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

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Stable carbon (δ13C) and nitrogen (δ15N) 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 δ13C and δ15N values among different sources of organic matter and thus in the tissues of the organisms that feed on them. Consumer δ13C values closely resemble those of their diet, and much of the variation in the δ13C of animal tissues can be ascribed to the differential use of C4/CAM and C3 plant-based food chains (Lajtha and Michen 2007). Additionally, the tissues of consumers tend to be significantly enriched in 15N compared to their diets, with each trophic step accompanied by an increase of 2–4‰ in δ15N (Minagawa and Wada 1984). Hence, tissue δ15N can be used to infer consumer trophic level (Bearhop et al. 2004).

The objectives of this study were to quantify the relative contributions of C3 and C4/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 δ13C and δ15N 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 C3 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 C3-based food webs. In contrast, the waxbill inhabits more open habitats with a greater proportion of C4 plants and feeds on grass seeds (Fry and Keith 2004), and was thus predicted to exhibit more enriched δ13C values. We further expected feathers of the frugivore and the granivore to be depleted in 15N 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 1992).

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 N2 and CO2 gases were separated by gas chromatography and admitted into the inlet of an isotope ratio mass spectrometer (Micromass Isoprime, Manchester) for determination of 15N/14N and 13C/12C ratios. Measurements are reported in δ notation in permil (‰) relative to atmospheric nitrogen (AIR) for δ15N and Vienna PeeDee Belemnite (VPDB) for δ13C. Replicate assays of internal laboratory reference material (albumen) indicated that the typical precision of
analyses (SD) was ±0.5‰ for δ15N and ±0.2‰ for δ13C. The analyses were conducted at the School of Biological Sciences, Washington State University, USA.

The contribution of C3 and C4 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 δ13C 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 δ13C values reported for C3 (~27‰) and C4 (~13‰) plants (Lajtha and Michener 2007). Feather δ13C differed significantly among the four species (Kruskal-Wallis H = 30.3, P < 0.001; Figure 1). Multiple comparisons showed that except for the waxbill the study species differed significantly from each other in δ13C 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 C3 plant dietary sources, while the waxbill feather δ13C values reflected a relatively lower proportion of C3 plants (Table 2). Feather δ15N also differed among the study species (Kruskal-Wallis H = 36.0, P < 0.001). The δ15N 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 δ13C and δ15N 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 C3 plant dietary sources, whereas the waxbill’s diet originated from a lower proportion of C3 plant-based food chains. Feather δ13C 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 δ15N 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 δ15N 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 δ13C and δ15N 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 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.

Table 1: Results of non-parametric Behrens–Fisher multiple comparison tests of stable carbon (δ13C) and nitrogen (δ15N) isotope ratios among the studied species (P-values for δ13C above the diagonal, those for δ15N below the diagonal)

<table>
<thead>
<tr>
<th>Species</th>
<th>Greenbul</th>
<th>Sunbird</th>
<th>Waxbill</th>
<th>Cisticola</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ13C (‰ VPDB)</td>
<td>Min.</td>
<td>Max.</td>
<td>% C3</td>
<td>95% CI</td>
</tr>
<tr>
<td>Greenbul</td>
<td>-23.6</td>
<td>-21.3</td>
<td>78.3</td>
<td>73.7–83.0</td>
</tr>
<tr>
<td>Sunbird</td>
<td>-22.8</td>
<td>-20.5</td>
<td>82.2</td>
<td>77.8–86.7</td>
</tr>
<tr>
<td>Waxbill</td>
<td>-22.3</td>
<td>-10.3</td>
<td>38.4</td>
<td>19.8–57.0</td>
</tr>
<tr>
<td>Cisticola</td>
<td>-21.8</td>
<td>-16.5</td>
<td>66.5</td>
<td>58.6–74.3</td>
</tr>
</tbody>
</table>

Table 2: Ranges of feather stable carbon (δ13C) and nitrogen (δ15N) isotope ratios, with the percentage and 95% CI of C3 food sources predicted by two-endpoint linear mixing models

<table>
<thead>
<tr>
<th>Species</th>
<th>δ13C (‰ VPDB)</th>
<th>δ15N (‰ AIR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenbul</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
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</table>
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References
