**Stable Isotope**

**Introduction/Teacher Notes/Questions**

The isotopes of carbon and nitrogen are very useful in biogeochemistry and ecology. For example, the ratio of 13C:12C can be used to determine if trees are experiencing drought stress and the amount of 15N can be used to determine the structure of food webs within ecosystems. Much of the utility of isotopes comes from the process of fractionation. Due to differences in atomic weight the lighter isotopes often react faster. As a result organisms can become “enriched” with certain isotopes.

There are international standards to compare carbon and nitrogen isotope ratios. For carbon isotopic ratios that standard is the Pee Dee Belemnite (PDB) which was based on a marine fossil from the Cretaceous period that had an anomalously high 13C:12C ratio. For nitrogen isotopic ratio, that atmospheric ratio of 15N:14N is the international standard.

**Example 1: Carbon isotopes**.

Plants take up CO2 from the atmosphere for photosynthesis. Thus, when plants open their stomata (specialized openings between cells that allow gas exchange with the atmosphere), they take up both 12C and 13C. Due to the faster reaction times plants become enriched with 12C. That is, plant biomass is primarily made of 12C. However, when plants become water stressed they will not open their stomata as frequently so as to conserve water by lower rates of evapotranspiration. When the stomata are closed plants begin to use more 13C. Thus under drought and water stress conditions plant tissue will incorporate more 13C. This will increase the ratio of 13C:12C – becoming less negative.

δ13C = (((13C/12C)sample / (13C/12C)standard) -1) \* 1000 ‰

**Carbon Isotope Excel Sheet:** Carbon isotopic data is presented for two tree species from a normal precipitation year and an extreme drought year. These data are constructed to allow students to see the effect of interannual precipitation variation and to test if different species respond the same way to drought. (Note: data provided for this activity are “made-up” but reflect realistic values and trends).

1. Have students calculate means and standard errors for the two treatments and for both species: (1) plant tissue during normal precipitation and (2) plant tissue during drought conditions.
2. Have students create bar graph with appropriately labeled axes.
3. Have students test the hypothesis using a simple t-test in Excel.
4. Do trees growing during a drought show an isotopic signal that indicating drought stress?
5. Do both species respond in the same way to drought? Offer hypotheses to explain why you observed the pattern you did between species.
6. Compare these data to those published in specifically **Figure 2**: Zhang et al (1997) Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. Tree Physiology 17: 461-466.
7. Ask students: Do these data show that drought has any effect on plants?
8. Ask students to discuss how they would experimentally test to determine if drought actually did have an effect on plants.
9. How could understanding carbon isotopes be useful in identifying sources of carbon dioxide emitted to the atmosphere?

**Example 2: Nitrogen Isotopes**

When an organism consumes another organism’s tissue (heterotrophy) the consumer will use some of the nitrogen of its prey to build many important biomolecules (e.g. amino acids/proteins and nucleic acids). However, during the process of digestion, the lighter isotope of nitrogen (14N) is excreted at a faster rate than the heavier isotope (15N). Thus, organisms higher on the food chain are enriched with 15N. Because of this nitrogen fractionation, the structure of food webs can be determined by examining the amount of 15N in animal tissue. One of the very practical uses of nitrogen isotopes can be found in restoration ecology to see if certain practices fundamentally change the structure of a food web. For example, does the invasion by exotic and invasive species fundamentally change where native fish are feeding on the food web? It will be useful to point out to the students that a change of roughly 3.5 (e.g. Minagawa and Wada 1984: Stepwise enrichment of 15N along food chains: further evidence and the relation between 15 N and animal age; Adams and Sterner 2000: The effect of dietary nitrogen content on trophic level 15N enrichment) represents the change to the next higher trophic/feeding level.

δ15N = (((15N/14N)sample / (15N/14N)standard) -1) \* 1000 ‰

**Nitrogen Isotope Excel Sheet:** These data are based on Marks et al. (2010): Effects of flow restoration and exotic species removal on recovery of native fish: lessons from a dam decommissioning. Restoration Ecology 18:934-943.

1. Have students calculate means and standard errors for the two environments: (1) the stream food web without exotic fish (2) stream food web with exotic fish.
2. Have students draw in their notebooks the structure of the food web under both scenarios. (This will require one vertical axis with the organisms placed at associated 15N value.)
3. What is the effect of exotic fish on the structure of stream food webs?
4. Who does it appear that the exotic fish are feeding on?
5. Who would have higher 15N values: a vegan or a person who consumed meat?
6. What are some other applications of nitrogen isotopes? (e.g. diets of human ancestors).