AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters

PREPARED BY:	ORGANIZATION:	DATE:
Keli Goodman	AQU	11/21/2014

APPROVALS (Name):	ORGANIZATION:	APPROVAL DATE:
Dave Tazik	SCI	11/21/2014
Mike Stewart	PSE	11/21/2014

RELEASED BY (Name):	ORGANIZATION:	RELEASE DATE:
Stephen Craft	PSE	11/21/2014

See Configuration Management System for Approval History

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A	11/21/2014	ECO-02430	Initial release

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of Observatory protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices
- site-specific safety practices
- general equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

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

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

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.014051	Field Audit Plan; needs updated to include Observation Systems audit.
RD [05]	NEON.DOC.001152	NEON Aquatic Sample Strategy document
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.000694	Surface Water Chemistry Sampling in Wadeable Streams
RD [08]	NEON.DOC.001190	Lake and Non-Wadeable Surface Water Chemistry
RD [09]	NEON.DOC. 001219	Groundwater Chemistry Sampling From Observation Wells
RD [10]	NEON.DOC.001646	NEON General AQU Field Metadata Sheet
RD [11]	NEON.DOC.002211	Datasheets for AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters
RD [12]	NEON.DOC.002494	Datasheets for AOS Shipping Inventory

2.3 Acronyms

^{o}C	Degrees Celsius
cm	centimeter
POM	Particulate Organic Matter
L	Liter
mL	milliliter
Mm	millimeter
µm	micrometer
NEON	National Ecological Observatory Network
P&P	Procedure and Protocol
PPE	Personal Protective Equipment

2.4 Verb Convention

"Shall" is used whenever a statement expresses a convention that is binding. The verbs "should" and "may" express non-mandatory provisions. "Will" is used to express a declaration of purpose on the part of the design activity.

2.5 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

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 BACKGROUND AND OBJECTIVES

3.1 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).

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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, which are documented in the NEON Scientific Data Products Catalog (RD [03]), available on the NEON website.

4 PROTOCOL

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 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 stream and just downstream of the only sensor set in non-wadeable rivers. In streams with a shallow water column, technicians must be cautious not to disturb the benthic sediments when sampling. 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.

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[09]).

The size and depth of the ponds, lakes and non-wadeable streams, define the sampling location. 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. Care should be taken, however, to sample outside a 5 m radius from the infrastructure and, where possible, downwind or downstream of the infrastructure. Shallow aquatic ecosystems tend to not thermally stratify during the year, hence, an integrated water column sample is desirable (Figure 1). Should the system be stratified, an integrated sample of the epilimnion and an integrated sample of the hypolimnion are taken (see Section 4.1). At these inlet and outlet locations the samples are taken at the surface (integrated 1 m). In non-wadeable streams, integrated samples are taken from the water column, in the area representing the steam thalweg (the deepest part of the stream).

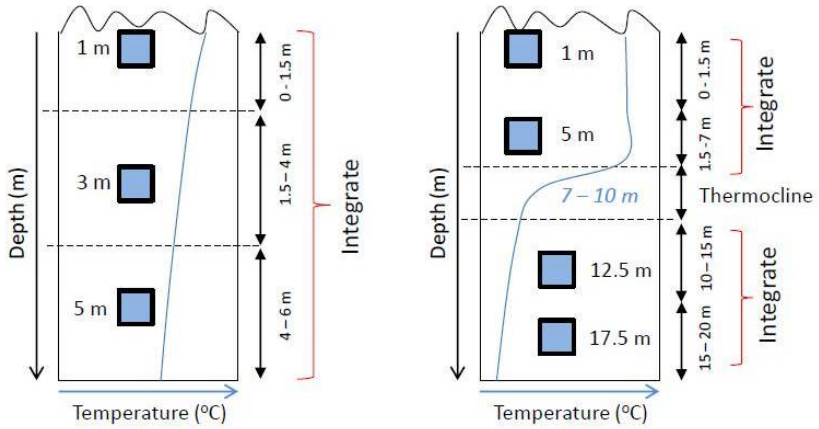

Figure 1. Example of non-stratified and stratified lake water column sampling depths with placement of thermocline.

4.1 Selecting sampling depths

1) Take one sample at a 1-meter depth.
2) Is the lake thermally stratified?
   a) If NO, and the lake is < 6 meters deep, then take a sample from midsection of the remaining water layer.
   b) If NO, and lake is ≥ 6 meters deep, then divide the remaining depth by 2 and take a sample in the midsection of both those layers (Figure 1).
      i) Integrate all samples.
   c) If YES, then calculate the remaining depth of the epilimnion and take a sample from the midsection of the water body.
   d) Calculate the depth of the hypolimnion.
      i) If <4 m then take a sample from the midsection of the hypolimnion depth.
      ii) If ≥4 m then divide the hypolimnion depth by 2 and take a sample in the midsection of both those layers.
   e) Integrate the samples from the hypolimnion into 1 sample and the samples from the epilimnion into another 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 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).

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD [04]). 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 (RD [06]). To ensure standardization and sample quality, isotope samples should be collected at water chemistry sampling locations, upstream of any solute injection work occurring on the same day as sampling, as well as upstream and upwind of any fieldwork disrupting the stream or lake bottom (e.g., morphology mapping, invertebrate collection, macrophyte collection, etc.).

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

If the water chemistry sampling location is too shallow 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. If no flow exists, sample in a nearby pool where the water is deep enough to obtain a clean, sediment free sample, and note this change in the field sheet. Be sure to note this on the field sheet as a "non-flowing sample." If the stream is entirely dry or frozen solid, note that on the field sheet. If the stream is ice-covered, but is still flowing, the ice should be broken so the stream can be sampled (following a few minute period to allow the water to clear). 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 (AD [06]). For lakes, also record new GPS position and total depth of the water column sampled with the water chemistry protocol (RD [08]). 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]).

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. GW 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 [09]).

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 at a base camp or Domain Lab (i.e., if weather (and safety) dictates 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[11]).

5.1 Decision Tree

1) Are you using a boat to sample?
   a) If No, go to 3
   b) If YES, go to 2
2) Are the weather conditions safe to be on a boat and to undertake water chemistry sampling including low wind speeds (<8 m/s) and safe wave heights (<1 m)?
   a) If YES, go to 3.
   b) If NO, Stop sampling.
3) Has there been any sampling that occurred upstream or upwind of the water chemistry sampling location?
   a) If YES, go to 4.
   b) If NO, continue sampling.
4) Did any of the previous work stir up sediments or added chemical constituents to the water column?
   a) If Yes,
      i) Allow water to clear and disturbance to pass
      ii) Sample in a different location, away from the disturbance
   b) If NO, continue sampling.

6 SAFETY

Personnel working at a NEON site must be compliant with safe fieldwork 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. Personnel working at a NEON Domain lab must be compliant with safe laboratory work practices as outlined in the NEON.DOC.000724 Domain Chemical Hygiene Plan and Biosafety Manual (AD [03]).

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 [01]). 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.

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

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 hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.)

7 PERSONNEL REQUIREMENTS

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 (depending on the number of wells sampled during each bout). Where applicable, personnel will be licensed to operate a boat and able to safely handle a motor and drive a boat safely.

8 TRAINING REQUIREMENTS

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

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

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

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

9.1.1 Criteria for Determining Sampling Dates

Stable isotope chemistry sampling occurs in conjunction with water chemistry sampling in streams, lakes and groundwater environment (RD [07], RD [08] and RD [09], respectively). 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 [05]). 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 [05]). 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.

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 will be used to define when the samples shall be collected, when available. 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 with 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.

9.1.2 Sampling Frequency

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.

In lakes, aquatic stable isotope sampling shall occur up to 12 times per year at each NEON location, and up to 4 times per year in groundwater wells, for a total of 8 wells per year.

9.1.3 Sampling Timing Parameters

Other than event based sampling, chemistry samples should be collected on Tuesday to coincide with other national chemistry sampling efforts (RD [05]). For lakes, the number of samples will vary with season, and depends on the timing of thermal stratification.

9.2 Equipment and Materials

Table 1. Equipment and Consumables for Surface and Subsurface Water isotope sampling

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
$^{18}O/^{16}O$, $^{2}H/^{1}H$ of H_2O Collection				
	15 mL glass vial with rubber closure	1		
	Syringe Filter, non-sterile, nylon, 0.2 µm pore size, 13 mm diameter	1		
	60 mL syringe (numbers covered with clear packing tape)	1		
	Plastic Paraffin film (e.g. Parafilm)	As needed		
$^{15}N/^{14}N$, $^{13}C/^{12}C$ of POM Filtering				
MX100386	Filter funnel (25 mm diameter)	1		
MX106350	GF/F filters (25 mm diameter, pre-ashed)	2		
MX100388	Vacuum filter flask	1		
	Vacuum pump	1		
	Filter forceps (flat ends)	1		
	Graduated cylinder, 250mL	1		
	Collection jug, 4L	1		
	Aluminum foil squares (~4 X 4 inches)	2		
Labeling				
	Isotope waterproof labels (1 * 2 $^{5}/_{8}$)	2		
	Permanent markers	1		

Note: Always take extras to the field.

a)

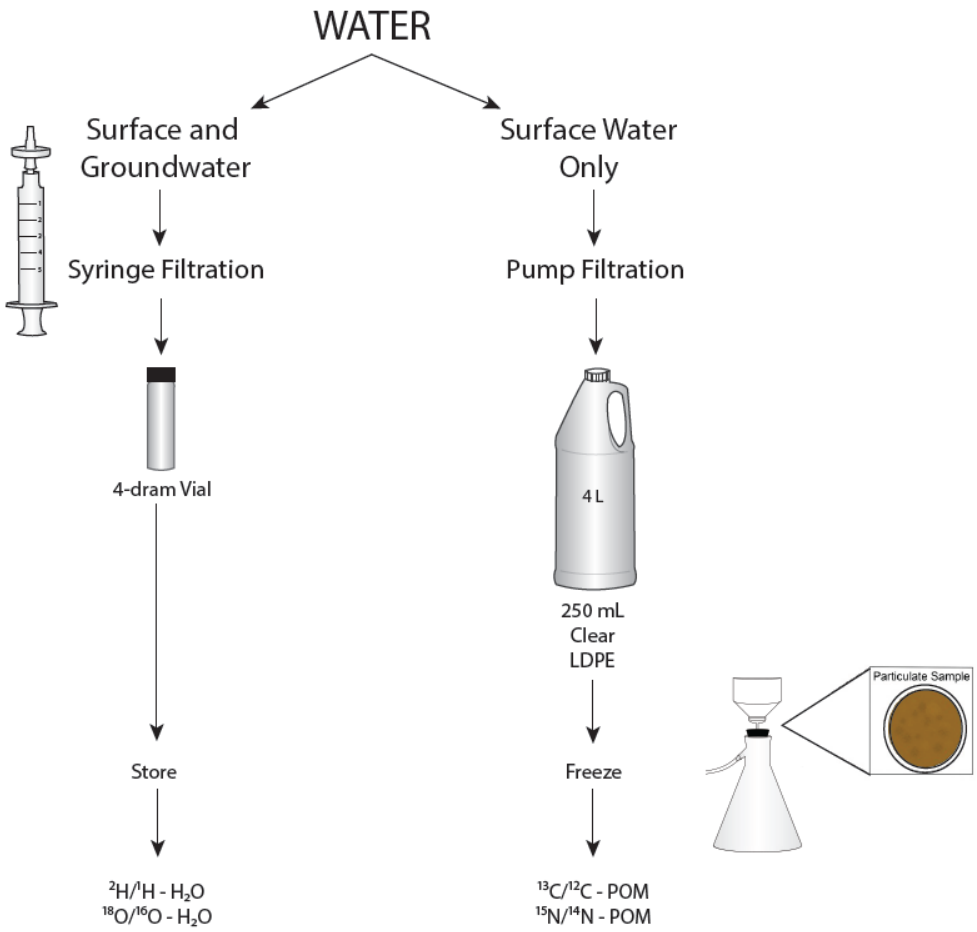

Figure 2. Diagram of isotope sample collection and equipment

Table 2. Additional Equipment and Consumables for surface water isotope sampling in lakes and rivers.

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Boat and oars	1		
	Safety kit	1		
	Kemmerer sampler	1		
	Tubing with flow control hose clamp	1		
	GPS	1		
	First Aid Kit	1		
	Waders or boots	2		
	Personal Flotation Devices	2		

	Ice auger	1		

Table 3. Additional Equipment and Consumables for sampling isotopes in groundwater.

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	4-L jug to collect sample water	1 per well sampled		
	Clean 5 Gallon Bucket	1		
	YSI Handheld Meter (YSI 30)	1		
	QED Sample Pro Pump	1		
	QED MP-50 Compressor / Controller	1		
	Key for unlocking well	1		
	Battery (12V, minimum of 3.6 Ah)	2		
	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.	1 per site, required for 1st sampling event.		
	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.	1 dedicated piece of tubing per well sampled (after 1st sampling event)		
	Water level tape	1		

Table 4. Field Equipment List: Sample Field Storage and Shipping

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Shipping cooler	1		
	Packing material	As needed		
	Resealable plastic bags	1		
	Ice or Ice packs	As needed		
	Clear Packing tape, roll	1		
	Laboratory paperwork	1		
	FedEx shipping labels	2		
	Dry ice	1 lb.		
	Dry ice shipping container (filters)	1		

9.3 Preparation

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 3) to sample bottles (Figure 2). Use a **Sharpie** to fill out labels before going into the field.

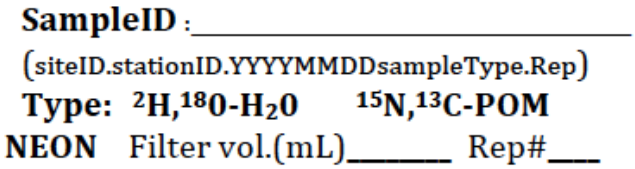

Figure 3. NEON aquatics stable isotope chemistry labels example.

9.4 Sample Collection in the Field

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[10]), and the Isotope Water Chemistry Field Sampling Datasheet (AD [06]) before collecting samples. You only need to fill out one General Field Sampling Datasheet per site, per day.
2) Collect Samples according to water chemistry sampling protocols in streams (RD [07]), rivers and lakes (RD [08]), or groundwater wells (RD [09]), as appropriate:
   a) Wadeable Streams (Follow RD [07]):
   Isotope sampling in wadeable streams will be completed in the same location as surface water chemistry, immediately downstream of sensor set 2 (RD [07]) and upstream of any stream disruption and in a flowing area of the stream, if possible.
      i) Collect stream isotope samples and metadata following the Wadeable Stream Chemistry Sampling Protocol (RD [07])
         (1) 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.

ii) Proceed to Section 9.4.1, Sample Collection and Processing.

b) Lakes and Rivers (Non-wadeable streams) (Follow RD [08]):

Isotope sampling in lakes and rivers will follow the Lakes and Non-Wadeable Streams Water Chemistry 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 Section 4.1).

i) Collect river and lake water samples and metadata following the Lake and Non-Wadeable Stream Chemistry Sampling Protocol (RD [08])

(1) Move to the sampling station note the station ID ("in", "out", "center", or "center1" or 'center 2" if stratified with "center1" being the top layer) on the field sheet (RD [08]; section 9.4.1).

(2) Sample using the Kemmerer bottle (Figure 4, RD [08]; section 9.4.2).

ii) Proceed to Section 9.4.1, Sample Collection and Processing.

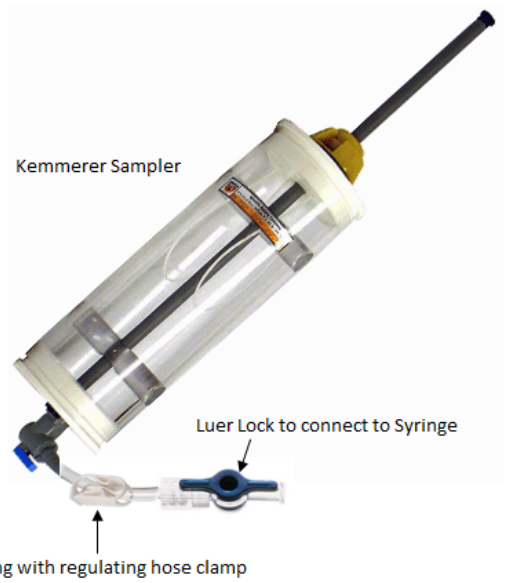

Figure 4. Example of tubing attachment with hose clamp and Luer-Lock to Kemmerer spigot.

c) Groundwater Wells (Follow RD [09]; section 9.4):

Groundwater isotope sampling will be completed in the same location as the groundwater chemistry sampling design (RD [09]).

i) Collect groundwater water and metadata from selected wells following the Groundwater Chemistry Sampling Protocol (RD [09], section 9.4).
   (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.

ii) Proceed to Section 9.4.1, Sample Collection and Processing.

9.4.1 Sample Collection and Processing

1) Process (filter and store appropriately) surface and subsurface isotope samples within 3 hours of sample collection:
   a) Record the SampleID (SiteID.StationID.YYYYMMDD.SampleType.Rep#) on the bottle labels (Figure 3). SiteID is the 4 letter site code. StationID is '1' in streams and rivers and is the well ID for groundwater well samples. In lakes the station ID is 'in', 'out', 'center', or if the lake is stratified 'center1' or 'center 2', with 'center1' being the top layer). SampleType is 'POM' or 'H2O'. Rep# is 1 for H_2O, Rep# is 1' or '2' for POM.
      i) You DO NOT need to complete a second General Field Sampling Datasheet unless aquatic stable isotope samples are collected on a different date than the water chemistry samples.

9.4.1.1 $^{18}O/^{16}O$ 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.

9.4.1.2 $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of POM from Surface Waters

1) Collect water using a 4 L jug to be filtered within 3 hours. 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 (**Error! Reference source not found.**5).
   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.

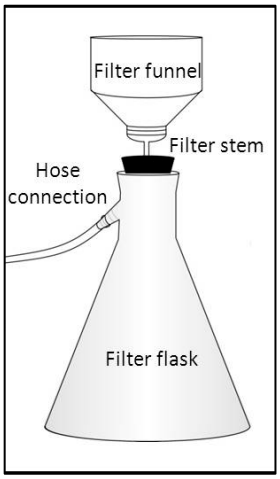

Figure 5. Filter apparatus setup

3) Remove the top of the filter funnel from apparatus, rinse with DI water.
4) Place pre-ashed GF/F filter on top of the filter stem (**Error! Reference source not found.**5), replace top of funnel.
5) Shake sample bottle vigorously for ~30 seconds to mix sample.
6) Filter known volume of sample.
   a) Measure using a graduated cylinder. Pour sample slowly in ≤100 mL increments into filter funnel.
      i) 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.
   b) **Keep track of the volume of sample filtered on the Lab Data Sheet (RD[11])**.
7) Draw suction on filter apparatus using the hand vacuum pump (or vacuum manifold, if available). Do not exceed 15 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[11]).**
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.
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 inches). Fold foil securely around the filter to form a packet.
   b) Label foil packet with adhesive sample label (1 * 2 $^{5}/_{8}$") (Figure 3)
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.

9.5 Sample Shipping

Shipments are to have a hardcopy of a shipping inventory (RD [12]) 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. Also include the shipment tracking # in the email. The hardcopy of a shipping inventory can be placed in a resealable plastic bag if moist conditions or dry ice may/does exist in the shipping container. If shipping materials are to be returned to the domain laboratory, a completed shipping form for return shipment and an explanatory note should be included with the shipping inventory.

Shipping information for the external facility that will receive samples can be found on the CLA SharePoint page. The file is named Shipping Information for External Facilities. The NEON Collections and Laboratory Analysis contact is also listed in this file.

1) Ship samples to an Analytical Laboratory following a schedule set forth by NEON Headquarters.
   a) **^{18}O/^{16}O and ^{2}H/^{1}H of H_2O samples**
      i) Ensuer you have wrapped plastic paraffin film around the lids to keep them secure during shipping
      ii) 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
      iii) Samples should be shipped Ground within 2 weeks of collection. Glass bottles can be packaged and shipped in a secure box to isotope lab. "Up" arrows can be affixed to the secure box.
   b) Filters (^{13}C/^{12}C and ^{15}N/^{14}N of POM)
      i) 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^{o}C freezer until shipping.

ii) Filters are shipped overnight on dry-ice in appropriate dry ice shipping containers.
iii) Place barrier (e.g. cardboard) between the dry ice and sample bag and place packing materials above the sample bag.
iv) Tape and label containers appropriately for shipping on dry ice and ship to algae chemistry lab.

9.6 Data Handling

1) General Field Sampling Datasheet_data ingest to be ingested into NEON database.
2) Aquatic Stable Isotope Sampling _data ingest to be ingested into NEON database.

9.7 Refreshing the Sampling Kit

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.

9.8 Equipment Maintenance, Cleaning and Storage

1) 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.
2) Rinse Filter Funnel apparatus with DI

10 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