Feather Hydrogen Stable Isotopes Reveal Migratory and Interhabitat Connectivity of North American Wintering Songbirds in Coastal Secondary Dry Forest on the South Coast of Puerto Rico

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Abstract. This project was established to (1) support and develop a recently established academic program in ornithology and avian conservation (the only such effort in Puerto Rico); (2) employ mist netting, color banding census, stable isotopic analysis, light-level geolocators and mitochondrial DNA (mtDNA) (for Northern Waterthrushes (Parkesia noveboracensis) techniques to establish links between breeding and wintering populations of migratory songbirds that nest in North America and winter, or stop over in Puerto Rico; and, (3) establish a long-term monitoring program for migratory and year-round resident species of terrestrial birds that utilize secondary coastal dry forest and mangroves in and around the area of the Jobos Bay National Estuarine Research Reserve (JBNERR). The project includes undergraduate and graduate student research focusing on long-distance migratory and interhabitat movement, habitat quality, and social structure of migrants and nesting residents, and promote and enhance the academic and professional development of ornithology and citizen science in Puerto Rico. We collected feather samples for stable isotope analysis and mtDNA analysis. Analysis of our first replicate of feather samples and comparison to the new feather isotope atlas for North America have already provided noteworthy results. For ground foragers (Northern Waterthrushes (Parkesia noveboracensis), Ovenbirds (Seiurus aurocapillus)), we found statistical differences overall between the isotopic signatures of wing (P1) and tail (R1) feathers, and discrepancies did occur between specific Isotopic Region assignments of individuals. Similar results were found for canopy foragers, Northern Waterthrush (Parkesia noveboracensis), Yellow Warbler (Setophaga petechia), Prairie Warblers (Setophaga discolor) and Bananaquits (Coereba flaveola), and there was statistical significance overall between wing and tail feather isotopic signatures of these species. Results also indicate that some individuals continued molting during migration and even upon arrival in Puerto Rico. Some individuals of the canopy foraging species displayed feather isotopic signatures indicative of the marine/mangrove environment, and about 13% of Yellow Warblers sampled appear to be migrants rather than local residents.

Keywords: Interhabitat connectivity, migration feather stable isotopes, Northern Watethrush (Parkesia noveboracensis), Yellow Warbler (Setophaga petechia), Bananaquit (Coereba flaveola).

Conectividad Migratoria e Interhabitat de las Aves Canoras Invernantes de América del Norte en el Bosque Seco Secundario Costero en la Costa Sur de Puerto Rico

Resumen. Este proyecto fue establecido para (1) apoyar y desarrollar un programa académico recientemente establecido en ornitología y conservación de
aves (el único esfuerzo en Puerto Rico); (2) emplear redes de niebla, análisis de bandas de color, análisis isotópico estable, geolocadores de luz y ADN mitocondrial (ADNmt) para establecer vínculos entre las poblaciones reproductoras e invernales de aves canoras migratorias que anidan en América del Norte (3) establecer un programa de monitoreo a largo plazo para las especies migratorias y residentes a lo largo de todo el año de aves terrestres que utilizan bosques secos costeros secundarios y manglares en y alrededor de la zona de la la Reserva Nacional de Investigación Estuarina de la Bahía de Jobos (JBNERR) El proyecto incluye la investigación de estudiantes de bachillerato y posgrado en el movimiento migratorio e interhabitat de larga distancia, la calidad del hábitat y la estructura social de los migrantes y residentes de anidación, y promover y mejorar el desarrollo académico y profesional de ornitología y ciencia de ciudadano en Puerto Rico. Las muestras de plumas se han recolectado para análisis de isótopos estables y ADNmt El análisis de nuestra primera réplica de muestras de plumas y la comparación con el nuevo atlas de isótopos de plumas para América del Norte ya han proporcionado resultados notables. Se encontraron diferencias estadísticamente significativas entre las firmas isotópicas de las plumas de ala (P1) y de cola (R1), y se observaron discrepancias entre las asignaciones específicas de la Región Isotópica de los individuos de la Pizpita de Mangle (Parkesia noveboracensis), Pizpita Dorada (Seiurus aurocapillus) Se encontraron resultados similares para las que aprovisionan en el dosel como el Canario de Mangle (Setophaga petechia), la Reinita Galana (Setophaga discolor) y la Reinita Común (Coereba flaveola), y hubo significancia estadística entre las concentraciones isotópicas de estas especies. Los resultados también indican que algunos individuos continuaron muda durante la migración e incluso a su llegada a Puerto Rico. Algunos individuos de las especies aprovisionan en el dosel mostraron signos isotónicos de plumas indicativos del medio ambiente marino / manglar, y alrededor del 13% de los Canarios de Mangle muestreados parecen ser migrantes en lugar de residentes locales.

**Palabras clave**: Conectividad interhabitat, isótopos estables de plumas, migración, Pizpita de Mangle (Parkesia noveboracensis), Canario de Mangle (Setophaga petechia) Reinita Común (Coereba flaveola).

**Introduction**

This project aims to (1) support and develop a recently established academic program in ornithology and avian conservation (the only such effort in Puerto Rico; (2) establish links between breeding and wintering populations of migratory songbirds that nest in North America and winter, or stop over in Puerto Rico; and, (3) establish a long-term monitoring program for migratory and year-round resident species of terrestrial birds that utilize secondary coastal dry forest and mangroves in and around the area of the Jobos Bay National Estuarine Research Reserve (JBNERR). All three of these objectives provide support for conservation and management efforts both in Puerto Rico for all species, and in the areas of nesting origin for migrants that do not nest in Puerto Rico.

This project expands undergraduate and community participation in the Universidad del Turabo’s growing mist netting and census performing effort related to interhabitat connectivity and habitat quality and use by Neotropical migrants and local residents during the entire year, focusing especially on Northern Waterthrushes (Parkesia noveboracensis), Ovenbirds (Seiurus aurocapillus), Yellow Warblers (Setophaga petechia), Prairie Warblers (Setophaga discolor), and if sufficiently abundant, Common Yellowthroats (Geothlypis trichas); and nesting resident birds Bananaquits (Coereba flaveola), as well as Common Yellowthroats (Geothlypis trichas),
Adelaide’s Warblers (*Setophaga adelaide*), and Black-Whiskered Vireos (*Vireo altiloquus*) in or adjacent to current secondary dry forests and mangroves.

Neotropical migrant species can be limited in size by factors such as reproductive success and parental survival rates during the breeding season, and by individual survival during the nonbreeding season (see Marra et al. 1993), as well as during the long distance migration between sites (see Paxton et al. 2007; 2008; Faaborg et al. 2010a,b, Hobson et al. 2014). Moreover, a species may occur in distinct populations that winter in different locations or whose departure and return dates may be quite different, and these differences may be key to understanding population trends. For example, Rubenstein et al. (2002), using stable isotopic methods demonstrated that northern populations of the Black-throated Blue Warbler (*Setophaga caerulescens*) with stable population trends wintered in Cuba and the Western Greater Antilles, while declining populations nesting in the southern portion of their range tended to winter in eastern Hispaniola.

Kelly et al. (2002) and Kelly (2006) used isotopic methods to examine migratory patterns and dispersal distances in multiple species and contrasted distinct patterns of migration timing of birds passing through their stop-over site in New Mexico: (1) “chain migration” (which may be typical of medium distance migrants) in which all nesting populations begin moving at about the same time such that southern nesters arrive before more northern nesters and “leap frog migration” (which may be characteristic of very long distance migrants) in which northern populations begin moving earlier, overflying southern populations. A similar pattern of leapfrog migration also has been recently revealed in Ovenbirds (*Seiurus aurocapilla*) by Hallworth and Marra (2015) using Miniaturized GPS tags.

As noted, management and conservation require knowledge on the timing and pattern of migration, details on demography and habitat use patterns on the wintering sites, and the awareness of, and will to conserve migratory species at their nesting, stop-over and wintering sites.

Participants in this project have continued to work hard to raise awareness amongst *Universidad del Turabo* (UT) students at all academic levels and among the community by including visitors and volunteers to Jobos Bay National Estuarine Research Reserve and the Puerto Rico Ornithological Society. This is a unique project in Puerto Rico and recognition of these efforts lead to an invitation to doctoral student Ivelisse Rodriguez-Colón to give a presentation on the project at the 20th Regional Meeting of Birds Caribbean in Kingston, Jamaica 25-29 July, 2015 (Figure 1).
Methodology

Intensive mist netting was conducted in mangrove night-roost areas beginning well before dawn in order to intercept birds before they leave the roosting areas and thus allow the capture of both despts and diurnal migrants. Mist netting also was conducted midday in both mangroves and dry forest, and opportunistically in the inter-habitat corridors. All birds captured were banded and color banded with unique 4-color band combinations to permit individual identification, and the time and specific capture location was recorded. Standard morphometric data including wing length and body mass, breast muscle size and fat scores, and body condition index parameters were collected.

Upon an individual’s first capture, feathers (P1 and R1) were collected for $\delta^D$ stable isotopic analysis to determine latitude and geographic origin. Previous genetic analyses of widespread North American species have indicated significant east-west differentiation (Boulet et al. 2006; Colbeck et al. 2008), and can be accomplished using the soft tissue associated with base of the removed feather. Hydrogen isotope ratios can be integrated with genetic markers to increase the resolution of connectivity (Kelly 2002, 2006; Kelly et al. 2005, 2008, also see Hobson et al. 2001; Hobson 2005; Paxton et al. 2007, 2008; Kelly et al. 2005, 2008, 2009; Langin et al. 2007; Robinson et al. 2010; Rohwer et al. 2011).

Unexpected complications with the university’s institutional animal care and use compliance procedures and requirements that resulted in the delay of the purchase of geolocators. However, this issue has now been resolved and geolocators are being purchased presently. Attachment of geolocators to up to 20 of Northern Waterthrushes and possibly migrant Yellow Warblers and others, will provide detailed information on the nesting origin and possible continued winter movement of these migrants. To distinguish whether our site is a stop-over site or the primary wintering ground, geolocaters deployed from September through November will be removed and replaced with new geolocators on birds, if recaptured in March and April.
Samples for mtDNA analysis (feather calamus) were collect with feathers collected for hydrogen stable isotope analysis and are awaiting laboratory analysis. We have thus far completed the laboratory analysis and summarized the results of a collection of feathers from about 130 individuals. An additional and larger replicate has also been collected and awaits laboratory analysis.

This project is conducted in the secondary (mesquite) coastal woodland and mangroves of the Jobos Bay National Estuarine Research Reserve (JBNERR – “the Reserve”) on the south coast of Puerto Rico (Figure 2).

![Figure 2. Jobos Bay National Estuarine Research Reserve (JBNERR) and adjacent agricultural lands. Short red lines indicate mist netting sites.](image)

Results and Discussion

During the period from January 2014 to May 2015 the project encountered (captured or recaptured) some 1,700 birds (Table 1). Moreover, for Northern Waterthrushes, banding efforts provided for increasing numbers of returning recaptures, with recaptures eventually outnumbering newly banded birds. These numbers are due in large part to the acquisition of sufficient equipment and supplies (funding support from the Blake-Nuttall Fund), enhanced outreach and program development. Additional data for summer residents and early-arriving migrants might have been possible were it not for an unfortunate traffic accident that disabled the project field vehicle. The vehicle was repaired and returned to service on 9 September 2015.
Table 1. Total numbers of encounters, new bands, recaptures and feathers collected for stable isotope analysis, as well as the number of Northern Waterthrush (NOWA) new captures (New Bands) and recaptures.

<table>
<thead>
<tr>
<th></th>
<th>Jan-Apr 2014</th>
<th>Jun-Dec 2014</th>
<th>Jan-May 2015</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Encounters</td>
<td>718</td>
<td>574</td>
<td>434</td>
<td>1726</td>
</tr>
<tr>
<td>Total Banded</td>
<td>485</td>
<td>401</td>
<td>242</td>
<td>1128</td>
</tr>
<tr>
<td>Total Recaptures</td>
<td>233</td>
<td>130</td>
<td>164</td>
<td>527</td>
</tr>
<tr>
<td>Unprocessed</td>
<td>47</td>
<td>25</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Feathers Collected for</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotope Analysis</td>
<td>100</td>
<td>151</td>
<td>133</td>
<td>384</td>
</tr>
<tr>
<td>NOWA New Bands</td>
<td>59</td>
<td>33</td>
<td>50</td>
<td>142</td>
</tr>
<tr>
<td>NOWA Recaptures</td>
<td>27</td>
<td>63</td>
<td>54</td>
<td>144</td>
</tr>
</tbody>
</table>

Previous findings from this project indicated that the first-year recapture rate for unbanded Northern Waterthrushes (banded for the first time at the Reserve) is nearly 50%, declining to 24% for second year recaptures.

These results compare favorably with those of Hallworth et al.’s (2013) geolocator study wherein they recaptured 20 of 50 (40%) Ovenbirds on the nesting grounds; and, they also recaptured 12 of 46 (26%) Ovenbirds in route or on the wintering grounds in Florida and Jamaica. Though not encumbered with a geolocator, the ability to predictably recapture Northern Waterthrushes at JBNERR makes these birds excellent candidates for a geolocator study of the nesting origins of the Reserve’s winter visitors, as well as possible winter movements further south.

The project has been able to host and benefit from more than 80 members of the general public to date, including students from other universities such as the InterAmerican University campuses in Ponce and San German, as well as members of the Puerto Rico Ornithological Society, who without this project would have no other opportunity to learn mist netting or see wild birds in the hand. This may help UT achieve a long-term goal of establishing an MAPS-style constant-effort monitoring program at Jobos Bay.

Stable Isotopic Analysis

Some studies seem to portray the timing of molt in North American songbirds as a very fixed, precisely predictable process with little inherent variability. Reading some descriptions of the distinct stages of molt (like Pyle 1997) in which statements such as “we studied species known to complete the molt on the breeding rounds prior to beginning migration southwards” tend to support the characterization of molt as a process with little variability. Yet, except for truly sedentary molters like waterfowl, that undergo a complete eclipse molt, the idea that new feathers are grown precisely on the nesting grounds can only be supported by actually capturing the birds and sampling newly grown flight feathers while the birds are still on the nesting grounds, and before migration begins.
Several studies, however (e.g., Marra et al. 1998; Hill 2004; Norris et al. 2004; Hallworth et al. 2013; others) have documented variability in the timing of molt, observing that some birds, especially those that finish nesting late in the season are unable to complete the molt prior to beginning the migration southwards, and therefore must the molt during migration, incurring a number of disadvantages.

Pyle (1997, p 136) also discusses an eccentric molt pattern, Hobson and Wassenaar (Wassenaar (2008)) also discuss the difference between sedentary molters (like many ducks) versus migration molters (eagles for example).

Stable isotope methods involving the use of the measurement of stable hydrogen isotope abundance in feathers ($\delta^{2}H_f$) of Neotropical migrant songbirds that breed in North America and molt (or begin the molt) prior to fall migration can be used to connect the breeding and wintering grounds of individual birds (migratory connectivity). While these methods do not constrain longitudinal assignments, they are substantially more economical than methods using geolocator and other PTT technologies.

The feather deuterium isoscape ($\delta^{2}H_f$, or $\delta^{D}f$) is derived from the precipitation deuterium isoscape ($\delta^{D}p$) Bowen et al. (2005), which itself originates from the normal process of Rayleigh Distillation in the global circulation model (GCM).

We can infer breeding origins in North America of Neotropical migrants wintering at Jobos Bay, on the south coast of Puerto Rico, applying a simple map lookup approach using the atlas of Isotopic Regions developed by Hobson et al. (2014).

Molt of flight feathers in the species studied here occurs after nesting and generally begins with the inner (medial) wing primary feather (Remix, P1) moving distally to the last Primary Remix (P9), and from the inner (medial) tail feather (Retrix, R1) moving distally to the outer Retrices.

Our chances of obtaining precise results are further enhanced because these species molt medially to distally and both P1 and R1 can be collected.

Remiges (wing flight feathers) are firmly connected to the bird’s wing by a strong tendon and muscles, while Retrices are less firmly attached to the bird and the timing of molt thus could be more variable than for Remiges. Retrices may be more easily lost as a result of mishaps, injuries or encounters with predators, thus initiating the formation of a new feather. Regardless of the timing of feather replacement, the isotopic signature of the new feather will be representative of the location in which the feather was formed. In the research team’s analysis of birds sampled in Puerto Rico, $\delta^{2}H_p$ values greater than -22‰ (Ground Foragers) and -32‰ (Canopy Foragers) are considered to be indicative of the coastal marine environment – foraging in mangroves or in coastal secondary dry forest immediately adjacent to mangroves; and, therefore assigned to Isotopic Region “M” to indicate potential influence of marine water.

Where discrepancies occurred between the Isotopic Region assignment of an individual’s P1 and R1 feathers (IRDs – Isotopic Region Discrepancies) the isotopically lighter value was chosen, and higher IR assignment as being representative of the location closest to the bird’s nesting grounds (birds are more likely to disperse south rather than north after nesting). These data were compared to known nesting distributions of these species (Breeding Bird Survey, among others).

Preliminary results from 2013-14 show that samples collected at Jobos Bay covered a broad isotopic range from -11.72‰ (the highest value) for YEWA R1 tail feather to a low of -151.39‰ for a NOWA P1 wing feather. Species means ranged from -31.45‰ for PRAW R1 tail feathers to -78.71‰ for NOWA R1 wing feathers (Table 2). Overall, significant differences were not found between the P1 wing and R1 tail feathers of ground foraging NOWA and OVEN (paired
one-tailed t-tests, \( p = 0.316 \) and \( 0.304 \), respectively), but P1 wing and R1 tail feathers were significantly different for PRAW, YEWA and BANA (paired one-tailed t-tests, \( p = 0.042, 0.001 \) and \( 0.043 \), respectively) (Table 2).

Table 2. Summary (mean, SD, 95% CI) of stable hydrogen analyses of feathers (\( \delta^{2}H \)) of birds captures at Jobos Bay, Puerto Rico. Shown are summary results for both P1 (wing) and R1 (tail) feathers of same individuals. Pairs of values marked with an asterisk indicate statistical significance (paired t-test). Four-letter Alpha Codes follow those used in the “Bandit” band management application. Bananaquits (BANA) are sedentary resident nesters, while Yellow Warblers (YEWA) may include both residents and migrants.

<table>
<thead>
<tr>
<th>Species</th>
<th>Code</th>
<th>Feather</th>
<th>N</th>
<th>Mean, (SD) (%)</th>
<th>95% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ground Foragers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Waterthrush</td>
<td>NOWA</td>
<td>P1 (wing)</td>
<td>72</td>
<td>-78.71, (19.49)</td>
<td>4.65</td>
</tr>
<tr>
<td><em>Parkesia novarboraceus</em></td>
<td>NOWA</td>
<td>R1 (tail)</td>
<td>72</td>
<td>-78.02, (11.90)</td>
<td>2.79</td>
</tr>
<tr>
<td>Ovenbird</td>
<td>OVEN</td>
<td>P1 (wing)</td>
<td>6</td>
<td>-39.53, (2.94)</td>
<td>7.55</td>
</tr>
<tr>
<td><em>Seiurus aurocapillus</em></td>
<td>OVEN</td>
<td>R1 (tail)</td>
<td>6</td>
<td>-37.77, (3.05)</td>
<td>7.45</td>
</tr>
<tr>
<td><strong>Canopy Foragers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prairie Warbler</td>
<td>PRAW</td>
<td>P1 (wing)</td>
<td>9</td>
<td>-38.54, (5.63)*</td>
<td>4.33</td>
</tr>
<tr>
<td><em>Setophaga discolor</em></td>
<td>PRAW</td>
<td>R1 (tail)</td>
<td>9</td>
<td>-31.45, (8.76)*</td>
<td>6.73</td>
</tr>
<tr>
<td>Yellow Warbler</td>
<td>YEWA</td>
<td>P1 (wing)</td>
<td>31</td>
<td>-43.36, (18.58)*</td>
<td>6.18</td>
</tr>
<tr>
<td><em>Setophaga petechia</em></td>
<td>YEWA</td>
<td>R1 (tail)</td>
<td>31</td>
<td>-32.22, (22.64)*</td>
<td>8.30</td>
</tr>
<tr>
<td>Bananaquit</td>
<td>BANA</td>
<td>P1 (wing)</td>
<td>10</td>
<td>-38.11, (4.70)*</td>
<td>3.15</td>
</tr>
<tr>
<td><em>Coereba flaveola</em></td>
<td>BANA</td>
<td>R1 (tail)</td>
<td>10</td>
<td>-32.45, (10.50)*</td>
<td>7.05</td>
</tr>
</tbody>
</table>

Irrespective of overall significance or lack of significance, individual differences between P1 wing and R1 tail values that were sufficient to provide different Isotopic Region assignments (Isotopic Region Discrepancies, IRDs) for individual birds of all species sampled, as discussed below.

**Ground Foragers.**

For Northern Waterthrushes (NOWA), 42 (58.3%) R1 wing feathers provided lower isotopic values while 30 (41.7%) R1 tail feathers provided lower values. There were 21 Isotopic Region Discrepancies between the P1 and R1 isotopic signatures that were sufficient to give assignments to different Isotopic Regions. Of these, P1 (wing) results provided more northerly assignments in 11 cases, while R1 (tail) results provided more northerly assignments in 10 cases.
Depending on the feather sampled, individual Isotopic Region Assignments covered from Region M (mangrove/marine) to F (boreal forest/tundra limit). In addition, at least 20% of birds grew their feathers in Regions B, A or M, where they do not nest; therefore, confirming the hypothesis that many birds mold during migration and may finalize their molt on the wintering grounds.

Table 3. Isotopic Region assignments for Northern Waterthrushes based on P1 wing, R1 tail or the “most northerly” of the two.

<table>
<thead>
<tr>
<th>Isotopic Region</th>
<th>P1 wing</th>
<th>R1 tail</th>
<th>Most Northerly</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>0</td>
<td>1.39% (1)</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>1.39% (1)</td>
<td>2.77% (2)</td>
<td>1.39% (1)</td>
</tr>
<tr>
<td>B</td>
<td>29.17% (21)</td>
<td>23.61% (17)</td>
<td>19.44% (14)</td>
</tr>
<tr>
<td>C</td>
<td>38.88% (28)</td>
<td>45.83% (33)</td>
<td>45.83% (33)</td>
</tr>
<tr>
<td>D</td>
<td>20.84% (15)</td>
<td>18.07% (13)</td>
<td>22.23% (16)</td>
</tr>
<tr>
<td>E</td>
<td>8.33% (6)</td>
<td>8.33% (6)</td>
<td>9.72% (7)</td>
</tr>
<tr>
<td>F</td>
<td>1.39% (1)</td>
<td>0.00% (0)</td>
<td>1.39% (1)</td>
</tr>
<tr>
<td>total</td>
<td>100.00%</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

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For Ovenbirds (OVEN), 4 (67.7%) R1 wing feathers provided lower isotopic values while 2 (33.3%) R1 tail feathers provided lower values. There were three (3) Isotopic Region discrepancies (RD) between the P1 and R1 isotopic signatures that were sufficient to give assignments to different Isotopic Regions. Of these, P1 (wing) results provided more northerly assignments in two (2) cases, while R1 (tail) results provided more northerly assignments in one (1) case (Table 4).
Table 4. Isotopic Region assignments for Ovenbirds based on P1 wing, R1 tail or the “most northerly” of the two.

<table>
<thead>
<tr>
<th>Isotopic Region</th>
<th>P1 wing</th>
<th>R1 tail</th>
<th>Most Northerly</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>66.67%  (4)</td>
<td>83.33% (5)</td>
<td>50.00% (3)</td>
</tr>
<tr>
<td>B</td>
<td>33.33%  (2)</td>
<td>16.67% (1)</td>
<td>50.00% (3)</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>100.00%</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Canopy Foragers

For Prairie Warblers (PRAW), seven (7) (78.8%) R1 wing feathers provided lower isotopic values while two (2) (22.3%) R1 tail feathers provided lower values. There were four (4) Isotopic Region discrepancies (RD) between the P1 and R1 isotopic signatures that were sufficient to give assignments to different Isotopic Regions. Of these, P1 (wing) results provided more northerly assignments in four (4) cases, while R1 (tail) results provided more northerly assignments in zero (0) cases. These data are indicative of molting or finishing molting en route or finishing the molt on the wintering grounds (Table 5, Figure 5).

Table 5. Isotopic Region assignments for Prairie Warblers based on P1 wing, R1 tail or the “most northerly” of the two.

<table>
<thead>
<tr>
<th>Isotopic Region</th>
<th>P1 wing</th>
<th>R1 tail</th>
<th>Most Northerly</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>11.11% (1)</td>
<td>55.56% (5)</td>
<td>11.11% (1)</td>
</tr>
<tr>
<td>A</td>
<td>88.89% (8)</td>
<td>44.44% (4)</td>
<td>88.89% (8)</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>100.00%</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

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Isotopic Region Assignments using the isotopically lightest feather, P1 or R1, for leaf-gleaning canopy foragers. Codes: PRAW=Prairie Warbler; NOPA=Northern Parula; YEWA=Yellow Warbler; BANA=Bananaquit (local reference).

For Bananaquits (BANA), nine (9) (81.82%) R1 wing feathers provided lower isotopic values while two (2) (18.18%) R1 tail feathers provided lower values, with five (5) Isotopic Region discrepancies (RD) between the P1 and R1 isotopic signatures that were sufficient to give assignments to different Isotopic Regions. Of these, P1 (wing) results provided more northerly assignments in five (5) cases, while R1 (tail) results provided more northerly assignments in zero (0) cases (Table 6).

Some individuals of the canopy foraging species displayed feather isotopic signatures indicative of the marine/mangrove environment, and about 13% of Yellow Warblers sampled appear to be migrants rather than local residents.

Figure 5. Isotopic Region Assignments using the isotopically lightest feather, P1 or R1, for leaf-gleaning canopy foragers. Codes: PRAW=Prairie Warbler; NOPA=Northern Parula; YEWA=Yellow Warbler; BANA=Bananaquit (local reference).
Table 6. Isotopic Region assignments for Bananaquits based on P1 wing, R1 tail or the “most northerly” of the two.

<table>
<thead>
<tr>
<th>Isotopic Region</th>
<th>P1 wing</th>
<th>R1 tail</th>
<th>Most Northerly</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>10.00%</td>
<td>60.00% (6)</td>
<td>10.00% (1)</td>
</tr>
<tr>
<td>A</td>
<td>90.00% (9)</td>
<td>40.00% (4)</td>
<td>90.00% (9)</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>100.00%</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

For Yellow Warblers (YEWA), 25 (80.65%) R1 wing feathers provided lower isotopic values while six (6) (19.35%) R1 tail feathers provided lower values.

There were 16 Isotopic Region discrepancies (RD) between the P1 and R1 isotopic signatures that were sufficient to give assignments to different Isotopic Regions. Of these, P1 (wing) results provided more northerly assignments in 12 cases, while R1 (tail) results provided more northerly assignments in four (4) cases. Birds with Isotopic Region assignments to Regions C and D are most certainly migrants (Table 7, Figure 5).

Table 7. Isotopic Region assignments for Yellow Warblers based on P1 wing, R1 tail or the “most northerly” of the two.

<table>
<thead>
<tr>
<th>Isotopic Region</th>
<th>P1 wing</th>
<th>R1 tail</th>
<th>Most Northerly</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>12.90% (4)</td>
<td>61.28% (19)</td>
<td>9.67% (3)</td>
</tr>
<tr>
<td>A</td>
<td>80.64% (25)</td>
<td>25.81% (8)</td>
<td>77.42% (24)</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>6.45% (2)</td>
<td>6.45% (2)</td>
</tr>
<tr>
<td>C</td>
<td>3.23% (1)</td>
<td>3.23% (1)</td>
<td>3.23% (1)</td>
</tr>
<tr>
<td>D</td>
<td>3.23% (1)</td>
<td>3.23% (1)</td>
<td>3.23% (1)</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0.00%</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>100.00%</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Because birds move around, P1 (wing) and R1 (tail) can give different results (post-nesting dispersal, more likely southwards than northwards). Therefore, in order to better identify nesting
origins, researchers in wintering areas and stop-over points should collect BOTH feathers and choose that value that provides the most “northerly” location, which is likely to be closest to the migrant’s real nesting location.

**Habitat Quality and Habitat Use**

Data collected on habitat use and bird abundance and body condition are currently being summarized and analyzed.

The research team’s conclusions thus far show six (6) terrestrial Isotopic Regions (A-F) and required the research team to invent a marine region, region “M”, which in the Reserve would represent birds foraging in areas influenced by marine/mangrove ecosystems rather than precipitation. Many birds, especially NOWA are molting during migration, including some that finalize the molt on the wintering grounds. Some YEWA, up to at least 13%, are migrants, rather than resident nesters.

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This study was conducted under the authorization of Animal Care and Use Committee (ACUC) Protocols A03-006-13 and A03-023-16 of the Ana G Mendez University System Office of Compliance.

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