δ^2H, δ^{13}C, and δ^{15}N Isotope Procedures

Cascade Project, Updated by G.M. Wilkinson (May 2014)

General Note: the amount of dry material needed for δ^2H analysis is 350-370 µg. The amount of dry material needed for combined δ^{13}C and δ^{15}N analysis is 1-2 mg depending on the C:N ratio

δ^2H POM

Equipment:

- 47mm membrane filter (0.8µm or 1.2µm pore size)
- 153µm pre- filter to remove zooplankton
- Filter tower
- Metal spatula
- Glass petri dishes
- 1L Erlenmeyer flask with side arm
- 1 mL dram vials with poly-seal caps
- Acetone

1. To remove any zooplankton from the sample, pour the water sample through the 153µm prefilter and into another clean bottle or beaker.
2. Filter the water through the 0.8µm or 1.2 µm membrane filters. Depending on the particle concentration, 1-4 L of sample water will need to be filtered. RESERVE THE FILTRATE FOR DOM.
3. When the membrane is full of particles, remove the membrane from the filter tower and place it into a small glass Petri dish. Cover the filter with DI water and gently scrape the particles off the filter.
4. When the sample has been scraped off the filter, remove the filter and rinse any particles back into the dish.
5. Continue filtering the rest of the water with more filters and scrape them into the same dish
6. Label the Petri dish with the Isotope ID number, cover it loosely with foil, and place it into the drying oven. Let dry for at least 24 hours or until it is dry.
7. When dry, scrape the sample out of the petri dish using a razor blade. Be sure to clean the razor blade and spatula with acetone before using. Place sample into a labeled 1 mL dram vial. Put vial into sample storage desiccator.

δ^{13}C, δ^{15}N POM

NOTE: POM for δ^{13}C and δ^{15}N analysis can either come from the method described above (scraping particles from the membrane filter) or filtering using 25mm GF/F filters. The procedure described above is not conservative in terms of material, and therefore particle concentration cannot be calculated using the δ^2H POM procedure. If POC concentration is needed, use the following method for POM δ^{13}C and δ^{15}N:

Equipment:

- 25 mm GF/F
- 153 µm prefilter mesh
- Petri dish
- graduated cylinder

1. Place an ashed 25 mm GF/F filters on a filter tower connected to an Erlenmeyer flask with side arm.

2. Prefilter the water sample using a 153 µm mesh and measure out a known volume using a graduated cylinder. We usually filter 200-250 mL of water for this analysis. The filter needs to be noticeably colored with particles after filtration (near clogged).
3. Record the volume filtered for POC concentration calculations later.
4. Using the vacuum, filter the particles onto the 25 mm GF/F.
5. Carefully remove the filter with clean tweezers when done and place in a labeled petri dish to dry in the drying oven.
6. Repeat steps 1-4 for the same water sample so that there are 2 filters for each sample.
7. The filters can be packaged in tin capsules (either at the isotope lab or do it yourself), combusted, and analyzed for $\delta^{13}C$ and $\delta^{15}N$.

δ^2H, $\delta^{13}C$, $\delta^{15}N$ DOM

Equipment:

- 47mm GF/F filter ashed
- (3) 300mL BOD bottles
- Food dehydrator

1. 350mL of filtrate from the δ^2H POM procedure outlined above is needed for one BOD bottle of DOM. In general, we use 3 BOD bottles (about 900 mL) in our lakes.
2. Rinse each BOD bottle three times with a small amount of filtrate.
3. Fill each BOD bottle to just below the neck with filtrate.
4. Add 0.333mL of 1N HCl to each BOD bottle. The acid will convert any DIC to CO_2 and it will off gas, insuring only DOM is left. This step is unnecessary if $\delta^{13}C$ will not be measured.
5. Label a large Petri dish and place it into the food dehydrator. Fill the dish with sample and allow it to dry. Continue adding more sample until it is all gone.
6. Store BOD bottles with sample in the fridge during the dehydration process.
7. Place the petri dish in the drying oven for 6-12 hours before beginning to scrape as the dehydrator does not completely dry the sample.
8. Scrape DOM with a razor blade as above and place sample into a labeled 1 mL dram.

δ^2H_2 and $\delta^{18}O$ of Water

Equipment:

- Filtrate
- 40 mL glass scintillation vial with polycone seal cap

1. Using filtrate reserved from the POM procedures, fill the scintillation vial to overflowing with filtrate.
2. Carefully cap the vial and check that there are no bubbles by turning it upside down.
3. Store the vial in the fridge until analysis.

$\delta^{13}C$-DIC

Use the CPSIL procedure. If you know DIC concentration and do not DOC, you can likely get away with one sample vial. Contact the lab before sampling to confirm.

http://www4.nau.edu/cpsil/General%20info_files/CPSIL%20DOC_DIC%20sampling%20protocol.pdf

Zooplankton

Equipment:

- 80 or 153 µm mesh conical net and dolphin bucket
- Curved forceps
- Clear, shallow dish
- Glass petri dish for each species to be picked
- Dissecting microscope
- 1mm mesh
- Mortar and pestal

1. Either do vertical or oblique tows, collecting enough organisms for a sample. For Daphnia, this is usually 300-400 individuals for all 3 isotopes, for Chaoborus, 50-100 depending on the instar.
2. Using a 1mm mesh, separate Chaoborus from the other zooplankton and store them in DI water in the fridge overnight.
3. Pour a small number of zooplankton into a shallow dish (like the square petri dishes in the lab) and separate the consumers into their respective petri dishes using the forceps.
4. When enough consumers have been separated to create a sample, dry the zooplankton in the drying oven for at least 2 days.
5. Using a clean razor, scrape the zooplankton from the dish and into a mortar and grind the biomass to a fine powder.
6. Scoop the powder into a 1 mL dram vial and store in a dessicator until analysis.