SEASONAL MIGRATION AND DIET USE IN A NEOTROPICAL COMMUNITY OF BIRDS AND BATS

by

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ABSTRACT

Temperature varies seasonally only slightly in the Neotropics but birds and bats show distinct seasonality in the timing of energetic events such as breeding, moulting and migration. This thesis explores the seasonality of bird diet, microhabitat use and altitudinal migration of birds and bats in a Nicaraguan cloud forest, using advanced methods in stable-isotope analysis to track diet and migration. I test several hypotheses proposed to explain the variation in stable-hydrogen isotope ratios (δD) in animal tissues. I used δD analyses of claws, feathers, arthropods, fruit and precipitation to show, for the first time, that micro-spatial and temporal factors, diet, species and body size all significantly influence δD values in a Neotropical cloud forest bird community. I also examined the utility of δD analyses for tracking altitudinal migration in bats. I conclude that broad local variation may limit the use of δD to track birds and bats over large distances but also suggests new applications for tracking seasonal micro-scale habitat use and moult in cloud forest and agroecosystems. Using mark-recapture methods I observed a Neotropical hummingbird (*Amazilia cynaura*) that migrates altitudinally to moult. Movement upslope is usually associated with breeding but this was not the case in *A. cyanura*. This is the first documented example of a hummingbird, or any Neotropical bird, migrating to moult. Using stable-nitrogen isotope analyses of bird tissues I conducted the first test of the diet complementation hypothesis in a community of free-living tropical birds. I predicted that birds would increase their protein consumption during breeding and moulting to meet their energetic and nutritional needs. I show that most species (11 of 14) increased their protein consumption during the breeding and moulting period. These results suggest that seasonal appetites for specific nutrients may influence food selection in Neotropical birds. My conclusions support studies and theoretical work that make a link between rainfall seasonality and the timing of migration, breeding and moulting in Neotropical birds and bats. Future research should be aimed at determining the link between the energetic and nutritive needs of birds and bats and the timing of these behaviours.

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CHAPTER 1: GENERAL INTRODUCTION

Most animals experience some degree of seasonal change in their environment in climate, food abundance and social factors (Hau *et al.* 2008). In the tropics, where the majority of vertebrate species live, seasonal variation was thought to be negligible, such that tropical animals existed in relatively stable physical environments (e.g. MacArthur 1972). However, several decades of work with birds in tropical forests have revealed a wealth of phenological change where seasonal temperature varies less than 5°C over an annual cycle (e.g. Moreau 1936, Belcher & Smooker 1937, Skutch 1950, Snow & Snow 1964, Ward 1969, Fogden 1972, Diamond 1974, Karr & Freemark 1983). While temperature may vary only slightly on an annual basis, other climatic factors such as precipitation may vary more dramatically and exert a greater influence (Fogden 1972, Hau *et al.* 2000, Stutchbury & Morton 2001). However, despite extensive research on phenology (seasonal change) in tropical forests we still lack a solid understanding of the link between the physical environment and the timing of annual events, such as breeding and migration, in tropical animals (Hau *et al.* 2008). The phenology of thousands of Neotropical bird and bat species has yet to be explored. A plethora of recent research has focused on seasonal cycles in Neotropical birds and bats that may time breeding or moult to coordinate with peak resource availability, track seasonal shifts in resources through migration, or may shift diets seasonally (e.g.s Hau *et al.* 2008, Mello *et al.* 2009, Becker *et al.* 2010, Boyle *et al.* 2010, Greeney 2010, Jahn *et al.* 2010).

Box 1: Definition of Neotropical resident birds and bats

In this thesis, a Neotropical bird or bat is a species that spends part or all of its life cycle in the Western Hemisphere between 23°26'N (tropic of Cancer) and 23°26'S (tropic of Capricorn). Bird and bat diversity reaches its peak in this region and most bird and bat families originate in the tropics (Levey & Stiles 1992, Popa-Lisseanu & Voigt 2009). There are more than 3500 species of Neotropical birds and 130 species of bats (Kricher 1997, Reid 1997).

While many Neotropical species spend only part of their lifecycle in the Neotropics (many migrate to north-temperate areas to breed), this thesis refers only to Neotropical 'resident' birds and bats, that is, species that spend all of their life cycle within the Neotropics even if migration within this region is a part of their lifecycle. 'Sedentary' birds and bats are species that show little or no seasonal movement, and spend their life cycle within or near a breeding area.

This thesis explores seasonal shifts in the behaviour of a Neotropical bird and bat community (Box 1) in a Nicaraguan cloud forest. It is also an exploration of stable isotope methods that may be used to track seasonal habitat use, diet and migration. In Chapter 2, I examine the influence of seasonal and spatial hydrological regimes on the stable isotope values of a cloud forest bird community through tests of several hypotheses relating to factors driving stable-hydrogen isotope variation in animal tissues. In Chapter 3, I study seasonal shifts in diet by testing the hypothesis that birds increase their protein consumption during periods of high energetic demand (breeding and moulting). In Chapters 4 and 5, I examine seasonal moult and migration in a hummingbird and a bat community. In Chapter 6, I discuss the implications of my findings and outline future work. The context for these investigations and rationale for study are detailed in the sections below. More general background information is included in textboxes (1-3).

Seasonality and the influence of the abiotic environment

The 'Tropics' are defined as the space framed between the tropics of Cancer and Capricorn (parallels at 23°26'). My work took place within the Neotropics, or New World tropics, in Central America (further details in Textbox 2). Of greater biological relevance, my study areas were within the 'humid tropics', defined generally as areas within the tropics that receive greater than 450-650 mm of rainfall annually (McGregor & Nieuwolt 1998). The humid tropics lack a 'cold' season, and there is little annual temperature variation; daily temperature ranges may be greater than annual ones (McGregor & Nieuwolt 1998). While the influence of temperature on the biotic environment may be slight, the influence of seasonal hydrological cycles is large. To varying degrees, areas in the Neotropics experience distinct dry and wet seasons, each lasting several months. These patterns are driven by shifts in the Intertropical Convergence Zone (ITCZ), a moving low-pressure zone associated with the seasonal influence of the sun. The movement of the zone northward is associated with the wet season; that generally occurs in my research area from May to November, sometimes with a brief pause in July and August (often referred to as the 'veranillo' or 'little summer'). The dry season, generally from December to April, is associated with the

Box 2: Study area

Fieldwork for my thesis took place in Nicaragua, Central America. My sites were located in northwestern Nicaragua, in the Cordillera Isabelia, at high elevation sites (greater than 1000m) in areas that can be classified as lower-montane forest (Holdridge 1967) (see Fig. 1-1). For more specific details on study location see Chapter methods.

More generically, the study sites can be classified as tropical rainforest or cloud forest. Cloud forests can be defined as areas that are frequently covered in cloud or mist and usually occur between 1500 and 3000m above sea level (Hamilton *et al.* 1995). They represent 2.5% of the total area of tropical forest (Cayuela *et al.* 2006), are storehouses of biodiversity, exhibit high rates of endemism and are a globally threatened habitat type (Cayeuela *et al.* 2006, Beiderweiden *et al.* 2007) as they are disappearing 'rapidly' (Bruijnzeel & Hamilton 2000). Cloud forests provide ecological services, the most important being that of water capture (Bruijnzeel 2001); however, these hydrological cycles may be interrupted by deforestation and global warming (Lawton *et al.* 2001). In the Neotropical bird and bat literature, far more attention has been paid to lowland forest, particularly lowland forests in Costa Rica. The movement and diet of many of the species in this thesis have never been studied, and these chapters contribute to the available information on cloud forest species.

southward movement of the ITCZ (Clark *et al.* 2000). Seasonal moisture availability may largely determine the phenology of the biotic environment. For example, in birds, it may drive prey availability, migration, breeding seasons, habitat selection and activity patterns on a spatio-temporal basis (e.g. Karr & Freemark 1983). However, the links between abiotic and biotic factors in tropical evergreen forests are still poorly understood and represent a significant gap in our knowledge (Clark *et al.* 2000). A major first step is to determine the seasonal changes in diet, movement and spatial habitat use that are characteristic of Neotropical bird and bat communities.

Unfortunately, it is logistically difficult to directly track diet and movements of birds and bats through multiple seasons. Instead, there is a pressing need for indirect indicators of past movements that can be interpreted from a single capture of an animal. One promising technique is the use of stable-hydrogen isotope analysis. In Chapter 2, I test several hypotheses for factors that drive δD (ratio of ^2H to ^1H) variation in animal tissues and in Chapter 5 use δD to detect altitudinal migration in bats. δD variation is strongly tied to spatial hydrological patterns. Much of the variation in stable-hydrogen isotope values can be explained by latitudinal or altitudinal variation in precipitation δD. These precipitation patterns are largely controlled by temperature-driven loss of the heavy isotope from air masses as they move to cooler areas at higher latitudes or elevation (Bowen *et al.* 2005). These values are incorporated into developing animal tissues through food webs and drinking water and may be used to track their movements at broad altitudinal (Hobson *et al.* 2003, Fraser *et al.* 2008, Hardesty & Fraser 2010) or latitudinal scales (reviewed in Hobson 2008) (Box 3). Indeed, the altitudinal structure of δD variation reported in previous studies (Poage & Chamberlain 2001, Hobson *et al.* 2003), provided the impetus for its use in my own explorations of bat migration (Chapter 5).

While factors influencing δD across broad geographic scales are well understood, much of the variation in stable-hydrogen isotope values at smaller scales remains unexplained (Langin *et al.* 2007, Betini *et al.* 2009) and may limit the utility of δD in migration studies (discussed in Farmer *et al.* 2008). One hypothesis proposed to explain this variation is that δD may vary at 'microgeographic' scales (e.g. within a single breeding population) through spatio-temporal variance in source water inputs to local food webs (Betini *et al.* 2009). This variation has important implications for migration studies, but it may also be used to understand more subtle patterns of spatio-temporal resource use and moult timing in animals. The seasonal and microgeographic hydrological patterns in cloud forest environments (described above) represent an ideal opportunity to test competing hypotheses for factors driving δD variation in animal tissues. In Chapter 2, I test the influence of diet, habitat use, and seasonal change in precipitation δD on the tissue values of a community of resident Nicaraguan birds. Understanding factors driving δD variation at small spatial and temporal scales may not only improve our ability to track animals over smaller distances, it may also reveal micro-scale patterns of habitat use and phenological change useful to animal ecologists working in these systems.

Phenology

Despite the relatively low variation in seasonal tempertures in the Neotropics most birds studied here have well-defined breeding seasons (Hau *et al.* 2000, Wikelski *et al.* 2000). The proximate factors controlling timing of breeding and other events may be

Box 3: Stable isotope analysis (SIA)

The recent decade has seen a marked increase in the use of stable-isotope analysis in animal ecology studies (Martinez del Rio *et al.* 2009). While uses and applications of the three most commonly used stable isotopes of the elements (H, N, C) vary widely and are constantly expanding, there are a few basic principles of variation that provide most of the substrate on which isotopic studies are based. The ratio of the heavy to light isotope is expressed in δ-notation as part-per-thousand (‰) deviations from a standard.

Stable-hydrogen isotopes

Stable-isotope ratios of hydrogen ($^2H/^1H$ expressed as δD) in water are influenced by temperature, amount of precipitation and evaporation leading to broad latitudinal and altitudinal trends in δD values (Clark & Fritz 1997). δD values of water entering the food web are transferred to the developing tissues of higher-order consumers such as birds and bats. Once incorporated, δD values may be used to track migratory animals back to the area of tissue synthesis, when they are caught and sampled at distant locations. However, the current geographic resolution of δD limits our ability to track animals at broad spatial scales (greater than 7 degrees of latitude, Farmer *et al.* 2008; greater than 1000 m altitude, Hardesty & Fraser 2010). Thus, there is great impetus to better understand factors that drive δD variability at smaller spatial scales in order to better track animal movement (Betini *et al.* 2009). Small-scale variation in δD may also be employed to study seasonal diet and resource use or moulting phenology in birds and bats, but this remains little explored (see Chapter 2 & 5).

Stable-nitrogen and carbon isotopes

Stable-isotope ratios of nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) and carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) are commonly used in food web studies (Post 2002). Typically, $\delta^{15}N$ values exhibit enrichment with increasing trophic level and are valuable for determining trophic structure in food webs (DeNiro and Epstein 1981). $\delta^{13}C$ values are not altered much by trophic transfer and can be used to determine primary sources of energy at the base of food webs (DeNiro and Epstein 1978). $\delta^{13}C$ values may also vary by photosynthetic pathway. Isotopic data are commonly mapped in $\delta^{13}C$-$\delta^{15}N$ biplots where comparisons between species are visualized in delta space.

Only $\delta^{15}N$ is used in this thesis in Chapter 3, where I am interested in seasonal trophic level change in the avian community. $\delta^{13}C$ is less useful in this regard, as it varies slightly with each trophic transfer (~<1‰), but strongly by photosynthetic pathway: CAM and C4 plants may be more enriched than C3 plants by greater than 10 permil (Smith and Epstein 1971). My study sites were composed of open scrub and coffee plantation where tropical grasses are common (C4) as well as adjacent primary forest dominated by C3 plants. Birds (and likely arthropods) move between these habitats (Fraser and Chavarria pers. obs., unpub. data) suggesting that any trophic level differences in $\delta^{13}C$ would likely be influenced by the much larger difference in $\delta^{13}C$ between photosynthetic pathways supporting the local food web.

subtle changes in photoperiod and/or other visual cues, such as presence of abundant food (Fogden 1972, Hau *et al.* 2000).

In a seasonally changing environment, animals should be expected to time energetically costly events in their annual cycle to coincide with good environmental conditions so as to maximize fitness (Lack 1968, Hau *et al.* 2000). This may include breeding when particular foods are most plentiful, switching resource use seasonally or migrating to areas that are more suited to reproduction. It is expected that birds will breed when preferred foods are most plentiful, so as to decrease the cost of territory defense, nest building, gamete production, and provisioning offspring. In Neotropical forests, the abundance of foods available to birds, influenced by rainfall seasonality, is likely the principal factor driving the timing of breeding (Fogden 1972, Hau *et al.* 2008). Indeed, visual food cues were shown experimentally to trigger breeding activity in a tropical rainforest bird. When spotted antbirds (*Hylophylax n. naevioides*) were shown live crickets in captivity, they progressed into breeding condition (enlarged gonads) and increased their song rate (Hau *et al.* 2000).

In the dry season, flower abundance peaks and it has been suggested that many species of hummingbird time migration and breeding events to correlate with peak flowering (Stiles 1980, Levey & Stiles 1992). In the early to mid-wet season, fruit availability may peak, potentially driving patterns of breeding, migration and abundance in birds that regularly consume fruit (Loiselle & Blake 1991, Levey & Stiles 1992, Boyle 2010). In the wet season, arthropods also peak (Janzen 1973), providing protein-rich foods that are fed to developing nestlings, even by birds considered largely frugivorous during the rest of the year (Morton 1973). However, little is known about the potential for seasonal change in adult Neotropical bird diets, particularly during periods of high energy expenditure and nutrient need. Recent studies examining seasonal resource use in Neotropical birds using stable isotope analysis have focused upon the relationship between peak availability of foods and their presence in the diet of birds (e.g. Hererra *et al.* 2005). Missing from these analyses is an examination of the role that particular foods might play in meeting protein and energy costs. The protein complementation hypothesis suggests that animals will complement their diets with foods that best help them to meet their nutritional and energy requirements. Birds and other animals may be expected to target food that best meets their physiological needs at periods of great expenditure, such as during breeding and moult (Murphy 1994). Many birds (and bats) in the Neotropics consume fruit and nectar as a regular component of their diet (Stiles and Skutch 1989). However, these foods have very low protein concentrations and may be inadequate to support reproduction or moult (Klasing 1998). In Chapter 3, I explore seasonal diet use through a test of the protein complementation hypothesis (Murphy 1994). I predict that birds will complement their diet with more protein-rich foods during breeding and moult, to compensate for greater expenditure during this period of the annual cycle. A better understanding of how birds respond to the high energy demands of breeding and moult through diet selection will allow us to better understand how birds partition and time periods of high energy expenditure within the context of phenological change in the forest.

Migration

Neotropical birds and bats may respond to changing micro- and macro-climatic conditions by seasonal movement. These movements can be seen on a continuum of scales from vertical shifts up into or down from the canopy, horizontal movements of less than 100 m (Karr & Freemark 1983), altitudinal migration of several hundred metres to several kilometres and long-distance migration out of the tropics (Levey & Stiles 1992). Causal factors for all of these gradations of movement may be driven by similar mechanisms, but these remain poorly known and rarely tested (Boyle & Conway 2007). Seasonality is likely an important mechanism driving bird movement; migration is more likely to evolve in habitats that are more seasonal (Levey & Stiles 1992, Chesser & Levey 1998, Boyle & Conway 2007, Griswold *et al.* 2010). However, a more detailed understanding of the link between seasonality, available resources and social factors is lacking, and so understanding intra-tropical bird migration remains an important new frontier of movement and migration research (Sekercioglu 2010).

The study of migration has been limited by our ability to study small (<100g) migratory animals. Birds of this size make up 65% of the world's bird species, but their migration patterns are poorly known (Wikelski *et al.* 2007). In tropical birds, thousands of species have yet to be studied and movement and migration patterns of many species remain undescribed (Sekercioglu 2010). We also lack movement information on bats, where the potential for intratropical migration has barely been considered in any species. More detailed information on the seasonal movements of tropical birds (and bats) will provide the ability to execute more robust tests of ecological correlates of migration, including the influence of seasonality.

The most commonly used technique to detect altitudinal movements remains the use of mark and recapture methods. The most detailed studies have been done in Costa Rica, where it is estimated that 10% of the entire avifauna are altitudinal migrants (Stiles

1985). In Chapter 4, I use data from mistnet monitoring in Nicaragua to detect and report on altitudinal migration (and moult-migration) in a hummingbird species (*Amazilia cyanura*, Blue-tailed Hummingbird).

Another method by which altitudinal migration may be detected is through the use of stable-isotope analysis. Recently, ratios of stable carbon ($^{13}C/^{12}C$) and hydrogen ($^{2}H/^{1}H$) have been used to detect and describe the altitudinal movements of bats and birds (Hererra 1997, Hobson *et al.* 2003, Fraser *et al.* 2008, Hardesty & Fraser 2010). In Chapter 5, I use stable-hydrogen isotope analysis of bat tissues (hair and claws) and rainwater to attempt to discern migrant from resident individuals. This represents the first time that potential altitudinal movement of Neotropical bats has been studied using stable-hydrogen isotopes; all previous investigations have involved birds.

The conservation of Neotropical altitudinal migrants may be more complicated than for more sedentary species because migrating individuals may require suitable habitat at both ends of migration as well as suitable migration corridors (Winker *et al.* 1997). Determining which species migrate and when thus has valuable conservation and management applications in the Neotropics. Considering that few Neotropical montane areas have been studied, and the migratory status of hundreds of bird and bat species remains unknown, describing patterns of altitudinal movement and determining factors that promote it remains an important challenge.

Hypotheses and predictions in Chapter 2 to 5

In Chapter 2, I simultaneously test several hypotheses to explain δD variation in bird tissues. I predict that foraging level (understory vs. overstory), foraging location (forest interior vs. coffee plantation), body size (weight), diet, sampling date, sampling year and species may all influence claw and feather δD values in a Nicaraguan cloud forest bird community.

In Chapter 3, I test the diet complementation hypothesis which suggests that animals will consume foods that best complement their energetic and nutritive needs. I predict that birds in a variety of foraging guilds (nectarivores to insectivores) will complement their diet with more protein-rich foods to support higher demand during breeding and moulting.

In Chapter 4, I test the hypothesis that the blue-tailed hummingbird (*Amazilia cyanura*) is an altitudinal moult-migrant. I predict that this species migrates from low elevation Pacific-slope forests to high elevation cloud forest in Nicaragua to complete its annual moult but not to breed.

In Chapter 5, I test the hypothesis that altitudinal migration in bats may be tracked using δD analysis of bat tissues. I predict that migrant bats can be identified by claw and hair δD that is more positive than that expected for the study site.

This thesis is presented in "articles" format. Each data chapter is formatted according to target journal: chapter 2, Journal of Animal Ecology; chapter 3, Journal of Avian Biology; chapter 4, Journal of Tropical Ecology (published); chapter 5, Biotropica (published).

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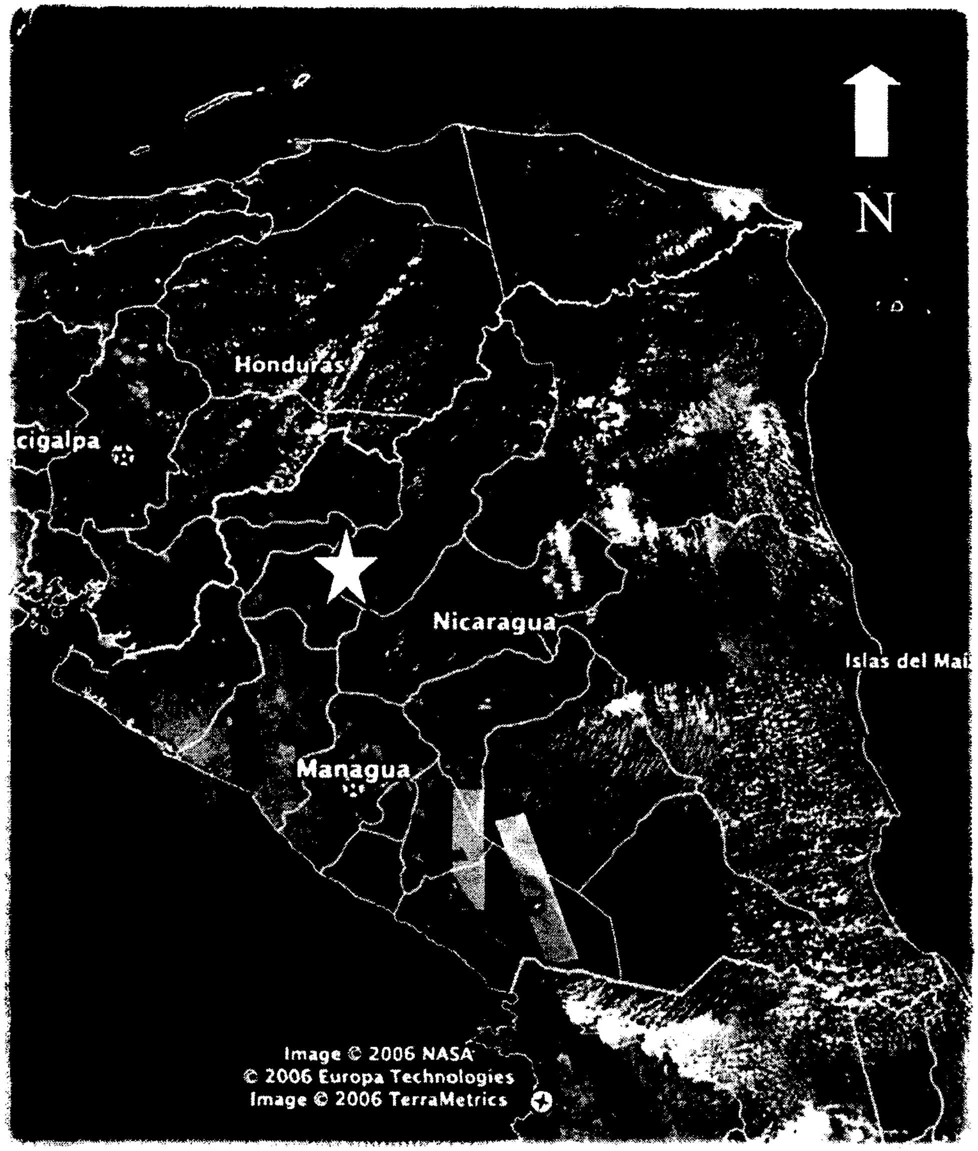

Figure 1-1. Map of Nicaragua. The primary field site (Finca y Reserva Silvestre Privada Nebliselva El Jaguar - Farm and Private Reserve El Jaguar Cloud Forest, hereafter 'El Jaguar'; 1350 masl; 13°10' N, 86°02' W) is marked with a star (Google Earth 2006).

CHAPTER 2: FACTORS DRIVING STABLE-HYDROGEN ISOTOPE VARIATION IN A NEOTROPICAL AVIAN FOOD WEB

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Summary

1. The application of stable-hydrogen isotope (δD) measurements to the study of animal movement, resource use, and physiology depends on understanding factors driving variation in δD in animal tissues. Broad geographical patterns of δD are often used in ecological studies, while the derivation and influence of within-site or micro-scale variation in δD is poorly known. Yet understanding drivers of micro-scale patterns of δD, which may include habitat use, climate, diet, and body size, could shed light on important ecological processes.

2. We explored variation in δD in a community of resident Neotropical birds inhabiting a high-elevation cloud forest in Nicaragua. The diversity of microhabitat use, trophic level, diet, and body size of Neotropical birds, and the strong seasonal precipitation patterns in the Neotropics, provide opportunity to test hypotheses regarding the relative influence of these factors on δD of bird tissues (feathers and claws) using linear and additive models.

3. Based on three years of data from year-round sampling, our results for 22 species suggest a complex interaction of competing factors influencing δD tissue values. The best models show that microhabitat, temporal shifts in precipitation δD, diet, and body size all significantly influence bird tissue values. Values of collected prey and precipitation supported spatial and temporal patterns in birds.

4. Our results reveal a previously undescribed microgeographical effect on δD, where foraging location (forest understory versus adjacent coffee plantation) and strata (on versus above the ground) were significant predictors of δD values in bird claws and feathers. Trophic level and body size hypotheses were supported by our best models, consistent with previous studies showing the influence of these factors on δD in tissues.

5. These results have implications for the tracking of long-distance altitudinal and latitudinal migration, since variation owing to microhabitat, body size, diet, and seasonal precipitation patterns may obscure broad geographical patterns of δD. However, our results suggest a role for δD in tracking small-scale habitat and resource use.

6. Relatively positive δD values in forest understory birds reveal a heavy reliance upon recycled moisture (fog). Fragmentation and climate change may result in increasingly desiccated cloudforest, and our results suggest that this may negatively affect several species that apparently rely heavily on these high-elevation hydrological cycles.

***Key-words*:** altitudinal migration, animal migration, deuterium, moult, movement studies, rain forest, seasonality, stable-nitrogen isotopes, tracking, trophic level.

Introduction

The use of stable isotope ratios of hydrogen (δD) in animal ecology studies has grown significantly in the previous decade. Precipitation δD shows strong and consistent spatial variation at broad scales (tens of degrees of latitude/longitude, or several hundred metres in altitude). Animal tissues generally reflect annual growing-season precipitation δD values of the geographic area where they were synthesized, and can thus be used to trace migratory origins or to estimate dispersal when sampled at another location (Hobson & Wassenaar 1997, Hobson 2008). δD analysis can be a particularly valuable tool in tracking migration over latitude (Hobson 2008) or altitude (Hobson et al. 2003, Hardesty & Fraser 2010). However, geographic resolution of δD is usually on a broad scale (Farmer, Cade, & Torres-Dowdall 2008, Hardesty & Fraser 2010), thus imposing limitations on the scale at which animal movements may be tracked. Many questions remain regarding factors driving variation within populations (Powell & Hobson 2006, Langin et al. 2007, Hobson 2008, Betini et al. 2009). In some cases, this variation may be substantial, precluding the use of δD to reliably track movement at any scale (e.g. Wunder et al. 2005, Rocque et al. 2006). More recent approaches to the use of δD have focused upon this variation as a potentially useful tool to track energy sources through food webs (Doucett et al. 2007) or to reconstruct consumer diets (Jardine, Kidd & Cunjak 2009). Despite the broad interest in improving upon geographic assignment in migration studies and the more recent development of δD analyses to reconstruct diet and energy use, many questions remain regarding factors driving δD variation in animal tissues.

Strong correlative patterns between precipitation and animal tissue values at broad latitudinal and altitudinal scales have been the principal handle supporting animal migration studies using δD (reviewed in Hobson 2008). However, the scale at which these movements may be tracked successfully using δD is often broader than desired, owing to the large, intra-population variation in δD that has often been observed (Farmer, Cade, & Torres-Dowdall 2008, Hardesty & Fraser 2010).

Small-scale spatial or temporal differences in food web δD may drive within-population δD variation in animal tissues (Betini et al. 2009). A spatial model best explained variation in feather δD within a single population of wood thrush (Hylocichla mustelina) (Powell & Hobson 2006). Betini et al. (2009) found that spatially stratified insect prey values were a significant predictor of tree swallow (Tachycineta bicolor) nestling blood δD values, suggesting an influence of microgeographic variation on animal tissue δD. Hydrological studies support the notion that δD may vary at small spatial scales in terrestrial ecosystems. For example, soil moisture in rainforest may become significantly enriched in deuterium, as compared with precipitation and groundwater, due to the influence of evaporative and fog drip cycles (Liu et al. 2007). Despite the potential value of these patterns for tracking spatial resource use, the influence of such microgeographical hydrological effects on animal tissues values has not been examined.

Temporal variation in precipitation δD may drive some of the variation observed at local scales in animal tissues. Fraser, McKinnon & Diamond (2010) suggested that the broad seasonal variation in precipitation δD they measured may explain differences in hair δD values of five species of Neotropical bats which moult in different seasons. Powell and Hobson (2006) suggested that uncoupling of wood thrush (Hylocichla mustelina) tail feather δD and expected δD based on local precipitation values may have been driven by the influence of deuterium-enriched May rainfall on the local food web.

Previous studies examining temporal effects on tissue δD directly have found no effect, however the short duration of these studies (~ 2 months) may have precluded the detection of temporal effects (Langin et al. 2007, Betini et al. 2009). Tests of seasonal precipitation effects on local food webs over expanded timescales are needed.

Physiological effects may also influence δD tissue values. Migratory studies often rely upon the use of a 'net isotopic discrimination value' (Wassenaar 2008) that is the sum total of all fractionation between δD values at the base of the foodweb (rainwater) and particular animal tissues. Most fractionation is assumed to occur between precipitation and plants, with negligible change at higher trophic levels (Hobson 2008). However, Birchall et al. (2005) found that animals feeding at a higher trophic level, as indicated by $\delta^{15}N$ values, had significantly higher δD. Similarly, Fraser, McKinnon & Diamond (2010) suggested that enriched δD in sanguinivorous bats may have been driven by a biomagnification effect. Other than the study by Birchall et al. (2005), there have been no direct tests in the field or laboratory of trophic discrimination effects on δD values in animal tissues (Hobson 2008, Martinez del Rio 2009), thus the relationship between trophic level and δD values remains largely unexplored.

Body size has also been implicated in δD variation in animal tissues. Larger birds may experience greater amounts of evaporative water loss (Williams & Tieleman 2005), and thus show more positive δD values owing to loss of the lighter hydrogen isotope. In a recent study, larger body size in tree swallow nestlings (Tachycineta bicolor) was associated with more positive δD blood values (Betini et al. 2009). This effect has not been explored in feather and claw tissue in adult birds.

We tested the above spatio-temporal and physiological hypotheses to explain variation in δD tissue values (claws and feathers) of 22 species of resident bird in a Nicaraguan cloud forest community. For spatial effects, we examined the microgeographic effects of foraging on the forest floor versus foraging above the ground and foraging within the forest versus in adjacent open scrub and coffee plantation. Considering the influence of successive evaporation and fog drip on forest soil moisture δD (Liu et al. 2007), we predicted birds foraging on the ground in the forest interior would have significantly more positive δD tissue values than those foraging above the ground and in adjacent open areas. For temporal effects on δD, we expected that claw values would be significantly correlated with sampling date. Claws grow continuously and thus may incorporate temporal changes in food web δD. For most birds, tail feathers are moulted once a year (Pyle 1997), but considering annual differences in precipitation δD (IAEA 2001, Hardesty & Fraser 2010), we predicted that year would be a significant factor influencing feather δD. For the trophic level hypothesis (Birchall et al. 2005), we predicted that birds feeding at a higher trophic level (reflected by $\delta^{15}N$ values) would show more positive δD tissue values. For the body size hypothesis, we predicted that larger birds would tend to have more enriched deuterium values. To complement these analyses, we collected precipitation in the study area over a year (to complement precipitation data in Fraser, McKinnon & Diamond 2010) and arthropod prey and fruit in forest and adjacent coffee plantation.

Materials and Methods

Field methods

Birds were sampled at three different locations in the Cordillera Isabella, Nicaragua. These were the Finca y Reserva Silvestre Privada Nebliselva El Jaguar (Farm and Private Reserve El Jaguar Cloud Forest, hereafter 'El Jaguar') (1350 masl; 13°10' N, 86°02' W), Datanli-el Diablo Natural Reserve (1390 masl, 13° 7' 60 N, 85° 55' 60 W, hereafter 'Datanli'), and Reserva Natural Sierra Quirragua (500-1300 masl, 12°50' N, 85°27' W., hereafter 'Quirragua'). All three sampling sites are lower montane forest and incorporate primary and secondary forest as well as, in the case of El Jaguar and Datanli, organic shaded coffee plantations. At the latter two sites, sampling efforts were divided between plantation and adjacent forest. Only one species was targeted (long-tailed manakin, Chiroxiphia linearis) at Quirragua. For more details on the capture locations see Fraser, McKinnon & Diamond (2010) and Martínez Salinas (2008).

Birds were captured using two to 27 mistnets (12 x 2.5 m, 36/30-mm mesh). Nets were set up at roughly 50 m intervals along trails and checked every 40 minutes. At El Jaguar, during two-day mist netting bouts, samples were collected in most months between December 2007 and September 2009 (with the exceptions of January, February and April 2008 and March, April, June and July 2009). Birds at Datanli were sampled in March 2008 and at Quirragua between February and April in 2007. A tail feather (third retrix, R3) and two claw clippings (1-3 mm depending upon species, middle claw of both feet) were collected and stored in paper envelopes until analysis. All birds, with the exception of hummingbirds, were banded. The chances of resampling an unbanded hummingbird were low, as trapping effort was rotated between areas and short-term recaptures at a particular site could be identified by evidence of sampling (missing R3 and blunt claws lasting ~2-4 months). All data collected in the field followed protocols outlined by Monitoreo de Sobrevivencia Invernal (De Sante et al. 2009).

Rain water was collected in 500 ml open-mouthed, polypropylene bottles, covered with screen and with 1 cm of mineral oil in the bottom (to prevent water evaporation) (USGS 2006). Two of these receptacles were secured off the ground, away from overhanging vegetation, in the coffee plantation at El Jaguar only. Every month, 5 ml of water was extracted and deposited in a sealed, polypropylene bottle using a syringe and the remaining water was discarded. We collected rainwater in 2009 to compare to that collected using the same location and protocols as in 2008 (presented in Fraser, McKinnon & Diamond 2010). Models of precipitation δD based on long-term (40 yr. +) growing-season means weighted by amount and corrected for altitude can be powerful tools for determing locally expected animal tissue values (Bowen *et al.* 2005). However, moisture contributed to the food web in this study likely arrives from multiple sources, including both precipitation and fog. Both of these sources were likely captured in our year-round sampling. We did not weight these sources by amount contribution as these moisture sources may have varying spatial influence within our study area. Instead, we present these values as an indicator of the range of moisture dD that is contributed to the study area over an annual cycle. We also collected two forest spring water samples in early March 2008 (end of dry season) at El Jaguar.

We hand-collected small fleshy fruits of a size (1-3 cm) commonly consumed by small (<100 g) frugivores (Moermond and Denslow 1985) opportunistically during netting bouts in 2008 at El Jaguar. We also hand-collected arthropods from the leaf litter and vegetation within the height of mistnets during the same period. We searched for fruit and arthropods in a way similar to a bird foraging and sampled items as encountered. Within 24 hours of collection, arthropod and fruit samples were dried in an oven at 60 degrees Celsius for 24 hours and then stored at room temperature until analysis.

Lab methods

Stable-isotope analyses of all bird tissues were performed at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick. Claws and feathers were washed to remove lipids and particles in a 2:1 chloroform:methanol solution for 24 hours and left to air dry. In order to account for potential air-tissue H exchange, we use a comparative equilibration technique with standards of known hydrogen isotope ratios (Wassenaar & Hobson 2000, 2003, Wassenaar 2008). Three standards [Bowhead Whale Baleen (BWB), Cow Hoof (CHS), and Chicken Feather (CFS)] were weighed into silver capsules at approximately 0.18-0.2 mg alongside samples and allowed to freely equilibrate with local water vapor for a minimum of 72 hours. Samples and standards were then loaded into a zero blank autosampler. After pyrolysis in a High Temperature Conversion/Elemental Analyzer (TC/EA) and analysis in a Thermo Finnigan Delta XP mass spectrometer, all values were adjusted to bring BWB, CHS and CFS to δD values of - 108‰, -187‰ and -147‰ respectively (Wassenaar & Hobson 2000, 2003, Wassenaar 2008). This technique adjusts all values relative to the Vienna Standard Mean Ocean Water (VSMOW) scale. To estimate analytical precision across runs, an internal laboratory keratin (feather) standard was used which yielded mean values of -116.6 ± 2.1‰. Rainwater samples were analyzed at Environment Canada's Stable Isotope Hydrology and Ecology Laboratory in Saskatchewan (see methods in Wassenaar & Hendry 2008).

Analysis

For data exploration, we followed protocols outlined in Zuur, Leno & Elphick (2010) and Zuur et al. (2009) where we tested for outliers and collinearity of explanatory variables. Explanatory variables examined for their potential effect on δD tissue values are described in Table 2-1. The effect of chosen explanatory variables on δD in feathers was explored using a general linear model (GLM). For claws, scatterplots of δD against $\delta^{15}N$ and sampling date suggested application of a linear model was inappropriate. Therefore, for claws, we used a generalized additive model (GAM). To rank models, we used Akaike's Information Criterion with a correction for small sample size (Burnham & Anderson 2002). We evaluated models where each variable was independent as well as all two-way interactions. Models were run separately for claws and feathers as these two tissues reflect different temporal periods of integration. Claws reflect a period of integration of 2 to 5 months (Bearhop et al. 2003, Fraser et al. 2008, Carleton et al. 2008, Fraser unpub. data). Individual tail feathers grow over a period of 20 to 25 days (Grubb & Pravosudov 1994) and reflect foraging around the time of moult (Hobson & Wassenaar 1997). After ranking models, we conducted significance testing of the best model and each main-effect term. We compared fruit and arthropod samples in open versus closed habitats using t-tests.

Considering that we collected fruit and arthropods as encountered during searches, the statistical variability we measured may not be correct for estimates of fruit or arthropod δD for comparison among groups because sampling may have included multiple samples of a particular species. However, our method does provide a suitable estimate of variability between the diet-available assemblages of fruit and arthropods in the two habitats. All analyses were conducted using R 2.11.1 (R Development Core Team 2010).

Results

Bird and model results

We sampled 211 individual birds of 22 species. Mean claw δD values (and standard deviation) varied from -83.23 ± 4.21‰ to -19.23 ± 14.51 ‰ and feather values ranged from -80.49 ± 7.67 to -24.24 ± 0.13 ‰ (Table 2-2). The most parsimonious model to explain δD variation in claws included foraging location (forest interior vs. exterior), foraging stratum (ground vs. above ground), body size, and sampling date. This model carried 38% of the AICc weights in claw δD (Table 2-3a). The second best claw model included two interactions (foraging level x sampling date and foraging level x species) and carried 36% of the AICc weights. The best AIC model of feather δD included foraging location, foraging stratum, δ^{15}N (trophic level), mass, and species, and carried 42% of the AICc weights (Table 2-3b). The second-best model included the addition of year and carried 27%. δD of claws and feathers, was more positive in ground foraging birds in the forest interior, in birds with larger size and those with higher δ^{15}N. Both foraging location and stratum were significant in both claw and feather models (Tables 2-4a and 2-4b).

Water and prey results

Rainwater values varied widely over the course of both years but followed a similar pattern, with more negative values obtained in the wet season (May-November) and more positive values in the dry season (December-April) (Figure 1). δD values varied from -46.5 to 11.5‰ between March 2007 and February 2008 (Fraser, McKinnon &

Diamond 2010) and between -130.8 and 3.51 ‰ between March 2008 and February 2009. Mean inter-annual δD varied by 14 ‰, however, yearly comparisons were not significantly different (t-test, $p=0.113$, $df=32.93$, $t=1.63$). Forest spring water samples collected at the end of the dry season (early March 2008) yielded values of -41 and -54‰. Arthropod prey values ranged from -159.6 to -58.7‰ and fruit ranged from -135.5 to 10.1‰. Arthropods and fruit collected in the study area differed significantly (t-test, $p<0.0001$, $df=45.92$, $t=-5.64$) (Figure 2). Arthropods collected inside and outside the forest also differed significantly (t-test, $p<0.0001$, $df=16.91$, $t=6.06$) and plant (fruit) samples approached significance (t-test, $p=0.05$, $df=32.05$, $t= 2.0$) (Figure 3a, 3b). Only plant samples collected in the forest had values greater than -40 ‰ (6 observations).

Discussion

Consistent with hypotheses proposed to explain variation in δD of animal tissues, we found that microgeographic and temporal effects, trophic level, body size, and species significantly influenced δD values in Neotropical bird tissues. Our best claw and feather models suggest there are multiple effects influencing claw and feather tissue δD.

In both the best claw and feather models, foraging stratum (forest floor or above) and foraging location (interior forest versus exterior coffee plantation/open scrub) significantly influenced δD values. Birds that commonly forage in forest leaf litter tended to have the most positive claw and feather values. This suggests sources of moisture available to the food web of these birds were more positive than those encountered by birds foraging in above-ground vegetation, and those in the coffee plantation. Species that dwell exclusively in the humid forest understory and forage on the ground such as the black-headed nightingale thrush (see Table 2-2 for scientific names) and scaled antpitta

(Stiles & Skutch 1989) had tissue values more positive than -20‰. Hydrological studies show that δD values become more positive with evapotranspiration and repeated evaporation and condensation of the same water source produces increasingly higher values (Lachniet & Patterson 2002). These effects may be particularly prevalent within tropical rainforest, where evaporated moisture, falling as fog drip within the forest, is more positive in δD than precipitation or groundwater. Most fog drip occurs in the dry season (as much as 86%; Liu et al. 2004), when it contains more recycled moisture and contributes to a significant δD increase of soil moisture (Liu et al. 2007). This provides a strong explanation for the patterns of claw deuterium enrichment that we observed, particularly in forest understory species. Considering that claws may reflect two to five months of integration time, dependent upon species (Bearhop et al. 2003, Fraser et al. 2008, Carleton et al. 2008, Fraser unpub. data), and that the dry season lasts for four months (Dec-Mar), it is not surprising that some understory species retain more positive claw values (> -30 ‰) throughout the year. Similar patterns of deuterium enrichment have been found in the tissues of forest understory birds in Belize and the Dominican Republic (Fraser & Diamond unpub. data, J. Nocera unpub. data).

In the wet season, soil moisture δD values are more influenced by precipitation and thus become more depleted in the heavier isotope (Liu et al. 2005). This is the season when most species in this study moult their tail feathers (Chavarria unpub. data). Our precipitation data show that values are much more depleted in deuterium in the period leading up to, and during moult. This may explain why the feathers of 16 out of 18 species, including understory species, generally exhibited δD values that were more negative than claw values. For example, mean black-headed nightingale thrush claw values were -19.23±14.51 ‰ whereas feathers were -59.25±11.92 ‰.

We can rule out ground water sources as directly providing enriched baseline δD to the forest food web, as analysis of forest spring water yielded values < -40 ‰ and groundwater sources show less seasonal variability and are recharged primarily by precipitation and not by evapotranspiration and recycled water sources (Liu et al. 2005). Temperature may also influence δD values, in that greater temperatures increase evapotranspiration and subsequently enrich animal tissue δD (McKechnie et al. 2004, Betini et al. 2009). However we may also rule out temperature as the driver of the enriched values in forest understory birds, as this habitat is buffered from radiative heat and experiences cooler daytime temperatures as compared to the adjacent open, coffee plantation (K. Fraser pers. obs).

Our study was not designed to exhaustively sample potential prey types and their location within and outside the forest. The grouping of multiple types of arthropods and plants into broad categories (e.g. forest interior versus exterior) is admittedly coarse. Thus, our results comparing these factors should be seen as preliminary. However, the initial patterns shown in prey, which are mirrored in the larger sample for the birds, suggest that there may be inherent differences in δD at smaller spatial scales than has generally been expected. Both arthropod and plant prey collected in the forest interior tended to be more positive in δD than prey collected in the open, coffee plantation and scrub areas. These patterns should be explored in greater detail in future studies.

Our results regarding the effect of trophic level ($\delta^{15}N$) on δD are consistent with other studies, where carnivores reportedly have more positive δD values than herbivores (Birchall et al. 2005, Fraser, McKinnon & Diamond 2010). For example, species in our study with the most positive δD feather values are reported to feed on arthropods and small vertebrates (scaled antpitta, speckled mourner, spotted woodcreeper, ruddy woodcreeper; Stiles and Skutch 1989). The potential biomagnification effect that we observe here has not been tested in controlled laboratory experiments (Hobson 2008, Martinez del Rio et al. 2009), thus the mechanism for such an effect remains unexplained. Several of the insectivorous species in our study exhibited δD tissue values more positive than arthropod, fruit or collected precipitation values. Alternatively, the link between high $\delta^{15}N$ and δD values may have been driven by other factors. In a study of spatial structure of $\delta^{15}N$ in a tropical rainforest, soil $\delta^{15}N$ values were significantly higher than canopy leaves (Hyodo et al. 2010). Considering that we found a link between ground foraging in the forest and more positive δD, this interaction could have driven the inclusion of $\delta^{15}N$ in the best feather model. Our results support the notion that we may predict different, potentially lower δD net isotopic discrimination values for animals at higher trophic levels, as birds in our study tended to have higher δD along with higher $\delta^{15}N$.

As was found in a recent study of nestlings (Betini et al. 2009), body size significantly influenced δD, with the largest birds having the most positive δD. Ours is the first study to find an influence of body size on feather and claw δD in adult birds. It was shown experimentally and in the field that heat stress or increased temperature causes enrichment of deuterium values (McKecknie et al. 2004, Betini et al. 2009). Heat stress during moult was suggested to be a potential driver of more positive feather δD in studies of a raptor and a passerine (Smith & Dufty 2005, Powell & Hobson 2006).

However, as described above, heat stress is an unlikely contributor to enriched deuterium values in forest understory bird feathers. The link between enriched tissue δD and body size warrants further investigation in this study system.

Our rainwater results show broad shifts in values over the year, with higher values during the drier season (December to March) suggesting a greater contribution of evapotranspirated water to our samples. Rainwater in both years tracked similar patterns of depletion and enrichment. The most depleted values were measured during the wetter part of the year (April to November), likely driven by the 'amount effect' (Dansgaard 1964) as values of tropical precipitation are not strongly driven by temperature (Fricke & O'Neill 1999). Unfortunately, we did not collect water within and outside the forest during this study, thus our values reflect those of rainwater and fog collected in the coffee plantation only. While forest fog drip could not have contributed to our collected precipitation values, enriched values in the dry season suggest some influence of overall recycled moisture from land sources. As predicted, sampling date significantly influenced claw values suggesting that shifts in precipitation δD were incorporated into growing claws. The second-best claw model also included an interaction between foraging level and sampling date, which may also reflect a seasonal shift in understory values between rainy and dry periods. We did not sample prey throughout the year to determine if these values track rainwater shifts but this is an important area for future investigation. Animal δD tissue values may be influenced by both drinking water (26-32%) and prey (Hobson, Atwell & Wassenaar 1999). The 14‰ difference in mean annual rainwater δD between 2008 and 2009 was not statistically significant and year was not retained in the best feather model. However it was included in the second-best feather model suggesting there may be an influence of year on feather δD values. Sampling feathers over a number of years in the same location would allow further exploration of the potential for year effects.

Conclusion

Our results are consistent with previous investigation of factors influencing δD on animal tissues, where microgeographic variation, temporal effects, trophic level, body mass, and species significantly influenced δD. We are the first to show that trophic level and body mass influence the tissue values of adult birds. We have also identified a pattern of microgeographic and temporal variation that has not previously been observed: that birds foraging in the forest understory tend to have more positive δD than those foraging in adjacent open areas and in the forest overstory. Not only will accounting and controlling for this variability improve attempts to track movement at altitudinal or latitudinal scales, but there is also the potential to use this variation to track movement and habitat use at a local scale. Our data suggest that δD may be used to differentiate forest use from adjacent open areas and scrub, which could be valuable in studies exploring habitat use in fragmented environments and agroecosystems. Our data highlight not only a way in which this variability may be used to track movement but also the importance of recycled moisture to the food webs of some forest understory species. Both climate change and patterns of fragmentation and deforestation may disrupt these forest hydrological cycles (Lachniet & Patterson 2002). Tracking δD in forest understory birds may be a reliable way in which to track moisture use in this food web, and how it may change over time.

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Table 2-1. Description of model variables examined to explain feather and claw δD values of a Neotropical forest bird community using general linear models (GLM) and general additive models (GAM).

Variable name	Description
Species	22 species were included in the study. See Table 2 for scientific names.
$\delta^{15}N$	Reflects trophic position. $\delta^{15}N$ and δD measured in same claw and feather samples. δD may become more positive with trophic level as ^2H is biomagnified through the food web (Birchall *et al.* 2005)
Interior versus exterior	Species were categorized by habitat association with forest interior or forest edge/scrub after Stiles and Skutch (1989), Fraser and Chavarría unpub. data. The moisture source supplying the interior versus exterior forest food web may differ driving variation in bird tissues between these locations.
Foraging level	Species were categorized as ground or above ground foragers after Stiles & Skutch (1989), Fraser and Chavarría unpub. data. Soil moisture δD may become more positive due to evapotranspirated water and subsequent fog drip (Liu *et al.* 2007). Birds feeding in the understory may be exposed to higher δD values than those foraging in the overstory leading to differences in tissue values.
Body size	Mean weight (g) for each species from Stiles & Skutch (1989). Larger birds have higher rates of evaporative water loss which may lead to more positive δD tissue values than smaller birds (Betini *et al.* 2009)
Sampling date	Ordinal date a claw sample was collected. Temporal differences in source moisture supplying the food web may influence tissue values (Fraser *et al.* 2010). Claws grow continuously and may incorporate these temporal differences.
Year	Year a feather or claw sample was collected. There may be annual differences in mean annual precipitation δD potentially driving variation in feathers grown in different years.

Table 2-2. Species included in the study as well as sample size and mean (±SD) δD values for claws and feathers. Species (American Ornithologists' Union 1983) are ordered from those with the lowest to highest tail feather δD values. Based upon observations at the study site and Stiles & Skutch (1989), species were categorized by foraging stratum (ground versus overstory; G and O in table) and foraging location (forest interior versus exterior; I and E in table).

Species	*N claw/ feather*	Claw δD (mean±SD)	Tail δD (mean±SD)	Foraging Stratum	Foraging Location
Common Bush-Tanager *Chlorospingus ophthalmicus*	2/10	-83.2±4.2	-80.5±7.7	O	E
Yellow-throated Euphonia *Euphonia hirundinacea*	2/9	-57.7±3.0	-80.0±7.4	O	E
Slate-colored Solitaire *Myadestes unicolor*	10/13	-59.4±6.7	-74.9±11.6	O	I
Ochre-bellied Flycatcher *Mionectes oleaginous*	9/48	-44.2±15.4	-73.4±9.3	O	I
Chestnut-capped Brushfinch *Atlapetes bruneinucha*	6/8	-40.6±12.4	-72.2±6.6	G	I
Orange-billed Nightingale Thrush *Catharus aurantiirostris*	1/1	-35.6	-72.0	G	E
Long-tailed Manakin *Chiroxiphia linearis*	0/22	NA	-71.2±7.8	O	I
Stub-tailed Spadebill *Platyrinchus cancrominus*	1/3	-40.8	-67.2±7.1	G	I
Long-billed Hermit *Phaethornis longirostris*	0/29	NA	-64.15±10.5	O	E
Olivaceous Woodcreeper *Sittasomus griseicappillus*	0/2	NA	-62.52±0.56	O	I
Clay-colored Thrush *Turdus grayi*	9/14	-36.6±11.7	-59.9±9.4	G	E
Black-headed Nightingale Thrush *Catharus mexicanus*	12/24	-19.2±14.5	-59.3±11.9	G	I
Eye-ringed Flatbill *Rhynchocyclus brevirostris*	2/3	-33.8±9.2	-58.7±4.6	O	I
Tawny-winged Woodcreeper *Dendrocincla anabatina*	2/5	-41.2±5.7	-57.8±8.2	O	I

Scaly-throated Foliage-Gleaner *Anabacerthia variegaticeps*	4/4	-43.6±9.0	-57.5±9.2	O	I
Golden-crowned Warbler *Basileuterus culicivorus*	1/1	-44.0	-56.2	G	I
Violet Sabrewing *Campylopterus hemileucurus*	1/3	-53.2	-52.6±10.4	O	E
White-breasted Wood-Wren *Henicorhina leucosticta*	3/5	-31.8±8.2	-52.7±12.4	G	I
Ruddy Woodcreeper *Dendrocincla homochroa*	0/1	NA	-49.75	O	I
Spotted Woodcreeper *Xiphorhynchus erythropygius*	2/3	-26.3±9.2	-46.3±1.8	O	I
Speckled Mourner *Laniocera rufescens*	1/1	-31.6	-34.3	G	I
Scaled Antpitta *Grallaria guatimalensis*	1/2	-15.2	-24.2±0.1	G	I

Table 2-3. Akaike's Information Criterion for **a)** top four competing generalized additive models (GAM) used to explain variation in claw (n=68, 22 species) and **b)** four general linear models (GLM) used to explain variation in tail feather (n=211, 22 species) δD. Models were ranked using Akaike's Information Criterion corrected for small sample size (AIC_c). ΔAIC_c is the difference between a model and the model with the lowest AIC_c; AIC_w is Akaike weight.

a)

Model	AIC_c	ΔAIC_c	AIC_w
Claw δD			
interior/exterior + foraging level + mass + sampling date	510.8	0.00	0.38
interior/exterior + foraging level x sampling date + foraging level x species + mass	510.9	0.1	0.36
interior/exterior + foraging level x sampling date + mass	512.4	1.6	0.17
interior/exterior + foraging level + mass + sampling date + $\delta^{15}N$	513.7	2.9	0.09

b)

Model	AIC_c	ΔAIC_c	AIC_w
Feather δD			
interior/exterior + foraging level + $\delta^{15}N$ + species + mass	1624.5	0.00	0.42
interior/exterior + foraging level + $\delta^{15}N$ + species + mass + year	1625.4	0.9	0.27
interior/exterior + foraging level x species + $\delta^{15}N$ + mass	1625.8	1.3	0.23
interior/exterior + foraging level x species + $\delta^{15}N$ + mass + year	1627.8	3.3	0.08

Table 2-4. **a)** Results of the most parsimonious general linear model (GLM) to describe variation in stable-hydrogen isotope values in feathers (n=211) of 24 Neotropical cloud forest species. **b)** Results of the most parsimonious generalized additive model (GAM) to describe variation in stable-hydrogen isotope values in claws (n=68) of 23 Neotropical cloud forest species. Bolded *P*-values show significance of smooth terms for continuous variables, unbolded show significance level for parametric terms (df = degrees of freedom, edf= estimated degrees of freedom).

a)

Parameter	Estimate	SE	*t*	*p*
Intercept	-69.789	6.133	-11.380	<0.0001
Interior vs. exterior	-9.132	2.138	-4.270	<0.0001
Foraging level	-5.042	2.140	-2.356	<0.0194
$\delta^{15}N$	1.822	0.466	3.907	<0.0001
Species	0.618	0.135	4.571	<0.0001
Mass	0.191	0.053	3.642	<0.0003

b)

Parameter	df/edf	F	*p*
Interior vs. exterior	1	10.69	<0.0019
Foraging level	1	17.91	<0.0001
Mass	6.28	4.72	**<0.0003**
Sampling date	6.20	10.53	**<0.0001**

Figure 2-1. Monthly δD rainwater values for the Nicaraguan cloud forest study site from March 2007 to February 2008 (solid line, from Fraser *et al.* 2010) and March 2008 to February 2009 (dashed line, this study).

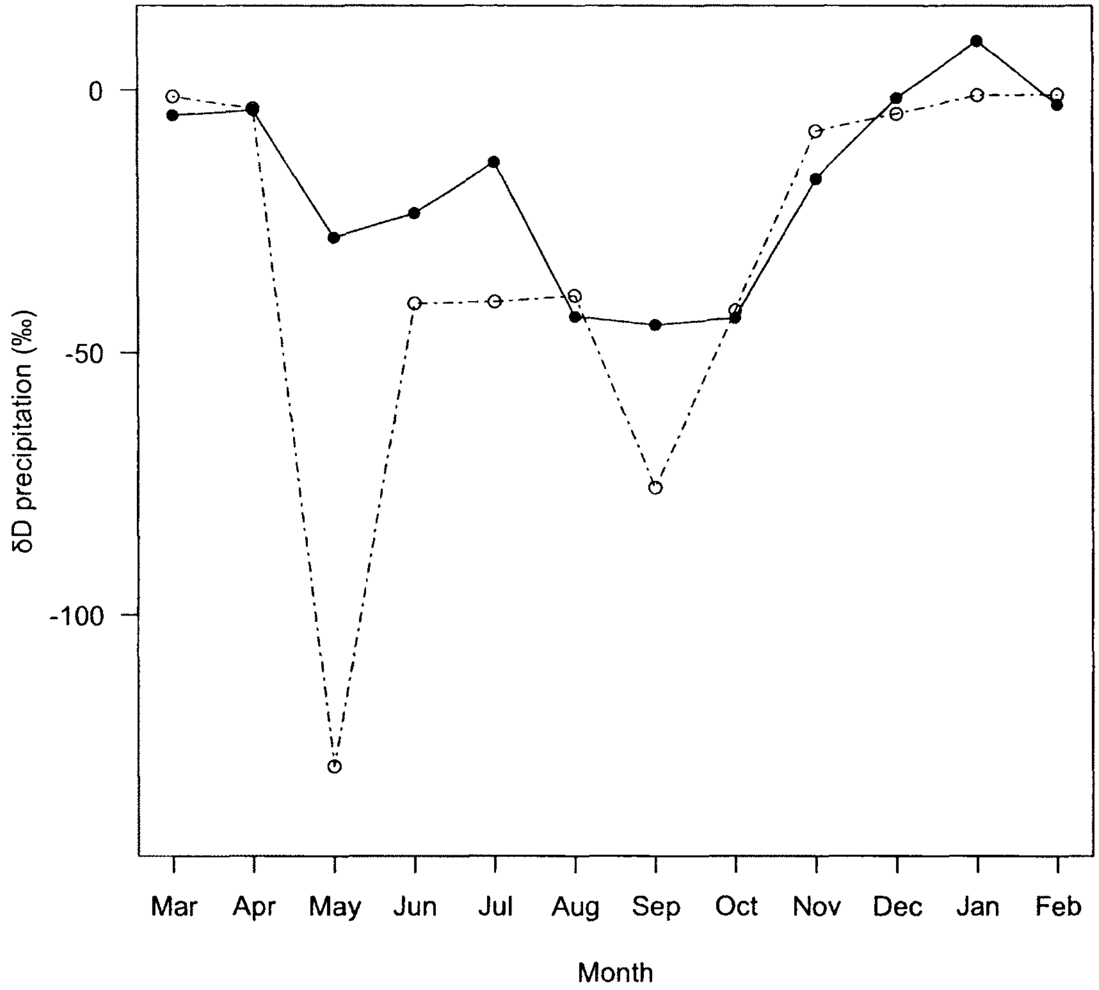


Figure 2-2. Boxplot comparisons of potential prey for birds in this study. Plots show median, 25th and 75th percentiles. Whiskers show maximum and minimum values. δD values were significantly different for arthropods and berries (t-test, $p<0.0001$, df=46, $t=-5.64$).

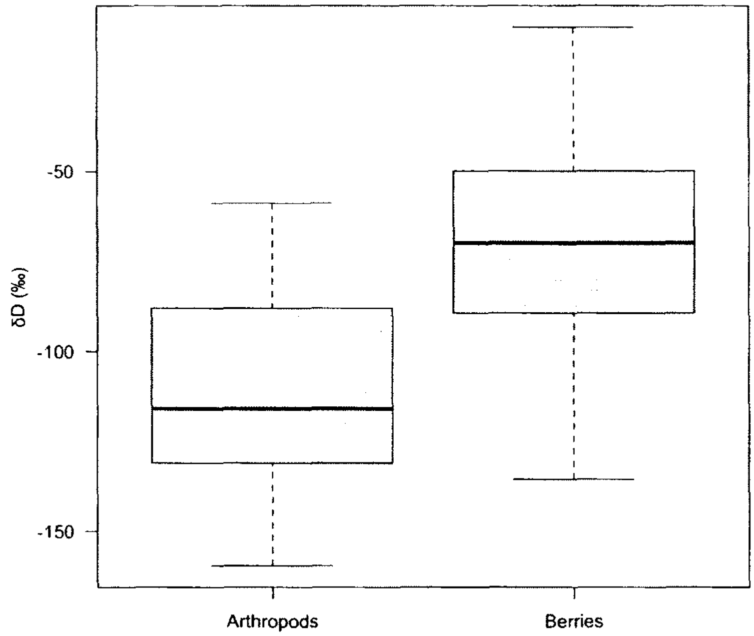
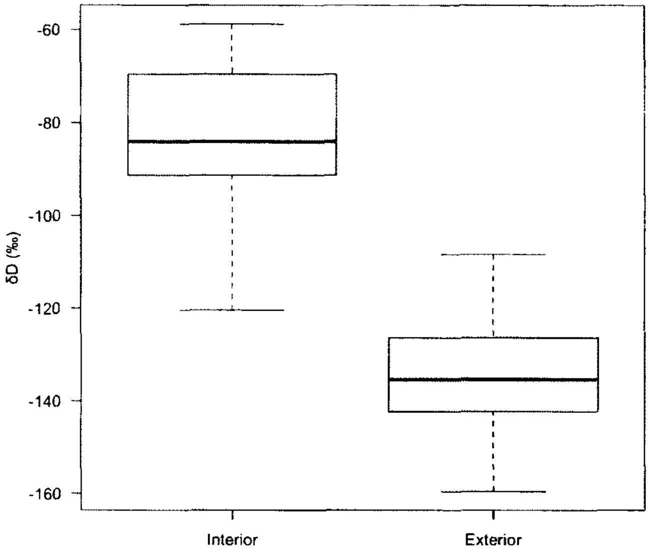
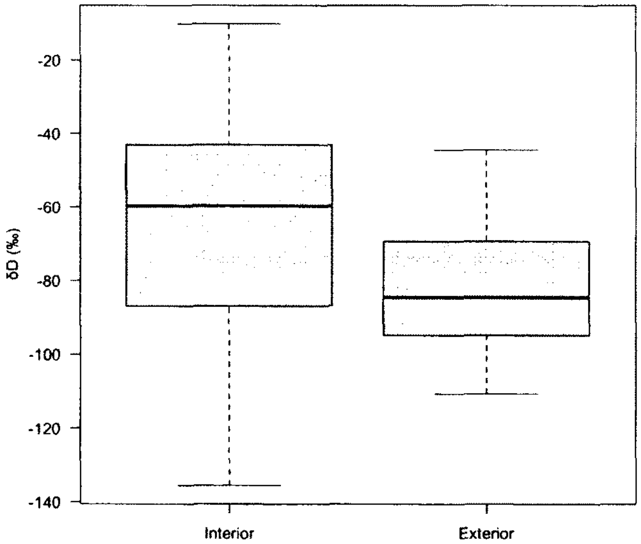


Figure 2-3. Boxplot (whiskers, interquartile) comparisons for arthropods (a) and fruit (b) sampled inside forested areas and in open areas/scrub. Arthropod values were significantly different between samples collected inside and outside the forest (t-test, $p<0.0001$, df=17, t=6.06) and fruit samples approached significance (t-test, p=0.05, df=32, t= 2.0).

a)



b)



CHAPTER 3: SOURCES OF DIETARY PROTEIN AND EVIDENCE OF SEASONAL DIET COMPLEMENTATION IN A NEOTROPICAL FOREST BIRD COMMUNITY

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[1] Kevin Fraser collected and analyzed data, interpreted results, and wrote manuscript, which has been submitted to Journal of Avian Biology. Antony Diamond supervised K.C.F's PhD thesis and edited manuscript.

[2] Liliana Chavarría collected data and edited manuscript.

Abstract

Animals may adjust their diet seasonally in response to shifting energetic and nutrient demand. Despite this, one-dimensional, qualitative dietary categories often provide the framework for theories of speciation, community structure, migration, and mating systems in tropical birds. Previous studies have not examined the potential for birds to seasonally complement their diets with more protein-rich foods during productive phases (diet complementation hypothesis). We tested this hypothesis in a cloud forest bird community in Nicaragua. We compared the stable nitrogen isotope ratios of avian tissues grown in different seasons to examine potential shifts in dietary sources of protein. We also conducted interspecies comparisons of dietary sources of protein (calibrated to species of known diets) during breeding/moulting. Our results support the diet complementation hypothesis: most species seemed to consume prey with significantly greater protein content during the breeding/moulting season. In some cases, this likely reflected an increase in arthropod consumption. Putative increases in protein consumption among insectivorous birds warrant further investigation. Interspecies comparisons reveal species that may have special adaptations to low-protein diets and those that rely upon higher protein sources, such as arthropods, during productive phases. Our results suggest complex adaptations and responses to the energetic and nutritive demands of productive phases and reveal previously underappreciated seasonal dynamics in diet that have broad import for our understanding of life history strategies in Neotropical birds and possibly other animals.

Keywords: breeding phenology, frugivory, moult, nectarivory, stable nitrogen isotopes, trophic position

Introduction

Productive phases of the annual cycle can pose energetic and nutritive challenges for animals. How birds respond to seasonal shifts in energy and nutrient demand through diet selection has received attention in laboratory studies (e.g. Murphy and King 1991a, 1992), but there have been few comparable studies of free-living birds (e.g. Witmer 2001), particularly in the tropics. Consequently, the way in which tropical birds respond to fluctuating energy and nutrient demands over an annual cycle remains largely unknown. Understanding how they do is important because many hypotheses in behavioural ecology, from migration to mating systems, are based upon assumptions about diet.

Laboratory experiments have revealed that energy and nutrient demand vary widely throughout the course of the annual cycle of birds (Murphy 1994a). During productive phases, such as in juvenile growth, gamete production, moult, or in premigratory fattening, nutrient and energy needs may be much greater and are additive to maintenance costs (Murphy 1996, Klasing 1998). There are several ways in which free-living birds may respond to such increases, but here we focus on active diet complementation, a strategy where consumers balance their changing nutrient needs through shifts in foraging activity and the selection of appropriate foods (Rapport 1980, Murphy 1994a). Diet complementation has been examined in many taxa, including birds (Rapport 1980, Witmer 2001), but has not been studied in wild tropical birds, where the diversity of consumer and prey interactions reach their zenith.

The diets of tropical birds include a wide variety of plant and animal foods. Plant-produced foods, such as fruit or nectar, are generally low in protein and lipids but high in carbohydrates compared to animal foods, such as arthropods (Jenkins 1969, Morton

1973, Moermond and Denslow 1985, Bosque and Pacheco 2000). This is particularly true for the small, watery, lipid- and protein-poor fruits commonly eaten by many small (<50g) birds (Moermond and Denslow 1985). Exclusive reliance on plant-produced foods may be extremely rare due to their generally low protein concentrations (Morton 1973, Moermond and Denslow 1985, Klasing 1998, Izhaki and Safriel 1989) and many tropical species consume plant and animal foods in different proportion (e.g. Levey and Stiles 1992).

Dietary protein supplies birds with essential amino acids and the nitrogen necessary to synthesize nonessential amino acids (Klasing 1998). Periodic complementation to supply peak protein requirements, such as during egg production and moult, may be particularly necessary in birds that are characterized by low-protein diets, such as frugivores or nectarivores (Moermond and Denslow 1985, Remsen *et al.* 1986,Witmer 1998, Klasing 1998, Bosque and Pacheco 2000). While some specialist frugivores show physiological and behavioral adaptations to a diet of low-protein fruit (e.g. Witmer 1998), most species characterized as mainly frugivorous may need to supplement their diets periodically (Bosque and Pacheco 2000). Many quantitative data exist for nestling provisioning in tropical birds but data on the diet of adult birds during the breeding and moulting period are relatively sparse. Comparisons of the diet of tropical adult birds in periods of high energetic or nutrient demand, such as during breeding and moult, with the nonbreeding period are rare.

Moulting birds require more protein, to supply both energy and the substrate for the production of feathers that are composed of 90% protein (Murphy and King 1991a, 1991b, 1992, Murphy 1996). Insufficient dietary protein can limit moult, by slowing its progress or leading to the development of poor quality plumage and may also cause birds to lose body mass (Murphy and King 1991a, 1991b). Due to the importance of feathers in thermoregulation, locomotion and communication, there should be strong selective pressure for the acquisition of adequate nutrition to support moult. Egg production and the energy required for territory defense, displays and nest provisioning are also additive to maintenance costs and may require compensatory adjustments in protein intake (King and Murphy 1985, Murphy 1994b). By comparing protein intake between non-productive and productive phases of the annual cycle, it is possible to measure individual and species-level responses to the increased protein demand of the breeding/moulting period.

We studied 40 small Neotropical resident bird species (< 80 g) in a cloud forest in northwestern Nicaragua. These species feed on arthropods, nectar, and small fruits or some combination of these foods (Stiles and Skutch 1989). We tested the diet complementation hypothesis by examining whether birds respond to the increased protein and energy demands of breeding and moult through shifts in diet towards protein-rich foods. Thus, our approach represents a natural experiment, where the foraging response of birds to seasonal change in nutrient demand was measured.

We used stable-isotope analysis to compare protein sources and content of foods. The ratio of stable isotopes of nitrogen ($\delta^{15}N$) becomes enriched through the food web and is thus useful in reconstructing the trophic position of consumer diets (DeNiro and Epstein 1981, Post 2002). There is a positive linear relationship between the protein content of food and $\delta^{15}N$ enrichment of animal tissues (Pearson *et al.* 2003, Martínez del Rio *et al.* 2009a). As discussed above, foods available to tropical birds vary widely in protein content and this is also true for $\delta^{15}N$. Arthropods have consistently higher $\delta^{15}N$ values than plant-produced foods (Herrera *et al.* 2003). We collected prey items in our study area to confirm patterns of $\delta^{15}N$ and protein content (%N) reported in other studies. Through intra- and interspecies comparisons of avian tissue $\delta^{15}N$ values it is possible to determine the relative contribution of various protein sources, and whether this reflects a diet of plant-produced or animal foods, or a mixture of the two. Different tissues incorporate $\delta^{15}N$ values derived from diet over different timescales, thus, multi-tissue sampling can allow comparisons of diet over broad temporal scales (Martínez del Rio 2009b, Carleton *et al.* 2008, Kurle 2009). We compared the $\delta^{15}N$ values of tissues deposited in the non-breeding period versus the breeding/moulting period. There have been few studies of seasonal diet change in Neotropical birds that incorporate stable-isotope analysis, but those that have been performed have focused on diet in relation to the seasonal abundance of food (Herrera *et al.* 2003, Herrera *et al.* 2005, Herrera *et al.* 2006, Herrera *et al.* 2009), not on diet complementation during productive phases of the annual cycle.

We compared $\delta^{15}N$ values of birds in our study community to values of sympatric species adapted to low-protein frugivorous diets. *Euphonia* spp. are one of few Neotropical groups known to be exclusive, or near-exclusive frugivores over the entire year, including the breeding period (Morton 1973, Sargent 1993). Two sympatric *Euphonia* species in our study area (yellow-throated euphonia *E. hirundinacea* and olive-backed euphonia *E. gouldi*) provided a control for our exploration of diet in other species. To determine $\delta^{15}N$ values that reflect a diet consisting only of arthropods, we used a species where diet is well known: an obligate arthropodivore, the southern house wren (*Troglodytes aedon*) (Skutch 1960). The diets of many of these species are being measured for the first time in this study. Mixing models cannot be appropriately applied to understand the relative proportions of dietary components because major dietary items, and their $\delta^{15}N$ values and expected diet-tissue fractionation, are not known for these species (Martínez del Rio *et al.* 2009a). As a first step in evaluating protein use and requirements at the time of breeding/moult we have evaluated the $\delta^{15}N$ values of the feathers of each species on a relative scale. We placed *Euphonia* and *T. aedon* as guide markers on a raw $\delta^{15}N$ scale (representing exclusive frugivory and arthropodivory, respectively) to assess the relative breeding/moulting diet of 38 other species. Such comparisons can yield insight into general patterns of protein consumption during breeding/moulting of species where diet is not known.

Methods

Bird and prey sampling:

We sampled birds at three different locations in the Cordillera Isabella, Nicaragua. These were the Finca y Reserva Silvestre Privada Nebliselva El Jaguar (Farm and Private Reserve El Jaguar Cloud Forest, hereafter 'El Jaguar') (1350 masl; 13°10' N, 86°02' W), Datanli-el Diablo Natural Reserve (1390 masl, 13° 7' 60 N, 85° 55' 60 W, hereafter 'Datanli'), and Reserva Natural Sierra Quirragua (500-1300 masl, 12°50' N, 85°27' W., hereafter 'Quirragua'). All three sampling sites incorporate primary and secondary forest as well as, in the case of El Jaguar and Datanli, organic coffee plantations. Only one species was targeted (long-tailed manakin, *Chiroxiphia linearis*) at Quirragua. For more details on the capture locations see Fraser *et al.* (2010) and Martínez Salinas (2008).

Birds were captured using two to 27 mistnets (12 x 2.5 m, 36/30-mm mesh). Nets were set up at roughly 50 m intervals along trails and checked every 40 minutes. In 2008 and 2009 at El Jaguar and Datanli, mistnetting took place for two days, once a month, except in April (2008) and June (2009). Birds at Quirragua were sampled between February and April in 2007. A tail feather (third retrix, R3) and two claw clippings (1-3mm depending upon species, middle claw of both feet) were collected and stored in paper envelopes until analysis. All birds, with the exception of hummingbirds, were banded. The chances of resampling an unbanded hummingbird were low, as trapping effort was rotated between areas and short-term recaptures at a particular site could be identified by evidence of sampling (missing R3 and blunt claws lasting ~2-4 months). All data collected from individuals in the field followed protocols for data collection outlined by Monitoreo de Sobrevivencia Invernal (De Sante *et al.* 2009). Due to the placement of our nets, our community study of diet use is restricted to species that are active within approximately three metres of the ground. The number of individuals sampled is not reflective of abundance at the sites, as birds were sampled opportunistically depending upon the number of captures in any given net-check interval.

While it has been frequently shown that plant and animal production differ consistently in their nitrogen content (%N) and $\delta^{15}N$ values, it was useful to confirm this within our study area and to determine referent baseline values. We hand-collected small fleshy fruits of a size (1-3 cm) commonly consumed by small (<100 g) frugivores (Moermond and Denslow 1985) opportunistically during netting bouts in 2008. We also hand-collected arthropods from the leaf litter and vegetation within the height of mistnets during the same period. We searched for fruit and arthropods in a way similar to a bird foraging and sampled items as encountered. Fruit was identified to family when possible and arthropods were identified to order. Within 24 hours of collection, arthropod and fruit samples were dried in an oven at 60 degrees Celsius for 24 hours and then stored at room temperature until analysis.

Laboratory methods: Bird and prey samples were analyzed at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick, Fredericton, New Brunswick, Canada. To remove surface oils and debris, feathers and claws were soaked in a 2:1 chloroform methanol solution for 24 hours, drained, and left to air dry under a fume hood for 72 hours. Feather samples were prepared by cutting from the distal end with dissection scissors. As values may differ slightly along the length of a single feather (Wassenaar *et al.* 2006) we prepared the tip of each feather for analysis. Claw samples were included whole, or were trimmed from the proximal end to reach optimal target weight. Fruit and arthropods were ground using mortar and pestle. Feathers, claws and arthropods were weighed to 0.2 ± 0.02 mg into tin capsules and fruit was weighed to 1.0 ± 0.02 mg. Target weights differed for plant and animal tissues, as analysis must be adjusted for relative amounts of $\delta^{15}N$ and $\delta^{13}C$. All samples were then combusted in a Carlo Erba NC2500 elemental analyzer. Resultant gases (CO_2 and N_2) were delivered via continuous flow to a Finnigan Mat Delta Plus isotope ratio mass spectrometer and were analyzed for isotopes of nitrogen and carbon. Data were brought to an international scale using commercially available and in-house standards (nicotinamide, BLS and SMB) that were calibrated using International Atomic Energy Association standards CH6(-10.4‰), CH7 (-31.8‰), N1 (0.4‰), N2(20.3‰). Samples taken from a group of unhomogenized clay-colored thrush (*Turdus grayi*) feathers were analyzed with each group of samples to assess analytical precision across runs (Jardine and Cunjak 2005) which yielded nitrogen isotope values of 9.81±2.3‰, n=8. Precision within runs was obtained by analyzing duplicate feather samples from long-tailed manakin (6.62±0.01‰), violet sabrewing (*Campylopterus hemileucurus*) (9.53±0.16‰), long-billed hermit (*Phaethornis longirostris*) (10.19±0.05‰), black-headed nightingale thrush (*Catharus mexicanus*) (6.19±0.23‰), golden-crowned warbler (*Basileuterus culicivorus*) (7.81±0.25‰), chestnut-capped brush-finch (*Atlapetes brunneinucha*) (8.47±0.09‰), clay-colored thrush (8.94±0.16‰) and yellow-throated euphonia (2.34±0.14‰).

Prey and consumer analysis: We compared mean fruit, insect and spider %N and $\delta^{15}N$ using Welch two sample t-tests. Considering that we collected fruit and arthropods as encountered during searches, the statistical variability we measured may not be correct for estimates of fruit or arthropod δD for comparison among groups because sampling may have included multiple samples of a particular species. However, our method does provide a suitable estimate of variability between the diet-available assemblages of fruit and arthropods in the two habitats. To compare protein sources in the non-breeding versus breeding/moulting period we compared claw and feather $\delta^{15}N$ values from each individual in paired-t tests. Claws and feathers are both made of beta (ß) keratin sheets, are mostly protein (~90%) with little lipid content (Fraser *et al.* 1972, Murphy and King 1991a, Fraser and Parry 1996, 2008) and are therefore strong indicators of sources of dietary protein. These tissues differ in their rates and timing of synthesis. Claws grow continuously and reflect dietary information from the previous two to over five months, dependent upon the species (Bearhop *et al.* 2003, Fraser *et al.* 2008, Carleton *et al.* 2008, Fraser unpub. data), thus reflecting long term averages of foraging events. For passerines, individual tail feathers grow over a shorter period of approximately 20 to 25 days (Grubb and Pravosudov 1994) and reflect foraging around the time of moult. Because many species tend to moult after breeding, feather $\delta^{15}N$ may also reflect foraging during the late breeding period. We therefore assume feather $\delta^{15}N$ reflects late breeding and moulting diet (hereafter referred to as breeding/moulting).

We pooled the values from the two *Euphonia* species and compared claw and feather $\delta^{15}N$ using paired t-tests. These served as our control, as we expected no complementation in these species regarding increased protein intake, as both are highly frugivorous birds adapted to a low-protein diet (Morton 1973, Sargent 1993, Herrera *et al.* 2009).

Interspecies comparisons of source protein: By comparing species $\delta^{15}N$ tail feather values with unknown diets to known obligate frugivores (*Euphonia spp.*) and obligate insectivore (southern house wren) we were able to estimate: a) which species rely more upon low-protein foods, such as those produced by plants and b) which rely more upon higher-protein foods, such as arthropods. There is likely a continuum of plant and animal food use by many species and it would be useful to distinguish the relative contribution of various components of mixed-diets (i.e. arthropods and fruit). Mixing models are often used to determine the fractional contribution of dietary sources to the isotopic composition of animal tissue (e.g. Herrera 2003). However, we chose not to employ mixing models, as these are used most successfully when the values of specific prey items and prey-specific discrimination are available for the species of interest (or a close relative) (Bond and Jones 2009, Martinez del Rio *et al.* 2009a). There are no experimental data on appropriate tissue to diet discrimination values for the diverse species and diets in this study. In addition, we anticipated that many of the species in this study consume both plant and animal foods and determining contributing fractions to diet is particularly problematic for omnivores (Caut *et al.* 2009, Martinez del Rio *et al.* 2009a). We therefore have relied upon relative comparisons between species with known (*Euphonia* and *T. aedon*) versus unknown diets to assess the relative degree of arthropod versus plant feeding. This may provide a baseline for more detailed studies of the annual diets of these species – some of which are studied here for the first time.

Where appropriate, we report means, standard deviation and 95% confidence intervals. All analyses were performed using R (version 2.10.1, R Foundation For Statistical Computing, 2009).

Results

Prey: We analyzed 25 individual arthropods of 19 species and 4 orders (Araneae, Coleoptera, Hemiptera and Orthoptera). Mean $\delta^{15}N$ (5.0 ±0.7‰) and %N (11.5 ±0.7) was slightly higher for spiders (*n*=6) than for other arthropods (*n*=19) ($\delta^{15}N$= 4.4 ±3.1‰, %N= 10.6 ±0.6), and was significantly different for %N (t.test: t=2.88, p<0.02, d.f.=2.89) and $\delta^{15}N$ (t.test: t=0.68, p<0.05,d.f.=19.53). We sampled fruit from 32 plant species from families including those known to be targeted by birds such as Moraceae, Solanaceae, Heliconiaceae, Rubiaceae, Sapotaceae and Onagraceae. $\delta^{15}N$ values ranged from -2.5 to 6.8 ‰ (2.4 ±2.6‰) and %N from 0.6 to 4.0 (1.8 ±0.8). As predicted, arthropod and fruit values were significantly different for both $\delta^{15}N$ (t-test: t=3.01, p<0.004, d.f.=47) and %N (t=42.73, p<0.0001, d.f.=50). A biplot of fruit versus arthropod %N and $\delta^{15}N$ values is shown in Figure 3-1. Our prey data are consistent with previous studies showing that arthropods have a higher %N and protein content than fruits collected in tropical forests (Morton 1973, Herrera *et al.* 2003, Jordano 2000, Barea and Herrera 2009).

Birds: We sampled 448 individual birds of 40 species, 12 families (Caprimulgidae, Trochilidae, Dendrocolaptidae, Formicariidae, Pipridae, Tyrannidae, Troglodytidae, Turdidae, Parulidae, Thraupidae and Emberizidae) and 3 orders (Caprimulgiformes, Apodiformes, Passeriformes). We collected tail feathers from 40 species and claw and feather pairings from 24 species. As predicted, in yellow-throated and olive-backed euphonia claw and feather values were not significantly different, paired t= 0.31, p=0.76 , d.f.=12 (Fig. 3-2). Where sample size for each species was greater than 7 (based on power-test for paired-t, delta=2.2, SD=1.8, power=0.8) for claw and feather pairings we conducted paired-t tests (14 species). Differences were significant in 11 species: long-billed hermit (t=2.88, p< 0.01, d.f.=17), violet sabrewing (t=6.08, p< 0.0005, d.f.=7), tawny-winged woodcreeper (*Dendrocincla anabatina*) (t=5.65, p< 0.0013, d.f.=6), long-tailed manakin (t=19.77, p< 0.0001, d.f.=62), ochre-bellied flycatcher (*Mionectes oleaginous*) (t=5.94, p< 0.0001, d.f.=25), plain wren (*Thryothorus modestus*) (t=3.72, p< 0.01, d.f. =6), slate-colored solitaire (*Myadestes unicolor*) (t=5.67, p< 0.0001, d.f.=19), black-headed nightingale-thrush (t=4.73, p< 0.0002, d.f.=19), clay-colored thrush (t=4.21, p< 0.0008, d.f.=15), chestnut-capped brush-finch (t=7.81, p< 0.0001, d.f.=10), variable seedeater (*Sporophila aurita*) (t=2.65, p< 0.026, d.f.=9). Values were not significantly different in three species: yellow-throated euphonia (t=0.99, p=0.36, d.f.=7), yellow-faced grassquit (*Tiaris olivacea*) (t=2.09, p=0.07, d.f.=7) and blue-black grassquit (*Volatinia jacarina*) (t=1.24, p=0.26, d.f.=6) (Table 3-1).

Interspecies comparisons of source protein: Mean nonbreeding foraging values (based on claw $\delta^{15}N$) ranged from 3.48 to 8.14 ‰ and breeding-moulting values (based on tail $\delta^{15}N$) ranged from 4.49 to 10.2 ‰ suggesting a broad range of nonbreeding and breeding/moulting diets in both seasons. The difference in $\delta^{15}N$ between non-breeding (claws) and breeding/moulting (feathers) ranged from -0.64 to +2.31 ‰. Twenty-three of 24 species showed a mean $\delta^{15}N$ increase from nonbreeding to breeding/moulting (Table 3-1). The continuous species-scale of mean feather $\delta^{15}N$ values reveals a broad gradient in dietary-source proteins at breeding/moulting for these species (Fig. 3-3). These comparisons reveal which species rely on foods with relatively low $\delta^{15}N$ (and likely low protein content) during breeding and moult and those that relied upon high $\delta^{15}N$-high protein sources. Species with values near *Euphonia* may indicate species that are particularly well adapted to low-protein diets during productive phases; 7 species had values lower than *Euphonia* which may indicate heavy reliance upon plant production. 10 species yielded higher values than *T. aedon* which may indicate that most feather protein was derived from arthropods.

Discussion

While it has long been known that many species of tropical bird consume a mixture of plant and animal prey, this is the first study to show that, for adult birds, this may be directed in the breeding/moulting period toward foods at a higher trophic level. This is consistent with birds targeting foods with more protein during the period of the annual cycle when nutrient and energetic demands peak, thus supporting the diet complementation hypothesis, where food selection is active and non-random (Murphy 1994a). Surprisingly, a breeding increase in $\delta^{15}N$ was evident in a variety of species, in both those considered highly frugivorous (e.g. long-tailed manakin, Foster 1976, Stiles and Skutch 1989) and mostly insectivorous, such as (e.g. black-headed nightingale-thrush, Stiles and Skutch 1989, Clement 2000). In some instances, we observed seasonal increases in $\delta^{15}N$ that may indicate potential switches from a plant-based source of protein toward an animal-based one, suggesting that the low-protein content of plant foods is limiting to some species at the time of breeding and moulting. We also identified birds that subsisted on low-protein foods year-round, suggesting the potential for special adaptations to low-protein diets in these species. Finally, our data show that one-dimensional diet categories (e.g. frugivore, nectarivore) may be inadequate for describing the complex diets of tropical birds.

Diet complementation hypothesis

In a captive setting, white-crowned sparrows (*Zonotrichia leucophrys*) were able to complement a diet deficient in balanced protein through active selection of an alternative food source such that mass and feather growth rates were similar to birds supplied with one diet of sufficient protein (Murphy 1994a). For the wild tropical birds in our study, most species (11 of 14 for which we had adequate power) displayed similar adjustments in protein-intake and targeted prey at a significantly higher trophic level during the breeding and moulting period. This suggests that these species are able to complement their diets to balance their nutrient needs in the breeding/moulting period. This is consistent with a field study of tropical bird diets, where there was a 'high occurrence' of arthropods and a decrease in vegetable matter in diets of birds during the breeding period in many species, including those traditionally classified as frugivores (Poulin *et al.* 1992). For the 'highly frugivorous' long-tailed manakin (Foster 1976, Stiles and Skutch 1989, Clement 2000), we found a large increase in $\delta^{15}N$ (2.31‰, Table 3-1), suggesting a switch in protein source from mostly fruit in the nonbreeding period to mostly arthropod consumption in the breeding/moulting period. For these birds, the low-protein diet of fruit in the nonbreeding period may be inadequate to supply the high protein demand of the breeding/moulting period. This was not the case for the frugivorous (Clement 2000) slate-colored solitaire (*Myadestes unicolor*). While *M. unicolor* did show a significant increase in trophic position between nonbreeding and breeding/moulting, all tissue values were lower than those of *Euphonia* spp., suggesting *M. unicolor* derives much or all of its protein from low-protein sources (possibly plants) year round. These data suggest a heretofore undescribed adaptation to a low-protein diet in this species. The seed-predators (yellow-faced and blue-black grassquit and variable seedeater) also showed little change in trophic level between nonbreeding and breeding/moulting periods. Seeds contain more protein than fruit or nectar (Klasing 1998), which may support the higher protein demand of breeding and moulting in these granivores.

Our results also support data suggesting that food availability, in this case arthropod foods, are influential in the timing of breeding seasons (Jenkins 1969, Poulin *et al.* 1992, Wikelski *et al.* 2000, Hau *et al.* 2008). Arthropod abundance may influence breeding phenology even in birds thought to be highly frugivorous or nectarivorous. This is supported by the observation that many frugivores, nectarivores and insectivores initiate breeding at similar times and that peak breeding overlaps with peak arthropod, not peak fruit, availability (Jenkins 1969, Poulin *et al.* 1992). While it is difficult from our data to determine the general proportions of arthropods in the diet, the increase in protein consumption in many species, with some remarkable increases such as in the long-tailed manakin (Foster 1976, Stiles and Skutch 1989) highlight the potential importance of arthropods in the breeding/moulting diets of birds of a variety of different foraging guilds.

Even though insectivory is a common dietary strategy, the nutrition of this guild is relatively understudied (Klasing 1998). It has generally been assumed that birds feeding at high trophic positions do not need to complement their diets (Murphy 1996). Surprisingly, in our study species that likely consumed arthropods in the non-breeding season such as the hummingbirds, wrens, and some woodcreepers, also showed higher $\delta^{15}N$ values in the breeding period. For example plain wren is thought to consume a diet composed of mostly insects and spiders (Stiles and Skutch 1989), yet our results show significantly higher $\delta^{15}N$ during the breeding and moulting period. This could be accomplished by preferentially consuming arthropods with more protein during this period, such as spiders. An alternative hypothesis are that differences in protein quality, not protein quantity, between breeding and non-breeding could have contributed to the differences we observed. While there is currently only comparative and not experimental evidence to support this idea, a decrease in protein quality in consumer diets could produce elevated $\delta^{15}N$ values in consumer tissues through changes in discrimination factor (i.e. the difference between diet and tissue values) (Robbins *et al.* 2005, Martínez del Rio *et al.* 2009a). It is unclear if this could have contributed to some of the larger differences we measured between claws and feathers such as in clay-colored thrush and tawny-winged woodcreeper for example. Whether we invoke protein quantity or quality to explain these differences, it is interesting that the pattern we observed in insectivores may reflect a diet shift during the breeding/moulting period and this warrants further, more detailed investigation.

We have also considered physiological explanations for $\delta^{15}N$ enrichment of feathers over claws. $\delta^{15}N$ values in animal tissues may become enriched with fasting time (reviewed in Martinez del Rio *et al.* 2009a). In a bird-specific study using captive quail, individuals fed *ad libitum* and those with diets simulating nutritional stress showed no significant difference in $\delta^{15}N$ feather values (Hobson *et al.* 1993), suggesting that fasting does not increase the $\delta^{15}N$ of developing feathers. Similarly Kempster *et al.* (2007) found no feather enrichment with fasting in captive song sparrow (*Melospiza melodia*). Thus, while it seems likely that birds rely on protein stored in muscle tissue during the day to support overnight feather growth (Murphy and King 1990, Murphy and Taruscio 1995) this likely has little if any effect on feather $\delta^{15}N$ values.

At the outset of this study, we presumed that because claws and feathers are composed of the same type of keratin (see Methods) there is unlikely to be differences in amino acid composition between these tissues. Such differences could promote varying discrimination factors and lead to inherent differences in $\delta^{15}N$ between these two tissues. This presumption seems to have been borne out in our data, where we found no difference in $\delta^{15}N$ between claws and feathers where this was predicted in obligate, year-round frugivores (i.e. *Euphonia spp.*). Unfortunately however, controlled laboratory tests comparing amino acid composition and $\delta^{15}N$ of claws and feathers are lacking. Our results must therefore be viewed with some degree of caution until we can estimate the potential influence of chemical differences between claws and feathers on the patterns that we have observed.

We also considered the potential influence of seasonal patterns of baseline $\delta^{15}N$ on enrichment in feather values we observed for several species. Unlike what is common in many aquatic systems, there is likely not a regime shift in primary production at the base of the food web in our study areas. In evergreen tropical wet forests, plants continue to produce foliage, and many produce fruit, year round. We therefore would not expect a seasonal shift in environmental baseline $\delta^{15}N$ that would be carried to higher order consumers such as birds. This is supported by previous studies in other Neotropical wet forests where $\delta^{15}N$ values at the base of the food web did not shift over the course of the year (Herrera *et al.* 2003, Herrera *et al.* 2009). However, such seasonal differences in $\delta^{15}N$ were observed in tropical dry forest (Herrera *et al.* 2006), suggesting that patterns may differ between these two systems. Birds in our study that showed a significant increase in $\delta^{15}N$ have breeding seasons that are asynchronous and collectively span 11 months, which strongly supports the notion that seasonal, baseline shifts in $\delta^{15}N$ are not responsible for the patterns we observed.

While $\delta^{15}N$ values of plant and animal prey collected in this study were significantly different, there was a range of values for both food types, owing to varying amounts of nitrogen in these foods (Fig 3-1.). Using two species as protein 'place-markers' (obligate frugivores, two *Euphonia* species; obligate insectivore, southern house wren) we were able to distinguish which species likely rely exclusively on plant protein during moult and which rely on animal protein (Fig. 3-3). These inter-species comparisons highlighted seven species that may rely exclusively on plant production and 10 that rely on animal foods during breeding/moulting. We obtained the surprising result that spotted woodcreeper, usually considered to feed mostly on arthropods and small vertebrates (Stiles and Skutch 1989), likely fed exclusively on plant-produced foods in our study system.

There are 23 species with $\delta^{15}N$ values between the obligate frugivore (*Euphonia*) and obligate insectivore (wren) that may have derived protein from a mixture of plant and animal sources, or have fed upon plants higher in $\delta^{15}N$ than *Euphonia*, or arthropods lower $\delta^{15}N$ than wren. Due to the limitations of mixing models as they pertain to our study (see Methods) we were unable to further distinguish the relative contributions of arthropods and fruit to the diets of these species using these methods. While these results are preliminary, they represent an important first step in determining sources of protein during breeding and feather production in this community of birds. Interspecies comparisons of feather $\delta^{15}N$, may enable us to determine which species may be particularly well adapted to low-protein diets during productive phases, such as we found in this study with slate-colored solitaire.

Implications for our understanding of tropical avian systems

In tropical birds, lek mating theory suggests that abundant plant-produced foods 'emancipate' males from parental care because females can successfully provision nestlings alone (Snow 1971, Snow and Snow 1979). It is noteworthy that the four species in our study that make the biggest increase in protein consumption between non-breeding and breeding/moulting diet are birds where females provide all the parental care (violet sabrewing, tawny-winged woodcreeper, long-tailed manakin, and ochre-bellied flycatcher). A fifth species, long-billed hermit, with sole female care also showed significant compensation (Table 3-1). Our inter-species comparisons of $\delta^{15}N$ values (Figure 3-3), also suggest that these species consume arthropods during the breeding and moulting period. While further investigation is required, these data suggest that diet complementation may be a means by which species exhibiting solo female parental care and lek mating systems compensate for higher energy expenditures of display (males) and caring for young (females). Detailed study of diet phenology in lekking species, including an exploration of male versus female diet, may yield new insights into the ecological correlates of mating systems.

It is not surprising that the hummingbird species in our study seemed to derive all of their feather and claw protein from arthropods (Fig. 3-4). Nectar is notoriously low in protein (Baker and Baker 1982, Klasing 1998) and it has long been observed that hummingbirds consume arthropods (e.g. Belt 1874, Stiles 1995, Remsen *et al.* 1986) presumably to meet their protein demands. Hummingbirds likely consume arthropods daily (Remsen *et al.* 1986) and females may also consume more arthropods and feed at higher trophic levels than males to support their greater investment in reproduction (Stiles 1995, Hardesty 2009). The notable contribution to our understanding of arthropod feeding in hummingbirds from our study is that birds targeted higher protein sources more during the breeding/moulting period than the non-breeding period. This has important implications for understanding breeding phenology and intratropical migration in hummingbirds. Most studies of Central American hummingbirds have focused on flower phenology to explain the timing of breeding, moulting and patterns of altitudinal migration (e.g. Feinsinger 1980, Stiles 1980, Levey and Stiles 1992). Our data show that patterns of arthropod consumption vary seasonally in the two hummingbird species in our study. This could be independent of seasonal availability of arthropods, reflecting a behavioral switch in foraging strategy only. However, it is plausible that altitudinal migrations observed in hummingbirds (Fraser et al. 2010) could result from individuals tracking abundance of preferred arthropod foods during periods of higher protein requirements. The ecological correlates of arthropod feeding in hummingbirds warrant further investigation.

Our results also highlight how dietary categories used to define Neotropical birds can be problematic. Classification is commonly categorical (e.g. frugivore) and qualitative (e.g. 'highly frugivorous') and is rarely attentive to the seasonal differences in diet composition that likely occur in many species. For example a bird classified as an 'omnivore' may be an obligate insectivore in one season and consume only fruit in another. Yet, these broad, qualitative characterizations of tropical bird diet have been used to support ideas about morphological evolution and the organization of community structure (e.g. Feinsinger 1980, Stiles 1985), altitudinal migration and the evolution of long-distance migration (e.g. Levey and Stiles 1992, Boyle and Conway 2007), breeding phenology (e.g. Stiles 1980), and the evolution of mating systems (e.g. Beehler 1987). This conceptual development has proceeded notwithstanding the lack of detailed information on the year-round diet of adult birds. Hummingbirds' heavy reliance on arthropods suggests that describing these species as nectarivores is misleading and perhaps inappropriate. So too for the long-tailed manakin which is considered a highly frugivorous species (Foster 1976, Stiles and Skutch 1989). A new structure for characterizing Neotropical bird diets may more appropriately direct research into the ecological correlates of life history strategies in these species.

The study of diet complementation and other temporal-based investigations of tropical bird diets has great potential for deepening our understanding of the interaction between seasonal ecological changes and physiological changes within individual birds. The role of the latter in shaping the natural history strategies of tropical birds has been largely neglected. Rather, the use of qualitative diet categories and an emphasis on food abundance have been the primary currency in explorations of many aspects of the behavioural ecology of tropical birds. While our data must be seen as preliminary and are restricted to one tropical avian community, we reveal an underlying complexity of seasonal diet use in this food web that may also be found in other systems. A study of resource use attentive to the specific seasonal needs and dietary strategies of birds may yield more powerful insights into factors that drive behavior of tropical birds.

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Table 3-1. Seasonal comparisons of $\delta^{15}N$ values based on claws and feathers. Species are ordered by the degree of $\delta^{15}N$ change between seasons (values bolded). Control obligate frugivores (*E. hurundinacea* and *E. gouldi*) are included for comparison. An asterisk (*) indicates significant difference in paired-t comparison of claw and feather $\delta^{15}N$ values (when sample size is 7 or greater).

Species	*n*	Claw $\delta^{15}N$ (mean±SD)	Tail $\delta^{15}N$ (mean±SD)	Difference in mean $\delta^{15}N$ from nonbreeding to breeding/moulting
Yellow-throated Euphonia - *Euphonia hirundinacea*	8	6.25±1.89	5.61±1.57	**-0.64**
Eye-ringed Flatbill- *Rhynchocyclus brevirostris*	2	6.0±0.19	6.04±0.14	**+0.04**
White-breasted Wood-Wren – *Henicorhina leucosticta*	5	5.96±1.38	6.44±0.88	**+0.48**
Yellow-faced Grasssquit – *Tiaris olivacea*	8	4.53±0.98	5.04±0.86	**+0.51**
Olive-backed Euphonia - *E.gouldi*	5	5.20±0.90	5.87±0.69	**+0.67**
Golden-crowned Warbler – *Basileuterus culicivorus*	6	6.4±1.03	7.11±0.42	**+0.71**
Scaly-throated Foliage-Gleaner – *Anabacerthia variegaticeps*	5	5.13±0.35	5.89±0.58	**+0.76**
Southern House Wren – *Troglodytes aedon*	4	8.09±0.28	8.86±0.63	**+0.77**
Plain Wren – *Thryothorus modestus*	7	8.14±0.90	8.97±0.82	**+0.83***
Long-billed Hermit – *Phaethornis longirostris*	18	7.78±1.06	8.66±0.75	**+0.88***
Common Bush-Tanager – *Chlorospingus ophthalmicus*	5	5.51±0.99	6.45±0.22	**+0.94**
Variable seedeater – *Sporophila aurita*	10	5.13±0.93	6.08±1.21	**+0.95***
Blue-black Grassquit – *Volatina jacarina*	7	6.34±1.76	7.31±1.27	**+0.97**
Crimson-collared Tanager – *Ramphocelus sanguinolentus*	5	5.56±0.48	6.54±1.26	**+0.98**

Olivaceous Woodcreeper – *Sittasomus griseicappillus*	2	6.14±0.33	7.14±1.04	**+1.00**
Slate-colored Solitaire – *Myadestes unicolor*	20	3.48±0.8	4.49±0.88	**+1.01***
Spotted Woodcreeper – *Xiphorhynchus erythropygius*	3	4.20±0.76	5.21±1.00	**+1.01**
Black-headed Nightingale Thrush – *Catharus mexicanus*	20	5.75±0.90	6.84±1.08	**+1.09***
Chestnut-capped Brushfinch – *Atlapetes bruneinucha*	11	6.83±1.14	7.93±1.1	**+1.1***
Clay-colored Robin – *Turdus grayi*	16	7.98±2.26	9.23±2.2	**+1.25***
Tawny-winged Woodcreeper – *Dendrocincla anabatina*	7	5.88±0.29	7.22±0.55	**+1.34***
Ochre-bellied Flycatcher – *Mionectes oleaginous*	26	6.31±0.81	7.75±1.27	**+1.44***
Violet Sabrewing – *Campylopterus hemileucurus*	8	8.08±0.81	9.74±1.32	**+1.66***
Long-tailed Manakin – *Chiroxiphia linearis*	63	4.77±0.66	7.08±0.85	**+2.31***

Figure 3-1. A comparison of $\delta^{15}N$ of claw (nonbreeding) and tail feather (breeding/moulting) in the obligate frugivores *Euphonia hirundinacea* and *E. gouldi* was nonsignificant as predicted.

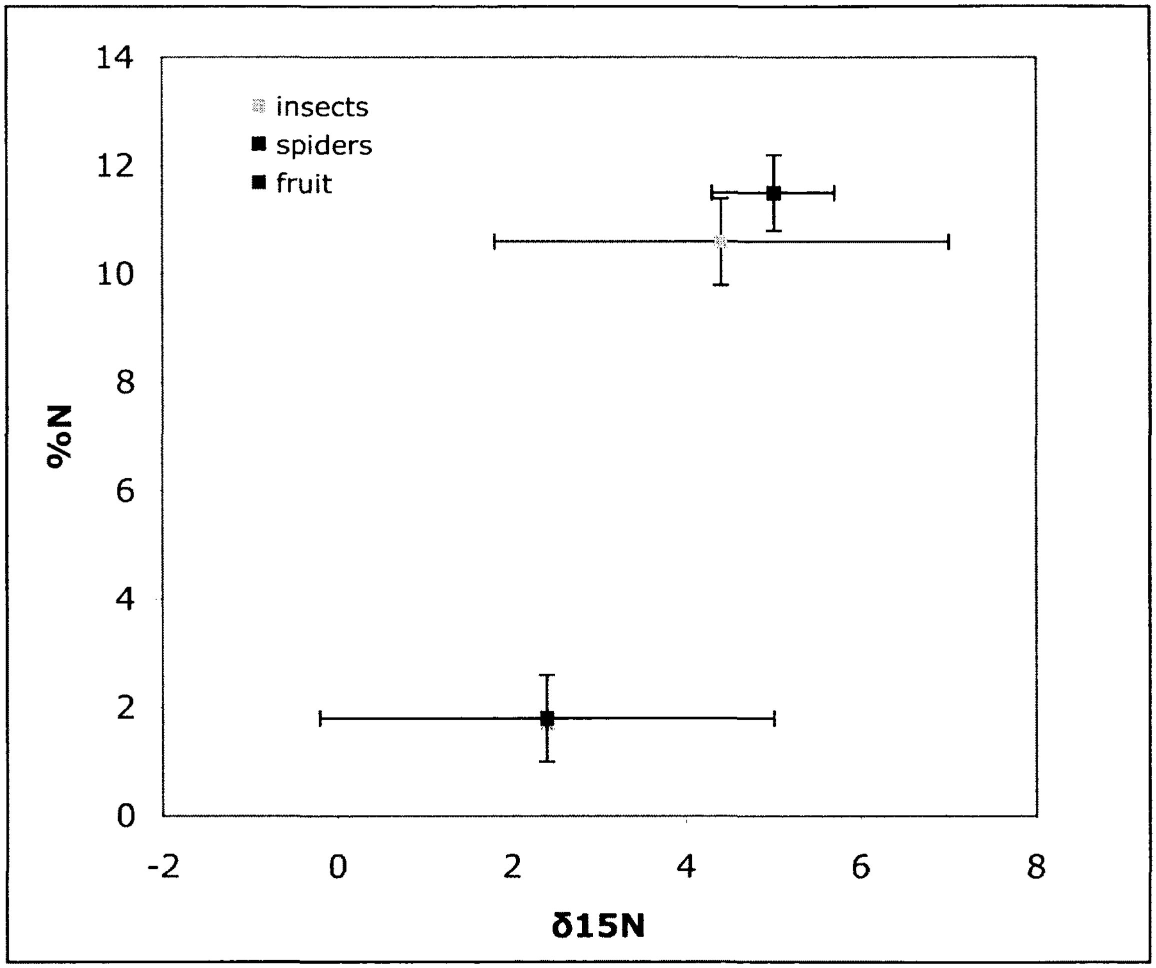


Figure 3-2. Boxplot (whiskers, interquartile) comparisons of δ^{15}N of claw (nonbreeding) and tail feather (breeding/moulting) in the obligate frugivores *Euphonia hirundinacea* and *E. gouldi* was nonsignificant as predicted (paired-t= 0.31, p=0.76 , d.f.=12).

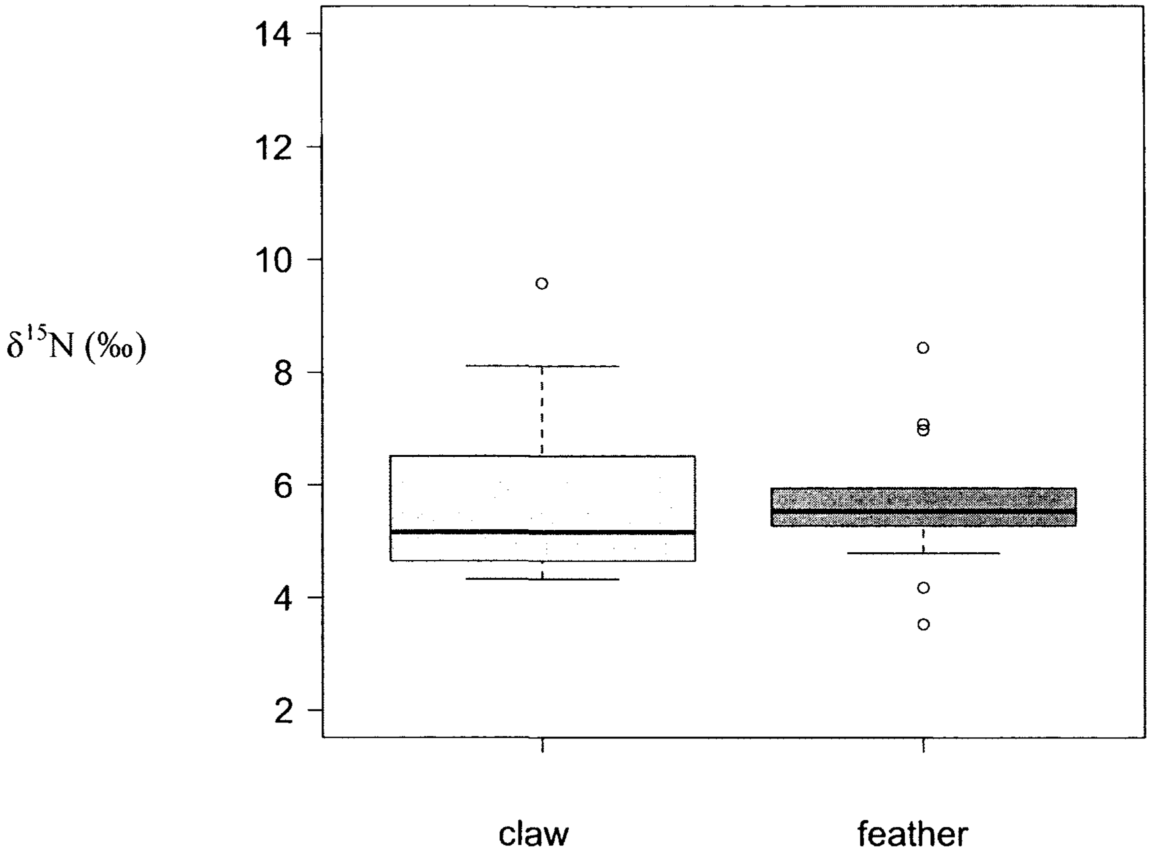
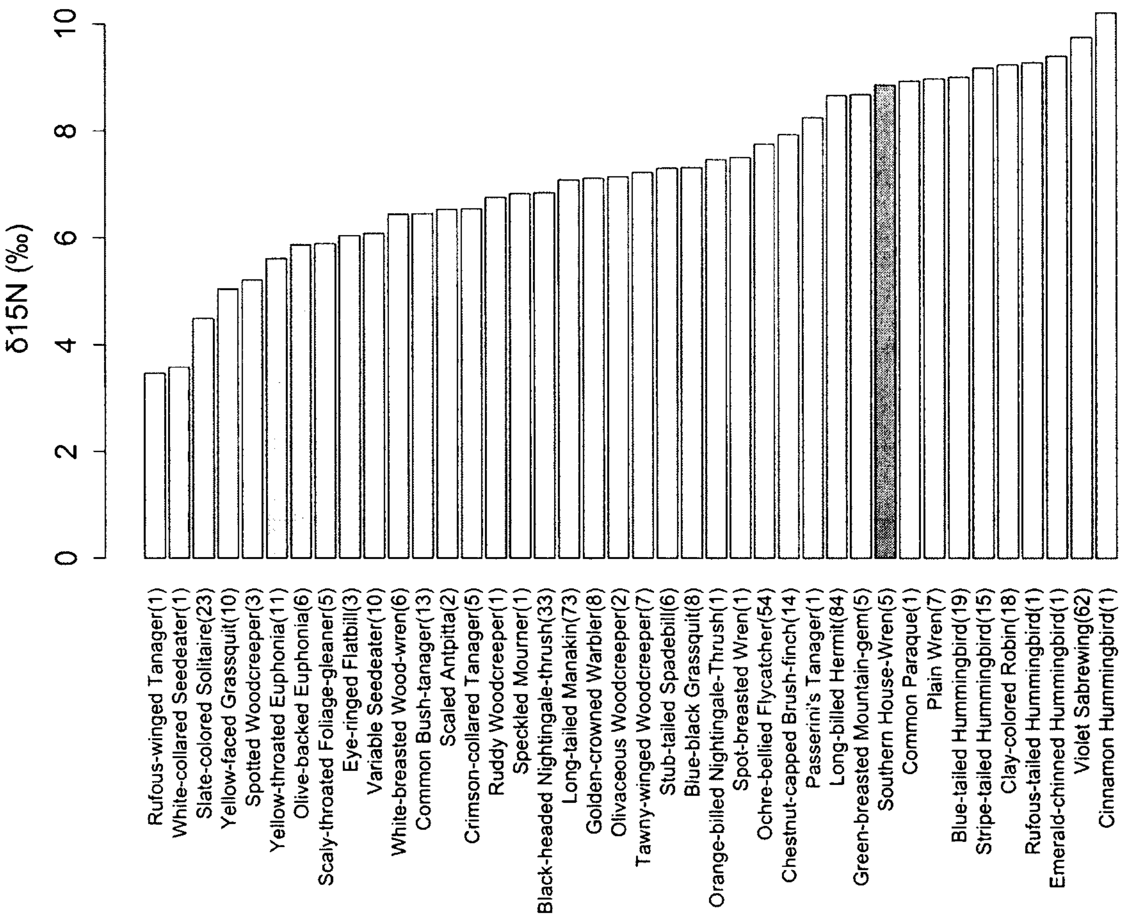


Figure 3-3. Barplot of tail feather δ^{15}N of 40 species of Neotropical birds. δ^{15}N values reflect the source of dietary protein at the time of breeding/moulting. Obligate frugivores (yellow-throated and olive-backed euphonia) are highlighted in light grey. An obligate arthropodivore (southern house-wren) is highlighted in dark grey. The diets of other species are measured in this study for the first time. Comparisons with euphonia and wren allow a general estimate of which species may have physiological adaptations to low-protein diets (such as plant-based) and which are more likely to consume foods with higher protein (such as arthropods) to support breeding/moulting. Sample size for each species in brackets.



CHAPTER 4: EVIDENCE OF ALTITUDINAL MOULT-MIGRATION IN A CENTRAL AMERICAN HUMMINGBIRD, *AMAZILIA CYANURA*

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Key Words: altitudinal migration, diet, intratropical migration, Neotropical birds, Nicaragua, resource tracking, seasonality

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[1] Kevin Fraser collected and analyzed data, interpreted results, and wrote manuscript, which is published in Journal of Tropical Ecology, 26, 645-648.. Antony Diamond supervised K.C.F's PhD thesis and edited manuscript.

[2] Liliana Chavarría initiated the study, collected data and edited manuscript.

For birds, moulting is an energetically costly endeavour (Murphy & King 1991), the timing and location of which may be flexible and governed by local ecological factors (Pyle *et al.* 2009, Rohwer *et al.* 2005). Some species or individuals may pause during long-distance migration, or migrate specifically to moult (Greenberg *et al.* 1974, Pyle *et al.* 2009, Rohwer *et al.* 2005, 2008). This strategy may be most common when food abundance reaches a nadir at the end of the breeding period, promoting movement to areas where food is more plentiful and the energetic and nutritive demands of moult may be met more suitably (Rohwer *et al.* 2005). This pattern is exemplified by insectivorous songbirds breeding in temperate, western North America that pause on southward migration to moult amidst the food flush that occurs following heavy rains in the Mexican monsoon region (Pyle *et al.* 2009, Rohwer *et al.* 2005, 2009), or more rarely, migrate upslope after breeding to moult in more moist, productive areas at higher elevation (Butler *et al.* 2002, Greenberg *et al.* 1974, Rohwer *et al.* 2008, Steele & McCormick 1995). Such altitudinal migration may be much more common in the Neotropics where many species engage in seasonal shifts in elevation. Hypotheses proposed to explain this behaviour, however, have focused on links made between migration and a principally frugivorous or nectarivorous diet (Levey & Stiles 1992, Stiles 1985, 1988) and not on moult requirements or phenology. Fruit and nectar availability may vary seasonally over an elevational gradient, and birds may migrate in order to track peak abundances (Levey & Stiles 1992, Loiselle & Blake 1991). Hummingbirds may also track arthropod (particularly spider) abundance, but this possibility remains little explored (Cotton 2007, Stiles 1980).

From 2005 to 2010, we observed a common but little studied Central American endemic hummingbird (*Amazilia cyanura,* Gould 1859, blue-tailed hummingbird, 4.5 g) at our Nicaraguan study site (El Jaguar; 1350 m asl; 13°10' N, 86°02' W) during the wet season, but at no other time of year. An individual captured during mist-net monitoring at the site in 2005 was in flight-feather and body moult, but no indicators of local breeding were observed. Considering that El Jaguar sits atop the highest peaks in the region, and that most observations of *Amazilia cyanura* are on the Pacific coast near sea level (L. Chavarría pers. obs., M. Torrez pers. comm., Stiles & Skutch 1989), we presumed that individuals arriving at the study area were derived from lowland populations. We hypothesized that *Amazilia cyanura* may be a moult-migrant that moves from lower elevation to the study site to complete moult, but not to breed. The closely related, and better-studied *A. saucerrottei* (De Lattre & Bourcier 1846; steely-vented hummingbird), breeds from December to April in Costa Rica (Stiles & Skutch 1989). If we presume similar timing of breeding for *Amazilia cyanura*, then arrival at El Jaguar is consistent with migration following breeding. From 2007 to 2010 we expanded mist-netting efforts to determine more specifically the period of tenure and activity of *Amazilia cyanura* at the study site.

El Jaguar can be classified as lower-montane moist forest (Holdridge 1967) and consists of mostly primary forest and second-growth (~80% of the site) as well as active coffee (*Coffea* spp.) plantation (~20%), shaded by banana, *Musa acuminata*. Capture effort was divided between the coffee plantation and adjacent forest. Birds were captured using 2-27 mistnets (12 × 2.5 m, 36-mm mesh). From October 2007 to May 2010, mistnetting took place for 2-6 d each month, except in April 2008, June 2009 and

February 2010. All data collected from individuals in the field followed protocols outlined by Monitoreo de Sobrevivencia Inveranal (De Sante *et al.* 2009). Body moult was scored on a four-point scale (0 = none to 4 = heavy). Flight feather moult was recorded as absent or present (which included adventitious, symmetric or juvenile growth); which specific feathers were moulting was recorded for some captures. Fat was scored visually on a scale of 0 (none) to 7 (excess) based on estimates of amount of fat in the furcular region of each individual. Breeding indicators were assessed visually and recorded on a number scale (brood patch: 0 = none to 5 = new feathers growing in; cloacal protuberance: 0 = none to 3 = large). We were not able to distinguish older from younger adults as no published accounts of visual age indicators exist for *Amazilia cyanura* or a closely related species, but plumage characters would allow the identification of juveniles (Howell & Webb 1995).

We logged 12968 cumulative net hours between October 2007 and May 2010 (Figure 4-1a), We captured 12 *Amazilia cyanura* between 22-23 May 2008, 8 between 12-13 May 2009, and three on 19 April 2010 (Figure 4-1b). We did not capture, or observe any *Amazilia cyanura* during the mist-netting bouts in any other months (June-March). In our pilot work for this project, we captured one *Amazilia cyanura* in July (2005) but from 2007 to 2010 we captured birds only in April and May. All individuals were caught in mist nets placed in the coffee plantation and no *Amazilia cyanura* was captured, or observed, in the adjacent forest. Although we captured many individuals of several hummingbird species smaller than 5 g, the mesh-size of the mist nets we used was large for birds of this size (36 mm). If this reduced our capture rate, our main results should not be affected, but it may preclude the use of our data for density estimates.

Of the 23 individuals captured, 17 (74%) were in active flight feather moult and 21 (91%) were in active body moult. When time at the ringing station allowed, specific feather tracts in moult were noted; four birds were growing primary feathers and one was growing retrices. Of those in body moult, 17 (81%) were scored as 'medium' or 'heavy' moult. All birds captured had adult plumage (Howell & Webb 1995, Stiles & Skutch 1989). Most birds (68%) had fat scores of 1 (n = 9) or 2 (n = 4). We did not observe any indications of breeding such as nests, breeding displays or the capture of juveniles or recently fledged young. No captured birds had brood patches or cloacal protuberances, although these may not be reliable indicators of breeding activity in hummingbirds (Diamond 1974). We did not ring individuals so have no data on inter-year recaptures. Missing tail feathers (due to sampling for a related project) would have identified intra-year recaptures but we did not observe any.

Our data suggest that *Amazilia cyanura* moves upslope to high-elevation cloud forest in Nicaragua to moult, but not to breed. This is the first observation of a moult-migration strategy in a hummingbird. Our captures from 2007 to 2010, along with an earlier observation, suggest that *Amazilia cyanura* tenure at El Jaguar is from late April to mid-July. Despite 9730 h of trapping effort between August and March, we caught no *Amazilia cyanura*. It is unlikely that this species breeds while at El Jaguar, as we found no signs of breeding activity (capture of fledged young, nests or displays, brood patch or cloacal protuberance). No nests or breeding activity have been observed during fieldwork at this site since 2004 (L. Chavarría, unpubl. data). Future research may show whether *Amazilia cyanura* captured at El Jaguar originate, and possibly breed, in lowland areas along the Pacific coast where they are more commonly observed. Little is known about breeding activity in *Amazilia cyanura* and the nest of this species is undescribed (Howell & Webb 1995).

Most (68%) *Amazilia cyanura* individuals were carrying relatively large amounts of fat and yielded scores of one or two. For comparison, two other hummingbird species that spend the year in the El Jaguar study area carried less fat; in *Campylopterus hemileucurus* (Deppe 1830; violet sabrewing) 34% of captures had fat scores of 1 or 2; in *Phaethornis longirostris* (Delattre, 1843; long-billed hermit), 23% of captures had a score of 1, 0% with a score of 2 (K. Fraser & L. Chavarría unpubl. data). Higher fat scores in *Amazilia cyanura*, as compared to known sedentary species, are consistent with patterns of fat deposition to support migration, but also patterns reported for hummingbirds in active moult (Stiles 1980).

Determining which factors favour upslope migration and moult in *Amazilia cyanura* is an interesting area for future research. Unproductive late-summer breeding areas seem to favour moult migration in some North American insectivorous passerines, which delay moult until arrival in Mexican monsoon region or at higher elevation within the U.S. where more moist conditions support a higher abundance of arthropods (Pyle *et al.* 2009, Rohwer *et al.* 2005, 2009). Tenure of *Amazilia cyanura* at El Jaguar coincides with the early rainy season, which generally corresponds to peak arthropod abundance and bird breeding activity in tropical forests (Poulin *et al.* 1992). Hummingbirds, including *Amazilia* species, commonly moult in the mid-rainy season (Stiles 1980) perhaps owing to the increased availability of arthropods. *Amazilia cyanura* and five other hummingbird species captured at the study site derive their feather protein principally from arthropods. Two of these species (*Campylopterus hemileucurus* and

Phaethornis longirostris) consumed prey with higher protein concentration in the breeding and moulting period than in the non-breeding period (Fraser *et al.* unpubl. data) which emphasizes the seasonal importance of arthropods. An exploration of seasonal arthropod availability over an elevational gradient may yield insight into factors driving a moult-migration strategy in *Amazilia cyanura.*

Species that migrate seasonally over an elevational gradient may pose a particular challenge to conservationists, in that altitudinal migrants often make use of multiple habitat types over a broader geographical range than more sedentary species (Powell & Bjork 1995, Winker *et al.* 1997). *Amazilia cyanura*, a Central American endemic, may require both suitable lowland habitat for breeding, as well as upland habitat for moulting. Further investigation may yield other altitudinal moult-migrants in this region.

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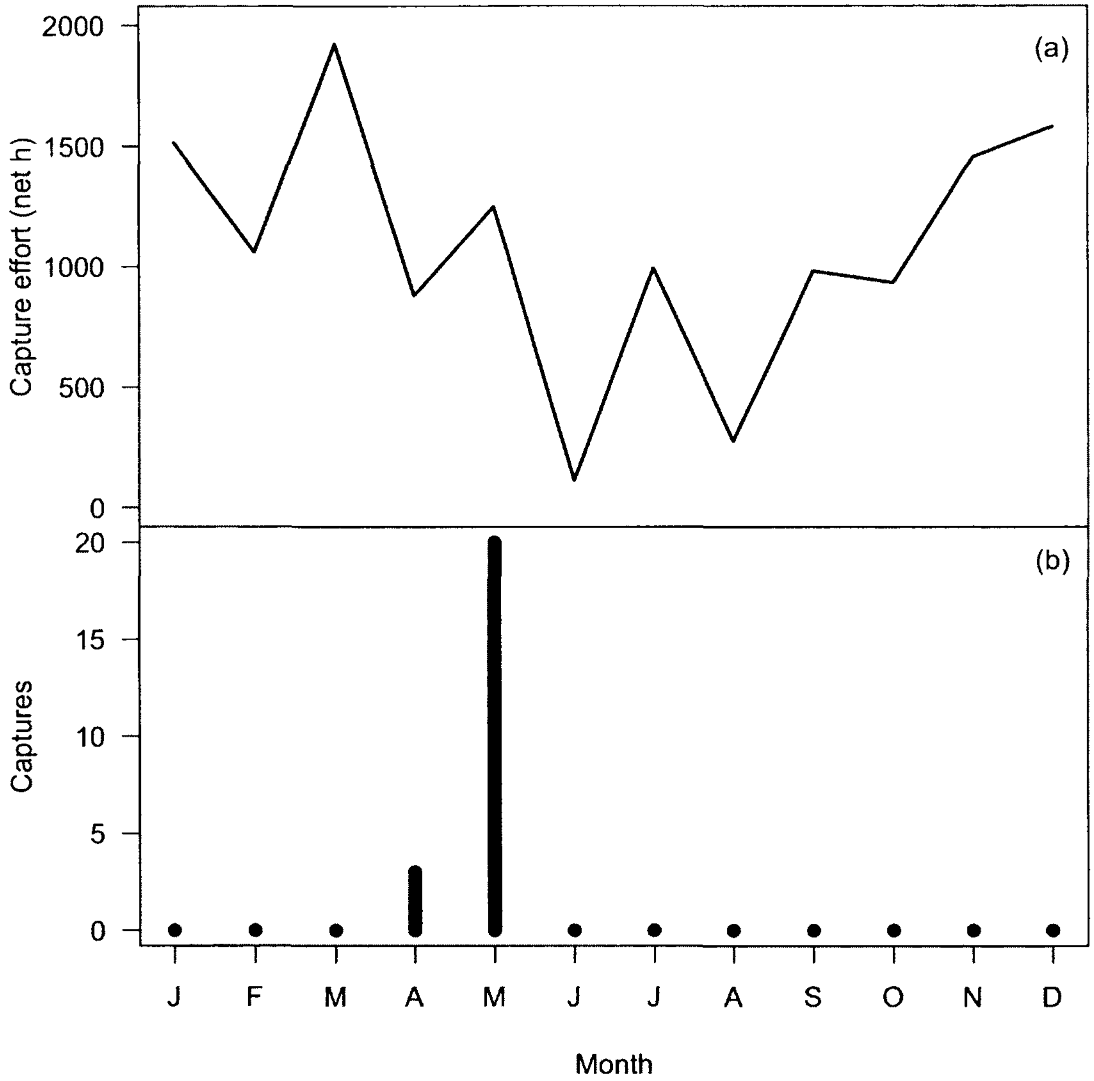
Figure 4-1. Netting effort (a) and captures (b) of *Amazilia cyanura* between October 2007 and May 2010 at 1350 m in north-western Nicaragua. Most captured individuals (22/23) were in active moult (91% in body moult, 74% in flight feather moult). No breeding activity has been observed at the capture site from 2004-2010.



CHAPTER 5: MIGRATION, DIET OR MOLT? INTERPRETING STABLE-HYDROGEN ISOTOPE VALUES IN NEOTROPICAL BATS

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[2] Emily McKinnon collected data, assisted with interpretation of results and edited manuscript.

Abstract

Migratory behavior in bats is poorly described, particularly in the Neotropics. Stable-hydrogen isotope (δD) analysis may allow tracking of altitudinal movements of bats but has not been explored. δD values in rainwater (δD_p) decrease linearly with altitude and are reflected in the keratinous tissues of animals through diet. A mismatch between keratin δD (δD_k) and that expected at the capture site based on δD_p can indicate prior migration. We collected rainwater, claws and hair from eight bat species at two lower-montane forest sites in Nicaragua. Claw δD for *Carollia brevicauda* and hair and claws for *Desmodus rotundus* (known to be non-migratory) fell within the predicted range based on rainwater (-17 to -60‰) suggesting these tissues were synthesized at the study site. Most δD tissue values for *Artibeus toltecus*, *Sturnira lilium*, *Glossophaga soricina*, *Anoura geoffroyi*, and hair for *C. brevicauda* were more negative than predicted for the capture site (-60‰) based on locally sampled rainwater and on values predicted based on long-term, mean annual growing-season precipitation suggesting tissue synthesis at higher elevation and migration downslope to the capture site. However, our study area represents the highest elevation in the region; the nearest appropriate higher elevations are 350-500km away and seasonal migration is expected to be less than 200 km. Thus we consider that seasonal shifts in δDp (9 to -45 ‰) may result in differences in species which molt at different times, and that diet may have driven differences in δD. Our results suggest that the effects of molt timing and diet may first need to be understood before δD may be successfully used to track bat movements.

Key Words: Altitudinal migration; elevational gradient; frugivorous bats; nectarivorous bats; lower-montane moist forest; Nicaragua.

MIGRATION LIKELY PLAYS AN IMPORTANT ROLE IN THE LIVES OF MANY species of tropical bats. Highly vagile animals that feed on fruit or nectar may be more prone to altitudinal movement as they track food that is available asynchronously at different elevations. This is a pattern hypothesized for both bats (Fleming & Eby 2003) and birds (Stiles 1988, Levey & Stiles 1992). While altitudinal migration in bats has not been studied in this context, it is postulated that seasonal fluctuations in food availability, and associated rainfall patterns, generally drive the movements of tropical bats (Fleming & Eby 2003). However, many lacunae exist regarding our knowledge of bat movements in the tropics and most species have yet to be classified as migratory or sedentary. Traditional methods of tracking bats over large distances have proven ineffective (Cryan *et al.* 2004). Banding studies in the tropics are rare (Fleming & Eby 2003) and few marked bats are ever recaptured (Cryan *et al.* 2004). Current radio-transmitter technology is also limiting; transmitters that are small enough for most bat species lack sufficient range or battery life to track bats over long distances (Cryan *et al.* 2004) and current geolocator technology, recently deployed on small passerines (Stuchbury *et al.* 2009) is still too large to be carried safely by many Neotropical bats.

Stable-isotope analysis may be a means to detect migration without the prior capture of individuals. Stable carbon ($^{13}C/^{12}C$) isotopes were used to identify bats that migrated altitudinally through the identification of an individual that had fed on an isotopically distinct carbon source not available at the capture site (Herrera 1997). The use of stable-hydrogen isotopes (H^2/H^1, δD, deuterium) to track bat movements has not been employed until a recent study of long-distance migration (Cryan *et al.* 2004) but may also allow the detection of altitudinal migration as it has for birds (Hobson *et al.* 2003, Fraser *et al.* 2008a).

δD values are expressed as the ratio of the heavy (^2H) to light isotopes (^1H), in units of permil (‰), in delta (δ) notation. When a vapour mass moves over an orographic barrier it cools as it rises. Through an equilibrium fractionation during cooling (Rayleigh distillation), the heavier isotope is lost preferentially through rainout, such that subsequent rainfall from the same vapor mass becomes progressively depleted in ^2H (Clark & Fritz 1997). This process results in a globally observed decrease in stable-hydrogen isotope values of precipitation (δD_p) of about -2.2‰ per 100 m increase in elevation (Poage and Chamberlain 2001, Hobson *et al.* 2003). Animals incorporate local δD_p through drinking water and diet. Keratinous tissues, such as fur and claws, incorporate δD_p during synthesis and are metabolically inert once grown (Hobson & Wassenaar 1997). Theoretically, if an animal synthesizes tissues at one elevation, then moves to another where it is captured and sampled, there should be a mismatch between keratin δD (δD_k) and that expected at the capture site based on δD_p.

Researchers often calculate locally expected values based on a 40-year data set publicly available through the IAEA (2001). Spatial models of δD_p, corrected for elevation and based on weighted averages of growing-season precipitation, provide a powerful tool for estimating locally expected animal tissue values (Bowen *et al.* 2005). However, there is monthly, seasonal and annual variance in δD_p and it is rare that an IAEA (2001) collection station is positioned near areas where animal tissues are collected. This variation could contribute to a reduced ability to detect or track migration when calculating expected δD_k based on δD_p. Despite a general understanding of this issue, the collection of rainwater in the temporal and geographical area of interest is rarely employed in animal migration studies. We therefore determined local δD_p based on long-term data available for our study area and on precipitation collected within the study site.

We applied isotopic methods to the identification of migration events in an understory bat community in two sites classified as lower-montane moist forest (Holdridge 1967) in Nicaragua. Our objective was to identify individuals that have migrated altitudinally by comparing hair, claw and locally expected δD values based on rainwater collected in our study region over an annual cycle. While isotopic turnover rates of claws have not been examined in bats (but are underway, E. Fraser, University of Western Ontario, pers. comm.), bird claws represent recent integration of δD (Bearhop *et al.* 2003, Mazerolle & Hobson 2005, Fraser *et al.* 2008b). We assume turn-over rates of bat claws to be similar to bird claws, and expect claw values to reflect growth over approximately16 weeks prior to capture (Fraser *et al.* 2008b). Bat hair likely represents values at the time of molt (Cryan *et al.* 2004), which may occur during a two-week period once a year (Quay 1970, Cryan *et al.* 2004). Differences in δD_k values between these two tissues and or locally expected values for the capture site may reflect their growth at different elevations and would identify an individual as an altitudinal migrant (Hobson *et al.* 2003, Fraser *et al.* 2008a). This will be most effective if a bat is captured fewer than 16 weeks after migration before new claw growth obscures spatial origins or if the last molt occurred prior to migration. Our study sites represent the highest elevation in the region; since δD depletes linearly with increasing altitude, we expect potential migrants to come from lower elevation and exhibit more enriched δD values than expected for the capture site.

Methods

FIELD METHODS – We sampled bats at two sites in Nicaragua in Jinotega province in the Cordillera Isabella, classified as lower-montane moist forest (Holdridge 1967). Finca Téchnica de la Reserva Silvestre Privada de Nebliselva El Jaguar (100 ha, 1350 masl; 13°10' N, 86°02' W) (hereafter, 'El Jaguar') incorporates mostly primary forest and second-growth (~80% of the site) as well as active coffee (*Coffea* spp.) plantation (~20%), shaded by banana trees (*Musa acuminata*). Datanli-el Diablo Natural Reserve (5848 ha, 1390 masl, 13° 7' 60 N, 85° 55' 60 W) (hereafter, 'Datanli') is similar to El Jaguar, with a mix of active coffee plantation and primary forest with some second-growth.

Sampling took place between 5 and 14 March 2008. Bats were captured using 10 mistnets (12 x 2.5 m, 36-mm mesh). Nets were set up at roughly 50 m intervals along trails. Nets were open for 2 nights at El Jaguar and 4 nights at Datanli from 1830 hrs to 0130 hrs and checked every 20 minutes. Bats were removed from the mistnets, weighed to the nearest 0.1 g with a pesola, forearms measured to the nearest mm using digital calipers and identified using keys (Reid 1997, Timm & Laval 1998). Approximately 2 mm of claw tip from the middle toe of both feet was clipped with scissors and placed in a small paper envelope. 1-2 mg of hair was cut from the scapular region using sharp dissection scissors and stored in paper envelopes. Handling and sampling followed guidelines for the capture and handling of wild bats by the American Society of

Mammalogists (Animal Care and Use Committee 1998) under permission from University of New Brunswick Animal Care Committee (permit # 09008).

Rain water was collected in 500 ml open-mouthed, polypropylene bottles, covered with screen and with 1 cm of mineral oil in the bottom (to prevent water evaporation) (USGS 2006). Two of these receptacles were secured off the ground, away from overhanging vegetation in El Jaguar only. Considering that the two study areas are 12.2 km apart, and the elevation is similar (1350 and 1390 masl), we expected rainwater δD_p between the two study areas to be similar. Every month, 5 ml of water was extracted and deposited in sealed, polypropylene bottles using a syringe and the remaining water discarded.

LAB AND DATA ANALYSIS –Stable-isotope analyses of all bat tissues were performed at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick. Claws and hair were washed to remove lipids and particles in a 2:1 chloroform:methanol solution for 24 hours and left to air dry. In order to account for potential air-tissue H exchange, we use a comparative equilibration technique with standards of known hydrogen isotope ratios (Wassenaar & Hobson 2000, 2003, Wassenaar 2008). Three standards [Bowhead Whale Baleen (BWB), Cow Hoof (CHS), and Chicken Feather (CFS)] were weighed into silver capsules at approximately 0.18-0.2 mg alongside samples and allowed to freely equilibrate with local water vapor for a minimum of 72 hours. Samples and standards were then loaded into a zero blank autosampler. After pyrolysis in a High Temperature Conversion/Elemental Analyzer (TC/EA) and analysis in a Thermo Finnigan Delta XP mass spectrometer, all values were adjusted to bring BWB, CHS and CFS to δD values of - 108‰, -187‰ and -147‰

respectively (Wassenaar & Hobson 2000, 2003, Wassenaar 2008). This technique brings all values relative to the Vienna Standard Mean Ocean Water (VSMOW) scale. To estimate analytical precision across runs, an internal laboratory keratin (feather) standard was used which yielded mean values of -116.6 ± 2.1‰. Replicate bat hair and claw samples varied less than 5‰. Rainwater samples were analyzed at Environment Canada's Stable Isotope Hydrology and Ecology Laboratory in Saskatchewan (see methods in Wassenaar & Hendry 2008).

δD_k does not reflect δD_p in a one to one ratio, but rather becomes more negative, due to biochemical interactions (fractionation) through the food web. The sum total of isotope fractionations between the source (water) and product compounds (hair and claws), can be referred to as the 'net isotopic discriminations' (Wassenaar 2008), hereafter referred to as NID. More specifically, NID can be defined as the degree to which a process (*i.e.* fractionation) 'preferentially' avoids the heavy isotope (Sulzman 2007), which results in δD_k that is more negative than δD_p. NID may be similar across a range of taxa but there can also be taxon- and site-specific differences (see review in Hobson 2008). The general approach in animal migration studies has been to use the most appropriate NID (species- or region-specific, or congener when available), to make predictions about expected δD tissue values, based on measured or interpolated δD_p. The estimate of NID currently available for bats is from the insectivorous *Lasiurus cinereus* (hoary bat) (Cryan *et al.* 2004) where hair δD_k reflected mean annual-growing season precipitation values in the following way: $\delta D_k = -24.81 + 0.7884[\delta D_p]$. We used this equation to calculate expected values ($\delta D_{k\text{-expected}}$) at our capture site in Nicaragua in two ways. The two rainwater samples collected monthly were used to determine mean annual

δD_p. To generate an estimated δD_p based on weighted precipitation, we retrieved a locally expected value (δD_p = -41‰) from the Online Isotopes In Precipitation Calculator (OIPC, http://www.waterisotopes.org). We expected the bats in this study to acquire most of their water through their respective diets of fruit, nectar, arthropods and blood with a smaller contribution derived from drinking water. The rainwater, and hence δD values, incorporated into bat diets may not have fallen during the period of bat tissue growth, but rather at some point over the annual cycle, thus mean annual δD_p values are most appropriate for analysis. $\delta D_{k\text{-expected}}$ was compared to claw and hair δD (for species with $\geq n = 3$) values using one-way ANOVA. Post-hoc pair-wise comparisons were made using Tukey's HSD test. All statistical analyses were performed using R (The R Foundation for Statistical Computing, Version 2.3.1).

Results

We captured 5 species that are mostly frugivorous: *Artibeus toltecus,* Toltec fruit-eating bat (*n* = 5), *Sturnira lilium*, little yellow-shouldered bat (*n* = 5), *Carollia brevicauda*, silky short-tailed bat (*n* = 4), *S. ludovici*, highland yellow-shouldered bat (*n* = 1) and *Chiroderma salvini*, Salvin's big-eyed bat (*n* = 1); two which are mostly nectarivorous: *Glossophaga soricina*, Palla's long-tongued bat (*n* = 3) and *Anoura geoffroyi*, Geoffroy's tailless bat (*n* = 1). We included samples from *Desmodus rotundus* (common vampire bat, *n* = 4), for comparison, as this species is not migratory (Fleming and Eby 2003). We did not include in the analysis species with fewer than three individuals. All but one of these species (*C. salvini*) are commonly found from the lowlands to our capture elevations in Central America (Reid 1997) and three have been captured at elevations ranging from 200-900 m in north-central Nicaragua (Medina *et al.* 2007). We therefore assume that there is potential for altitudinal migration in these species (excepting *D. rotundus*).

Values for all species (including those with $n < 3$) are shown in Table 5-1. Mean rainwater δD for the whole year was -19‰, and using the NID equation previously determined for hoary bat (Cryan *et al.* 2004), the mean expected δD for the capture site is -40‰ (range of -17 to - 60‰). Expected δD_p using the OIPC yielded a more depleted value (-57‰) than that based on local, unweighted precipitation. Most bat tissues collected in the site were significantly more depleted in δD_k than the two expected values with the exception of tissues of *D. rotundus* and *C. brevicauda* claws (Fig. 5-1).

δD_p (not adjusted with NID) varied widely throughout the year (9 to -45‰) (Fig. 5-2). The months with the most negative δD corresponded with the end of the rainy season (August and October). The most positive δD_p values occurred in the dry season (December to February).

δD_k values varied intra- and interspecifically. Claw versus hair comparisons were significantly different in *A. toltecus* (P<0.001) and *C. brevicauda* (P<0.01) but not in *G. soricina* (P=0.458), *S. lilium* (P=0.605), or *D. rotundus* (P=0.491). Comparisons of claw δD and hair δD across species were significantly different (claws: $F_{1,19}$=4.99, P<0.01; hair: $F_{1,19}$=29.259, P<0.001).

Discussion

We predicted that migrant bats could be identified by more positive δD_k, reflecting tissue synthesis in lowland areas, and migration upslope prior to capture at our study areas. However, no bat tissues collected in this study showed this pattern of values. Migratory frugivorous and nectarivorous birds may travel upslope to time breeding with peak resource availability (Stiles 1988). If bats were synchronous with birds in this respect, many of the claws we analyzed would likely have reflected growth at lower elevation prior to migration and capture. Rather than the predicted enrichment we expected for migratory bat tissues, most were more depleted than expected (based on locally collected precipitation during the study and long-term, weighted growing-season precipitation) with the exception of *C. brevicauda* claws and *D. rotundus* claws and hair. This suggests at least two potential interpretations: (1) Tissues more negative in δD were grown at an elevation higher than the capture location, because δD values become more negative with increasing elevation, or, (2) the predicted value for the study area based upon values from rainwater and the NID value from Cryan *et al.* (2004) is not appropriate for the bats in our study.

If for interpretation (1), we consider that the difference between mean expected δD (based upon local, unweighted precipitation) and δD_k for bats with more negative tissues is -28.8 ‰, and assuming a 2.2‰ decrease in δD per 100 m of elevation (Poage & Chamberlain 2001, Hobson *et al.* 2003), bats would have to have grown tissues at a site greater than approximately 2700 masl. El Jaguar and Datanli-Diablo (1350 and 1390 masl respectively) represent some of the highest elevation in Nicaragua. The closest peaks with elevation greater than 2700 masl are 350-500 km away in Honduras and Costa Rica. For bat species known to track seasonal abundance of food through intratropical migration, one-way distances tend to be less than 200 km (Fleming & Eby 2003). It therefore seems unlikely that the δD_k values we obtained for four out of five species reflects growth at higher elevation more than 350 km away, although we cannot rule out the possibility that bats migrate further distances than previously expected.

The range of δDk for most bats (excepting *D. rotundus*) is similar to δD_k values for birds believed to be sedentary and captured in the same study site in 2007 (bats: -50 to -97‰; birds: -42 to -82‰) (Fraser *et al.* 2008a), suggesting that these data may represent a local range of values. Despite this, we cannot rule out the possibility that these bats are migratory at a point in the year that is not reflected in the tissues we sampled. If δD turn-over rates in claws determined for songbirds (Fraser *et al.* 2008b) can be applied to bats, we would be able to detect altitudinal migration only within the period of about 16 weeks prior to capture. Hair δD would allow us to detect prior migration only if it grew at an elevation other than the capture site. If these bats do migrate from lowland areas, our data suggest that the migration does not occur in the same period most common for birds that shift elevation seasonally. Future sampling of bats (hair, claws, blood) through an annual cycle would allow us to confirm if these species are indeed sedentary. For interpretation (2), we reconsider the appropriateness of our methods for calculating expected δD for our capture sites. The net isotopic discrimination value we used to calculate expected site values was derived from an insectivorous bat (*L. cinereus*, Cryan *et al.* 2004). The mostly frugivorous and nectarivorous bats in our study likely consume some arthropod prey (Reid 1997), but this probably represents a smaller component of their diet. While further research is needed to determine the effect of trophic level on δD values, the potential mismatch between our expected and measured δD_k values requires further consideration in this context. Birchall *et al.* (2005) reported higher δD in carnivorous animal tissues relative to herbivorous animal tissues. It is possible that trophic differences drove a lack of fit between the frugivorous and nectarivorous bat tissues in our study and those expected based on the Cryan *et al.* (2004) NID. If so, we can calculate an appropriate

NID for frugivorous and nectarivorous bats in our study. Considering the mean δD_k of all bat tissues (with the exception of *D. rotundus*), and mean annual δD_p, then NID (mean δD_k - mean annual δD_p) may be close to -50 ‰. The use of an inappropriate NID may be the most parsimonious explanation for the mismatch between expected δD and the frugivorous and nectarivorous bats in our study.

Both hair and claw values for *D. rotundus* (sanguinivore) were significantly more positive than those for all other bats (nectarivores and frugivores), and all *D. rotundus* values fell within those predicted for the study area using an insectivore-derived NID value. It is possible that this enhanced 'fit' is due to a greater similarity in trophic level between *D. rotundus* and *L. cinereus* compared to other bats in this study (Fig. 5-1). This also strongly suggests that trophic level may be driving the large differences in δD_k between *D. rotundus* (obligate sanguinivore) and the rest of the bats sampled in this study (frugivores/nectarivores).

As the relationship between δD and trophic level is poorly understood, ratios of stable nitrogen ($^{15}N/^{14}N$) are more commonly used to discriminate between animals feeding at different trophic levels (*e.g.* Herrera *et al.* 2002). To explore the potential effect of trophic level on the δD values we obtained in this study, we analyzed the same hair samples for $\delta^{15}N$ for *A. toltecus* (mostly frugivorous) and *D. rotundus* (obligate sanguinivore) for comparison with δD values. Hair $\delta^{15}N$ values for *D. rotundus* were higher, and the difference was significant (student's *t*-test, $t = -6.5$, $P<0.001$). The direction of differences in $\delta^{15}N$ and δD between *D. rotundus* and the other species in this study (i.e. both higher for *D. rotundus*) is consistent with the results of Birchall *et al.* (2005), where carnivorous animals showed significantly more positive δD tissue values than those feeding at lower trophic levels. More positive δD tissue values for *D. rotundus* are likely driven by trophic differences in diet, and not synthesis of tissue at a lower elevation, since *D. rotundus* is not migratory. This has implications for how δD values are interpreted in migratory studies of bats where there are differences in diet within or between species.

δD_k for frugivorous bats was generally similar in value to nectarivorous species (Fig 5-1); however, there were some significant differences between claws and hair of the same species (*A. toltecus* and *C. brevicauda*), and various differences between species (*e.g. A. toltecus* hair more depleted than all other bat δD_k). The δD_k range for hair is much larger than for claws: -47 ‰ spread for claws versus -68 ‰ spread for hair. We predicted that differences between claw and hair δD would suggest growth at different elevations and indicate altitudinal migration, similar to claw and feather comparisons previously used to detect elevational movement in birds (Fraser *et al.* 2008a). However, if we presume that bats synthesized tissues within the study area, we must consider that the differences between these tissues have been driven by other factors.

The expected δD_k value for the study site based on locally collected preciptation during the study was more positive (-40‰) than that based on long-term, weighted growing-season precipitation (-57‰). In addition to precipitation, our collection containers likely accumulated fog water that is known to be more positive in δD than precipitation (Liu *et al.* 2007). This moisture source could contribute to bat tissue values but is not represented in the precipitation data set used by the OIPC. We also did not weight our locally collected precipitation by the amount of rain (or fog). Both of these factors likely contributed to the difference in expected values between our sampling and that generated using the OIPC. Most bat tissues were more negative than expected values calculated using either method. If we assume that tissues were synthesized within the study area (and not at higher elevation), this suggests that predicting expected δD_k may require improved estimates of local δD_p supplying the food web of these bats.

Laboratory studies with captive quail (Hobson *et al.* 1999) showed that δD values of metabolically inactive, inert tissues such as claws and feathers may be influenced significantly by drinking water δD during synthesis (26-32% of δD_k was derived from drinking water) (Hobson *et al.* 1999). In our study site, δD_p fluctuated broadly over the year (9 to -45 ‰), with the most negative values between August and October and the most positive December to February (Fig. 5-2). Such seasonal patterns of δD_p are well known and described, and the depletion of values that we measured in the rainy season are likely driven by the 'amount effect' (Dansgaard 1964). This seasonal fluctuation in δD_p (values spanning 36‰) could present a drinking water and food supply to bats that changes significantly over the annual cycle.

If the bat tissues we sampled were synthesized at different points in the year, the influence of shifts in drinking water and food δD could contribute to differences between claws and hair of individual bats or between individuals or species. In this sense, differences in values may reflect temporal differences in tissue synthesis, and not differences in synthesis location. For example, while molt patterns for *A. toltecus* and *C. brevicauda* are unknown, if they molted their pelage at different times of the year, this may have contributed to the differences within and between these species in δD_k in hair that we measured. If so, the use of δD to determine molt timing in non-migratory bat species may be a valuable future application of δD analyses. Molting phenology is little known, but bats may be expected to molt into new dorsal pelage once a year (Constantine 1957) and this may take about two weeks (Cryan *et al.* 2004). Considering that bats may obtain much of their water from the foods they consume, we recommend the sampling and analysis of fruit and nectar (in addition to rainwater) over an annual cycle to detect potential within-site shifts in dietary δD. Sampling water and prey items over a range of elevations may allow researchers to separate within-site from between-site variation in δD.

Bats sampled in this study are reported to have breeding peaks that span the year, with no overlap in peak breeding between some species (Table 5-2). If molting is tied to breeding cycles, this suggests that molt may occur in different species at different times of year. As a proxy for molt timing, we compared known temporal breeding peaks for bats in this study (Table 5-2). For species that partially share a temporal breeding peak, such as *A. toltecus* and *G. soricina,* and also *G. soricina* and *S. lilium*, hair δD values were similar. We did not have a large enough sample for intraspecific comparisons of males and females. Future work, with larger sample sizes and site-specific breeding peaks may more fully delineate the link between hair δD values and timing of breeding. It could be expected that when claw and hair δD values are closer together, then sampling has occurred closer to the molt period. Interestingly, *D. rotundus* breeds year round and hair values (time of moult) were similar to claws (recent integration).

We assumed that claws sampled in the same period would reflect a similar period of growth, and would generally show the same δD values if grown in the same location. In contrast, we found a broader range of δD_k claw values than would be expected if claws were grown in the same location (Fig. 5-1). As with hair, the differences could be ascribed to unanticipated differences in the timing of synthesis between species. Studies of claw growth rates in bats have not been performed, and our data may reflect differences in growth rates between species. Considering that claw tissue of bird species of different sizes (warblers vs. sparrows, Fraser *et al.* 2008b, Carleton *et al.* 2008) showed roughly similar isotopic turn-over rates, we expected differences in growth rate to be negligible. Alternative explanations, such as relative differences in the effects of drinking water and diet will need to be considered in future studies.

The description of the complex altitudinal migratory patterns in the Resplendent Quetzal (*Pharomachrus moccino*) has underlined the importance of the creation and development of an interconnected network of reserves to encompass their intrannual movements (Powell & Bjork 1995). Very little is known about altitudinal migration in bats, but for those species that migrate, conservation initiatives may be complicated by seasonal movements. This study represents a first examination of potential elevational movement in Neotropical bats using stable-hydrogen isotopes. While our small sample size precludes strong interpretation, our results point to methods by which future researchers may use stable isotope analysis to track intratropical bat movements. An expansion of our approach to include more species, more regions, a larger sample size, and extended temporal sampling over the annual cycle may yield valuable insights into the potential for intratropical bat migration, with broad implications for bat conservation and reserve design in the Neotropics. However, our results suggest that tracking altitudinal migration in bats using δD may be complicated by the effects of molt timing and diet. While not the initial focus of this study, our results show support for trophic enrichment of δD and the future potential for determining little-known molt patterns of bats using stable isotopes. We caution that researchers using δD to track altitudinal migration in bats should consider all factors that may drive δD variation in keratinous tissues.

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Table 5-1. *δDk values (mean±SD) of Nicaraguan bat claws and hair for all individuals captured in this study. Species with* n *≥3 were used in analysis.*

Species (*n*)	δD_k claw (‰)	δD_k hair (‰)
Artibeus toltecus (5)	-60.6±5.4	-82.4±8.9
Sturnira lilium (5)	-67.6±11.6	-64.6±3.5
Carollia brevicauda (4)	-50.9±1.3	-63.5±6.4
Glossophaga soricina (3)	-68.7±2.7	-71.65±5.5
Desmodus rotundus (4)	-43.3±8.5	-37.4±5.3
Chiroderma salvini (1)	-85.6	NA
Anoura geoffroyi (1)	-69.2	-75.2
S. ludovici (1)	-78.8	-80.7

Table 5-2. *Breeding peaks (from Reid 1997) as a proxy for molt timing linked to δD hair values for 5 species in this study. Species that do not share a lower-case letter did not have >1 shared peak birth month and those not sharing an upper-case letter had significantly different δD hair values.*

Species	**Breeding peak(s)**	**Share >1 peak birth month?**	**Shared hair δD?**
A. toltecus	Apr-May, Aug-Sep	a	A
S. lilium	Feb-Mar, Jun-Jul	b	B
C. brevicauda	Unknown	?	B
G. soricina	Apr-Jun, Dec-Feb	ab	AB
D. rotundus	Year round	ab	C

Figure 5-1. Boxplots of bat hair, claw, and monthly rainwater δD. Plots show median, 25th and 75th percentiles. Whiskers show maximum and minimum values excepting *S. lilium* claw which show 1.5 times the interquartile range of the data. Rainwater δD with net isotopic discrimination values added (so it is comparable to bats) tended to be more enriched than frugivorous and nectarivorous bat tissues. The dashed line indicates a locally expected value (-57‰) based upon long-term, weighted growing-season precipitation.

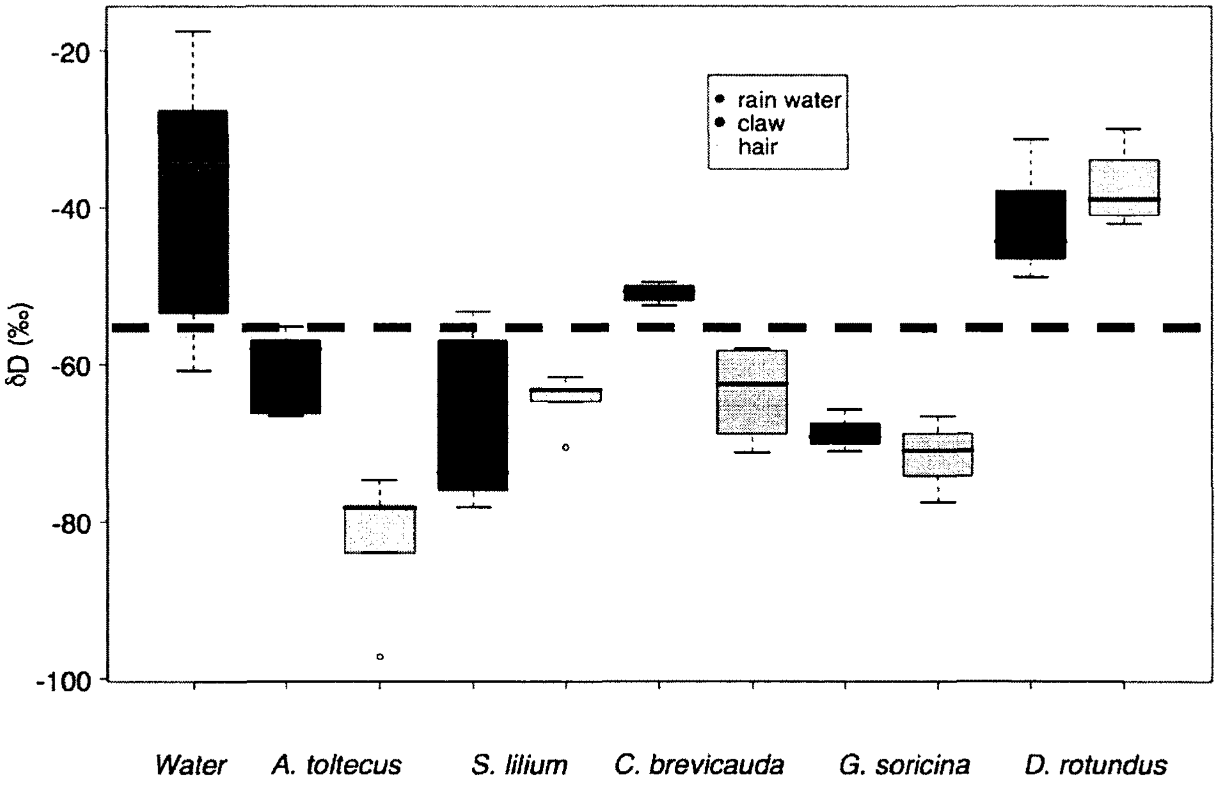
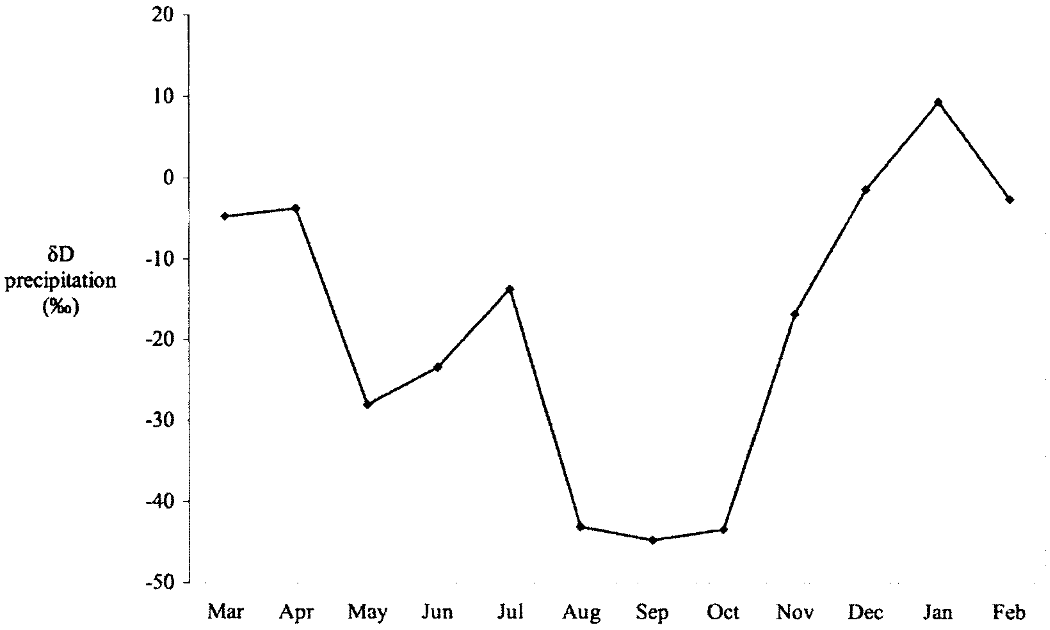


Figure 5-2. δD values for rainwater collected in our study site over one year. Values vary by 54 ‰ with the lowest values occurring between August and October.



CHAPTER 6: GENERAL DISCUSSION

This thesis examines diet and migration in Neotropical birds and bats in a seasonal context. It also explores new stable-isotope based methods for tracking altitudinal migration and diet use in a cloud forest vertebrate community. The implications of this work and priorities for future research are outlined below.

Determining what factors drive δD variability in animal tissues within one breeding area or at 'micro-scales' (Betini *et al.* 2009) has been a problem tackled primarily by those studying animal migration. This is because micro-scale variability (e.g. variation within one breeding population) limits the resolution at which migration and dispersal may be tracked (e.g. Farmer *et al.* 2008, Hardesty & Fraser 2010). Understanding the causes of this variability may enhance the ability of the migration ecologist to track animal movement at smaller scales than is currently possible.

The results presented in chapter 2 provide important insight into factors that influence δD variability in animal tissues. This work shows that micro-scale spatial and temporal variation, diet and body size are all significant influences on δD values. To track animals successfully, researchers may need to consider, and/or control for, the potential influence of all of these factors on the δD tissue values of the animals they study. The species that may be most easily tracked using δD is one that has an unvaried diet, whose movements can be tracked within one season, and that migrates between areas with similar hydrological patterns. Such a scenario may exist for only a small number of species. However, understanding how changes in diet, season, or microhabitat use influence the values of a species of interest may allow researchers to determine when δD tissue values denote migratory movement.

Recent work with δD in a non-migratory context has focused upon other uses of micro-scale variability to track sources of energy flow through foodwebs (Doucett *et al.* 2007) or as a 'food source tracer' (Jardine *et al.* 2009). Work in chapter 2 suggests that bird tissue values may reflect seasonal micro-scale hydrological patterns and provide insight into the structure of the avian community as it pertains to water sources at the base of the food web. Bird tissue values suggest that during the dry season, individuals foraging within the same general area, and caught in the same nets, depend upon different sources of water entering the system at different times of year. For example, I show that foraging location (interior forest or adjacent coffee plantation) and stratum (ground foraging or above) significantly influence stable-hydrogen isotope values in bird tissues. The foraging behaviour of many of these species has long been known from observational studies (e.g. Stiles & Skutch 1989). However, data presented in chapter 2 show for the first time that the food webs of these cloud forest birds are supplied by different moisture sources depending upon when and where they forage. Understory forest birds tended to have more positive tissue values than those feeding above the ground in vegetation or in surrounding open areas. These enriched values suggest that the food web of these birds is based upon recycled sources of moisture, as these tend to become increasingly negative as water is continually evaporated, condensed and falls as fog or canopy drip (Liu *et al.* 2007). Birds with the most positive values were understory insectivores such as black-headed nightingale thrush (*Catharus mexicanus*), chestnut-capped brushfinch (*Atlapetes brunneinucha*) and scaled antpitta (*Grallaria guatimalensis*). These birds commonly forage by walking on the ground and searching for arthropods in the leaf litter (Stiles & Skutch 1989). Soil moisture in the dry season in tropical wet forest is significantly more positive in δD compared to rain, groundwater and other sources (Liu *et al.* 2007). The enrichment of soil moisture in the understory in the dry season likely influences the values of low lying vegetation with shallow roots and other sources of food for the understory arthropod community on which the birds listed above depend.

These patterns of enrichment were less evident for birds such as the common bush tanager (*Chlorospingus ophthalmicus*) that feed in the coffee plantation or in above-ground vegetation. Birds that most commonly forage in these locations tended to have more negative δD values. The rainy season in tropical forests replenishes groundwater sources. Groundwater is released slowly (recharge) through the dry season (Liu *et al.* 2005). If the larger trees and taller vegetation that provide substrate for these foraging birds draw upon groundwater sources (which they likely do) then this could explain how birds that forage above the ground in larger trees have more depleted tissue values. While the above is highly speculative, data presented in chapter 2 do suggest a micro-scale hydrological structure in this montane forest bird community.

Moisture seasonality and availability exerts an important influence on tropical bird phenology, migration and community structure (Diamond 1974, Karr & Freemark 1983, Hau *et al.* 2000, Jahn et. al 2010). It cannot be determined from present data if the understory, ground-foraging bird community is critically dependent upon the recycled moisture sources that are evident in their δD tissue values. However, dividing up the bird (and possibly bat) community by hydrological inputs may provide a handle with which to examine their influence upon phenology, migration, population trends and other aspects of community structure.

Moisture patterns may vary from year to year and can be influenced by anthropogenic change. Cloud forests are extremely sensitive to climatic oscillations (Ponette-Gonzales *et al.* 2010) and hydrological changes are estimated to have large affects on species abundance and diversity (Enquist 2002). In a Costa Rican cloud forest, a decrease in cloud moisture driven by lowland deforestation influenced bird distribution and community structure and contributed to the extinction of anurans (Pounds *et al.* 1999). The main influence and benefit of cloud mist and subsequent fog drip is in the dry season, when these inputs are critical to the hydrology of cloud forest systems (Pounds *et al.* 1999, Ponette-Gonzalez *et al.* 2010). In a long-term study of forest understory birds, moisture gradients, humidity and rainfall amount were shown to significantly influence habitat use and activity level on a temporal-spatial scale. It was suggested that these patterns were independent of food availability but rather indicated a physiological constraint on birds and their activity and foraging (Karr & Freemark 1983). However, population trends and other aspects of community structure have not been examined in the context of the source of water that supplies the food webs within cloud forest. Work presented in chapter 2 reveals some of this structure in the avian community.

Considering this structure, we might expect changes in hydrological patterns to effect forest food webs differently. An expanded exploration of long-term trends of bird abundance and community structure in relation to hydrological trends and source water influences revealed by stable isotopes may be a worthy area for future research. It is important to note that in many Neotropical locations, a negative population trend for understory insectivorous birds has been reported (Sigel *et al.* 2005). Interactions between fragmentation and patch size, small populations and restricted dispersal have been implicated as factors potentially driving these trends (discussed in Sekercioglu *et al.* 2002). Despite this, the specific proximate causes of these declines are largely unexamined and evidence for the influence of other factors is lacking (Sigel *et al.* 2005). Exploration of trends in dry-season moisture may provide an important contribution to these analyses. For example, small forest patches in a fragmented environment may experience reduced evapotranspiration due to the reduction of cover and greater exposure at edges. This could lead to a reduction in the source moisture for the understory food web that includes ground foraging birds. Considering that moisture input is a much more seasonal contributor to montane sites than to low elevations on the Caribbean slope (Cavelier *et al.* 1996), stable-hydrogen isotope values of bird species that span an elevational transect may be instructive in estimating potential variation in source water influence on different food webs.

Stable-hydrogen isotope results for both birds and bats indicate that there are strong within-site patterns of variation (chapter 2 & 5). Claw and feather comparisons for birds and claw and hair comparisons for bats both reveal that tissues deposited at different times of year may differ widely in δD, even when these tissues have grown within the same area (i.e. the elevation of El Jaguar). These differences are likely driven, in part, by seasonal differences in precipitation δD (see results chapter 5). Thus, tracking the migration of either birds, or bats, using δD may be most effective when tissues deposited in a similar season are used. Even then, however, it may only be possible to track altitudinal movements that span more than 1000 m, as was shown in an Andean community of birds (Hardesty & Fraser 2010). The within-site variation of δD at El Jaguar was approximately 80‰ for birds and 70‰ for bats. If δD is expected to deplete linearly by -2.2‰ per 100 m of elevation (Hobson *et al.* 2003), then within-site variability exceeds that which could be expected over the 1350 m elevational gradient in Nicaragua (29.7‰).

More promising is that micro-scale patterns of habitat use may be determined for birds using δD (described above). However, this may be limited in its utility for tracking micro-scale patterns of habitat use for bats. The largest differences in δD values of birds were those between ground foragers and those that forage in the overstory. The δD tissue values of insectivorous, frugivorous and nectarivorous bats (see chapter 5) are much more negative than those of ground foraging birds (see chapter 2). Neotropical bats do not forage on the ground (Reid 1997) so are not exposed to the relatively high δD values that are present there in the dry season. No comparable structure of δD variation was found in the bat community that could be used to distinguish source water or foraging locations as was possible with birds. However, there is one bat species whose foraging habitat can be distinguished from the other species using δD. The carnivorous bat, *Desmodus rotundus* (common vampire bat) is a ground forager by proxy – it feeds mostly upon domesticated cattle (Reid 1997) which in turn have consumed low-lying tropical grasses. δD values of *D. rotundus* (range -29 to -48‰) are more similar to those of ground-foraging birds. Interestingly, $\delta^{13}C$ claw and hair values of *D. rotundus* reflect a tropical grass 'signature' (K. Fraser unpub. data).

Work presented in chapter 3 addresses how birds may respond to the energetic and nutritive challenges of breeding and moulting by consuming appropriate foods. The rationale of this approach is that the manner in which birds respond to energetic and nutritional challenges can reveal important insights into physiological and dietary limitations and how these are negotiated within the context of phenological change (Murphy 1994). Wild tropical birds observed to feed heavily on low-protein diets such as small, watery fruits or nectar should, perforce, supplement these diets periodically with more protein-rich foods, such as arthropods (Moermond and Denslow 1985, Remsen *et al.* 1986, Witmer 1998, Klasing 1998, Bosque and Pacheco 2000). However, the degree to which fruit and nectar-eating tropical birds supplement their diets with protein has never been measured. In chapter 3, I show that many species employ a protein-complementation strategy. This is the first time that this has been observed in a wild tropical bird community.

A lek mating system is one in which males display, often communally, to attract females for mating. In tropical birds, lek theory suggests that abundant plant-produced foods 'emancipate' males from parental care because females can provision nestlings alone (Snow 1971, Snow and Snow 1979). The trophic level at breeding and moult of the three lekking species discussed in chapter 3 (violet sabrewing, *Campylopterus hemileucurus*; long-tailed manakin, *Chiroxiphia linearis*; and ochre-bellied flycatcher, *Mionectes oleagineus*) suggests that arthropods are an important food source for adult birds during this period. These three species showed the greatest increase in $\delta^{15}N$ between nonbreeding and breeding-moulting periods. A fourth lekking species (long-billed hermit, *Phaethornis longirostris*) also showed a large increase. $\delta^{15}N$ values delineate sources of assimilated protein, therefore our data do not rule out the possibility that these birds are simultaneously targeting fruit and nectar for carbohydrates and lipids. Indeed, seasonal abundance of plant foods could fuel increased arthropod foraging to satisfy protein requirements during this period. However, our data emphasize the little-considered contribution of higher protein foods for adult birds in the breeding and moulting periods. The protein derived from arthropods could be a critical limiting factor during these periods. Detailed study of diet phenology in lekking species, including an exploration of male versus female diets, may yield new insights into the ecological correlates of mating systems. Males and females in lek mating systems may require different food resources dependent upon season. For example, arthropod availability may be more important than fruit at certain times of year, particularly for females that require protein as a substrate for egg production and for energy during nest building, incubation and nestling provisioning.

Work in chapter 3 emphasizes the importance of examining the interaction between food availability and the shifting physiological needs of birds. Fruit abundance has commonly been examined as a driver of food choice, migration and breeding phenology in Neotropical birds (e.g.s. Loiselle & Blake 1991, Herrera *et al.* 2005, Boyle 2010). Many altitudinal migrants, for example, may be largely frugivorous (Levey & Stiles 1992), thus fruit abundance has been explored as a correlate of migratory patterns and the timing of breeding (Boyle 2010). However, few studies have found links between peak tropical plant production and bird phenology. The role of fruit abundance in promoting altitudinal migration in the Neotropics has not been largely supported (discussed in Boyle & Conway 2007). Fruit abundance did not correlate with the timing of migration and the initiation of the breeding period in a manakin species (Boyle 2010). There may be several reasons why such a link has not been found: 1) More fruit biomass may not mean that more fruit is available to birds. For example, fruit consumption may be more limited by digestive constraints than by availability (Moermond & Denslow 1985); 2) Peak plant production may be incidental and not necessarily a driver of bird phenology, as plants may increase production to promote greater seed dispersal (Jenkins 1969). No studies have demonstrated that fruit availability is seasonally limiting to birds; 3) Protein derived from arthropods may be more important than fruit availability during breeding and moulting. The production of eggs and feathers represents a considerable increase in the demand for dietary protein (discussed in chapter 3).

The majority of Neotropical birds, even those considered largely frugivorous, provision nestlings with protein-rich arthropods to support growth (Morton 1973). Arthropod availability may be inherently more limiting than fruit. While fruit 'wants' to be eaten so as to promote seed dispersal, arthropods do not. Thus the time required to capture arthropods is greater than it is for fruit (Morton 1973). This is supported by recent community ecology studies. For example, closely related and sympatric tanager species in the Andes overlapped in the species of fruit they consumed, but displayed unique foraging niches with respect to arthropod prey (Naoki 2006). In chapter 3, my data support the notion that birds increase their dietary protein intake during the breeding-moulting period. In many cases, this may have been achieved by greater consumption of arthropods (which are higher in protein than fruit or nectar). Do birds time their breeding season to coincide with peak arthropod availability? There is evidence that points to this.

Poulin *et al.* (1992) found a correlation between peak arthropod availability and breeding in Neotropical bird species of a number of foraging guilds, including birds considered to eat mostly fruit. Diamond (1980) observed a strong correlation between insect availability and breeding in Seychelles Warblers (*Acrocephalus sechellensis*). Hau *et al.* (2000) found that visual cues of arthropod availability triggered breeding condition in a Neotropical forest insectivore. However, Boyle (2010) did not find a link between overall arthropod availability and the timing of migration and breeding in a manakin. Clearly more work is needed in a variety of habitat types and with a greater number of species in the Neotropics to determine the potential link between arthropod abundance and the timing of breeding and altitudinal migration.

It is also possible that the link between food abundance and timing of breeding does not exist for any food type. Factors other than abundance may drive seasonal change in bird diets (Murphy 1996). Seasonally heterogeneous diets may reflect changes in individual bird preferences, dependent upon nutritional need. Hypotheses linking condition, growth and mortality to factors other than food abundance are becoming more common (Murphy 1996). Seasonal diet may be driven by environmental or organismal factors as well as interactions between the two (Murphy 1994). A recent study (Arnold *et al.* 2007) with blue tits (*Cyanistes caeruleus*) showed that adults fed spiders preferentially to developing nestlings at a particular age. Spiders contain high amounts of the amino acid taurine, which plays a critical role in brain development. Nestling blue tits fed a taurine supplement experimentally, were more likely to take risks in exploring novel objects and performed better in spatial learning tasks than controls. This illustrates the subtle but important role of a particular food, in this case spiders, on behavioural phenotypes in birds, and presumably, on their fitness. Interestingly, the timing of breeding and spider provisioning in wild blue tits was unrelated to spider availability. This sort of detailed study focused upon both availability of food and the physiological need of birds during various stages of the annual cycle is mostly lacking for wild Neotropical birds. A good first step is work examining seasonal diet change in birds in the context of periods of higher energetic demand such as during breeding or moulting as presented in chapter 3. Through such studies we may gain a greater understanding of the link between the abiotic environment and the timing of seasonal activities in Neotropical birds.

We may glean similar information from studies of bats. Bats, like birds, have defined breeding and moulting periods (Quay 1970, Reid 1997, Cryan *et al.* 2004). Neotropical bats span all of the dietary guilds that birds do. Bat species that commonly consume low-protein foods such as fruit or nectar, may be expected, like birds, to supplement their diets periodically with more protein-rich food sources such as arthropods. As with birds, this may be more critical during periods of peak nutritive and energy demand, such as during breeding and moulting. However, we may also anticipate some differences between birds and bats in the duration of diet complementation to support increases in protein demand. Tropical passerine birds for example tend to have a clutch of just two eggs that may be laid over a period of two days (Stutchbury & Morton 2001). The duration of gestation in bats varies widely but greatly exceeds that of egg production in birds – at one extreme female *D. rotundus* may gestate for 205 days and at the other extreme *Pipistrellus hesperus* gestate for 40 days (Barclay & Harder 2003). Both the production of eggs in birds and pregnancy in bats likely represent an annual peak in protein demand. However, if frugivorous and nectarivorous bats complement their diet with more protein–rich foods such as arthropods, we may expect this to occur over a longer period for bats, owing to the comparatively longer gestation period of bats as compared to the egg laying period of birds.

For female bats, we may also expect this period of high demand to extend into the provisioning of young – female bats must supply their young with protein-rich milk derived from their own diet. Lactation may last between one and ten months (Barclay & Harder 2003). While female birds may use protein as an energy substrate during nestling provisioning (Murphy 1996), they likely have more flexibility in the amount of protein they consume during this period because they can eat a variety of foods, including fruit, while supplying their young with arthropod food (Morton 1973). In sum, if nectarivorous and frugivorous bats complement their diet with arthropods during breeding, we might expect this to occur over a much longer period than is expected for birds. In this scenario, we may also predict larger differences between the sexes and breeding versus nonbreeding individuals in bats than would be expected for birds.

Regarding the link between rainfall seasonality and the timing of breeding and other productive periods in bats, we may expect these to be similar between birds and bats that consume arthropods. During the rainy season in the Neotropics, arthropods that are active in the day (when birds most commonly forage) and at night (when bats forage) become more common (Janzen 1973, Skutch 1950). Bat species that rely upon arthropods to support the demands of breeding may breed during periods when arthropod abundance is greatest, such as has been suggested for birds (Hau *et al.* 2000).

The seasonal changes in diet revealed in chapter 3 also suggest that current dietary categories applied to birds (and many other animals) may be problematic. Dietary categories (e.g. frugivore) belie the dietary complexity, temporal variation and digestive variability that is common in the avian world and is discussed in chapter 3.

A new multi-level approach to dietary categorization may provide a solution.

Birds are typically classified into handy dietary categories (example frugivore, nectarivore, ominivore, etc.). These classifications provide an important support for many theories, from morphological adaptation to mating systems theory. However, there are several problems with these one-dimensional categories, such as: 1) they are often qualitative, not quantitative. A bird that eats 'much' fruit may be variously classified as 'highly frugivorous', 'mostly frugivorous' or an 'obligate frugivore' (e.g. Moermond & Denslow 1985). The data on which these qualitative categories are based are often not presented in a way that allows a reader to fully evaluate methods and data (e.g. Beehler & Pruett-Jones 1983, Levey & Stiles 1992). 2) Diet classification is often based on small sample size. For example, various species of birds of paradise exhibit a variety of mating systems, from social monogamy to polygyny. They seem to reflect the classic notion that these patterns are driven by diet: more frugivorous species tend toward polygyny because abundant fruit 'emancipates' males from parental care as females can provision nestlings alone. In more insectivorous species, males and females must share parental duties. However, this pattern was not a perfect fit and is based on only one diet study with relatively small sample size (Buehler and Pruett-Jones 1983). This study also highlights another problem with one-dimensional diet categories, which is, 3) classification often does not take a temporal component into account. Work presented in chapter 3 shows that diets may change significantly with season, and should be taken into account when measuring diet. A final limitation, is 4), that one-dimensional categories do not consider diet at the specific nutrient or macro- molecule level – i.e. what foods are targeted for what nutrients, what are they used for and when. Such an approach neglects the way in which birds may use different foods, such as arthropods for protein, and plant production for carbohydrates and lipids. An example is provided above where taurine in spiders is likely important in nestling development (Arnold *et al.* 2007).

It has long been recognized that one-dimensional diet categories are not only in error, but may mislead and limit progress (Table 6-1). A new approach to characterize the diet of birds, and possibly other animals such as bats, based on quantitative measurements of diet, incorporating temporal and physiological measurements, may be much more instructive. Such a system may be divided into levels, from the use of specific macromolecules to the relationship between diet and the broader ecosystem. For example, a hummingbird may be a year-round, protein arthropodivore that times breeding to peak availability of a preferred protein source, such as spiders. One study observed a female broad-tailed hummingbird (*Selasphorus platycercus*) that consumed only spiders (and no nectar) over the course of incubation (Montgomerie & Redsell 1980). But of course, as for all hummingbirds, nectar is an important source of food as well. Thus we must take into account diet needed to support protein requirements (arthropods) and diet needed for energy (nectar); each need may interact with ecological and social factors to contribute to observed behaviour. A more complex system of dietary categorization may improve hypothesis testing wherever food is an important factor. This level of analysis was missing from many of the food-based underpinnings of early theory development that still forms the basis for much research on birds today, from niche to migration theory. We will need to reassess these associations based on a quantitative, multi-tiered approach to the study of diet in Neotropical birds.

Table 6-1. Quotes from papers on Neotropical birds that highlight the limitation of one-dimensional diet categories.

Quote	Source
"Nearly all of the so-called frugivorous birds eat other types of foods as well (usually insects or other invertebrates) and should be properly referred to as omnivores. However, the term "frugivore" has been commonly used for such frequent but not exclusive fruit-eaters as tanagers."	Moermond & Denslow 1985
"..blanket assignment of species to diet categories based solely on family membership, a practice widely used in current research on community ecology of Neotropical forest birds, is incorrect."	Remsen *et al.* 1993
"Many bird species, however, eat more than one food type to various degrees. This is especially true for so-called 'frugivorous' birds, most of which supplement their fruit diet with protein-rich foods, such as seeds, insects, and vertebrates."	Naoki 2007
"Cedar Waxwings and thrushes show digestive specialization to the utilization of sugars and lipids, respectively, as dominant dietary nutrients. These traits explain patterns of food selection by these birds in the laboratory and in nature. Variation in the value of particular fruit nutrients to different birds renders the dietary descriptor of 'frugivore' ambiguous in a nutritional context."	Witmer & VanSoest 1998

There are several important implications of the descriptive account of altitudinal migration in blue-tailed hummingbirds presented in chapter 4. First, moulting is an energetically costly activity for birds (Murphy & King 1991). Birds rarely overlap moult with other costly activities such as breeding or migrating (Murphy & King 1991). However, there may be some instances where birds moult on migration (Leu & Thompson 2002), or migrate specifically to an area that is more productive to moult (e.g. Rohwer *et al.* 2008). Extended or late breeding effort may also promote the overlap of energetically costly activities such as moulting and migration (Norris *et al.* 2004) although there may be other explanations for putative moult-migrants (Reudink *et al.* 2008). Such energetic trade-offs have rarely been investigated, and represent an active area of current research, fueled in part by new technologies that allow the tracking of small-bodied (< 100g) animals and methods to detect moult location.

Detecting which species may moult on migration or migrate from a home range to an area specifically for moult is an important first step in determining which factors promote this behaviour. That the blue-tailed hummingbird is the first Neotropical species to be shown to engage in this behaviour is significant. As discussed in chapter 4, the next step is to determine what factors promote moult away from breeding areas in this and other species. The blue-tailed hummingbird data is also significant because it suggests an alternative causal mechanism that favours altitudinal migration in hummingbirds. In previous work, the emphasis has been on the seasonal and asynchronous abundance of nectar as the principal factor promoting altitudinal migration in hummingbirds (Levey & Stiles 1992). Moult data presented in chapter 4 and details of moult diet in chapter 2 suggest that protein requirements may also promote migration in hummingbirds.

It is not known if protein requirements for moult could promote the movements of bats. Bats may moult during a defined period once a year (Quay 1970, Cyran *et al.* 2004) just as many birds do. However, bird plumage serves a greater number of purposes than does bat pelage – birds use feathers not just for thermoregulation but also, of course, for flight. Thus the replacement of worn feathers and the development of good quality plumage may impose a stronger selective pressure on moult strategies in birds as compared to bats. The relative protein requirements for a typical bat versus bird moult are unknown, but these data may indicate the relative cost of moult for bats versus birds. If moult requirements reflect an energetic peak in the annual cycle of bats, it is possible that like birds, bats may incur the risks of migration in order to reach suitable moult habitat.

The data in chapter 4 also have conservation implications for the blue-tailed hummingbird. Altitudinal migrants may pose a conservation challenge, or complicate conservation initiatives (Winker *et al.* 1997, Powell & Bjork 1995). There are several unique conservation needs that have been identified for birds that migrate altitudinally: 1) Altitudinal migrants make seasonal changes in habitat use, thus have broader habitat needs than species that are more sedentary. This has direct implications for reserve design. 2) Some tropical forest birds may need an interconnected network of corridors to facilitate migratory movements and to promote genetic diversity through population mixing. 3) Possibly the biggest need is further research and data on tropical altitudinal migration. Many lacunae still exist in our knowledge of migratory behaviour, and some species have yet to be identified as migratory, sedentary, or partial migrants. Data in chapter 4 contribute to this last conservation need, and may promote the study of other altitudinal migrants in the region.

In chapter 5, altitudinal migration in Neotropical bats was explored using stable-hydrogen isotopes. This is the first time that these methods have been employed to examine altitudinal migration in bats, and one of the first studies to explore these methods in any taxa (for other examples see Hobson *et al.* 2003, Fraser *et al.* 2008, Hardesty & Fraser 2010). In this chapter, I show that the use of stable-hydrogen isotopes to track altitudinal migration in bats may be complicated by within-site variation in δD. This is important, as there is burgeoning interest in using δD to track animals that migrate altitudinally (Hobson *et al.* 2003, Fraser *et al.* 2008, Hardesty and Fraser 2010). The data presented in chapter 5 suggest that before δD may be used successfully to track altitudinally migrating animals, we must first have a better understanding of factors that drive this variation at local scales. This is a notion echoed in much of the more recent literature dealing with the use of δD to track long-distance or latitudinal migration (e.g. Langin *et al.* 2007, Farmer *et al.* 2008, Betini *et al.* 2009). Indeed, the work presented in chapter 2 points out factors that drive δD variation at a local/microgeographic scale in the very system where the work in chapter 5 was carried out. Further explorations of this micro-scale variation in δD will not only improve our abilities to track migratory animals over smaller distances, but as both chapters 2 and 5 reveal, it may be useful to understand moult phenology, seasonal habitat use and diet.

Since the completion of the work in chapter 5, several large review papers have been published (Popa-Lisseanu & Voigt 2009, Holland & Wikelski 2009, McGuire & Guglielmo 2009) and an international symposium on bat migration has been held (1st International Symposium on Bat Migration, Berlin, January 2009). Bat migration research has generally lagged far behind that of birds (Popa-Lisseanu & Voigt 2009). Knowledge gaps were highlighted that pertain to my investigation of bat migration, i.e. we know next to nothing about the migration of tropical bat species (Popa-Lisseanu & Voigt 2009). This review also points out that the development of methods to track bat movements is one of the largest limiting factors; the so-called 'small animal problem' (Holland & Wikelski 2009). New developments in geolocator technology, used successfully to track long-distance migration in birds (Stutchbury *et al.* 2007, Stutchbury *et al.* 2010) may not be applicable to bats, despite an interest expressed in the bat literature (Holland & Wikelski 2009). The successful use of geolocators relies on accurate recording of sunrise and sunset. Roosting patterns of bats are likely to interrupt accurate recording of these essential parameters. Therefore, other methods currently being used to track migration in small-bodied bats (e.g. Cryan *et al.* 2004 chapter 5 in this thesis) may prove more useful until other tracking technologies (such as satellite tags, see review in Holland & Wikelski 2009) are small enough to be deployed on bats. In this regard, the work presented in chapter 5 represents an important contribution to the development of methods to study bat migration.

Previous work on correlates of migratory behaviour in birds may be useful to consider in future studies of bats. There has been little work on bats in this regard, but bat migration may be tied to ecosystem seasonality (Popa-Lisseanu & Voigt 2009). As such, we may expect similar patterns in Neotropical bats as in birds, where seasonality of food resources (arthropods, nectar, fruit) may drive migration. In tropical areas that are more seasonal, such as open areas and edge, we may expect to find more migratory bat species than in areas described as less seasonal, such as tropical forest understory (however see chapter 2 on ways in which this habitat too is seasonal). However, the ecological correlates of bat migration were not discussed in the recently published review papers on bat migration (Popa-Lisseanu & Voigt 2009, Holland & Wikelski 2009, McGuire & Guglielmo 2009), suggesting such an exploration is warranted and may help to direct future research. Comparative studies with bats may even yield new insights into factors driving migration in birds. Migratory patterns may be driven by seasonality in both bats and birds that consume similar food resources. However, other factors differ strongly between bats and birds, such as breeding systems and other social behaviour. Comparative analyses between bats and birds may yield greater insight into how these factors interact to drive migratory patterns.

Conclusion

This thesis shows that hydrological patterns in cloud forest drive variation in δD in both bird and bat tissues on a seasonal and micro-spatial scale. δD has most commonly been used in animal ecology studies to track migration and the results of this thesis have several important implications for such studies. Results for birds and bats suggest that several factors drive δD variation at micro-scales (i.e. within one study area or population); these are rainfall seasonality, foraging location (ground versus overstory and closed-canopy forest versus open areas), and trophic level. Species that show little variability in food type and foraging location and can be studied over single seasons may be the best candidates for tracking studies. However, such species may be rare, suggesting that δD may have limited utility in tracking animals over elevational gradients in Neotropical systems.

The notion that few species feed upon one food type is supported by results presented in chapter 3, where even birds considered highly frugivorous and nectarivorous increase their level of protein consumption during breeding and moulting. It seems likely in some cases that this is accomplished through a seasonal increase in the consumption of protein-rich arthropods. This is the first time that protein diet complementation has been observed in a wild community of birds. This observation is compatible with breeding phenological studies in the tropics, which show that the timing of breeding in many birds may be intimately tied to rainfall seasonality and its effects on arthropod abundance (e.g. Poulin et al. 1992). Results in chapter 3 for 'frugivores' and 'nectarivores', suggest that the validity of such dietary categories may be questionable considering the potential for seasonal diet switching and ominivory in even 'highly' frugivorous or nectarivorous birds.

One such nectarivore, the blue-tailed hummingbird (discussed in chapter 4) is the first Neotropical and hummingbird moult migrant that has been documented. The timing of moult migration in this species and its $\delta^{15}N$ feather values suggest that seasonal arthropod abundance should be investigated as a potential factor promoting altitudinal moult migration. Altitudinal migration in the Neotropics has been little studied and future research may yield more moult migrant species.

While the use of δD to track altitudinal migration may be limited by within-site effects, results in chapter 2 suggest that δD may reveal previously unexamined hydrological structure, microhabitat use and moult phenology in Neotropical bats and birds. There is also great potential here for δD to be developed to track habitat use in cloud forest and agroecosystems (particularly in tandem with $\delta^{13}C$ analyses). While annual moult represents a critical event in the annual cycle of Neotropical birds (and possibly bats), moult phenology is poorly known. δD analyses of bird and bat tissues may be developed to explore these patterns in Neotropical birds and bats.

In 2001, Stutchbury and Morton urged for further research on tropical bird systems. Their reasoning was that most of our understanding of the behavioural ecology of birds comes mainly from the relatively small percentage of birds that migrate to north temperate areas in Europe and North America. They argued that we would do well to develop and test theories in the tropics where the majority, by far, of birds species live. The same could be said for Neotropical bats.

While behavioural ecology research on birds (and bats) in the tropics appears to have risen in the recent decade, we are still lacking basic natural history information for thousands of species. Such knowledge will allow us to test hypotheses developed in temperate systems. It was recently shown that hypotheses proposed to explain partial migratory behaviour in temperate species (Ketterson & Nolan, Gauthreaux) may not explain migration in Neotropical systems (Boyle & Conway 2007, Boyle *et al.* 2010, Jahn *et al.* 2010). This may be because rainfall is the predominant factor driving tropical seasonality and exerts different selective pressures than those experienced by birds in the temperature zone where seasonality is driven to a greater degree by variation in temperature (Jahn *et al.* 2010). This observation illustrates arguments made by Stutchbury and Morton (2001) that lessons learned in north-temperate systems may not apply to tropical ones (where most of the world's species actually live). Unraveling these patterns is the challenge of contemporary behavioural ecologists working in the tropics.

This thesis makes an important contribution to our knowledge of the migration, breeding, diet and moulting ecology of Neotropical birds and bats. Our data are the first to show that free-living birds increase their protein consumption during breeding and moult. We also document the first example of a hummingbird migrating altitudinally to moult. Our conclusions support studies and theoretical work that suggest a link between rainfall seasonality and the timing of migration breeding and moulting in Neotropical birds and bats. Future research should be aimed at determining the link between the energetic and nutritive needs of birds and bats and the timing of these behaviours. This thesis also provides important exploration and development of stable isotope methods with which to track altitudinal migration, seasonal diet and microhabitat use. While local δD variability may complicate our ability to track migration in cloud forest animals, our data reveal previously unknown hydrological structure in a cloud forest bird community. This structure can be used to explore the link between seasonal hydrology and bird and bat phenology. It may also be used to explore the link between anthropogenic influence on hydrological cycles and cloud forest bird populations. It is my hope that this thesis will stimulate further research in cloud forest systems, both to better understand them, and to promote their conservation.

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Curriculum Vitae

Education

Sept 2006 – Dec 2010	Ph.D., Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada
Sept 2004 – 2006	M.Sc., Department of Biology, Queen's University, Kingston, Ontario, Canada.
Sept 2002 - 2004	M.F.A., Mel Oppenheim School of Cinema, Concordia University, Montreal Québec, Canada.
Sept 1993 – May 1997	B.F.A., School of Art, Drama and Music, McMaster University, Hamilton, Ontario, Canada.

Publications

Fraser, K.C., Diamond, A.W. & Chavarría, L. (2010) Evidence of altitudinal moult-migration in a Central American hummingbird. *Journal of Tropical Ecology* 26:1-4.

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FRASER, K.C., BRODERS, H., CUNJAK, R.A., DOUCETT, R.R. & FORBES, G. Foraging location drives isotopic niche partitioning of two sympatric bat species and reveals an unexpected carbon source. *In prep. for Journal of Mammalogy*

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Presentations

FRASER, K.C. Migration in the 3rd dimension: what we know about tropical altitudinal migration and why we need to know more (2009) In *symposium*: Movers and shakers in the mountains: New research on tropical altitudinal migration (**organized by Fraser K.C.**). American Ornithologists' Union 127th annual meeting. Philadelphia, PA

MCKINNON, E.A., **FRASER, K.C.,** & DIAMOND, A.W. (2009) Bicknell's and Swainson's thrushes in regenerating New Brunswick clearcuts: niche separation by nest site and diet. American Ornithologists' Union annual meeting. Philadelphia, PA

FRASER, K.C., CUNJAK, R.A. & DIAMOND, A.W. (2008) Detecting altitudinal migration and moult patterns in Neotropical birds using stable hydrogen isotopes. 6th International Conference on Applications Of Stable Isotope Techniques to Ecological Studies (ISOECOL), Honolulu, Hawaii

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FRASER, K.C., ROBERTSON, R.J., KYSER, T.K. & RATCLIFFE, L.M. 2006. Stable-hydrogen isotope values in Ontario Golden-winged Warbler (*Vermivora chrysoptera*) claws: Utility for determining migratory origins. Inaugural meeting for Canadian Society for Evolution and Ecology. Montréal, QC. (poster)

Teaching Experience

Lecturer

Ornithology 2008. 4th year undergraduate course, Dept. of Biology, University of New Brunswick.

Teaching Assistantships

BIOL 4723 Ornithology. 2007-2008. University of New Brunswick.
BIOL 3383 Aquatic and Terrestrial Field Ecology Course. 2007. University of New Brunswick.
BIOL 4722 Ornithology. 2006. University of New Brunswick.
BIOL 302 Population and Evolutionary Ecology. 2005. Queen's University.
BIOL 303 Community and Ecosystem Ecology. 2005. Queen's University
BIOL 210 Biology of Sex. 2004. Queen's University

Grants and Awards

2008-2009	Natural Sciences and Engineering Research Council of Canada (NSERC), Doctoral Scholarship (PGS-D)
2008	Kathleen S. Anderson Award, Manomet Centre for Conservation Sciences
2008	Bat Conservation International, Student Research Scholarship
2008	Board of Governor's Award, University of New Brunswick
2006-7	Magee Third Century Graduate Award, University of New Brunswick
2003-4	Ontario Graduate Scholarship, Queen's University

Related Experience

January-Sept 2008	**Manager of Science** for the Stable Isotopes in Nature Laboratory (SINLAB), University of New Brunswick.
2007-present	**Co-chair** of the research sub-committee, International Bicknell's Thrush Conservation Group
2008	**Student representative** University of New Brunswick student for the Atlantic Cooperative Wildlife Ecology Research Network (ACWERN)

Research highlighted in popular press

Mt. Moult: A hummingbird's climb for new feathers (October 27, 2010) D. Malakoff. **Conservation Magazine** - http://www.conservationmagazine.org/2010/10/mt-moult/

Reviewer for:

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