Assigning birds to geographic origin using feather hydrogen isotope ratios ($\delta^2$H): importance of year, age, and habitat

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Abstract: In North America, gradients in the ratio of stable hydrogen isotopes in amount-weighted, growing-season mean precipitation ($^2$H/$^1$H; depicted as $\delta^2$H$_{po}$) form a largely latitude-sensitive isoscape that can be used to estimate the geographical origin of animals. Feathers are metabolically inert following growth and $\delta^2$H$_{po}$ values retain information on geographical origins. However, there are important assumptions underlying this approach that can only be tested using birds of known origin. Here, we investigated sources of variation in $\delta^2$H$_{po}$ measurements from Ovenbirds (Seiurus aurocapilla (L., 1766)) associated with year, age class, feather type, season, and habitat type in New Brunswick, Canada. The observed $\delta^2$H$_{po}$ generally followed that predicted from the Global Network of Isotopes in Precipitation database. However, we found a strong year × age interaction on $\delta^2$H$_{po}$. Season, habitat type, and feather type explained only a small portion of the overall variation in $\delta^2$H$_{po}$. These results show the advantages of using annual $\delta^2$H$_{po}$ isoscapes and age-specific corrections when converting $\delta^2$H$_{po}$ to $\delta^2$H$_{f}$. We submit that the interaction effect can be modeled by accounting for interannual variation in the amount of precipitation during the breeding season. These procedures will allow for more precise estimates of the geographical origin of individual birds, especially for studies conducted near oceanic coasts, which are subject to significant annual variations in growing season $\delta^2$H$_{po}$.

Key words: Ovenbird, Seiurus aurocapilla, stable isotopes, geographic assignment, isoscapes.

Introduction

Recent studies aiming to assign the origin of individuals or populations of migratory animals have emphasized the advantages of intrinsic markers that circumvent the need for re-capturing previously marked individuals (Hobson 2011). Contaminants, parasites, pathogens, trace elements, and genetic markers have all been suggested as potential intrinsic markers (reviewed by Hobson and Norris 2008; Hobson 2008; Bensch et al. 2009; Coiffait et al. 2009; Rodríguez et al. 2011). Stable isotope measurements of animal tissues are frequently used to quantify animal movement because biogeochemical information inherent in isotope values is transferred from the base of food webs to animal tissues (Hobson...
and Wassenaar 2008). Assignment of an individual to geographic origin using this approach is based on the premise that spatial patterns of several elements (i.e., isoscapes) are known or can be modeled (West et al. 2010).

The stable isotope ratios of hydrogen ($^{2}$H:\(^{1}\)H; depicted as $^{2}$H) are particularly useful for assigning individuals to approximate location because the processes that create variance in these isotopes in the hydrosphere are relatively well understood at continental scales (Clark and Fritz 1997; Bowen et al. 2005; West et al. 2010). For many migratory bird species in North America, moult occurs on the breeding grounds (Pyle 1997) prior to migration and $^{2}$H value of feathers ($^{2}$Hf) obtained elsewhere can potentially be used to place birds on $^{2}$Hf isoscapes (e.g., Hobson et al. 2009a, 2009b). Recent advances in analytical approaches such as Bayesian or likelihood-based assignment techniques now integrate several sources of error (Wunder 2010) when determining the spatial origin of birds as a function of their isotopic signature. However, which covariates should be included in such models remains unclear for most species owing to limited studies looking at within and between individual variance in $^{2}$Hf from birds of known origin (Smith et al. 2008; Hobson 2011).

Numerous assumptions are made when using isotopes to assign birds to specific moulting locations. First, the underlying amount-weighted mean growing season $^{2}$Hf in precipitation from the long-term Global Network of Isotopes in Precipitation (GNIP; $^{2}$Hf) database (IAEA–WMO 2001) is assumed to correctly model $^{2}$H. Compared with studies conducted in coastal regions, those based on feather samples collected in central North America tend to more closely match predictions from the GNIP model (Lott et al. 2003; Wunder et al. 2005; reviewed by Hobson 2008). Although this difference may reflect the fact that studies conducted closer to oceanic coasts have mainly featured “problematic groups of birds” (i.e., shorebirds and raptors; Lott et al. 2003; Wunder et al. 2005), it seems more likely that some coastal effects on $^{2}$Hf have not been properly modeled owing to more important annual variations in $^{2}$Hf. A second assumption is that differences among individuals from the same part of a breeding range do not reflect differential timing in growth (Wassenaar and Hobson 2006; Reudink et al. 2008). Understanding the amount of within and between feather variation in individual birds is important for testing this assumption (Wassenaar and Hobson 2006; Paxton et al. 2007; Smith et al. 2008; Bortolotti 2010; but see Kelly et al. 2002 and Mazerolle et al. 2005). A third assumption is that all individuals of a species show the same isotopic signature if they grew their feathers in the same region. However, differences between adults and juveniles (i.e., hatch-year) have been shown (Meehan et al. 2003; Langin et al. 2007; Betini et al. 2009), suggesting that this assumption needs to be explicitly tested, especially when using stable isotopes for studying age-specific dispersal questions.

In this study, our objectives were to determine factors influencing intra- and inter-individual variability in $^{2}$Hf from known origin Ovenbirds (Seiurus aurocapilla (L., 1766)) in a coastal forest of eastern Canada. From 2006 to 2011, we have monitored a subpopulation of individually marked Ovenbirds in New Brunswick to study the effects of selection harvesting on demographic parameters including recruitment and site fidelity (Haché and Villard 2010). To confirm the validity of using $^{2}$Hf measurements to study recruitment and dispersal dynamics in Ovenbird populations, we tested for age (nestling vs. adult) and year (2005–2011) effects. Based on results from Langin et al. (2007) on American Redstarts (Setophaga ruticilla (L., 1758)), we predicted that nestlings will have a lower $^{2}$Hf than adults and that this effect will vary among years owing to differential departures of local climate and $^{2}$Hf from the long-term GNIP mean value (Van Wilgenburg et al. 2012). We also tested for effects of microclimate. Because single-tree selection harvesting opens the canopy, which in turn can increase mean temperatures on the forest floor (Mladenoff 1987; Vance and Nol 2003), we hypothesized that nestlings from harvested stands would experience greater evapotranspiration than those from control stands. Betini et al. (2009) found that nestling Tree Swallows (Tachycineta bicolor (Vieillot, 1808)) from sites with higher ambient temperatures had higher $^{2}$Hf values. Hence, we predicted that nestlings from harvested stands (i.e., grown under semi-open canopies) will have a higher $^{2}$Hf than nestlings from untreated (closed canopy) stands. We also hypothesized that $^{2}$Hf would vary among feathers types (here, primaries vs. rectrices) for a given individual, because in the Ovenbird, flight feathers are typically grown in the nest, whereas tail feathers are grown once the young have fledged, altering the microclimate and energetic regime under which the different feathers are grown (Betini et al. 2009). Therefore, we predicted that $^{2}$Hf from primaries will be lower than $^{2}$Hf from rectrices. Adults moult in August and September (Pyle 1997), whereas nestlings grow their feathers in June and July. Hence, if age-specific differences in $^{2}$Hf are correlated with within breeding season variation in precipitation amount, we predicted that $^{2}$Hf would decrease with increasing amount of rainfall, as expected from continental patterns (Clark and Fritz 1997). Additionally, we compared predictions from the continental model linking $^{2}$Hf with predicted $^{2}$Hf (Bowen et al. 2005; Clark et al. 2006) to the observed $^{2}$Hf in a study area located close to the Atlantic coast. This comparison aimed to determine whether $^{2}$Hf assignment predictions based on the long-term GNIP mean values are as accurate for coastal compared with more inland regions (Langin et al. 2007; Coulton et al. 2009; Van Wilgenburg et al. 2012).

**Materials and methods**

**Study area and experimental design**

The study was conducted in the Black Brook district (47°23'N, 67°40'W), a 2000 km² land base owned by J.D. Irving Ltd. located in northwestern New Brunswick, Canada (approximately 200 km from the Gulf of St. Lawrence). The region is characterized by a mosaic of relatively small management units of spruce plantations (37%) and deciduous (25%), coniferous (20%), and mixedwood (18%) forest stands. Mean patch size per stand type ranges from 16.1 to 107.0 ha (Etheridge et al. 2005, 2006).

In 2006, an experiment was designed to monitor the demographic response of the Ovenbird to single-tree selection harvesting (Haché and Villard 2010). The treatment removed 30%–40% of the basal area (cross-sectional area at breast height (1.35 m) of all trees with a diameter ≥10 cm) every 20–25 years, 20% of which is associated with the creation of
skid trails (5 m wide and 20 m apart). The remaining 10%–20% is harvested from the residual stand between skid trails. Specifically, five pairs of study plots (25 ha each) located in untreated mature deciduous stands (≥30 years since last disturbance) were used in a before–after control–impact paired design (BACI). One plot of each pair was harvested by single-tree selection harvesting during the winter between the first and the second year of the study. The distance between the most distant study plots was 40 km. We marked most territorial males each year and conducted intensive nest searching (Pérot and Villard 2009; Haché and Villard 2010). Some nest searching also was performed in 2005 in control stands. Males banded in the study area in year x + 1 were assumed to have moulted in the study area prior to fall migration (Pyle 1997). In 2008 (n = 7), 2009 (n = 21), 2010 (n = 23), and 2011(n = 14), the two 3rd rectrices were collected from returning males. When nestlings were 6–9 days old, two 6th primaries were plucked from two randomly chosen nestlings per nest. Primaries were collected in both treated plots (i.e., single-tree selection) and controls in 2005 (n = 5), 2006 (n = 10), 2008 (n = 7), 2009 (n = 19), 2010 (n = 26), and 2011 (n = 21).

In nestlings, rectrices do not grow significantly until they have fledged. To determine whether variation in δ2Hf reflects the different feather types taken from nestlings and returning males, both primaries (p1) and rectrices (r3) were collected from 49 hatch-year birds captured during the 2010 fall migration at the Lesser Slave Lake Bird Observatory (LSLBO; 55°20′N, 114°40′W), Alberta, Canada. Prior to fall migration (prebasic moult), hatch-year birds replace their coverts, body feathers, and rarely tertials, whereas the flight feathers and rectrices are only replaced after their first breeding season (Pyle 1997).

Stable isotope analysis

All feathers were cleaned using 2:1 chloroform:methanol solution to remove surface oils. Samples were prepared according to Wassenaar (2008) and analysed at the Stable Isotope Hydrology and Ecology Laboratory of Environment Canada (2005–2010) and the Colorado Plateau Stable Isotope Laboratory (2011). An H2 pulse generated from high-temperature (1350 °C) flash pyrolysis for each sample (350 ± 20 μg) was used to obtain stable hydrogen measurements by continuous-flow isotope-ratio mass spectrometry (CFIRMS). To account for exchangeable hydrogen in keratins, we used comparative equilibration with in-house keratin working standards (BW: ~108‰, CFS: ~147.7‰, CHS: ~187‰), for which the δ2H value of nonexchangeable H had been previously established (Wassenaar and Hobson 2003). Both laboratories used the same standards and analyzed samples the same way. All results are expressed as nonexchangeable deuterium isotope ratio (δ2Hf) in units of per mil (‰) and normalized according to the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW–SLAP) standard scale. Based on within run analyses of keratin standards, we assume measurement error to be approximately ±2‰.

Statistical analyses

Interpolated amount-weighted mean δ2Hf for the growing season for our study area estimated by the GNIP model (Bowen et al. 2005; available from http://www.waterisotopes.org, accessed 10 August 2011) were converted to a predicted range of δ2Hf using the calibration equation for songbirds suggested by Clark et al. (2006, 2009). To test for the effects of age, year, and age × year interaction on δ2Hf, we used a two-way ANOVA. A generalized linear mixed model was used to determine whether δ2Hf from nestlings was significantly influenced by year, treatment, and year × treatment interaction (fixed effects) while controlling for the nested experimental design (i.e., nest(plot) and plot(pair of plots); random effects). This analysis only included values for nesting samples collected in 2009–2011, owing to low sample sizes in treated plots during previous years. Returning males could not be assigned to a given study plot, as they often move substantially during the postfledging period (S. Haché, M.-A. Villard, and E.M. Bayne, unpublished data). Hence, because individuals may grow their feathers in different habitat types than those where they held a territory, the effect of harvest treatment on δ2Hf from returning males could not be tested and all individuals were considered to be a random sample from our study area to test for a year effect (one-way ANOVA). We tested for a within individual difference in δ2Hf by comparing mean values from primaries and rectrices collected from hatch-year birds using a paired t test. An ANCOVA was used to determine whether δ2Hf was influenced by the Julian date at which nestling primaries were collected, and whether the observed pattern changed among breeding seasons (2009–2011). A similar analysis could not be performed for returning males because their moulting dates could not be determined. Lastly, linear regressions were performed to investigate potential causal relationships between δ2Hf in nestlings and returning individuals and the amount of precipitation in months prior to feather growth or moult. Hence, different combinations of the total amount of precipitation (mm) in May and June were considered for nestlings (three models), whereas combinations of monthly precipitation values during the breeding season (May–July; six models) in the previous year (hereafter, year x − 1) were considered for returning individuals. Precipitation data for our study area (St-Leonard weather station) were obtained from Environment Canada (http://wwwclimate.weatheroffice.gc.ca, accessed 20 November 2011).

Results

In our study area, the estimated growing season mean δ2Hf ranged from −62.4‰ to −64.8‰ (Bowen et al. 2005; note that three 20 min × 20 min grids overlapped our study area, each with a δ2Hf value). These values calibrated in δ2Hf corresponded to a range of −80.8‰ to −82.9‰, close to the mean δ2Hf of −86.2‰ ± 12.6‰ (mean ± SD; n = 153; range: −45.5‰ to −111.9‰) measured from the feather samples. Age significantly affected δ2Hf as shown by a significant age × year interaction (F[3,143] = 7.51, p < 0.001; Figs. 1a, 1b). The most important departures from the predicted long-term mean δ2Hf were observed in 2005 for nestlings (−65.5‰) and in 2010 for returning males (−67.6‰; Fig. 1b). When analyses were conducted by age class, a significant year effect was observed in δ2Hf for both returning males (F[3,61] = 61.68, p < 0.001) and nestlings (F[2,52] = 46.56, p < 0.001; Figs. 1a, 1b). Furthermore, there was no significant difference between the δ2Hf of nestlings in treated
plots (−94.6‰ ± 7.0‰) and controls (−93.3‰ ± 8.8‰; $F_{[2,52]} = 2.35, p = 0.132$). Note that the latter analysis was performed using only data from 2009, 2010, and 2011. There was a significant variation in $\delta^{2}H_f$ within breeding season for nestlings ($F_{[1,65]} = 4.20, p = 0.045$). However, only 10% ($R^2$) of this variation was explained by Julian date (Fig. 2). Finally, mean $\delta^{2}H_f$ varied slightly with feather type (rectrices: −134.0‰ ± 7.6‰; primaries: −135.9‰ ± 8.3‰) plucked from the same hatch-year birds captured in Alberta during fall migration ($t_{[48]} = -2.42, p = 0.020$). In nestlings, a linear model including the annual variation in the total amount of precipitation in May and June was the best predictor of the negative relationship between yearly variation of $\delta^{2}H_f$ ($F_{[1,3]} = 5.80, R^2 = 0.59, p = 0.074$; Fig. 3a). The second-best model (total amount of precipitation in June) explained 19% less of the observed variation. For returning birds, the only model showing the predicted negative relationship was that which included the amount of precipitation in June in year $x - 1$ ($F_{[1,3]} = 2.90, R^2 = 0.59, p = 0.231$; Fig. 3b).
Discussion

We found an age effect on δ²H in the Ovenbird that varied significantly among years (age × year interaction), whereas only a small fraction of the overall variation was explained by habitat type, feather type, and Julian date. Most years, δ²H values from nestlings were lower than those from returning males (19.0‰, 15.9‰, and 10.9‰ lower for 2009, 2010, and 2011, respectively). In 2008, δ²H was similar for both age classes, though it should be noted that sample sizes were small that year. Hence, researchers should account not only for age class but also for a year-specific age effect when using δ²H to assign the origin of individuals. This finding has especially important implications for studies assigning geographic origins of birds using mean δ²H from long-term databases.

Several nonexclusive hypotheses related to evaporative water loss in individuals can be proposed to explain the observed age-specific differences in δ²H. Body water lost through evaporation is depleted in deuterium relative to basal body water and this can result in progressive enrichment of the body H pool (Wolf and Walsberg 1996; McKechnie et al. 2004). Adults may have a greater metabolic rate during feather growth than juveniles and this may result in higher δ²H in adults owing to increased evaporative water loss. This hypothesis is consistent with the observed difference between songbirds and raptor species. In raptors, moult and breeding activities coincide, which would result in a disproportionately high metabolic rate during feather growth owing to increased work during that period (Langin et al. 2007).

Within songbirds, differences in nestling–adult δ²H values among species could be due to the different levels of heat stress to which each age group is typically exposed (McKechnie et al. 2004). The American Redstart is an open-cup nester and the conditions experienced by nestlings and adults may be closer than in the Ovenbird, which builds a domed nest on the ground that may provide a more stable microclimate than that experienced by adults. Feather growth rate can also differ among individuals of different ages (Bortolotti 1984), and Holmes 1996; Stodola et al. 2010). Lastly, differences in hydrogen budgets of individuals. We would be hesitant, for example, to generalize our results away from coastal regions. Our findings suggest that
coastal effects on $\delta^2$H can be modeled for our focal species based on rainfall amount. Similar studies simultaneously testing assumptions underlying the stable isotope approach ($\delta^2$H$_2$) for “problematic species” (e.g., shorebirds and raptors) are important to shed some light on the relevance of this method for these species (Hobson 2011). Other authors have called for laboratory experiments to better understand the physiological ecology of focal species and to identify proximate causes of variation in $\delta^2$H$_2$ (Hobson et al. 1999; Martínez del Río et al. 2009; Hobson 2011). This would likely further improve the spatial resolution of $\delta^2$H$_2$ and increase our ability to assign geographical origins.

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**References**


