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Commentary

Aloe nectar, birds and stable isotopes: opportunities for quantifying trophic interactions

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A common issue associated with studies of animal diets and resource use is the difficulty of estimating the contribution of a dietary source to the nutrient budget of individual animals. In southern Africa, the tree-like succulent *Aloe marlothii* makes available to the animal community its abundant energy and water resources in the form of floral nectar in exchange for pollination services. The copious production of floral nectar (50–100 L/ha, yielding 100 000–200 000 kJ/ha in energy) and the observation that at least 73 species of birds have been observed visiting aloe flowers suggests that this plant may be of significant energetic (and/or osmoregulatory) importance to the bird community. In this issue, Symes *et al.* (2011) attempt to quantify these interactions using carbon stable isotopes to track aloe nectar use by the bird community. Given a suitable isoscape and a few caveats, stable isotope methods can provide direct estimates of resource use by consumers. Here, we provide an overview of the opportunities and constraints that stable isotope methods offer, using the aloe nectar/bird community system as an example. In many ecosystems, stable isotope approaches can provide powerful insights into the depth and breadth of species interactions and the movement of energy and materials through individuals and foodwebs.

In the past, estimating the contribution of a certain food source to the nutritional ecology of a particular species or community has been time and labour intensive, and has been accomplished through observations of feeding activities (e.g. Cecere *et al.* 2010), stomach sampling (e.g. Tsipoura & Burger 1999) and fecal analysis (e.g. Durst *et al.* 2008). Although these approaches provide snapshot insights into the use of specific dietary items, they do not provide quantitative information on the resources that are actually assimilated. Stable isotope analysis focuses on the nutrients assimilated by animals and relies on the old adage that 'you are what you eat', which is both isotopically and literally true. The isotopic composition of an animal's tissues generally reflects that of its diet, with some predictable offset due to biochemical and physical processes that tend to favour the lighter and more abundant isotopes of a given element. Thus, for carbon isotopes (^{13}C and ^{12}C), animals and their tissues tend to be enriched in the ^{12}C isotope compared with their dietary sources. These differences are represented using the delta notation (δ) and reported on a parts-per-thousand or per mil (‰) basis compared with a standard reference material for a given element (i.e. $\delta^{13}C$ – ‰ VPDB (Vienna Pee Dee Belemnite)). If an animal's dietary sources differ appreciably in their carbon isotope ratios ($\delta^{13}C$), then estimates of carbon incorporation can be made from measurements of tissue isotope ratios ($\delta^{13}C$).

Stable isotope approaches do not provide a magic bullet for divining the sources of nutrients flowing into any consumer. For the stable isotope approach to be useful, the dietary source or sources of interest must differ isotopically (by a few ‰ or more) from the background isotopic landscape. The Suikerbosrand Nature Reserve in South Africa, where Symes *et al.* conducted their study, is a savannah ecosystem and provides a particularly informative example because plants using all three common photosynthetic pathways occur there. How does photosynthetic pathway come into play? In arid ecosystems, plants use C_3, C_4 or CAM photosynthesis and the photosynthetic chemistry of these differing pathways leads to differences in the isotopic composition of the tissues they produce. Globally, C_3 plants represent the bulk of plant biodiversity and terrestrial biomass, accounting for approximately 95% of all species and about 70% of global plant biomass. They include most flowering trees, shrubs and annual plants found in temperate and tropical climates. Isotopically, their tissues tend to be greatly depleted compared with plants using C_4 or CAM photosynthesis (e.g. at Suikerbosrand values averaged: C_3 $\delta^{13}C = -27.2$‰ VPDB; C_4 $\delta^{13}C = -14.7$‰ VPDB and CAM $\delta^{13}C = -12.6$‰ VPDB). Globally, C_4 species account for approximately 3% of total plant biodiversity and a surprising 25% of global plant biomass. C_4 plants are dominated by warm season tropical and sub-tropical grasses and are greatly enriched isotopically compared with C_3 plants (see above). The third photosynthetic pathway type found on the Suikerbosrand reserve are the CAM plants (crassulassian acid metabolism), which include *A. marlothii* (Asphodelaceae) and represent a diverse group (26 families), many of which are succulents. Well-known families include the Cactaceae, Crassulaceae, Euphorbiaceae, Orchidaceae,

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Bromeliaceae and Liliaceae. CAM plants usually grow where water is in short supply and frequently occupy deserts, semi-arid regions, salt marshes and epiphytic sites. Isotopically, their tissues are similar to those of C_4 plants (see above).

The Suikerbosrand isoscape thus provides both opportunities and challenges. For obligate and opportunistic nectarivores, the nectar resource landscape is made up of floral nectar produced by C_3 plants and nectar from *A. marlothii*. Isotopically, the resources are separated by 14.7‰ (machine accuracy is typically better than 0.3‰ for measurements of carbon), and this large difference provides for robust estimates of the contribution of aloe nectar to the nutrient budgets of avian consumers using a simple mixing model (see Martínez del Río & Wolf 2005 for an overview of mixing models and their use). For the granivorous birds that are also opportunistic nectarivores, *Symes et al.* note that the isoscape is more complex. Both C_3 plants and C_4 grasses produce seeds eaten by granivores and the C_4 plant $\delta^{13}C$ values are close to the values for *A. marlothii* nectar. As a consequence of these overlapping values, there is a potential for the C_4 grass signal to mask the use of aloe nectar. In addition, insectivorous birds may eat insects that have been feeding on C_4 plant material, which would also mask the use of aloe nectar. How did Symes *et al.* deal with these specific challenges? Because different animal tissues have differing carbon turnover rates, sampling specific tissues or materials allows for estimates of resource use that encompass different temporal windows. Using this approach the authors were able to show that birds used the carbon from *A. marlothii* nectar over different time scales. The $\delta^{13}C$ of feathers, which are grown and replaced when aloe nectar is not available, established a dietary baseline free from the $\delta^{13}C$ signal of nectar. This contrasted with the $\delta^{13}C$ of exhaled CO_2, which represents the fuel that animal is actually metabolizing at that time (Hatch *et al.* 2002, Bauchinger & McWilliams 2009) and was found to be essentially identical to the $\delta^{13}C$ of aloe nectar for African Red-eyed Bulbuls *Pycnonotus nigricans* and Cape White-eyes *Zosterops capensis*. The values for whole blood show the inclusion of nectar in the diet of these two species may have started a few days to a week or more before the birds were sampled (Podlesak *et al.* 2005, Voigt *et al.* 2008). Thus, the three different samples taken during a single capture event provided evidence that these two species were not feeding on nectar during the moulting season and were dependent primarily on aloe nectar to fuel metabolism at the time of capture. Furthermore, the transition to relying upon nectar must have occurred within a few days or weeks of capture. This approach (sampling multiple tissues or pools) when combined with sampling each species using a time series that encompasses the period before aloe nectar was available provides very strong evidence of a facultative shift to aloe nectar in species where blood and breath CO_2 $\delta^{13}C$ values advanced towards the aloe nectar $\delta^{13}C$ values.

Symes *et al.* also discuss isotope routing and the problems associated with determining how different nutrients are routed or directed to different tissues after they are assimilated. The inability of the authors to determine whether tissue carbon turnover rates, nutrient routing, or a combination of the two, was responsible for the $\delta^{13}C$ values observed in the whole blood of African Red-eyed Bulbuls and Cape White-eyes demonstrates the importance of sampling carbon from multiple pools. Had the authors analysed the $\delta^{13}C$, the red blood cells and the plasma portion of the blood separately, they might have been able to determine whether nectar carbon was being routed primarily for energy production and not protein synthesis, or whether the lack of a nectar signal in the blood was due to the slow turnover of red blood cells. Blood plasma turns over rapidly, on the order of hours to days for passerines (Pearson *et al.* 2003, Bauchinger & McWilliams 2009), and a lack of a nectar signal in blood plasma carbon would strongly suggest that nectar carbon is routed towards metabolism and not protein synthesis. By contrast, if the blood plasma were found to have an isotopic signal similar to that of the nectar, this would strongly suggest that nectar carbons are also used in protein synthesis. It might also suggest that the inclusion of nectar in the diet was too recent for much aloe nectar carbon to have been used in the synthesis of red blood cells, which have a turnover rate on the order of days to weeks in passerines (Pearson *et al.* 2003, Bauchinger & McWilliams 2009). Thus, while sampling from some pools can provide information on time scale and sampling from others can provide information on nutrient routing, sampling from the right combination of pools can also help distinguish between these two potentially confounding factors.

Symes *et al.* illustrate many of the strengths and weaknesses of stable isotope approaches. Many of the weaknesses can be overcome by including other strategies of identifying and quantifying nutrient transfer. Expert knowledge of the natural history of the system is important and stable isotope methods are not a substitute for this insight. Another obvious answer is to include traditional methods such as observation and stomach sampling. Isotopic sampling of multiple pools also helps. Symes *et al.* are able to make a convincing argument that the increase in $\delta^{13}C$ that they observed in the breath and tissues of the species they studied was due to the addition of aloe nectar to the diet because (1) behavioural observations supported this conclusion (Symes *et al.* 2008), (2) they had a time series of isotopic samples before and during the period of flowering, and (3) they had measured the isotopic value of multiple pools. In addition to sampling from multiple pools, analysing samples for more than one stable isotope can often aid in quantifying nutrient transfer. In this system, as the

authors suggest, we expect aloe nectar water to be enriched in ^{2}H (deuterium or D) and ^{18}O, and measuring of the isotope ratios (δD and $\delta^{18}O$) of nectar water and body water contained in the plasma would have potentially provided additional tracers for estimating aloe nectar use (Wolf *et al.* 2002, McKechnie *et al.* 2004, Rosenstock & Wolf 2010). Enriched δD values in body water accompanied by enriched $\delta^{13}C$ values in blood cells would have provided unambiguous evidence of nectar use and, at the same time, provided insight into the contribution of aloe nectar to the water balance of the bird community.

Symes *et al.* provide an elegant starting point for examining the functional importance of nectar to a community of consumers and further research may indicate that *A. marlothii* is a 'keystone' resource in this ecosystem. If we use a simple mixing model, the $\delta^{13}C$ of blood samples from the 11 most common species of opportunistic nectarivores suggests that approximately 25% of their incorporated carbon was derived from *A. marlothii* nectar. Although breath sampling was limited to only a few species, the observation that the $\delta^{13}C$ of exhaled CO_2 was virtually identical to the $\delta^{13}C$ of aloe nectar strongly suggests that, during the flowering period of *A. marlothii*, Cape White-eyes and African Red-eyed Bulbuls rely almost exclusively on nectar for metabolizable energy. Newer technologies with enhanced capabilities including field portability (Isotope ratio CO_2 analysers by Campbell Scientific, Picarro, and Los Gatos Research) can make real-time measurements of breath CO_2 $\delta^{13}C$ and $\delta^{18}O$ in animals as small as grasshoppers, honey bees and ants (Engel *et al.* 2009), and lower per sample costs (US$0.5) suggest that studies of this type will become increasingly important. Because stable isotope methods trace atoms through organisms, this approach provides insights into processes at the physiological, individual, population, community and ecosystem scales, as is nicely demonstrated by this study.

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