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The nutritional significance of a winter-flowering succulent for opportunistic avian nectarivores

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The winter-flowering succulent *Aloe marlothii* provides nectar for many opportunistic avian nectarivores in southern African savannas. We assessed the importance of *A. marlothii* nectar sugar for opportunistic nectarivores by analysing temporal changes in stable carbon isotope ratios ($\delta^{13}C$) in the tissues of birds in Suikerbosrand Nature Reserve, South Africa. The blood of the 11 most common non-granivorous opportunistic nectarivores at our site was enriched in ^{13}C by 3.4 ± 1.5‰ during the flowering period of *A. marlothii*, reflecting the enriched crassulacean acid metabolism (CAM) isotopic signature of nectar (−12.6 ± 0.5‰). This relatively small contribution of *A. marlothii* nectar to assimilated carbon in whole blood contrasted with that of exhaled CO_2 in African Red-eyed Bulbuls *Pycnonotus nigricans* and Cape White-eyes *Zosterops capensis*. In both these species, the $\delta^{13}C$ of breath samples was significantly enriched compared with blood and feathers, and closely resembled that of the nectar, revealing combustion of ingested nectar rather than assimilation. Although our analysis was complicated by the presence of C_4 grasses, whose $\delta^{13}C$ values are similar to those of CAM photosynthesizers, when considered with previously published feeding observations our data reveal that opportunistic nectarivores feeding on *A. marlothii* nectar obtain a relatively small fraction of their assimilated carbon, but most of their metabolized carbon, from this seasonally available carbohydrate food resource. Because the $\delta^{13}C$ values of insects associated with C_3 plants also became enriched during the flowering season, some insect-eating opportunistic nectarivores may have assimilated *A. marlothii* carbon indirectly from insects. This study highlights the importance of understanding isotopic routing when assessing the nutritional significance of specific dietary items to consumer communities.

Keywords: *Aloe marlothii*, bulbul, isotope routing, stable isotope, succulent, sunbird, $\delta^{13}C$, $\delta^{15}N$.

Many plants produce large quantities of nectar and/or fruit to attract pollinators or seed dispersers (Herrera & Pellmyr 2002). In dry ecosystems, succulent plants that store water in their tissues are often able to produce nectar and or fruit during dry parts of annual cycles when few other such resources are available to consumers. These energy resources can have major landscape-scale consequences for consumers, and in some cases these plants function as keystone species, being critical for survival and reproduction in many resident species (Wolf & Martínez del Rio 2000, 2003), as well as for consumers migrating through dry desert areas (Fleming 1992, Fleming *et al.* 1993, 1996).

Most ecological studies of avian nectarivory have focused on the major nectarivorous families that have evolved independently in the Neotropical, Afrotropical and Australasian regions, namely the hummingbirds (Trochilidae), sunbirds (Nectariniidae) and honeyeaters (Meliphagidae), respectively (Maclean 1990, Gartrell 2000,

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Nicolson & Fleming 2003). In contrast, relatively few studies have investigated the interactions between flowering plants and opportunistic nectarivores, species that usually feed on other food resources but switch to partly nectarivorous diets during periods of high nectar availability (Symes *et al.* 2008). In Afrotropical savannas, for instance, many species of *Aloe* (Asphodelaceae) produce copious amounts of nectar during cool, dry winters in summer-rainfall regions, with diverse avian consumer communities taking advantage of these nectar resources (Oatley 1964, Skead 1967, Reynolds 1969, Oatley & Skead 1972, Pettet 1977, Botes *et al.* 2008, 2009, Symes *et al.* 2008, Forbes *et al.* 2009). For many birds in these habitats, the incorporation of nectar into their diet seasonally supplements their typical diets of insects, fruit and/or seeds, and a switch to nectar-feeding may coincide with a seasonal shortage of their usual food (Symes *et al.* 2008).

Aloe marlothii is widespread in mesic southern African savannas where it occurs in stands of up to several thousand individual plants (Reynolds 1969, Bredenkamp & Van Vuuren 1987, Glen & Hardy 2000, Van Wyk & Smith 2005). It favours dry, rocky north-facing slopes and produces large numbers of flowers during the dry austral winter months (June–September) (Reynolds 1969, Glen & Hardy 2000, Van Wyk & Smith 2005). Flowering is highly synchronous between individuals, although not all plants flower in each year (Symes & Nicolson 2008). Flowers produce large quantities (approximately 250 μL per flower) of dilute nectar (approximately 12% w/w) on large, yellow–orange inflorescences that attract a diverse suite of avian consumers (Symes & Nicolson 2008, Symes *et al.* 2009). At least 77 bird species representing 26 families, including granivores, frugivores, insectivores and omnivores, have been recorded feeding on the nectar of *A. marlothii* (Oatley 1964, Oatley & Skead 1972, Botes *et al.* 2008, Symes *et al.* 2008, Symes 2010). At Suikerbosrand Nature Reserve, avian abundance and diversity increase significantly during the flowering period, with *c.* 46% of species recorded during transects regularly observed feeding on nectar (Symes *et al.* 2008).

Despite the well-documented association between *A. marlothii* and occasional nectarivore communities, the significance of this suite of animal–plant interactions for avian nutrition and energy balance has not been quantitatively investigated. As *A. marlothii* is a crassulacean acid metabolism (CAM) photosynthesizer (Denius & Homan 1972, Kluge *et al.* 1979, Eller *et al.* 1993), with $\delta^{13}C$ values distinct from those of C_3 plants (Vogel *et al.* 1978, J. Vogel unpubl. data), we used an isotopic approach to document avian dietary shifts that coincided with feeding on *A. marlothii* nectar. Because of the overlap in $\delta^{13}C$ values between *A. marlothii* and C_4 grasses, we focused on non-granivorous bird species whose diets are at least predominantly C_3-based, and which were recorded feeding regularly on *A. marlothii* nectar (Symes *et al.* 2008). We hypothesized that feeding on *A. marlothii* by opportunistic avian nectarivores is associated with significant incorporation of carbon from this source into the tissues of avian consumers, and expected that the onset of flowering in *A. marlothii* would coincide with a shift in the $\delta^{13}C$ of consumer tissues towards the enriched values associated with CAM metabolism.

A complication in the assessment of the importance of nectar carbohydrates is that they consist largely of rapidly metabolizable hexose sugars (Van Wyk *et al.* 1993). Thus, carbon ingested during nectar feeding is not necessarily incorporated into tissues, but instead can be routed directly to catabolic pathways, in which case the isotopic signature of nectar is evident immediately in exhaled CO_2 (Carleton *et al.* 2004, 2006, Voigt *et al.* 2008a, 2008b). Because of the potential for such isotopic routing in birds feeding on *A. marlothii* nectar, we investigated tissues with differing turnover rates, namely feathers, blood and breath, in a subset of species to examine in more detail the metabolic fate of ingested nectar.

METHODS

Study site

This study was conducted during 2005–2007 in Suikerbosrand Nature Reserve (SNR), a 19 779-ha reserve 60 km southeast of Johannesburg, South Africa, with sampling spanning the months before (May–July), during (August–September) and after (October) flowering of *A. marlothii*. Fieldwork took place in an *A. marlothii* forest consisting of several thousand plants in the western part of the reserve (26°31′S, 28°10′E; 1600–1700 m asl). Vegetation in SNR is dominated by grassland and savanna biomes, with *A. marlothii* growing predominantly on rocky north-facing slopes. Rainfall is highly seasonal, falling mainly during summer

months (October–March), and winters are dry with circadian variation in air temperature of *c.* −5 to 25 °C. Another *Aloe* species, *Aloe greatheadii* var. *davyana*, occurring in the reserve, flowers earlier than *A. marlothii* and is visited less frequently by birds (Symes *et al.* 2009). Full descriptions of the flowering phenology of *A. marlothii* and the occurrence of opportunistic avian nectarivory at this site are provided by Symes and Nicolson (2008) and Symes *et al.* (2008), respectively.

Sampling of avian dietary items

As in most of South Africa, trees and shrubs in Suikerbosrand typically use C_3 photosynthesis, whereas over 90% of grass cover consists of C_4 species (Vogel *et al.* 1978). During winter 2006, representative C_3 and C_4 vegetation samples, as well as insects associated with each vegetation type, were collected each month from May to September. Grass and insect samples were collected at five sites in the aloe forest with 10 sweeps of a handheld net per sample. Leaf samples were collected from five common tree species (*Acacia karroo*, *Ziziphus mucronata*, *Tarconanthus camphoratus*, *Gymnosporia heterophylla*, *Rhus leptodictya*) and an insect net (diameter = 42 cm) placed beneath each tree was used to catch invertebrates shaken from the tree (10 shakes per tree). Sample collection occurred during mid-afternoon (14:00–16:00 h). The invertebrates collected (henceforth referred to as C_3 and C_4 insects) were stored in ethanol (75%) and plant samples were placed in labelled envelopes. Samples were transported to the University of Pretoria, where they were oven-dried at 50 °C to constant mass and fine-ground using a mortar and pestle in preparation for isotope analysis. *A. marlothii* nectar was sampled during peak flowering in August 2006, with approximately 2 mL of nectar collected from flowers of nine individual plants, using disposable haematocrit tubes (75 μL). Two additional nectar samples were collected during 2005. In the laboratory, the nectar samples were oven-dried at 50 °C to constant mass.

Blood sampling

We caught birds in mist-nets during May–October for 3 years (2005–2007) in the western portion of the aloe forest. A disused vehicle track through a large stand of *A. marlothii* facilitated the erection of nets in areas where aloes were abundant, and where birds were most active and observed feeding on aloe nectar. The majority of sampling took place during 2006, with additional samples collected during 2005 as part of a pilot study, and further limited sampling during August 2007. All captured birds were ringed with SAFRING rings (University of Cape Town) to prevent the sampling of recaptured birds. A blood sample (10–50 μL) was collected from each bird using a 27-gauge needle to prick the brachial vein and a 75-μL non-heparinized haematocrit tube to collect blood. The blood samples were then transported to a laboratory and dried to constant mass in the tube at 50 °C in a drying oven (Hobson & Clark 1993).

Breath sampling

Breath samples were collected over 4 days (from first light at *c.* 06:00 h) during peak flowering of *A. marlothii* in August 2007. Blood and feather (rectrix) samples were also collected from all birds for which breath samples were obtained. Feathers were cleaned in a mix of 2 : 1 chloroform/methanol (Mizutani *et al.* 1992) and dried. Sections of feather were then cut and weighed (0.100–0.350 mg) into tin cups for isotopic analysis (see below). Breath samples were collected using a sealed chamber with an empty volume of 205 mL. The chamber volume was reduced by up to 60% by the presence of a bird in the chamber. The chamber was constructed using two modified ground Pyrex glass cone and socket parts (B45) with a 2-mm Teflon stopcock to control gas flow at each end. One end was connected to a carbon-free gas supply (78–80% nitrogen, 20–22% oxygen; maximum impurities = 0.5 p.p.m. CO_2, 0.5 p.p.m. CO, 3 p.p.m. H_2O, 0.5 p.p.m. total hydrocarbons as CH_4; Afrox® Instrument Grade Zero, Johannesburg, South Africa). The bird was placed in the chamber, as soon after capture as possible, for approximately 60 s with a steady flow (100–200 mL/min) of gas through the chamber, to clear it of any residual atmospheric carbon gases (i.e. CO and CO_2). The chamber was then sealed for 40–60 s to accumulate exhaled CO_2 from the bird. The time allowed for CO_2 build-up in the chamber was also sufficient to minimize the effect of any possible atmospheric CO_2 contamination (C. Martínez del Rio pers. comm.). The bird's condition was continually monitored through the glass chamber. A breath sample was then collected by

displacement in a 10-mL borosilicate gas-tight glass Exetainer® vial (Labco Ltd, High Wycombe, UK), by continuing the gas supply flow for another 50–60 s. The sample entered the Exetainer® via a needle pierced through the airtight seal, displacing CO_2-free gas that escaped through a second needle open to the atmosphere. The breath samples were labelled and returned to the isotope laboratory for analysis within 2 days of sampling.

Isotopic analyses

The stable carbon isotope ratios ($\delta^{13}C$) of all materials collected were measured at the Natural Resources and the Environment isotope laboratory at the Council for Scientific and Industrial Research (CSIR), Pretoria. Representative blood, feather and plant samples (0.150–0.300 mg) were weighed in tin cups (pre-cleaned in toluene) and combusted at 1020 °C in a Flash Elemental Analyzer (1112 Series, Thermo™; Thermo Fisher Scientific, Bremen, Germany). The $^{13}C/^{12}C$ isotope ratios were then determined using a Delta V Plus continuous-flow isotope ratio mass spectrometer (CFIRMS) (Thermo Finnigan, Bremen, Germany), plumbed in-line with the elemental analyser by means of a Conflo III device (Thermo™). Two aliquots of a laboratory standard (homogenized dried chicken blood; mean $\delta^{13}C \pm sd = -17.87 \pm 0.15‰$; $n = 331$) were used for every six unknowns in sequence, with duplicates run for each sample (to correct for equipment drift). The laboratory standard was standardized against C652 ANU sucrose, 1577b bovine liver (National Institute of Standards and Technology) and SRM 1547 peach leaves (NIST). Isotope ratios are expressed in δ notation in permil (‰) relative to Vienna Pee Dee Belemnite (VPDB).

The breath samples were placed on a GC PAL gas bench connected to the CFIRMS where the $^{13}C/^{12}C$ ratio was determined. A laboratory gas standard (CO_2; mean $\delta^{13}C \pm sd = -31.41 \pm 0.22‰$, $n = 8$) was used for every five unknowns in sequence. Water was removed from breath samples in-line.

Data analysis

Two key limitations in many isotopic studies of consumer communities, including ours, are (i) the lack of species-specific tissue–diet discrimination factors, and (ii) unknown extents of metabolic routing for different diet components. Because our study dealt with a wide range of species differing in their diets, and because the similarity in the $\delta^{13}C$ values of *A. marlothii* and C_4 grasses at the study site precluded the unambiguous distinction between carbon obtained from each of these sources, we were cautious about using mixing models to estimate the proportion of nectar carbon assimilated by birds (Phillips & Gregg 2003).

In light of the limitations discussed above, and the small sample sizes for many species, we restricted our interpretation of isotopic data to the assessment of temporal changes in $\delta^{13}C$, using Mann–Whitney *U*-tests to compare isotopic values between non-flowering and flowering periods. Data for most species were not normally distributed (Shapiro–Wilk *W*-test for normality). For comparing $\delta^{13}C$ among tissues, we used repeated-measures analyses of variance (RM-ANOVA), after testing for normality using the Shapiro–Wilk *W*-test. A Kruskal–Wallis test was used to compare $\delta^{13}C$ values among different feeding guilds. All statistical analyses were conducted using STATISTICA 6.0 (1984–2004). Unless otherwise stated, values are presented as mean ± 1 sd.

The mean $\delta^{13}C$ values for blood samples during the pre-flowering and flowering period were calculated for each species. The change between these periods was then calculated as the difference of these two means.

RESULTS

Dietary items

The $\delta^{13}C$ of *A. marlothii* nectar averaged $-12.6 \pm 0.5‰$ VPDB ($n = 9$), and was isotopically distinct from the C_3 plants we sampled (pooled C_3 $\delta^{13}C = -27.2 \pm 1.4‰$ VPDB, $n = 30$). The C_4 grasses we sampled were slightly, but significantly, depleted in ^{13}C relative to nectar, with pooled C_4 $\delta^{13}C = -14.7 \pm 2.5‰$ VPDB ($n = 30$; Mann–Whitney, $U = 37.0$, $P = 0.001$). Although the $\delta^{13}C$ values of C_3 plants did not differ significantly between the pre-flowering and flowering periods (Mann–Whitney $U = 52.0$, $P = 0.202$), the corresponding values for insects became significantly enriched during the flowering season (Mann–Whitney $U = 21.0$, $P = 0.02$; Table 1). C_4 plants were significantly enriched in ^{13}C during the flowering period (Mann–Whitney $U = 33.0$, $P = 0.020$; Table 1),

Table 1. Monthly $\delta^{13}C$ values (mean ± sd) of C_3 and C_4 vegetation, and of insects collected in each vegetation type, at our study site in Suikerbosrand Nature Reserve during pre-flowering (May–July), flowering (August–September) and post-flowering months in 2006. Monthly sample $n = 5$, unless indicated in parentheses.

Month	C_3 plant	Total	C_4 grass	Total	C_3 insects	Total	C_4 insects	Total
May	−27.5 ± 1.6	−27.8 ± 1.2	−13.5 ± 0.4	−15.7 ± 2.9	−23.8 ± 1.7	−23.0 ± 3.1	−17.7 ± 4.4	−17.0 ± 4.5
Jun	−27.9 ± 1.2		−14.9 ± 2.5		−22.5 ± 3.7		−18.2 ± 5.9	
Jul	−28.1 ± 1.0		−18.8 ± 1.9		−22.5 ± 4.8 (3)		−15.1 ± 3.0	
Aug	−26.7 ± 1.6	−26.9 ± 1.5	−12.6 ± 0.5	−13.4 ± 1.2	−18.9 ± 3.8 (4)	−19.5 ± 3.8	−15.8 ± 3.7	−17.5 ± 4.9
Sep	−27.0 ± 1.6		−14.3 ± 1.1		−20.1 ± 4.3 (4)		−19.2 ± 5.8	
Oct	−26.1 ± 1.2		−14.3 ± 1.9		−19.2 ± 6.1 (4)		−17.4 ± 2.1	
Total	−27.2 ± 1.4		−14.7 ± 2.5		−21.3 ± 4.2		−17.2 ± 4.2	

although this may be an artefact of sampling technique that may have included C_3 plants during sweeping. In contrast, $\delta^{13}C$ values for C_4 insects did not change between seasons (Mann–Whitney $U = 71.1$, $P = 0.82$; Table 1). Insects collected from C_3 and C_4 vegetation had pooled $\delta^{13}C$ values of −21.3 ± 4.2 and −17.2 ± 4.2‰ VPDB, respectively ($n = 25$ and 30, respectively), revealing that neither group obtained carbon exclusively from the vegetation type in which they were sampled (Table 1).

Incorporation of nectar carbon into avian blood

We obtained blood samples from 402 birds representing 41 species during 2006, and a further 56 samples from 19 species during 2005. Our blood sampling included 32 of the 38 species recorded feeding on *A. marlothii* nectar at this site (Symes *et al.* 2008). Many species exhibited a significant enrichment in blood $\delta^{13}C$ during the *A. marlothii* flowering period (Table 2, Fig. 1A–E), but exclusively granivorous species did not (Fig. 1F). For the 11 most common opportunistic nectarivores, blood $\delta^{13}C$ values were enriched by 3.4 ± 1.5‰ during the flowering period compared with the pre-flowering period. This enrichment was similar for the three feeding guilds that may each have had different intakes of nectar and insects, namely frugivores (which may occasionally feed on insects), insectivores and omnivores (Kruskal–Wallis $H_{2,11} = 0.77$, $P = 0.68$).

$\delta^{13}C$ of feathers, blood and breath

We obtained feather, blood and breath samples from four or more individuals for only three species, namely African Red-eyed Bulbul *Pycnonotus nigricans* ($n = 30$), Cape White-eye *Zosterops capensis* ($n = 4$) and Southern Masked-Weaver *Ploceus velatus* ($n = 6$). In the bulbuls and white-eyes, $\delta^{13}C$ varied significantly among these tissues (RM-ANOVA: bulbuls, $F_{2,55} = 146.80$, $P < 0.001$; white-eyes, $F_{2,6} = 83.19$, $P < 0.001$) with the $\delta^{13}C$ of exhaled CO_2 being significantly enriched compared with blood and feathers (Figs 2 and 3). In contrast, the $\delta^{13}C$ values of the three tissues in Southern Masked-Weavers did not vary significantly (RM-ANOVA: $F_{2,10} = 1.78$, $P = 0.217$; Fig. 2). In African Red-eyed Bulbuls, exhaled CO_2 was enriched in ^{13}C relative to blood throughout the day and neither breath $\delta^{13}C$ (Pearson's $r = 0.031$; $P > 0.05$) nor blood $\delta^{13}C$ (Pearson's $r = -0.214$; $P > 0.05$) was correlated with time of day (Fig. 3). We did not expect a correlation for blood because of the slow turnover of stable isotopes in this tissue. Because carbon stable isotopes in breath represent immediately metabolized energy, we expected values to be more depleted in the morning, particularly if values for blood did not represent a significant proportion of nectar in the diet. However, sampling began at first light (*c.* 06:00 h), so it is possible that birds had already fed on nectar by the time they were captured and sampled.

DISCUSSION

The availability of *A. marlothii* nectar at our study site coincided with small but significant shifts towards enriched blood $\delta^{13}C$ values in most of the non-granivorous species from which we obtained data, indicating a greater proportion of assimilated carbon derived from C_4/CAM sources than during the pre-flowering period. Our data do not prove

Table 2. Mean blood $\delta^{13}C$ in selected bird species captured at the *Aloe marlothii* forest in Suikerbosrand Nature Reserve during pre-flowering (May–July) and flowering months (July–August). Mann–Whitney U-test; *U* and *P* values given where appropriate. Significant differences ($P < 0.05$) between pre-flowering and flowering months are highlighted in bold.

Species	Guild	$\delta^{13}C$ (‰ VPDB)		*U*	*P*
		Pre-flowering	Flowering		
Indicatoridae					
Lesser Honeyguide *Indicator minor*[a]	Ins	−21.2 ± 0.9 (3)	−21.5 (2)	–	–
Lybiidae					
Acacia Pied Barbet *Tricholaema leucomelas*	Fr	−22.7 ± 0.3 (6)	−19.7 (2)	–	–
Black-collared Barbet *Lybius torquatus*	Fr	−22.4 (2)	−19.3 ± 1.7 (4)	–	–
Coliidae					
Red-faced Mousebird *Urocolius indicus*	Fr	−24.6 (2)	−22.4 ± 0.7 (13)	–	–
Columbidae					
Laughing Dove *Streptopelia senegalensis*[a]	Gr	−15.1 ± 2.7 (30)	−12.8 ± 1.6 (13)	98.0	**0.01**
Malaconotidae					
Brown-crowned Tchagra *Tchagra australis*[a]	Ins	−20.9 (2)	−20.5 (2)	–	–
Pycnonotidae					
African Red-eyed Bulbul *Pycnonotus nigricans*	Om	−24.0 ± 0.5 (6)	−19.5 ± 1.9 (31)	2.0	**<0.001**
Sylviidae					
Chestnut-vented Tit-Babbler *Parisoma subcaeruleum*	Ins	−24.4 ± 0.9 (6)	−19.0 ± 1.2 (3)	0.0	**0.02**
Zosteropidae					
Cape White-eye *Zosterops capensis*	Om	−25.2 ± 0.5 (3)	−21.3 ± 1.4 (13)	0.0	**0.009**
Cisticolidae					
Black-chested Prinia *Prinia flavicans*	Ins	−23.8 ± 1.2 (10)	−21.0 ± 2.0 (4)	4.0	**0.02**
Bar-throated Apalis *Apalis thoracica*	Ins	−22.9 ± 1.7 (6)	−16.8 ± 0.9 (5)	0.0	**0.006**
Muscicapidae					
Fiscal Flycatcher *Sigelus silens*	Ins	−19.4 ± 1.3 (15)	−17.3 ± 1.2 (14)	25.0	**<0.001**
Cape Robin-Chat *Cossypha caffra*	Ins	−20.7 ± 1.3 (13)	−19.5 ± 1.4 (15)	48.0	**0.02**
Ploceidae					
Southern Masked-Weaver *Ploceus velatus*	Om	−17.5 ± 3. 4 (11)	−14.6 ± 2.7 (8)	26.0	0.14
Estrildidae					
Black-faced Waxbill *Estrilda erythronotos*	Gr	−13.0 ± 0.5 (4)	−15.3 ± 2.7 (3)	3.0	0.29
Violet-eared Waxbill *Granatina granatina*	Gr	−12.3 ± 0.4 (7)	−12.7 ± 0.7 (3)	6.0	0.31
Green-winged Pytilia *Pytilia melba*	Gr	−12.0 ± 0.4 (48)	−12.3 ± 1.1 (17)	401.0	0.92
Jameson's Firefinch *Lagonosticta rhodopareia*	Gr	−11.4 ± 0.2 (4)	−11.7 ± 0.3 (4)	4.0	0.25
Passeridae					
Southern Grey-headed Sparrow *Passer diffusus*	Gr	−12.3 (2)	−13.7 ± 0.9 (3)	–	–
Fringillidae					
Cape Bunting *Emberiza capensis*	Gr	−14.3 ± 1.1 (5)	−17.9 (2)	–	–

Major feeding guilds: Ins, insectivore; Fr, frugivore; Gr, granivore; Om, omnivore (data from Hockey *et al.* 2005). Where statistics values are missing, we were unable to conduct tests because of small sample sizes.
[a]Indicates species not recorded feeding on nectar. Sample sizes given in parentheses.

that the enrichment of avian blood $\delta^{13}C$ was a direct result of feeding on *A. marlothii* nectar. There are several additional possibilities that cannot be ruled out. First, the birds could have obtained the enriched $\delta^{13}C$ signal by feeding on insects that fed on *A. marlothii* nectar. In this scenario, a dietary shift to nectar by insects would result in a corresponding enrichment in the blood of the birds that fed on them, with enrichment of avian blood $\delta^{13}C$ thus occurring indirectly. A related possibility is that the $\delta^{13}C$ of insects increased at this time of year via diet changes unrelated to the availability of *A. marlothii* nectar. Secondly, the insect component of avian diets could have shifted to include a greater proportion of insects associated with C_4 vegetation. A third possibility is, of course, that the enrichment of avian blood $\delta^{13}C$ reflected a combination of increased nectar feeding as well as a shift in the $\delta^{13}C$ of insect tissues. However, observations of regular feeding by a variety of bird species on *A. marlothii* nectar, and significant increases in abundance of these species during the flowering period (Symes *et al.* 2008), support the view that at least some of the observed enrichment in blood

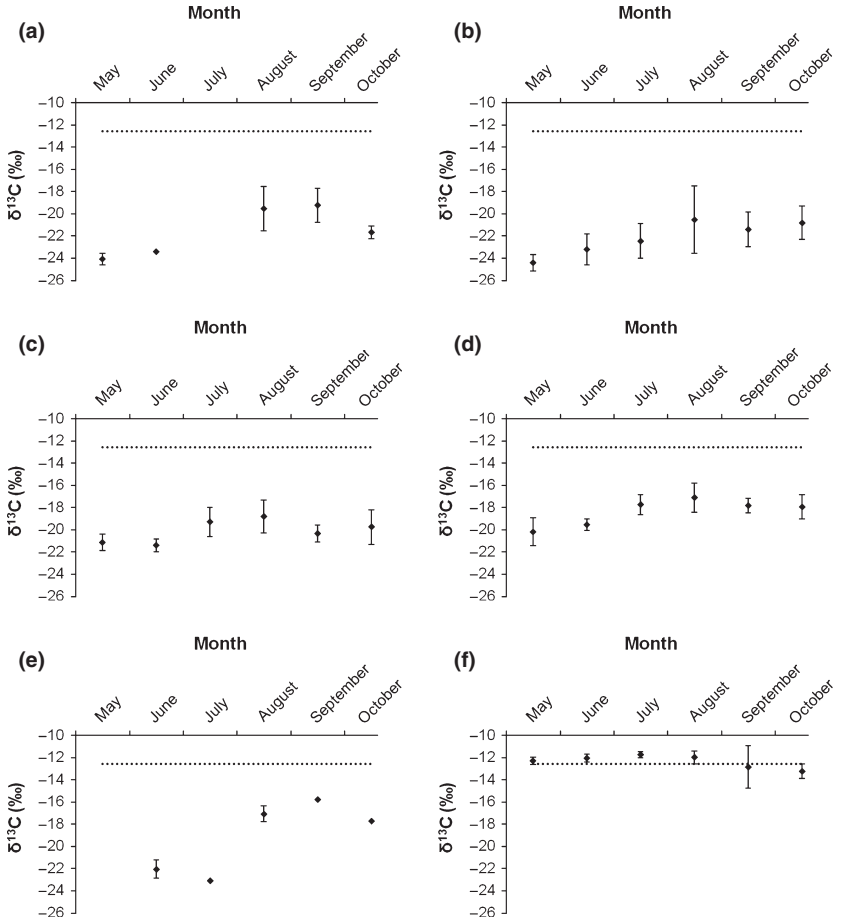

Figure 1. Mean monthly $\delta^{13}C$ values (‰ VPDB, ±sd) for whole blood samples of opportunistic nectarivores (a–e), and a granivore (f), feeding on *Aloe marlothii* in Suikerbosrand Nature Reserve. Blood samples were collected during pre-flowering (May–July), flowering (August–September) and post-flowering (October) periods (abbreviated on *x*-axis). Comparisons of monthly means (Kruskall–Wallis) indicated for (a) African Red-eyed Bulbul ($P = 0.034$), (b) Black-chested Prinia *Prinia flavicans* ($P = 0.082$), (c) Cape Robin-Chat *Cossypha caffra* ($P = 0.029$), (d) Fiscal Flycatcher *Sigelus silens* ($P = 0.002$), (e) Bar-throated Apalis *Apalis thoracica* ($P = 0.035$), (f) Green-winged Pytilia *Pytilia melba* ($P = 0.079$). The horizontal dashed line indicates the mean $\delta^{13}C$ value of nectar (−12.6‰ VPDB). See Table 2 for feeding guild details.

$\delta^{13}C$ can be directly attributed to nectarivory. Also, a protein-rich food source (insects) is more likely to lead to a significant isotopic shift in whole blood than a protein-poor food source (nectar), further supporting the idea that the similar enrichment of avian blood from different guilds largely reflects nectar feeding. Although carbon routed from insects is likely to have a greater influence on the $\delta^{13}C$ of whole blood than nectar, significant enrichment in breath $\delta^{13}C$ values reiterates the importance of taking isotopic routing into account. In light of our behavioural observations (Symes *et al.* 2008), we believe that the blood $\delta^{13}C$ data greatly underestimate the nutritional significance of *A. marlothii* nectar during this time of year. This is because carbohydrates are routed directly to metabolism with little routing to storage and body tissues (Voigt *et al.* 2008a). The marked isotopic routing we observed in African Red-eyed Bulbuls and Cape White-eyes suggests that these species, and very likely other opportunistic nectarivores, obtain the majority of their metabolized carbohydrates from *A. marlothii* nectar. A further source of uncertainty concerns our analyses of whole blood. Red blood cells have a longer isotopic half-life than plasma (Hobson & Clark 1992a,b, Carleton *et al.* 2006), and where diet shifts occurred recently, analysis of whole blood is likely to underestimate the contribution of the more recent diet to an animal's carbon pool.

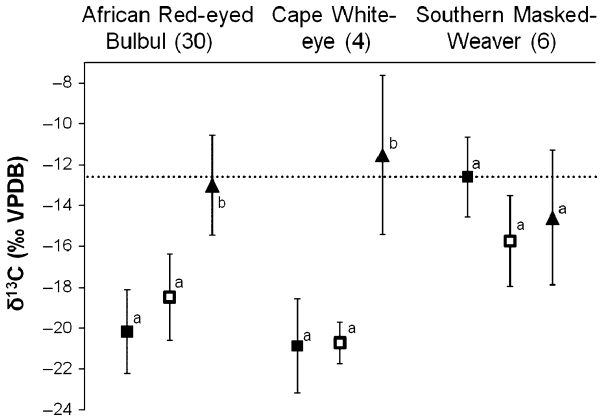

Figure 2. Mean $\delta^{13}C$ values (‰ VPDB, ±sd) for feathers, whole blood and breath of three nectar-feeding species, African Red-eyed Bulbul *Pycnonotus nigricans*, Cape White-eye *Zosterops capensis* and Southern Masked-Weaver *Ploceus velatus*, sampled during peak flowering in *Aloe marlothii* in Suikerbosrand Nature Reserve. Sample sizes given in parentheses. Intra-species comparisons indicated by letters. The horizontal dashed line indicates the mean $\delta^{13}C$ value of nectar (–12.6‰ VPDB). Feathers = solid square; blood = open square; breath = open triangle.

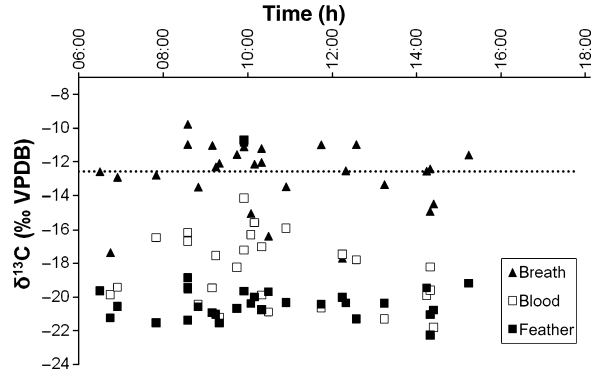

Figure 3. $\delta^{13}C$ values of feather, blood and breath samples collected in a day during peak flowering of *Aloe marlothii* for African Red-eyed Bulbul *Pycnonotus nigricans* at Suikerbosrand Nature Reserve.

Significance of *A. marlothii* nectar as a seasonal food resource

The nectar of *A. marlothii* is consumed by members of several avian feeding guilds, including frugivores, insectivores, omnivores and granivores (Oatley 1964, Skead 1967, Oatley & Skead 1972, Botes *et al.* 2008, Symes *et al.* 2008, Forbes *et al.* 2009, Symes 2010). Whereas we could not use the stable carbon isotope approach to examine the significance of opportunistic nectarivory in granivores in SNR, because of the overlap in $\delta^{13}C$ values between C_4 and CAM photosynthesizers, enriched blood $\delta^{13}C$ values reveal the incorporation of nectar carbon (either directly from nectar or indirectly from insects that assimilated nectar) by a diverse suite of non-granivorous avian consumers. Most species that feed on *A. marlothii* nectar in SNR are non-migrant, year-round residents, but the winter-flowering period coincides with an increase in avian diversity, with several species appearing in the area at this time, including Wattled Starlings *Creatophora cinerea* (Symes *et al.* 2008). Sturnids are unable to digest sucrose (Martínez del Rio & Stevens 1989, Martínez del Rio *et al.* 1992); however, the sugars in *Aloe* nectars predominantly consist of hexoses (Van Wyk *et al.* 1993). Similar associations with opportunistic nectarivores have been reported for other *Aloe* species, including *Aloe ferox*, *Aloe speciosa*, *Aloe africana*, *Aloe pluridens*, *Aloe lineata* var. *muirii*, *Aloe barberae*, *Aloe pruinosa* and *Aloe vryheidensis* (Oatley 1964, Oatley & Skead 1972, Johnson *et al.* 2006, Botes *et al.* 2008, 2009, Forbes *et al.* 2009, Wilson *et al.* 2009). In South Africa, Oatley and Skead (1972) recorded at least 73 bird species (in 24 families) that are not specialist nectarivores, feeding on 14 *Aloe* species and eight other flowering plants.

Our study provides further evidence that succulent plants that occur in moderate to high densities can significantly contribute to the food requirements of animals if they produce nectar and/or fruit during the dry season. In terms of the importance of nectar as a resource for a diverse assemblage of avian consumers, striking similarities exist between *A. marlothii* in southern Africa and the Saguaro cactus *Carnegiea gigantea*, a CAM succulent in the Sonoran Desert of North America (Wolf & Martínez del Rio 2000, 2003, Wolf *et al.* 2002). In both these systems, a seasonal pulse of food resources by a succulent species results in broad-scale diet switching in avian communities. However, the timing of these resource pulses is different; flowering and fruiting of the Saguaro occurs during hot summer months (Wolf & Martínez del Rio 2003), whereas flowering in *A. marlothii* occurs during winter. In both systems, resources become available when conditions are dry and food and/or water resources are possibly limited; in both cases these resources result in significant shifts in the $\delta^{13}C$ values of consumers' tissue (Wolf & Martínez del Rio 2003).

We were unable to test whether the water in nectar of *A. marlothii* was important for birds or whether alternative sites provided water for birds. Further analysis of stable hydrogen isotopes would provide evidence of this. *A. marlothii* nectar is relatively dilute and produced in copious amounts (Symes & Nicolson 2008) (nectar production estimated at 50–100 L/ha; C. T. Symes unpubl. data), and because it is consumed by species that do not characteristically feed on nectar (Symes *et al.* 2008), and because very little rain falls during the flowering period and ground water sources become scarce, we suggest that it is an important water source for many species. Nearby (approximately 1 km) to the *A. marlothii* forest was an artificial water drinking trough that was observed during field trips to the site (C. T. Symes pers. obs.). During the flowering period there appeared to be a greater number of birds that drank there (C. T. Symes pers. obs.). Despite the abundance of nectar produced by *A. marlothii*, birds still visited the drinking trough for water. A related observation is that, during ringing efforts, an African Red-eyed Bulbul and Acacia Pied Barbet *Tricholaema leucomelas* that had previously been caught in the *A. marlothii* forest were subsequently recaptured at the drinking trough, suggesting that birds utilizing nectar nevertheless sought water sources elsewhere (C. T. Symes unpubl. data).

Isotopic routing: consequences for understanding plant–animal interactions

The routing of ingested nutrients to metabolism is a topic of considerable current interest among ecologists and physiologists (Hatch *et al.* 2002, Podlesak *et al.* 2005, Carleton *et al.* 2006, Voigt *et al.* 2008a, 2008b). Broad-tailed Hummingbirds *Selaphorus platycercus* have been shown to fuel their metabolism largely (*c.* 90%) from assimilated sugars, although when birds were losing mass they fuelled their metabolism from endogenous reserves (Carleton *et al.* 2004, 2006). Similarly, nectar-feeding bats (*Glossophaga soricina*) use recently ingested sugars (carbohydrates) to fuel a large proportion of their metabolism (Voigt & Speakman 2007, Welch *et al.* 2008). In an omnivorous bat, *Carollia perspicillata*, isotope analysis of breath and tissue confirmed that ingested carbohydrates were routed directly to metabolism, whereas ingested protein was routed to body synthesis (Voigt *et al.* 2008b). Our data for African Red-eyed Bulbuls and Cape White-eyes provide an instructive example of what such metabolic routing can mean for consumers feeding on seasonally available food resources. Whereas the blood $\delta^{13}C$ values of avian opportunistic nectarivores in SNR indicate that only a relatively small fraction of the carbon assimilated into blood originates from *A. marlothii*, the $\delta^{13}C$ values of exhaled CO_2 reveal that, at least in some species, carbohydrates obtained from this resource probably represent the bulk of metabolized carbon. If the relative contributions of C_3 and C_4 carbon remained unchanged during the pre-flowering and flowering periods, and we assume that the fractionation factor from diet to blood is 1‰, then the enrichment in blood $\delta^{13}C$ is equivalent to an average carbon contribution from nectar of 15%. However, a key source of uncertainty in this calculation concerns the relative contributions of red blood cells and plasma to the $\delta^{13}C$ values of whole blood. Because the carbon atoms in red blood cells turn over more slowly than those in plasma, a recent diet switch may be reflected in plasma but not in red blood cells. In diet-switch experiments, for instance, turnover times of 15–16 days for red blood cells have been reported in House Sparrows *Passer domesticus* (Carleton & Martínez del Rio 2005), whereas turnover times for blood plasma were just 0.4–0.7 days in Yellow-rumped Warblers *Dendroica coronata* (Pearson *et al.* 2003), emphasizing the different time scales over which these two components of blood incorporate dietary information. With respect to breath samples, the interpretation is different. Considerable variability has been reported in diet–breath discrimination factors (Perkins & Speakman 2001, Hatch *et al.* 2002, Podlesak *et al.* 2005, Voigt *et al.* 2008a, 2008b), and we estimate, with caution, contributions of *A. marlothii* nectar to metabolized carbon. However, the similarity in $\delta^{13}C$ between nectar and breath samples in African Red-eyed Bulbuls and Cape White-eyes suggests that close to 100% of their metabolized carbohydrates originated from this source (Figs 2 and 3). It therefore appears that sugars of *A. marlothii* nectar are more important as an income energy resource than are capital resources that can be deposited as fat and used during subsequent months.

One observation in the African Red-eyed Bulbul data is that breath $\delta^{13}C$ values were not correlated with the time of day, and that even early in the

morning the bulbuls were metabolizing carbon derived from *A. marlothii* nectar. If the enrichment of exhaled CO_2 solely reflected the routing of ingested nectar to metabolism, then we would have expected the Bulbuls to exhibit early-morning breath $\delta^{13}C$ values similar to those of blood, as the nectar $\delta^{13}C$ signature would only appear in their breath some time after the start of feeding (Voigt *et al.* 2008a, 2008b). Thus, our comparisons of breath and blood $\delta^{13}C$ values do not preclude the possibility that the Bulbuls synthesized lipid stores from *A. marlothii* nectar, and it is these reserves that they metabolized when not feeding on nectar. However, another possibility is that Bulbuls captured early in the morning had already been feeding on *A. marlothii* nectar long enough for it to appear in their exhaled CO_2. In fasted Broad-tailed Hummingbirds the carbon isotope signature of ingested nectar with a unique isotopic signature was detected in breath soon after feeding (Carleton *et al.* 2006).

The lack of significant differences between the $\delta^{13}C$ values of breath, blood and feathers in Southern Masked-Weavers does not necessarily indicate that isotopic routing of nectar carbon did not occur in this species, but instead simply reflects the large C_4 component of their diet (Hobson & Clark 1992a, 1992b). Likewise, in granivorous species that regularly fed on nectar, but which did not exhibit any changes in blood $\delta^{13}C$, the routing of nectar carbon may similarly have been masked by the C_4 isotopic signature associated with a diet of grass seeds.

In the Sonoran Desert, White-winged Doves *Zenaidia asiatica* feeding extensively on Saguaro nectar exhibited no detectable change in liver $\delta^{13}C$ values, and the isotopic shift towards a CAM signature occurred only after fruit became available (Wolf & Martínez del Rio 2000). These authors argued that the lack of a change in liver $\delta^{13}C$ probably reflected the routing of nectar carbon directly into metabolism and not tissue synthesis. Our data for two passerines feeding on *A. marlothii* nectar support this idea, and confirm that the nutritional significance of seasonally available food resources may be underestimated if exhaled CO_2 is not sampled.

Oatley (1964) suggested that birds feeding on *A. marlothii* nectar in northern KwaZulu-Natal, South Africa, were not food stressed and used the winter-flowering period to supplement already plentiful food supplies in preparation for the accumulation of reserves prior to breeding. Our observations that ingested nectar carbon is largely routed to fuelling metabolism in at least two species, and that relatively little nectar carbon is incorporated into their blood, argue against the possibility that nectar resources are accumulated as reserves. Instead, the use of *A. marlothii* nectar to fuel current metabolic demands may mean that body stores of carbohydrates and lipids are depleted more slowly than they would have been otherwise, with potential benefits in terms of future investment in reproduction. However, as discussed above, the lack of correlation between breath $\delta^{13}C$ and time of day may mean that the reality is more complex.

In summary, our data reveal that the availability of *A. marlothii* nectar is associated with a distinct shift in the blood $\delta^{13}C$ of a suite of avian opportunistic nectarivores, but that the contribution of this food resource to blood carbon is relatively modest, reflecting little contribution to capital energy reserves from nectar carbohydrate sugars. Analyses of blood $\delta^{13}C$ greatly underestimate the nutritional importance of *A. marlothii* nectar to avian consumers, as in at least some species the bulk of metabolized income carbohydrate originates, directly or indirectly, from the nectar. This use of a high-energy carbohydrate resource may have implications for the movements of birds across the landscape, particularly where the availability of food resources differs significantly between seasons (Symes *et al.* 2008). Our findings thus reiterate the consequences that seasonally available nectar resources can have for consumer communities, and provide further insights into the ecological roles of succulent plants in dry winter periods.

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