

Mesoscale patterns of altitudinal tenancy in migratory wood warblers inferred from stable carbon isotopes

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Abstract. We analyzed carbon isotope ratios ($\delta^{13}\text{C}$) of liver and pectoral muscle of Black-throated Blue Warblers (*Dendroica caerulescens*) to provide a mesoscale perspective on altitudinal tenancy in the Appalachian Mountains, North Carolina, USA. Movements of males are poorly understood, particularly the degree to which yearlings (first breeding season) and older males (second or later breeding season) wander altitudinally during the breeding season. Liver and muscle $\delta^{13}\text{C}$ values of warblers exhibited significant year and altitude effects, but yearling and older males were isotopically indistinguishable. Liver $\delta^{13}\text{C}$ values increased with altitude at the rate of $\sim 0.5\%$ per 1000 m. The altitudinal lapse rate of muscle $\delta^{13}\text{C}$ ($\sim 1.1\%$ per 1000 m) was nearly identical to the average rate of increase reported in several groups of C_3 plants ($\sim 1.1\%$ per 1000 m). This suggests that the majority of males foraged within relatively narrow altitudinal zones during the breeding season. We caution, however, that the discrimination of altitudinal trends in carbon isotope ratios depends on relatively large multiyear samples. Given the scatter in data, it is unlikely that individuals can be accurately assigned to a particular altitude from carbon isotope values. Rapid adjustment of liver and muscle $\delta^{13}\text{C}$ values to local altitudinal environments is consistent with the results of experimental dietary studies that show carbon turnover rates are relatively rapid in small migratory passerines. In a broader context, carbon isotope data have been increasingly used as proxies for wintering habitat use of Nearctic–Neotropical migratory passerines. However, tissues with high metabolic rates are unlikely to retain much isotopic signal of wintering habitat use by the time migrants reach their breeding territories.

Key words: altitudinal tenancy; Appalachian Mountains, North Carolina, USA; Black-throated Blue Warbler; *Dendroica caerulescens*; isotope turnover rates; migratory birds; stable carbon isotopes.

INTRODUCTION

The capacity to track the movements of migratory birds in real time throughout their annual cycle is arguably the Holy Grail of avian ecology. Although satellite telemetry and archival tags promise to revolutionize knowledge of dispersal and migratory behavior of larger avian species such as albatross (Jouventin and Weimerskirch 1990) and geese (Gudmundsson et al. 1995), the vast majority of migratory songbirds are too small to carry powerful transmitters or data recorders. Banding programs have been the principal method for studying dispersal and migration of songbirds for the past century (Børnøkke et al. 2006). The substantial up-front investment of time and financial resources required to mark individuals is a major drawback of mark–recapture protocols, but the principal weakness is that population-level patterns of dispersal and connectivity between breeding and wintering areas are rarely

discernable because recovery rates of songbirds are vanishingly low away from initial capture sites. Thus there is an urgent need to develop new methods of tracking population movements of migratory passerines.

Stable isotope analysis has shown some promise as a tool for elucidating dispersal patterns (Graves et al. 2002, Hobson et al. 2003, Hobson et al. 2004, Hobson 2005b) and the geographic origins of avian populations from field sampling of unmarked individuals (Chamberlain et al. 1997, Hobson and Wassenaar 1997, Hobson 1999, Bearhop et al. 2005). The power of stable isotope analysis in ecological research lies in the predictable relationship between assimilated food and water resources and the isotopic composition of biological tissues (DeNiro and Epstein 1978, Estep and Dabrowski 1980, DeNiro and Epstein 1981, Macko et al. 1983, Hobson et al. 1999). In migration studies, the assignment of individuals to a particular geographic locality depends on the robust mapping of isotopic gradients (Poage and Chamberlain 2001, Meehan et al. 2004, Bowen et al. 2005) as well as the spatial distribution of isotopically distinctive food resources within the potential geographic range of the species

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(Romanek et al. 2000, Wassenaar and Hobson 2000, Chamberlain et al. 2005, Cerling et al. 2006). Despite recent methodological advances in isotope ecology, continental-scale analyses of migratory birds continue to yield no better than coarse isotopic discrimination of populations, even when combinations of elements are examined (Rubenstein et al. 2002, Royle and Rubenstein 2004, Bowen et al. 2005, Hobson 2005a, Passey et al. 2005, Wunder et al. 2005, Rocque et al. 2006, Kelly et al. 2008, Wunder and Norris 2008). Failure to achieve finer spatial resolution may stem, in part, from the confounding effects of topography on continental isotope gradients of hydrogen, oxygen, and carbon, the most commonly investigated elements in avian migration studies (Hobson 2005a, West et al. 2006). Precipitation and surface water exhibit progressively lower δD (ratio of deuterium to protium) and $\delta^{18}O$ values with increasing altitude and latitude and distance from coastlines (Dansgaard 1964, Siegenthaler and Oeschger 1980, Poage and Chamberlain 2001, Meehan et al. 2004, Bowen et al. 2005). $\delta^{13}C$ values increase with altitude and latitude in C_3 plants as a consequence of pressure and temperature effects on carboxylation efficiency and other factors during photosynthesis (Körner et al. 1988, Vitousek et al. 1990, Körner et al. 1991, Marshall and Zhang 1994, Sparks and Ehleringer 1997, Hultine and Marshall 2000). While more detailed isotope base maps for topographically complex regions will improve the specificity of isotopic interpretations, one impediment to achieving finer isotopic resolution of breeding bird populations is the difficulty of obtaining sufficient numbers of specimens stratified by sex, age class, molt, and geographic location. Intensive multiyear investigations conducted in relatively small catchments suggest that finer isotopic resolution at mesoscales may be possible if altitudinal effects and other within-population sources of isotopic variation are identified (Graves et al. 2002).

The Black-throated Blue Warbler (*Dendroica caerulescens*) breeds in cool deciduous and mixed deciduous-coniferous forests in eastern North America and winters in the Caribbean basin, primarily in the Greater Antilles (Holmes 1994, Rubenstein et al. 2002). Populations breeding south of 40° N latitude are restricted to higher altitudes in the Appalachian Mountains. Graves et al. (2002) examined altitudinal variation of carbon isotope ratios ($^{13}C/^{12}C$) in feathers of males breeding in the Big Santeetlah Creek watershed, a relatively small but topographically complex catchment in western North Carolina, USA. The Big Santeetlah Creek population is part of a larger metapopulation in the southern Appalachian Mountains (10^5 – 10^6 individuals; G. R. Graves, unpublished data), where it is one of the most common breeding species between 800 m and 1450 m above sea level (a.s.l.) (Wilcove 1988, Graves 1997b, Haney et al. 2001). In the southern Appalachians, males return to breeding territories (0.75–3.0 ha) at lower altitudes (<800 m a.s.l.) as early as 1 May, but

settlement at higher altitudes (>1300 m a.s.l.) may be delayed by two to three weeks. The staging areas for individuals that eventually occupy territories at higher altitudes are unknown. The diet of nestlings and adults during the breeding season is predominately lepidopteran larvae gleaned from the foliage and twigs of trees and shrubs (Rodenhouse and Holmes 1992, Holmes 1994).

This small migrant (9–10 g) undergoes a complete molt in July and August before fall migration to the Caribbean basin, such that flank feathers sampled during the current breeding season were grown on or near their breeding or natal territories during the previous year (Holmes 1994, Graves 1997a). In theory, carbon isotope profiles of feather keratin reflect the diet ingested during June and July and provide a tool for tracking the year-to-year movements of individuals along altitudinal gradients in C_3 forests. Carbon isotope ratios for yearling males (first breeding season) and older males (second or later breeding season) exhibited divergent altitudinal patterns. Whereas feather $\delta^{13}C$ values from yearling males were uncorrelated with altitude, most likely reflecting natal dispersal (i.e., movement from hatching site to first breeding territory), feather $\delta^{13}C$ values for older males increased with altitude at the rate of $\sim 1.3\text{‰}$ per 1000 m, which approximates the lapse rate ($\sim 1.1\text{‰}$ per 1000 m) observed in several groups of C_3 plants along altitudinal gradients (Körner et al. 1991). This suggests that older males exhibit a significant degree of breeding season philopatry to narrow altitudinal zones, if not to individual territories. However, the range of feather $\delta^{13}C$ values reported from the watershed nearly bracketed the range of feather $\delta^{13}C$ values observed in breeding populations sampled from Georgia to New Brunswick (11° of latitude). These findings suggest that the isotopic detection of long-distance dispersal, a process facilitated by the identification of statistical outliers or higher isotopic variance in local populations (Hobson et al. 2004, Møller and Hobson 2004, Møller et al. 2006) may be complicated by local isotopic variation in topographically complex regions. On the other hand, the lapse rate of $\delta^{13}C$ values observed along altitudinal gradients in C_3 plants and feather keratins suggests that under certain circumstances the carbon isotope profiles of metabolically active tissues may provide useful information on altitudinal tenancy patterns in local populations.

Knowledge of tenancy patterns of Black-throated Blue Warblers during the peak of the breeding season has been limited to observations of marked individuals on small, comparatively level, study plots in the Hubbard Brook Experimental Forest, New Hampshire, USA (Holmes et al. 1992, Marra and Holmes 1997, Webster et al. 2001, Sillett et al. 2004). This species, however, reaches its greatest abundance in the southern Appalachian Mountains where the altitudinal amplitude of local breeding populations may exceed 800 m (Graves 1997b, Graves et al. 2002). Male behavior in the

TABLE 1. Summary statistics for Black-throated Blue Warblers (*Dendroica caerulescens*) collected during eight consecutive breeding seasons in the Big Santeetlah Creek watershed, western North Carolina, USA.

Year	Collection date	Altitude, mean \pm SD (m)	No. yearling males	No. older males
1995	18–23 June	1119 \pm 168	4	35
1996	12–20 June	1167 \pm 185	11	36
1997	11–19 June	1152 \pm 191	9	34
1998	10–18 June	1152 \pm 187	12	23
1999	10–20 June	1115 \pm 207	19	24
2000	10–18 June	1128 \pm 185	20	25
2001	9–17 June	1148 \pm 198	26	24
2002	11–18 June	1160 \pm 185	22	24

Note: Mean altitude is expressed as meters above sea level.

topographically rugged Appalachians is poorly understood; particularly the degree to which yearlings (first breeding season) and older males (second or later breeding season) wander altitudinally during the nesting season after the establishment of territories. Differences in the breeding biology of yearling and older males suggest that patterns of altitudinal tenancy may be age related. For instance, yearlings arrive later on the breeding grounds than older males (Hubbard 1965); they are behaviorally subordinate (Holmes et al. 1996) and less likely to occupy high-quality territories than older males (Holmes et al. 1996). Once settled, both yearling and older males defend small territories and are thought to be relatively sedentary, except for a small subset of unmated males (“floaters”) that search for females and undefended territories (Holmes et al. 1992, Marra and Holmes 1997). In most passerine species, floaters are more likely to be yearling males (Smith and Arcese 1989), which implies that the pattern of altitudinal tenancy in yearling warblers may differ fundamentally from that of older males.

In this study, we present the results of an eight-year investigation of $\delta^{13}\text{C}$ values in muscle and liver samples of the Black-throated Blue Warbler, a study designed to examine mesoscale patterns of altitudinal tenancy during the peak of the breeding season (June) in the southern Appalachian Mountains. Because animal tissues exhibit a range of isotopic turnover rates, depending on the metabolic rate of the tissue and the element of interest, analyses of two or more tissue types from an individual may provide information on foods assimilated over different time scales. Analyses of liver and pectoral muscle $\delta^{13}\text{C}$ values provide differential profiles of isotope assimilation from warbler diets over comparatively short time frames (days to weeks). We hypothesized that liver and muscle of yearlings and older males would display similar isotopic patterns of variation if altitudinal movements (i.e., patterns of altitudinal tenancy) of the two age classes were similar. By sampling territorial males over eight breeding seasons from a relatively small catchment, we were able to examine age-

specific and year effects while controlling for the influence of altitude on carbon isotope signatures.

METHODS

Study site

This study was conducted in the Big Santeetlah Creek watershed (35°21' N, 84°00' W) on the eastern slope of the Unicoi Mountains, the highest subsidiary mountain range in the Appalachians south of the Little Tennessee River (Graves et al. 2002). This forested watershed (680–1689 m a.s.l., 5350 ha) in Graham County, North Carolina, is embedded in the largest contiguous tract of montane forest in eastern North America and supports one of the most taxonomically diverse woody floras north of Mexico (Lorimer 1980). The terrain is steep, and slopes commonly vary from 20% to 40%. The greatest dimensions of the study area are ~11.3 km (east–west axis) \times ~8.0 km (north–south axis).

Forestry practices since the 1920s have resulted in a mosaic of relict old-growth trees and even-aged stands (\bar{x} = 70 \pm 41 years [\bar{x} = mean age of forest stands \pm SD]; n = 100 stands) of hardwood–hemlock forest dominated by C_3 plants. Cove and streamside forest in the Big Santeetlah watershed is dominated by hemlock (*Tsuga canadensis*), tulip poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), beech (*Fagus grandifolia*), northern red oak (*Quercus rubra*), yellow birch (*Betula alleghaniensis*), sweet birch (*Betula lenta*), silverbell (*Halesia carolina*), and white basswood (*Tilia heterophylla*). Concentrations of chestnut oak (*Quercus prinus*) are found on drier slopes and ridges. American chestnut (*Castanea dentata*) was a major component in this community prior to the chestnut blight (Braun 1950, Lorimer 1980). Witch hazel (*Hamelis virginiana*), striped maple (*Acer pensylvanicum*), mountain maple (*Acer spicatum*), flowering dogwood (*Cornus floridana*), and alternate-leaf dogwood (*Cornus alternifolia*) are important subcanopy species. The understory is dominated by “evergreen” thickets of rosebay (*Rhododendron maximum*) and mountain laurel (*Kalmia latifolia*). Important components of the shrub layer that provide nesting sites for Black-throated Blue Warbler include hydrangea (*Hydrangea arborescens*), mapleleaf viburnum (*Viburnum acerifolium*), hobble bush (*Viburnum alnifolium*), smooth allspice (*Calycanthus fertilis*), and flowering raspberry (*Rubus odoratus*), as well as tree saplings. Grassy balds occur at the summit of peaks (>1600 m), but C_4 crops have not been cultivated in the watershed in >70 years.

Population sampling

Males (n = 348) were collected under state and federal licenses for multiple research purposes (Graves 1997b, Graves et al. 2002, Rubenstein et al. 2002, Graves 2004, Fallon et al. 2006, Grus et al. 2009) during eight consecutive breeding seasons (1995–2002) along transects spanning the altitudinal range (750–1545 m) of the

species in the Big Santeetlah Creek watershed (Table 1). The altitude of all collecting sites was determined with a Thommen altimeter (Revue Thommen, Waldenburg, Switzerland) calibrated from landmarks on U.S. Geological Survey 7.5-minute topographic maps. Year-to-year variation in the altitudinal distribution of collected specimens was statistically insignificant (ANOVA, $F_{7,340} = 0.46$, $P = 0.87$; Table 1). Males collected in this study responded to playback of recorded songs and exhibited territorial behavior. Although females were observed on many of the territories, we did not systematically attempt to locate females or nests. The sampling period (9–23 June) coincided with the overlap of the fledging period for the first brood and nest-building and egg-laying for the second brood. Older males were preferentially collected from 1995 through 1998, whereas a more even balance of yearlings and older males was obtained in the latter years of the study. Annual standardized censuses along a 14.5-km altitudinal transect indicated that the removal of males in the watershed had no demonstrable effect on the census population. Specimens were packaged whole in multiple layers of insulating tissue paper and aluminum foil and frozen within 30 min of death in liquid nitrogen. Voucher specimens (rounded skins, partial skeletons, tissues, stomach contents) were deposited in the research collection of the National Museum of Natural History, Smithsonian Institution.

Two age classes of males were distinguished by plumage characters (Graves 1997a): (1) yearlings hatched the previous year (first alternate plumage, or SY in banding terminology), and (2) older individuals in their second or later breeding season (definitive alternate plumage, or ASY in banding terminology) (Pyle 1997). Yearling males were identified by the olive-green (rather than blue) edges of their alulae and primary coverts and a suite of other characters (Graves 1997a).

Isotopic turnover rates

Feeding trials and isotope assays of tissues have been conducted on several large-bodied avian species (Hobson and Clark 1992, 1993, Haramis et al. 2001, Bearhop et al. 2002). However, only three studies of isotope turnover rates have been conducted on small migratory passerines (<20 g) with higher metabolic rates (Hobson and Bairlein 2003, Pearson et al. 2003, Podlesak et al. 2005), and these studies were limited to examinations of carbon and nitrogen isotopes of whole blood or blood components and feathers. Experimental dietary studies with captive quail (*Coturnix japonica*, ~115 g) concluded that the half-lives of carbon isotopes were ~2.7 d for liver, ~11.4 d for whole blood, and ~12.4 d for pectoral muscle (Hobson and Clark 1992). Whole blood of smaller, captive Yellow-rumped Warblers (*Dendroica coronata*, 11 g; Pearson et al. 2003) and Garden Warbler (*Sylvia borin*, 20 g; Hobson and Bairlein 2003) had carbon half-lives of 3.9–6.1 and 5.0–5.7 d, respectively. These studies suggest that carbon isotopes in the whole

blood of wood warblers may exhibit nearly complete turnover (>98%) in as little as 24 days (~6 half-lives). Carbon isotope turnover rates in liver and pectoral muscle of small migratory passerines are unknown. However, given the observation that turnover rates for whole blood and pectoral muscle in larger birds are roughly equivalent and turnover rates for liver are about four times faster than for muscle (Hobson and Clark 1992), we estimated a turnover rate of ~1.0–1.5 d for liver and ~4–6 d for pectoral muscle of small migratory passerines. As such, the carbon isotope signatures of liver and pectoral muscle of the warblers sampled in this study undoubtedly reflect the diet assimilated on breeding territories rather than during migration or on the wintering grounds.

Isotope analyses

Liver and pectoral muscle samples were freeze-dried, soaked in a 2:1 chloroform:methanol mixture for 24 h to remove lipids (Hobson and Welch 1992), rinsed with methanol, and then oven-dried at ~45°C. Prior to isotope analyses, samples were homogenized by grinding with a mortar and pestle. After grinding, 2–3 mg of each sample were weighed to the nearest ± 1 μg in a precleaned tin capsule. Capsules were then sealed and placed in the autosampler of a Carlo Erba Elemental Analyzer (NA 2500; Thermo Electron, Milan, Italy) attached to a continuous-flow isotope ratio mass spectrometer (Finnigan Delta^{PLUS} XL, Finnigan-MAT, San Jose, California, USA) for carbon and nitrogen isotope analysis. Samples were converted to CO₂ and N₂ in oxidation–reduction furnaces, separated by gas chromatography, and then measured for ¹³C/¹²C and ¹⁵N/¹⁴N ratios on the mass spectrometer.

An internal N_{2(g)} working standard was admitted prior to the introduction of each sample and a CO_{2(g)} standard was admitted at the conclusion of each combustion for calibration to AIR (nitrogen) and Vienna PeeDee Belemnite (V-PDB) (carbon) international standards (Mariotti et al. 1980, Coplen 1996). Stable nitrogen isotope profiles of warbler tissues were uncorrelated with altitude (Appendix A) and will not be discussed further.

Stable isotope ratios are reported in per-mil units (‰) using standard delta (δ) notation (Craig 1961). External working standards of dogfish muscle and liver (DORM-2) were reproducible to <0.2‰ (SD) per run for $\delta^{13}\text{C}$. All isotope analyses were performed at the University of Georgia's Savannah River Ecology Laboratory.

The $\delta^{13}\text{C}$ values were tested for goodness of fit to a normal distribution with the Lilliefors test. We used analysis of covariance (ANCOVA) to investigate the effects of categorical (age class, year) and continuous variables (altitude, day of year) on isotope values for pectoral muscle and liver (ANOVA module of SYSTAT Version 11). We used paired *t* tests to compare mean isotope values of liver and pectoral muscle. We used ordinary least squares regression (OLS) to investigate the

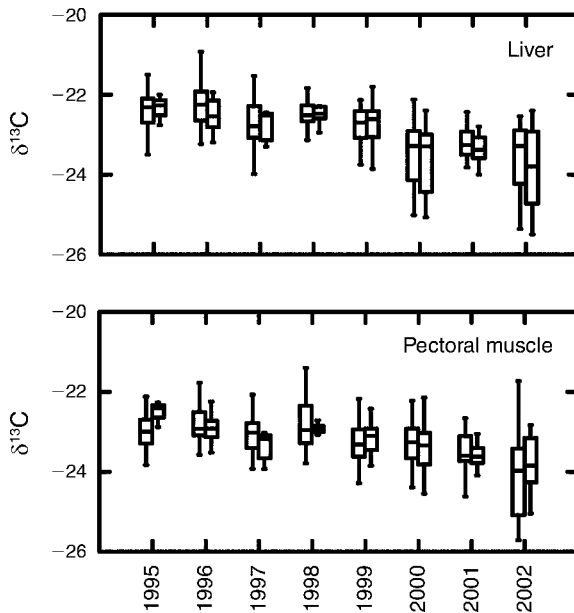


FIG. 1. Box plots depicting the annual fluctuation of $\delta^{13}\text{C}$ values for liver and pectoral muscle in yearling males (right-hand box for each year) and older males (left-hand box) of the Black-throated Blue Warbler (*Dendroica caerulescens*) from the Big Santeetlah Creek watershed, western North Carolina, USA. Horizontal lines within boxes represent the median, and whiskers represent the range of data values. A few outliers were omitted from the figure.

relationship between isotope values and selected independent variables. All P values are two tailed ($\sigma = 0.05$).

RESULTS

Stable carbon isotopes of liver

The liver $\delta^{13}\text{C}$ values from the pooled sample of yearling and older males ($n = 8$ yr) ranged from -25.5 to -19.6 ($x = -23.0\% \pm 0.8\%$ [mean \pm SD]; $n = 330$). Year-to-year differences (e.g., 1997 to 1998) in mean liver $\delta^{13}\text{C}$ values ranged from 0.1% to 0.9% (Fig. 1). Mean liver $\delta^{13}\text{C}$ values were significantly higher than muscle $\delta^{13}\text{C}$ values for both yearling and older males (Table 2, Fig. 2). Liver and muscle $\delta^{13}\text{C}$ values were positively correlated in yearlings (OLS: $R^2 = 0.27$, $P < 0.0001$; $n = 114$) and in older males (OLS: $R^2 = 0.30$, $P < 0.0001$; $n = 212$). The relationship between liver $\delta^{13}\text{C}$ values and altitude showed considerable year-to-year variation, and significant correlations (OLS) were observed in only three of eight years (Fig. 3, Appendix

B). In the pooled sample ($n = 8$ yr), liver $\delta^{13}\text{C}$ values increased with altitude at the rate of $\sim 0.5\%$ per 1000 m (liver $\delta^{13}\text{C} = -23.54 + 0.0005$ [altitude, m]). In ANCOVA, liver $\delta^{13}\text{C}$ values exhibited significant altitude ($P = 0.005$) and year effects ($P < 0.0001$) but no age class or seasonal (day of year) effects (Table 3). Liver $\delta^{13}\text{C}$ values were uncorrelated with feather $\delta^{13}\text{C}$ values reported in Graves et al. (2002) for feathers grown during the previous molt in yearling (OLS: $R^2 < 0.01$, $P = 0.69$; $n = 115$) and older males (OLS: $R^2 < 0.01$, $P = 0.23$; $n = 215$).

Stable carbon isotopes of pectoral muscle

The muscle $\delta^{13}\text{C}$ values for the pooled sample of yearlings and older males ranged from -25.7% to -19.8% ($x = -23.2\% \pm 0.7\%$ [mean \pm SD]; $n = 331$). Year-to-year differences in mean muscle $\delta^{13}\text{C}$ values ranged from 0.1% to 0.5% (Fig. 1). The relationship between muscle $\delta^{13}\text{C}$ values and altitude exhibited substantial year-to-year variation (Fig. 3, Appendix C). Slope coefficients (OLS) were positive during all breeding seasons, but significant correlations were observed in only five of eight years. In the pooled sample ($n = 8$ yr) of yearling and older males, muscle $\delta^{13}\text{C}$ values increased at a rate of $\sim 1.1\%$ per 1000 m (muscle $\delta^{13}\text{C} = -24.46 + 0.0011$ [altitude, m]). ANCOVA indicated that muscle $\delta^{13}\text{C}$ values were significantly influenced by altitude ($P < 0.0001$) and year effects ($P < 0.0001$) but not by age class or seasonal effects (Table 3). Muscle $\delta^{13}\text{C}$ values were uncorrelated with feather $\delta^{13}\text{C}$ values reported in Graves et al. (2002) for feathers grown during the previous year in yearlings (OLS: $R^2 < 0.01$, $P = 0.53$; $n = 116$) and in older males (OLS: $R^2 = 0.02$, $P < 0.06$; $n = 215$).

DISCUSSION

Altitude effects in isotope signatures

Significant altitude effects for liver and muscle $\delta^{13}\text{C}$ values were consistent with the relationship previously described for feather $\delta^{13}\text{C}$ from older males in the Big Santeetlah Creek watershed (Graves et al. 2002). In the pooled sample of yearling and older males, liver $\delta^{13}\text{C}$ values increased with altitude at the rate of $\sim 0.5\%$ per 1000 m, whereas muscle $\delta^{13}\text{C}$ values increased at a rate of $\sim 1.1\%$ per 1000 m. The difference in the altitudinal lapse rates of muscle and liver $\delta^{13}\text{C}$ values may be related to the disparity in isotopic turnover rates of liver and muscle. The lapse rate of muscle $\delta^{13}\text{C}$ values with

TABLE 2. Summary statistics for $\delta^{13}\text{C}$ values of liver and pectoral muscle (mean \pm SD) of male Black-throated Blue Warblers collected in the Big Santeetlah Creek watershed.

Age class	Liver	Pectoral muscle	t †	P
Yearlings	$-23.1\% \pm 0.9\%$ (115)	$-23.3\% \pm 0.7\%$ (116)	-2.34	0.02
Older males	$-22.8\% \pm 0.8\%$ (215)	$-23.2\% \pm 0.8\%$ (215)	-7.26	<0.0001

Note: Parenthetical values represent n , the number of individual birds.

† Paired t test.

altitude was comparable to that observed in feather $\delta^{13}\text{C}$ values in older males ($\sim 1.3\%$ per 1000 m) and nearly identical to the average rate of increase reported in several groups of C_3 plants ($\sim 1.1\%$ per 1000 m) along altitudinal transects (Körner et al. 1991). The significantly lower F ratios observed for altitudinal effects in liver $\delta^{13}\text{C}$ ($F = 7.92$) compared to muscle $\delta^{13}\text{C}$ ($F = 33.33$) suggest that liver data may more closely track fluctuating isotope signals in lepidopteran prey at any given altitude. Temporal variability in the isotopic composition of diet is also suggested by the surprisingly low correlation between liver and muscle $\delta^{13}\text{C}$ values in yearlings ($R^2 = 0.27$) and older males ($R^2 = 0.30$) and the year-to-year fluctuations in correlation coefficients (Appendices B, C).

Regardless of the nuances of the carbon isotope record, the rapid adjustment of $\delta^{13}\text{C}$ values to local altitudinal environments is consistent with the hypothesis that carbon turnover rates are relatively rapid in liver and pectoral muscle of small migratory passerines. Liver and muscle $\delta^{13}\text{C}$ values (measured in year $t + 1$) were uncorrelated with feather $\delta^{13}\text{C}$ values (feathers grown in year t) in yearlings and older males. This finding is unsurprising because yearling wood warblers rarely breed near their natal territories (Nolan 1978, Holmes 1994) and because annual mortality rates of adults may exceed 40% (Nolan 1978, Holmes 1994, Holmes et al. 1996). Consequently, as few as half the territories in any given breeding season are occupied by incumbents.

Altitudinal tenancy during the breeding season

Similar altitudinal trends of $\delta^{13}\text{C}$ variation in liver and pectoral muscle of yearlings and older males suggest that the majority of territory holders of both age classes are relatively sedentary during the peak of the breeding season. In other words, yearlings appeared to be no more likely than older males to wander altitudinally in the days and weeks leading up to the collection date. However, there was considerable scatter in the data and some curious outliers among yearlings and older males (see Fig. 3). We suspect the majority of scatter in $\delta^{13}\text{C}$ data is related to annual variation in climate (expressed as year effects) and the topographic complexity of territories. Owing to the steep terrain in the Santeetlah Creek watershed, the altitudinal range of male territories typically spans 10–20 m and may exceed 50 m on some territories. A multitude of factors that affect $\delta^{13}\text{C}$ values in C_3 plants (and isotope signatures of lepidopteran larvae), such as moisture availability and irradiance, vary with slope and aspect (Ehleringer et al. 1986, Zimmerman and Ehleringer 1990, Hanba et al. 1997, Saurer et al. 1997). We consider several other potential sources of data scatter as less likely. For example, some outliers may represent territorial males that were drawn significantly upslope or downslope beyond the boundaries of their territories by the broadcast of taped songs. Alternatively, these data points may represent wander-

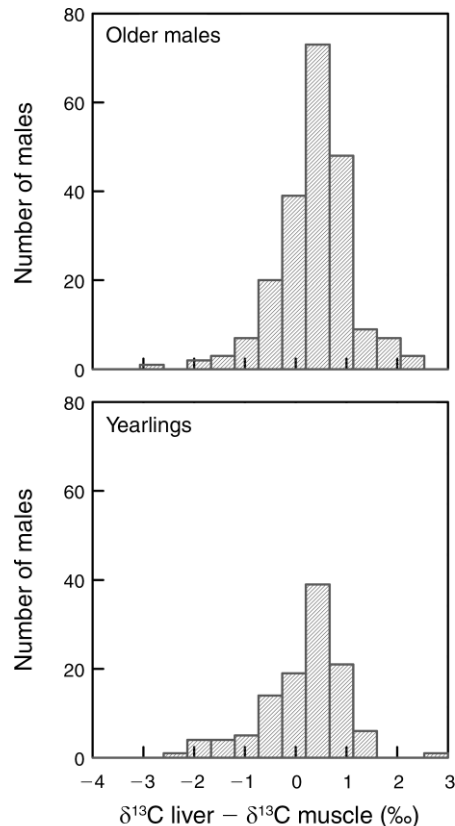


FIG. 2. Distribution of differences in carbon isotope values ($\delta^{13}\text{C}$ for liver – $\delta^{13}\text{C}$ for muscle) within individual Black-throated Blue Warblers of two age groups.

ing floaters that responded to playback of songs and briefly exhibited territorial behavior at the collection site. A fourth possibility is that outliers represent territorial males that were intercepted as they briefly explored outlying territories, perhaps searching for extra-pair copulations (Webster et al. 2001). Such reconnaissance flights traversing 100–200 m of altitude would require only a few minutes to complete and would be very difficult to detect through direct observation, standard mark–recapture methods, or radio telemetry.

Despite the many sources of isotopic noise in the data, the significant correlation between $\delta^{13}\text{C}$ values of metabolically active tissues and altitude suggest that carbon isotopes may have some use in evaluating aggregate patterns of tenancy along altitudinal gradients. We caution, however, that the altitudinal signal of stable carbon isotopes in warbler tissues is relatively weak and that the discernment of mesoscale patterns depends on relatively large sample sizes obtained over multiple breeding seasons. Our data indicate that analyses limited to a single breeding season can yield results that are at odds with isotopic patterns that emerge over time periods equivalent to the maximum life span of passerine birds. Given the scatter in data, it is unlikely that individuals can be accurately assigned to a

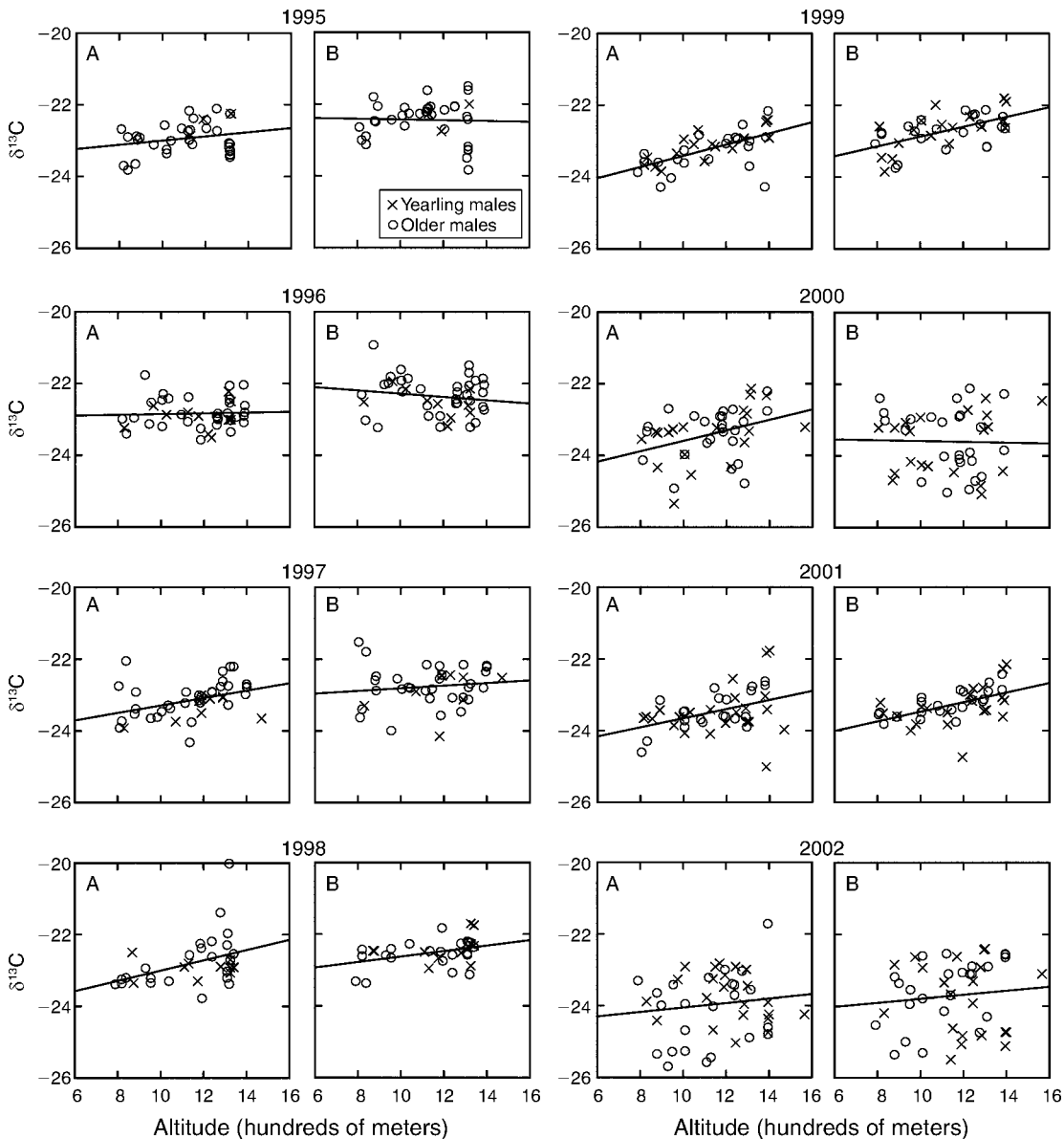


FIG. 3. Relationship between (A) $\delta^{13}\text{C}$ muscle and (B) $\delta^{13}\text{C}$ liver values, paired for each year, with altitude of breeding territories of Black-throated Blue Warblers. Yearlings and older males are represented respectively by \times 's and open circles. See Appendix B (liver $\delta^{13}\text{C}$) and Appendix C (muscle $\delta^{13}\text{C}$) for slope coefficients and intercepts of least squares regression lines. Altitude is expressed as meters above sea level.

particular altitude solely from carbon isotope values. Future studies should explore the feasibility of combining carbon, oxygen, and hydrogen isotope data from a variety of tissues in altitudinal assignment models.

Implications for connectivity analysis of wintering and breeding populations of migratory birds

Stable isotope data are frequently used as proxies for wintering habitat quality of birds sampled on breeding territories or captured during migration (Marra et al. 1998, Norris et al. 2003, Bearhop et al. 2004). The validity of such studies depends on the isotope turnover

rates of assayed tissues and the duration of migration. Feeding experiments indicate that isotopic turnover rates in whole blood and plasma of small migratory birds (<20 g) are rapid, perhaps too rapid to provide much isotopic signal of wintering habitat for species sampled on breeding territories at high latitudes (Hobson and Bairlein 2003, Pearson et al. 2003, Hobson 2005a, Mazerolle and Hobson 2005). Nearctic–Neotropical migratory passerines, such as the Black-throated Blue Warbler, typically take several weeks to reach their breeding grounds (Cooke 1888, 1915). For example, median capture dates for 15 species of

TABLE 3. Independent analyses (ANCOVA) for $\delta^{13}\text{C}$ values of liver and pectoral muscle of Black-throated Blue Warblers collected in the Big Santeeetlah Creek watershed (1995–2002).

Dependent and independent variables	df	MS	F ratio	P
$\delta^{13}\text{C}$ liver ($R^2 = 0.41$)				
Age	1	0.17	0.39	0.53
Year	7	9.90	23.13	<0.0001
Age \times year	7	0.20	0.47	0.86
Altitude	1	3.39	7.92	0.005
Day of year	1	0.01	0.02	0.89
Error	312	0.43		
$\delta^{13}\text{C}$ pectoral muscle ($R^2 = 0.35$)				
Age	1	0.00	0.00	0.95
Year	7	5.12	13.56	<0.0001
Age \times year	7	0.51	1.34	0.23
Altitude	1	12.57	33.33	<0.0001
Day of year	1	0.05	0.13	0.72
Error	313	0.38		

migratory passerines at a banding station on the Gulf Coast of Louisiana were 22 days earlier than those recorded for the same species at two stations located approximately 2500 km to the north in Pennsylvania and Ontario (Marra et al. 2005). Nocturnally migrating passerines characteristically refuel at diurnal stopover locations, which could result in a stepwise change in the isotopic composition of metabolically active tissues during the transit (Hobson 2005a). The lengthy spring migration of Neartic–Neotropical migrant passerines suggests that the bulk of the carbon isotope signal in blood, liver, and muscle of individuals at the time of their arrival on their breeding territories reflects food consumed en route. This implies that tissues with high metabolic rates sampled from migratory species on breeding territories have limited value as isotopic proxies of wintering habitat use. Tissues that lock in isotopic signals over a limited time frame such as toenail keratins and feathers, for species that molt on the wintering grounds, are much more likely to reflect the isotopic composition of wintering-ground diet and habitats (Chamberlain et al. 2000, Bearhop et al. 2004, Mazerolle and Hobson 2005).

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APPENDIX A

Independent analyses (ANCOVA) for $\delta^{15}\text{N}$ values of liver and pectoral muscle of male Black-throated Blue Warblers, *Dendroica caerulescens*, collected during eight consecutive breeding seasons, 1995–2002, in the Big Santeetlah Creek watershed (*Ecological Archives* A019-049-A1).

APPENDIX B

Intercept and slope coefficients, by year, for the relationship between altitude and liver $\delta^{13}\text{C}$ values of Black-throated Blue Warblers in the Santeetlah Creek watershed (*Ecological Archives* A019-049-A2).

APPENDIX C

Intercept and slope coefficients, by year, for the relationship between altitude and muscle $\delta^{13}\text{C}$ values of Black-throated Blue Warblers in the Santeetlah Creek watershed (*Ecological Archives* A019-049-A3).

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Appendix A. Independent analyses (ANCOVA) for $\delta^{15}\text{N}$ values of liver and pectoral muscle of male Black-throated Blue Warblers (*Dendroica caerulescens*) collected during eight consecutive breeding seasons (1995-2002) in the Big Santeetlah Creek watershed.

Dependent variable	Independent variables	df	MS	F-ratio	P
$\delta^{15}\text{N}$ Liver ($R^2 = 0.20$)	Age	1	0.16	0.38	0.54
	Year	7	1.99	4.69	0.0001
	Age X Year	7	0.30	0.71	0.67
	Altitude	1	0.69	1.62	0.20
	Julian date	1	0.42	1.00	0.32
	Error	314	0.42		
$\delta^{15}\text{N}$ Pectoral ($R^2 = 0.26$)	Age	1	0.06	0.20	0.66
	Year	7	1.42	5.00	< 0.0001
	Age X Year	7	0.45	1.57	0.14
	Altitude	1	0.67	2.36	0.13
	Julian date	1	1.31	4.60	0.03
	Error	315	0.28		

Appendix B. Intercept and slope coefficients (by year) for the relationship between altitude and $\delta^{13}\text{C}_{\text{liver}}$ values of Black-throated Blue Warblers in the Santeetlah Creek watershed (see Fig. 3).

1995 ($n = 37$) $R^2 = 0.00$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-22.32	0.59	-37.63	
Altitude	-0.0001	0.0005	-0.20	0.84

1996 ($n = 45$) $R^2 = 0.00$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-22.10	0.66	-33.24	
Altitude	-0.0002	0.0006	-0.31	0.76

1997 ($n = 41$) $R^2 = 0.02$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-23.18	0.53	-44.01	
Altitude	0.0004	0.0005	0.81	0.42

1998 ($n = 33$) $R^2 = 0.14$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-23.39	0.39	-59.42	
Altitude	0.0008	0.0003	2.27	0.03

1999 ($n = 37$) $R^2 = 0.00$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.25	0.36	-66.85	
Altitude	0.0014	0.0003	4.27	0.0001

2000 ($n = 45$) $R^2 = 0.00$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-23.47	0.81	-64.61	
Altitude	-0.0001	0.0007	-0.16	0.88

2001 ($n = 43$) $R^2 = 0.29$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.82	0.28	-64.61	
Altitude	0.0013	0.0003	4.04	<0.001

2002 ($n = 46$) $R^2 = 0.01$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.35	0.91	-26.80	
Altitude	0.0006	0.0008	0.72	0.48

Appendix C. Intercept and slope coefficients (by year) for the relationship between altitude and $\delta^{13}\text{C}_{\text{muscle}}$ values from Black-throated Blue Warblers in the Santeetlah Creek watershed (see Fig. 3).

1995 ($n = 37$) $R^2 = 0.05$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-23.59	0.47	-50.18	
Altitude	0.0006	0.0004	1.41	0.17

1996 ($n = 43$) $R^2 = 0.002$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-22.96	0.43	-53.38	
Altitude	0.0001	0.0004	0.29	0.77

1997 ($n = 53$) $R^2 = 0.17$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.80	0.62	-40.18	
Altitude	0.0015	0.0005	2.86	0.007

1998 ($n = 34$) $R^2 = 0.15$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.44	0.69	-35.23	
Altitude	0.0014	0.0006	2.42	0.021

1999 ($n = 39$) $R^2 = 0.38$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.97	0.37	-67.21	
Altitude	0.0016	0.0003	4.76	< 0.0001

2000 ($n = 45$) $R^2 = 0.14$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-25.04	0.64	-39.35	
Altitude	0.0015	0.0006	2.61	0.012

2001 ($n = 44$) $R^2 = 0.15$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.92	0.53	-46.74	
Altitude	0.0013	0.0005	2.77	0.008

2002 ($n = 46$) $R^2 = 0.017$

Effect	Coefficient	Standard error	t	P (two-tailed)
Constant	-24.67	0.84	-29.26	
Altitude	0.0006	0.0007	0.86	0.39