Elemental Ecology Exercise #2 (50 points)

This exercise is designed to teach you how to (1) calculate trophic discrimination factors; (2) determine tissue-specific discrimination factors; and (3) use stable isotope mixing models to quantify dietary proportions. All data are provided below or in the attached Excel spreadsheet (Exercise #2.xls); both are available on the course website. **This exercise is due by midnight on Thursday, November 19th.** Email your homework to Seth Newsome (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 is available for questions and assistance.

Problem #1: Leopard Shark Trophic Discrimination!

One important aspect of animal isotopic ecology is determining the isotopic offset between a consumer's tissues and its diet, which is often called a trophic discrimination or trophic enrichment factor. As part of her dissertation at UC-Santa Cruz (Go Slugs!), Dr. Sora Kim conducted a 4+ year captive feeding experiment on leopard sharks (*Triakis semifasciata*) to determine trophic discrimination factors and isotopic incorporation rates for a variety of tissues commonly sampled from sharks in the wild. For the first part of this lab exercise, please determine the diet-to-tissue trophic discrimination factors for these leopard shark tissues.

What is the equation for a trophic discrimination factor?

What are the average $\delta^{13}C$ and $\delta^{15}N$ values for muscle? C:N?

What are the average $\delta^{13}C$ and $\delta^{15}N$ values for liver? C:N?

What are the average $\delta^{13}C$ and $\delta^{15}N$ values for squid? C:N?

What are the average trophic discrimination factors for muscle? For liver?
(Propagate the error between the tissue and prey isotope values)

Why do you think the discrimination factors are different between tissues?

Why are C:N ratios important? Now plot $\delta^{13}C$ vs. C:N. Why are the liver C:N ratios concerning?

Problem #2: Thresher Shark Tissue-Specific Discrimination

Tissue-specific isotopic discrimination is a poorly understood phenomenon in animal isotope ecology. Our colleague, Dr. Carlos Polo, studies sharks in Ecuador and Mexico. Carlos uses $\delta^{13}C$ and $\delta^{15}N$ to characterize trophic relationships, habitat use, and large-scale (i.e., ocean basin) movement within and among shark species in the eastern Tropical Pacific Ocean; as an aside, his data have some major implications for conservation and management of a ruthlessly exploited group of fish. A recent dataset from pelagic thresher sharks (*Alopias pelagicus*) collected off coastal Ecuador showed an intriguing difference between $\delta^{15}N$ of muscle and vertebrae tissues (see below). One of the possible explanations for this dataset is tissue-specific discrimination.

a. Explain what tissue-specific discrimination is and what types of data are needed to test the explanation that this phenomenon is the reason why Carlos' shark tissues differ in their $\delta^{15}N$ isotopic composition. Using the data from the literature (see papers below by Mizuta et al. 2001, Kittiphattanabawom* et al. 2010, and Popp et al. 2007), determine whether or not tissue-specific discrimination is driving the observed trend. *This is his/her real name.

b. Why can we use data for tuna (Popp et al. 2007) as a surrogate for thresher sharks?

c. Tissue-specific discrimination aside, what is another possible explanation for the observed $\delta^{15}N$ difference between muscle and vertebrae tissue?

Shark Tissue	δ^{13}**C**	δ^{15}**N**
Muscle	-16.0	13.8
Vertebrae	-16.8	9.2

REFERENCES

Kittiphattanabawon P, Soottawat B, Wonnop V, Fereidoon S (2010) Isolation and characterization of collagen from the cartilages of brownbanded bamboo (*Chiloscylliumpunctatum*) and black tip (*Carcharhinus limbatus*) shark. Food Science and Technology 43:792-800.

Mizuta S, Hwang J, Yoshinaka R (2001) Molecular species of collagen from wing muscle of skate (*Raja Kenojei*). Food Chemistry 100(3):921-925.

Popp BN, Graham BS, Olson RJ, Cecelia CSH, Lott MJ, López-Ibarra GA, Galván-Magaña F, Fry B (2007) Insight into the Trophic Ecology of Yellowfin Tuna, *Thunnus albacares*, from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids. (download at*:* http://www.soest.hawaii.edu/GG/FACULTY/POPP/bpopp-publ.html*)*

Problem #3: Linear and Concentration-Dependent Stable Isotope Mixing Models

Mixing models are an often used but rarely understood component of many applied studies in animal isotope ecology. Briefly describe how mixing models work, the type of data required for their use, and most importantly the major assumptions that are implicit in their accurate use and interpretation.

a. Using the isotopic dataset from Alaskan black and brown bears below, determine the relative proportion of the two general prey sources (salmon and berries) to each populations diet using a simple 2-source, 1-isotope linear mixing model. Be sure to write and describe the variables in the equations for both $\delta^{13}C$ and $\delta^{15}N$ mixing models:

$$\delta^{15}N_M = f_x(\delta^{15}N_x + \Delta^{15}N_x) + f_y(\delta^{15}N_y + \Delta^{15}N_y)$$
$$1 = f_x + f_y$$

b. Using the same isotopic dataset, determine the relative proportions of the three general prey sources with a concentration-dependent mixing model using the [C] and [N] data supplied below:

$$\delta^{15}N_x + \Delta^{15}N_x = \delta^{15}N'_x$$
$$\delta^{15}N_y + \Delta^{15}N_y = \delta^{15}N'_y$$
$$(\delta^{15}N'_x - \delta^{15}N_M)[N]_x f_x + (\delta^{15}N'_y - \delta^{15}N_M)[N]_y f_y = 0$$
$$1 = f_x + f_y$$

c. What are the major physiological mechanisms that are likely driving the observed differences in the prey proportions between the model results in part A and B?

d. In some instances, when you draw mixing triangle by connecting isotope values of three sources, some of the consumers (e.g., bears) have isotope values outside the mixing triangle. Give one or more reasons that consumers can have isotope values outside the mixing envelope defined by the measured sources.

Consumer/Prey	$\delta^{15}N$	$\Delta^{15}N$	**[N]**
Brown Bear (sympatric)	10.9	–	–
Black Bear (sympatric)	4.9	–	–
Black Bear (allopatric)	7.6	–	–
Salmon	13.2	2.3	12.0
Berries	-0.9	4.1	1.0

Problem #4: Sea Lion SIAR

My close colleague Dra. Maritza Sepulveda (University of Valparaiso) studies sea lions along the southern coast of Chile just south of Puerto Montt. This area is home to one of the most productive salmon aquaculture regions in the world. Salmon are raised in floating pens in coastal bays and fjords, but are fed a specially formulated fishmeal that calorically is dominated by soy, a C_3 plant. Maritza and her colleagues are trying to figure out how often sea lions consume farmed salmon by exploiting the gradient in isotopic composition between the sea lions natural fish prey and that of the (hippy dippy) farmed salmon largely raised on tofu. To do so, Maritza collects samples of potential natural prey items and farmed salmon obtained directly from pens; these data are listed in the worksheet entitled "Sea Lion SIAR" in the Excel spreadsheet that accompanies this exercise. In addition, Maritza and her students use crossbows to collect two types of tissue (hair and skin) from each of 15 individuals at a sea lion haul-out that is in close proximity to an area that contains a high density of salmon farms. She conducts her fieldwork at the end of the austral winter when it is believed that sea lions consume more farmed salmon than during the productive spring/summer months.

a. Use these data to construct a mixing model in SIAR (Parnell et al. 2010) for the two types of tissues collected from each sea lion. Given the observed amount of variation in isotope values among individual sea lions, its best to quantify the diet for each individual rather than using mean values for each tissue type. You will have to search the literature for $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors (TDFs) and decide whether you should be using the same or different TDFs for hair versus skin. Report your findings in a table showing the relative proportion of each prey type consumed by each individual.

b. What potential mechanisms (there are at least two) that could explain the difference in isotope values and associated mixing model output for sea lion hair versus skin tissue. Hint: think about the time of the year represented by each tissue type and what each tissue is composed of when formulating your answer.

c. During the second year of the study, abnormal conditions lead to an increase in the occurrence of southern sardines (*Sprattus fueguensis*) in the coastal bays and fjords where the sea lions forage. Usually, sardines are an open ocean forage fish that are rarely observed inshore. Maritza opportunistically collects a sample of sardines and measures their isotopic composition; their mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values are -15.6±0.4 and 15.3±0.2 respectively. How does the inclusion of this novel prey source change your results concerning what sea lions consume in this region. More importantly, how does the inclusion of this prey affect the use of stable isotopes as a tool to quantify sea lion diets given its distribution in $\delta^{13}C$ versus $\delta^{15}N$ space relative to the other prey.