

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

S. Haché, K.A. Hobson, M.-A. Villard, and E.M. Bayne

Abstract: In North America, gradients in the ratio of stable hydrogen isotopes in amount-weighted, growing-season mean precipitation ($^2\text{H}:^1\text{H}$; depicted as $\delta^2\text{H}_p$) form a largely latitude-sensitive isoscape that can be used to estimate the geographical origin of animals. Feathers are metabolically inert following growth and $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ measurements from Ovenbirds (*Seiurus aurocapilla* (L., 1766)) associated with year, age class, feather type, season, and habitat type in New Brunswick, Canada. The observed $\delta^2\text{H}_f$ generally followed that predicted from the Global Network of Isotopes in Precipitation database. However, we found a strong year \times age interaction on $\delta^2\text{H}_f$. Season, habitat type, and feather type explained only a small portion of the overall variation in $\delta^2\text{H}_f$. These results show the advantages of using annual $\delta^2\text{H}_p$ isoscapes and age-specific corrections when converting $\delta^2\text{H}_p$ to $\delta^2\text{H}_f$. 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 $\delta^2\text{H}_p$.

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

Résumé : En Amérique du Nord, les gradients du rapport d'isotopes stables d'hydrogène en fonction des moyennes pondérées de la quantité de précipitations durant la période de croissance ($^2\text{H}:^1\text{H}$; dénoté $\delta^2\text{H}_p$) constituent un paysage isotopique principalement sensible à la latitude qui peut être utilisé pour estimer l'origine géographique des animaux. Après leur croissance, les plumes sont métaboliquement inertes, et leurs valeurs de $\delta^2\text{H}_p$ procurent de l'information sur l'origine géographique. Cela dit, d'importants postulats qui sous-tendent cette approche ne peuvent être vérifiés qu'avec des oiseaux dont l'origine est connue. Nous avons étudié les sources de variabilité des mesures du rapport isotopique de plumes ($\delta^2\text{H}_f$) de parulines couronnées (*Seiurus aurocapilla* (L., 1766)) liées à l'année, la classe d'âge, le type de plumes, la saison et le type d'habitat, au Nouveau-Brunswick, Canada. Les valeurs observées de $\delta^2\text{H}_f$ coïncident généralement avec les prédictions produites à partir des données du Réseau mondial de mesure des isotopes dans les précipitations, mais nous avons toutefois relevé une forte interaction année \times âge sur les valeurs de $\delta^2\text{H}_f$. La saison, le type d'habitat et le type de plumes n'expliquent qu'une petite partie de la variabilité totale de $\delta^2\text{H}_f$. Ces résultats démontrent l'importance d'utiliser des paysages isotopiques annuels de $\delta^2\text{H}_p$ et des corrections en fonction de l'âge pour la conversion de $\delta^2\text{H}_p$ en $\delta^2\text{H}_f$. Nous arguons que l'effet d'interaction peut être modélisé en tenant compte de la variabilité interannuelle de la quantité de précipitations durant la période de reproduction. Ces procédures permettront des estimations plus précises de l'origine géographique d'individus, notamment pour les études menées près des côtes océaniques, des régions exposées à des variations annuelles significatives de $\delta^2\text{H}_p$ durant la période de croissance.

Mots-clés : paruline couronnée, *Seiurus aurocapilla*, isotopes stables, assignation géographique, paysages isotopiques.

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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 recapturing previously marked individuals (Hobson 2011). Contaminants, parasites, pathogens, trace elements, and ge-

netic 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

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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 ($^2\text{H}:^1\text{H}$; depicted as $\delta^2\text{H}$) 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, moulting occurs on the breeding grounds (Pyle 1997) prior to migration and $\delta^2\text{H}$ value of feathers ($\delta^2\text{H}_f$) obtained elsewhere can potentially be used to place birds on $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}$ in precipitation from the long-term Global Network of Isotopes in Precipitation (GNIP; $\delta^2\text{H}_p$) database (IAEA–WMO 2001) is assumed to correctly model $\delta^2\text{H}$. 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 $\delta^2\text{H}_p$ have not been properly modeled owing to more important annual variations in $\delta^2\text{H}_p$. 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ than adults and that this effect will vary among years owing to differential departures of local climate and $\delta^2\text{H}_p$ 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 $\delta^2\text{H}_f$ values. Hence, we predicted that nestlings from harvested stands (i.e., grown under semi-open canopies) will have a higher $\delta^2\text{H}_f$ than nestlings from untreated (closed canopy) stands. We also hypothesized that $\delta^2\text{H}_f$ would vary among feather 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 $\delta^2\text{H}_f$ from primaries will be lower than $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ are correlated with within breeding season variation in precipitation amount, we predicted that $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ with predicted $\delta^2\text{H}_p$ (Bowen et al. 2005; Clark et al. 2006) to the observed $\delta^2\text{H}_f$ in a study area located close to the Atlantic coast. This comparison aimed to determine whether $\delta^2\text{H}_f$ 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 (BACIP). 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 that returned 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 $\delta^2\text{H}_f$ 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; $55^\circ 20' \text{N}$, $114^\circ 40' \text{W}$), 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 H_2 pulse generated from high-temperature (1350°C) flash pyrolysis for each sample ($350 \pm 20 \mu\text{g}$) 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 (BWB (-108‰), CFS (-147.7‰), CHS (-187‰)), for which the $\delta^2\text{H}$ 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 isotopic ratio ($\delta^2\text{H}_f$) 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 $\pm 2\text{‰}$.

Statistical analyses

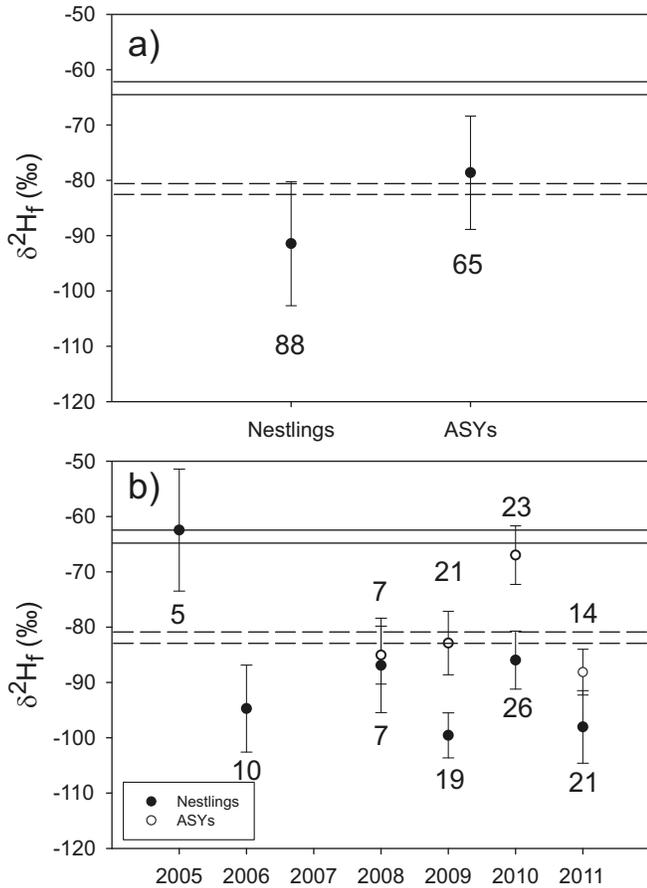
Interpolated amount-weighted mean $\delta^2\text{H}_p$ for the growing season for our study area estimated by the GNIP model (Bo-

wen et al. 2005; available from <http://www.waterisotopes.org>, accessed 10 August 2011) were converted to a predicted range of $\delta^2\text{H}_f$ using the calibration equation for songbirds suggested by Clark et al. (2006, 2009). To test for the effects of age, year, and age \times year interaction on $\delta^2\text{H}_f$, we used a two-way ANOVA. A generalized linear mixed model was used to determine whether $\delta^2\text{H}_f$ from nestlings was significantly influenced by year, treatment, and year \times 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 nestling 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ 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://www.climate.weatheroffice.gc.ca>, accessed 20 November 2011).

Results

In our study area, the estimated growing season mean $\delta^2\text{H}_p$ ranged from -62.4‰ to -64.8‰ (Bowen et al. 2005; note that three $20 \text{ min} \times 20 \text{ min}$ grids overlapped our study area, each with a $\delta^2\text{H}_p$ value). These values calibrated in $\delta^2\text{H}_f$ corresponded to a range of -80.8‰ to -82.9‰ , close to the mean $\delta^2\text{H}_f$ of $-86.2\text{‰} \pm 12.6\text{‰}$ (mean \pm SD; $n = 153$; range: -45.5‰ to -111.9‰) measured from the feather samples. Age significantly influenced $\delta^2\text{H}_f$ as shown by a significant age \times year interaction ($F_{[3,143]} = 7.51$, $p < 0.001$; Figs. 1a, 1b). The most important departures from the predicted long-term mean $\delta^2\text{H}_f$ were observed in 2005 for nestlings (-65.5‰) and in 2010 for returning males (-67.0‰ ; Fig. 1b). When analyses were conducted by age class, a significant year effect was observed in $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ of nestlings in treated

Fig. 1. Differences in $\delta^2\text{H}_f$ (‰; mean \pm SD and sample size) (a) between after second year (ASY) males and nestling Ovenbirds (*Seiurus aurocapilla*) and (b) between ASY males and nestlings in different years. In each figure, solid horizontal lines represent the range of amount-weighted mean $\delta^2\text{H}_p$ for the growing season of the study area and the broken lines correspond to $\delta^2\text{H}_f$ values corrected using the calibration equation suggested by Clark et al. (2006). All feathers were collected in the Black Brook district, New Brunswick, Canada. Note that the mean values in panel a are not from a normal distribution.



plots ($-94.6\text{‰} \pm 7.0\text{‰}$) and controls ($-93.3\text{‰} \pm 8.8\text{‰}$; $F_{[2,52]} = 2.35$, $p = 0.132$). Note that the latter analysis was performed using only data from 2009, 2010, and 2011. There was a significant variation in $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ varied slightly with feather type (rectrices: $-134.0\text{‰} \pm 7.6\text{‰}$; primaries: $-135.9\text{‰} \pm 8.3\text{‰}$) 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 $\delta^2\text{H}_f$ ($F_{[1,5]} = 5.80$, $R^2 = 0.59$, $p = 0.074$; 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).

Fig. 2. Relationship between $\delta^2\text{H}_f$ in primaries from nestling Ovenbirds (*Seiurus aurocapilla*) and Julian date at which feathers were collected for two breeding seasons. All feathers were collected in the Black Brook district, New Brunswick, Canada.

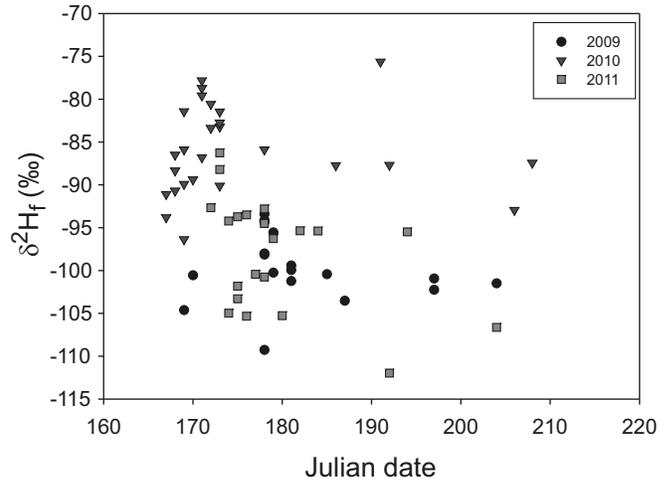
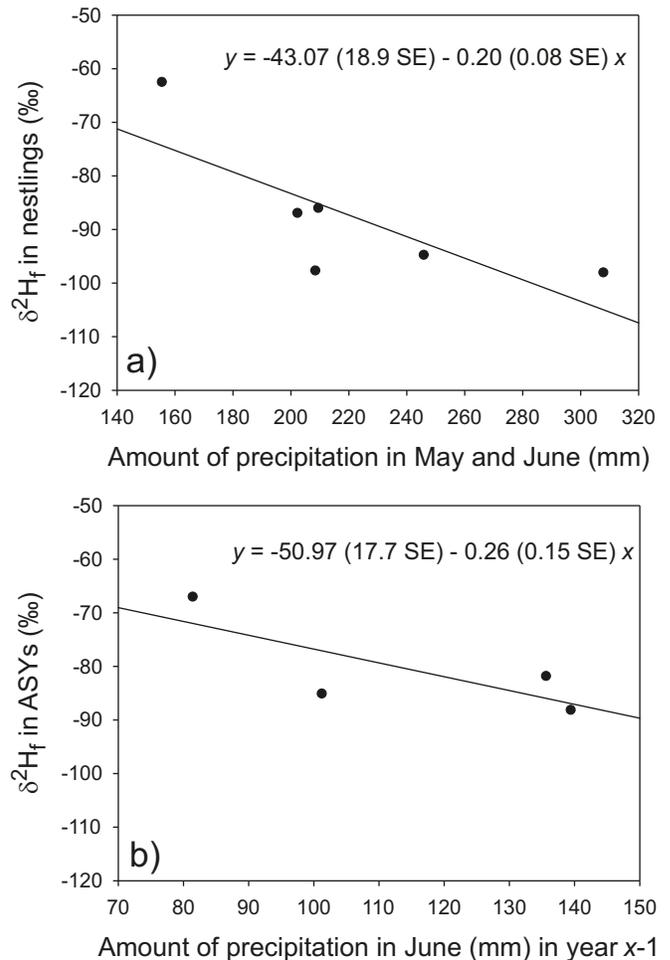


Fig. 3. Linear relationships (a) between the annual variation of $\delta^2\text{H}_f$ in nestling Ovenbirds (*Seiurus aurocapilla*) and the amount of precipitation in our study area and (b) between the annual variation of $\delta^2\text{H}_f$ in after second year (ASY) males and the amount of precipitation in our study area during the previous year.



Discussion

We found an age effect on $\delta^2\text{H}_f$ in the Ovenbird that varied significantly among years (age \times year interaction), whereas only a small fraction of the overall variation was explained by habitat type, feather type, and Julian date. Most years, $\delta^2\text{H}_f$ values from nestlings were lower than those from returning males (19.0%, 15.9%, and 10% lower for 2009, 2010, and 2011, respectively). In 2008, $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ to assign the origin of individuals. This finding has especially important implications for studies assigning geographic origins of birds using mean $\delta^2\text{H}_p$ 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 $\delta^2\text{H}_f$. 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 $\delta^2\text{H}_f$ 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 $\delta^2\text{H}_f$ 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), which might result in different $\delta^2\text{H}_f$ irrespective of levels of physical activity or microenvironment because isotopic compositions are dictated by rate-limiting processes. The observed discrepancy in $\delta^2\text{H}_f$ between adults and nestlings may also reflect differences in hydrogen budgets of individuals. Langin et al. (2007) attributed the differences in $\delta^2\text{H}_f$ they found between adult and nestling American Redstarts to the fact that adults ingest drinking water, whereas nestlings acquire water through soft food items (e.g., Lepidoptera larvae in insectivorous birds; Omland and Sherry 1994; Goodbred and Holmes 1996; Stodola et al. 2010). Lastly, differences in $\delta^2\text{H}_f$ between feathers from juveniles and adults might be expected in species using a capital breeding strategy, whereby stored nutrients that differ isotopically from those available in the local environment are transferred to offspring and incorporated into their feathers (Klaassen 2003). This explanation is unlikely to apply here, given that songbirds are income breeders (Langin et al. 2006).

On average, nestlings that grew their primaries in study plots treated by single-tree selection harvesting had slightly lower $\delta^2\text{H}_f$ (2.5%) than those hatched in control plots. However, given that the measurement error of $\delta^2\text{H}_f$ analyses is of

the order of 2%, we cannot make strong inferences from this result. The dome-shaped nest of the Ovenbird may have reduced some of the effects of abiotic factors such as heat stress, resulting in a substantially lower effect on $\delta^2\text{H}_f$ than that observed in an experimental manipulation of nest-box temperature on nestling Tree Swallows (Betini et al. 2009). Experimental manipulations of ambient temperature may be required to determine the causes of differences between adult and nestling $\delta^2\text{H}_f$ values in open-cup nesting species.

The difference in $\delta^2\text{H}_f$ between the 3rd rectrix and the 1st primary of hatch-year birds captured during fall migration (1.9%) also fell within measurement error. This suggests that all flight feathers were grown at the same location and could potentially be used to assign birds to geographic regions. This result contrasts with the extensive within feather variation in $\delta^2\text{H}_f$ reported in some raptors (Smith et al. 2009).

Most of the variation in $\delta^2\text{H}_f$ in New Brunswick Ovenbirds reflected year and age effects. By generating mean $\delta^2\text{H}_f$ values for both age classes in different years with relatively small variations, we were able to account for the large range in the observed values (66.5%). Interestingly, the maximum departure from calibrated $\delta^2\text{H}_p$ values predicted from the GNP data set for both age classes reported in this study occurred in different years. Nestlings grow most of their feathers in July, whereas adults are thought to undergo a prebasic moult in August or September (Pyle 1997). This time lag in feather growth and variable year effects between age classes challenged our understanding of how year-specific weather patterns influence $\delta^2\text{H}_f$ (Smith et al. 1979; Hobson 2011). It is also interesting to note that the annual variation in the amount of precipitation in May and June was correlated with variations in $\delta^2\text{H}_f$ of nestlings. A similar pattern was observed between the amount of precipitation in June in year $x - 1$ and $\delta^2\text{H}_f$ from returning males. These correlations are consistent with an effect of amount of precipitation within and among breeding seasons on $\delta^2\text{H}_f$, as well as with the observed age \times year interaction. However, the limited number of years available prevented us from making strong inferences regarding a potential causal relationship, especially for returning males.

This study, along with that of Langin et al. (2007), suggests that much of the variation in $\delta^2\text{H}_f$ in songbirds of known origin and, potentially, in other species groups having a similar moulting pattern (e.g., waterfowl; Coulton et al. 2009) can be accounted for by modeling year- and age-specific isoscapes (e.g., Van Wilgenburg et al. 2012). Year-specific isoscapes will soon become available through a new Web-based tool, IsoMap (available from <http://isomap.stanford.edu/>, accessed 5 February 2012). Extensions of such tool could also allow accounting explicitly for other factors (e.g., age or feather type) that would reduce the number of assumptions that are currently underlying the use of stable hydrogen isotopes to estimate animal movements, presumably increasing assignment accuracy.

Future work should focus on investigating the cause of the year \times age interaction observed in this study to determine the generality of our findings and to account for the remaining unexplained variation in $\delta^2\text{H}_f$. Without such information, this year \times age interaction cannot be included in predictive models. We would be hesitant, for example, to generalize our results away from coastal regions. Our findings suggest that

coastal effects on $\delta^2\text{H}_f$ can be modeled for our focal species based on rainfall amount. Similar studies simultaneously testing assumptions underlying the stable isotope approach ($\delta^2\text{H}_f$) 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 $\delta^2\text{H}_f$ (Hobson et al. 1999; Martínez del Río et al. 2009; Hobson 2011). This would likely further improve the spatial resolution of $\delta^2\text{H}_f$ and increase our ability to assign geographical origins.

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