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## Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community

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**Abstract** Altitudinal migration and dispersal is an important component of the life history of several temperate and tropical birds but remains poorly understood due to the limited success of mark and recapture techniques. Stable isotopes of hydrogen ( $\delta\text{D}$ ) in rainfall, and to a lesser extent, carbon ( $\delta^{13}\text{C}$ ) in plants are known to change with altitude and hence may provide the basis of a technique for tracking the altitudinal movements in birds and other wildlife. We investigated the potential for this technique by measuring  $\delta^{13}\text{C}$ ,  $\delta\text{D}$ , and  $\delta^{15}\text{N}$  values in tail feathers of eight species of hummingbirds (*Phaethornis malaris*, *P. syrmatophorus*, *P. guy*, *Adelomyia melanogenys*, *Coeligena torquata*, *C. lutetiae*, *Metallura baroni*, *M. williami*) along an altitudinal gradient (300–3,290 m asl) in the Andes Mountains of Ecuador. Feather  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values were correlated and each changed significantly with elevation above 400 m. In general, we found good agreement between feather  $\delta\text{D}$  values and those predicted from a generalized relationship of precipitation and surface water  $\delta\text{D}$  with altitude. Similarly, feather  $\delta^{13}\text{C}$  values showed an enrichment of  $\sim 1.5\text{‰}$  per 1,000 m over the linear portion of the elevational response. Stable-

nitrogen isotope values were variable, and so did not provide useful information on elevation in birds, apart from trophic effects. Overall there appears to be good potential for using the ( $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ) stable isotope approach to track altitudinal movements and to elucidate previously unrecognized patterns of life history variation in both temperate and tropical species that migrate across elevational isotopic gradients.

**Keywords** Altitudinal migration · Carbon-13 · Deuterium · Elevational gradients · Nitrogen-15

### Introduction

Avian migration and dispersal, the respective periodic and permanent movement of individuals among areas, are compelling and often poorly understood aspects of the life history of numerous species throughout the world (Berthold 2001). While such movement patterns are often considered in two dimensions, typically as large-scale seasonal north-south geographical movements, the periodic or permanent vertical movements of birds inhabiting montane regions represents a third dimension of migration and dispersal (Johnston-Stewart and Hammer 1988). Such altitudinal movements are known to occur in both temperate (Dixon and Johnson 1964; Rabenold and Rabenold 1985; Laymon 1989) and tropical (Loiselle and Blake 1991; Johnson and Maclean 1994; Powell and Bjork 1994) avifauna, and are likely associated with climate and food availability at various altitudes in montane settings. In fact, altitudinal movements have long been suggested to be common among avian species at critical stages in their life history (Baldwin 1953; Moreau 1966). Moreover, recent work on connectivity of migrant birds indicates that an understanding of demographic conditions at all stages of the annual life cycle is necessary for interpreting population trends (Webster et al. 2002). To date, however, our understanding of these movements has been hindered due to the limitations of

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conventional approaches to tracking bird movements through mark and recapture techniques (Hobson 2002).

The more recent development of stable isotope techniques to track migratory animals represents a powerful alternative to these traditional approaches for cases where it can be demonstrated that individuals move between and feed in isotopically distinct regions (Hobson 1999a). In North America, the predictable continental pattern of deuterium ( $\delta D$ ) and  $\delta^{18}O$  in rainfall (e.g. Bowen and Wilkinson 2002) have been linked to food web resources (Hobson and Wassenaar 1997), and thereby used to infer geographical origins and migratory connections of several species by measuring hydrogen isotope ratios in key body tissues (Hobson and Wassenaar 1997, 2001; Chamberlain et al. 1997; Hobson et al. 2001; Kelly et al. 2001; Meehan et al. 2001; Wassenaar and Hobson 2000a, 2001; Rubenstein et al. 2002). Recently, it was suggested that altitudinal gradients in foodweb  $\delta D$  values could be used to evaluate philopatry and dispersal in Bicknell's thrush (*Catharus bicknelli*), now restricted to a few mountainous sites in northeastern USA and Canada (Hobson et al. 2001). Investigations on the effects of altitude on feather  $\delta^{13}C$  and  $\delta^{15}N$  values in second-year and after-second year male black-throated blue warblers (*Dendroica caerulescens*) breeding in the southern Appalachians, USA (Graves et al. 2002) showed no effects of altitude on  $\delta^{15}N$  values and a small ( $R^2=0.09$ ) but significant ( $P<0.0001$ ) effect of altitude on  $\delta^{13}C$  values of feathers of older males. That study provided some evidence that  $\delta^{13}C$  analyses might be used to examine philopatry and dispersal in birds breeding in mountainous areas. However, the large variance in their data unexplained by altitude and age clearly raises questions as to how useful the stable isotope approach might be in avian altitudinal investigations in general. Clearly, more investigations are required in other systems, over larger elevational gradients and using other isotopes and tests.

Altitudinal gradients in plant  $\delta^{13}C$  values have been demonstrated by several authors and are thought to be associated with plant physiological adaptations to changes in growing conditions and  $pCO_2$  with elevation. Other factors influencing isotopic changes in plant tissues include water-use efficiency, soil moisture, ambient humidity, and vapor pressure (Körner and Diemer 1987; Marshall and Zhang 1994; Sparks and Ehleringer 1997; Hultine and Marshall 2000). The combined result of these processes is typically, but not always, a slight enrichment in plant  $^{13}C$  content with elevation by a few per mil over a 1,000 or more meters (Körner et al. 1988, 1991). Far less is known about altitudinal patterns of  $\delta^{15}N$  in foodwebs, if any, but the high spatial variability in this isotope due to a variety of other factors such as plant community, climate, soil type, and soil processes makes its potential use for tracing altitudinal movements of birds problematic (Nadelhoffer and Fry 1994; Hobson 1999a). However, there has been limited evidence that soil  $\delta^{15}N$  values may become more negative with altitude at a given location (Mariotti et al. 1980) and that these patterns may also be reflected in local foodwebs (Gröcke et al. 1997).

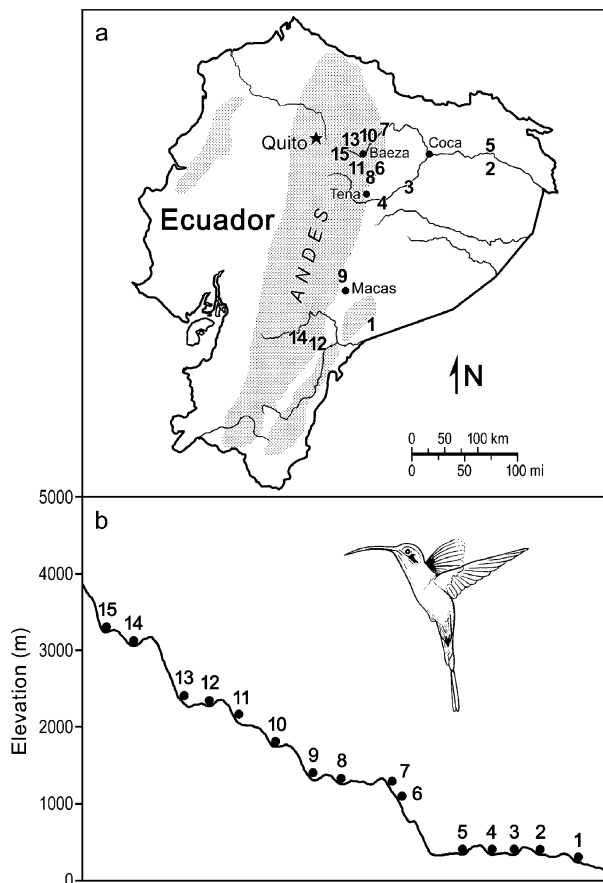
Plants ultimately obtain their hydrogen from rainfall, ground and surface source waters. Strong altitudinal gradients in precipitation  $\delta D$  and  $\delta^{18}O$  values have long been known for mountain ranges throughout the world (Dansgaard 1964; Siegenthaler and Oeschger 1980; Poage and Chamberlain 2001; Bowen and Wilkinson 2002). This depletion effect results in part from Rayleigh distillation and the depletion of  $^{18}O$  and deuterium in residual moisture as an air mass rises orographically and loses moisture to rainfall and to decreasing temperature. In general, one would anticipate a relative depletion in plant  $^{18}O$  and deuterium content and an enrichment in  $^{13}C$  with altitude at a given latitude or geographic area. If these expected patterns are passed on to upper trophic-level consumers through consumption of plants or herbivorous insects over an altitudinal gradient, then one may expect certain animal tissues (e.g. feathers) to reflect the isotopic values ( $\delta D$ ,  $\delta^{13}C$ ) of the forage and diet at the elevation of feeding. Such an elevational isotopic marker is precisely what is required to trace birds that undergo altitudinal migration or dispersal. Feathers are particularly useful records of isotopic signatures with elevation since they are expected to represent such information during the relatively short period of feather growth that typically occurs annually in most species (Hobson 1999b).

Here we measured  $\delta^{13}C$ ,  $\delta^{15}N$ , and  $\delta D$  values of feathers of eight species of hummingbirds (*Phaethornis malaris*, *P. syrmatophorus*, *P. guy*, *Adelomyia melanogenys*, *Coeligena torquata*, *C. lutetiae*, *Metallura baroni*, *M. williami*) distributed along a large altitudinal gradient in the Ecuadorean Andes. We hypothesized that these birds would show increasing  $\delta^{13}C$  values and decreasing  $\delta D$  values corresponding to the altitude at which they were captured. We also looked for evidence of altitudinal migration using departures of feather isotope values from those values expected from locations where birds were sampled.

## Materials and methods

### Study area

Our study area covers an altitudinal (300–3,290 m) gradient in the eastern slope of the Andes of Ecuador (Fig. 1). Sampling localities, elevation and dates for the 15 sites were: (1) Miazal, S2°38.160' W77°47.880', 300 m, 8–12 August 2000; (2) Tiputini, S0°38.220' W76°8.940', 400 m, 16–18 January 2000; (3) Loreto, S0°41.640' W77°18.660', 400 m, 27–29 August 2000; (4) Jatun Sacha, S1°4.320' W77°37.260', 400 m, 28–31 December 1999; (5) Pañacocha, S0°23.880' W76°7.440', 400 m, 25–29 January 2000; (6) Hollin, S0°41.340' W77°43.620', 1,200 m, 3–4 January 1999; (7) San Rafael, S0°6.060' W77°35.040', 1,300 m, 19–22 August 2000; (8) Hollin2, S0°43.320' W77°44.400', 1,320 m, 24–26 August 2000; (9) Sangay, S2°5.940' W78°9.120', 1,350–1,400 m, 9–12 August 2000; (10) Santa Maria, S0°15.360', W78°51.480', 1,800 m, 20–21 December 1999; (11) Yanayacu, S0°35.931' W77°53.372', 2,160 m, 22–26 August 2000; (12) Cerro Bosco, S3°0.161' W78°30.583', 2,340 m, 2–6 September 2000; (13) Chalpi, S0°15.360' W77°57.96', 2,400 m, December 1999; (14) Patacocha, S3°0.267' W78°38.842', 3,120 m, 8–10 September



**Fig. 1** Study site showing **a** location where hummingbirds were sampled in Ecuador and **b** the relative locations of sites with respect to the elevational gradient. 1 Miazal, 2 Tiputini, 3 Loreto, 4 Jatun Sacha, 5 Pañacocho, 6 Hollin, 7 San Rafael, 8 Hollin2, 9 Sangay, 10 Santa Maria, 11 Yanayacu, 12 Cerro Bosco, 13 Chalpi, 14 Patacocho, 15 Papallacta

2000; (15) Papallacta, S0°20.588' W78°9.344', 3,290 m, 28–29 August 2000 (Table 1).

Birds were captured by mistnetting, banded, mensural measurements taken, and sampled for blood and feathers prior to release. Feathers were obtained by collecting the two outer rectrices from each individual. Samples were kept at ambient temperatures until they were returned to the laboratory, when they were archived at 20°C. We obtained tail feathers from eight species of hummingbirds representing 128 individuals (Table 1).

#### Stable isotope measurements

Hummingbird feathers were cleaned of surface oils using a 2:1 chloroform:methanol solution and then dried in a fume hood. For  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analyses, we subsampled approximately 1 mg by weighing feather material into small tin cups. These samples were then analyzed on a Europa 20:20 continuous-flow isotope-ratio mass spectrometer interfaced with a Robo Prep elemental analyzer. Measurements are reported in  $\delta$ -notation relative to the PDB standard in parts per thousand deviation. Measurement error is estimated at  $\pm 0.1\text{‰}$  and  $0.3\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively.

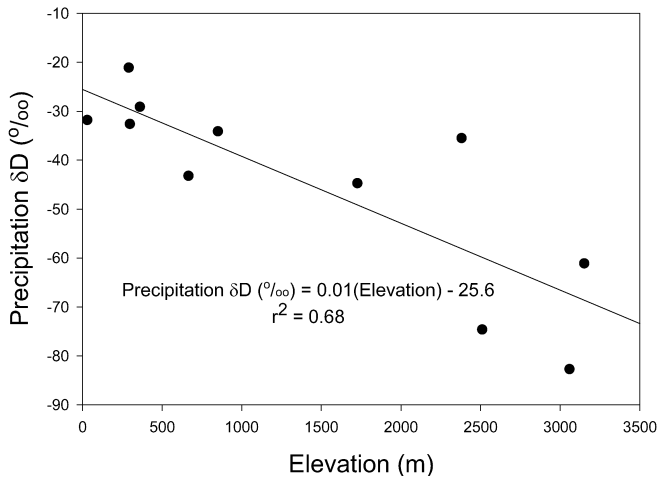
Stable-hydrogen isotope analyses of feathers are complicated compared to  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  due to the problem of uncontrolled isotopic exchange between a percentage of non-carbon bound hydrogen in the samples and ambient water vapor (Wassenaar and Hobson 2000b). Elsewhere we describe how we routinely use keratin standards and comparative equilibration as a means of correcting for this effect so that the values reported here are non-exchangeable feather hydrogen (Wassenaar and Hobson 2003).

Stable hydrogen isotope measurements on feathers and keratin standards were performed on  $\text{H}_2$  derived from high-temperature flash pyrolysis of feathers and CF-IRMS. Pure  $\text{H}_2$  was used as the sample analysis gas and the isotopic reference gas. A Eurovector 3000 high temperature elemental analyzer with autosampler was used to automatically pyrolyse feather samples to a single pulse of  $\text{H}_2$  gas (and  $\text{N}_2$  and  $\text{CO}$  gas). The resolved  $\text{H}_2$  sample pulse was then introduced to the isotope ratio mass spectrometer (Micromass Isoprime with electrostatic analyzer) via an open split capillary. All  $\delta\text{D}$  results are expressed in the typical delta notation, in units of per mil (‰), and normalized on the VSMOW-SLAP standard scale. Repeated analyses of hydrogen isotope reference material IAEA-

**Table 1** Ecuadorean hummingbird sample analyzed and results (mean $\pm$ SD) of stable carbon and hydrogen isotope analysis. For  $\delta\text{D}$  analyses, values in parentheses are sample sizes used for that

isotope where this differs from that of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analyses. Elevation Elevation of capture, NA not analyzed. Site number corresponds to Fig. 1b

Location	Elevation (m)	Species (n)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta\text{D}$ (‰)
1. Miazal	300	<i>Phaethornis malaris</i> (13)	8.0 $\pm$ 0.7	-26.25 $\pm$ 0.36	-82.7 $\pm$ 7.7
2. Tiputini	400	<i>P. malaris</i> (6)	10.0 $\pm$ 0.2	-26.1 $\pm$ 0.3	-75.9 $\pm$ 11.8
3. Loreto	400	<i>P. malaris</i> (11)	9.8 $\pm$ 0.8	-26.3 $\pm$ 0.4	-82.7 $\pm$ 7.7
4. Jatun Sacha	400	<i>P. malaris</i> (5)	9.2 $\pm$ 1.0	-25.6 $\pm$ 0.6	NA
5. Pañacocho	400	<i>P. malaris</i> (12)	9.5 $\pm$ 0.6	-26.2 $\pm$ 1.2	-83.3 $\pm$ 6.3 (5)
6. Hollin	1,100	<i>P. guy</i> (2)	8.7, 7.5	-26.1, -26.3	NA
7. San Rafael	1,300	<i>P. syrmatophorus</i> (5)	5.2 $\pm$ 0.4	-26.2 $\pm$ 0.4	NA
		<i>P. guy</i> (10)	5.5 $\pm$ 0.8	-26.0 $\pm$ 0.5	-83.3 $\pm$ 18.4 (5)
		<i>Adelomyia melanogenys</i> (3)	4.9 $\pm$ 0.6	-25.2 $\pm$ 0.5	-91.0 $\pm$ 2.7
8. Hollin2	1,320	<i>P. guy</i> (2)	7.4, 7.2	-26.5, -26.1	NA
9. Sangay	1,350–1,400	<i>A. melanogenys</i> (7)	6.8 $\pm$ 0.4	-25.8 $\pm$ 0.9	-71.9 $\pm$ 8.9
10. Santa Maria	1,800	<i>P. syrmatophorus</i> (11)	6.8 $\pm$ 0.8	-25.4 $\pm$ 0.6	-87.6 $\pm$ 7.8
		<i>A. melanogenys</i> (6)	5.7 $\pm$ 1.3	-24.1 $\pm$ 0.77	-92.1 $\pm$ 5.2
11. Yanayacu	2,160	<i>P. syrmatophorus</i> (2)	9.5, 9.5	-25.9, -25.6	-104.2, -102.2
		<i>A. melanogenys</i> (6)	6.8 $\pm$ 1.3	-24.4 $\pm$ 0.6	-100.9 $\pm$ 6.4
		<i>Coeligena torquata</i> (4)	8.0 $\pm$ 0.4	-23.8 $\pm$ 0.5	-105 $\pm$ 4.7
12. Cerro Bosco	2,340	<i>C. torquata</i> (5)	6.8 $\pm$ 1.1	-24.3 $\pm$ 0.25	-90.4 $\pm$ 4.7
13. Chalpi	2,400	<i>A. melanogenys</i> (6)	7.4 $\pm$ 0.9	-24.2 $\pm$ 0.5	-90.8 $\pm$ 5.6
14. Patacocho	3,120	<i>Metallura baroni</i> (4)	9.3 $\pm$ 1.3	-23.6 $\pm$ 0.5	-106.4 $\pm$ 5.1
15. Papallacta	3,290	<i>C. lutetiae</i> (4)	8.0 $\pm$ 0.8	-24.3 $\pm$ 0.5	-108.0 $\pm$ 3.9
		<i>M. williami</i> (4)	6.7 $\pm$ 0.4	-24.0 $\pm$ 0.5	-111.5 $\pm$ 2.8



**Fig. 2** Relationship between weighted mean annual  $\delta D$  in precipitation and elevation for Ecuador. Data based on IAEA data publicly available through the Global Network of Isotopes in Precipitation (IAEA 2000)

CH-7 ( $-100\text{‰}$ ), routinely included as a check, yielded an external repeatability of better than  $\pm 1.5\text{‰}$ .

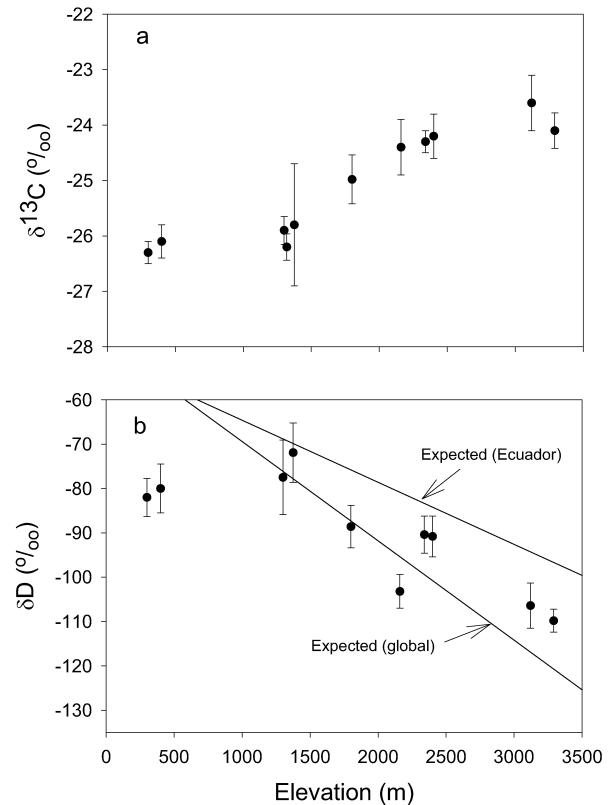
Expected deuterium response with elevation

Empirical relationships between elevation and the isotopic composition of precipitation and surface waters throughout the world over the past five decades have been summarised recently (Poage and Chamberlain 2001; Bowen and Wilkinson 2002). These reviews describe the isotope depletion and trends in  $^{18}\text{O}$  with altitude ( $\sim 0.28\text{‰}$  per 100 m) and other factors. Existing precipitation stable isotope data for Ecuador includes 12 sites covering elevations from sea level to 3,150 masl (IAEA 2001). A plot of mean weighted annual  $\delta D$  versus elevation is shown in Fig. 2. We used these data to derive an expected mean  $\delta D$  value of  $-25.6\text{‰}$  near sea level and a depletion trend of  $\delta D = -0.014 \times \text{altitude (masl)} - 25.6$ . We then applied an isotopic fractionation factor of  $-25\text{‰}$  between water available to the local plant community and feathers ( $\delta D_f$ ; Wassenaar and Hobson 2001) to obtain the expected relationship between elevation ( $E$ ) and feather  $\delta D$  values across our altitudinal gradient:

$$\delta D_f = -25.6 + E(-0.014) - 25\text{‰} \quad (1)$$

Equation 1 should predict the expected  $\delta D$  value of feathers of hummingbirds grown at a specific altitude in Ecuador. We used currently available precipitation data and assume here the  $-25\text{‰}$  isotope fractionation between feather and water previously determined for red-winged blackbirds (*Agelaius phoeniceus*) is valid for these hummingbirds. The overall  $\sim 3^\circ$  latitudinal spread between our sampling stations would only have a very minor effect on our expected altitudinal relationships (i.e. potentially up to  $4\text{‰}$ ), based on Eq. 3 in Bowen and Wilkinson (2002). We also considered the expected feather  $\delta D$  vs. altitude pattern derived from the global database as described by Poage and Chamberlain (2001). Here,  $\delta^{18}\text{O}$  values of about  $-4\text{‰}$  at sea level corresponded to an expected mean  $\delta D$  value of  $-22\text{‰}$  (Craig 1961), and a depletion trend for  $\delta D$  of  $-2.2\text{‰}/100$  m. Applying again the assumed net trophic isotopic fractionation factor of  $-25\text{‰}$  between feather and water ( $\delta D_f$ ), we obtained the following predicted relationship between  $E$  and feather  $\delta D$  values across our altitudinal gradient:

$$\delta D_f = -22 + E(0.0224) - 25\text{‰} \quad (2)$$



**Fig. 3** Relationship between **a** stable-carbon and **b** deuterium isotope values in hummingbird feathers along an elevational gradient in Ecuador. Stable isotope values are pooled for all species occurring at a particular altitude (see Table 1) and are expressed as  $\text{mean} \pm 2\text{SE}$ . Only those sites with at least three individuals are presented. The expected relationships are based on empirical studies relating precipitation and surface water  $\delta D$  values and elevation for Ecuador and other regions (see Materials and methods) and the application of a  $-25\text{‰}$  net isotope fraction between feather and precipitation

## Results

Stable-isotope values of hummingbird feathers showed a considerable range of variation in elements among locations (Table 1). Stable-carbon isotope values varied significantly with elevation ( $F_{6,126}=4.8$ ,  $P<0.001$ ) and with species ( $F_{4,126}=5.7$ ,  $P<0.001$ ) with no interaction effects ( $F_{3,126}=1.2$ ,  $P=0.31$ ). We found a steady rise in  $\delta^{13}\text{C}$  values from 1,300 to 3,120 m ( $R^2=0.54$ ,  $P<0.001$ ) with a slope of  $1.5\text{‰}$  per 1,000 m in this elevational range. We also observed evidence of a possible plateau effect at the highest and lowest elevations (Fig. 3a).

Deuterium values of hummingbird feathers similarly showed a significant response with elevation ( $F_{6,102}=5.4$ ,  $P<0.001$ ) and species ( $F_{4,102}=2.9$ ,  $P=0.05$ ). Above 400 m elevation,  $\delta D$  values declined with altitude ( $R^2=0.53$ ,  $P<0.001$ ). Feather  $\delta D$  and  $\delta^{13}\text{C}$  values were correlated ( $R^2=0.34$ ,  $P<0.001$ ) but no correlation was found among all other isotope combinations involving  $\delta^{15}\text{N}$  ( $R^2<0.03$ ,  $P>0.05$ ). We compared our observed  $\delta D$  measurements with those values that were expected at each location



based on the linear relationships derived from Eqs. 1 and 2. Currently, error values associated with the expected linear relationship of  $\delta D_f$  with elevation are not available and so we could not adequately test for statistically significant departures from the expected relationships. However, by graphical inspection alone, we noted that bird feathers obtained at the two highest elevations tended to show more positive values than that expected from the global average relationship, and the birds at the lowest elevation (300–400 m) were more negative than expected (Fig. 3b).

For  $\delta^{15}N$  measurements, significant influences of both elevation ( $F_{6,126}=19.5$ ,  $P<0.001$ ) and species ( $F_{4,126}=5.6$ ,  $P<0.001$ ) were found together with a significant interaction effect ( $F_{3,126}=3.8$ ,  $P=0.012$ ) driven by the fact that *Phaethornis malaris* was present exclusively at the lowest elevations that were among the most positive. The association between  $\delta^{15}N$  values and elevation was weak ( $R^2=0.14$ ,  $P<0.001$ ) with the most positive values occurring at both low (400 m) and high (3,120 m) elevations (Fig. 3). By excluding the two lowest elevations (300–400m), the relationship improved ( $R^2=0.28$ ,  $P<0.001$ ) with some evidence of a small positive altitudinal effect on  $^{15}N$  above these lower regions.

At the Loreto site (400 m) *P. malaris* was sampled during August ( $n=6$ ) and December ( $n=5$ ). However, for all isotopes, we found no significant difference in measurements between these two time periods ( $t<1.0$ ,  $P>0.35$  in all cases).

## Discussion

We found a strong relationship between hummingbird feather  $\delta^{13}C$  and  $\delta D$  values and elevation in the Ecuadorean Andes. These patterns follow from previously established elevational patterns of  $\delta^{13}C$  values in plants (Lajtha and Marshall 1994) and  $\delta D$  values of precipitation and surface water values throughout the mountain ranges of the world (Poage and Chamberlain 2001; Bowen and Wilkinson 2002). Less elevational coupling was found for feather  $\delta^{15}N$  values, with a weak positive trend being counter to the findings of the few previous studies on soils [Mariotti et al. 1980; Gröcke et al. 1997; see also Dawson et al. (2002) for an overview of complexities associated with plant  $\delta^{15}N$  values]. Our results confirm that stable-carbon and hydrogen isotope analyses may be used to track altitudinal migration and dispersal in birds inhabiting mountainous regions.

The unambiguous isotopic association of an individual feather sample with altitude may be complicated by the fact that we generally have poor predictive power for most stable isotopes for any given region and across landscapes. Variability among altitudinal transects depend on a variety of climatic and edaphic factors that covary with altitude. Most previous research has focussed on plant  $\delta^{13}C$  response to altitude and here previous research reports about a 1.1‰ enrichment per 1,000 m for  $C_3$  plants (Körner et al. 1991). We found a slightly higher

value of 1.5‰ per 1,000 m for hummingbird feathers over the 1,300–3,120 m gradient but we have no information on the relative contributions of  $C_3$  and  $C_4$  plants to hummingbird diet along this transect, although  $\delta^{13}C$  values support a primarily  $C_3$ -based foodweb. Graves et al. (2002) similarly found a higher feather  $\delta^{13}C$  response to altitude of ~1.3‰ per 1,000 m). The general similarity in feather elevational  $\delta^{13}C$  response over the linear portion of our data with previous isotopic studies on plants and feathers is encouraging.

Above 400 m, we found a remarkably good fit between feather  $\delta D$  values and those expected from a model based on global patterns in precipitation and precipitation results reported for Ecuador and elsewhere (Eq. 2 in Fig. 2b; IAEA 2001; Poage and Chamberlain 2001; Bowen and Wilkinson 2002). The relationship based on the actual Ecuadorean precipitation dataset (Eq. 1) generally showed that birds had more depleted  $\delta D$  values compared to that predicted from Eq. 1. Overall, this would suggest that feathers were grown at higher elevations than where birds were captured. However, the Ecuadorean isotopic precipitation dataset is relatively sparse (IAEA 2001). Presumably, the expected feather vs. precipitation  $\delta D$  relationship could be modified regionally to provide predicted feather  $\delta D$  values as a first approximation to identifying movements of altitudinal migrants. Currently, without a good estimate of the error associated with the expected regression of  $\delta D_f$  and elevation, we are unable to statistically determine true departures of individuals and species from this regression line. However, some patterns are apparent. For example, all three isotope measurements suggest that *P. malaris* captured at 300–400 m had feather isotope values that were inconsistent with the elevational trends. Notably,  $\delta^{15}N$  and  $\delta D$  values were more positive and  $\delta^{13}C$  values more negative than expected. Taken together, this suggests that these birds may have molted their feathers at lower elevation than where they were captured (i.e. 1,400–2,000 m). This species is described as occurring generally below 1,000 m (Ridgely and Greenfield 2001). Whether isotope values for this species represent altitudinal migration or some other factor will require further research. It is also possible that anthropogenic influences may have caused this isotopic effect. High  $\delta^{15}N$  values are associated with agriculture (Hebert and Wassenaar 2001; Hobson 1999b) and depleted  $\delta D$  values might correspond with the use of water derived from runoff from higher elevations. We note that *P. malaris* was typically captured along streams and rivers at these low elevations.

At higher altitudes, the  $\delta^{15}N$  and  $\delta^{13}C$  values suggest that *Metallura baroni* at 3,290 m molted their feathers at lower elevation than their capture site. Comparison of feather  $\delta D$  with those expected for this species showed a less obvious effect at that altitude. However feather  $\delta D$  also generally suggested that hummingbirds captured at higher elevation (i.e. above 2,400 m) may molt at lower elevations. Molting is likely an energetically demanding period for hummingbirds (Lindstrom et al. 1993) and they may be forced to move to elevations where nectar

resources are most abundant during periods in which they molt. On the other hand, *P. syrmatophorus* was sampled at 1,300, 1,800 and 2,160 m and isotopic values differed at those elevations and matched those of other species found at those elevations. This suggests that this species did not molt at elevations other than those at which it was sampled.

We conclude that there is good potential for using the stable isotope approach ( $\delta D$ ,  $\delta^{13}C$ ) to track altitudinal migration and dispersal in both temperate and tropical species and believe the likelihood of elucidating previously unrecognized patterns of life history is high. Ideally, elevational gradients in stable isotope values of foodwebs driving nutritional requirements of birds will be determined first so that more accurate expected feather isotope values can be established. For species like hummingbirds that may specialize on a few plant species, such an approach could result in a reasonably robust model. Other, generalist species or species that change their diets seasonally or with elevation may prove to be more challenging. In addition, while feather samples provide a convenient means of establishing elevation during the molt period, metabolically active tissues such as blood serum or plasma could provide year-round elevational information for those species that can be captured routinely.

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