Excerpts from: Stable Isotopes as Tools in Ecological Research

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Stable isotope analysis has proved to be an extremely useful tool in elucidating many eco- logical problems, with stable isotope ecology comprising the theme of a series of international conferences (http://www.isoecol.org). Stable isotopes can be used as biological tracers in the following ways: (1) to identify sources, for instance in determining the identity of basal carbon in a food web; (2) to distinguish sources, for example to determine whether a breeding animal is using local resources or its own reserves, or when an animal migrates/disperses from one location to another and (3) to quantify relative inputs in a system, for example determining the proportions of different prey items to a consumer's diet. When utilised carefully, stable isotope analysis provides some advantages over conventional methods and an additional device for the ecologist.

Introduction and Background

Recently, ecologists have added storeys to the theoretical foundations of applied stable isotope geochemistry, constructing a large database over the past 30 years to enable the study of the interactions among plants, animals and their environment. Following an account of isotope terminology and measurement, the natural variations of stable isotope ratios of the light elements are described, followed by some examples of how stable isotope approaches are applied to several broad ecological themes.

Isotope terminology

Isotopes of a given element differ in the number of neutrons they contain and are loosely described as 'heavy' if they are neutron-rich and 'light' if they are neutron-poor. Isotopes are divided into three types based on their stability and origin: (1) radioactive isotopes, (2) radiogenic stable isotopes and (3) nonradiogenic stable isotopes. Radioactive isotopes are those which spontaneously decay into 'daughter' isotopes. Stable isotopes are those which do not, but may form as products of radioactive decay (i.e. radiogenic stable isotopes), in which case their abundance is purely a function of time, or alternatively their abundance may be determined by stellar nucleosynthesis at the birth of the solar system (i.e. nonradiogenic stable isotopes). Nonradiogenic stable isotopes are most ecologically useful, and particularly those of the light elements: H, C, N, O and S, which are the major constituents of organic materials, as there are large relative mass differences between isotopes of the same element. This mass difference between a heavy and light isotope of a given element causes different physical and chemical behaviour during natural processes and thus changes the isotope ratio. It is this property which

makes them useful to many branches of applied science. Stable isotope ratios of heavy to light isotopes are usually measured using stable isotope mass spectrometry, which comprises a gas-source isotope ratio mass spectrometer with a peripheral instrument which prepares samples into a form (i.e. a gas) suitable for mass spectrometry.

As absolute isotope ratios (R = heavy isotope/light isotope) are difficult to quantify accurately and precisely, the isotope ratio (R_x) of a sample x is compared against that of an internationally agreed standard (R_{std}). Thus, the delta (δ) notation is used for describing isotope ratios (McKinney *et al*., 1950), where $\delta X = (R_x / R_{\text{std}} - 1)$, where X is the notation of the heavy isotope of a pair. For clarity, delta values are multiplied by a thousand to give units in ‰ (per- mil). It follows from this equation that each element has a primary international standard with a value of 0‰. Deviations towards the positive and negative imply enrichments and depletions in the heavy isotope *with respect to the standard*.

Mixing and fractionation

In terms of modelling/tracing various ecological processes, there are two themes – mixing and fractionation. Mixing is self-explanatory, and mixing of two or more components with different isotope ratios can be modelled using a mass balance approach, in an identical manner to mixing solutions of differing concentrations:

$$\delta_{\text{mix}} = \frac{\delta_1 m_1 + \delta_2 m_2 + \cdots + \delta_n m_n}{m_1 + m_2 + \cdots + m_n}$$

where $\delta_1, \delta_2, \ldots, \delta_n$ and $m_1, m_2, \ldots, m_n$ are, respectively, the delta values and mass of that element in separates 1, 2, … , n in the mix.

Isotope fractionation, which is essentially partitioning of isotopes into different phases/compounds/reservoirs in a system, results from the subtle differences in the properties of two isotopes of a given element. Two rules may be determined:

1. In kinetic reactions, light isotopes usually react faster
2. In equilibrium reactions, heavy isotopes preferentially partition into components involving strong chemical bonds.

Fractionations may be practically quantified by subtracting the delta value of a reactant from that of a product.

Natural Variations Carbon and $\delta^{13}C$

Carbon has two stable isotopes: the more common ^{12}C and the minor isotope ^{13}C, with the primary international standard being V-PDB, derived from the carbonate skeleton of a Cretaceous cephalopod (PDB). The natural $\delta^{13}C$ variations of terrestrial material span a 100‰ range, from biogenic methane and other reduced carbon compounds with very negative values (Grey, 2016), through soft animal and plant tissues, to carbonates with $\delta^{13}C$ values just into the positive portion of the $\delta^{13}C$ scale. One of the seminal discoveries in stable isotope ecology was that terrestrial plants using disparate photosynthetic pathways could be differentiated using $\delta^{13}C$ (Smith and Epstein, 1971). C3 plants generally have values of around −35‰ to −20‰, whereas C4 plants have a $\delta^{13}C$ range of about −18‰ to −7‰, with the resulting soil organic $\delta^{13}C$ reflecting the respective photosynthetic process. The disparity between the $\delta^{13}C$ of C3 and C4 plants is stark, given that the precursor for both photosynthetic pathways is atmospheric CO_2 . The difference is caused by the fact that the ribulose-1,5-bisphosphate carboxylase (Rubisco) catalyst involved in the C3 pathway strongly discriminates against $^{13}CO_2$ compared with phosphoenolpyruvate (PEP) carboxylase, the C4 catalyst. The δ^{13} C of CAM (crassulacean acid metabolism) plants overlaps with C3 and C4 plants, with a range −10‰ to −22‰ (O'Leary, 1988).

Nitrogen and δ^{15} N

Nitrogen has two isotopes, the more common ^{14}N and the minor isotope ^{15}N. By far, the largest reservoir of nitrogen above the geosphere is atmospheric nitrogen, and its uniform isotope ratio is the reason for its choice as the international standard for $\delta^{15}N$, atmospheric isotope reservoir (AIR). Nitrogen fixation involves very little nitrogen isotope fractionation, such that N- fixing plants have $\delta^{15}N$ values very close to 0‰. Non-nitrogen- fixing plants have a slightly more varying range of δ^{15} N compositions, depending on their particular source of nitrogen.

Determining diet/food webs

Although some animals' diets may readily be determined by observation alone, more elusive species may require a more invasive approach. In some animals, it may be possible to obtain regurgitated dietary material to study, whereas in others, only gut content analysis (GCA) will be possible, which is obviously a destructive technique. The main disadvantage of these conventional techniques is that they all comprise 'snapshot analyses': they only provide a measure of what an animal has been feeding on most recently. Stable isotope analysis integrates diet over a longer time period and offers an alternative approach which is often nondestructive, at least for large taxa, and in many cases, noninvasive.

It is possible to use a theoretical approach to calculate isotope fractionations for many simple chemical reactions; however, in the real, complex, biological world, an experimental/ observational approach is more feasible. Merely subtracting the original delta value from the final delta value in a particular process gives a workable approximation, Δ of the total

fractionation of the process. For instance, the long-held maxim of 'you are what you eat, plus a few ‰' (DeNiro and Epstein, 1976) describes the general fact that the $\delta^{15}N$ of a consumer is a few ‰ 'heavier' than those of its diet (**Figure 1**) and is the basis of all food-web ecology investigations using nitrogen isotopes. The effect for $\delta^{13}C$ is much less, with carbon isotope ratios reflecting more the source of carbon, such that $\delta^{15}N$–$\delta^{13}C$ plots (**Figure 1**) are a useful graphical representation of simple food webs. The arithmetic difference in the nitrogen isotope ratio of consumer and diet ($\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{diet}$, cf. **Figure 1**) has been termed the 'trophic enrichment factor' (TEF). TEFs have been calculated for many taxa, and although mean $\Delta^{15}N$ for several compilations of taxa averages around +3‰ (Minagawa and Wada, 1984; Post, 2002; Vanderklift and Ponsard, 2003; Vander Zanden and Fetzer, 2007) in agreement with the earliest work (DeNiro and Epstein, 1981), the range of $\Delta^{15}N$ is quite wide and varies with the biochemical mode of excretion and diet C:N (Vanderklift and Ponsard, 2003). In some cases, for example large cetaceans, it is impractical to measure TEFs directly, and assumptions are made regarding a probable TEF for the consumer/diet in question. It is thus possible to determine the trophic level of a consumer from a bulk stable isotope analysis if one has knowledge of the TEFs; however, this requires collection of primary production samples from the habitat under investigation, as the 'baselines' of $\delta^{13}C$ and $\delta^{15}N$ will differ from one habitat to another and can be affected by local (e.g. anthropogenic) effects.

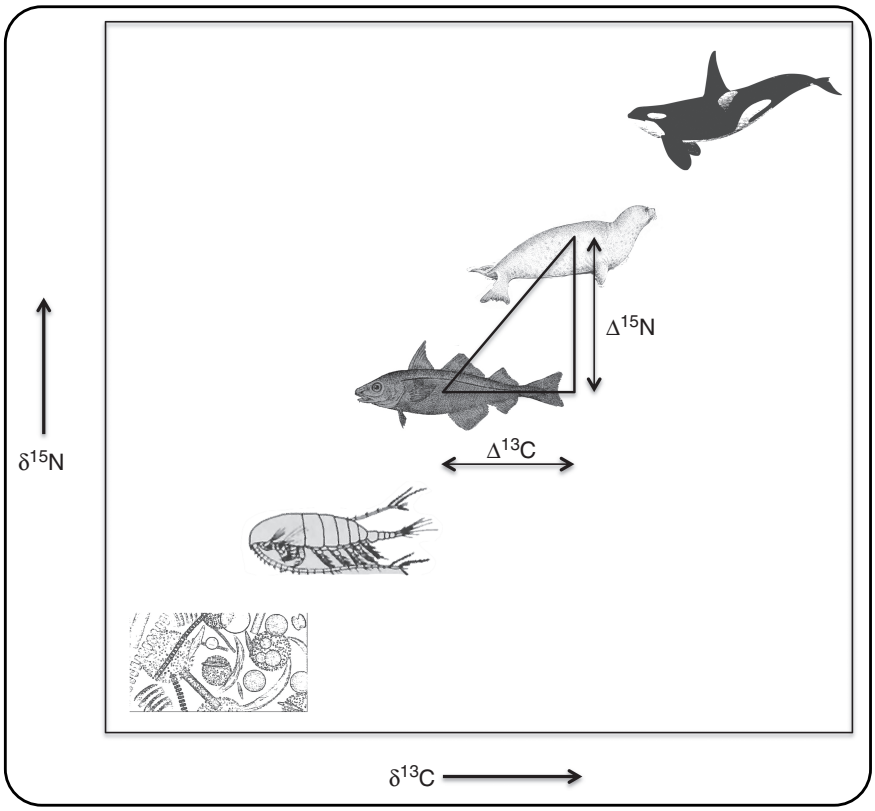

Figure 1 Schematic marine food web in which $\delta^{15}N$ (and $\delta^{13}C$) represent the trophic enrichment factors. See text for further explanation.

Diet is generally determined by stable isotope analysis of specific tissues, rather than that of the whole animal, and different tissues will partition isotopes by differing amounts, hence tissue-specific TEFs (i.e. $\delta^{15}N_{tissue} - \delta^{15}N_{diet}$) have to be taken into account. Despite these complications, $\Delta^{15}N$ has been used to make direct inferences about the diet of many consumers, and nitrogen stable isotopes have also been used to infer a variety of ecological parameters. For instance, the variance of stable isotope ratios has been used to derive a measure of trophic niche width (*sensu* Bearhop *et al*., 2004). However, this measure might better be described as 'isotopic niche width'; Yeakel *et al.* (2016) discuss the ecological interpretation of such data. The $\delta^{15}N$ value of a consumer relative to that of the base of the food web combined with the knowledge of TEFs provides a quantitative measure of the food chain length (Vander Zanden *et al*., 1999), which is not subject to the kind of errors involved in estimating the number of trophic levels based on the presence/absence of intermediate consumers.