Using stable isotopes to trace resource acquisition and trophic position in four Afrotropical birds with different diets

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Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analysis has become increasingly used to identify basal food web macromolecular sources and define trophic positions in diverse ecosystems. The use of this technique relies on variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among different sources of organic matter and thus in the tissues of the organisms that feed on them. Consumer $\delta^{13}\text{C}$ values closely resemble those of their diet, and much of the variation in the $\delta^{13}\text{C}$ of animal tissues can be ascribed to the differential use of C_4/CAM and C_3 plant-based food chains (Lajtha and Michener 2007). Additionally, the tissues of consumers tend to be significantly enriched in ^{15}N compared to their diets, with each trophic step accompanied by an increase of 2–4‰ in $\delta^{15}\text{N}$ (Minagawa and Wada 1984). Hence, tissue $\delta^{15}\text{N}$ can be used to infer consumer trophic level (Bearhop et al. 2004).

The objectives of this study were to quantify the relative contributions of C_3 and C_4/CAM plants to assimilated nutrients and compare trophic levels among four tropical bird species. We chose the Western Mountain Greenbul *Andropadus tephrolaemus* (hereafter greenbul), Northern Double-collared Sunbird *Cinnyris reichenowi* (hereafter sunbird), Black-crowned Waxbill *Estrilda nonnula* (hereafter waxbill) and Chubb's Cisticola *Cisticola chubbi* (hereafter cisticola), representing frugivorous, nectarivorous, granivorous and insectivorous species, respectively. Since the isotopic composition of keratinous tissues, such as feathers, reflects the diet of the organism during the period when these structures were synthesised (Hobson 1999), we expected feather $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to reflect the isotopic composition of carbon and nitrogen sources during moult, and to differ among species according to their diets. We predicted that the greenbul (an arboreal forest feeder subsisting on tree fruits; Keith et al. 1992), the sunbird (feeding on the nectar of C_3 plants, and insects in open forest with clearings; Fry and Keith 2000), and the cisticola (preying on insects in grassy clearings and bushy

forest edges; Urban et al. 1997) derive most of their carbon from C_3 -based food webs. In contrast, the waxbill inhabits more open habitats with a greater proportion of C_4 plants and feeds on grass seeds (Fry and Keith 2004), and was thus predicted to exhibit more enriched $\delta^{13}\text{C}$ values. We further expected feathers of the frugivore and the granivore to be depleted in ^{15}N relative to the insectivore and the nectarivore. This prediction is based on the assumption that nectarivorous birds derive most of their nitrogen from arthropods, because of the low protein content of nectar (Gottsberger et al. 1984).

The field work was conducted in the Bamenda Highlands near Big Babanki (North West Province, Cameroon; 06°05'26" N, 10°18'09" E; 2 135 m above sea level). For a detailed description of the study area see Reif et al. (2006).

From 23 November to 3 December 2008, we mist-netted birds along a 120 m transect across a forest–savanna boundary and sampled left innermost primary feathers: greenbul ($n = 13$), sunbird ($n = 15$), waxbill ($n = 15$) and cisticola ($n = 13$). Samples were stored dry in sealable bags until analysis. Since all four species are resident at the study site, we assume that the feathers were grown locally between March and May during the main moulting period (Eyckerman and Cuvelier 1982).

Feather samples were rinsed in a 2:1 chloroform:methanol solution and air-dried in a fume hood. Subsamples (c. 0.2 mg each) were weighed into small tin cups to the nearest 0.001 mg and then combusted in an elemental analyser (Eurovector, Milan, Italy). The resulting N_2 and CO_2 gases were separated by gas chromatography and admitted into the inlet of an isotope ratio mass spectrometer (Micromass Isoprime, Manchester) for determination of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios. Measurements are reported in δ -notation in permil (‰) relative to atmospheric nitrogen (AIR) for $\delta^{15}\text{N}$ and Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$. Replicate assays of internal laboratory reference material (albumen) indicated that the typical precision of

analyses (SD) was $\pm 0.5\text{‰}$ for $\delta^{15}\text{N}$ and $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$. The analyses were conducted at the School of Biological Sciences, Washington State University, USA.

The contribution of C_3 and C_4 plants to the carbon isotope composition of bird feathers was estimated using two-endpoint linear mixing models in IsoError 1.04 (Phillips and Gregg 2001). We corrected feather $\delta^{13}\text{C}$ values by diet–tissue discrimination factors associated with the expected trophic position of the species: 1.5‰ for the frugivore and granivore, and 3.0‰ for the insectivore and nectarivore (Symes and Woodborne 2009). As endpoints, we used mean $\delta^{13}\text{C}$ values reported for C_3 (-27‰) and C_4 (-13‰) plants (Lajtha and Michener 2007).

Feather $\delta^{13}\text{C}$ differed significantly among the four species (Kruskal–Wallis $H_{3,56} = 30.3$, $P < 0.001$; Figure 1). Multiple comparisons showed that except for the waxbill and cisticola, the study species differed significantly from each other in $\delta^{13}\text{C}$ values (Table 1). By using discrimination factors and mixing models we found that the greenbul, the sunbird and the cisticola depended on a relatively higher proportion of C_3 plant dietary sources, while the waxbill feather $\delta^{13}\text{C}$ values reflected a relatively lower proportion of C_3 plants (Table 2). Feather $\delta^{15}\text{N}$ also differed among the study species (Kruskal–Wallis $H_{3,56} = 36.0$, $P < 0.001$). The $\delta^{15}\text{N}$ values of the waxbill and the greenbul were significantly lower (by 3.5‰) than those of the sunbird and the cisticola (Table 1). Both the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of waxbill feathers showed relatively higher ranges and variances compared to the other species (Table 2, Figure 1).

As predicted, the mixing models revealed that the greenbul, sunbird and cisticola relied mostly on C_3 plant dietary sources, whereas the waxbill's diet originated from a lower proportion of C_3 plant-based food chains. Feather $\delta^{13}\text{C}$ measurements are thus in broad agreement with the known dietary habits and habitat preferences of the study species (Keith et al. 1992, Urban et al. 1997, Fry and Keith 2000, 2004, Reif et al. 2007).

Feather $\delta^{15}\text{N}$ values in the greenbul and waxbill differed by 3.5‰ from those in the sunbird and cisticola, which corresponds to approximately one trophic step in a food web (Minagawa and Wada 1984). We did not measure the $\delta^{15}\text{N}$ of local plants, but assume that plant amino acids constitute the primary source of assimilated nitrogen in the greenbul and the waxbill, whereas both the cisticola and the sunbird occupy a higher trophic level, deriving most of their nitrogen from invertebrates. The sunbird feeds both on nectar and invertebrates; however, as nectar contains only trace amounts of nitrogen (Gottsberger et al. 1984), its contribution to nitrogen assimilation is most likely negligible.

Most interesting is the large range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in waxbill feathers compared to the other species (Table 2), implying a wider isotopic niche during moult (Newsome et al. 2007). This illustrates interindividual variation in assimilated diet within a species, which may be increased by the fact that waxbills undergo a complete moult in the non-breeding period when they range over large areas (Fry and Keith 2004), visiting isotopically varied habitats. Previously, Reif et al. (2006) estimated bird abundance (using a point-count method) at this study site and quantified niche breadth of these four species within the local avian community. Their

Figure 1: Mean feather stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios of four sympatric passerine species: Western Mountain Greenbul *Andropadus tephrolaemus*, Northern Double-collared Sunbird *Cinnyris reichenowi*, Black-crowned Waxbill *Estrilda nonnula*, and Chubb's Cisticola *Cisticola chubbi*. Error bars = SD

Table 1: Results of non-parametric Behrens–Fisher multiple comparison tests of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios among the studied species (P -values for $\delta^{13}\text{C}$ above the diagonal, those for $\delta^{15}\text{N}$ below the diagonal)

Species	Greenbul	Sunbird	Waxbill	Cisticola
Greenbul		0.002	<0.001	<0.001
Sunbird	<0.001		0.014	0.005
Waxbill	0.308	<0.001		0.851
Cisticola	<0.001	0.578	<0.001	

Table 2: Ranges of feather stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios, with the percentage and 95% CI of C_3 food sources predicted by two-endpoint linear mixing models

Species	$\delta^{13}\text{C}$ (‰ VPDB)		% C_3	95% CI	$\delta^{15}\text{N}$ (‰ AIR)		n
	Min.	Max.			Min.	Max.	
Greenbul	-23.6	-21.3	78.3	73.7–83.0	5.1	7.4	13
Sunbird	-22.8	-20.5	82.2	77.8–86.7	8.3	10.3	15
Waxbill	-22.3	-10.3	38.4	19.8–57.0	2.5	9.9	15
Cisticola	-21.8	-16.5	66.5	58.6–74.3	8.2	10.3	13

results indicate that the waxbill and cisticola have narrower local niche breadths among the four species, while the sunbird and greenbul have broader niches. This contrasts markedly with the isotopic results reported here, suggesting that habitat and isotopic niche breadths may describe largely different aspects of the ecology of a species.

Our results illustrate the advantages of stable isotope analyses for the study of diet and niche segregation without the need for time-consuming observation and/or faecal or crop contents analyses. Additional analyses of stable isotope ratios through more extensive sampling may provide further insights into seasonal changes in food composition and individual dietary strategies.

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References

- Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* 73: 1007–1012.
- Eyckerman R, Cuvelier D. 1982. The moult of some bird species on Mount Cameroon. *Malimbus* 4: 1–4.
- Fry CH, Keith S (eds). 2000. *The birds of Africa*, vol. 6. London: Academic Press.
- Fry CH, Keith S (eds). 2004. *The birds of Africa*, vol. 7. London: Christopher Helm.
- Gottsberger GJ, Schrauwen J, Linskens HF. 1984. Amino acids and sugars in nectar, and their putative evolutionary significance. *Plant Systematics and Evolution* 145: 55–77.
- Hobson KA. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120: 314–326.
- Keith S, Urban EK, Fry CH (eds). 1992. *The birds of Africa*, vol. 4. London: Academic Press.
- Lajtha K, Michener RH (eds). 2007. *Stable isotopes in ecology and environmental science* (2nd edn). Oxford: Blackwell.
- Minagawa M, Wada E. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48: 1135–1140.
- Newsome SD, Martínez del Río C, Bearhop S, Phillips DL. 2007. A niche for isotope ecology. *Frontiers in Ecology and the Environment* 5: 429–436.
- Phillips DL, Gregg JW. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127: 171–179.
- Reif J, Hořák D, Sedláček O, Riegert J, Pešata M, Hrázský Z, Janeček Š, Storch D. 2006. Unusual abundance-range size relationship in an Afrotropical bird community: the effect of geographical isolation? *Journal of Biogeography* 33: 1959–1968.
- Reif J, Sedláček O, Hořák D, Riegert J, Pešata M, Hrázský Z, Janeček Š. 2007. Habitat preferences of birds in a montane forest mosaic in the Bamenda Highlands, Cameroon. *Ostrich* 78: 31–36.
- Symes CT, Woodborne SM. 2009. Trophic level delineation and resource partitioning in a South African afrotropical forest bird community using carbon and nitrogen stable isotopes. *African Journal of Ecology*, doi:10.1111/j.1365-2028.2009.01201.x.
- Urban EK, Fry CH, Keith S (eds). 1997. *The birds of Africa*, vol. 5. London: Academic Press.