceed with sampling. The thickness of the ice shall be tested on the nearshore environment prior 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 sampling stirred up sediments or added chemical constituents to the water within the past hour, allow the water to clear and disturbance to pass or sample in a different location.	No adverse outcome.
	If weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>20 mph) and has unsafe wave heights (<1 m) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	No adverse outcome.

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Table 2. Contingent decisions for 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 3-4 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 the base of the well is surrounded by standing water. 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.
	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.
	For sites which 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.

4.5 Sampling Specific Concerns

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 and total depth of the water column sampled with the water chemistry protocol (RD[08]).

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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, as such, conducting the groundwater chemistry sampling event over two days is also acceptable, though 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 and/or frozen) as soon as possible. If necessary, surface water may be collected in a large container, kept on ice at 4℃, and filtered and/or preserved within 3 hours (maximum of 6 hours allowable for lake sites) at a base camp or Domain Lab (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 on the Aquatic Isotope Sampling Data Sheet (RD[05]).

Table 3. 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.

5 SAFETY

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

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

Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s) (AD[02]). When working around ice, use caution and good judgment to carefully evaluate site conditions including ice strength. Do not continue if the risk is too great.

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

1. Due to site-specific hazards that may be encountered, technicians may perform GPS positioning around the lake, and measurements for inflow and outflow, without dismounting from the vessel. In addition, technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All personnel must be wearing a personal flotation device prior to entering the boat, and in wadeable streams when conditions are approaching the allowed wading limit (AD[02]).
3. All employees shall have access to a form of communication with other team members such as a two-way radio.
4. Technicians should be aware of any site-specific water hazards of that particular location (i.e., current status, tidal charts, etc.).

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6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 4. Equipment list – Surface and subsurface water isotope sampling

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	60 mL syringe (numbers covered with clear packing tape)	$^{18}O/^{16}O$, $^{2}H/^{1}H$ of H_2O Collection	1	N
MX100386	R	Filter funnel (25 mm diameter)	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	1	N
MX100388	R	Vacuum filter flask	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	1	N
	R	Vacuum pump	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	1	N
	R	Filter forceps (flat ends)	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	1	N
	R	Graduated cylinder, 250mL	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	1	N
	R	Collection jug, 4L	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	1	N
	R	Handheld Temperature and Conductivity Meter	Measuring temperature and conductivity	1	N

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	First Aid Kit		1	N
Consumable items					
	R	15 mL glass vial with rubber closure	$^{18}O/^{16}O$, $^{2}H/^{1}H$ of H_2O Collection	1	N
	R	Syringe Filter, non-sterile, nylon, 0.2 µm pore size, 13 mm diameter	$^{18}O/^{16}O$, $^{2}H/^{1}H$ of H_2O Collection	1*	N
	R	Plastic Paraffin film (e.g., Parafilm)	$^{18}O/^{16}O$, $^{2}H/^{1}H$ of H_2O Collection	As needed	N
MX106350	R	GF/F filters (25 mm diameter, pre-ashed)	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	2	N
	R	Aluminum foil squares (~4" X 4")	$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering	2	N
	R	Isotope waterproof labels (1" x 2 $^{5}/_{8}$")	Labeling samples	2	N
	R	Permanent markers	Labeling samples	1	N

R/S=Required/Suggested

* May require more than 1 per sample in turbid waters

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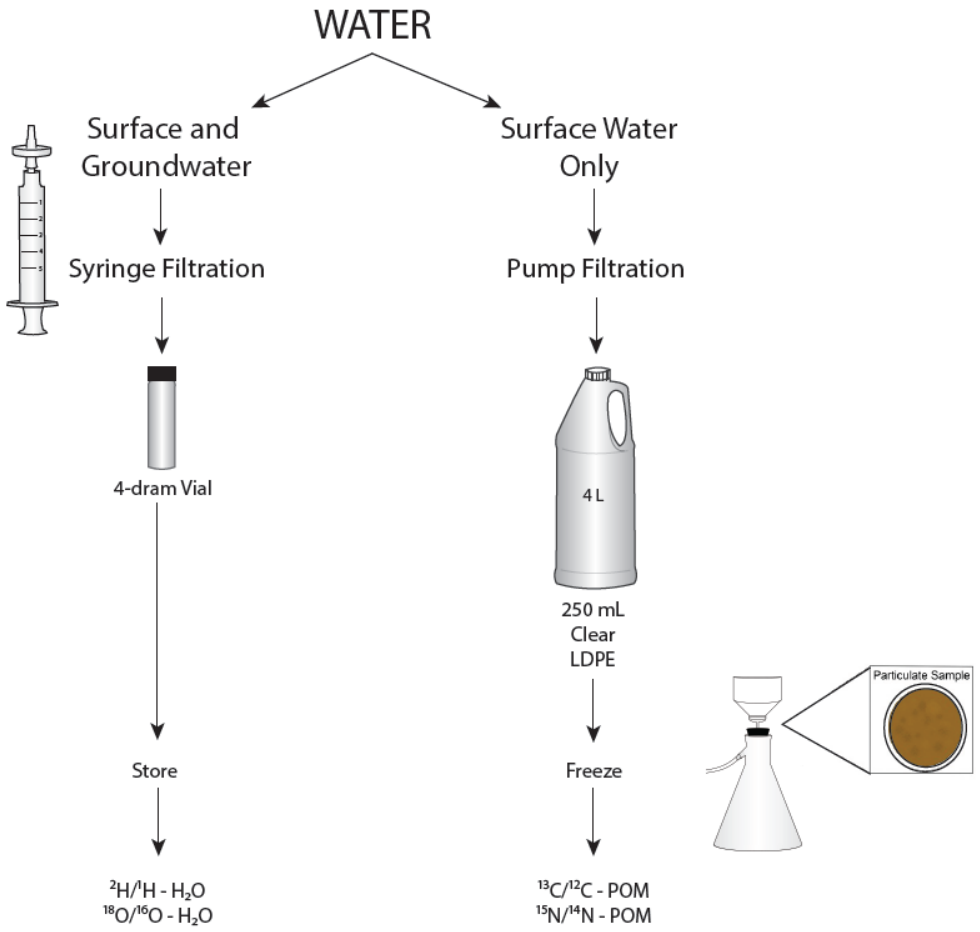

Figure 2. Diagram of isotope sample collection and equipment. 2 filters are collected at each collection event.

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Table 5. Equipment list – Additional equipment and consumables for surface water isotope sampling in lakes and rivers

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Kemmerer sampler	Collecting samples	1	N
	R	Tubing (1/8” ID)	Sample transfer from Kemmerer to syringe	1	N
	R	Tubing (3/8” ID)	Sample transfer from Kemmerer to syringe	1	N
	R	Flow control hose clamp	Sample transfer from Kemmerer to syringe	1	N
	R	3/8” to 1/8” tubing adaptor	Sample transfer from Kemmerer to syringe	1	N
	R	Ice auger	Drilling a hole in the ice	1	N
	R	Boat		1	Y
	R	Anchor with rope		1	N
	R	Oars		2	N
	R	Trolling Electric Motor		1	Y

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Battery (12 volt)		1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		1	Y
	R	Personal Flotation Devices (PFDs)		1 per person	N
	R	Waders or boots		1 per person	N
Consumable items					
		(none)			

R/S=Required/Suggested

Table 6. Equipment list – Additional equipment and consumables for sampling isotopes in groundwater

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Water level tape	Measuring water level	1	N

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Clean 5 Gallon Bucket	Pumping sample water	1	N
	R	4-L jug	Collecting sample water	1 per well sampled	N
	R	QED Sample Pro Pump	Pumping sample water	1	N
	R	QED MP-50 Compressor / Controller	Pumping sample water	1	N
	R	Key	Unlocking well	1	N
	R	Battery (12V, minimum of 3.6 Ah)	Pumping sample water	2*	N
	R	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 1st sampling event.	N
	R	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 1st sampling event)	N
Consumable items					
		(none)			

R/S=Required/Suggested *Always take Extra batteries into field

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Table 7. Equipment list – Sample field storage and shipping

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Shipping cooler	Shipping samples	1	N
	R	Dry ice shipping container	Shipping filters	1	N
Consumable items					
	R	Cardboard box	Shipping H2O Glass Vials	As needed	N
	R	Packing material	Filling up extra space and adding absorbent material	As needed	N
	R	Resealable plastic bags (gallon and quart size)	Separately enclosing the shipping labels, ice packs and samples	1	N
	R	Ice or ice packs	Keeping the samples cool in field	As needed	N

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Clear Packing tape, roll	Labeling shipment	1	N
	R	Shipping labels	Labeling shipment and cooler return	2	N
	R	Dry ice	Shipping filters	1 lb.	Y

R/S=Required/Suggested

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6.2 Training Requirements

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

All personnel required to operate a boat shall be trained through a NEON EHS approved program. All others shall be aware of boating safety procedures.

6.3 Specialized Skills

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

6.4 Estimated Time

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

We estimate sampling requires 1-2 technicians for 1-2 hours each surface water sampling day plus travel to and from the site. We estimate 1 technician for 4-16 hours for groundwater wells (depending on the number of wells sampled during each bout).

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7 STANDARD OPERATING PROCEDURES

SOP A Selecting Sampling Depth in Lakes

1. Take one sample at a 0.5-meter depth at all stations.
2. Is the lake thermally stratified at buoy station?
   a. If NO, do not take any more samples.
   b. If YES, calculate the depth of the **hypolimnion** section at the buoy. Ensure you are calculating the hypolimnion section depth, NOT the maximum lake depth (**Figure 3**)
      1) If ≤4 m, then collect a sample from the midsection of the hypolimnion depth.
      2) If >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.

In lakes, 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 non-wadeable streams, the anchor and boat are within the permitted reach. If sediments are disrupted, wait until the area has cleared before sampling (~15 mins).

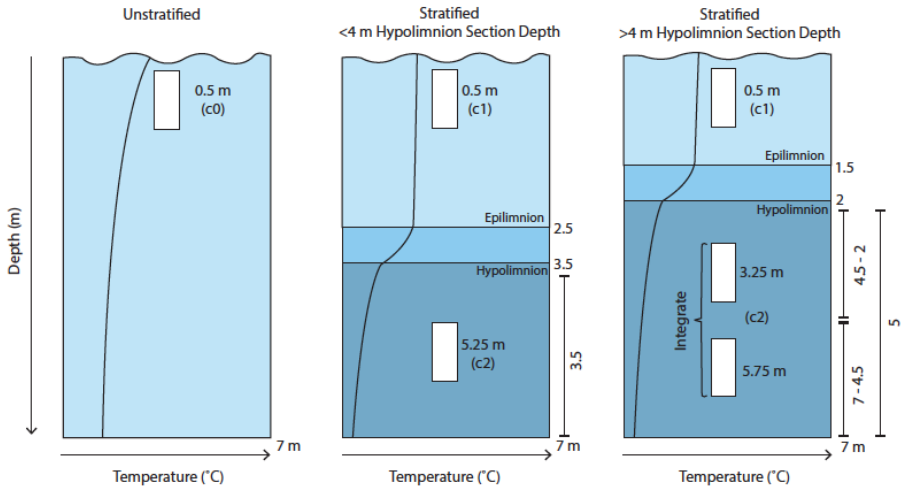

Figure 3. Examples of unstratified and stratified lake water column sampling depths with placement of thermocline.

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SOP B Preparing for Sampling

1. 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 ^{o}C) for 6 hours.
   c. After 6 hours, remove from furnace, 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.
2. Check the aquatic isotope field sampling kit to make sure all supplies are packed.
3. **Labels are waterproof but should be filled out before getting wet to ensure ink will stick to the labels.**
4. Attach pre-printed labels (Figure 4) to sample bottles (Figure 2). Use a **Sharpie** to fill out labels before going into the field.

SampleID :_____
(siteID.stationID.YYYYMMDDsampleType.Rep)
Type: $^{2}H,^{18}0\text{-}H_20$ $^{15}N,^{13}C\text{-}POM$
NEON Filter vol.(mL)_____ Rep#___

Figure 4. NEON aquatics stable isotope chemistry labels example.

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SOP C Field Sampling

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

1. In the field, fill out the General AQU Field Metadata Sheet (RD[06]) and the Isotope Water Chemistry Field Sampling Datasheet (RD[05]) before collecting samples. You only need to fill out one General Field Metadata Datasheet per site per day.
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.
3. Station ID is the 2-digit station code where sample was taken (i.e., Station ID for streams ="ss", non-wadeable streams/rivers = 'rs'; in Lakes, inlet="in", outlet="ot", center="c0", if center is stratified: "c1", "c2", "c3" with one being the top layer; "w1"-"w8" for groundwater wells).

C.1 Wadeable Streams (Follow RD[08]):

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.

1. Collect stream isotope samples and metadata following the Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]).

2. 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.
3. Proceed to SOP D, Sample Collection and Processing.

C.2 Lakes and Rivers (Non-wadeable streams) (Follow RD[08]):

Isotope sampling in lakes and rivers will follow the Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]). Determine the depths for sampling lakes according to the data downloaded from the real-time data on the website or using the latest profiling data available acquired by the buoy. Look at the temperature profile and determine if and where stratification occurs and how many samples will be taken (See SOP A).

1. Collect river and lake water samples and metadata following the Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]).
   a. Move to the sampling station and note the station ID on the field sheet (RD[08]; SOP B.2).

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b. Sample using the Kemmerer bottle (Figure 6, RD[08]; SOP B.2). Depth should be measured from the bottom of the Kemmerer.

2. Proceed to SOP D, Sample Collection and Processing.

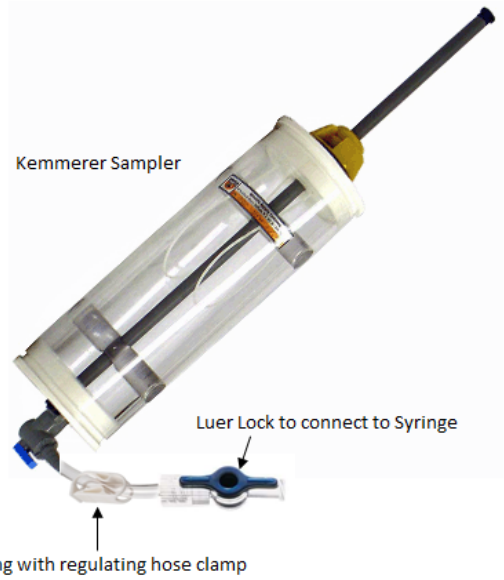

Figure 5. Example of tubing attachment with hose clamp and Luer-Lock to Kemmerer spigot.

C.3 Groundwater Wells (Follow RD[08]; SOP B.3):

Groundwater isotope sampling will be completed in the same location Water Chemistry Sampling in Surface Waters and Groundwater Protocol (RD[08]).

1. Collect groundwater water and metadata from selected wells following the Groundwater Chemistry Sampling Protocol (RD[08], SOP B.3).
   a. Locate well and measure water depth.
   b. Remove groundwater well sensor from the well.
   c. Extract groundwater from well, using appropriate pump method.
   d. Collect water samples.
2. Proceed to SOP D, Sample Collection and Processing.

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SOP D Sample Collection and Processing

1. Process (filter and store appropriately) surface and subsurface isotope samples within 3 hours (maximum of 6 hours in lake sites) of sample collection:
   a. Record the SampleID (SiteID.StationID.YYYYMMDD.SampleType.Rep#) on the bottle labels (Figure 5). SiteID is the 4 letter site code. Stations ID is the 2-digit station code where sample was taken (i.e. Station ID for streams ="ss", non-wadeable streams/rivers = 'rs'; in Lakes, inlet="in", outlet="ot", center="c0", if center is stratified: "c1", "c2", "c3" with one being the top layer; "w1"-"w8" for groundwater wells). SampleType is 'POM' or 'H2O'. Rep# is 1 for H_2O, Rep# is 1' or '2' for POM.
      1) You DO NOT need to complete a second General Field Metadata Datasheet unless aquatic stable isotope samples are collected on a different date than the water chemistry samples.

D.1 $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of H_2O samples from Surface and Ground Water

1. Rinse a 60 mL syringe 2 times with stream water. Discard rinse water downstream or on the bank.
2. Attach a 13mm, 0.2 µm syringe filter.
3. Rinse collection bottle (15 mL glass vial with rubber closure) with filtered sample water 2 times.
4. Collect sample in a 15 mL glass bottle (Figure 2). Sample bottle should be **~90% full** to minimize evaporation, but should have enough headspace to ensure bottle will not be broken if freezing occurs.
5. Securely attach lid and **wrap plastic paraffin film** around the lid and vial to secure the lid and to reduce air movement.
6. No additional preservation or special shipping is required.

D.2 $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of POM from Surface Waters

1. Collect water using a 4 L jug to be filtered within 3 hours (maximum of 6 hours for lake sites). 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. To reduce biological activity, keep jug on ice and in the dark until you are able to process the sample.
2. Set up filter funnel, filter flask, and vacuum pump (Figure 6).
   a. Attach flexible tubing to 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.

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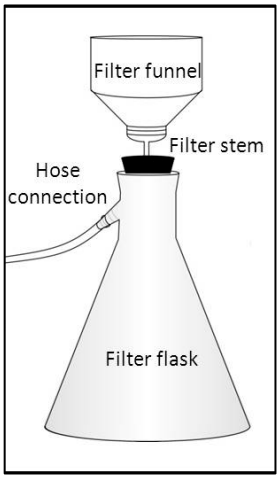

Figure 6. Filter apparatus setup

3. Remove the top of the filter funnel from apparatus, rinse with de-ionized (DI) water.
4. Place pre-ashed GF/F filter on top of the filter stem (Figure 6), replace top of funnel.
5. Shake sample bottle vigorously for ~30 seconds to mix sample.
   a. Filter known volume of sample.
      1) Measure using a graduated cylinder. Pour sample slowly in ≤100 mL increments into filter funnel.
   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.
6. **Keep track of the volume of sample filtered on the Lab Data Sheet (RD[05])**.
7. 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 hand 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.**
8. 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 Lab Data Sheet (RD[05]).**
   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.
9. Rinse inside walls of funnel using wash bottle of DI.
   a. Do not include DI rinse water in the volume of sample filtered.

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10. 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.
11. Remove top of filter funnel, release suction using the release valve on the hand pump.
12. Carefully remove the filter from the stem using filter forceps (forceps with flat ends). Take care not to touch the filter with your fingers.
    a. Fold filter in half and place on a clean square of aluminum foil (~4x4"). Fold foil securely around the filter to form a packet.
    b. Label foil packet with adhesive sample label (1 x 2 $^{5}/_{8}$") (Figure 4).
13. Repeat above steps until you have 2 replicate filters from the same sample.
    a. Two (2) ^{13}C, ^{15}N POM isotope filters
14. Place all foil packets 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" ($^{13}C/^{15}N$).
    a. Place all filters in a -20^{o}C freezer until shipping.

D.3 Ending the Day

1. Refreshing the sampling kit
    a. Restock the sampling kit (shipping cooler) with new isotope chemistry sampling bottles with new labels attached, filters, resealable plastic bags, etc. Refer to 9.2 Equipment and Materials.
2. Equipment maintenance, cleaning and storage
    a. Peristaltic Pump:
        1) Run clean water through the peristaltic pump to rinse tubing. Make sure to pump all water out of tubing before storage.
        2) Charge batteries.
    b. Rinse Filter Funnel apparatus with DI

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SOP E Data Entry and Verification

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

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SOP F Sample Shipment

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

Shipments are to have a hardcopy of the "per Sample" tab of the shipping inventory (RD[09]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. ShipmentID must be included in the electronic version of the shipping inventory, but is not necessary for the hard copy. Also include the shipment tracking # in the email.

F.1 Handling Hazardous Material

N/A

F.2 Supplies/Containers

1. $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of H_2O samples
   a. Ensure you have wrapped plastic paraffin film around the lids to keep them secure during shipping.
   b. Pack glass bottles for **$^{18}O/^{16}O$ and $^{2}H/^{1}H$ of H_2O samples** in absorbent packing material for protection from breaking and leakage. After bottles have absorbent material packed securely around them, any remaining space can be filled with regular packing material.
   c. Place 'per sample' tab of AOS shipping inventory (RD[09]) in a resealable plastic bag into shipping container.
   d. Glass bottles can be packaged and shipped in a secure box to isotope lab. "Up" arrows can be affixed to the secure box.
2. Filters ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ of POM)
   a. Filters are shipped overnight on dry-ice in appropriate dry ice shipping containers.
   b. Place barrier (e.g., cardboard) between the dry ice and sample bag and place packing materials above the sample bag.
   c. Place 'per sample' tab of AOS shipping inventory (RD[09]) in a resealable plastic bag into shipping container.
   d. Tape and label containers appropriately for shipping on dry ice and ship to algae chemistry lab.

F.3 Timelines and Conditions

1. $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of H_2O samples
   a. Samples should be shipped Ground within 2 weeks of collection.
2. Filters ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ of POM)

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a. Filters shall be shipped in batches every 2 months or at the same time as the biology bout shipment. Filters shall be stored in a -20°C freezer until shipping.

F.4 Grouping/Splitting Samples

Organize by Site ID, if applicable.

F.5 Return of Materials or Containers

N/A

F.6 Shipping Inventory

Fill out the AOS Sample Shipping Inventory (RD[09]). Each box sent should have a copy of the 'per sample' tab of the shipping inventory of its contents. The 'Shipment ID' does not need to be filled out on the hardcopy. The electronic shipping inventory that includes ShipmentIDs and IDs of all samples shipped should be emailed to the appropriate contact at the receiving analytical laboratory as well as the NEON CLA contact on the day that samples ship. Include shipping IDs and estimated arrival date(s)/time(s) in the email as well.

F.7 Laboratory Contact Information and Shipping/Receipt Days

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

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8 REFERENCES

Fry , B. 2008. *Stable Isotope Ecology.* 308 p. Springer.

Gandhi, H. T.N. Wiegner, P.H. Ostrum, L.A. Kaplan, and N.E. Ostrom. 2004. Isotopic (^{13}C) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. Rapid Communications in Mass Spectrometry 18:903-906

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 8. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC. 002211	Datasheets for AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002494	Datasheets for AOS Shipping Inventory

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

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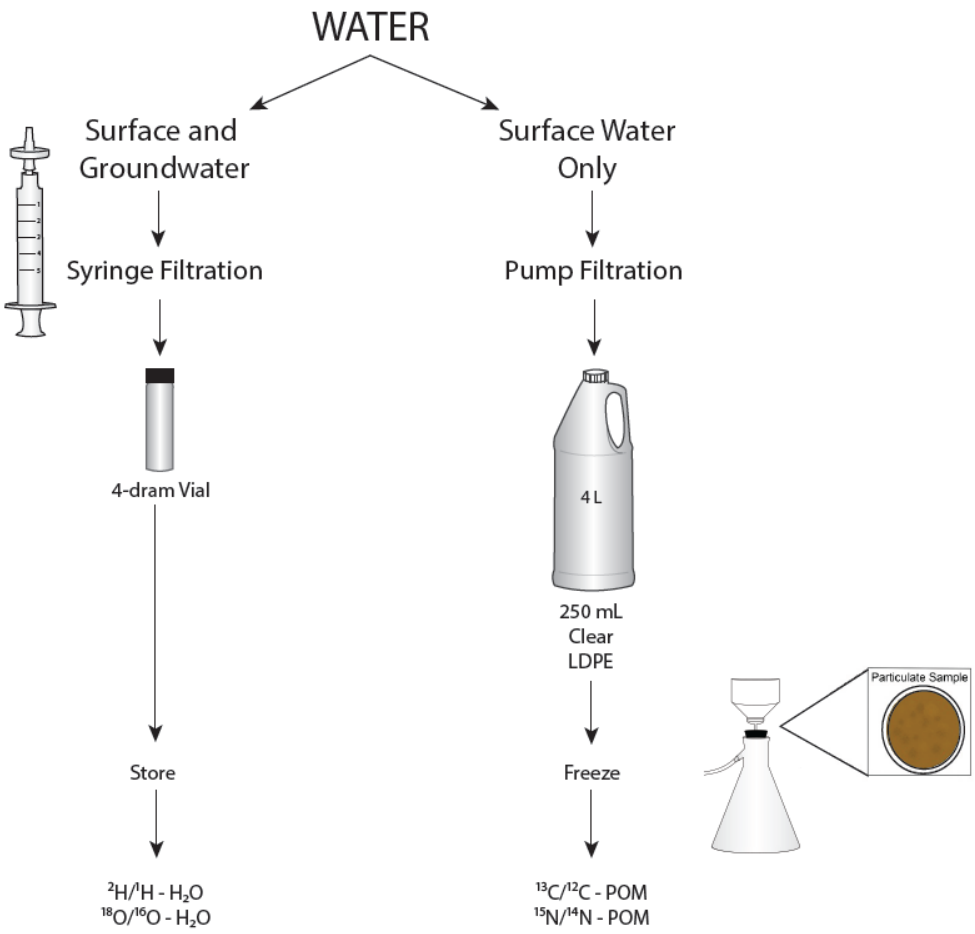
APPENDIX B QUICK REFERENCES

B.1 Flowchart of Sample Collection and Filtration



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B.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.YYYYMMDDsampleType.Rep)
Type: $^{2}H,^{18}0\text{-}H_20$ $^{15}N,^{13}C\text{-POM}$
NEON Filter vol.(mL)_____ Rep#___

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 3 hours of sample collection (maximum of 6 hours allowable for lake sites):

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

Step 9 – Ship samples:

1. $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of H_2O samples: Ship samples Ground within 2 weeks of collection.
2. Filters ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ of POM): Ship filters every 2 months or at the same time as the biology bout shipment. Filters shall be stored in a -20^{o}C freezer until shipping. Ship on dry ice.

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APPENDIX C 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 Lab 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 D 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 E SITE-SPECIFIC INFORMATION

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

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