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USING STABLE ISOTOPES TO UNDERSTAND SEASONAL INTERACTIONS IN A
LONG-DISTANCE MIGRATORY SONGBIRD

A Thesis Presented

by

Traynor G. Biasioli

to

The Faculty of the Graduate College

of

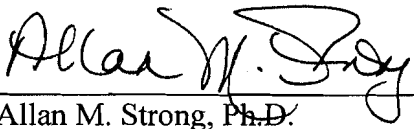
The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Master of Science
Specializing in Natural Resources


May, 2008

Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Master of Science, specializing in Natural Resources.

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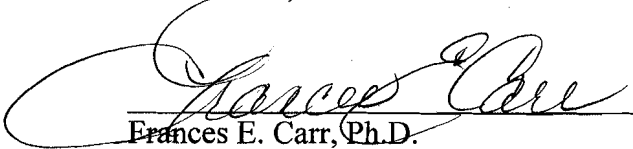
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ABSTRACT

Recent advances in the analysis of intrinsic markers, particularly stable isotopes, have allowed great insight into interactions between different stages of the annual cycle of migratory birds. Hydrogen isotope ratios, because of strong latitudinal trends in their distribution, have been widely used to address long-distance movements of migratory birds. Likewise, carbon isotope ratios have been useful in examinations of habitat quality because of their responsiveness to changes in plant community composition. However, basic assumptions underlying the use of certain isotopes have yet to be adequately examined. Additionally, much of the research regarding seasonal interactions in migratory songbirds comes from study of a single species, and it is unclear whether these findings are applicable to a wider range of migrant songbirds. In this study, I collected tissue samples from black-throated blue warblers (*Dendroica caerulescens*) at a breeding site in New Hampshire to address two important questions regarding stable isotopes and the investigation of seasonal interactions. First, using feather samples from both adult and juvenile birds, I investigated the influence of age, molt timing, and reproductive effort in determining the stable hydrogen isotope ratios that are incorporated into tissue samples. Secondly, I took claw samples from adult birds to examine the importance of winter habitat quality, as inferred through carbon isotope ratios, in determining subsequent reproductive success.

I found that hydrogen isotope ratios in feather samples were significantly influenced by both age and molt timing, though not by reproductive effort. The mechanism underlying age-related isotopic variation is unclear, but may be widespread among passerines. In addition, this study is the first to note a significant seasonal trend in feathers grown throughout a breeding season, although the mechanism for this pattern is also unclear. Taken together, these findings have important implications for the use of hydrogen isotope ratios for purposes of geographic assignment. Further research is needed to determine the prevalence and magnitude of age-related and seasonal trends in hydrogen isotope ratios. I recommend that future studies note the age class of birds when sampling for hydrogen isotopes, and researchers should attempt to collect feathers grown early in the molt cycle.

Analysis of carbon isotope ratios from claw samples indicated that winter habitat quality did not directly influence subsequent reproductive success. However, winter habitat quality may have an indirect influence on reproductive output. Females from higher quality wintering sites were in significantly better body condition on the breeding grounds, and settled on more insect-rich breeding territories. Both body condition on the breeding grounds and breeding territory quality have previously been shown to influence subsequent reproductive success, in this and other songbird species. These results indicate that winter habitat quality may be important in determining future reproductive success for black-throated blue warblers, and interactions between events during the wintering and breeding periods may need to be incorporated into future population models for this species.

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CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

Population limitation in migratory birds

Factors limiting populations of migratory birds have received considerable attention, in part due to declining populations of many species of migrants (Hagan and Johnston 1992). Unlike non-migratory species, the factors that affect migratory species are spatially segregated, often at continental scales. Depending on the species and specific conditions encountered, population limitation may occur at any stage of the annual cycle, including breeding (Chase et al. 1997, Rodenhouse et al. 2003), wintering (Hill 1988, Peach et al. 1991, Sherry et al. 2005), or during migration (Baker et al. 2004). However, studies focused on population dynamics within a single portion of the annual cycle are based on the assumption that events during these distinct stages of the annual cycle act independently of one another (Newton 2004). Indeed, most research on population dynamics of long-distance migrants has focused on within-season events (Webster et al. 2002). However, ecologists have long argued that population dynamics in migratory species result from the integration of events that occur across seasons and continents (Fretwell 1972), an assertion that has been supported by more recent empirical research (Marra et al. 1998, Gill et al. 2001, Bêty et al. 2003, Norris et al. 2004a). Understanding the strength of seasonal interactions depends on the ability to understand the factors affecting individuals and populations during multiple stages of the annual cycle. Conventional mark-recapture techniques have proved useful in studies of seasonal interactions in larger species, many of which form large aggregations which allow for easier resighting or recapture (Rubenstein and Hobson 2004). However, these

conventional techniques are not practical in studies involving smaller, less conspicuous species. In this review I will first summarize the findings of early research investigating seasonal interactions with mark-resight techniques. I will then address more recently developed techniques involving analysis of stable isotope ratios, which can facilitate investigation of seasonal interactions. Finally, I will discuss recent projects which have utilized stable isotope analysis in studies of seasonal interactions in migratory songbirds, and summarize their relevance to understanding population dynamics in these species.

Seasonal interactions at the population level

Research on seasonal interactions has often focused on the importance of these interactions to population dynamics, and researchers have endeavored to determine population-level consequences of potential interactions (Norris and Taylor 2006). In a few studies, researchers have been able to assess the influence of various seasonal interactions at the level of the population. Cabot and West (1973) observed that female Barnacle Geese (*Branta leucopsis*) were less likely to breed, and breeding females laid smaller clutches, in years with poor food supply at migratory stopover sites. Davies and Cooke (1983) found similar results for Lesser Snow Geese (*Chen caerulescens caerulescens*), indicating that for these two species of waterfowl, food supply during migration provided essential resources for clutch formation in distant breeding grounds. Nilsson (1979) found a positive correlation between mean winter temperature and numbers of juvenile Whooper Swans (*Cygnus cygnus*) in flocks during the subsequent winter, suggesting that milder winters allowed increased overwinter survival of adults

and/or increased allocation of resources in preparation for breeding. The results of the above studies are not surprising, given that many species of waterfowl, particularly those breeding at high latitudes, utilize endogenous resources for egg formation (Ankney and MacInnes 1978, Choinière and Gauthier 1995). Thus, seasonal interactions are implicit in the life history strategies of these species, as breeding activity directly relies upon resources gained at a previous stage in the annual cycle. In a more recent study involving a long-distance passerine migrant, Saino et al. (2004) demonstrated that Barn Swallows (*Hirundo rustica*) bred earlier and laid larger clutches following winters with more favorable conditions (as measured through the normalized difference vegetation index, an indicator of primary productivity). Winter conditions had a measurable impact upon Barn Swallow populations, as populations were 15% larger in years following favorable winter conditions as opposed to years with poor winter conditions.

The above studies have shown that environmental conditions during winter and spring migration can determine subsequent reproductive performance and, ultimately, population sizes of long-distance migratory birds. However, research on seasonal interactions at the population level may not be possible for many species of long-distance migrants, and some factors that influence events in subsequent seasons may only be observable at the individual level. For example, in species where individuals occupy territories of differing quality, differences in resource abundance may be particularly influential in shaping events during subsequent seasons at the individual level, but this territory-level variability may be obscured at the population level. Furthermore, population-level studies may be confounded in species with diffuse migratory

connectivity. Where migratory connectivity is weak, members of the same breeding population often migrate to widely separated wintering populations, and thus may experience variable conditions outside of the breeding season. Therefore, the influence of even large-scale factors may be attenuated in subsequent seasons. Currently, the degree of migratory connectivity is unknown for most species (Webster et al. 2002).

Seasonal interactions investigated via conventional techniques at the individual level

As noted above, investigations of seasonal interactions at the population level may not be possible for many migratory species. Furthermore, taking a population-level view may obscure numerous interactions that operate at a smaller scale. In many instances, investigation of seasonal interactions at the individual level is both more feasible and provides greater insight. As a result of these potential advantages, many researchers have utilized individually-based studies of seasonal interactions. Most of these projects have focused on understanding the determinants of reproductive success. In particular, timing of and condition upon arrival on the breeding grounds have been shown to influence both choice of territory and subsequent reproductive success for a wide range of species (Ankney and MacInnes 1978, Lanyon and Thompson 1986, Lozano et al. 1996, Currie et al. 2000, Smith and Moore 2003). While these studies demonstrated that breeding season events were influenced by events during previous stages of the annual cycle, they were not able to establish the timing or specific events responsible for influencing breeding season events. Although these studies are important in demonstrating the presence of seasonal interactions, they are of limited use in developing

more precise models of migrant population dynamics. Moreover, these studies were unable to determine if these interactions were primarily caused by mechanistic processes, such as differences in habitat quality, or stochastic events, such as random weather events on migration. Thus, it remains unclear whether these interactions can be influenced by management actions.

Although most studies of seasonal interactions have been unable to observe individuals throughout multiple stages of the annual cycle, this limitation has been overcome in a few studies of larger and more conspicuous species. In a study of Brent Geese (*Branta bernicla*), Ebbinge (1992) was able to demonstrate the role of foraging conditions at migratory stopover sites in influencing subsequent reproductive success. Geese that obtained access to higher-quality foraging sites during stopover were more likely to fledge young, and fledged significantly more young than geese that used lower quality stopover habitat. Bêty et al. (2003) also demonstrated significant connections between events during migration and the breeding season. Female Snow Geese in better body condition at a migratory stopover site in southern Quebec arrived earlier on the breeding grounds and laid larger clutches. Finally, Gill et al. (2001) found that Black-tailed Godwits (*Limosa limosa islandica*) that occupied high quality wintering sites arrived earlier on the breeding grounds than did birds from lower quality wintering sites, thus establishing a link between winter foraging conditions and events during the breeding season.

All of the above studies involved relatively large and conspicuous species. These attributes allow for increased probability of resighting color-banded individuals, as

well as for attachment of large remote sensing units (e.g. radio transmitters). However, the majority of migratory birds are relatively small and secretive. Mark-resight studies are only effective when individuals concentrate at predictable sites during at least one stage of the annual cycle. Species with highly specific habitat requirements, such as waterfowl or shorebirds, generally fulfill this condition. However, species that are less restricted in their habitat requirements are much more difficult to locate during multiple stages of the annual cycle. Additionally, only relatively large birds can accommodate telemetry units with sufficient battery life and range to allow tracking over migrations that can extend thousands of kilometers. Until the technology exists to follow smaller migrants over these distances, researchers will have to utilize indirect markers in order to study seasonal interactions at the individual level.

Stable isotopes and the investigation of seasonal interactions

Stable isotope ratios have shown great promise and have garnered much research interest for investigations of seasonal interactions. Ratios of stable isotopes of certain elements respond predictably to various ecological factors, such as changes in temperature, differences in plant communities, and variation in plant water use efficiency (Rubenstein and Hobson 2004). As these elements are passed on to higher order consumers and incorporated into animal tissues during synthesis, isotopic ratios are conserved, or are altered in a predictable manner, depending on the element in question. Stable isotope ratios persist in tissues for time periods ranging from days to years, depending on the turnover rate of the tissue in question, or remain fixed in tissues that are

inert following synthesis (Hobson 1999). Because of their responsiveness to important ecological factors, stable isotopes have proven useful at identifying the geographic location or habitat type in which a particular tissue was synthesized. These characteristic variations in isotope ratios have allowed use of stable isotopes to investigate seasonal interactions and movement patterns of long-distance migrants (Rubenstein and Hobson 2004).

Because of strong latitudinal trends in stable hydrogen isotope ratios (δD), stable hydrogen isotopes have been frequently used as a geographical marker (Dansgaard 1964, Hobson 1999). Hydrogen isotopes have been used to investigate numerous aspects of avian long-distance movements, including migration patterns of different populations (Kelly et al. 2002), frequency of long-distance dispersal (Hobson et al. 2004), and degree of connectivity between wintering and breeding populations (Hobson and Wassenaar 1997, Hobson et al. 2001, Rubenstein et al. 2002). More recently, Norris et al. (2004b) used δD to demonstrate an interaction between events during the breeding season and the timing and location of molt. As determined from δD values in tail feathers, male American redstarts (*Setophaga ruticilla*) that fledged young later in the breeding season were more likely to molt during migration at locations south of the breeding grounds. Furthermore, males that molted on migration had reduced carotenoid levels in their feathers, indicative of reduced immune function and individual quality. These results highlighted the energetic trade-offs between late-season reproductive effort, molt, and migration, and demonstrated the consequences for individuals that combine these activities.

Stable carbon isotopes have also proven instrumental in many studies of seasonal interactions involving long-distance migrants. Carbon isotope ratios are strongly influenced by differences in plant communities, particularly by differences in the proportion of C₃ and C₄ plants in a given habitat (Lajtha and Marshall 1994). C₃ plants are more typical of mesic sites, whereas C₄ plants become increasingly common at drier sites. These differences in plant composition generally result in more negative $\delta^{13}\text{C}$ values for birds from mesic sites. Furthermore, differences in plant water use efficiency between wet and dry sites also leads to differences in $\delta^{13}\text{C}$ fractionation, which generally leads to further isotopic differentiation between birds from wet versus dry habitats (Lajtha and Marshall 1994). The isotopic differentiation between wet and dry habitats is important because habitat quality for wintering migratory birds is often linked with relative moisture level of their wintering site. Mesic sites generally support a greater abundance of the insect prey favored by many species of wintering migratory birds (Parrish and Sherry 1994, Studds and Marra 2005). Differences in $\delta^{13}\text{C}$ values have been particularly useful at determining quality of winter territories, and have been exploited by numerous researchers investigating the role of winter habitat quality in shaping events during subsequent seasons. Marra et al. (1998) were the first to exploit stable isotopes in investigations of seasonal interactions. In that study, the authors demonstrated that winter habitat quality had significant impacts on time of departure for spring migration, as American redstarts from moist forest sites departed earlier than individuals from dry forest sites. Furthermore, the authors found that these differences carried over to the breeding season. Stable carbon isotope samples from individuals at a breeding site in

New Hampshire indicated that individuals from more mesic winter habitat (more negative $\delta^{13}\text{C}$ values) arrived earlier on the breeding grounds. These results, taken in combination with the results of previous studies demonstrating that early arrival on the breeding grounds often leads to higher reproductive success (e.g., Lozano et al. 1996), suggest that reproductive success may be significantly influenced by winter habitat occupancy. Further research with American redstarts confirmed this connection. Norris et al. (2004a), using stable carbon isotopes from cellular blood samples, found that birds from high quality mesic wintering habitat arrived earlier and fledged more young than did birds from dry forest sites. This study indicated that the influence of winter habitat quality could be substantial, as modeling suggested that redstarts from high quality winter habitat would fledge on average two additional young per year relative to redstarts from poor quality sites. Thus, not only did events during the wintering period influence subsequent reproductive success, but were also shown to have consequences for population dynamics that would not be evident simply by studying events during that single stage of the annual cycle. Research with black-throated blue warblers (*Dendroica caerulescens*) also indicated that winter habitat quality can influence events in subsequent seasons. Bearhop et al. (2004) also used $\delta^{13}\text{C}$ values to demonstrate that birds originating from mesic winter territories were in better body condition during migration. It is plausible that the benefits of enhanced body condition during migration may carry over to the breeding season, though this has not been explored.

Despite the potential applications of stable isotopes in research of seasonal interactions, there still remain numerous limitations and uncertainties surrounding their

use. The most serious concerns regard the reliability of δD as a latitudinal marker. While hydrogen isotopes from feather samples have shown significant latitudinal trends with certain songbird species (Hobson and Wassenaar 1997, Rubenstein et al. 2002), many studies with other avian groups have suggested that δD may not function effectively as a latitudinal marker (Meehan et al. 2003, Smith and Dufty 2005, Rocque et al. 2006). Some of the variability in δD values has been attributed to known factors, such as age (Meehan et al. 2003), while in other cases the causes of observed variation have not been satisfactorily explained (Rocque et al. 2006). Another factor limiting the reliability of conclusions drawn from hydrogen isotope data is that past studies which endorsed δD as an effective geographic marker did not use known-origin feathers. That is, these studies assumed that the location of molt in year x was relatively nearby the sampling location (usually the breeding site) in year $x+1$. However, many of the studies that utilized feathers with known molt locations have questioned the effectiveness of δD as an indicator of geographic origin (Meehan et al. 2003, Smith and Dufty 2005, Rocque et al. 2006). Thus, although past research with passerines has suggested that δD has tremendous potential to advance understanding of migration patterns and seasonal interactions in passerines, future research using passerine feathers of known origin will be required to test the assumptions required to use δD as a geographical marker.

Study objectives

One of the main factors limiting modeling of population dynamics in migratory birds has been the difficulty of understanding if, and to what degree, events in different

stages of the annual cycle interact with one another. Models that have simply assumed events in different stages are independent of one another are at odds with much recent research. In addition, most research on seasonal interactions in passerines has been conducted on a single species, the American redstart. While the findings from these studies have greatly advanced our understanding of seasonal interactions, it is uncertain whether these results can be applied to a wider range of migratory songbirds. In this study, I will examine the prevalence and importance of seasonal interactions in the black-throated blue warbler, a Nearctic-Neotropical migrant passerine. Relative to most other migratory passerines, both the wintering and breeding ecology of black-throated blue warblers have been extensively studied (Holmes et al. 1989, Holmes et al. 1992, Wunderle 1995, Rodenhouse et al. 2003), which will facilitate investigation of seasonal interactions in this species. Specifically, I will first examine potential sources of variation in δD values using feathers of known origin. Second, assuming that my results demonstrate that δD may be effectively used as a geographical marker, I will examine the role of reproductive effort in determining molt latitude. Finally, I will investigate the importance of winter territory quality on events during the breeding season via use of $\delta^{13}C$ values. This study will enhance understanding of the importance of seasonal interactions in migratory songbirds, and will also assess the validity of using δD as a latitudinal marker and assist in further refinement of this technique.

CHAPTER 2: TRACKING MIGRATORY BIRDS USING INTRINSIC MARKERS: INVESTIGATING VARIATION OF STABLE HYDROGEN ISOTOPE RATIOS IN FEATHERS OF KNOWN ORIGIN

ABSTRACT. -- Stable hydrogen isotope ratios (δD) in feathers have become increasingly important in understanding animal migration patterns and connectivity between breeding and wintering populations of migratory species. Predictable latitudinal variation in δD has allowed researchers to use δD values as a geographic marker of tissue growth. However, many of the assumptions underlying the use of δD have not been thoroughly examined with songbird feathers of known geographic origin. Here, I analyzed feather samples from black-throated blue warblers (*Dendroica caerulescens*) to examine variation in δD as a function of age, molt date, and reproductive effort. Using feathers of known geographic origin, I found that primary feathers of adult birds were significantly elevated in δD relative to juvenile primaries, and juvenile δD values declined significantly with later hatching dates. Analysis of tail feathers from returning adult birds indicated that δD values were not influenced by reproductive effort, although some of these returning adults molted south of their breeding site. While the mechanisms responsible for the observed variation are not clear, these results carry important implications for future studies utilizing feather δD values. Age-related differences in feather δD values may be widespread in passerines, and researchers may need to assess this variability in their analyses. I suggest that future studies analyzing feather δD note the age class of each bird sampled, and that researchers aim to sample feather types that are molted early in the molt cycle.

INTRODUCTION

Populations of migratory animals are influenced by events in multiple locations and in multiple stages of the annual cycle (Newton 2004). Until recently, researchers lacked tools with which they could effectively track migratory animals during different stages of the annual cycle, and thereby investigate fundamental aspects of the ecology of migratory organisms. Traditional approaches to studying movement patterns, such as mark/recapture techniques, fail to provide sufficient data for all but the largest and most conspicuous species (Hobson 1999). Advanced remote-sensing technologies, such as radio- or satellite-telemetry, provide valuable data, though cost and weight considerations will continue to limit their use (Rubenstein and Hobson 2004). However, recent advancements in the application and analysis of intrinsic markers, particularly the ratios of ecologically relevant stable isotopes, have allowed investigators to address numerous previously intractable questions.

Stable hydrogen isotope ratios (δD) have been particularly useful in studying the ecology of migratory animals. Fractionation of hydrogen isotopes is largely temperature dependent, which results in large-scale latitudinal patterns in δD (Dansgaard 1964). This variation in δD is incorporated into animal tissues during tissue synthesis, which allows use of tissue δD (δD_t) to estimate latitude of tissue growth (Hobson 1999). Analysis of δD_t has proven particularly useful with tissues that are metabolically inert following synthesis, such as hair and feathers, as these δD_t values remain constant after growth and thus can retain information about locations inhabited many months prior to tissue sampling (Hobson 1999). Within the past decade, δD_t has been used extensively in

studies of migratory organisms, including birds (Hobson and Wassenaar 1997), mammals (Cryan et al. 2004), and insects (Dockx et al. 2004). This technique has been used to address a variety of long-distance movement patterns, including degree of connectivity between wintering and breeding populations (Rubenstein et al. 2002, Norris et al. 2006), population-specific patterns in migration timing (Kelly et al. 2002, Kelly 2006), and breeding and natal dispersal patterns (Hobson et al. 2004, Clark et al. 2006).

Despite the breadth of questions that have been addressed using hydrogen isotopes, many questions remain regarding the reliability of δD_t as a latitudinal marker of tissue growth. Numerous assumptions underlie the use of δD_t for purposes of geographic assignment. Temporal variation (both seasonal and inter-annual) in δD_t is assumed to be minimal at a given site. Additionally, different individuals at the same location are assumed to incorporate similar isotopic ratios, irrespective of age or sex. Further, if δD_t values are to be used for purposes of assignment to a particular breeding population, it must be assumed that the tissue in question was grown on or near the breeding site. Many of the early studies which utilized δD_t values for geographic assignment did not explicitly test these assumptions (Chamberlain et al. 1997, Hobson and Wassenaar 1997). More recent studies, using feathers of known geographic origin from a range of avian taxa, have highlighted potential limitations of this technique. Research on two species of North American raptors demonstrated significant age-related variation in δD_t , as adult feathers were substantially more enriched in deuterium (more positive δD_t values) than juvenile feathers (Meehan et al. 2003, Smith and Dufty 2005). The cause of this age-related variation remains unclear, but appears to be physiological in origin (Smith and Dufty

2005). In a related study investigating variation in δD_t in songbirds, Langin et al. (2007) also found that adult feathers were elevated in δD_t relative to juveniles, though the age-related difference was less pronounced than in raptors. Additional studies involving known-origin feathers of shorebirds (Wunder et al. 2005, Rocque et al. 2006) and passerines (Powell and Hobson 2006) have found high and unexplained levels of variability in δD_t which were sufficient to preclude reliable geographic assignment using δD_t values. Similarly, Norris et al. (2004b) found that molt location of American redstarts (*Setophaga ruticilla*) was influenced by level of reproductive effort, with birds expending greater effort having δD_t values indicative of molt several hundred kilometers south of the breeding grounds. While this study did not dispute the effectiveness of δD_t as a geographic marker, it did suggest that δD_t may not be a reliable indicator of breeding origin.

Given the growing importance of δD_t in understanding the ecology of migratory organisms, it is essential that the assumptions behind this technique are rigorously tested. With feather samples from black-throated blue warblers (*Dendroica caerulescens*), a long-distance Nearctic-Neotropical migrant, I addressed three potential factors which may limit use of δD_t values to track migratory animals. Using feathers of known geographic origin, I tested for age-related and seasonal variation in δD_t . Furthermore, using feather samples from returning adult birds of known breeding history, I examined the influence of reproductive effort on molt latitude, as inferred through δD_t values.

METHODS

Field methods

I conducted field work at Hubbard Brook Experimental Forest, New Hampshire, USA, where black-throated blue warblers have been studied intensively since 1986 (Holmes et al. 2005). Feathers were collected at two sites spanning distinct elevational gradients: a low elevation site (Main) ranging from 500-580m, and a high elevation site (Ridge) ranging from 750-820m. To examine potential age-related and seasonal variation in δD_t values, I collected freshly molted primary feathers from juvenile and adult black-throated blue warblers from June to August 2006. Because δD_t values may vary with feather type (Smith and Dufty 2005, Langin et al. 2007) and because adult black-throated blue warbler's prebasic molt is initiated in their primaries (Pyle 1997, T.G.B., personal observation), I collected incoming primary feathers from both adults and juveniles. Feathers were collected from juveniles throughout the breeding season, 0-2 days following fledging (age 9-11 days, $n = 44$, from 34 nests). Adults frequently initiate molt after termination of breeding activities, but will occasionally begin molt while provisioning nestlings at late-season nests (T.G.B., personal observation). Therefore, I captured adults at late-season nests (after 1 August), and if they had begun molt, I collected the most recently grown primary ($n = 9$). To address the possible influence of reproductive effort on δD_t values, I collected tail feathers of returning adults with known breeding history (birds that bred at Hubbard Brook the summer prior to sampling) from May to August 2005 and 2006 ($n = 43$).

Isotopic analysis

Feathers were cleaned of surface contaminants using a 2:1 chloroform:methanol solution and rinsed using deionized water, after which they were dried under a fume hood for 72 h. After drying, I clipped 0.35 ± 0.02 mg of feather vane material from the feather tip (Wassenaar and Hobson 2006) and packaged the samples in 3.5 x 5.0 mm isotope grade silver capsules. Stable isotope analysis was conducted at the Colorado Plateau Stable Isotope Laboratory in Flagstaff, Arizona. Keratin standards were used to correct for isotopic exchange between samples and ambient water vapor; thus, the δD_t values reported here reflect nonexchangeable hydrogen in the feather samples (Wassenaar and Hobson 2003). Stable hydrogen isotope ratios were determined on H_2 gas, produced by high-temperature flash pyrolysis of feathers using a high-temperature conversion elemental analyzer ($1,400^\circ C$) coupled with a continuous-flow isotope-ratio mass-spectrometer (Finnigan Delta Plus XL). All δD_t values are reported in per mil notation (‰) relative to the VSMOW-SLAP scale. One laboratory standard was analyzed for every three unknown samples, and yielded a measurement repeatability of $\pm 2\%$. Duplicates of the same feather sample had a mean standard deviation of 1.5% ($n = 13$).

Data analysis

To test for seasonal trends in δD_t , I performed a linear regression with molt date as the predictor and δD_t as the response variable. Only juvenile feathers were used for this analysis, as all adult feather samples were molted within a short time period (< 2 weeks) which would likely be too brief to detect any potential seasonal trends. Furthermore, as elevation is also known to influence δD_t values (Araguas-Araguas et al. 2000), separate regressions were performed for juvenile feathers collected on each study site. The effect

of age on δD_t was examined via a three-way ANOVA, with age, site, and date as model factors. For the purposes of the three-way ANOVA, molt date was divided into three 17-day “molt periods” (25 Jun - 11 July, 12 July - 28 July, and 29 July - 14 August).

Additionally, the effect of age was also examined via a paired *t*-test with a subset of feathers, in cases where both an adult and one of its offspring were sampled. In instances where multiple nestlings were sampled from a single nest, one nestling was chosen at random for the paired *t*-test. Feather samples from molting adults were collected within three days of sampling of their offspring, thus minimizing any potential seasonal effects.

To test for a relationship between δD_t and reproductive effort, I performed two linear regressions with different measures of breeding effort (*z* scores of latest fledging date and number of young fledged; both standardized by year) as predictors and δD_t as the response variable. Furthermore, because female black-throated blue warblers often provide uni-parental care at late-season nests (Holmes et al. 2005), I repeated the same regressions and considered each sex independently.

RESULTS

Seasonal and age-related variation in δD_t

The mean δD_t value for all known-origin feathers was $-94.8 \pm 6.9\text{‰}$ (mean \pm SD, $n = 53$), and ranged from -107.4 to -80.0‰ . As expected, feathers from Ridge plot were more depleted in deuterium ($-99.8 \pm 5.5 \text{‰}$, range -107.4 to -91.0‰ , $n = 17$) than feathers from Main plot ($-92.4 \pm 6.2\text{‰}$, range -104.9 to -80.0‰ , $n = 36$). Juvenile δD_t values declined significantly throughout the breeding season on both sites (Ridge: $r^2 =$

0.80, $p < 0.001$, $n = 13$; Main: $r^2 = 0.38$, $p < 0.001$, $n = 31$; Fig. 1). The slope of the fitted regression line was similar for both sites (Ridge: $-0.23\%/d$, Main: $-0.24\%/d$).

Results from a three-way ANOVA indicated that δD_t was significantly influenced by age ($F_{1,52} = 25.2$, $p < 0.001$), molt timing ($F_{2,52} = 18.1$, $p < 0.001$), and elevation ($F_{1,52} = 17.6$, $p < 0.001$), with site used as a proxy for elevation. No interaction terms were significant (all $p > 0.5$). Adult feathers were significantly more enriched in deuterium than juvenile feathers grown during the same molt period (Fig. 2). However, because of the seasonal decline in juvenile δD_t values, adult feathers (all grown late in the breeding season) were similar in isotopic composition to juvenile feathers grown at the same elevation earlier in the breeding season (Fig. 2). A comparison of adult feathers and feathers of their offspring also indicated that adult feathers had significantly more positive δD_t values ($\Delta = 7.2 \pm 6.8\%$; paired t -test, $t = 2.9$, $p = 0.03$, $df = 6$).

Variation in δD_t with reproductive effort

Tail feathers of birds returning to breed at Hubbard Brook showed considerably more variability in δD_t values than did the sample of known-origin feathers. The mean (\pm SD) δD_t for feathers of returning birds was $-87.9 \pm 11.0\%$ ($n = 43$), and ranged from -101.4 to -38.9% . Most of the feather δD_t values fell within the range of the known-origin feathers, though there were three outliers (7% of returning adults; Figure 3) which appear to have molted at least one tail feather south of the breeding grounds. Tail feather δD_t was not significantly related to either number of offspring fledged ($r^2 = 0.00$, $p = 0.90$) or fledging date ($r^2 = 0.07$, $p = 0.10$). Additionally, all relationships were non-significant when I examined females and males separately (all $p > 0.1$).

DISCUSSION

Stable hydrogen isotopes have been widely used as a tool to track migratory organisms. However, applications of this novel technique have outpaced attempts to critically examine its underlying assumptions. Recent studies in a variety of avian taxa have highlighted certain limitations of this technique. Here, using passerine feathers of known geographic origin, I demonstrate that δD_t values can vary significantly based on age-related and seasonal factors. Furthermore, using feathers from returning adult birds, I found no evidence that δD_t values were influenced by reproductive effort. While the results of this study carry clear implications for future research, the mechanisms responsible for the observed variation in δD_t values remain uncertain.

Age-related variation in δD_t

Previous studies, involving both raptors (Meehan et al. 2003, Smith and Dufty 2005) and passerines (Langin et al. 2007), have noted significant differences in δD_t values between adult and juvenile birds. In raptors, this age-related variation is apparently due to physiological stress during molt (Smith and Dufty 2005). In many species of raptors, adults initiate molt during incubation. The combination of these two energetically demanding activities likely results in the use of evaporative cooling by adults during molt, which leads to elevated δD_t values relative to juveniles. This mechanism could also be responsible for the observed difference between juvenile and adult black-throated blue warbler feathers. Due to the difficulty of trapping molting adults that were no longer on their breeding territories, I was only able to collect molting feathers from adults that

combined molt with reproduction. Therefore, the elevated δD_t of adult feathers could be the result of energetic stress caused by overlapping molt with reproduction. However, if this mechanism was indeed responsible for the observed age-related differences in δD_t values, I would have expected δD_t values from feathers of returning adults to vary in relation to reproductive effort and timing of reproduction during the previous breeding season. My results indicate that reproductive effort did not significantly influence δD_t values, thus suggesting that the mechanism responsible for age-related variation in raptors is not in operation consistently with black-throated blue warblers.

In a previous study which found similar age-related variation in passerines, Langin et al. (2007) suggested that age-related variation in diet, and in particular access to isotopically enriched surface drinking waters, was responsible for elevated δD_t values in adults. As opposed to Langin et al.'s (2007) research site, there are no large open sources of drinking water at Hubbard Brook, and given the lack of late-season rainfall in 2006, most streams near the study plots at Hubbard Brook were dry at the time that adults initiated molt. Thus, it is unlikely that the elevated δD_t values of adult feathers are the result of differential access to surface waters. In addition, there is no evidence to suggest that the diet of adult black-throated blue warblers differs substantially from that of juveniles. However, in the absence of further data on diet, it remains possible that adults may preferentially consume certain prey types, which could lead to the observed differences in δD_t values.

The seasonal decline in δD_t values observed in this study may be responsible for age-related differences in δD_t . While the δD_t values of adults were elevated relative to

juveniles that were molting during the same time period, the adult δD_t values were similar to those of juveniles which molted earlier during the breeding season (Fig. 2). Thus, if adults synthesize feathers using energy stores that were accrued earlier in the breeding season, the observed age-related differences may simply be an artifact of differential timing of energy storage between adults and juveniles. However, previous research suggests that molt is fuelled by dietary intake during molt or from very short-term reserves (Murphy 1996), though the possibility remains that certain nutrients are derived from longer-term stores (Bearhop et al. 2002).

Finally, there may be some basic underlying differences in molt physiology between adult and juvenile birds that have yet to be explained. Irrespective of the mechanism, adult δD_t values may be elevated relative to juveniles in a wide variety of passerines. I found that adult δD_t values were elevated by 7-10‰ relative to juveniles molting during the same time period, consistent with the findings of the only other study which has investigated age-related δD_t variation in passerine feathers of known origin (Langin et al. 2007).

Seasonal variation in δD_t

I also observed a significant seasonal trend in juvenile feather δD_t values, with juvenile δD_t values declining across the breeding season at both study sites. The rate of decline in δD_t values was virtually identical on the two plots, lending further confidence to the observed seasonal trend. I anticipated that I might find seasonal trends in juvenile δD_t values, given that δD values of precipitation regularly show seasonal variation (Araguas-Araguas et al. 2000). However, I expected that if a trend was present, juvenile

δD_t values would increase throughout the breeding season, whereas the observed values declined significantly during the sampling period.

There are multiple factors which can result in seasonal trends in δD values of precipitation or animal tissues. In temperate regions, precipitation δD values increase substantially during summer months, due to temperature-dependent fractionation of hydrogen isotopes (Araguas-Araguas et al. 2000). At Hubbard Brook, mean daily temperatures increased slightly throughout the 2006 breeding season (Bailey 2007), which, in the absence of confounding factors, should lead to an increase in δD values of precipitation. Given that δD_t values decreased over the breeding season, it appears that the seasonal trend was not driven by temperature-dependent isotopic fractionation.

Water stress is also known to influence animal δD_t values, as increases in evaporative water loss can lead to increases in δD_t values (McKechnie et al. 2004). Thus, seasonal changes in water availability may lead to corresponding changes in δD_t values. Indeed, precipitation did show a seasonal trend during the breeding season. However, the quantity of precipitation declined during the breeding season (Eager and Bailey 2007), which, if it resulted in water limitation, should lead to a seasonal increase in δD_t . Given that δD_t values declined, it is unlikely that water availability was responsible for the observed trend.

Finally, seasonal variation in δD_t may also be driven by seasonal changes in the sources of precipitation (Araguas-Araguas et al. 2000). Precipitation that originates from colder, high latitude sources is likely to have more negative δD values than precipitation originating from warmer mid-latitude waters. Thus, the observed seasonal decline in δD_t

values could be explained if the proportion of precipitation at Hubbard Brook derived from high latitude sources increased over the breeding season. Unfortunately, data on air mass mixing for this region is not available, and I am unable to adequately assess this hypothesis.

Regardless of the mechanism responsible for the decline in δD_t values across the breeding season, a seasonal trend of the magnitude observed here can have important implications for geographic assignment. Black-throated blue warblers have a relatively lengthy breeding season, and juveniles from early nests at Hubbard Brook can fledge 10 or more weeks earlier than juveniles from late nests (Holmes et al. 2005). Given a rate of decline of -0.24‰ per day, this can result in a difference of 16.8‰ between juveniles from early v. late-season nests. This degree of variation would be equivalent to a 5-6 degree difference in latitude, according to Meehan et al.'s (2004) isotopic base map.

Influence of reproductive effort on δD_t

Contrary to the findings of Norris et al. (2004b) with American redstarts, I found no significant influence of reproductive effort on molt latitude (as inferred through δD_t). Neither the number of young fledged nor the timing of fledging was significantly related to δD_t values from tail feathers of returning adults. However, some adults from my sample apparently molted at locations south of the breeding site. Three of the 43 adults sampled had δD_t values that were substantially more positive than the range for known origin feathers (> 10‰ difference). Given that there were no outliers from the large sample of known origin feathers, nor were there substantial deviations from expected values for the laboratory standards, I feel that it is highly unlikely that these more positive

δD_t values were the result of measurement error. It is unclear whether these individuals undertook a complete molt south of the breeding grounds, or if they simply replaced a few feathers during migration. Unfortunately, I do not have feather samples from other feather tracts for these individuals, so I cannot determine the extent of the molt that occurred south of their breeding site.

Implications for future research

Analysis of δD_t values from migratory animals has become an essential tool in advancing our knowledge of patterns of migration, dispersal, and population connectivity. While this innovative technique has enabled investigation of previously intractable questions, many of the assumptions underpinning valid use of δD_t values are only beginning to be critically addressed. The results of this study carry important considerations for future applications of δD_t analysis.

The present study is apparently the first to detect a seasonal trend in δD_t values. The observed daily rate of change in δD_t clearly limits the precision with which geographic origin can be inferred, particularly for species with extended breeding seasons. To the best of my knowledge, only one other study has examined feather δD_t values for potential seasonal trends (Langin et al. 2007). They did not observe any seasonal variation, though potential trends may have been masked by lumping δD_t values from multiple years in their analysis. Future analysis of feathers from juveniles born throughout an entire breeding season would help understand the frequency and magnitude of seasonal variation in δD_t values, and would help determine the degree of latitudinal precision possible via δD_t analysis.

Consistent with the findings of multiple previous studies, I found that feathers from adults had significantly more positive δD_t values than did juvenile feathers. Given the seasonal trend in δD_t values found in this study, the observed age-related variation would have been of little consequence in terms of geographic assignment. While adult feathers were significantly enriched relative to those of juveniles grown during the same time period, the adult feather δD_t values closely matched those of juveniles from early in the breeding season. However, there is no evidence to suggest that seasonal trends in δD_t should be present every year, or indeed that they should be in the same direction or of the same magnitude as that observed here. Thus, depending on the strength and direction of seasonal trends in δD_t , the observed differences between age classes could have important consequences for geographic assignment. Taken together with the results of Langin et al. (2007), it appears that adult passerine feathers may regularly be elevated in δD_t relative to juvenile feathers by 7-10%. Further research is clearly needed to determine if this is a widespread pattern. Additionally, I recommend that future feather sampling efforts should record the age class of those birds that are sampled.

Finally, while I did not detect any influence of breeding season events on molt location, I did observe that a small number (7%) of returning adult birds had tail feather δD_t values inconsistent with molt on their breeding grounds. It is unclear with what frequency other species may molt their tail feathers south of their breeding or natal sites. However, even a relatively small number of outliers may be sufficient to alter or mask relationships in research using δD_t values to determine breeding origins. Tail feathers are commonly collected for analysis of δD_t (Smith et al. 2003), though they may not be the

most appropriate feather type for inferring latitude of breeding or natal sites. Tail feathers may be lost more frequently than other feather types during stressful situations such as predator avoidance (Møller et al. 2006), and thus may be replaced at locations hundreds of kilometers from the breeding grounds. Furthermore, molt of tail feathers often occurs later in the molt cycle for many passerines (Pyle 1997). Thus, if molt is interrupted prior to departure from the breeding grounds, tail feather δD_t values will likely not carry a signal from the breeding location. Sampling of feathers grown earlier in the molt sequence may be more likely to carry δD_t values indicative of breeding locations. In samples of primaries from 42 returning adult American redstarts, Langin et al. (2007) found no evidence of molt south of the breeding site. However, in a previous study using tail feathers from American redstarts at the same breeding location, Norris et al. (2004b) found considerable evidence of tail feather molt south of the breeding grounds. I suggest that future sampling efforts should attempt to collect feather types grown early in the molt cycle of the species of interest.

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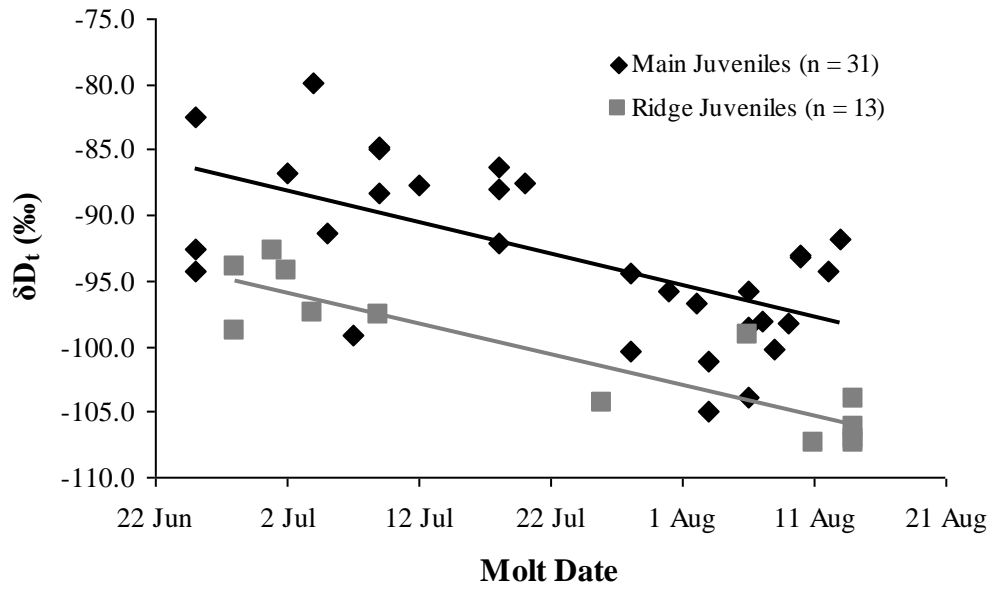


Fig. 1. δD_t in juvenile black-throated blue warbler feathers as a function of molt timing. Feather samples were collected at Hubbard Brook Experimental Forest, New Hampshire, USA, during summer 2006. The elevation of Main plot ranges from 500 - 580 m, and Ridge ranges from 750 - 820 m.

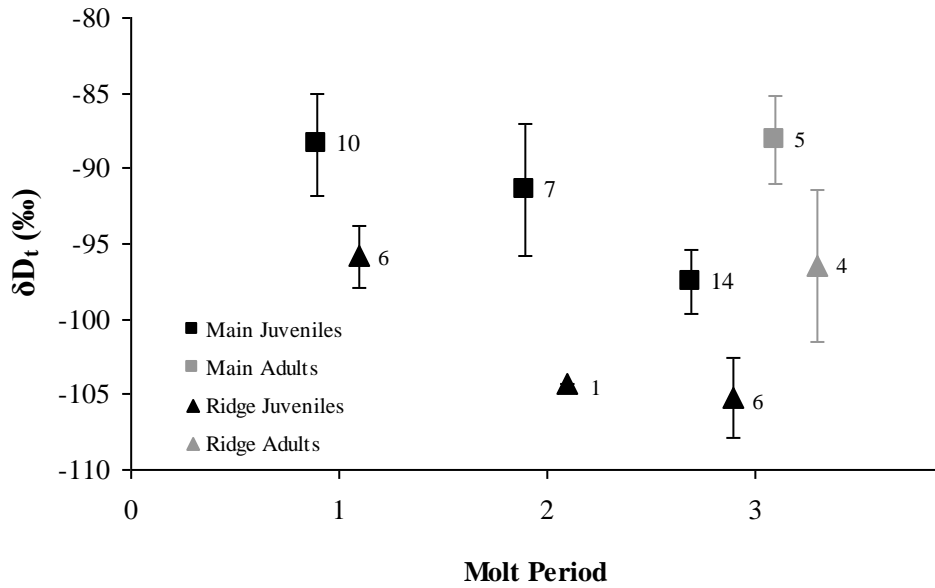


Fig. 2. δD_t (mean \pm 2 SE) in black-throated blue warbler feathers as a function of age,

molt timing, and elevation. Feather samples were collected at Hubbard Brook

Experimental Forest, New Hampshire, USA, during summer 2006. Dates for molt periods

are: 1 = 25 Jun - 11 Jul; 2 = 12 Jul - 28 Jul; 3 = 29 Jul - 14 Aug. The elevation of Main

plot ranges from 500 - 580 m, and Ridge ranges from 750 - 820 m.

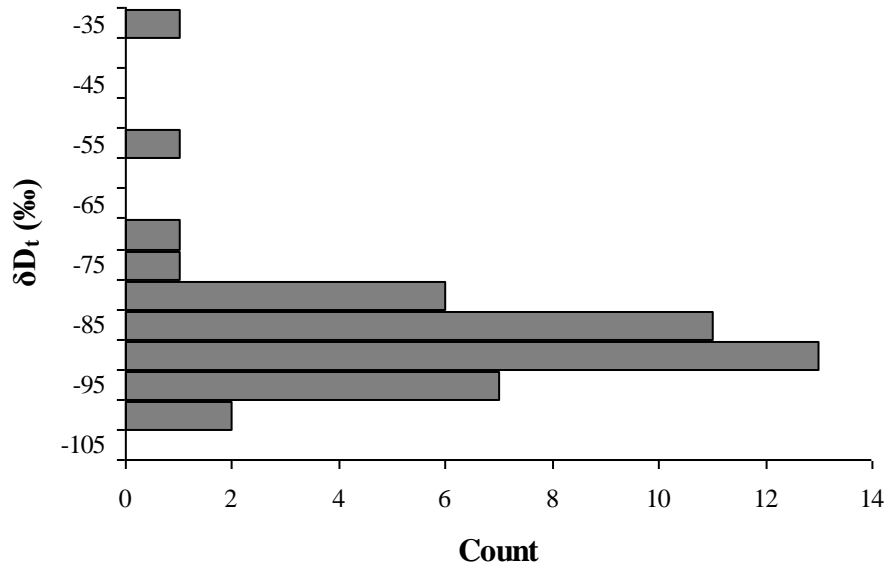


Fig. 3. δD_t values for tail feathers of returning black-throated blue warblers at Hubbard Brook Experimental Forest, New Hampshire, USA. Tail feathers were grown in 2004 and 2005, and were sampled in the subsequent summer. The more positive values of the outliers indicate molt south of the breeding site.

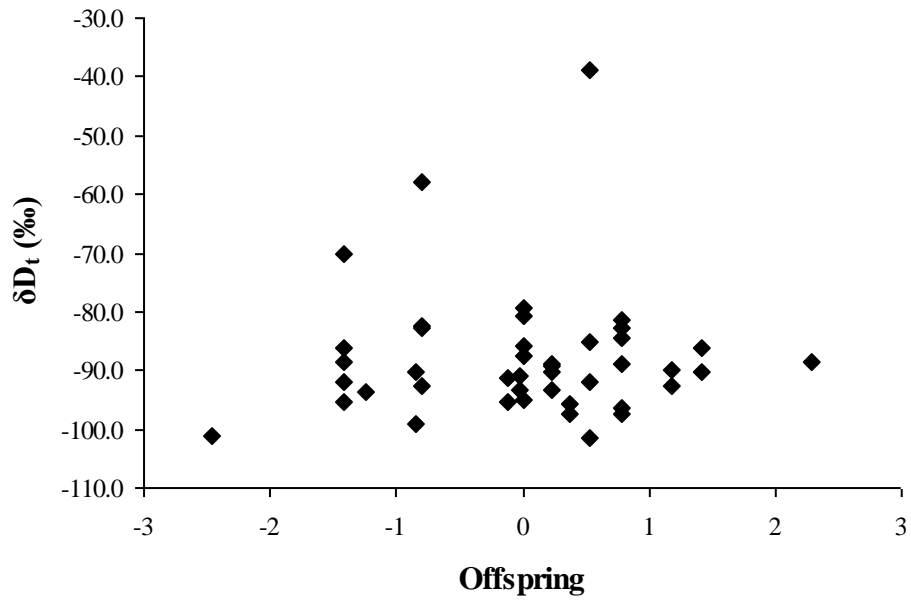


Fig. 4. δD_t values from tail feathers of returning black-throated blue warblers as a function of number of offspring fledged. Feathers were grown in 2004 and 2005, and were sampled at Hubbard Brook Experimental Forest during the subsequent breeding season. Number of offspring is from the year of feather growth, and was normalized within year.

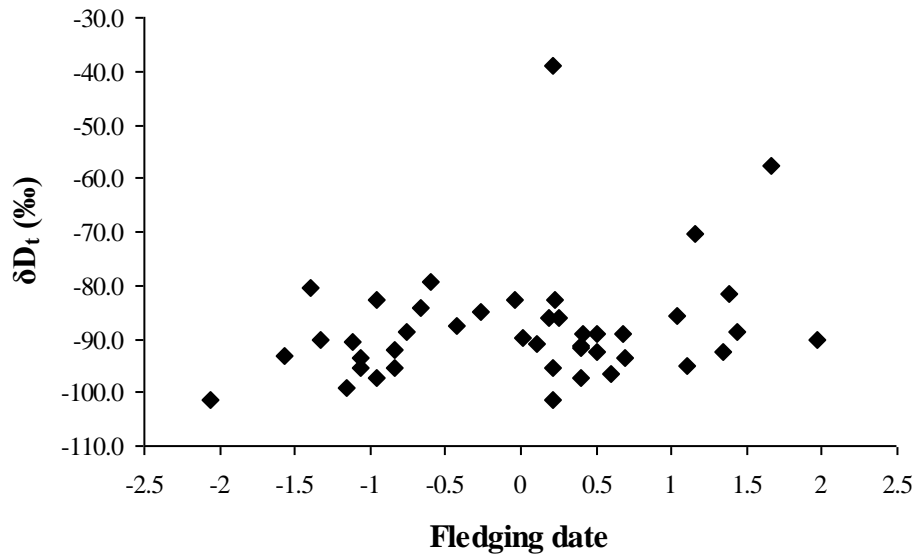


Fig. 5. δD_t in tail feathers of returning adult black-throated blue warblers as a function of fledging date. Feathers were grown in 2004 and 2005, and were sampled at Hubbard Brook Experimental Forest during the subsequent breeding season. Fledging date is from the year of feather growth, and was normalized within year. In instances when the birds fledged two broods, fledging date from their second nest was used.

CHAPTER 3: VARIATION IN WINTER HABITAT QUALITY AFFECTS BODY CONDITION AND BREEDING TERRITORY QUALITY IN A MIGRATORY SONGBIRD

ABSTRACT. -- Migratory birds are influenced by events encountered throughout their annual cycle. Until recently, ecologists have been unable track individuals throughout different stages of the annual cycle, or understand how different stages of the annual cycle may interact at the individual level. Here, using stable carbon isotope ratios in claw samples obtained from black-throated blue warblers (*Dendroica caerulescens*) during the breeding season, I demonstrate that winter habitat quality can influence important fitness correlates during the subsequent breeding season. Female black-throated blue warblers from mesic wintering sites were in better body condition during the breeding season and selected more insect-rich breeding territories relative to females from more xeric wintering sites. These results demonstrate that the benefits of high quality winter habitat are not limited to the wintering period, and that reproductive success may be influenced by events that occur hundreds of kilometers from the breeding grounds.

INTRODUCTION

Migratory birds are influenced by events throughout their annual cycle. Conditions experienced during one stage of the annual cycle may have repercussions that influence events during subsequent stages. However, for long-distance migrants, these events may be spatially segregated by thousands of kilometers. The great distances covered by these migrants has traditionally precluded investigation of how individuals

are influenced by interactions between events spanning different stages of the annual cycle (Webster et al. 2002).

Recent advances in the analysis and use of stable isotopes have enabled researchers to begin to investigate potential seasonal interactions. In particular, stable carbon isotopes have proven useful in addressing the influence of winter habitat quality on events in subsequent seasons. Carbon isotope ratios ($\delta^{13}\text{C}$) are strongly influenced by differences in plant communities, particularly by differences in the proportion of C_3 and C_4 plants at a given site (Lajtha and Marshall 1994). Because of differences in photosynthetic pathways, C_3 plants have more negative $\delta^{13}\text{C}$ values than do C_4 plants. Mesic sites, which generally have higher arthropod abundance and represent higher quality wintering habitat (Parrish and Sherry 1994, Studds and Marra 2005), are dominated by C_3 plants, while C_4 plants become increasingly common in drier sites. These differences in plant composition result in correspondingly distinct $\delta^{13}\text{C}$ values for organisms inhabiting these sites (Hobson 1999). Marra et al. (1998) used stable carbon isotopes to demonstrate that American redstarts (*Setophaga ruticilla*) from mesic, high-quality winter habitat (birds with more negative $\delta^{13}\text{C}$ values) tended to arrive on the breeding grounds earlier than individuals from more xeric winter habitat. A subsequent study with American redstarts demonstrated that the benefits of high quality winter habitat can extend throughout the breeding season, as individuals from mesic wintering sites were likely to fledge more young than individuals from xeric sites (Norris et al. 2004). Additional research with black-throated blue warblers (*Dendroica caerulescens*) indicated that winter habitat quality, as inferred through stable carbon isotopes, can

influence events during spring migration, as birds from high quality winter sites were in significantly better body condition than birds from poorer quality sites (Bearhop et al. 2004).

Despite these intriguing findings, there remain many important questions regarding the investigation of interactions between stages of the annual cycle. Seasonal interactions between the wintering and breeding periods have been demonstrated with only a single Nearctic-Neotropical migrant passerine, the American redstart (Marra et al. 1998, Norris et al. 2004). While these results may be relevant to a wider range of migrants, the applicability of this research has not yet been examined with other species. It is unclear to what degree these findings may vary based on differences in species ecology.

Here, I test whether variation in winter habitat quality of black-throated blue warblers can influence events during the subsequent breeding season. Black-throated blue warblers are Nearctic-Neotropical migrants that winter in the northern Caribbean and breed primarily in northeastern North America, and have been well studied on both the wintering and breeding grounds (Wunderle 1995, Sillett and Holmes 2002, Holmes et al. 2005). Because of the substantial knowledge regarding its wintering and breeding ecology, this species is well suited to a study of seasonal interactions. During the wintering period, individuals defend individually-based territories of differing quality, with dominant individuals (generally older birds, and males within a given age class) occupying more mesic, insect-rich territories (Wunderle 1995). These differences in winter territory quality are largely tied to moisture regime, which enables use of stable

carbon isotopes to infer winter territory quality. I used carbon isotope ratios to examine whether variation in winter habitat can influence subsequent reproductive success.

However, because of the likely influence of stochastic events between departure from the wintering grounds and successful fledging of a nest (Norris et al. 2004), I did not expect to find a direct relationship between winter habitat quality and reproductive success.

Therefore, I also examined the relationship between winter habitat quality and variables known to influence reproductive success, such as arrival timing, body condition, and breeding territory quality.

METHODS

Field methods

I conducted field work at the Hubbard Brook Experimental Forest, New Hampshire, USA from May to August 2006. Black-throated blue warblers have been studied intensively at this site since 1983 (Holmes et al. 2005). Adult birds were captured using 6 m mist nets. Males were captured using conspecific playbacks and a mounted decoy. However, females do not defend territories during the breeding season, and are thus not responsive to simulated conspecific intruders. Therefore, females can only be reliably captured during periods of nest attendance. To minimize the possibility of nest abandonment, females were not captured until after the sixth day of incubation. Nests were located and checked every 2 days. Each individual was given a unique color band combination to allow future identification. To examine body condition, birds were weighed (to the nearest 0.1 g) and measured (tarsus length and unflattened wing chord,

both to the nearest 0.1 mm). To determine body condition, I calculated the scores of a principal-component analysis (PCA) based on wing chord and tarsus measurements (Marra and Holberton 1998). PC1 scores were used to estimate body size. I then regressed body mass against PC1 scores and used the residuals as an estimate of body condition.

A 1-2 mm claw sample was taken from the hallux for $\delta^{13}\text{C}$ analysis. Bearhop et al. (2003) estimated that the outermost 1-2 mm of claw material should represent tissue synthesized 2-5 months prior to sampling. Black-throated blue warblers spend between 1 and 1.5 months on spring migration (Holmes et al. 2005). To minimize the possibility that claw samples contained tissue synthesized after departure from the wintering grounds, all birds included in this study were captured within 30 days of their arrival at Hubbard Brook.

To determine whether winter habitat quality influenced events throughout the breeding season, I measured numerous variables known to influence reproductive success. Arrival date was determined through daily surveys of the study site. Clutch initiation date was defined as the date on which the first egg was laid in the first nest of the breeding season. Number of young fledged was defined as the number of nestlings present 1-2 days prior to fledging. Fledging was confirmed by the presence of adults provisioning young outside of the nest. For a subset of birds, breeding territory quality was assessed via four biweekly counts of food abundance. During each count, 800 leaves each of American beech (*Fagus grandifolia*), hobblebush (*Viburnum alnifolium*), and striped maple (*Acer pennsylvanicum*) were visually surveyed for prey items, and the

length of each caterpillar and spider detected was recorded. Biomass of each prey item was determined using length-weight regressions (Rodenhouse and Holmes 1992).

Isotopic analysis

I conducted isotopic analysis of claws in the Environmental Stable Isotope Laboratory at the University of Vermont. Claw samples were cleaned of surface contaminants using a 2:1 chloroform:methanol solution and rinsed using deionized water, after which they were dried under a fume hood for 72 h. I then packaged claw samples in quartz tubes containing 0.6 g CuO and 0.5 g granular Cu and sealed the tubes on a vacuum line. Stable carbon isotopic analysis was conducted on CO₂ gas, produced by combustion of claw samples at 900° C for 2 h. CO₂ was extracted from the combusted samples on a gas extraction line, and analyzed for δ¹³C on a VG/Fisons SIRA series II stable isotope ratio mass spectrometer. Sample δ¹³C values are reported in per mil notation (‰) relative to the Pee Dee Belemnite standard. One laboratory standard was analyzed for every five samples, and yielded a measurement repeatability of ± 0.05‰.

Data analysis

I assessed the influence of winter habitat quality on reproductive success via a linear regression, with δ¹³C as the independent variable and number of young fledged as the response. However, as winter habitat likely influences reproductive success primarily through indirect pathways (Norris et al. 2004), I also used linear regression to test for relationships between δ¹³C and multiple factors that are known to influence reproductive success, including arrival date, clutch initiation date, body condition, and breeding

territory quality (as measured through arthropod biomass). Because of known sex-related winter habitat segregation (Wunderle 1995), I conducted separate analyses for males and females.

RESULTS

The mean claw $\delta^{13}\text{C}$ value was $-22.8 \pm 0.8\text{‰}$ (mean \pm SD; $n = 46$), and ranged from -24.4 to -21.1‰ . This closely matched the $\delta^{13}\text{C}$ values observed by Norris et al. (2004) for American redstarts sampled on the breeding grounds. The mean value observed here was slightly more negative than that observed by Bearhop et al. (2004) for black-throated blue warblers on migration, though the range of $\delta^{13}\text{C}$ values was similar.

I found no significant relationship between claw $\delta^{13}\text{C}$ values and number of young fledged (males: $r^2 = 0.05$, $p = 0.27$; females: $r^2 = 0.11$, $p = 0.20$). Likewise, there was also no apparent influence of winter habitat quality on timing of either arrival (males: $r^2 = 0.07$, $p = 0.19$; females: $r^2 = 0.07$, $p = 0.30$) or clutch initiation (males: $r^2 = 0.04$, $p = 0.35$; females: $r^2 = 0.01$, $p = 0.78$). However, females from more mesic winter habitat were in better body condition on the breeding grounds ($r^2 = 0.35$, $p = 0.01$; Fig. 1), though this relationship was not present in males ($r^2 = 0.01$, $p = 0.94$). Although the sample size was limited ($n = 6$), females from high quality winter territories also acquired higher quality breeding territories, as indicated by arthropod biomass ($r^2 = 0.75$, $p = 0.03$; Fig. 2). There were insufficient assessments of male breeding territory quality to adequately examine this relationship for males ($n = 2$).

DISCUSSION

Winter habitat quality did not appear to directly influence either reproductive success or factors involved in the timing of reproduction, including arrival date and clutch initiation date. Given the numerous stochastic events that can occur between departure from the wintering grounds and successfully fledging a nest, it is not surprising that winter habitat quality did not directly influence reproductive success. Furthermore, the lack of a significant relationship between winter habitat and timing of arrival or clutch initiation may simply be the result of the limited temporal scope of this study. Previous studies which have found a significant relationship between winter habitat quality and arrival timing noted that this relationship was not present in all years or in all age/sex classes (Marra et al. 1998, Norris et al. 2004), suggesting that stochastic events (e.g., significant storm events during migration) may overwhelm the benefit of winter habitat quality in certain years. Thorough evaluation of these relationships will require sampling over the course of multiple breeding seasons.

However, winter habitat quality did significantly influence some important fitness correlates during the subsequent breeding season. Females from more mesic winter territories were in significantly better body condition during incubation on the breeding grounds. Given the extended time period between arrival and measurement of body condition, it is possible that the relationship between winter territory quality and body condition may be mediated by intervening factors. However, Bearhop et al. (2004) found that black-throated blue warblers from more mesic winter habitats were in significantly better body condition during spring migration, demonstrating that winter

habitat quality can influence body condition during subsequent stages of the annual cycle. Thus, I suggest that body condition during the breeding season, at least in female black-throated blue warblers, can be influenced by habitat quality during the wintering period. Furthermore, while there was no relationship between winter habitat quality and body condition for males, this result might be a result of variation in capture timing for males. Whereas all females were captured and measured at similar stages of the breeding season (6-8 days after initiating incubation), males were captured more opportunistically. Some males were captured as early as two days following arrival on the breeding grounds, while others were captured 3-4 weeks after arrival. Previous research with other avian species has demonstrated that male body condition varies considerably during the first month following arrival, generally based on the demands of territory acquisition and defense, and mate guarding (Hohman 1986, Romero et al. 1997). Proper consideration of the influence of winter territory quality on male body condition would necessitate capture of all males at similar stages after arrival. Given logistical constraints during my project, this was not possible.

In addition, my analysis suggests that winter territory quality may also influence the quality of breeding territory acquired, at least for females. Females from high quality winter territories inhabited breeding territories with significantly higher prey biomass. Although females do not actively defend territories, it is likely that territory quality is a significant cue in their selection of a social mate. The cues used for mate selection have not been investigated with black-throated blue warblers, but evidence from other

songbird species suggests that females use information regarding both male quality and territory quality during mate selection (Jennions and Petrie 1997, Candolin 2003).

While I did not find any direct relationship between winter territory quality and subsequent reproductive success, when taken together with the results of previous research, my findings suggest that winter territory quality may indirectly influence reproductive output. Past research has demonstrated that body condition on the breeding grounds is an important determinant of reproductive success (Chastel et al. 1995, Smith and Moore 2003). In addition, prior studies with black-throated blue warblers have indicated that fecundity is strongly correlated with prey availability on breeding territories (Rodenhouse et al. 2003, Nagy and Holmes 2005). My results indicate that both female body condition and breeding territory quality are significantly influenced by winter habitat quality, thus suggesting mechanisms through which winter habitat quality may ultimately influence fecundity.

Previous studies have clearly demonstrated that winter habitat quality has important consequences during the breeding season for American redstarts (Marra et al. 1998, Norris et al. 2004). The present research is the first project to extend these findings to a Nearctic-Neotropical migrant other than the American redstart, suggesting that variation in tropical winter habitat quality may have important fitness consequences for a wide range of long-distance migrants. Future population models and management plans will need to consider the importance of these seasonal interactions in the population dynamics of long-distance migratory songbirds.

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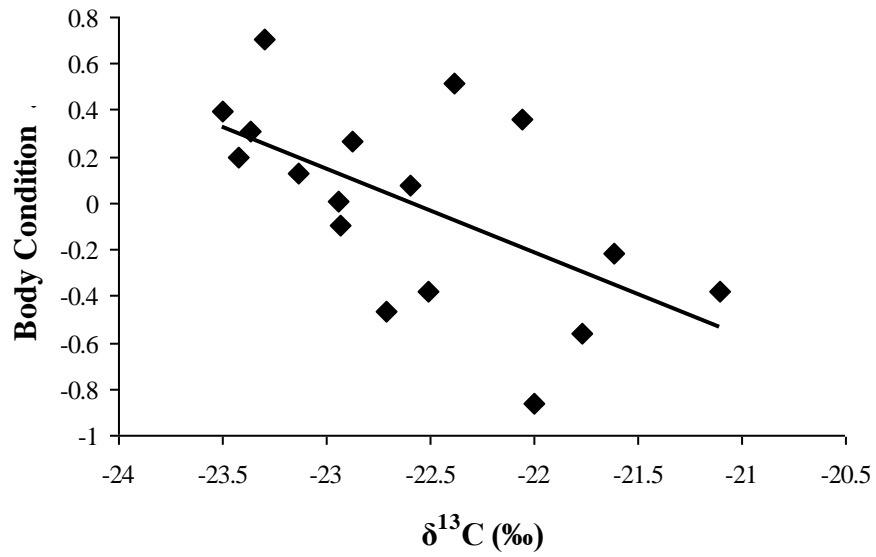


Fig. 1. Body condition of female black-throated blue warblers on the breeding grounds was significantly related to quality of wintering habitat (as inferred through $\delta^{13}\text{C}$ values; $R^2 = 0.35$, $p = 0.01$). Samples were collected at Hubbard Brook Experimental Forest, New Hampshire, USA, in June 2006.

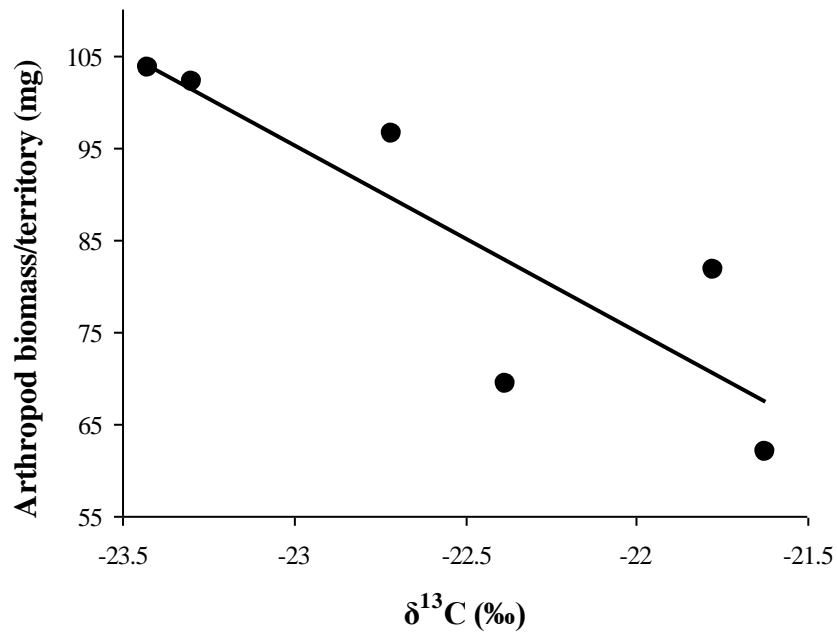


Fig. 2. Female black-throated blue warblers from more mesic wintering sites (as indicated by $\delta^{13}\text{C}$ values) inhabited breeding territories with significantly greater prey biomass ($R^2 = 0.75$, $p = 0.03$). Samples were collected at Hubbard Brook Experimental Forest, New Hampshire, USA, during summer 2006.

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