Using Stable-isotopes in Bird Feathers to Assess the Benefits of Local Conservation Actions

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Project Overview

Natal dispersal, the movement from a natal site (place of birth) to a new breeding site, is probably the most important and least understood life history trait in wild animals. It is fundamental to the ecological understanding of landscapes, populations and organisms, and a necessary consideration when devising conservation plans. Despite the fact that natal dispersal (or lack thereof) drives population biology, colonization and range expansion, metapopulation and source-sink population dynamics, and the genetic structure in populations, we know very little about it. We know particularly little about natal dispersal in Neotropical migratory birds, many of which are high on conservation priority lists. Therefore, it has been difficult to assess the full impact of local conservation actions designed to benefit breeding populations of Neotropical migratory birds. Recent developments in stable-isotope techniques allow us to now use feathers to determine the origins of migratory birds. By studying the stable-isotope signature of feathers collected from the Neotropical migratory Prothonotary Warblers (*Protonotaria citrea*) that were newly recruited into local breeding populations in the Cache River watershed in Illinois, we can better assess the effectiveness of local conservation actions that increase the nesting success of birds (e.g. land acquisition and reforestation, wetland restoration), and measure the benefits of local conservation actions to the local breeding bird community. In collaboration with Dr. Keith Hobson, a world-renowned expert in stable-isotopes and animal migration, and with additional assistance from the Florida Museum of Natural History (Dr. Scott Robinson), this research addressed the questions: 1) Does local reproduction maintain local populations in a migratory species (warblers produced in the Cache return to the Cache to breed); and 2) to what extent are local populations maintained (“rescued”) by birds that are dispersing
into the system from distant sources? The answers to these related questions have profound implications for how we evaluate the effectiveness of conservation actions.

**Project Justification**

Natal dispersal, the movement from a natal site (place of birth) to a new breeding site, is probably the most important and least understood life history trait (Clobert et al. 2001). It is fundamental to the ecological understanding of landscapes, populations and organisms, and a necessary consideration when devising conservation plans. Natal dispersal has remained a mystery in migratory songbirds, largely because few of the nestlings banded on study sites ever return to those sites in subsequent years (Weatherhead and Forbes 1994; Winkler et al. 2005). This has left researchers and conservation practitioners wondering if the birds not returning are dead or if they have dispersed to locations (nearby or distant) outside of the study area. Similarly, we know little or nothing about the origin of migratory birds immigrating into our study populations each year. Despite the fact that natal dispersal (or lack thereof) drives population biology, colonization and range expansion, metapopulation and source-sink population dynamics, and the genetic structure in populations, we know very little about it, particularly in the Neotropical migratory birds that are high on priority lists of conservation organizations (Clobert et al. 2001; Hobson 2005a; Webster and Marra 2005). Therefore, the full impact of local conservation actions designed to benefit breeding Neotropical migratory birds has been difficult to assess. We are left with the question: How much do local conservation actions benefit local populations of Neotropical migratory birds?

The recent development of stable-isotope analysis techniques allows us to now attempt to answer this question (Hobson 2005b; Kelly et al. 2005). Stable-hydrogen isotope ratios (also
referred to as deuterium) in birds’ feathers reflect those of local precipitation (Chamberlain et al. 1997; Hobson and Wassenaar 1997). Because there is a strong north to south gradient in the hydrogen isotope ratios (Fig. 1) this measure has been useful in assessing the origins of migratory species (Hobson 1999, 2005a). The stable-isotope signature in feathers is that of where the feather was grown (Hobson 2005b). The stable-isotope ratios in a given area accumulate in feathers as they are grown and are assimilated into feathers through the food chain: rain filters through soil, plants take up water in soil, insects eat plants, and birds eat insects while growing their feathers. Once the feather is fully grown, its stable-isotope signature does not change. Therefore, a feather grown by a nestling/juvenile Neotropical migratory bird on the breeding grounds (at its birthplace) should bear the stable-isotope signature of that location. However, one difficulty is that most migratory birds molt their natal (juvenile) feathers prior to returning to breed for the first time. The Prothonotary Warbler is an exception to this rule. They are one of only a few migratory songbirds that retain their natal tail feathers (grown at their place of birth) through their first breeding season (Pyle et al. 1987), therefore allowing us to possibly determine their point of origin.

By studying the stable-isotope signature of feathers collected from the Neotropical migratory Prothonotary Warblers that are newly recruited into local breeding populations in the Cache River watershed in Illinois, we can better assess the effectiveness of local conservation actions that increase the nesting success of birds (e.g. land acquisition and reforestation, wetland restoration), and measure the benefits of local conservation actions to the local breeding bird community. In collaboration with Dr. Keith Hobson, a world-renowned expert in stable-isotopes and animal migration, and with matching funds from the Florida Museum of Natural History (Dr. Scott Robinson), our goal was to address the questions: 1) Does local reproduction maintain
local populations in a migratory species (warblers produced in the Cache return to the Cache to breed); and 2) to what extent are local populations maintained (“rescued”) by birds that are dispersing into the system from distant sources? The answers to these related questions have profound implications for how we evaluate the effectiveness of conservation actions. The results of this study, in conjunction with close monitoring of songbird populations in the Cache River watershed, should aid in determining the extent to which conservation actions within Illinois actually affect populations of a migratory songbird.

**Project Objectives**

1. **Develop a stable-isotope profile for Prothonotary Warbler feathers grown in the Cache River watershed.**

2. **Compare the actual values of the stable-isotopes in Prothonotary Warbler feathers which were grown in the Cache River watershed with predicted values of stable-isotopes given the latitude where samples were collected** (Figure 1 shows precipitation values for hydrogen, values of -19 to -25 are added to these precipitation values to estimate what the hydrogen values should be in bird feathers grown at a particular location; Wassenaar and Hobson 2001, Bowen et al. 2005). This comparison will be one of the first of its kind and will test the relatively new technique of using stable-isotopes in bird feathers to determine where the feather was grown (point of origin). For the warblers in this group, we know that their tail feathers were grown on site in the Cache River watershed, we know what the predicted isotope levels should be given the latitude of the watershed, and we can now test to see how closely actual values match predicted values. The results of this analysis will
validate the stable-isotope technique, and also allow us to determine how precisely (within 10s versus 100s of kilometers) we can estimate “point of origin”. One potential challenge is that the technique of using hydrogen isotopes in feathers to determine point of origin has been championed in bird species that use habitats that are more terrestrial (uplands). The Prothonotary Warblers used as the focal species of this study are tightly associated with forested wetlands, and the transfer of hydrogen from the water through the food chain to the bird feathers in this more-aquatic system may be quite different than for a more-terrestrial species of bird (K. Hobson, personal communication).

3) **Determine what proportion of the one-year-old warblers in the breeding population were produced locally the previous year based on the stable-isotope signature of their tail feathers.** To accomplish this we will compare the stable-isotope values of tail feathers of one-year-old Prothonotary Warblers that originated in the Cache River watershed (warblers that were banded as nestlings, grew their original tail feathers in the watershed, and were captured the next year still bearing their original tail feathers), with one-year-old warblers from an unknown origin (unbanded, grew their original tail feathers at an unknown location the previous year while they were nestlings/juveniles).

4) **Catalog the stable-isotope profiles of Prothonotary Warbler feathers grown in the Cache River watershed for future comparisons with feathers collected from Prothonotary Warblers spending their first winters in Central America and northern South America.** Feathers collected on wintering grounds in future years will allow us to link breeding and wintering areas for this, and possibly other, Neotropical migrant birds.
(5) **Use this information to determine whether local conservation efforts to improve nesting success** (e.g. land acquisition and habitat restoration that reduces nest predation and cowbird parasitism, off-channel wetland restoration that increases reproductive output) **actually have a positive effect on the population dynamics of the migratory songbirds that are supposed to benefit.** In other words, we will be able to answer the question: Do locally produced migratory birds tend to return to breed near where they were produced, or are local populations maintained (“rescued”) by birds that are dispersing into the system from distant sources? The answer to this question has direct application to how we evaluate the effectiveness of conservation actions.

**Project Methodology**

**Study system**- Feathers were collected from Prothonotary Warblers breeding within the Cache River watershed in southern Illinois (37° 18’ N, 88° 58’ W; Fig. 2). The Cache River has a total length of 176 km and meanders through the southern tip of Illinois to the Ohio River, draining 1,537 km² of land. Breeding habitat for these warblers consists of wet floodplain forests, forested sloughs and backwaters, and bald cypress (*Taxodium distichum*) and tupelo (*Nyssa aquatica*) swamps located within a 16x12 km (19,200 ha) portion of the watershed.

**Study organism**- The Prothonotary Warbler is a migratory bird that winters in the Neotropics and breeds in forested wetlands throughout much of the eastern half of the United States (Petit 1999). This species is territorial and socially monogamous (Petit and Petit 1996). Prothonotary warblers build their nests in secondary cavities, and associate closely with standing water in bottomland and swamp forests (Petit 1999; Askins 2000). These warblers readily use nest
boxes when available (Petit 1989; Blem and Blem 1994; Hoover 2003) and can be studied in great
detail during the breeding season (e.g., Petit and Petit 1996; Hoover 2003, 2006). Because the
breeding habitat (forested wetlands) of the prothonotary warbler typically occurs in discrete
patches in the study area, we knew the identity of >95% of individuals breeding on our study sites
every year (Hoover 2003, 2006).

Prothonotary Warblers are one of the few Neotropical migratory songbirds that breed in
North America that have only a partial molt during their first year of life (Pyle et al. 1987). What
this means is that some of the feathers grown by Prothonotary Warbler nestlings (and newly
fledged offspring) on their natal site (their birthplace) are retained through their first breeding
season the following year. The tail feathers of Prothonotary Warblers are not molted until after
their first breeding season. Therefore, these tail feathers possess the chemical signature of the
latitude where the feather was grown (their birthplace) (Hobson 2005b). Prothonotary Warblers
can also be accurately aged during the breeding season and placed into one of two categories based
on plumage, feather wear, and feather length: 1) one year old; or 2) at least 2 years old (Pyle et al.
1987). The combination of a partial first molt, along with the ability to distinguish individuals that
are one year old makes the Prothonotary Warbler one of the only species of Neotropical migratory
songbirds whose feather isotopes can be used in an attempt to determine where birds breeding for
the first time were produced the previous year. Therefore, the focus of this proposal is on one-year-
old Prothonotary Warblers breeding in the Cache River watershed.

Banding nestlings and recapturing those that return- During 2003-2005, 2,223
Prothonotary Warbler nestlings were banded with a USFWS aluminum band when they were 8-9
days old (approx. 2-3 days before they leave the nest). We captured 156 of these banded nestlings
that returned the very next year to breed (one-year-olds) and collected a single tail feather from
each. Males were captured using a mist net, decoy, and taped playback of a male song. Females were captured while in their nest boxes. These 156 feather samples were from individuals produced in the Cache River watershed the previous year (feathers of known origin) and the stable-isotope values for these feathers were used to create the hydrogen isotope profile for Prothonotary Warblers produced in the forested wetlands of the Cache River watershed.

**Catching unbanded one-year-old adults of unknown origin** - All adults breeding on study sites in the Cache River watershed (Figure 2) were captured during 2004-2006. We collected single tail feathers from 196 individuals that were determined to be one-year-old birds, and that were unbanded when we initially captured them. This group of one-year-old birds originated (i.e. were born) at locations unknown to us (feathers of unknown origin). We banded each unmarked individual with a unique color-band/USFWS aluminum band combination. Males were captured using a mist net, decoy, and taped playback of a male song. Females were captured while in their nest boxes.

**Lab techniques** - Tail feathers collected from Prothonotary warblers were stored in glassine envelopes until they were sent to the lab of Dr. Keith Hobson in Canada, processed and analyzed. At the lab, feathers were cleaned of surface oils using a 2:1 chloroform:methanol solution. Single tail feather sub-samples (0.31-0.37 mg) were weighed and placed into 4 x 6 mm silver capsules. Stable-isotope analyses of the non-exchangeable hydrogen of feathers were then conducted with continuous-flow isotope ratio mass spectrometry as described in detail by Wassenaar and Hobson (2003). Hydrogen isotope (deuterium) values are expressed in the delta notation, in units of parts per thousand (‰), normalized on the VSMOW-SLAP standard scale. Deuterium has been recently discovered as the most significant isotope for tracking migratory wildlife (Bowen et al. 2005). The latitudinal isotopic pattern of deuterium in precipitation (Fig.
is the most useful isoscape available to studies of migratory connectivity for terrestrial species (Hobson 2005b), particularly for species (like the Prothonotary Warbler that breed from the Gulf of Mexico to central Wisconsin and Michigan) with breeding ranges that extend across vast latitudes (Kelly et al. 2002; Hobson et al. 2004).

**Statistical comparisons**- Predicted deuterium (i.e. hydrogen) values for feathers from the Cache River watershed are approximately –46 to –52 (adding -19 to -25 to the precipitation value of -27 from Fig. 1). I compared the mean value (and its 95% confidence limit) for feathers of known origin to these values (–46 to –52) to determine if, as expected, the deuterium values of the feathers of known origin are statistically indistinguishable from these predicted values. I then compared the deuterium values of the feathers from individuals of unknown origin to the distribution of values from the Cache River watershed. Those feathers of unknown origin that were statistically similar to the feathers from the Cache were presumed to be from the same approximate latitude. Feathers of unknown origin with deuterium values statistically greater or less than Cache feathers would indicate individuals whose origins (place of birth) were located at latitudes north or south of the Cache (Fig. 1). Feathers of unknown origin that are vastly different from Cache feathers could be indicative of individuals coming from possibly hundreds of kilometers away.

**Additional data collection**- I have included additional information in this report that is pertinent to the issue of how local conservation actions affect local populations of birds. This additional data was collected as part of our ongoing long-term research of Prothonotary Warblers in the Cache River watershed. Since 1995 we have been banding warbler offspring produced on our study sites. To date, over 6,000 have been banded, and over 500 have returned to breed in the watershed. For each of these warbler offspring that has returned, I calculated their natal dispersal
distance (distance from their birth location to first breeding location in a subsequent year) and determined median natal dispersal distances for males and females.

During 2008 we took another approach to determine how close to home these warbler offspring return by surveying our study populations and non-study populations in our core study area, as well as populations of breeding warblers at varying distances (in 5-km increments) out to 30 km surrounding our core study area. The purpose of this survey was to document in each 5-km distance category the proportion of the breeding population that was made up of banded offspring from our core study population. We predicted that if natal dispersal distances in this migratory warbler are small, then the proportion of banded offspring in the breeding population should decrease with increasing distance away from our core study population. However, if natal dispersal distances are large, then we should find a number of banded offspring in the outer distance categories, and the proportion of banded offspring in the breeding population should not necessarily decrease with increasing distance away from our core study population.

The study of Prothonotary Warblers and collection of tail feathers was permitted under Animal Use Protocol no. 04092 from the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign, and Federal Fish and Wildlife Permit no. MB815400-0 from the United States Fish and Wildlife Service.

**Results and Discussion**

**Feather Isotope Values** - I obtained 156 tail feathers of known origin and 196 of unknown origin that were subsequently analyzed for hydrogen isotopes. I also collected 35 water samples from study sites (forested wetlands, swamps, and the mainstream channel of the Cache River) where the tail feathers of known origin were grown. Predicted deuterium (i.e. hydrogen) values for
feathers from the Cache River watershed are approximately –46 to –52, given what has been published to date from studies throughout North America in dryer terrestrial systems (e.g. grasslands, upland forest; Wassenaar and Hobson 2001, Bowen et al. 2005). The range of actual values for the feathers of known origin was -5 to -65, for feathers of unknown origin was -20 to -70, and for the water samples was -5 to -25 (Fig. 3). The mean (± 95% Confidence Level) deuterium value for feathers of known origin was -22.7 (±2.1) and was substantially different than the predicted values (–46 to –52). The mean deuterium value for feathers of unknown origin was -41.6 (±1.5) and was closer to the predicted values, but still different. The mean deuterium value of feathers of known origin was significantly less than that of feathers of unknown origin ($t_{300} = 14.45, P < 0.001$). One explanation for this difference is that the feathers of unknown origin are from birds that are produced hundreds of kilometers to the north and are immigrating into the Cache River watershed. However, alternative explanations exist that do not involve immigration of individuals from distant origins (see alternative explanations below).

Even though the mean hydrogen values were significantly different between feathers of known and unknown origin, it is important to note that range of values for the feathers of known origin included nearly the entire distribution of values from the feathers of unknown origin. The range of values for feathers of known origin included values expected over a several hundred kilometer range of latitude even though the feathers were grown within a 10-km range of latitude. This result indicates that the hydrogen isotope values in Prothonotary Warbler feathers will not be a useful tool in determining the amount of long-distance natal dispersal into the Cache River watershed from distant sources.

The results of the isotope analyses did not assist in determining whether locally-produced songbirds return to breed near their birthplace, or if breeding populations are bolstered or
maintained by immigrants (young birds dispersing into the system) coming from distant locations hundreds of kilometers away. The isotope work did yield a cautionary tale in that aquatic systems may be problematic for deuterium analyses because it appears that the hydrogen may pass through the food chain to the birds’ feathers differently than for birds breeding in terrestrial (e.g. upland forests) habitats at the same latitude. This issue makes it difficult to say whether many of our birds of unknown origin came from hundreds of kilometers to the north of the Cache River watershed (explanation 1), from the Mississippi and Ohio Rivers at the same latitude as the Cache (hydrogen values in the big rivers at the latitude of the Cache River watershed could be higher than expected because of the transfer of water downriver from hundreds of kilometers to the north; explanation 2), or some of both.

It is also possible that within the Cache River watershed there are vast differences in hydrogen isotope values among Prothonotary Warblers. Given the hydrogen values from the water samples collected from our forested wetland study sites, those birds originating from forested wetlands and swamps may have lower than expected hydrogen values in their feathers compared to what was predicted by current scientific literature (Hobson 2005a, 2005b). It is also possible that some Prothonotary Warblers originating from within the same watershed could have significantly higher hydrogen values in their feathers if their natal territory included dry bottomland or adjacent upland habitat (explanation 3). This explanation seems plausible given that the range of deuterium values for feathers of known origin (from just within the Cache River watershed) was much larger than expected (from -5 to -65). In general, the isotope values of the feathers of known origin were similar to values obtained from water samples taken from the forested wetlands that serve as study sites (Fig. 3). This result indicates that the standard way of using the precipitation hydrogen values and then scaling them by adding -19 to -25 for the
changes that occur in hydrogen as it works through the food chain and into bird feathers does not work for Prothonotary Warblers produced in these wet bottomland habitats.

The use of feather isotopes to determine origin remains a highly valuable tool for those birds that use dryer terrestrial or upland habitat (Hobson 2005a, Kelly et al. 2005, Langin et al. 2007), but the technique needs further refinement for use with birds that breed in aquatic or semi-aquatic habitat. Until those techniques are worked out, detailed long-term field studies of bird populations remain the best way to document the response of birds to conservation actions and to determine whether local conservation efforts benefit local populations of birds.

Additional information on natal dispersal from the study system – Two lines of evidence suggest that Prothonotary Warblers that are produced in the Cache River watershed return to breed close to where they were produced (even though they migrate to Central and northern South America for the winter before returning for their first breeding season). First, to date we have documented the natal dispersal distances of 515 warbler offspring in our study system (258 males and 257 females). Remarkably, the median natal dispersal distances were very similar and relatively small and were 1405 m for females and 1450 m for males. In general, natal dispersal distances of over 70% of these individuals were less than 3 km. Given the extensiveness of our study system (16x12 km) and our survey efforts of non-study populations of warblers up to 30 km away from our core study area, we were capable of documenting natal dispersal events of much greater distances had they occurred. Second, our 2008 survey of breeding adult warblers documented that 1) a relatively high proportion (0.14) of individuals in the core study area (and a 5-km buffer around it) were warbler offspring we had banded in a previous year, 2) this proportion dropped to 0.01 just 5-10 km away from the core study area, and 3) the proportion
was zero beyond 10 km (Fig. 4). These results do not suggest that natal dispersal distances of
tens to hundreds of kilometers do not occur, but the data do strongly suggest that larger natal
dispersal distances may be relatively rare for Prothonotary Warblers.

The extensive and long-term monitoring of the warbler populations in the Cache River
watershed is proving valuable on many fronts by allowing us to better assess the effectiveness of
local conservation actions that increase the nesting success of birds (e.g. land acquisition and
reforestation, wetland restoration), and measure the benefits of local conservation actions to the
local breeding bird community. For example, wetland restoration in the Cache River watershed
that resulted in an increase in the reproductive success of Prothonotary warblers not only
increased local warbler densities through the following pathway: greater reproductive success =
greater adult site fidelity = greater attraction of other adults (Hoover 2003, Hoover in press); but
it also obviously put more warbler offspring out in the environment. If these offspring returned
the following year to breed, they usually did so within 3 km of where they were produced
(Hoover and Reetz 2006, this report). This is another factor that can contribute to an increase in
warbler densities within highly productive restored wetlands (Hoover in press). Thus, I submit
that conservation practices, applied locally within the Cache River watershed in Illinois, are
benefiting local populations of prothonotary warblers. Conservation efforts that promote low or
reduced rates of nest predation and high annual fecundity, such as the deep-water areas of
restored wetlands or core areas of bottomland forests that are far removed from non-forest
habitat, are important to local population dynamics and may be critical to the maintenance of
populations over larger geographic scales (Robinson et al. 1995, Hoover 2003).
The isotope component of this research resulted in more questions than answers, but will likely now serve as the catalyst to new research directions exploring hydrogen assimilation into the feathers of birds that use aquatic and semi-aquatic habitats. This will provide new insights and have broad application to the growing body of research on stable-isotopes and animal migration. A continuation of the long-term bird research in the Cache River watershed, particularly in association with conservation efforts, will further expand our knowledge and increase our ability to effectively and efficiently restore and manage floodplain forests, and will ultimately provide guidelines to promote restoration and management practices that will provide the greatest benefit to local avian communities residing in bottomland forest ecosystems.

**Literature Cited**


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Figure 1. Map depicting the latitudinal gradient in Hydrogen isotopes across North America. The star represents the location of where Prothonotary Warbler feather samples were taken in southern Illinois.
Figure 2. Circles indicate general locations of sites where Prothonotary Warbler feathers have been sampled during 2004-2006.
Figure 3. Distributions of hydrogen isotope (deuterium) levels in feathers of birds from known and unknown origin, and from water samples collected from study sites (e.g. swamps, off-channel wetlands, Cache River main channel).
Figure 4. Birds produced locally return to breed locally. Proportion of individuals in sample observed during 2008 that were produced on study sites in the Cache River watershed prior to 2008. The “0-5” category represents the core study area and a 5-km buffer around it. Other categories represent 5-km-wide bands at ever increasing distances away from the core study area.