**Biology 302: Ecology Laboratory**

**Stable Isotopes: Sample Preparation in the Laboratory**

*In today’s laboratory exercise, we will be preparing the samples we obtained last week for stable isotopes analysis.*

IT IS IMPORTANT TO WEAR GLOVES AND NEVER TOUCH THESE SAMPLES OR ANY LAB SUPPLIES WITH YOUR BARE HANDS. Oils from your hands could contaminate the samples, and result in skewed values.

**Sorting**

You already sorted samples by trophic level as best as you could in the field, but it is critically important to the success of this study to ensure that samples are isolated from those of other trophic levels. This will require picking through samples under a dissecting scope and making accurate identifications.

Using plastic forceps and weigh boats (do not touch either the sample or the boats bare-handed), sort samples into the following categories:

* Detritus (sticks, leaves, etc.)
* Algae (will likely have diatoms attached, look under scope to determine)
* Midges
* Mayflies
* Stoneflies
* Mysis shrimp
* Fish fin clips

Not all groups will have representatives of all these categories, but separate what you did get into these categories. Our goal is to include 0.5-1.0 g of dried tissue, after washing and drying (about the size of a painted lady butterfly wing), so it is important to get as much of some of these samples (e.g., midges) as possible.

LABEL EACH SAMPLE AS INSTRUCTED!!

A proper label should include a group number, a trophic level, and a sample number. These will be assigned in lab. Write these on the weigh boats you sort samples onto using a sharpie, and again on lab tape that you will attach to the glass Petri dishes when you wash the samples.

**Washing**

THE WASHING STEPS MUST BE CONDUCTED UNDER A FUME HOOD!! DO NOT REMOVE THE CHLOROFORM:METHANOL SOLUTION FROM THE HOOD UNDER ANY CIRCUMSTANCES. BREATHING CLOROFORM FUMES CAN CAUSE UNCONSCIOUSNESS (OR EVEN DEATH).

GLOVES AND SAFETY GOGGLES ARE NECESSARY.

NOTE: The chloroform:methanol wash solution will MELT PLASTIC. Do not use plastic tools of any kind. It will also remove Sharpie and dissolve lab tape adhesive. Be very careful not to spill the solution!

* Place samples into glass Petri dishes, according to trophic level. Petri dishes will be marked into quadrants, and each group will be responsible for ensuring that their sample is placed into one of these and labelled appropriately, taking care to only place samples belonging to the same trophic level onto the same Petri dish (i.e., only midges go with midges, only algae goes with algae, etc.). Write your sample ID numbers on lab tape and attach them to each of your quadrants ON THE OUTSIDE OF THE PETRI DISH. **If you have questions, ask! We must be able to track our samples through this process.**
* Wash samples in a 2:1 chloroform:methanol solution – Transfer 10 ml of this solution into the glass Petri dish containing the sample using a glass transfer pipette.
* Using metal tweezers, gently agitate the samples for 30 seconds to remove lipids and other surface contaminants (e.g., dirt, pollen) according to previously published methods (Wassenaar and Hobson 1998). (Plastic tweezers will melt onto the samples and ruin them.)
* Using a glass transfer pipette, remove the wash solution and pipette it into the waste container in the fume hood.
* Using a CLEAN glass transfer pipette, repeat the washing steps twice more, gently agitating the sample for 30 seconds each time.
* Dispose of as much of the cleaning solution as you can pipette off the samples in the waste container in the fume hood after each wash.
* Dispose of all used glass pipettes in the broken glass waste.

**Drying**

All samples must air dry on glass Petri dishes in the fume hood (a dust free environment) for a minimum of three days.

Once dry, samples will be transferred by your instructor to a low-temperature drying oven, where they will be dried for 24-48 hours at 50-60°C. Hence, the reason why properly labelling your samples is so important.

Dried samples will then be transferred to 1-dram glass vials and shipped to the Stable Isotope Paleo Environments Research Group (SIPERG) Laboratory at Iowa State University for further processing. Metal spatulas will be used to scrape samples into the glass vials, and these must be washed with ethanol and dried with a kim-wipe between samples.

**ISU SIPERG Laboratory**

Once received, samples will be placed into tin capsules and immediately sealed. Carbon (δ13C ) and nitrogen (δ15N ) stable isotopes analyses will be conducted on a Costech EA attached to a Finnigan MAT Delta Plus SL. δ13C will be corrected to a Vienna Pee Dee Belemnite (VPDB) standard, and δ15N will be corrected to an air standard.

**Statistical Analysis**

Statistical analyses and graphing will be conducted in R v3.2.2 (R Core Team 2015) using packages ggplot2 v.2.1.0 (Wickham 2009) and mgcv v.1.8 (Wood 2006).

**Literature Cited**

R Core Team. 2015. R: A language and environment for statistical computing.

Wassenaar, L. I., and K. A. Hobson. 1998. Natal Origins of Migratory Monarch Butterflies At Wintering Colonies in Mexico : New Isotopic Evidence. PNAS **95**: 4.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. New York, NY: Springer-Verlag.

Wood, S. N. 2006. Generalized Additive Models: An Introduction with R, C. and Hall [ed.]. CRC.