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plea[34] for this kind of work has gone unheeded except in a few areas (e.g. rodents[35]; intertidal organisms[36]). However, field experiments carried through several generations with natural populations are essential for advancement of knowledge about the mechanisms of population change. Variance and vagueness in density relationships and stochasticity in population dynamics need more rigorous demonstration than simple deterministic relationships, and only well-replicated experiments can yield critical information about population change.

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Stable Isotopes in Physiological Ecology and Food Web Research

James R. Ehleringer, Philip W. Rundel and Kenneth A. Nagy

Mention the word 'isotopes' and most people will think of short-lived radioactive isotopes. Yet far more abundant than the radioactive forms and perhaps much more useful for ecological studies are the stable isotopes. Early interest in stable isotope analyses developed in the geological sciences, and their application in environmental biology has developed slowly until recently. Over the past few years, innovative applications of stable isotope analyses to studies of biological processes have been expanding rapidly, and there is every indication that stable isotope approaches will lead to major advances over the next decade in our understanding of physiological processes and fluxes through ecological systems. Studies of plant and animal physiological ecology and food webs will benefit greatly.

Virtually all elements of biological importance have at least two stable isotopes, although one form may be far more common than others[1]. For example, there are two stable isotopes of hydrogen, ^{1}H and ^{2}D (deuterium), which are present under natural conditions at abundances of 99.9844% and 0.0156%, respectively. Similarly, there are two stable isotopes of carbon, ^{12}C (98.89%) and ^{13}C (1.11%), and four stable isotopes of sulfur, ^{32}S (95.02%), ^{33}S (0.75%), ^{34}S(4.21%) and ^{36}S (0.02%). As a consequence of differences in physical properties and enzymatic-based discrimination, there are natural differences in the stable isotopic composition of biotic and abiotic compounds of ecological interest.

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The purpose of this review is to point out how the differences in isotopic composition, both between biotic and abiotic compounds and between biotic compounds derived from different biochemical processes, have become useful as powerful indicators of physiological activities as well as providing a valuable tool for understanding the movement of compounds and energy through ecosystems. This brief review cannot cover all of the areas of ecological research where stable isotopes are used. Many of these areas are by themselves worthy of reviews, and include marine ecology, historical ecology and biogeochemical cycling, where stable isotopes are providing information about long-term fluctuations in temperature, global CO_2 and precipitation.

Stable isotope analysis

Differences in stable isotope composition at natural abundance levels are measured using an isotope ratioing mass spectrometer. It is inconvenient to express stable isotope ratios as absolute values, especially when we are concerned

with small differences in these ratios. Therefore, isotope ratios are expressed relative to a standard. The unit of isotopic ratio measurements is the delta value (δ) and is expressed as the deviation per mil (‰) from an arbitrary standard. Thus,

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R is the absolute isotopic ratio of either the sample or standard. For the light elements of ecological interest, the standards are: H – Standard Mean Ocean Water (SMOW), C – *Belemnitella americana* from the Cretaceous Peedee Formation (PDB), N – atmospheric air, O – SMOW, and S – Triolite (FeS) from the Canyon Diablo (CD) iron meteorite[1].

Carbon isotopes in plant physiological ecology

Carbon isotope ratios were first used to distinguish the different photosynthetic pathways (C_3, C_4 and CAM) found in terrestrial plants[2,3]. It is now known that plants possessing the conventional Calvin cycle (C_3 pathway) have $\delta^{13}C$ values of −20 to −35‰, plants possessing the Hatch-Slack cycle (C_4 pathway) −7 to −15‰, and plants possessing Crassulacean acid metabolism (CAM) −10 to −22‰. Carbon isotope ratios have been widely used as a tool for investigating photosynthetic pathway changes among species associated with environmental gradients of temperature, elevation and precipitation[3–5]. From these and other studies, it became clear that there could be significant changes in the frequency of a photosynthetic pathway with changes in habitat (Fig. 1). Furthermore, other studies have revealed that some species could shift back and forth from C_3 to CAM photosynthesis as environmental conditions changed[3,6]. In yet other species, the leaves appeared to have one photosynthetic pathway (C_3), while the stems possessed another (CAM)[3].

Large variations in carbon isotope composition of the order of 3 to 5‰ can occur within a single species. While some of this variation is associated with environmental gradients such as salinity[7], temperature[8] and light[9], other studies indicate that large genetically based differences may also be common[8,10,11].

Leaf carbon isotopic analyses show great promise as a tool to understand integrated plant behavior now that the causes of variations in carbon isotope ratios of C_3, C_4 and CAM pathway plants are mostly understood[3,12,13]. The basis for discrimination against the heavier isotope in C_3 plants is associated with the primary photosynthetic carboxylation reaction (catalysed by ribulose-1,5-bisphosphate carboxylase) and by diffusion rate differences between ^{13}C and ^{12}C. The theoretical relationship between carbon isotope ratio and intercellular CO_2 concentration predicted by Farquhar *et al.*[12] has been supported by observations on a large number of plant species[9,10,11,14].

Carbon isotope ratios become more negative as intercellular CO_2 concentrations increase (Fig. 2). Since intercellular CO_2 concentration is quantitatively related to leaf water use efficiency[12] (the ratio of photosynthesis to transpiration) then carbon isotope ratios become a potential measure of plant water use patterns[10,11,12,14]. Farquhar and Richards[10] and Hubick, Farquhar and Shorter[11] have verified that carbon isotope ratios are a reliable measure of patterns of water use by whole plants and as such are useful in plant breeding programs as well as in understanding ecological systems. Ehleringer *et al.*[14] have taken one step in the application to ecological systems by using carbon isotope ratio differences to interpret variations in water use patterns between parasitic mistletoes and their hosts and the mineral nutrition factors which regulate host–parasite interactions. The applications of stable carbon isotopes to understanding leaf intercellular CO_2 levels and water use efficiency are expanding rapidly and much progress will be made in the next few years.

PEP carboxylase, the initial carboxylating enzyme in C_4 and CAM plants, appears not to discriminate against ^{13}C. Variations in the carbon isotope ratios of C_4 plants appear to be related to leakage rates of CO_2 from the bundle sheath cells. The physiological and ecological significance of these isotopic variations are at present poorly understood. In CAM plants, however, variations in isotopic composition represent the relative percentages of photosynthesis contributed by C_3 and CAM pathways and as such reveal much about the interactions between photosynthesis and drought stress[3,6]. For example, Winter *et al.*[6] have shown that *Mesembryanthemum crystallinum* will switch from C_3 to CAM modes as soils dry out and leaf water potentials decline, and will switch back to C_3 when water is re-supplied.

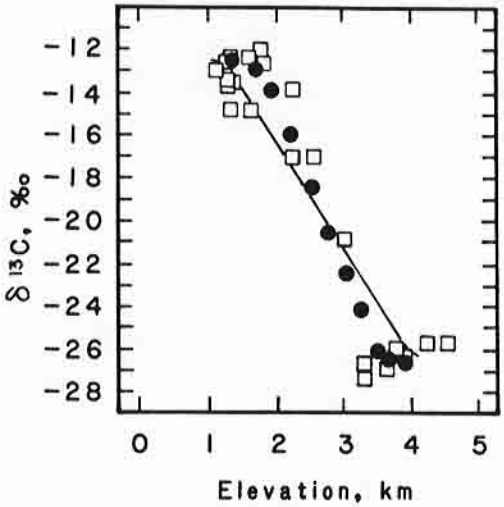

Fig. 1. Average leaf carbon isotope ratios of grassland vegetation (mixtures of C_3 and C_4 species) along an elevational transect in East Africa. r = 0.94; the line drawn is $\delta^{13}C = 6.6–0.48$ km. Redrawn from Ref. 5.

Nitrogen, hydrogen and oxygen

Symbiotic nitrogen fixation in natural ecosystems is difficult to estimate by conventional means and it is even more difficult to

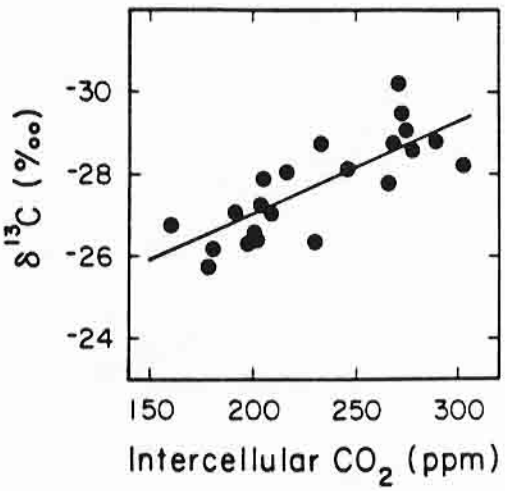

Fig. 2. Leaf carbon isotope ratios from different plant species in central Australia as a function of the average intercellular CO_2 concentration measured during the day. The regression line is $\delta^{13}C = -22.54 - 0.0226\ c_i$, $P < 0.01$. Standard error of slope is 0.0042. Redrawn from ref. 14.

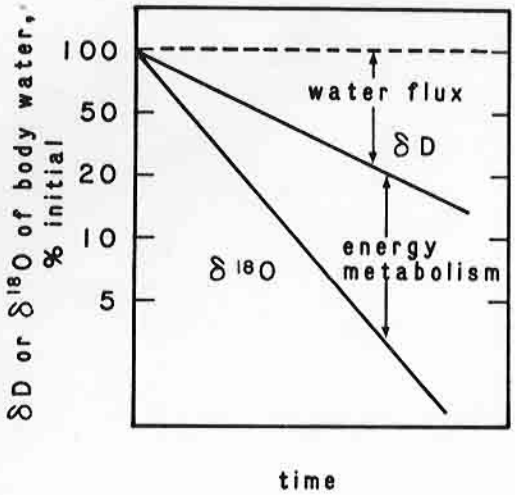

Fig. 3. Time dependence of the loss of δD and $\delta^{18}O$ from body fluids of animals injected with doubly labeled water[20,21].

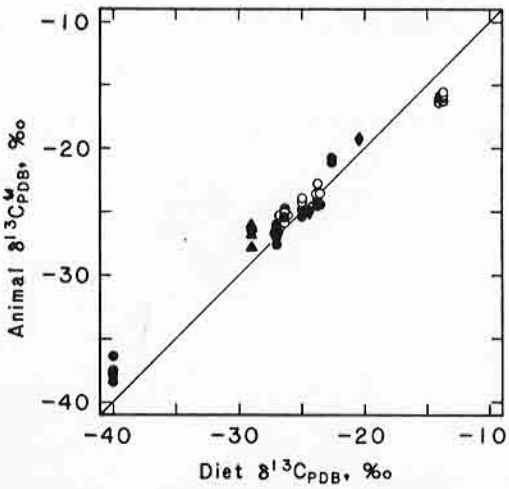

Fig. 4. $\delta^{13}C$ values of whole bodies of animals and their diets. The line represents a 1:1 slope. Redrawn from Ref. 25.

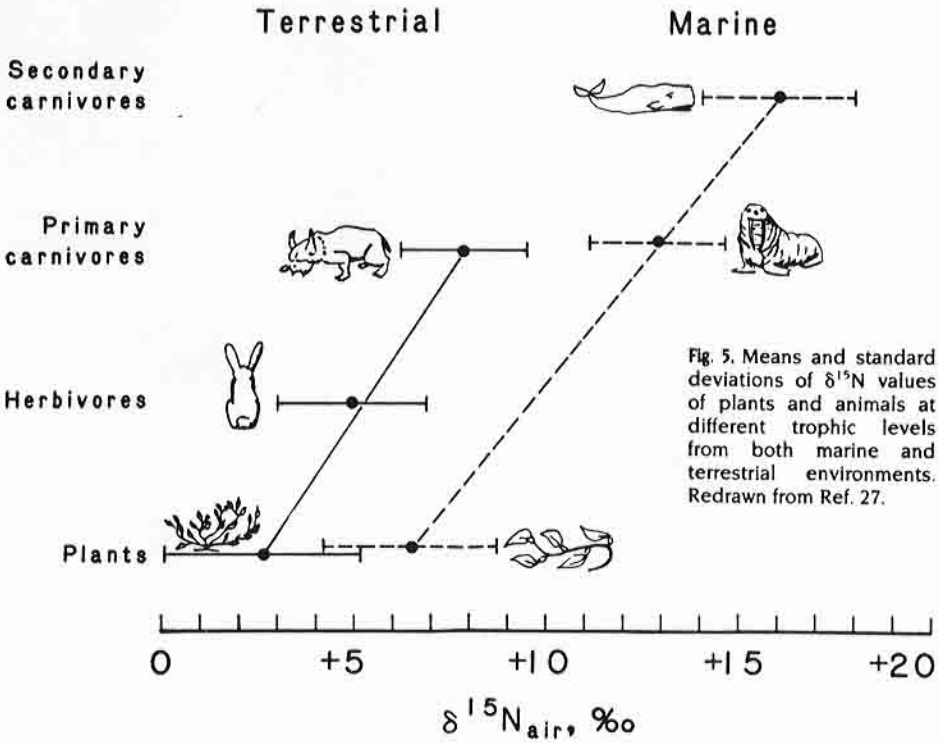

Fig. 5. Means and standard deviations of $\delta^{15}N$ values of plants and animals at different trophic levels from both marine and terrestrial environments. Redrawn from Ref. 27.

obtain long-term estimates of the contribution of fixed nitrogen to the total nitrogen content of a plant. However, stable nitrogen isotopes can provide integrated estimates of nitrogen sources for plants. This is because there are small differences between the natural abundance of ^{15}N between atmospheric N_2 and soil sources of nitrogen[15]. Soils tend to be enriched in ^{15}N (mean surface value[16] of $\delta^{15}N$ = 9.2 ‰) and the nitrogen derived from bacterial fixation processes is lighter, reflecting atmospheric N_2 values ($\delta^{15}N$ = 0‰). Thus, legumes with nitrogen-fixation capabilities have lower $\delta^{15}N$ values than species that do not have nitrogen fixation capabilities[15]. The fraction of the nitrogen in a legume derived from nitrogen fixation activity can be estimated as the ratio of the difference between the $\delta^{15}N$ of the leaf minus the $\delta^{15}N$ values expected if nitrogen were derived solely from the atmosphere ($\delta^{15}N$ = 0) divided by the difference in $\delta^{15}N$ of non-nitrogen fixing plants minus the atmospheric value. Such approaches indicate that legumes can differ widely in the proportion of nitrogen derived from soil versus nitrogen fixation sources[15].

There has been less research in physiological ecology on hydrogen and oxygen stable isotopes. Fractionation of these isotopes does occur in plants[17,18]; for instance the δD values of cellulose in CAM plants are more positive than those in C_3 and C_4 plants[18]. Recent evidence suggests that isotopic analyses of xylem sap for either element may provide a signature of the source of soil moisture which a plant is using; this approach has been used in studies of water balance in pines where the objective was to separate the uptake of ground water from recent precipitation during the growing season[19].

Animal physiological ecology

In free-living animals whose body water is experimentally enriched with doubly labeled water ($DH^{18}O$), the different wash-out rates of the oxygen and hydrogen isotopes can provide accurate measures of both water loss and energy metabolism[20,21]. After injection of the doubly labeled water, the carbonic anhydrase in animals rapidly brings ^{18}O from the water into an equilibrium with the CO_2 derived from metabolism. While D will be lost from the animal only through water loss, the ^{18}O will be washed out at a faster rate both as part of water and of CO_2 (Fig. 3). Thus the wash-out curves become measures of both water loss and metabolic activity. Current applications of such approaches include investigations of the requirements of animals for water, energy and food under native habitat conditions, and also studies of the metabolic requirements of humans. One clear advantage of this approach is that metabolic parameters can be estimated for animals that are free-ranging rather than caged.

At higher levels of biological organization, studies incorporating doubly labeled water methods to estimate metabolic costs have revealed much about the ecological energetics of populations of both reptiles and birds[22,23]. Lizards contribute more to the energy and material flow through ecosystems than previously estimated[22]. A study of penguins from southern Africa[23], in which doubly labeled water was used to determine the metabolic rates of nesting birds and to estimate their annual food requirements, revealed the scale of the pressure on the penguins' food resources from commercial fisheries. Fishermen in the region were

estimated to catch more than 12 times as many anchovies (the penguins' principal food) as the penguin population.

Given the diversity of potential applications of doubly labeled water, and the likelihood of exciting discoveries yet to be made, it is not surprising that interest in this method has increased significantly over the last several years. In the past, doubly labeled water techniques have been limited to small animals because of high isotope costs, but the increased levels of analytical precision available in new isotope ratioing mass spectrometers should allow future applications to larger animals.

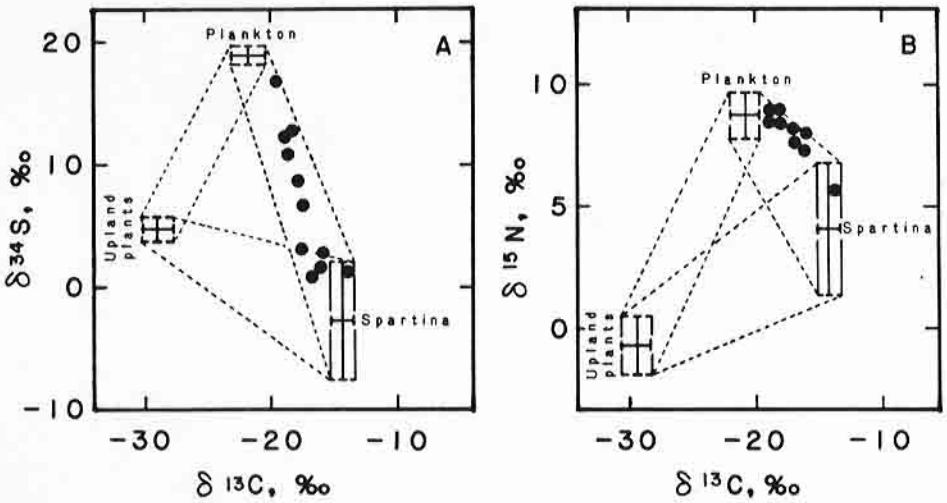

Fig. 6. Plots of multiple isotope ratios (means and standard deviations) of C, N and S for plankton, upland plants and marsh grass from a salt marsh and the stable isotope signatures of a ribbed mussel (solid dots) feeding on detritus within this ecosystem. Redrawn from Ref. 30.

Food web studies

A variety of single and multiple isotope signatures have been used to study patterns of plant–herbivore interactions and energy transfer along food chains. The majority of these have involved the use of carbon isotope ratios to investigate patterns of food selection in the diet of animals[24–29]. Since the carbon isotope ratio in animal tissues closely parallels the ratio of the food eaten (Fig. 4), diet selectivity in a habitat between foods of different isotopic composition can be assessed. DeNiro and Epstein[24,25] in documenting the influence of diet on carbon isotope ratios pointed out that 'you are what you eat (plus a few o/oo)'. Recent studies analysing vertebrate herbivore food preferences have further illustrated the utility of isotopic analyses for quantitatively determining the feeding preferences of different species over time[26,27]. In particular, the study of large herbivores in Kenyan grasslands[26] demonstrated that reliable estimates of both long and short-term feeding preferences can be obtained for large numbers of animals with limited sampling efforts.

Carbon isotope ratios have also been used to explore the nature of ancient human diets. Bone collagen provides a permanent record of the diet at the time it was laid down. Studies of carbon isotope ratios of human bone collagen have yielded direct information on the relative amounts of marine and terrestrial foods in prehistoric diets[28,29] and have been used to trace the introduction of corn among different tribes of North American Indians.

To date there has been only limited interest in the use of nitrogen isotope ratios in food chain studies. $\delta^{15}N$ values in animals reflect the composition of their diets, but are characteristically 2–4‰ more positive at each trophic level (Fig. 5). This characteristic elevation in nitrogen isotope ratios along food chains is thought to be due to isotopic fractionation associated with catabolic metabolism.

The most exciting future developments in food chain studies using stable isotope ratios will almost certainly come from multiple element studies. Organisms that are similar in isotope ratios for one element may well differ in another (Fig. 6). Using stable isotopes of C, N and S, Peterson *et al.*[30] were able to trace the flow of organic matter within a salt marsh ecosystem and to indicate clearly the detrital substances utilized by mussels downstream (Fig. 6). This approach may require sophisticated treatment of mathematical data in addition to the analytical needs, but it shows great promise for a wide variety of applications.

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