Elemental Ecology Exercise #1 (50 points)

This homework exercise will introduce you to the basic calculations required for correction and normalization of raw data from the mass spectrometer and determination of isotopic fractionation. In addition, you will begin to work with real data and learn how to plot data in δ^{13}C versus δ^{15}N isotope space and interpret isotopic patterns associated with trophic discrimination. All data are provided and solutions are to be reported in the workbook file "Exercise #1.xls". **This exercise is due in class on Tuesday October 13th.** Email your completed assignment as an excel file to newsome@unm.edu. Rename and save this workbook file by adding your last name to the filename. You can work collaboratively on this assignment, and the course instructor and teaching assistant is available for questions and assistance.

1) Correcting Isotopic Data Using Linear Regression

Use the data in the worksheet "Question 1" for this problem.

a) Normalize the raw delta values to the V-PDB (for carbon) and AIR (for nitrogen) scales using the observed and known δ values of "normalization standards" and report the normalized data in new data columns. Generate graphs on the same sheet showing the linear regression (with equation) used for the normalization.
b) Calculate the standard deviation and mean offset from the calibrated V-PDB and AIR values for the "check standard" samples.

For this problem, you will need to know the following:

Standard	Known δ^{13}C, V-PDB	Known δ^{15}N, AIR
UWSIF11 (Peptone)	-15.1	5.3
UWSIF23 (Acetalinide)	-33.7	-0.8
UWSIF01 (Liver)	-17.6	6.9

2) Calculating δ Values

Use the data in the worksheet "Question 2" for this problem. Calculate the δ values in per mil (‰) from the isotopic ratio (R) values and report relative to the required isotopic standard listed in the worksheet. Also, in the next column, calculate the isotopic ratio (R) of the sample from the reported δ value. Refer to Clark and Fritz (1997) Table 1-1 for the isotopic abundance ratio of standards. Report these δ and R values in highlighted cells on the spreadsheet. Also calculate and report the atom percent (Atom%) values for each and record in the highlighted cells.

3) V-PDB versus V-SMOW

Use the data in the worksheet "Question 3" for this problem. Convert the δ^{18}O values between V-PDB and V-SMOW scales and report the converted values in a new column on the spreadsheet.

4) 1000lnα Madness

Calculate the isotopic difference (approximated by 1000lnα) for ^{18}O in water in equilibrium with vapor at 5° and 40°C using both the Majoube (1971) and Bottinga and Craig (1969) relationships listed on the inside front cover of Clark and Fritz (1997), which can be downloaded from the class website. Then calculate the fractionation factor (α) and enrichment factor (ε, in ‰) from these values. Report these values along with your calculations on the worksheet entitled "Question 4" and show your work for full credit.

5) What Cows Drink

The inorganic portion of bone is bioapatite, a biological form of hydroxyapatite [$Ca_5(PO_4)_3OH$]. Within this mineral matrix, carbonate (CO_3) can substitute for phosphate (PO_4). Archeologists and paleontologists analyze this CO_3 for $\delta^{13}C$ and $\delta^{18}O$ values.

5A. Compare the organic and inorganic $\delta^{13}C$ values. Are they similar? Why or why not.

5B. The $\delta^{18}O$ value of an animal's body water is dependent on the $\delta^{18}O$ value of potential sources (food and drinking water) and temperature. However, mammals keep their bodies at constant temperature, so the ambient environmental temperature is not a major factor in determining the $\delta^{18}O$ value of biogenic inorganic substrates (e.g., tooth enamel). Instead, we can use mammalian $\delta^{18}O$ values to determine the $\delta^{18}O$ value of body water that is assumed to represent local meteoric water since tooth/bone carbonate is formed in equilibrium with body water. The isotopic value of body water ($\delta^{18}O_w$) can be calculated with the equations below:

$$10^3 \ln \alpha_{CaCO3-w} = 2.78 \times 10^6/T^2 - 2.89$$

$$\alpha_{CaCO3-w} = (\delta^{18}O_{CaCO3} + 10^3)/(\delta^{18}\,O_w + 10^3)$$

where T is temperature in Kelvin and $\delta^{18}O_{CaCO3}$ is the oxygen isotope value of calcium carbonate. These cows were raised near Columbia, Missouri. Does the $\delta^{18}O_w$ match the modeled precipitation $\delta^{18}O$ value? Describe why or why not? Isoscapes of $\delta^{18}O$ and $\delta^{2}H$ in precipitation can be downloaded at:

http://wateriso.utah.edu/waterisotopes/index.html

6) Sandia Mountains Survivor

In this problem you will practice interpreting carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes by analyzing data collected during a popular reality television show, produced right here in the Sandia Mountains of New Mexico (in the summer of course). You will need to make some graphs and tables for this exercise, and then we will review the answers with the entire class. We begin by gathering a few samples from the set – a few leaves on the ground, mushrooms, a worm and a centipede from the soil, spiders from the bushes and bird feathers from sparrows and hawks. We dry the samples overnight at 60°C, grind them to a fine powder with a mortar and pestle, weigh them into tin capsules, and analyze them in an isotope ratio mass spectrometer. The $\delta^{13}C$ and $\delta^{15}N$ results arrive in your email inbox a month later, and now the work begins to interpret the data.

Step 1. Make a table and an isotope biplot (x,y) graph with the following stable isotope data:

Sample ID	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**
Juniper Leaves	2.0	-27.2
Mushroom	3.2	-24.1
Worm	4.2	-26.3
Centipede	8.1	-21.5
Spider #1	5.4	-24.0
Spider #2	10.3	-21.2
Sparrow Feather	8.5	-23.7
Hawk Feather	12.8	-18.2

Questions: Look at your $\delta^{13}C$ versus $\delta^{15}N$ plots – are the axes correctly labeled? Which isotope system did you decide to plot on the x-axis ($\delta^{13}C$ or $\delta^{15}N$), and why did you choose one or the other? Can you make an initial assessment of who is eating whom?

<u>Step 2.</u> Assign trophic levels to your plot, using a 3‰ increase in $\delta^{15}N$ increase indicate an increase of one trophic level (TL). Use the plant isotope value as TL 1, and see how many consumer animal TLs beyond 1 are indicated; there are usually less than 8 TLs in natural food webs. The general formula for calculating trophic levels starting with plants at TL1 is:

$$TL = 1 + (\delta^{15}N_{CONSUMER} - \delta^{15}N_{PLANT})/3$$

Note that in this formula the "3" is the per mil (‰) increment in $\delta^{15}N$ that occurs on average per each trophic level, and the "1" in the equation ensures that trophic levels start at TL = 1 at the plant level. Questions: How many trophic levels are there? Is there any sample whose trophic level does not make sense?

<u>Step 3.</u> During the second month of the show (to insure full incorporation) we were able to collect fingernails from all of the cast members, and prepared this tissue for $\delta^{13}C$ and $\delta^{15}N$ analysis; see attached spreadsheet. We also did a bit more "fieldwork" and found a few smuggled items in the camp on the set, including some Frito corn chips ($\delta^{13}C$: -13.5‰, $\delta^{15}N$: 2‰) and a pig bone ($\delta^{13}C$: -12.5‰, $\delta^{15}N$: 5.5‰). Update your table and make another isotope biplot graph with the combined data.

Questions: Does including these new points change your impressions of which food sources support the cast members on New Mexico Survivor? Do you think the juniper leaves are the only primary producer of nutrition in this food web? Which food sources are most important? Which cast members appear to be cheating the most?

<u>Step 4:</u> Make corrections to the cast member $\delta^{13}C$ data, factoring out discrimination effects related to TL, and leaving source inputs as the sole reason for the animal $\delta^{13}C$ variations. This correction gives you the $\delta^{13}C$ value of the inferred plant diet for each consumer, needed for direct comparison to the possible plant foods. The increase in $\delta^{13}C$ per trophic level averages near 0.5‰, so use the following formula to calculate $\delta^{13}C$ values corrected for trophic level effects:

$$\text{Corrected } \delta^{13}C = \text{Measured } \delta^{13}C - 0.5 * (TL - 1)$$

where TL is estimated from the $\delta^{15}N$ data in step 2 above. Add the corrected $\delta^{13}C$ values to your data table. Questions: Do these TL corrections change the $\delta^{13}C$ data very much? Which food sources are most important for the overall food web? Which cast members appear to be cheating the most?