



# VCU

Virginia Commonwealth University  
VCU Scholars Compass

---

Theses and Dissertations

Graduate School

---

2017

## A Range-wide Assessment of Migratory Connectivity for the Prothonotary Warbler Using Stable Hydrogen Isotopes

Jessica Reese  
*Virginia Commonwealth University*

Follow this and additional works at: <https://scholarscompass.vcu.edu/etd>



Part of the [Ecology and Evolutionary Biology Commons](#)

© The Author

---

Downloaded from

<https://scholarscompass.vcu.edu/etd/4871>

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact [libcompass@vcu.edu](mailto:libcompass@vcu.edu).

**A range-wide assessment of migratory connectivity for the  
prothonotary warbler using stable hydrogen isotopes**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science  
at Virginia Commonwealth University

By

Jessie Reese  
Bachelor of Science, James Madison University, 2009

Advised by

Lesley Bulluck, Ph.D.  
Department of Biology, Center for Environmental Studies  
Virginia Commonwealth University

Virginia Commonwealth University  
Richmond, VA  
April, 2017

## Table of contents

Acknowledgement.....	i
List of tables.....	ii
Chapter 1.....	ii
Chapter 2.....	ii
List of figures.....	iii
Chapter 1.....	iii
Chapter 2.....	iii
Abstract.....	1
Chapter 1.....	3
Introduction.....	3
Methods.....	6
Results.....	8
Discussion.....	9
Literature Cited.....	16
Tables and Figures.....	20
Chapter 2.....	23
Introduction.....	23
Methods.....	27
Results.....	33
Discussion.....	35
Literature Cited.....	44
Tables and Figures.....	49
Vita.....	57

## Acknowledgement

First and foremost, I wish to thank my advisor, Lesley Bulluck, who dedicated herself to this project and to my development as a scientist with unwavering positivity and enthusiastic support. I am humbled to be mentored by someone whose energy and generosity I hope to emulate as scientist, conservationist, and person. I wish to thank all of my committee members, and especially Cathy Viverette, Rodney Dyer, and Chris Tonra. Cathy's positive attitude encouraged me through the (many) roadblocks, and I thank her for teaching me how to fundraise. I thank Rodney for making me an R wizard (in training), and for teaching me to think spatially. I am grateful to Chris Tonra for all his guidance, including help with study design and analysis, for facilitating lab work, and for always suggesting a creative solution when problems arose. I wish to thank Gabe Bowen and all the lecturers at the SPATIAL short course at the University of Utah who taught me so much about isotopes and large-scale spatial data analysis. I thank the American Association of University Women, who provided financial support which allowed me to attend conferences and the SPATIAL course. I thank Nick Bayly for inviting me to do fieldwork in Colombia, and Angela Caguazano and Alessandro Molina for their help in the field. I thank all my former fieldwork supervisors who shaped my development as an avian field biologist, especially Erin Rowan, Sarah Stock, Dan Albrecht-Mallinger, and Jenny Smetzer. I wish to thank all my collaborators in the Prothonotary Warbler Working Group for their camaraderie. I thank all past and present members of the Bulluck lab, who I feel lucky to know and who are a testament to the culture Lesley promotes in her work, and I especially thank Dan Albrecht-Mallinger, Nik Moy, Matt DeSaix, and Liz Schold for their friendship. I thank Josephine Lush for her listening ear and her gracious friendship. Finally, I thank my parents for their support and encouragement.

## List of Tables

### Chapter 1

Table 1: Comparison of the effect of age, sex, year, and feather type on stable hydrogen isotope values in feathers from the literature

### Chapter 2

Table 1: Measured and calibrated stable hydrogen isotope values for prothonotary warbler feathers collected during the breeding season

Table 2: Accuracy and precision estimates for spatially-explicit assignment to origin for known-origin feather samples

## List of figures

### Chapter 1

Figure 1: Comparison of feather stable hydrogen values between primary and rectrix feathers

Figure 2: Variation in feather stable hydrogen values by age and year

### Chapter 2

Figure 1: Range map of the prothonotary warbler with feather sampling locations

Figure 2: Median feather hydrogen isotope values by sampling location for the breeding and nonbreeding season

Figure 3: The relationship between stable hydrogen values measured in precipitation and feathers

Figure 4: Relative abundance maps for prothonotary warblers based on data from the Breeding Bird Survey or from spatio-temporal exploratory models

Figure 5: Maps of predicted probability of origin for known-origin feather samples from Louisiana, South Carolina, Virginia, and Ohio for assignments with and without prior information

Figure 6: Maps of predicted probability of origin for feather samples from Central America, western Colombia, eastern Colombia, and the Caribbean islands for assignments with and without prior information

## Abstract

### **A range-wide assessment of migratory connectivity for the prothonotary warbler using stable hydrogen isotopes**

by Jessie Reese, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science  
at Virginia Commonwealth University

Virginia Commonwealth University, 2017

Advisor:

Lesley Bulluck, Ph.D., Department of Biology, Center for Environmental Studies

Populations of many species of migratory birds are declining, and an understanding of how populations are linked between the breeding and nonbreeding grounds is necessary in order to determine drivers of declines. While all current tracking technologies to study the movements of small songbirds are limited by their coarse resolution, tracking birds by measuring stable isotopes in keratin offers an advantage because it requires only a single capture and is cost-effective, which leads to robust sample sizes. While this tracking method is accurate, stable hydrogen isotope values measured in feathers ( $\delta^2\text{H}_f$ ) are known to be variable within a site, and may be influenced by the hydrology of a site. In this study, we assessed sources of variation in  $\delta^2\text{H}_f$  values in a wetland-associated Neotropical migratory bird, the prothonotary warbler (*Protonotaria citrea*), by comparing  $\delta^2\text{H}_f$  values among ages, sexes, years and feather type. We found that age and year significantly influence  $\delta^2\text{H}_f$  values, and that differences between primaries and rectrices are statistically significant but generally small. We also tested the accuracy and precision of models to assign prothonotary warblers to their breeding origin using known-origin feathers. We assigned birds in a spatially-explicit manner using an interpolated surface of stable hydrogen values measured in precipitation ( $\delta^2\text{H}_p$ ), and developed a species-

specific calibration equation to account for the offset between  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$ , which explained 51% of the variation in  $\delta^2\text{H}_f$  values. We incorporated breeding bird abundance as prior information in our assignment model, and compared two sources of abundance data: the Breeding Bird Survey and a spatio-temporal exploratory model developed with eBird data. The assignment model with no prior information was accurate (83% of birds correctly assigned to their true location of origin), but imprecise (50% of grid cells assigned as likely locations of origin). Incorporating abundance as prior information led to a decrease in accuracy (9-14% of birds correctly assigned) but higher precision (1% of grid cells assigned as likely). We also assigned prothonotary warblers to their breeding origin using feathers collected from across the nonbreeding range. We found that all nonbreeding sampling locations contained a mixture of birds whose origins spanned the majority of the breeding range, with no evidence of strong connectivity between the seasons. In the absence of strong connectivity, the influence of events occurring at one nonbreeding location will be widespread and diffuse across the breeding range. For prothonotary warblers, understanding connectivity can help conservation planners understand how nonbreeding season habitat destruction and other processes influence population dynamics. We recommend that future studies account for age and year variation in  $\delta^2\text{H}_f$  values when possible, and continue to examine the trade-off between precision gained and accuracy lost when using abundance as prior information.



## Chapter 1

The influence of age, sex, year, and feather type on hydrogen isotope variation in a wetland-associated songbird

### Introduction

Migratory animals can be tracked using intrinsic markers such as stable isotopes, genetic markers, or trace elements (Webster et al. 2002). Measuring ratios of stable isotopes in animal tissues has been useful for provenance studies because isotope ratios can exhibit predictable geographic trends, and can be measured from a single capture (Hobson 1999). The isotope ratio of the local environment is assimilated into animal tissue, and for keratinous tissues such as hair or feathers, the ratio doesn't change for the life of the tissue (i.e., until it is regrown or molted). The location of feather growth is known for many species of migratory birds, and feathers are often retained for an entire migration cycle (Pyle 1997). Stable isotopes, especially stable hydrogen, have been used widely to assign migratory birds captured on the nonbreeding grounds to their location of feather growth on the breeding grounds. Stable hydrogen isotopes are useful because patterns of atmospheric circulation and temperature create a strong latitudinal gradient of stable hydrogen values in precipitation ( $\delta^2\text{H}_p$ ) across North America (Hobson and Wassenaar 2008). Fractionation occurring between phase changes such as evaporation and condensation depletes precipitation of the heavy isotope of hydrogen (Bowen 2010). More positive  $\delta^2\text{H}_p$  values in the southeast become more negative with increasing latitude towards the northwest, which is well documented by continent-wide precipitation sampling (Bowen et al. 2005). Studies typically take advantage of the strong correlation between  $\delta^2\text{H}_p$  and stable hydrogen values measured in bird feathers ( $\delta^2\text{H}_f$ ) by correcting an interpolated  $\delta^2\text{H}_p$  map (isoscape) using the regression equation between the two values and assigning birds of unknown origin to this surface

in a spatially-explicit manner (Bowen et al. 2005, West et al. 2010). However,  $\delta^2\text{H}_f$  is often variable among individuals from a single sampling location (Powell and Hobson 2006, Ooppel et al. 2011), indicating that large-scale processes like continental precipitation patterns do not fully describe the mechanisms that result in a given individual's  $\delta^2\text{H}_f$  value.

In order for studies using stable isotopes and precipitation patterns to measure migratory connectivity in an accurate and meaningful way, Wunder et al. (2005) suggests that  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$  should have a linear relationship that does not depend on species, age, location, or time, and calls for more direct tests of these assumptions. Langin et al. (2007) further highlighted several assumptions of such studies, including that all birds at a given location should have similar  $\delta^2\text{H}_f$ , regardless of age or sex, and that a given location should have a similar  $\delta^2\text{H}_f$  within and between seasons. However, previous studies have found that  $\delta^2\text{H}_f$  can vary significantly among individuals within a site (see Table 1 for summary). Specifically,  $\delta^2\text{H}_f$  can vary among birds of different ages (Meehan et al. 2003, Langin et al. 2007, Gow et al. 2012, Hache et al. 2012), and between years at the same site (Hache et al. 2012, Van Dijk et al. 2014). An interaction between year and other sources of variation has been found in some studies, with a year-specific effect on age (Gow et al. 2012, Hache et al. 2012) or species (Nordell et al. 2016). Most studies have not investigated differences between sexes, but those that did have been inconclusive (Langin et al. 2007) or found no effect (Nordell et al. 2016). A further assumption is that  $\delta^2\text{H}_f$  values should not vary between feather types in the same individual, but differences have been found between wing and tail feathers (Hache et al. 2012) and different wing feathers (Meehan et al. 2003, Table 1). With the increasing amount of studies investigating sources of variation in  $\delta^2\text{H}_f$  values, we are beginning to see trends that may be applicable across a wide range of taxa, and despite these sources of variation, it is still possible to accurately assign birds to their geographic origin

(Chabot et al. 2012, Hobson et al. 2012). However, Nordell et al. (2016) found that the best predictor of  $\delta^2\text{H}_f$  was species, which suggests that species may respond to sources of variation differently, and indicates that species-specific transfer functions linking  $\delta^2\text{H}_p$  with  $\delta^2\text{H}_f$  may be warranted.

Differences in habitat, migration distance, and foraging pattern have also been considered as sources of variation in  $\delta^2\text{H}_f$  values (Hobson et al. 2012, Nordell et al. 2016). Hobson et al. (2012) found that migration distance and foraging guild influence  $\delta^2\text{H}_f$  values, and many studies now use guild-specific transfer functions to relate  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$ , which classify species as short-distance, long-distance, or non-migratory and whether they forage on the ground or in the canopy. While Hobson et al. (2012) did not find support for an effect of aquatic versus upland habitat on  $\delta^2\text{H}_f$  values, hydrologic processes occurring within aquatic habitats may distort the relationship between  $\delta^2\text{H}_p$  and  $\delta^2\text{H}_f$  (Hobson et al. 2004, Clark et al. 2006). Some studies have excluded aquatic-associated species from analyses (Hobson et al. 2004) or justified the exclusion of outliers for this reason (Hobson et al. 2012), but few studies have explicitly investigated sources of variation in  $\delta^2\text{H}_f$  values of wetland-associated birds. Bortolotti et al. (2009) found that variation in  $\delta^2\text{H}$  values in wetland-associated tree swallows (*Tachycineta bicolor*) were not related to seasonal changes in  $\delta^2\text{H}$  values in water from local wetlands and aquatic insects, which were influenced by evaporation and timing of precipitation. Understanding how different sources of variation affect  $\delta^2\text{H}_f$  values of wetland-associated species can help us understand if measured  $\delta^2\text{H}_f$  values may be decoupled from expected  $\delta^2\text{H}_f$  values in these species.

In this study, we assessed factors responsible for variation in  $\delta^2\text{H}_f$  values in a population of prothonotary warblers (*Protonotaria citrea*) breeding in eastern Virginia, including age, sex, year, and feather type. The prothonotary warbler is a Neotropical migratory songbird, and a

habitat specialist of forested wetlands such as bald cypress-tupelo swamps, riparian corridors, and flooded bottomland hardwood forests (Petit 1999). Their breeding range encompasses the southeastern United States, extending through the Atlantic Coastal Plain and the Mississippi Alluvial Valley north to Ontario, Canada (Petit 1999). They nest in cavities and readily use abandoned woodpecker holes, natural cavities, or man-made nest boxes, and often produce two broods of young each breeding season. Many other species of long-distance migratory birds rely on wetlands, and there is a need to explicitly assess both how much variation in  $\delta^2\text{H}_f$  values occurs in these species, and if any patterns of variation can be identified and accounted for when using stable isotope analysis to assess dispersal and migratory movements.

## **Methods**

### *Feather samples*

We collected feather samples from adult male and female prothonotary warblers between 2013-2016 at long-term study sites in the Lower James River Important Bird Area (Blem and Blem 1994, Bulluck et al. 2013). The majority of samples used in this study were from Deep Bottom Park (37°24'N, -77°18'W), with additional samples from Presquile National Wildlife Refuge (37°21'N, -77°15'W), both in Henrico County, VA. At both sites, we obtained most samples from birds nesting in nest boxes positioned over water on metal conduit poles near the shore of tidal creeks and the mainstem of the river. We captured males using simulated territorial intrusion consisting of playing conspecific songs and calls near a mist net containing a decoy. We captured females using a hand held net placed over the cavity entrance hole during incubation. We determined age for each bird as after second-year (ASY, birds that hatched at least two summers prior) or second-year (SY, first-time breeders that hatched the previous summer) following molt criteria outlined in Pyle (1997), and we determined sex using plumage

characteristics and presence of breeding condition. We collected one outermost rectrix (tail feather) in all years, and in 2015 and 2016, we also collected one innermost primary (wing feather). In prothonotary warblers, timing and location of feather growth varies with age class. Nestlings begin growing wing and tail feathers in the nest, and retain these through their second summer, which means that feathers of SY birds were grown in the nest. Beginning with their second summer, they molt all feathers at the end of the breeding season, so that feathers of ASY birds were not grown in the nest. We used a mixture of primary and rectrix feathers for comparisons between age classes and sexes; all samples used in analysis from 2013 and 2014 were rectrices, and all samples used in analysis from 2015 and 2016 were primaries. Of the birds used in this study, 58% were known to have been present on the study site the previous year (i.e., the year the feather was grown), and 71% were known to have been present at the study site in at least one of the previous sampling years. The majority of birds that were known to be present any previous year but not the year prior to sampling were males, which were not sampled intensively at our study site. Of those birds that were not known to be previously present at the study site, 11 (12%) were SY females, 14 (15%) were ASY males, and 3 (3%) were SY males. We followed all applicable institutional and/or national guidelines for the care and use of animals, and we conducted work under Institutional Animal Care and Use Committee protocol #10230, U.S. Geologic Survey permit #23486, and Virginia Department of Game and Inland Fisheries permit #053965.

### *Stable isotope analysis*

We conducted stable isotope analysis at the Smithsonian Institute Stable Isotope Mass Spectrometry Laboratory in Suitland, MD in August, 2016. We cleaned all feathers in a 2:1 chloroform:methanol solution to remove oil and debris, then dried the feathers in a fume hood

for 48 hours. Subsamples of feather vane from the distal end (0.3-0.4 mg) were analyzed via high-temperature combustion in an elemental analyzer (Thermo TC/EA; Thermo Scientific, Waltham, MA, USA) and an isotope ratio mass spectrometer (Thermo Scientific Delta V Advantage). We corrected measurements of stable hydrogen for exchangeable atmospheric hydrogen using the comparative equilibrium method (Wassenaar and Hobson 2003) using three in-house keratin reference standards (USGS42: -78.5‰, CBS: -197‰, KHS: -54.1‰).

Analytical error of laboratory measurements was  $\pm 2\%$ . We present stable isotope values in units of per mil (‰) with non-exchangeable hydrogen reported in relation to the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation standard scale.

### *Statistical analysis*

We used a paired t-test and a linear regression to compare within-individual differences in  $\delta^2\text{H}_f$  between 15 paired rectrix and primary feathers sampled in 2016 from adult (10 ASY and 5 SY) female prothonotary warblers. We performed a two-way ANOVA to look for differences in  $\delta^2\text{H}_f$  between age class, year, and an interaction between age class and year. We used a Kruskal-Wallis rank sum test to examine differences in  $\delta^2\text{H}_f$  between the sexes because a Levene's test indicated unequal variances between male and female birds ( $F_{94, 1} = 4.68$ ,  $p = 0.03$ ). We conducted all statistical analyses in R version 3.3.0 (R Core Team 2016), and we report all results as mean  $\pm$  SD.

### **Results**

The mean  $\delta^2\text{H}_f$  value for the study area across all ages, sexes, years, and feather types was  $-57.17 \pm 10.12\%$  (n = 96; range = -85.27 to -38.09‰, 95% CI = -59.2, -55.14‰). The predicted  $\delta^2\text{H}_f$  value for our study site based on the growing-season  $\delta^2\text{H}_p$  isoscape of Bowen et al. (2005)

calibrated using the Hobson et al. (2012) equation for long-distance non-ground foraging migratory songbirds was -60.13‰.

Primary feathers ( $-57.89 \pm 10.91\%$ ,  $n = 15$ ) had more negative  $\delta^2H_f$  values than rectrices ( $-54.914 \pm 10.12\%$ ,  $n = 15$ ) in paired samples ( $t_{14} = -2.66$ ,  $p = 0.02$ , Figure 1), but their values were also significantly correlated ( $R^2 = 0.84$ ,  $F = 69.7$ , Figure 1). In other words, individuals with more negative  $\delta^2H_f$  values in their primary feathers tended to have more negative  $\delta^2H_f$  values in their rectrices. Age ( $F_{1,88} = 23.6$ ,  $p < 0.0001$ ) and year ( $F_{3,88} = 6.39$ ,  $p = 0.0006$ ) had a significant influence on  $\delta^2H_f$  values, but an interaction between age and year was not significant ( $F_{3,88} = 0.66$ ,  $p = 0.58$ , Figure 2). SY birds had more negative  $\delta^2H_f$  values than ASY birds, and feathers sampled in 2015 had significantly more negative  $\delta^2H_f$  than feathers sampled in any other years.  $\delta^2H_f$  values were not different in male and female birds ( $\chi^2 = 2.27$ ,  $df = 1$ ,  $p = 0.13$ ).

## Discussion

In this study, we sought to explore different sources of variation in  $\delta^2H_f$  in a wetland-associated songbird, and considered age, sex, year, and feather type as predictors of  $\delta^2H_f$  value. We found strong support for age and year effects on  $\delta^2H_f$  value, limited support for a difference between feather types, and no difference between the sexes. Primary feathers tended to have more negative  $\delta^2H_f$  values than rectrices for paired samples of SY and ASY female prothonotary warblers; however, the average difference between the two feather types was -2.98‰, which is similar to the analytical error ( $\pm 2\%$ ) for the laboratory analysis. Hache et al. (2012) also found that primaries were on average slightly more negative (-1.9‰) than rectrices in nestling ovenbirds. A less negative  $\delta^2H_f$  value in rectrices could indicate that these feathers, which are replaced later in the molt sequence than primaries, may be molted further south than the breeding territory, in an isotopically enriched (i.e. less negative  $\delta^2H$  value) region. Indeed, range-wide

monitoring data from the Monitoring Avian Productivity and Survivorship (MAPS) program indicate that prothonotary warblers can move off their breeding territory to molt, as evidenced by low recapture rates of individuals undergoing molt at the same banding station where they were captured breeding (Peter Pyle, pers. comm). However, it is not known whether these individuals are simply moving off their breeding territory, which may put them out of the MAPS station study area (usually ~20 ha), or making larger movements that would be indicative of a true molt-migration and actually influence  $\delta^2\text{H}_f$  values. Out of the 15 birds for which we made feather type comparisons, three were ASY individuals that had primaries that were 6.7-12.9‰ more negative than rectrices, and we cannot rule out the possibility that these individuals may have molted their rectrices in an area isotopically distinct from where they molted their primaries. However, because this difference was neither large nor systematic, it does not provide evidence prothonotary warblers regularly make large flights south prior to molting. While we suggest that future studies continue to test for differences between feather types, we conclude that primary and rectrix feathers in prothonotary warblers do not differ enough to preclude their simultaneous use in studies assessing migratory connectivity.

We found that the  $\delta^2\text{H}_f$  of younger birds was more negative than older birds, which is consistent with several other studies (Meehan et al. 2003, Langin et al. 2007, Hache et al. 2012). However, Gow et al. (2012) found that this relationship varied between years, with younger birds having more negative  $\delta^2\text{H}_f$  than older birds in one year, and the opposite the next year. Another study found no effect of age on  $\delta^2\text{H}_f$  (Hobson et al. 2012). Several explanations for the difference in  $\delta^2\text{H}_f$  between younger and older birds have been suggested, and we address how each one may be relevant for prothonotary warblers below.



Differences in body size between adult and nestling birds may lead to differences in  $\delta^2\text{H}_f$  because larger birds have higher rates of evaporative water loss (Tieleman and Williams 2000), leading to more enriched  $\delta^2\text{H}_f$  because the light isotope of hydrogen is evaporated preferentially (McKechnie et al. 2004). This is consistent with our observations of less negative  $\delta^2\text{H}_f$  values in ASY birds compared to SY birds, because SY birds retain flight feathers that were grown as nestlings until they are replaced during the first complete post-breeding molt (Pyle 1997).

Differences in microhabitat of molt location could cause differences in  $\delta^2\text{H}_f$  values because birds in warmer environments tend to experience higher evaporative water loss (Betini et al. 2009, Hache et al. 2012). Prothonotary warblers nest in cavities, which may have a different microclimate than the surrounding environment (Betini et al. 2009, Hache et al. 2012). Betini et al. (2009) found that tree swallow nestlings in nest boxes with higher temperatures had more enriched  $\delta^2\text{H}_f$ . Blem and Blem (1994) found that prothonotary warblers in our study area select higher temperature nest boxes early in the summer (early clutches) and lower temperature boxes later in the summer (late clutches), indicating that there may be a temporal component to microclimate differences as well. In other words, at low ambient temperature, nest boxes were warmer than ambient temperatures, but at high ambient temperatures, nest boxes were cooler than ambient temperatures (Blem and Blem 1994). Additionally, Bortolotti et al. (2013) and Hache et al. (2012) found that nestling  $\delta^2\text{H}_f$  values became more negative as the breeding season continued for tree swallows and ovenbirds, respectively. We were not able to test for an effect of either location or timing of molt on  $\delta^2\text{H}_f$  value because timing of molt in the previous year was not known for all birds sampled.

Differences in sources of hydrogen available through prey items and drinking water may lead to systematic variation between younger and older birds if the prey items and drinking water

have different  $\delta^2\text{H}$  values and/or the age classes consume those items in different quantities (Langin et al. 2007, Betini et al. 2009). These different sources of consumed hydrogen may also lead to individual variation *within* age classes, because diet/resource availability may vary among individuals. Langin et al. (2007) found that precipitation and standing water  $\delta^2\text{H}$  values were less negative than  $\delta^2\text{H}_f$  values of American redstarts, while insects had more negative  $\delta^2\text{H}$  values compared to redstarts. They suggest that their observation of less negative  $\delta^2\text{H}_f$  values in adults compared to nestlings is a result of adults incorporating both prey items and drinking water into their diet, and nestlings deriving all water from the prey items they consume (Langin et al. 2007). For wetland-associated birds, the quantity of aquatic versus terrestrial prey consumed may be especially important if those prey sources have different  $\delta^2\text{H}$  values. Betini et al. (2009) suggests that the contribution of aquatic insects in the diet of nestlings from a riparian site could have led to a more negative  $\delta^2\text{H}$  value measured in nestling blood samples. On the other hand, Bortolotti et al. (2013) found that aquatic insects had slightly more enriched  $\delta^2\text{H}$  values compared to terrestrial insects. To date, no studies have examined  $\delta^2\text{H}$  values in prothonotary warbler food webs, but an analysis of carbon and nitrogen stable isotope ratios in prothonotary warbler nestling and adult breast muscle indicated that adults likely consume more terrestrial prey (L.P.B. unpublished data). More study is needed to understand both the contributions of aquatic and terrestrial prey to adult and nestling prothonotary warbler diet as well as how those prey items differ in isotopic composition.

We examined  $\delta^2\text{H}_f$  values from four years of data, and found that one year (2015) had a more negative mean  $\delta^2\text{H}_f$  value compared to all other years. While the samples from 2015 were primaries, which were demonstrated to have slightly more negative  $\delta^2\text{H}_f$  values than rectrices, we do not think differences in  $\delta^2\text{H}_f$  values between feather types is the source of between year

variation in this study. Feather samples from 2016 were also primaries but this year was not more negative than other years, and the magnitude of the difference between years was greater than the difference between feather types. Other studies have also found yearly differences both in  $\delta^2\text{H}_f$  (Hache et al. 2012, Van Dijk et al. 2014) as well as  $\delta^2\text{H}_p$  (Van Wilgenburg et al. 2012). In contrast, Gow et al. (2012) and Langin et al. (2007) did not find evidence of yearly difference in  $\delta^2\text{H}_f$ , in their studies spanning two and three years of sampling, respectively. Yearly variation in  $\delta^2\text{H}_f$  values may be related to large-scale variation in climate processes such as the North Atlantic Oscillation or the El Nino-Southern Oscillation which cause deviations from long-term averages in  $\delta^2\text{H}_p$  (Hobson et al. 2012). Van Wilgenburg et al. (2012) found that isoscapes based on long-term average of  $\delta^2\text{H}_p$  values were accurate for predicting the mean  $\delta^2\text{H}_p$  across all years at their study sites, but that yearly variation in  $\delta^2\text{H}_p$  ranged as much as 40%. In order to account for these common yearly variations, two studies used year-specific isoscapes developed using the online tool IsoMap (Bowen et al. 2014), but neither study found that these year-specific isoscapes improve assignment accuracy, likely because they rely on fewer precipitation monitoring stations for interpolation (Vander Zanden et al. 2014, Tonra et al. 2014). Alternatively, a correction could be applied for anomalous years, and Van Dijk et al. (2015) found that a calibration equation which corrected for age and year had the highest accuracy when testing assignment of mallards (*Anas platyrhynchos*) of known origin. Correcting  $\delta^2\text{H}_f$  values of anomalous years may be a valid method when using  $\delta^2\text{H}_f$  values to determine migratory connectivity for prothonotary warblers, but would depend on the consistency of the anomalies in space (i.e. across the whole breeding range) (Studds et al. 2012).

We tested for differences in  $\delta^2\text{H}_f$  values between males and females. We found no evidence that  $\delta^2\text{H}_f$  varies by sex, which was consistent with the results of Nordell et al. (2016).

Langin et al. (2007) did not find a difference between sexes when evaluating all samples as a whole, but when evaluating a subsample of feathers that had been isotopically analyzed at a different time than the rest of their samples, they found that males have more enriched  $\delta^2\text{H}_f$  values relative to females. Differences in  $\delta^2\text{H}_f$  values between males and females could occur if timing or location of molt differed between the sexes, or if there were sex-based differences in diet, but our results do not support either of these hypotheses.

In conclusion, we found that age and year effects on  $\delta^2\text{H}_f$  values are important sources of variation in prothonotary warblers breeding in southeastern Virginia, USA. Our results support the growing body of literature that have also found similar effects of these two sources of variation. More study is needed to understand the mechanisms behind these effects, especially how the influence of local precipitation and surface water  $\delta^2\text{H}$  values (e.g. differences among tidal rivers, bald cypress-tupelo swamps, reservoirs) interact with individual and/or age related differences in diet and physiology to produce an individual's  $\delta^2\text{H}_f$  value. In studies of migration or dispersal which seek to assign birds to geographic origin, variation resulting from age and year differences in  $\delta^2\text{H}_f$  value can be controlled by limiting sampling and geographic assignment to a single age class and single year. Realistically, this would require a costly and logistically challenging international sampling effort and would eliminate the option of using previously collected samples. Instead, correction factors to account for the offset between age and/or anomalous years may be appropriate for some studies. Variation relating to age, year and feather type is a concern for studies of migratory connectivity using stable isotopes, and should be accounted for whenever possible, which can lead to more precise estimates of origin (Hobson et al. 2012). However, the range of variation that we observe in this study is similar to that of other

studies that have successfully determined migratory connectivity using stable isotopes (Paxton et al. 2007, Tonra et al. 2014).

## Literature Cited

- Betini, G. S., Hobson, K. A., Wassenaar, L. I., & Norris, D. R. (2009). Stable hydrogen isotope ( $\delta D$ ) values in songbird nestlings: effects of diet, temperature, and body size. *Canadian Journal of Zoology*, 87(9), 767–772. <http://doi.org/10.1139/Z09-069>
- Blem, C. R., & Blem, L. B. (1994). Composition and Microclimate of Prothonotary Warbler Nests. *The Auk*, 111(1), 197–200. <http://doi.org/10.2307/4088523>
- Bortolotti, L. E., Clark, R. G., & Wassenaar, L. I. (2013). Hydrogen isotope variability in prairie wetland systems: Implications for studies of migratory connectivity. *Ecological Applications*, 23(1), 110–121. <http://doi.org/10.1890/12-0232.1>
- Bowen, G. J. (2010). Isoscapes: Spatial Pattern in Isotopic Biogeochemistry. *Annu. Rev. Earth Planet. Sci*, 38, 161–87. <http://doi.org/10.1146/annurev-earth-040809-152429>
- Bowen, G. J., Liu, Z., Vander Zanden, H. B., Zhao, L., & Takahashi, G. (2014). Geographic assignment with stable isotopes in IsoMAP. *Methods in Ecology and Evolution*, 5(3), 201–206. <http://doi.org/10.1111/2041-210X.12147>
- Bowen, G. J., Wassenaar, L. I., & Hobson, K. a. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, 143(3), 337–348. <http://doi.org/10.1007/s00442-004-1813-y>
- Bulluck, L., Huber, S., Viverette, C., & Blem, C. (2013). Age-specific responses to spring temperature in a migratory songbird: Older females attempt more broods in warmer springs. *Ecology and Evolution*, 3(10), 3298–3306. <http://doi.org/10.1002/ece3.673>
- Chabot, A. A., Hobson, K. A., van Wilgenburg, S. L., McQuat, G. J., & Lougheed, S. C. (2012). Advances in linking wintering migrant birds to their breeding-ground origins using combined analyses of genetic and stable isotope markers. *PLoS ONE*, 7(8). <http://doi.org/10.1371/journal.pone.0043627>
- Clark, R. G., Hobson, K. A., & Wassenaar, L. I. (2006). Geographic variation in the isotopic ( $\delta D$ ,  $\delta^{13}C$ ,  $\delta^{15}N$ ,  $\delta^{34}S$ ) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. *Canadian Journal of Zoology*, 84, 1395–1401. <http://doi.org/10.1139/z06-135>
- Gow, E. A., Stutchbury, B. J. M., Done, T., & Kyser, T. K. (2012). An examination of stable hydrogen isotope ( $\delta D$ ) variation in adult and juvenile feathers from a migratory songbird. *Canadian Journal of Zoology*, 90(5), 585–594. <http://doi.org/10.1139/z2012-024>
- Haché, S., Hobson, K. A., Villard, M., & Bayne, E. (2012). Assigning birds to geographic origin using feather hydrogen isotope ratios ( $\delta^2H$ ): importance of year, age, and habitat. *Canadian Journal of Zoology*, 90(6), 722–728. <http://doi.org/10.1139/z2012-039>

- Hobson, K. A. (1999). Tracing Origins and Migration of Wildlife Using Stable Isotopes: A Review. *Source: Oecologia*, *120*(3), 314–326. Retrieved from <http://www.jstor.org/stable/4222394>
- Hobson, K. A., Bowen, G. J., Wassenaar, L. I., Ferrand, Y., & Lormee, H. (2004). Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia*, *141*(3), 477–488. <http://doi.org/10.1007/s00442-004-1671-7>
- Hobson, K. A., Van Wilgenburg, S. L., Wassenaar, L. I., & Larson, K. (2012). Linking hydrogen isotopes in feathers and precipitation: Sources of variance and consequences for assignment to isoscapes. *PLoS ONE*, *7*(4), 1–9. <http://doi.org/10.1371/journal.pone.0035137>
- Hobson, K., & Wassenaar, L. I. (2008). *Tracking Animal Migration with Stable Isotopes*. Academic Press.
- Langin, K. M., Reudink, M. W., Marra, P. P., Norris, D. R., Kyser, T. K., & Ratcliffe, L. M. (2007). Hydrogen isotopic variation in migratory bird tissues of known origin: Implications for geographic assignment. *Oecologia*, *152*(3), 449–457. <http://doi.org/10.1007/s00442-007-0669-3>
- McKechnie, A. E., Wolf, B. O., & Martínez del Rio, C. (2004). Deuterium stable isotope ratios as tracers of water resource use: an experimental test with rock doves. *Oecologia*, *140*(2), 191–200. <http://doi.org/10.1007/s00442-004-1564-9>
- Meehan, T. D., Rosenfield, R. N., Atudore, V. N., Bielefeldt, J., Rosenfield, L. J., Stewart, A. C., ... Bozek, M. a. (2003). Variation in hydrogen stable-isotope ratios between adult and nestling cooper's hawks. *The Condor*, *105*(3), 567–572. <http://doi.org/10.1650/7283>
- Nordell, C. J., Haché, S., Bayne, E. M., Sólymos, P., Foster, K. R., Godwin, C. M., ... Hobson, K. A. (2016). Within-Site Variation in Feather Stable Hydrogen Isotope ( $\delta^2\text{H}_f$ ) Values of Boreal Songbirds: Implications for Assignment to Molt Origin. *Plos One*, *11*(11), e0163957. <http://doi.org/10.1371/journal.pone.0163957>
- Oppel, S., Pain, D. J., Lindsell, J. A., Lachmann, L., Diop, I., Tegetmeyer, C., ... Flade, M. (2011). High variation reduces the value of feather stable isotope ratios in identifying new wintering areas for aquatic warblers *Acrocephalus paludicola* in West Africa. *Journal of Avian Biology*, *42*(4), 342–354. <http://doi.org/10.1111/j.1600-048X.2011.05252.x>
- Paxton, K. L., Van Riper III, C., Theimer, T. C., & Paxton, Eben, H. (2007). Spatial and Temporal Migration Patterns of Wilson's Warbler (*Wilsonia Pusilla*) in the Southwest As Revealed By Stable Isotopes. *The Auk*, *124*(1), 162–175. [http://doi.org/10.1642/0004-8038\(2007\)124](http://doi.org/10.1642/0004-8038(2007)124)

- Petit, L. J. (1999). Prothonotary Warbler (*Protonotaria citrea*). *The Birds of North America Online*, 26818421. <http://doi.org/10.2173/bna.408>
- Powell, L. A., & Hobson, K. (2006). Enriched feather hydrogen isotope values for Wood Thrushes sampled in Georgia, USA, during the breeding season: implications for quantifying dispersal. *Canadian Journal of Zoology*, 84(9), 1331–1338. <http://doi.org/10.1139/z06-114>
- Pyle, P. (1997). *Identification Guide to North American Birds: Part 1*. Slate Creek Press.
- Studds, C. E., McFarland, K. P., Aubry, Y., Rimmer, C. C., Hobson, K. A., Marra, P. P., & Wassenaar, L. I. (2012). Stable-hydrogen isotope measures of natal dispersal reflect observed population declines in a threatened migratory songbird. *Diversity and Distributions*, 18(9), 919–930. <http://doi.org/10.1111/j.1472-4642.2012.00931.x>
- Tieleman, B. I., & Williams, J. B. (2000). The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiological and Biochemical Zoology: PBZ*, 73(4), 461–79. <http://doi.org/10.1086/317740>
- Tonra, C. M., Both, C., & Marra, P. P. (2014). Incorporating site and year-specific deuterium ratios ( $\delta^2\text{H}$ ) from precipitation into geographic assignments of a migratory bird. *Journal of Avian Biology*, 46(3), 266–274. <http://doi.org/10.1111/jav.00553>
- van Dijk, J. G. B., Meissner, W., & Klaassen, M. (2014). Improving provenance studies in migratory birds when using feather hydrogen stable isotopes. *Journal of Avian Biology*, 45(1), 103–108. <http://doi.org/10.1111/j.1600-048X.2013.00232.x>
- Van Wilgenburg, S. L., Hobson, K. A., Brewster, K. R., & Welker, J. M. (2012). Assessing dispersal in threatened migratory birds using stable hydrogen isotope ( $\delta\text{D}$ ) analysis of feathers. *Endangered Species Research*, 16(1), 17–29. <http://doi.org/10.3354/esr00383>
- Vander Zanden, H. B., Wunder, M. B., Hobson, K. A., Van Wilgenburg, S. L., Wassenaar, L. I., Welker, J. M., & Bowen, G. J. (2014). Contrasting assignment of migratory organisms to geographic origins using long-term versus year-specific precipitation isotope maps. *Methods in Ecology and Evolution*, 5(9), 891–900. <http://doi.org/10.1111/2041-210X.12229>
- Wassenaar, L. I., & Hobson, K. A. (2003). Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies*, 39(3), 211–217. <http://doi.org/10.1080/1025601031000096781>
- Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S., & Holmes, R. T. (2002). Links between worlds: unraveling migratory connectivity. *Trends in Ecology & Evolution*, 17(2), 76–83. [http://doi.org/10.1016/S0169-5347\(01\)02380-1](http://doi.org/10.1016/S0169-5347(01)02380-1)



West, J. B., Bowen, G. J., Dawson, T. E., & Tu, K. P. (2010). *Isoscapes: Understanding movement, pattern, and process on earth through isotope mapping*. Springer.  
<http://doi.org/10.1007/978-90-481-3354-3>

Wunder, M. B., Kester, C. L., Knopf, F. L., & Rye, R. O. (2005). A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia*, *144*(4), 607–617.  
<http://doi.org/10.1007/s00442-005-0071-y>

Table 1. Comparison of the effect of age, sex, year, and feather type on stable hydrogen isotope variation as reported in six studies. Age is denoted as HY (a bird in its hatching year), SY (a bird in its second year, i.e., first breeding season), or ASY (a bird in at least its second year, i.e., at least its second breeding season). Feather type is denoted as P (primary, i.e., wing feather) or R (rectrix, i.e., tail feather). The direction of the effect is reported if known, otherwise it is reported as present (yes) or absent (no). A dash denotes comparisons that were not performed in that study. See Langin et al. (2007) for details about their conclusions regarding the effect of sex.

<b>Study</b>	<b>Species</b>	<b>Age</b>	<b>Year</b>	<b>Age*year</b>	<b>Feather type</b>	<b>Sex</b>
<i>This study</i>	prothonotary warbler	SY < ASY	yes	no	P slightly < R	no
<i>Hache et al. 2012</i>	ovenbird	nestling < ASY	yes	yes	P slightly < R	-
<i>Gow et al. 2012</i>	wood thrush	yes	no	yes	no	-
<i>Langin et al. 2007</i>	American redstart	nestling < ASY	no	-	P < contour	no*
<i>Meehan et al. 2003</i>	Cooper's hawk	nestling < adult	-	-	P ≠ P	-
<i>Van Dijk et al. 2014</i>	mallard	HY < adult	yes	-	-	-

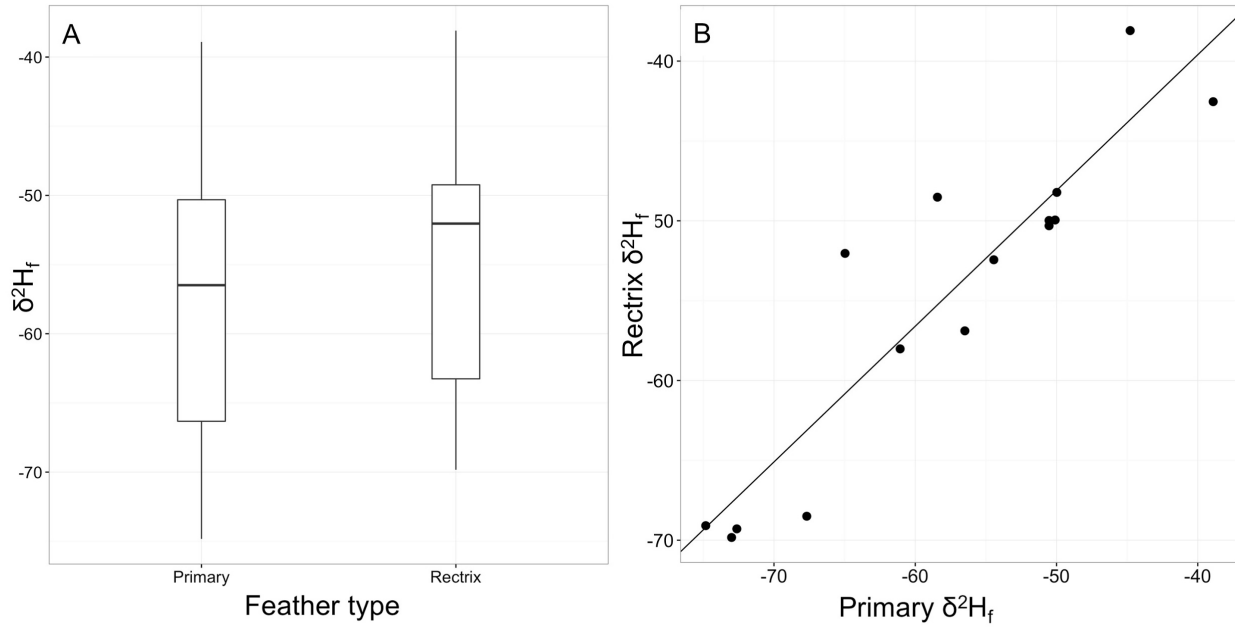


Figure 1. A) Primary feathers have significantly more negative  $\delta^2H_f$  values than rectrix feathers from paired samples of 15 female prothonotary warblers from Henrico County, VA ( $t = -2.6$ ,  $p = 0.018$ ). B) These  $\delta^2H_f$  values are also correlated ( $R^2 = 0.84$ ,  $p < 0.001$ ).

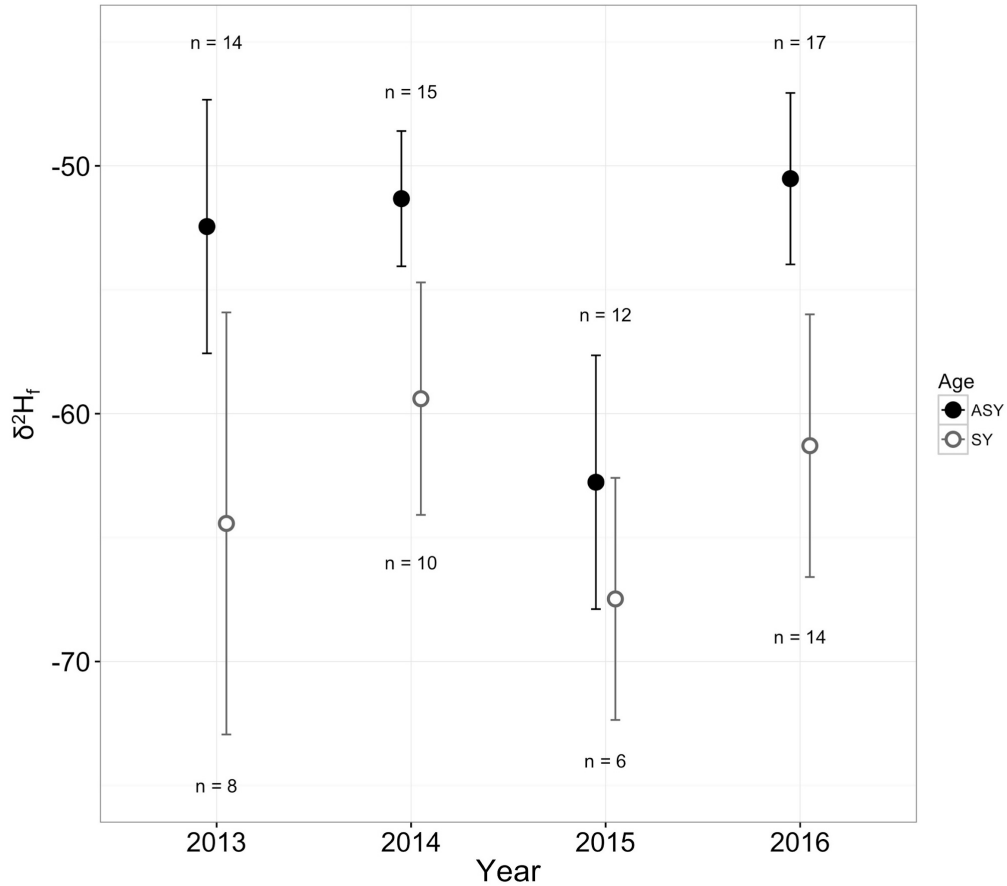


Figure 2.  $\delta^2H_f$  values vary significantly across year and age class ( $p < 0.001$ ) for 96 feather samples from prothonotary warblers collected in Henrico County, VA, but an interaction between age and year was not significant. Birds in the first year of breeding (SY) had significantly more negative  $\delta^2H_f$  value than birds in their second year of breeding or older (ASY). Samples collected in 2015 had significantly more negative  $\delta^2H_f$  values than those collected in 2014 or 2016.

## Chapter 2

### Assessing migratory connectivity in the prothonotary warbler using stable hydrogen isotopes: comparing models with and without prior information on abundance

#### Introduction

In North America, many species of migratory birds are experiencing rapid population declines (Sauer et al. 2013). Our ability to identify the cause of these declines and attempt to reverse them is limited by incomplete knowledge of factors affecting populations throughout their annual cycle (Faaborg et al. 2010, Marra et al. 2016). Integrated population models offer a solution to find out when and where in the annual cycle populations are limited (Hostetler et al. 2015), but a prerequisite to using these models is an understanding of how populations are spatially linked between the breeding and wintering grounds, known as migratory connectivity (Webster et al. 2002).

The distribution of long-distance migratory bird species between the North American breeding grounds and the wintering grounds in Latin America has consequences for population dynamics (Martin et al. 2007, Mills and Weir et al. 2007, Finch et al. 2017). When migratory connectivity is strong, individuals that are geographically segregated on the breeding grounds also exhibit geographic partitioning during the winter, and when migratory connectivity is weak, individuals from separate populations on the breeding grounds intermix on the nonbreeding grounds (Webster et al. 2002). Processes that negatively affect populations at a given nonbreeding location, such as those leading to low survival, will disproportionately influence certain breeding populations when connectivity is strong, but the effect will be spread diffusely across breeding populations when connectivity is weak (Finch et al. 2017). In addition to informing full annual cycle population models, accounting for migratory connectivity when

designing conservation plans can help ensure adequate protection for regional sub-populations of migratory birds (Martin et al. 2007).

Studying migratory connectivity in passerines is challenging because many individuals are too small to carry satellite tracking devices. Miniaturization of tracking devices like archival light-level geolocators has recently allowed for rangewide tracking of passerine species (Stutchbury et al. 2009, McKinnon et al. 2013), but these devices are expensive and require recapture of individuals to download data, which often results in low sample sizes. However, intrinsic markers that exhibit geographic variation, like stable isotopes and genetic markers, offer a cost effective solution to determine geographic origin that does not rely on recapturing individuals (Hobson 1999, Hobson and Wassenaar 2008) or satellite tracking. Stable isotope analysis relies on predictable variation across landscapes in ratios of isotopic forms, which is assimilated into animal tissue through the local food web (Vander Zanden et al. 2016). Hydrogen isotopes are widely used for geographic assignment in avian migration studies because the ratio of heavy to light hydrogen isotopes ( $^2\text{H}:^1\text{H}$ ) forms a latitudinal gradient across North America (Hobson and Wassenaar 2008). Bird feathers are well suited for studying migratory connectivity using hydrogen isotopes, because they assimilate the local isotope value at their location of molt, are usually grown at a known location (e.g., near the breeding grounds), and are retained for an entire year. The isotope ratio of a feather sampled from a bird at any location other than where it was grown (e.g., on the wintering grounds) can then be compared to known (or predicted) values to assess where that bird spent the previous summer through a geographic assignment process.

Spatially-explicit geographic assignments have been performed by probabilistically assigning birds to spatially interpolated base maps of predicted feather hydrogen isotope values ( $\delta^2\text{H}_f$ ) (Royle and Rubenstein 2004, Bowen et al. 2005). Rather than interpolating  $\delta^2\text{H}_f$  values

directly, studies using stable hydrogen analysis to determine migratory connectivity take advantage of the relationship between hydrogen isotopes measured in feathers and local precipitation ( $\delta^2\text{H}_p$ ), because there are usually many more locations with stations measuring  $\delta^2\text{H}_p$  than sample sites measuring  $\delta^2\text{H}_f$  (Bowen et al. 2005). The equation from the linear regression between  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$  is used to create a calibrated  $\delta^2\text{H}_f$  isoscape, which accounts for the offset between  $\delta^2\text{H}_p$  and  $\delta^2\text{H}_f$  that occurs as the heavy hydrogen isotope is progressively enriched through transpiration in plants and trophic accumulation in the food web (Hobson and Wassenaar 2008). Calibration equations have been developed at the order, guild, and species level for migratory birds (Clark et al. 2006, Hobson et al. 2012, Tonra et al. 2014). Hobson et al. (2012) refined calibration equations for passerines by incorporating foraging and migratory guild, and these are now used widely to infer migratory connectivity (Gonzalez-Prieto et al. 2011). However, species is an important predictor of  $\delta^2\text{H}_f$  variation (Nordell et al. 2016), and guild-level transformations may not adequately describe the relationship between  $\delta^2\text{H}_p$  and  $\delta^2\text{H}_f$  for all species. This may be especially true for birds associated with wetland or riparian habitats, where hydrological processes may influence the correlation between  $\delta^2\text{H}_p$  and  $\delta^2\text{H}_f$ .

While stable hydrogen isotopes have been successfully used to make spatially-explicit assignments of birds to their geographic origin, the precision of the assignments is usually low (Hobson et al. 2015). This reflects the nature of the gradient in  $\delta^2\text{H}_p$  values in North America, which varies little along the east-west axis, and changes gradually with latitude. More precise assignments can be achieved by adding other isotopes (Hobson et al. 2014b), morphometric information (Rushing et al. 2014) or genetic markers (Clegg et al. 2003, Chabot et al. 2012, Rundel et al. 2013) to the assignment model, or by incorporating prior information in a Bayesian framework into the assignment model (Royle and Rubenstein 2004). Relative abundance on the

breeding grounds has been incorporated as prior information because a bird randomly sampled from the wintering grounds has a greater chance of originating from an area of high abundance on the breeding grounds than an area of low abundance (Royle and Rubenstein 2004). Several studies have incorporated relative abundance data from the Breeding Bird Survey (BBS) as prior information (Royle and Rubenstein 2004, Gonzalez-Prieto et al. 2011, Hallworth et al. 2013). The BBS is a citizen-science bird monitoring program that has collected abundance and species richness data at defined roadside routes across North America every spring since 1966 (Sauer et al. 2013). While the BBS is a rich source of data, the surveys occur along roads and so may poorly sample certain species, locations, and habitats, such as wetlands. For species poorly sampled by the BBS, alternative sources of abundance data have been considered as prior information, such as species distribution models built from broad-scale citizen science bird occurrence data compiled in the eBird database (Pekarsky et al. 2015, Fournier et al. 2016). Recently, spatio-temporal exploratory models (STEMs) have been developed using eBird count data and remote sensing data to predict abundance and distribution for several species of Neotropical migratory birds (Fink et al. 2010, Johnston et al. 2015). These spatial models of abundance may be a good alternative for species undersampled by the BBS, but have not yet been tested as prior information in a Bayesian assignment model. Comparing the performance of different sources of abundance data like BBS data with STEM data has yet to be done and will demonstrate the utility of each dataset for improving our understanding of migratory connectivity.

Royle and Rubenstein (2004) suggest that accounting for abundance across a species' range when making probabilistic assignments may be especially important in cases where there is low geographic structure in isotope values or when the species of interest has a highly non-



uniform distribution in abundance. The prothonotary warbler is a Neotropical migratory songbird that breeds in the southeastern United States (Figure 1), a region characterized by a relatively small gradient in geographic variation in hydrogen isotope values. While they are found in small numbers as far north as Ontario, Canada, the majority of prothonotary warblers occur in more southern locations along the Mississippi Alluvial Valley and the Atlantic Coastal Plain. Prothonotary warblers are habitat specialists which depend on mature forested wetlands throughout their annual life cycle, and their population has declined 40% since 1966 (Sauer et al. 2013). Loss of mature forested wetland habitat, particularly widespread clearing of coastal mangroves on their wintering grounds, is considered the greatest threat to their recovery (Petit 1999). Determining the strength of connectivity and the geographic relationships between populations on the breeding and nonbreeding grounds is a high priority for this species because of its habitat specificity, which makes it especially vulnerable to habitat loss and degradation across its range. In this study, our objectives are to 1) develop a species-specific isotope calibration equation for prothonotary warblers, 2) test model performance by assigning known-origin birds, and compare the performance of models with and without prior information of abundance (using BBS and STEM data as priors), and 3) assign overwintering birds to breeding origin in order to determine the strength of migratory connectivity.

## **Methods**

### *Feather samples*

We obtained feather samples from collaborators in the Prothonotary Warbler Working Group, the Smithsonian Migratory Bird Center, and the UCLA Conservation Genetics Resource Center from 13 sites on the breeding grounds and eight sites on the wintering grounds between 2001-2016 (Figure 1). Birds were captured passively using mist nets, actively by simulated

territorial intrusion consisting of playing conspecific songs and calls near a mist net containing a decoy, or by using a hand held net placed over cavity nest entrance hole during incubation. One outermost rectrix or one innermost primary was sampled from each individual; both rectrices and primaries are retained for one year following the post-breeding molt in this species (Pyle 1997), and  $\delta^2\text{H}_f$  value between these feather types are correlated within the same individual (see Chapter 1).

### *Stable isotope analysis*

We conducted stable isotope analysis at the Smithsonian Institute Stable Isotope Mass Spectrometry Laboratory in Suitland, MD in August 2016. We washed all feathers in a 2:1 chloroform:methanol solution to remove surface oil and debris, then dried them in a fume hood for 48 hours. We loaded subsamples of feather vane (0.3-0.4 mg) from the distal end into silver capsules and analyzed them via high-temperature combustion in an elemental analyzer (Thermo TC/EA; Thermo Scientific, Waltham, MA, USA) and an isotope ratio mass spectrometer (Thermo Scientific Delta V Advantage). We corrected measurements of stable hydrogen for exchangeable atmospheric hydrogen using the comparative equilibrium method (Wassenaar and Hobson 2003) using three in-house keratin reference standards (USGS42: -78.5‰, CBS: -197‰, KHS: -54.1‰). We measured analytical error ( $\pm 2\%$ ) as the standard deviation of the keratin standards, averaged across all runs. We present stable hydrogen ratios ( $^2\text{H}:^1\text{H}$ ) in delta notation ( $\delta$ ) in units of per mil (‰) with non-exchangeable hydrogen reported in relation to Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation standard scale.

### *Isoscape and calibration equation*

We developed and downloaded a  $\delta^2\text{H}_p$  isoscape using the IsoMAP online tool (Bowen et al. 2014). We used precipitation and the absolute value of latitude as predictors in the IsoMAP

model with data from 50 stations between 1960-1999, and restricted the temporal range of the input data to the months of feather growth, which we defined as June-August (IsoMap job #59795, available to download at <<http://isomap.org>>). In order to create a calibrated isoscape for  $\delta^2\text{H}_f$ , we modeled the relationship between  $\delta^2\text{H}_p$  and  $\delta^2\text{H}_f$  using a linear regression, with the average  $\delta^2\text{H}_f$  value measured at each site regressed against the  $\delta^2\text{H}_p$  isoscape-predicted value for each site (Hobson and Wassenaar 2008). We split the known-origin feather samples from the breeding grounds into a build set used to create the calibration equation, and a validation set used to assess model accuracy and precision. All sites with <5 observations were included in the build set, and all sites with >5 observations were randomly divided such that 50% of samples from each site were assigned to the build set ( $n = 72$ ) and 50% to the validation set ( $n = 52$ ). For most sample sites, we did not have information about whether individuals were present at that location the previous year. In order to minimize the chance of sampling birds that had dispersed from a different breeding site the year prior (the year of feather growth), we only used samples from after second year birds (adults in at least their second breeding season), because they tend to have higher site fidelity than first-time breeders (Cline et al. 2013). We produced a calibrated  $\delta^2\text{H}_f$  isoscape by correcting every cell in the  $\delta^2\text{H}_p$  isoscape using the calibration equation:

$$\delta^2\text{H}_{f(\text{predicted})} = m * \delta^2\text{H}_p + b, \text{ where } m \text{ is the slope and } b \text{ is the intercept.}$$

#### *Assignment of feather samples to geographic origin*

To assign feather samples to their geographic origin, we used a normal probability density function to generate a spatially-explicit assignment test (Royle and Rubenstein 2004, Hobson et al. 2014a). This equation incorporates the likelihood that an individual with given values for  $\text{H}_f$  originated from a given raster cell in the feather isoscape:

$$f(y^*|\mu_c, \sigma_c) = (1/\sqrt{(2\pi\sigma_c)}) \exp [-1/2\sigma_c^2(y^*-\sigma_c)^2] \quad [\text{Equation 1}]$$

where  $f(y^*|\mu_c, \sigma_c)$  is the probability that a bird ( $y^*$ ) has a potential origin in a given raster cell, given that  $\mu_c$  is the predicted mean  $\delta^2H_f$  value for that cell (based on the calibrated  $\delta^2H_f$  isoscape), and  $\sigma_c$  is the expected standard deviation of  $\delta^2H_f$  (Royle and Rubenstein 2004, Bowen et al. 2005). We estimated  $\sigma_c$  as 10.6, which was the standard deviation of the residuals of the linear relationship between  $\delta^2H_p$  and  $\delta^2H_f$ .

We also conducted assignments in a Bayesian framework where breeding season abundance was incorporated into the assignment model as prior information:

$$f_x = f(y^*|\mu_c, \sigma_c)f_p / (\sum_i f(y^*|\mu_c, \sigma_c)f_p) \quad \text{[Equation 2]}$$

where  $f(y^*|\mu_c, \sigma_c)$  is determined from the previous equation and  $f_p$  is the spatially explicit prior probability of origin from a raster of relative abundance of prothonotary warblers across the breeding range (Royle and Rubenstein 2004). We developed the BBS abundance probability surface using BBS count data from 1994-2002 (Sauer et al. 2011), which we downloaded from the USGS Patuxent Wildlife Research Center website ([https://www.mbr-pwrc.usgs.gov/bbs/geographic\\_information/Instructions\\_abundance\\_grid.htm](https://www.mbr-pwrc.usgs.gov/bbs/geographic_information/Instructions_abundance_grid.htm)) as a smooth-grid abundance shapefile. We converted the shapefile to a raster, and because some of our sampling locations fell outside the bounds of the BBS data (i.e., no prothonotary warblers were detected by the BBS in Wisconsin), we mosaicked a range map raster (Ridgely et al. 2003) to the BBS raster and assigned the non-overlapping cells to the lowest abundance value from the BBS raster using ArcGIS 10.3.1 (ESRI, Redlands, California, USA). We developed the STEM abundance probability surface using a raster of relative abundance derived using the STEM method (Fink et al. 2010, Johnston et al. 2015), which combines eBird count data with remotely sensed habitat variables to produce a predictive species distribution model. Because the STEM abundance map contained no data values for parts of the breeding range, we mosaicked a range map to the STEM

data in the same manner as above. Finally, we converted the resulting BBS and STEM abundance maps to probability surfaces by dividing every cell in each raster by the sum of all the cells in each raster. Because the precipitation isoscape and abundance maps were different resolutions, all rasters used in the analysis were rescaled to  $0.19^\circ$  (~20km).

#### *Model validation of known-origin samples*

We evaluated the accuracy and the precision of our assignment models with and without prior abundance information by assigning individuals of known origin from the validation sample to their geographic origin. We measured accuracy as the proportion of birds correctly assigned to their true location of origin under a 2:1 odds ratio (Tonra et al. 2014). We first converted the probability surface into a binary map by assigning cells as “likely” or “unlikely” based on 2:1 odds of correctly assigning a bird to its true location of origin, which selected the upper 67% of cells (Ashley et al. 2010). We considered an assignment as correct if the pixel corresponding to the true location of origin was coded as “likely”. We initially compared the accuracy using the odds ratios typically used in the literature for this type of study (3:1 versus 2:1) and found no difference in accuracy, so we used the more stringent 2:1 odds, which increases precision (Van Wilgenburg and Hobson 2011). We calculated precision as the number and proportion of cells in the binary raster assigned “likely” for each of the three models (Chabot et al. 2012, Tonra et al. 2014). We summed the resulting raw probability maps for all individuals at a given site for each of the three models (no prior abundance information, BBS abundance information, and STEM abundance information), and rescaled each to range between 0 and 1 by dividing each raster by its maximum value.

#### *Assignment of overwintering birds*

We assigned samples from the nonbreeding season to geographic origin using Equation 1 for the model with no prior information on abundance and Equations 1 and 2 for the models incorporating prior information of abundance from BBS and STEM data. We pooled data from each nonbreeding season sampling location into four geographic regions (Figure 2), and summed the raw probability surfaces for samples from Central America, western Colombia, eastern Colombia, and the Caribbean islands.

#### *Additional measures of connectivity*

In addition to the geographic assignment process, we looked for evidence of weak or strong connectivity by comparing the mean and variance of the  $\delta^2H_f$  values of samples from each nonbreeding season location and tested for a relationship between mean  $\delta^2H_f$  value and longitude. We tested for differences in  $\delta^2H_f$  between the nonbreeding sampling locations using an ANOVA and Tukey's post hoc test. Differences in the mean could indicate that sampling locations have different source populations of birds and therefore would indicate strong connectivity. We tested for equal variances in the nonbreeding season samples using a Levene's test of homogeneity (Hobson et al. 2014a). Unequal variance could indicate that some sampling locations have stronger connectivity, and are therefore comprised of individuals from fewer breeding locations relative to other locations. Lastly, we tested for a relationship between the longitude of nonbreeding season capture location and  $\delta^2H_f$  value for each individual using a linear regression because the core of the nonbreeding season range of the prothonotary warbler varies more longitudinally than latitudinally (Figure 1). We excluded sample sites in the Caribbean for this analysis because we expected that birds may exhibit a chain or leapfrog migration pattern across the continental portion of their nonbreeding range, two common migration strategies of songbirds (Bell 2005). A positive relationship between  $\delta^2H_f$  value and

longitude would be evidence of a chain migration, because birds with northern origins would spend the winter further west than birds with southern origins, while a negative relationship between  $\delta^2\text{H}_f$  value and longitude would be evidence of a leapfrog migration, because birds with more southern origins would winter further west than birds with northern origins (Holmgren and Lundberg 1993). We conducted all assignments, validations, and statistical analyses using the R statistics program (R Core Team 2016).

## Results

We measured hydrogen isotope ratios in 124 samples at 13 sites on the breeding grounds and 123 samples at nine sites on the nonbreeding grounds (Figure 1). Stable hydrogen values measured in feathers from birds on the nonbreeding grounds (range -82.48‰ to -20.10‰) were consistent with the range of values measured from the breeding grounds (range -83.61‰ to -20.38‰, Figure 2).

### *Calibration equation*

We found that  $\delta^2\text{H}_p$  was a significant predictor of  $\delta^2\text{H}_f$  across all breeding season samples ( $p < 0.0001$ ,  $R^2 = .51$ ). The relationship between  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$  can be explained by the equation  $y = 2.36x + 5.43$  (Figure 3). After correcting the precipitation isoscape using this calibration equation, the predicted  $\delta^2\text{H}_f$  isoscape values ranged between -97.35‰ and -9.34‰.

### *Model validation of known-origin samples*

Assignment accuracy and precision for known origin samples in the breeding grounds varied between the assignment model with no prior abundance information and the models using BBS or STEM data as prior information (Table 1). Accuracy, which was measured as percentage of birds whose true location was assigned as likely under a 2:1 odds ratio, was high for the model without priors (83% of birds assigned correctly, Table 1), and decreased when either BBS

abundance or STEM abundance was incorporated (9% of birds assigned correctly in the BBS model, and 14% in the STEM model, Table 1). Precision was low for the assignment model with no prior information (50% of cells in the raster assigned as “likely”, Table 1) compared to either assignment model using prior information (1% assigned “likely” for either model, Table 1).

The BBS model of abundance predicted high densities of birds in two main hotspots: southern Louisiana and the Atlantic coast of southern Virginia and northern North Carolina (Figure 4). The STEM model predicted greatest abundance in a larger area spanning the Mississippi Alluvial Valley and surrounding regions as well as the eastern Atlantic Coastal Plain (Figure 4). As a result, the Bayesian assignment models tended to produce assignment maps with predictions concentrated in those regions (Figure 5). In the model with no prior information, samples from the extreme southern (such as LA) and northern (such as OH) ends of the breeding range tended to result in assignment maps that were more precise (i.e., predicted a smaller portion of the breeding range as likely) compared to sampling locations in the central portion of the breeding range (such as VA) (Figure 5).

#### *Assignment of overwintering birds*

Assignment of overwintering birds to their breeding origin varied between the three assignment models (Figure 6). Assignment using no prior information was the most imprecise, with 51% of grid cells assigned as “likely”, followed by the BBS model with 1% and the STEM model with 0.6% of cells assigned as “likely”. Assignments made with either the BBS or STEM model were similar, with the majority of birds being assigned to the same areas of high abundance that were found in the known-origin validation assignments.

When comparing assignment maps from the model with no priors, all nonbreeding regions contained birds that originated from the south-central portion of the breeding range, and



none of the regions contained birds exclusively from the northern or southern part of the breeding range (Figure 6). Central America contained more birds with southern origins relative to other regions we sampled, while western Colombia contained more birds with northern origins relative to other regions.

#### *Additional measures of connectivity*

We found evidence that mean  $\delta^2H_f$  values were different across the nonbreeding season sampling locations ( $F_{8,114} = 2.37$ ,  $p = 0.02$ ), but no pairwise comparisons of sampling locations were significantly different. Variance in  $\delta^2H_f$  value did not differ between nonbreeding season sampling locations (Levene's test,  $F_{8,114} = 1.15$ ,  $p = 0.34$ ). The relationship between longitude and  $\delta^2H_f$  was significant for birds overwintering in mainland Central & South America, such that for every degree moving east,  $\delta^2H_f$  decreased by 0.69‰; however, only 5% of the variation in  $\delta^2H_f$  was explained by this relationship ( $t = -2.4$ ,  $p < 0.0001$ ,  $R^2 = 0.05$ ).

#### **Discussion**

In this study, we sought to investigate patterns of migratory connectivity in the prothonotary warbler by measuring  $\delta^2H$  values in feather samples from locations that spanned most of the breeding and wintering range. We developed a species-specific calibration equation which indicated that  $\delta^2H_p$  explains 51% of the variation in  $\delta^2H_f$  for prothonotary warblers. We tested the accuracy and precision of geographic assignment using the calibration equation and found that our model was highly accurate but imprecise when assigning birds of known origin. We compared different sources of abundance data as prior information for refining assignment models in a Bayesian framework by comparing accuracy and precision for known origin samples. Whether using BBS or STEM abundance data, we found that incorporating abundance as a prior led to more precise but less accurate assignments. Finally, we assigned overwintering

prothonotary warblers to their predicted geographic origin on the breeding grounds, and found limited support for weak migratory connectivity.

#### *Calibration equation*

Our species-specific calibration equation explained 51% of the variation in  $\delta^2\text{H}_f$  values for samples from across the prothonotary warbler breeding range in the eastern United States. This equation has a lower coefficient of determination than several other studies which created single species equations (e.g., Hobson et al. 1999:  $R^2 = 0.69$ , Paxton et al. 2007:  $R^2 = 0.91$ , Hobson et al. 2009:  $R^2 = 0.64$ , Tonra et al. 2014:  $R^2 = 0.80$ ) or multi-species equations (e.g., Clark et al. 2006:  $R^2 = 0.80$ , Hobson et al. 2012:  $R^2 = 0.83$ ). One explanation may be the influence of yearly variation in  $\delta^2\text{H}_p$  that appears have been reflected in some of our samples. Differences in  $\delta^2\text{H}_p$  can occur between years at a given site (Van Wilgenburg et al. 2012) and year of sampling has been shown to be an important predictor of variation in  $\delta^2\text{H}_f$  (Hache et al. 2012, Van Dijk et al. 2014, Chapter 1 of this study). Specifically, three of our sampling locations (Louisiana, Arkansas, and Ohio) had more negative  $\delta^2\text{H}_f$  values than expected, and these samples were all collected in 2015. Additionally, birds sampled from Alabama in 2016 had less negative  $\delta^2\text{H}_f$  values than predicted. Van Dijk et al. (2014) found that correcting a species-specific calibration equation to account for yearly variation in  $\delta^2\text{H}_f$  produced more accurate assignment results than an uncorrected equation; however, they sampled feathers in only two years and found no interaction between location and year. Our study uses samples that were collected over sixteen years and further, deviations from predicted  $\delta^2\text{H}_f$  are not consistent spatially; in contrast to what is reported above, samples collected in 2015 from South Carolina and 2016 from Wisconsin are consistent with predicted values. Tonra et al. (2015) and Vander Zanden et al. (2014) attempted to account for yearly variation in  $\delta^2\text{H}_f$  by developing year-specific

isoscapes using the IsoMap tool, but found assignment accuracy and precision were not improved, likely due to the reduction in precipitation sampling stations that occurred when restricting the inputs to only one year. Recommendations have been put forth to collect all samples from both the breeding and wintering grounds in a single year so as to minimize the risk of yearly variation (Hobson et al. 2014a), but in practice, this would require an enormous multi-national sampling effort and would preclude the use of archived feather samples or museum specimens (Hobson et al. 2012). Annual variation in  $\delta^2\text{H}_f$  values may therefore impact our ability to use these isotopes for geographic assignments.

Another explanation for the marginal fit between  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$  in this study may be the influence of local (rather than latitudinal) hydrologic processes that decouple  $\delta^2\text{H}_f$  values from the landscape-scale gradient in  $\delta^2\text{H}_p$  values, including the mixing of  $\delta^2\text{H}_p$  values with  $\delta^2\text{H}$  values of groundwater, lakes and rivers, and evaporation of surface water (Vander Zanden et al. 2016). These processes may vary locally and regionally with wetland type, and samples for this study were collected from a variety of habitats, including riparian corridors, flooded bald cypress-tupelo swamps, and reservoirs. Sampling locations with high potential for evaporative water loss (e.g., standing bodies of water or areas with high temperature/low humidity) may have a less negative  $\delta^2\text{H}_f$  value than would be expected based on  $\delta^2\text{H}_p$  because the light isotope of hydrogen is preferentially evaporated (Bowen et al. 2011). However, our only sampling site at a reservoir (Ohio), where we might expect high evaporative water loss, had a more negative  $\delta^2\text{H}_f$  value than expected. Sampling sites under the influence of the Mississippi River, especially in Arkansas, may have a more negative  $\delta^2\text{H}_f$  value due to the influx of isotopically depleted water associated with the north-south flow of this river (Bowen et al. 2011). More study is needed in order to

understand the influence of hydrologic mechanisms which lead to deviations from expected  $\delta^2\text{H}_f$  values for prothonotary warblers and other species inhabiting aquatic environments.

*Validation of known-origin samples with and without priors*

We found that a spatially-explicit assignment model that did not incorporate the prior probability of abundance was accurate but imprecise when used to validate provenance assignment of known-origin samples. The accuracy of the no priors assignment (83%) was similar to or higher than the accuracy of other studies that also used a 2:1 odds ratio (e.g., Hobson et al. 2012: 64%, Chabot et al. 2012: 71%, Tonra et al. 2014: 82-86%). However, many studies do not measure accuracy, precluding comparisons. Similarly, precision estimates, though often not reported, in our no priors assignment model were similar in precision (50% of cells assigned as likely) to that of Tonra et al. (2014). Stable hydrogen isotope analysis is acknowledged as being a low spatial resolution technique, but poor assignment precision in our study may be a result of a smaller breeding range that covers a narrower isotopic gradient (38‰) of  $\delta^2\text{H}_p$  values relative to other studies (e.g., Hobson and Wassenaar 1997: 109‰, Paxton et al. 2007: 54‰, Hobson et al. 2012: 120‰, Tonra et al. 2014: 60‰). Low precision could also potentially be a consequence of a large amount of within-site variance (i.e., the value of sigma used in the assignment model, Hobson et al. 2014a), but our sigma value (10.6) was similar to what other studies have used (Hobson et al. 2012: 9.7, Chabot et al. 2012: 10.5, Tonra et al. 2014: 11.9, Hallworth et al. 2013: 16.4). While low precision may not impact our ability to assign birds to general areas of the breeding range (e.g., southern versus northern) for birds that span a large isotopic gradient, it may be problematic for those birds with smaller ranges. Qualitatively, the results suggest that our assignment model with no priors performs reasonably well to define feather origin for a bird from the extreme northern or southern end of the breeding

range, but for a large isotopically-homogenous area in the south-central portion of the range, assignments precision limits inferences about origins. Future work to increase precision and accuracy for assignment of prothonotary warblers could incorporate morphological data that varies geographically (Rushing et al. 2014) or genetic markers into the assignment model (Clegg et al. 2003, Chabot et al. 2012, Rundel et al. 2013). Future studies should report precision estimates relative to the size of the breeding range (i.e., proportion of range) so that comparisons can be made between studies.

We found that assigning birds to geographic origin conditional on the prior probability of breeding season abundance led to a 50-fold increase in precision but a six-fold decline in accuracy. Other studies that have used abundance as prior information also observed an increase in precision when assigning birds to geographic origin, but the magnitude of the increase is difficult to compare with these studies, which do not report precision estimates (González-Prieto et al. 2011, Hallworth et al. 2013, Hobson et al. 2014a). For the BBS model, we found that samples originating from across the breeding range were predominantly assigned to one of the two population hotspots -- south central Louisiana and northern North Carolina/southern Virginia. Population estimates derived from BBS abundance data suggest that Louisiana supports ~25% of the global population of prothonotary warblers, with North Carolina and Virginia together supporting another ~20% (Partners in Flight Science Committee 2013). The STEM model assigned birds to broader regions that included the Mississippi Alluvial Valley and surrounding areas, and the eastern Atlantic Coastal Plain. This suggests that the STEM data may be less biased than the BBS data, which is known to poorly sample habitats which the prothonotary warbler prefers.

To our knowledge, there has not been another study which tests the accuracy of an assignment model using abundance data as prior information for assignment of known-origin samples from multiple geographic regions. More such studies are needed, especially that compare species with non-uniform breeding distributions to those with dispersed distributions, to determine when and if abundance data are weighted too heavily and lead to erroneous assignments. A model that is based on feather samples from low density, peripheral breeding populations that incorporates prior information on abundance will inevitably weight high density, core breeding populations as more likely origins in most cases. However, our assessment is useful because it reveals that birds from widely separated areas of the breeding range get assigned to similar locations. In contrast to what Royle and Rubenstein (2004) suggest, adding abundance data as a prior for a bird species with a breeding range that has low isotopic variation and a highly non-uniform abundance pattern may not be useful because birds are assigned exclusively to the areas of high abundance, which overrides the underlying variation in  $\delta^2\text{H}_f$ .

#### *Assignment of overwintering birds*

We assigned overwintering birds from four regions in Central America, western Colombia, eastern Colombia, and the Caribbean islands to geographic origin using models with and without priors. We examined histograms of the  $\delta^2\text{H}_f$  value for each region, which showed potentially multi-modal distributions for birds from Central America, eastern Colombia, and the Caribbean. This may indicate that birds from multiple geographic origins are present at these locations, suggesting diffuse connectivity. Our assignment maps do not indicate that any of the sampling locations contain birds exclusively from the extreme southern or northern end of the breeding range, which may also suggest weak connectivity. The two areas of highest abundance on the breeding grounds are widely separated and have distinct  $\delta^2\text{H}_f$  values, so if any regions on

the nonbreeding grounds contained exclusively birds from one or the other of the hotspots (i.e., strong connectivity), they would likely be distinguishable.

Further, we found that mean  $\delta^2\text{H}_f$  values were not different between the nonbreeding season sampling sites, which contrasts with significant differences between sites on the breeding grounds (Welch's ANOVA,  $F_{31,10} = 54.4$ ,  $p < 0.0001$ ). The presence of geographic structure to  $\delta^2\text{H}_f$  values on the breeding grounds but not the nonbreeding grounds also suggests weak connectivity. We found that the variance between all of the nonbreeding season sampling locations was similar, which suggests that the degree of connectivity is similar across all sites (Hobson et al. 2014a); that is, there is no evidence that some sites have stronger connectivity than others. We found limited support for a relationship between longitude and  $\delta^2\text{H}_f$  values, such that birds from the western part of the nonbreeding range had more southern breeding origins on average than birds from the eastern part of the wintering range. The nonbreeding season range of the prothonotary warbler varies more longitudinally than latitudinally, and this relationship could suggest a potential commonly observed leap-frog migration (Holmgren and Lundberg 1993), with southern birds migrating a shorter distance to overwinter in Central America, and northern birds continuing east. However, this relationship explained little of the variation in  $\delta^2\text{H}_f$  values.

These data indicate a high degree of mixing on the non-breeding grounds, which is consistent with results from a concurrent study using archival light-level geolocators to track prothonotary warblers through their annual cycle (Tonra et al. unpublished data). Geolocators were deployed in Virginia, South Carolina, Ohio, Louisiana, and Arkansas, and we found that some individuals from each breeding population converged in an area in north-central Colombia, likely in the Magdalena River valley. The Magdalena River delta forms an extensive mangrove forest at one of the sampling locations in this study, Vía Parque Salamanca, which is located on

the eastern coast of Colombia. STEM maps of prothonotary warbler abundance during spring migration suggest that they follow river courses when migrating inland, and if they exhibit this pattern during the nonbreeding season as well, it would make sense that there would be a high degree of mixing at Salamanca in addition to what we observe further up the river valley.

Prothonotary warblers exhibit low migratory dispersion, i.e., their nonbreeding range is smaller than their breeding range (Gilroy et al. 2016). Because of this contraction in range size, high mixing between populations would be expected (Finch et al. 2017). Low connectivity can have implications for population dynamics because events occurring at any one site on the wintering grounds can have consequences that impact the global population and not just one breeding population (Martin et al. 2007). For prothonotary warblers, understanding connectivity is important for conservation because habitat loss during the nonbreeding season may be driving population declines (Petit 1999). However, if nonbreeding season habitat loss is the primary driver of population declines and prothonotary warblers have diffuse connectivity, we would expect the resulting population declines to occur evenly across the breeding grounds. Population trends vary spatially on the breeding grounds, with some increasing or stable and others decreasing (De Sante et al. 2016), so our data suggest that declines are not due exclusively to population-specific nonbreeding season habitat loss.

In conclusion, we found that despite geographic structure in  $\delta^2\text{H}_f$  values of the prothonotary warbler, high variance in  $\delta^2\text{H}_f$  values at individual sampling locations combined with homogeneity in  $\delta^2\text{H}_p$  values across much of the breeding range resulted in an assignment model with low predictive ability due to lack of precision. Conducting assignments conditional on the prior probability of abundance did not improve predictive ability because models had low accuracy whether based on either BBS or STEM data. We suggest that future studies using stable



hydrogen isotope analysis to conduct assignment to geographic origin would be improved by testing and reporting accuracy as well as precision, and suggest that more studies should be undertaken to understand the trade-off between precision gained and accuracy lost when incorporating prior information in a Bayesian framework.

## Literature Cited

- Ashley, P., Hobson, K. a, Van Wilgenburg, S. L., North, N., & Petrie, S. a. (2010). Linking Canadian Harvested Juvenile American Black Ducks to Their Natal Areas Using Stable Isotope ( $\delta D$ ,  $\delta^{13}C$ , and  $\delta^{15}N$ ) Methods. *Avian Conservation and Ecology*, 5(2). <http://doi.org/7>
- Bell, M. (2005). Towards Rigorous Cross-National Comparison of Internal Migration : Who Collects What ? In *International Union for the Scientific Study of Population Twenty-Fifth Conference Session 401: Data and Methods in the Study of Internal Migration*.
- Bowen, G. J., Kennedy, C. D., Liu, Z., & Stalker, J. (2011). Water balance model for mean annual hydrogen and oxygen isotope distributions in surface waters of the contiguous United States. *Journal of Geophysical Research: Biogeosciences*, 116(4), 1–14. <http://doi.org/10.1029/2010JG001581>
- Bowen, G. J., Liu, Z., Vander Zanden, H. B., Zhao, L., & Takahashi, G. (2014). Geographic assignment with stable isotopes in IsoMAP. *Methods in Ecology and Evolution*, 5(3), 201–206. <http://doi.org/10.1111/2041-210X.12147>
- Bowen, G. J., Wassenaar, L. I., & Hobson, K. a. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, 143(3), 337–348. <http://doi.org/10.1007/s00442-004-1813-y>
- Chabot, A. a., Hobson, K. a., van Wilgenburg, S. L., McQuat, G. J., & Lougheed, S. C. (2012). Advances in linking wintering migrant birds to their breeding-ground origins using combined analyses of genetic and stable isotope markers. *PLoS ONE*, 7(8). <http://doi.org/10.1371/journal.pone.0043627>
- Clark, R. G., Hobson, K. a., & Wassenaar, L. I. (2006). Geographic variation in the isotopic ( $\delta D$ ,  $\delta^{13}C$ ,  $\delta^{14}N$ ,  $\delta^{34}S$ ) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. *Canadian Journal of Zoology*, 84, 1395–1401. <http://doi.org/10.1139/z06-135>
- Clegg, S. M., Kelly, J. F., Kimura, M., & Smith, T. B. (2003). Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson. *Molecular Ecology*, 50(4), 308–830. <http://doi.org/10.1046/j.1365-294X.2003.01757.x>
- Cline, M. H., Strong, A. M., Sillett, T. S., Rodenhouse, N. L., & Holmes, R. T. (2013). Correlates and Consequences of Breeding Dispersal in a Migratory Songbird. *The Auk*, 130(4), 742–752. <http://doi.org/10.1525/auk.2013.12244>
- Desante, D. F. (2015). *Vital Rates of North American Landbirds*. Retrieved from [www.VitalRatesOfNorthAmericanLandbirds.org](http://www.VitalRatesOfNorthAmericanLandbirds.org)

- Finch, T., Butler, S., Franco, A., & Cresswell, W. (2017). Low migratory connectivity is common in long-distance migrant birds. *Journal of Animal Ecology*, *38*(1), 42–49. <http://doi.org/10.1111/1365-2656.12635>
- Fink, D., Hochachka, W. M., Zuckerman, B., Winkler, D. W., Shaby, B., Munson, M. A., ... Kelling, S. (2010). Spatiotemporal exploratory models for broad-scale survey data. *Ecological Applications*, *20*(8), 2131–2147. <http://doi.org/10.1890/09-1340.1>
- Fournier, A. M. V., Sullivan, A. R., Bump, J. K., Perkins, M., Shieldcastle, M. C., & King, S. L. (2016). Combining citizen science species distribution models and stable isotopes reveals migratory connectivity in the secretive Virginia rail. *Journal of Applied Ecology*, 1–10. <http://doi.org/10.1111/1365-2664.12723>
- Gilroy, J. J., Gill, J. A., Butchart, S. H. M., Jones, V. R., & Franco, A. M. A. (2016). Migratory diversity predicts population declines in birds. *Ecology Letters*, *19*(3), 308–317. <http://doi.org/10.1111/ele.12569>
- González-Prieto, A. M., Hobson, K. A., Bayly, N. J., & Gómez, C. (2011). Geographic Origins and Timing of Fall Migration of the Veery in Northern Colombia. *The Condor*, *113*(4), 860–868. <http://doi.org/10.1525/cond.2011.100245>
- Haché, S., Hobson, K. a, Villard, M., & Bayne, E. (2012). Assigning birds to geographic origin using feather hydrogen isotope ratios ( $\delta^2\text{H}$ ): importance of year, age, and habitat. *Canadian Journal of Zoology*, *90*(6), 722–728. <http://doi.org/10.1139/z2012-039>
- Hallworth, M. T., Studds, C. E., Sillett, T. S., & Marra, P. P. (2013). Do archival light-level geolocators and stable hydrogen isotopes provide comparable estimates of breeding-ground origin? *The Auk*, *130*(2), 273–282. <http://doi.org/10.1525/auk.2013.13037>
- Hobson, K. A. (1999). Tracing Origins and Migration of Wildlife Using Stable Isotopes: A Review. *Source: Oecologia*, *120*(3), 314–326. Retrieved from <http://www.jstor.org/stable/4222394>
- Hobson, K. A., Wassenaar, L. I., & Taylor, O. R. (1999). Stable isotopes (  $\text{dD}$  and  $\text{d}^{13}\text{C}$  ) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia*, *120*(3), 397–404. <http://doi.org/10.1007/s004420050872>
- Hobson, K. a., Van Wilgenburg, S. L., Dunn, E. H., Hussell, D. J. T., Taylor, P. D., & Collister, D. M. (2015). Predicting origins of passerines migrating through Canadian migration monitoring stations using stable-hydrogen isotope analyses of feathers: a new tool for bird conservation. *Avian Conservation and Ecology*, *10*(1), 3. <http://doi.org/10.5751/ACE-00719-100103>
- Hobson, K. A., Van Wilgenburg, S. L., Faaborg, J., Toms, J. D., Rengifo, C., Sosa, A. L., ... Brito Aguilar, R. (2014). Connecting breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopes: A call for an isotopic atlas of migratory

- connectivity. *Journal of Field Ornithology*, 85(3), 237–257.  
<http://doi.org/10.1111/jofo.12065>
- Hobson, K. a., Van Wilgenburg, S. L., Wassenaar, L. I., & Larson, K. (2012). Linking hydrogen isotopes in feathers and precipitation: Sources of variance and consequences for assignment to isoscapes. *PLoS ONE*, 7(4), 1–9. <http://doi.org/10.1371/journal.pone.0035137>
- Hobson, K. a., Wilgenburg, S. L. Van, Wesolowski, T., Maziarz, M., Bijlsma, R. G., Grendelmeier, A., & Mallord, J. W. (2014). A Multi-Isotope ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) Approach to Establishing Migratory Connectivity in Palearctic-Afrotropical Migrants: An Example using Wood Warblers *Phylloscopus sibilatrix*. *Acta Ornithologica*, 49(1), 57–69. <http://doi.org/10.3161/000164514X682896>
- Hobson, K. a., Wunder, M. B., Van Wilgenburg, S. L., Clark, R. G., & Wassenaar, L. I. (2009). A method for investigating population declines of migratory birds using stable isotopes: Origins of harvested lesser scaup in North America. *PLoS ONE*, 4(11). <http://doi.org/10.1371/journal.pone.0007915>
- Hobson, K., & Wassenaar, L. I. (2008). *Tracking Animal Migration with Stable Isotopes*. Academic Press.
- Holmgren, N., & Lundberg, S. (1993). Despotic behaviour and the evolution of migration patterns in birds. *Ornis Scandinavica*, 24(2), 103–109.
- Hostetler, J. a., Sillett, T. S., & Marra, P. P. (2015). Full-annual-cycle population models for migratory birds. *The Auk*, 132(2), 433–449. <http://doi.org/10.1642/AUK-14-211.1>
- Johnston, A., Fink, D., Reynolds, M. D., Hochachka, W. M., Sullivan, B. L., Bruns, N. E., ... Kelling, S. (2015). Abundance models improve spatial and temporal prioritization of conservation resources. *Ecological Applications*, 25(7), 1749–1756. <http://doi.org/10.1890/07-1650.1>
- Marra, P. P., Cohen, E. B., Loss, S. R., Rutter, J. E., & Tonra, C. M. (2015). A call for full annual cycle research in animal ecology. *Biology Letters*, 11(8), 2015.0552. <http://doi.org/10.1098/rsbl.2015.0552>
- Martin, T. G., Chadès, I., Arcese, P., Marra, P. P., Possingham, H. P., & Norris, D. R. (2007). Optimal conservation of migratory species. *PLoS ONE*, 2(8), 3–7. <http://doi.org/10.1371/journal.pone.0000751>
- Mckinnon, E. a., Fraser, K. C., & Stutchbury, B. J. M. M. (2013). New discoveries in landbird migration using geolocators, and a flight plan for the future. *The Auk*, 130(2), 211–222. <http://doi.org/10.1525/auk.2013.130.2.12226>

- Mills, A. M., & Weir, C. J. (2007). Winter commingling of populations of migratory species can cause breeding range underpopulation. *Oikos*, *116*(12), 2062–2070. <http://doi.org/10.1111/j.2007.0030-1299.15964.x>
- Nordell, C. J., Haché, S., Bayne, E. M., Sólymos, P., Foster, K. R., Godwin, C. M., ... Hobson, K. A. (2016). Within-Site Variation in Feather Stable Hydrogen Isotope ( $\delta^2\text{Hf}$ ) Values of Boreal Songbirds: Implications for Assignment to Molt Origin. *Plos One*, *11*(11), e0163957. <http://doi.org/10.1371/journal.pone.0163957>
- Paxton, K. L., Van Riper III, C., Theimer, T. C., & Paxton, Eben, H. (2007). Spatial and Temporal Migration Patterns of Wilson’s Warbler (*Wilsonia Pusilla* ) in the Southwest As Revealed By Stable Isotope
- Pekarsky, S., Angert, A., Haese, B., Werner, M., Hobson, K. A., & Nathan, R. (2015). Enriching the isotopic toolbox for migratory connectivity analysis: A new approach for migratory species breeding in remote or unexplored areas. *Diversity and Distributions*, *21*(4), 416–427. <http://doi.org/10.1111/ddi.12306>
- Petit, L. J. (1999). Prothonotary Warbler (*Protonotaria citrea*). *The Birds of North America Online*, *26818421*. <http://doi.org/10.2173/bna.408>
- Pyle, P. (1997). *Identification Guide to North American Birds: Part 1*. Slate Creek Press.
- Ridgely, R. S., Allnutt, T. F., Brooks, T., McNicol, D. K., Mehlman, D. W., Young, B. E., & Zook, J. R. (2003). Digital distribution maps of the birds of the western hemisphere. Arlington, VA: NatureServe.
- Royle, J. A., & Rubenstein, D. R. (2004). The Role of Species Abundance in Determining Breeding Origins of Migratory Birds with Stable Isotopes. *Ecological Applications*, *14*(6), 1780–1788.
- Rundel, C. W., Wunder, M. B., Alvarado, A. H., Ruegg, K. C., Harrigan, R., Schuh, A., ... Novembre, J. (2013). Novel statistical methods for integrating genetic and stable isotope data to infer individual-level migratory connectivity. *Molecular Ecology*, *22*(16), 4163–4176. <http://doi.org/10.1111/mec.12393>
- Rushing, C. S., Ryder, T. B., Saracco, J. F., & Marra, P. P. (2014). Assessing Migratory Connectivity for a Long-Distance Migratory Bird Using Multiple Intrinsic Markers. *Ecological Applications*, *24*(3), 445–456. <http://doi.org/10.1890/13-1091.1>
- Sauer, J. R., Link, W. a., Fallon, J. E., Pardieck, K. L., & Ziolkowski, D. J. (2013). The North American Breeding Bird Survey 1966–2011: Summary Analysis and Species Accounts. *North American Fauna*, *79*(79), 1–32. <http://doi.org/10.3996/nafa.79.0001>

- Stutchbury, B. J. M., Tarof, S. a, Done, T., Gow, E., Kramer, P. M., Tautin, J., ... Afanasyev, V. (2009). Tracking Long-Distance Songbird Migration by Using Geolocators. *Science*, 323(5916), 896. <http://doi.org/10.1126/science.1166664>
- Tonra, C. M., Both, C., & Marra, P. P. (2014). Incorporating site and year-specific deuterium ratios from precipitation into geographic assignments of a migratory bird. *Journal of Avian Biology*, 46(3), 266–274. <http://doi.org/10.1111/jav.00553>
- van Dijk, J. G. B., Meissner, W., & Klaassen, M. (2014). Improving provenance studies in migratory birds when using feather hydrogen stable isotopes. *Journal of Avian Biology*, 45(1), 103–108. <http://doi.org/10.1111/j.1600-048X.2013.00232.x>
- Van Wilgenburg, S. L., & Hobson, K. A. (2011). Combining stable-isotope and band recovery data to improve probabilistic assignment of migratory birds to origin. *Ecological Applications*, 21(4), 1340–1351. <http://doi.org/10.1890/09-2047.1>
- Van Wilgenburg, S. L., Hobson, K. A., Brewster, K. R., & Welker, J. M. (2012). Assessing dispersal in threatened migratory birds using stable hydrogen isotope analysis of feathers. *Endangered Species Research*, 16(1), 17–29. <http://doi.org/10.3354/esr00383>
- Vander Zanden, H. B., Soto, D. X., Bowen, G. J., & Hobson, K. A. (2016). Expanding the Isotopic Toolbox: Applications of Hydrogen and Oxygen Stable Isotope Ratios to Food Web Studies. *Frontiers in Ecology and Evolution*, 4(March), 1–19. <http://doi.org/10.3389/fevo.2016.00020>
- Vander Zanden, H. B., Wunder, M. B., Hobson, K. A., Van Wilgenburg, S. L., Wassenaar, L. I., Welker, J. M., & Bowen, G. J. (2014). Contrasting assignment of migratory organisms to geographic origins using long-term versus year-specific precipitation isotope maps. *Methods in Ecology and Evolution*, 5(9), 891–900. <http://doi.org/10.1111/2041-210X.12229>
- Wassenaar, L. I., & Hobson, K. A. (2003). Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies*, 39(3), 211–217. <http://doi.org/10.1080/1025601031000096781>
- Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S., & Holmes, R. T. (2002). Links between worlds: unraveling migratory connectivity. *Trends in Ecology & Evolution*, 17(2), 76–83. [http://doi.org/10.1016/S0169-5347\(01\)02380-1](http://doi.org/10.1016/S0169-5347(01)02380-1)
- Partners in Flight Science Committee Population Estimates Database. (2013). Retrieved April 19, 2017, from <http://rmbo.org/pifpopestimates>

Table 1. Breeding season sampling locations of prothonotary warbler feathers.  $\delta^2\text{H}_p$  values are extracted at each location from a long-term precipitation isoscape developed using the IsoMap tool. Calibrated  $\delta^2\text{H}_f$  values were calculated using the equation  $\delta^2\text{H}_{f(\text{calibrated})} = 2.36 * \delta^2\text{H}_p + 5.43$ . Measured  $\delta^2\text{H}_f$  values are the mean of all samples at each site.

<b>Location</b>	<b>N</b>	<b>Latitude</b>	<b>Longitude</b>	<b><math>\delta^2\text{H}_p</math></b>	<b>Calibrated <math>\delta^2\text{H}_f</math></b>	<b>Measured <math>\delta^2\text{H}_f</math></b>
LA	10	29.85624	-92.13187	-12.86	-24.91	-29.41
AL	4	32.34805	-85.88846	-18.86	-39.07	-27.79
TX	9	33.15167	-96.60000	-18.73	-38.77	-28.46
SC	10	33.21908	-80.34773	-16.37	-33.20	-33.42
AR	10	34.35805	-91.09088	-18.48	-38.17	-51.97
NC	10	35.39861	-78.28944	-21.18	-44.55	-37.32
Western KY	4	36.92778	-88.46417	-20.09	-41.98	-39.21
VA	39	37.41212	-77.29948	-26.76	-57.72	-53.87
Eastern KY	3	37.97722	-86.02861	-22.77	-48.31	-44.82
Southern IN	4	38.87028	-86.90194	-22.50	-47.66	-45.93
Northern IN	3	39.30306	-86.73389	-23.08	-49.04	-51.79
OH	10	40.10720	-82.88641	-28.23	-61.19	-74.95
WI	8	42.52765	-89.31818	-28.83	-62.60	-67.03

Table 2. Accuracy and precision estimates for spatially-explicit geographic assignments of known-origin prothonotary warblers. Accuracy is measured as the proportion of individuals whose assignment map overlaps the true location of origin under a 2:1 odds ratio. Precision is measured as the number of cells assigned as likely under the 2:1 odds scenario, with proportion of total cells in parentheses.

<b>Accuracy</b>				
	n	No priors	BBS priors	STEM priors
LA	5	1	0.6	0
TX	5	0.6	0	0
AR	5	0.8	0	0.4
WI	4	0.75	0	0
SC	5	1	0	0
NC	4	1	0	0
VA	19	0.89	0.16	0.74
OH	5	0.6	0	0
All sites	52	0.83	0.09	0.14
<b>Precision</b>				
Mean		3781 (50)	67 (1)	55 (1)
SD		520	12	47



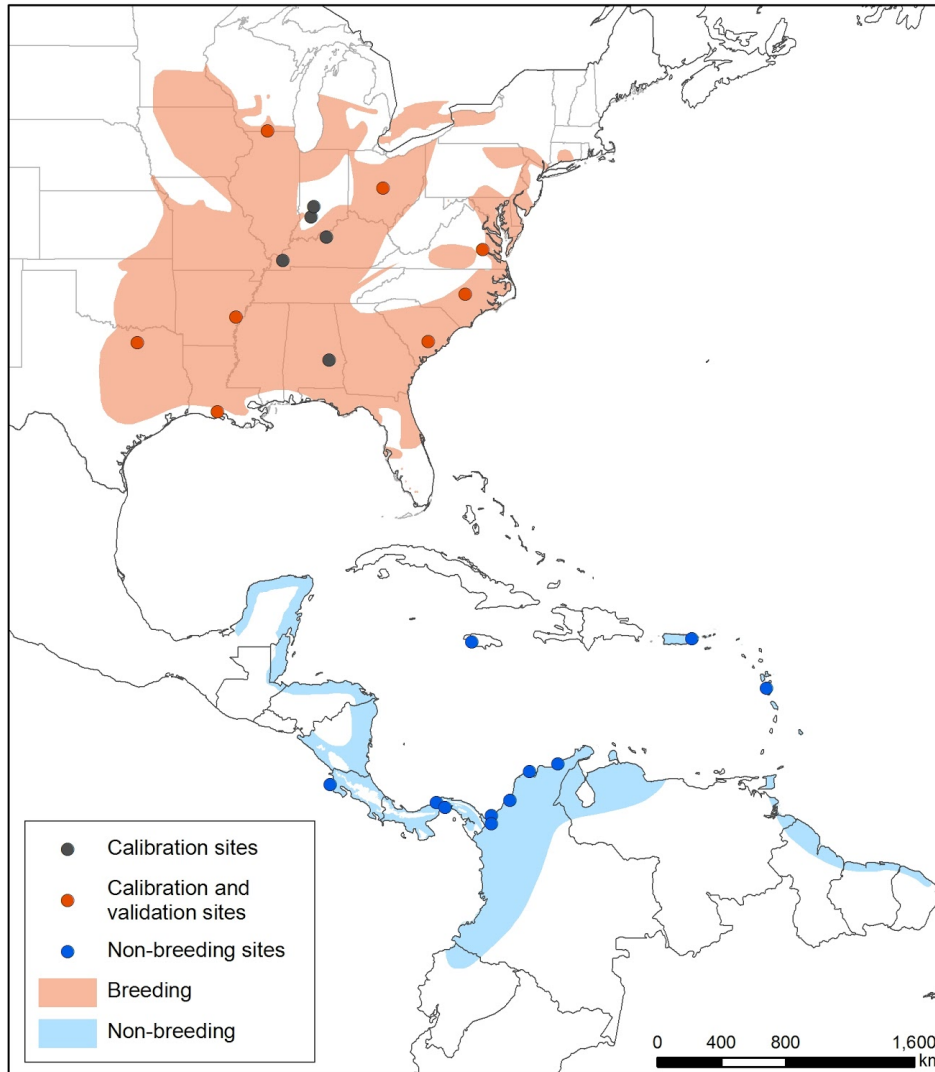


Figure 1. Range of the prothonotary warbler (adapted from Ridgely et al. 2003). Calibration sites were used to build a calibration equation to describe the relationship between  $\delta^2\text{H}_f$  and  $\delta^2\text{H}_p$ , and validation sites were used to assess the accuracy of spatially-explicit geographic assignments based on that relationship. Feathers collected from nonbreeding sites were assigned to their geographic origin on the breeding grounds.

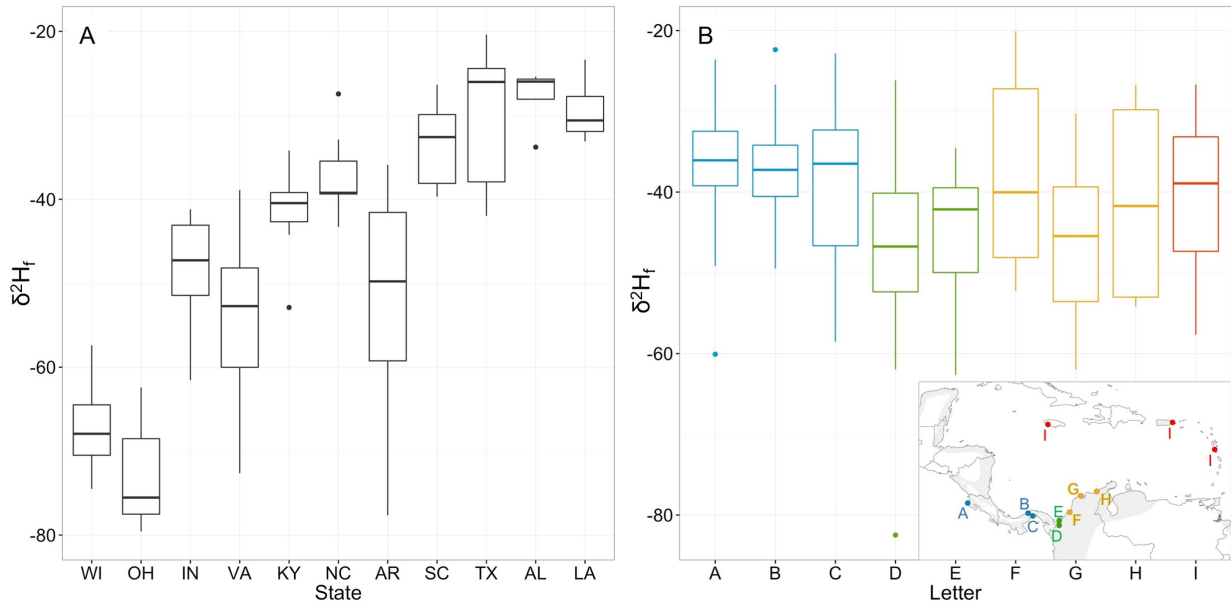


Figure 2.  $\delta^2\text{H}_f$  values for breeding season samples (A) and nonbreeding season samples (B) of prothonotary warblers. Map inset shows the location of each nonbreeding season sampling location, which are grouped by region: blue - Central America, green - western Colombia, yellow - eastern Colombia, red - Caribbean islands. Grey shading in the map indicates the extent of the prothonotary warbler nonbreeding season range.

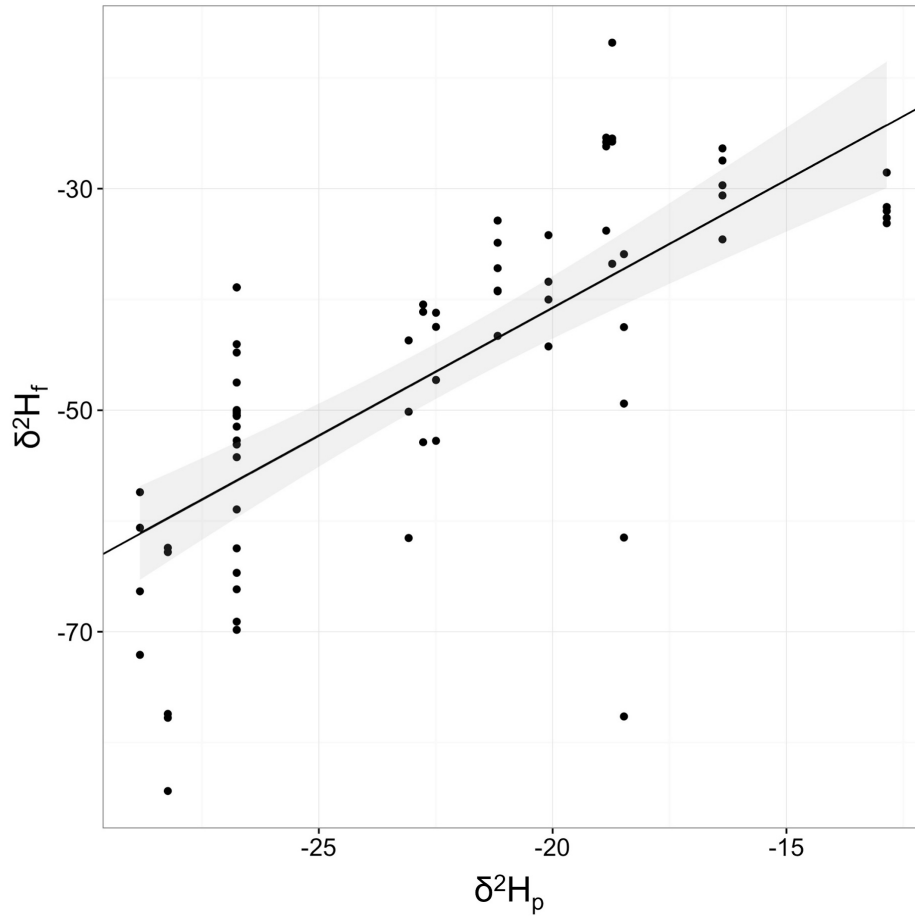


Figure 3. The relationship between  $\delta^2H_p$  and  $\delta^2H_f$  for 72 prothonotary warbler feathers collected from after-second year individuals at 13 sites on the breeding grounds. The equation of the linear model was  $y = 2.31x + 5.43$  ( $p < 0.0001$ ,  $R^2 = .51$ ).

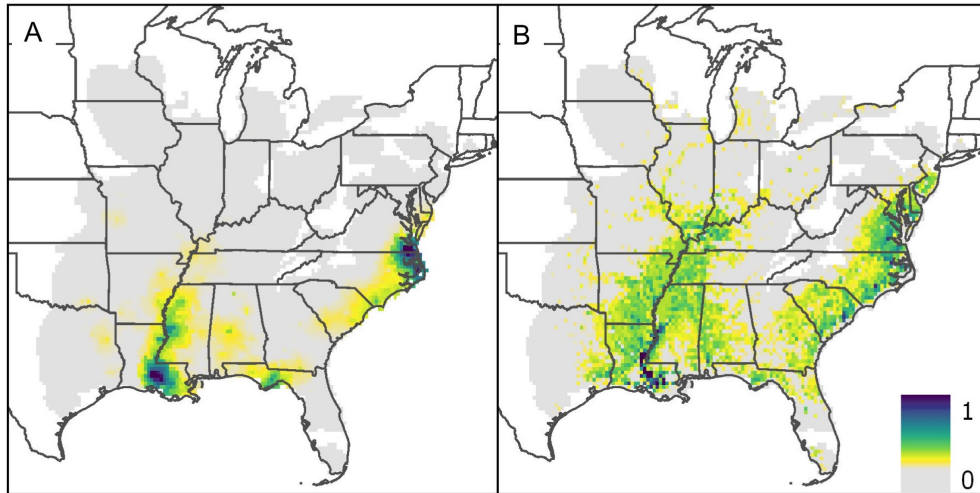


Figure 4. Relative abundance of prothonotary warblers on the breeding grounds as estimated by A) Breeding Bird Survey data and B) a spatio-temporal exploratory model developed using eBird count and remote sensing data (Fink et al. 2010, Johnston et al. 2015).

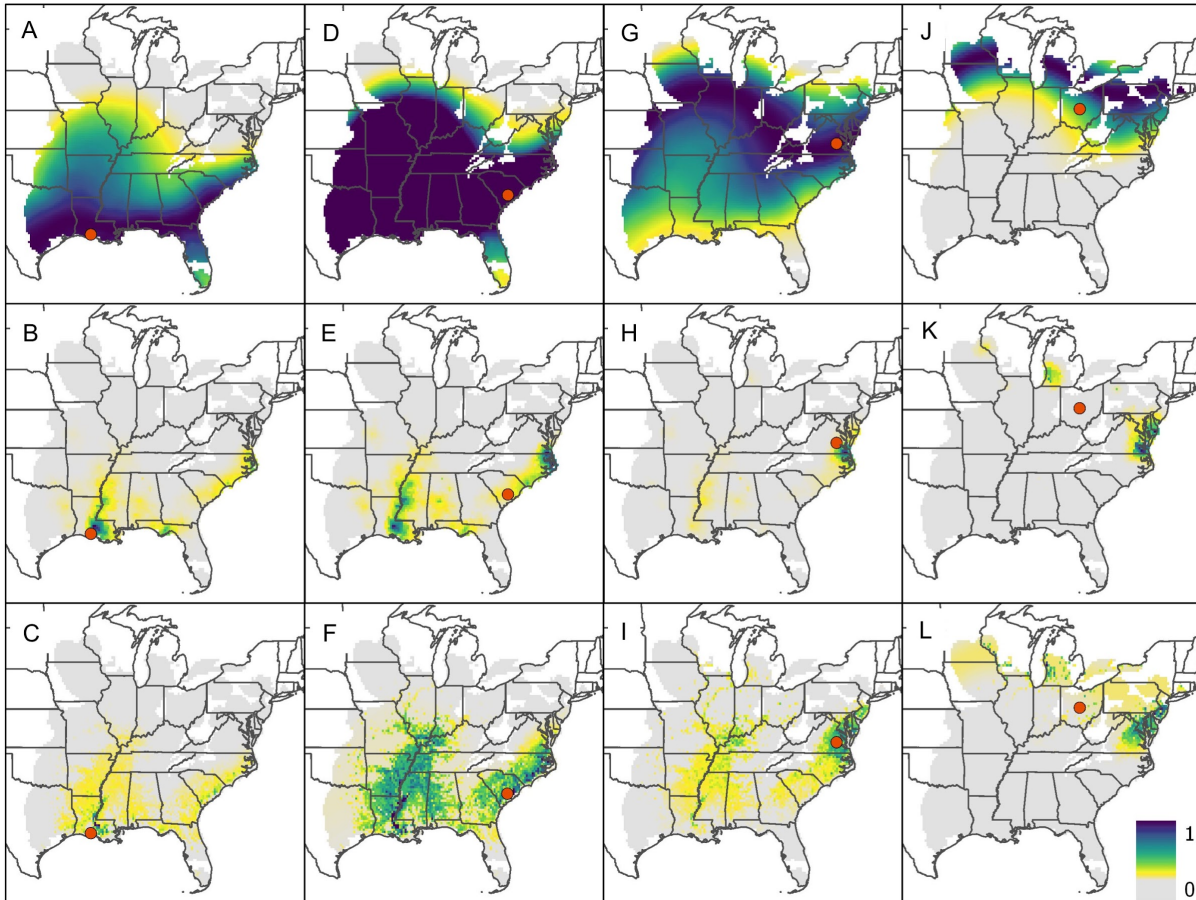


Figure 5. Predicted probability of origin using stable hydrogen isotopes for known-origin feather samples from prothonotary warblers from A-C) Louisiana, D-F) South Carolina, G-I) Virginia, and J-L) Ohio. The first row shows assignments based only on the relationship between hydrogen isotopes measured in feathers and precipitation, while the second and third rows show assignments conducted in a Bayesian framework with BBS or STEM abundance data as a prior, respectively.

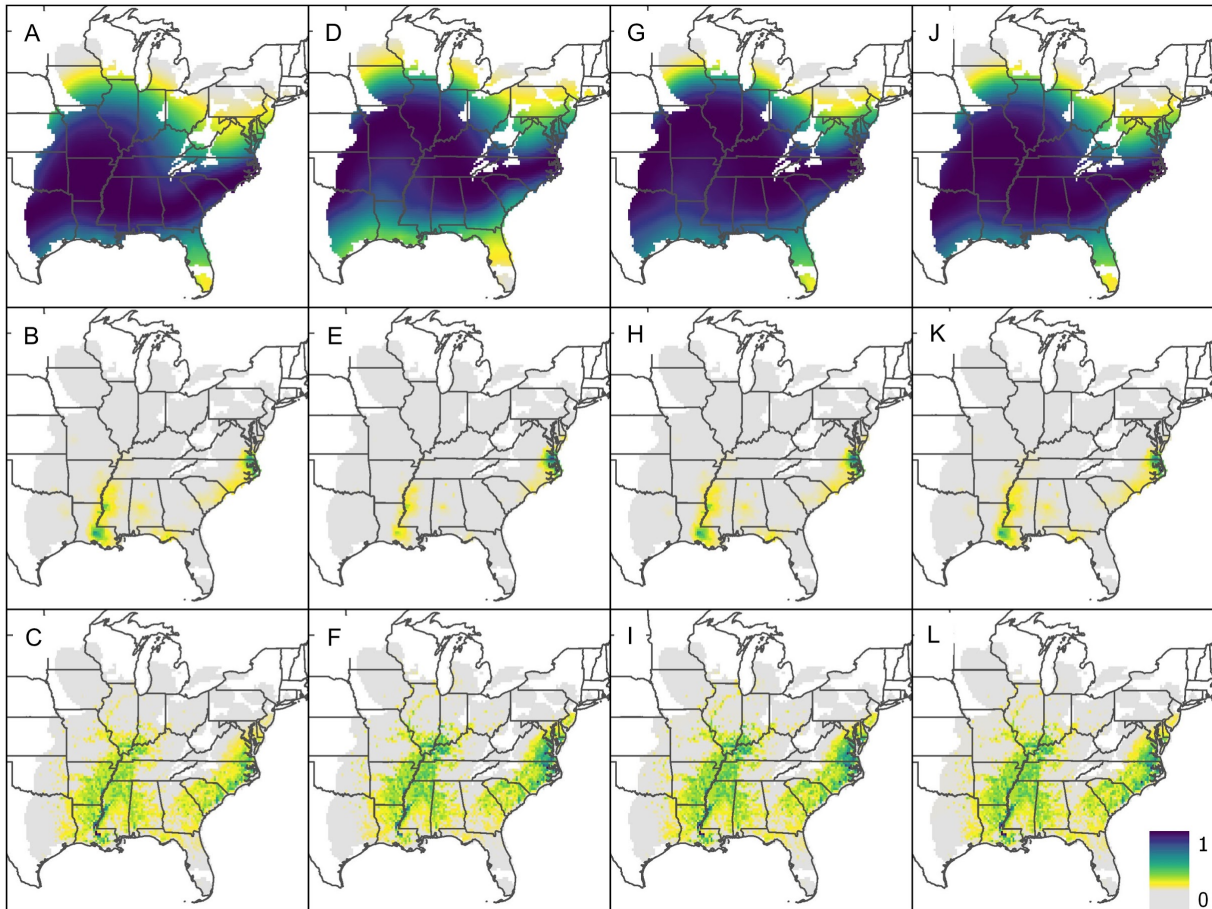


Figure 6. Predicted probability of origin using stable hydrogen isotopes for feather samples collected from prothonotary warbler from four regions of the nonbreeding grounds. A-C) Central America, D-F) western Colombia, G-I) eastern Colombia, J-L) Caribbean islands. The first row shows assignments based only on the relationship between hydrogen isotopes measured in feathers and precipitation, while the second and third rows show assignments conducted in a Bayesian framework with BBS or STEM abundance data as a prior, respectively.

## Vita

Jessie Reese was born in Philadelphia, Pennsylvania on June 6, 1987 and graduated from Turner Ashby High School in 2005. She earned a Bachelor of Science in Biology with a concentration in Ecology and Environmental Biology from James Madison University in 2009. She pursued an undergraduate research project under the guidance of Dr. Cheryl Talley. Following graduation, she worked in the lab of Dr. Roger Hanlon at the Marine Biological Laboratory and served for one year in AmeriCorps as an environmental educator at the Roger Williams Park Zoo. Next, she spent four years conducting avian field research for organizations such as The Institute for Bird Populations and various universities studying population dynamics, breeding ecology, migratory stopover ecology, and tropical avian ecology. After graduating from Virginia Commonwealth University, she plans to combine avian field research with outreach and mentorship to promote conservation.