Detecting Altitudinal Migration Events in Neotropical Birds Using Stable Isotopes

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ABSTRACT

We evaluated the utility of stable-hydrogen isotope ratios in tropical bird tissues for detecting altitudinal migration events. Our results identified two of five species as altitudinal migrants in Nicaragua. This approach may circumvent the current limitations of mark–recapture techniques and enhance our ability to study this poorly characterized behavior.

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ALTITUDINAL MIGRATION IS THOUGHT TO PLAY AN IMPORTANT ROLE in the seasonal distribution of tropical birds over an elevational gradient and is important to consider in conservation efforts (Stiles 1988, Powell & Bjork 1995, Winker *et al.* 1997), but remains a little studied and poorly understood phenomenon. This is particularly true for small passerines, where nearly all knowledge has been derived from seasonal fluctuations in observations and capture-rates at different elevations (Stiles 1985a, Dasilva 1993, Johnson & Maclean 1994, Winker *et al.* 1997, Burgess & Mlingwa 2000, Wright 2005, Brown 2006) and occasional band recoveries (Loiselle & Blake 1991, Burgess & Mlingwa 2000). These methods may be limiting for at least five reasons: (1) seasonal increases or decreases in abundance at capture sites are not often equated with concomitant shifts at other elevations; (2) marked birds are rarely recaptured elsewhere (Berthold 2001); (3) seasonal shifts in abundance due to altitudinal migration are difficult to separate from lateral, seasonal habitat shifts at the same elevation; (4) parallel shifts in movement at different elevations may obscure the detection of some movements (Loiselle & Blake 1991); (5) intrapopulation migration patterns, *e.g.*, where one portion of the population is migratory, the other sedentary, cannot be studied without information on the movement of individuals.

Stable-hydrogen isotope analysis may provide a means by which we can detect altitudinal migration without prior capture of individuals (Hobson *et al.* 2003). Metabolically inert, keratinous tissues such as feathers and claws incorporate local precipitation signatures through diet (Hobson & Wassenaar 1997) that are 'locked-in' upon synthesis (Bearhop *et al.* 2003, Mazerolle & Hobson 2005) with little to no additional species level effects (Hobson *et al.* 2004; K. Fraser, pers. obs.). Recent studies show a highly significant correlation between δD, $\delta^{13}C$, and $\delta^{13}N$ in feathers and claws (Bearhop *et al.* 2003, Hobson *et al.* 2006), and studies using both tissues

have assumed that keratinous tissues grown in the same locale share δD values (Bearhop *et al.* 2005). Thus, comparisons of δD values in various tissues may provide clues about the prior movement of organisms.

Globally, hydrogen isotopic values in meteoric waters (δD_p) show a depletion trend of about -2.2 per mil per 100 m elevation (Poage & Chamberlain 2001, Hobson *et al.* 2003). Hobson *et al.* (2003) found strong correlation between hydrogen isotope values in feathers (δD_k) collected along an elevational gradient with expected capture site values based on annual mean precipitation. Few previous studies have compared hydrogen isotope values of multiple keratinous tissues from individual birds to determine geographic movement or origins (*e.g.*, Mazerolle & Hobson 2005, Hobson *et al.* 2006). Our objective in this study was to compare feather and claw δD_k to detect potential altitudinal migration. Claws represent recent integration of hydrogen isotopes (Bearhop *et al.* 2003, Mazerolle & Hobson 2005; Fraser *et al.* 2008) whereas feathers represent values at the time of molt (Hobson & Wassenaar 1997). In resident Neotropical birds, differences in δD_k values between these two tissues are likely to reflect their growth at different elevations.

We looked for altitudinal migration in two partial-frugivores (black-headed nightingale thrush *Catharus mexicanus*, ochre-bellied flycatcher *Mionectes oleaginous*) and three nectarivores (long-billed hermit *Phaethornis longirostris*, stripe-tailed hummingbird *Eupherusa eximia*, violet sabrewing *Campylopterus hemileucurus*). We chose these species because frugivores and nectarivores are thought to migrate altitudinally as they track seasonal fluctuations in food availability (Levey & Stiles 1992). We asked: (1) Are δD_k values in claws (recent integration) and tail feathers (postbreeding) significantly different for the individuals of each species? (2) Do our data support or refute the findings of other methods used to detect altitudinal movements in these species?

We sampled adult individuals of four *C. hemileucurus*, six *P. longirostris*, three *E. eximia*, seven *M. oleaginous*, and three *C. mexicanus* between 20 February and 6 March 2006 at the Finca Téchnica de la

Reserva Silvestre Privada de Nebliselva El Jaguar (13°10′ N, 86°02′ W; 1350 m asl), a lower-montane forest in western Nicaragua. Claw tips were collected (1–2mm depending on species) from the middle toe of both feet using sharp scissors and deposited in a small paper envelope. A single tail feather (R3) was pulled from each individual and stored in the same envelope. Capture and sampling were carried out under permits from Ministerio del Ambiente y los Recursos Naturales (MARENA, No.010–03007), and Environment Canada (CA 0106), and field methods were approved by Queen's University Animal Care Committee (Protocol-2005–017-R1). All individuals were released without injury.

One claw per individual (from left or right foot chosen randomly) and tail feather were washed to remove lipids and particles in a 2:1 chloroform:methanol solution for 24 h and left to air-dry. To account for potential exchangeable hydrogen effects between ambient laboratory water vapor and tissue, we used an equilibration technique. Claws and feathers were stored in open containers in a fume hood for at least one week at the Queen's Facility for Stable Isotope Research (QFIR). Moisture and temperature at QFIR were monitored and kept constant.

Mineral and keratin standards and claw and feather samples were weighed (0.08–0.22 mg, weight varied with species), placed in silver capsules and oven-dried for 24 h at 100°C to remove any absorbed water, which can be a substantial source of exchangeable hydrogen (Algie & Watt 1962; Bowen *et al.* 2005).

Samples were crushed and loaded into an autosampler connected to a Finnigan TC/EA (1450°C) reduction furnace where they passed online to a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer for analysis. Stable-hydrogen isotope ratios ($^2H/^1H = R$) are expressed in units of per mil (%), where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$ and $R_{standard}$ is the hydrogen isotope ratio of Vienna Standard Mean Ocean Water (V-SMOW). For every five claw and feather samples, we ran two in-house mineral standards with very fine grain sizes (Georgia clay $\delta D = -58\%$ and brucite $\delta D = -96\%$) and an in-house keratin standard (chicken feather $\delta D = -95 \pm 5\%$). A series of standards with different degrees of exchangeability is necessary to evaluate the effect of absorbed water and the linearity of the mass spectrometer.

Potential effects of source nonlinearity were compensated for by analysis of the effect of H^{3+} and by varying the amounts of standards and duplicate samples. Nonlinearity is constantly monitored at QFIR by varying the amount of standards and by calibrating to the international standards, VSMOW and VSLAP. Our method of calculating δD values uses a single keratin standard and two in-house mineral standards, the latter to monitor instrument linearity and adsorbed moisture. This differs from that of other labs who use a 3-point keratin calibration curve to estimate nonexchangeable δD (Wassenaar & Hobson 2003). Hence, some values of δD from this study may not be directly comparable with those from labs using the latter approach.

All standards and samples were treated in the same way and values are reproducible to $\pm 3\%$. δD_k values of feather versus claws were compared using paired *t*-tests. Statistical analyses were performed using R (v. 2.3.1, The R Foundation For Statistical Computing).

Stable-hydrogen claw and feather values were significantly different for *C. mexicanus* ($t_2 = -14.7$, $P = 0.004$), and *M. oleaginous* ($t_6 = 5.2$, $P = 0.002$; Fig.1). Values were not significantly different for *P. longirostris* ($t_6 = 0.9$, $P = 0.4$), *E. eximia*, ($t_2 = 0.03$, $P = 0.9$), and *C. hemileucurus* ($t_3 = 0.51$, $P = 0.6$; Fig. 1).

Significant differences between claw and feather δD_k values for *C. mexicanus* suggest that sampled individuals had made a recent elevational shift. δD_k values in claw tips (mean $= -37\%$) were less negative than feathers (mean $= -63\%$). We captured *C. mexicanus* individuals at the beginning of the breeding period (Stiles & Skutch 1989) at a known breeding site (K. Fraser, pers. obs.). If tail feather δD_k represents breeding altitude the previous season and claw δD_k represents *ca* 16 weeks prior to collection (Fraser *et al.* 2008), then our data indicate migration upslope in the weeks prior to breeding. This pattern has been reported for other species (Loiselle & Blake 1991). The difference between feather and claw δD_k is 26 per mil, which given a -2.2 per mil per 100 m elevation (Poage & Chamberlain 2001, Hobson *et al.* 2003), represents a shift of 1182 m and suggests that *C. mexicanus* migrated from lowland areas in Nicaragua.

Catharus mexicanus was reported as sedentary in Costa Rica (Stiles 1985a, Stiles & Skutch 1989, Loiselle & Blake 1991; Table 1). However, it is possible that migrating individuals escaped detection in prior study as many sedentary birds have later been reclassified as migratory (Winker *et al.* 1997, Berthold 2001).

Mionectes oleaginous was categorized as a partial altitudinal migrant (Loiselle & Blake 1991; Table 1). Significant differences between claws and feathers in this study (Fig. 1) suggest that all captured individuals were migratory. As with *C. mexicanus*, we caught *M. oleaginous* at the beginning of the breeding period (Stiles & Skutch 1989), thus an 18 per mil difference between claws and feathers reflecting 818 m of movement, likely represent upslope migration prior to breeding.

It has been suggested that intraspecies differences in diet can have an effect on δD values (Langin *et al.* 2007) therefore we wanted to be sure that differences in δD between feathers and claws reflect synthesis at different elevation and not dietary shifts at the same elevation. If intraspecies diet shifts were responsible for the differing δD_k values between feathers and claws, we would expect to find interspecies differences in feather δD_k reflecting known differences in prey items. However, tail feather δD_k values were not significantly different for our five study species ($F_{4,18} = 1.4$, $P = 0.27$, $N = 23$), suggesting known dietary differences between species did not have a measurable effect on δD values. To confirm differences in dietary intake between species, we analyzed the same feathers for $\delta^{15}N$, where the relative contribution of insects (higher $\delta^{15}N$) or fruit/nectar (lower $\delta^{15}N$) could be determined (Herrera *et al.* 2003). We found significant differences in $\delta^{15}N$ values in the same tail feathers ($F_{4,18} = 4.9$, $P < 0.05$, $N = 23$), reflecting expected dietary differences between species.

While it is reported that both frugivory and nectarivory commonly correlate with altitudinal movement (Levey & Stiles 1992) we did not detect altitudinal migration events in hummingbird species *P. longirostris*, *E. eximia*, or *C. hemileucurus*. However, date of capture (20 Feb–6 Mar) may have limited our ability to detect
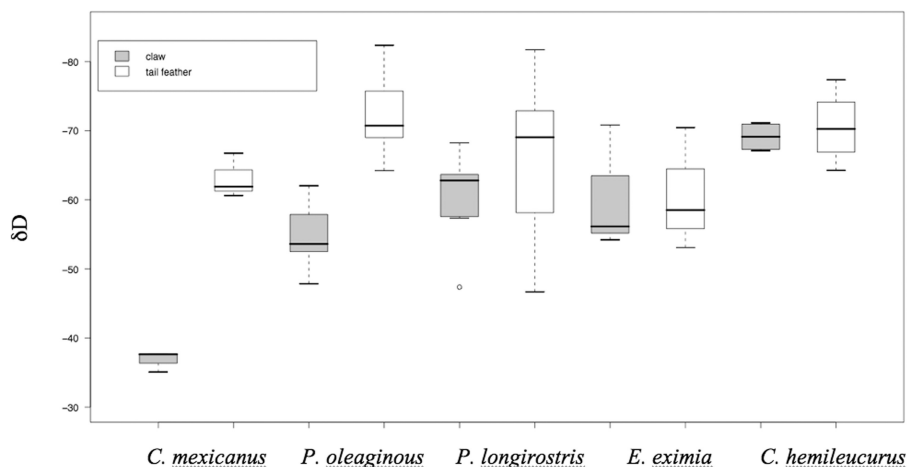
FIGURE 1. Boxplot (median, interquartile ranges and outliers) comparisons of claw versus tail for five Nicaraguan species (*C. mexicanus*, N = 3; *M. oleaginous*, N = 7; *P. longirostris*, N = 7; *E. eximia*, N = 3; *C. hemileucurus*, N = 4). Significant differences between feather and claw δD_k for *C. mexicanus* and *M. oleaginous* suggest these individuals are altitudinal migrants.

migration in these species. While the annual breeding period for most Neotropical species is April to August (Loiselle & Blake 1991), peak breeding and molting periods for hummingbirds in this region are November and December (Stiles 1988). If we expect migration upslope to occur in the weeks before breeding (Loiselle & Blake 1991) and claws retain premigratory δD values for ~16 weeks (Fraser *et al.* 2008), then potential δD_k values representing prebreeding origins may have been obscured by new growth. However, the large range of δD values for tail feathers in *P. longirostris* suggests that these individuals, while captured at the same elevation, may have molted at different elevations. Further sampling at different points in the annual cycle could further elucidate potential movement patterns in this species.

Claw δD was generally less variable than feather δD for four of the five species (Fig. 1). This suggests that there is greater intraspecific variability in molting altitude than nonbreeding altitude where claws were synthesized. This may indicate that altitudinal movements begin premolt in these species or that breeding occurs over a broader range of altitude than nonbreeding sites; however, further investigation is required.

Campylopterus hemileucurus is reported as an altitudinal migrant (Table 1) that breeds at high elevation (1500–2400 m) and may descend to 400 m postbreeding (Stiles & Skutch 1989). This may explain the more negative δD values we found for this species, which indicate that the tissues may have been grown at higher breeding elevation before descent and capture at lower elevation.

In summary, (1) comparisons of claw and tail δD values from individual birds can be used to detect altitudinal migration, and (2) this approach may reveal movement not detected by other methods. We emphasize that these comparisons may not be effective if molt, claw growth, and capture are close temporally (in these cases, sampling at various points in the annual cycle may be preferred). We found that partial-frugivores examined were altitudinal migrants, while nectarivores seemed to be more sedentary. Future work with larger sample size and more dietary guilds will enable us to further test the link between dietary preference and altitudinal migration.

TABLE 1. *Detection of altitudinal migration using two different methods.*

Species	Comparison of δD claw vs. tail	Seasonal fluctuations in capture rates/mark–recapture/pers. obs.
Catharus mexicanus	Yes	No (Stiles & Skutch 1989, Loiselle & Blake 1991)
Mionectes oleaginous	Yes	Yes/partial (Loiselle & Blake 1991)
Phaethornis longirostris	No	No, (Levey & Stiles 1992, Stiles & Wolf 1979)
Eupherusa eximia	No	Possible (Stiles & Skutch 1989)
Campylopterus hemileucurus	No	Yes (Skutch 1967, Stiles 1985b)

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