Appendix 1

Study Sites

Coastal woodlands provide some of the first habitat where northward songbird migrants can stopover to rest and refuel after the flight across the GOM. Because it is the first available habitat after the, many species and populations that segregate across breeding or wintering ranges occur together in high densities at coastal GOM stopover sites and it is possible to sample individuals from across the latitudinal distribution of species (Langin et al. 2009). The western stopover site is located in scrub forest along the inter-coastal canal of the mid-Texas coast on The Nature Conservancy’s Clive Runnells Family Mad Island Marsh Preserve (28° 37’N, 96°6ʹW; see Cohen et al. 2015b). The central stopover site is located within a Chenier, a narrow strip of forested wetland, about 1.5 km inland from the coast surrounded by marsh near Johnson’s Bayou, Louisiana (29°45ʹN, 93°37ʹW; see Cohen et al. 2015a). The eastern stopover site is located in scrub forest on a barrier island in the Apalachicola National Estuarine Research Reserve St. George Island, on the panhandle of Florida (29°40’N, 84°50ʹW; see Lester et al. 2016).

Estimation of breeding latitude

Stable isotope analyses of feather (δ²H) and claw (δ¹³C) samples were conducted at The Smithsonian Institution’s Stable Isotope Mass Spectrometry Facility using the methods described in Rushing et al. (2016). The latitudinal gradient of abundance of hydrogen isotopes (δ²H) in North American precipitation is incorporated into feathers grown at those latitudes (Hobson et al. 2012). For birds that retain those feathers throughout the year, the stable δ²H abundance in feathers sampled during migration reflects the geographic origin of the bird from the previous breeding season. To assign migrating birds to breeding destination, we first created 0.33’x0.33’ raster basemaps for each species’
breeding range obtained from the Breeding Bird Survey (Sauer et al. 2017) describing spatial variation in expected feather δ²H values from amount-weighted growing-season δ²H values for non-ground (American Redstart) or ground (Ovenbird, Wood Thrush) foraging birds (Bowen et al. 2005, Hobson et al. 2012). For each raster cell, we calculated the likelihood that any given cell represented the breeding destination of each migrating individual using a normal probability density function with mean equal to the expected δ²H value for the cell and standard deviation of 12‰ (Rushing et al. 2017). Next, we converted the likelihoods to posterior probabilities using Bayes’ rule, treating each raster cell’s relative abundance as a prior probability (Royle and Rubenstein 2004). We used data from the North American Breeding Bird Survey (Sauer et al. 2013) to create species-specific base maps of breeding abundance (Rushing et al. 2017). Raw abundance estimates were then converted into a probability surface by dividing the abundance of each cell by the total abundance of all cells. To ensure that the abundance data did not overwhelm the isotope data in the assignments, we raised the likelihood estimates and prior probabilities to species-specific powers as described by Rushing et al. (2017). We built probability surfaces using the methods described in Rushing et al. (2017) and estimated the likely breeding latitude of each individual by calculating the mean latitude of all ‘likely’ cells weighted by their posterior probability and used the delta method to estimate the standard error of the latitude estimates. To determine the likelihood of cells, we used a smoothing spline function to estimate the probability threshold that defined the upper 67% of the cumulative probability and classified any cell with posterior probability greater than this threshold as "likely" and all other cells as "unlikely" origins (Hobson et al. 2009, Chabot et al. 2012, Rushing et al. 2014). These assignments assume that individuals return to the latitude where their feather was grown and, therefore, that the magnitude and direction of natal and breeding dispersal are within the margin of uncertainty of our assignments, given that long-distance breeding dispersal is generally rare (Paradis et al. 1998, Rushing et al. 2016).
Strength of migratory connectivity

We estimated the strength of migratory connectivity from probabilistic based stable hydrogen isotope assignments sampled from feathers of three migratory songbirds (American Redstart, Ovenbird & Wood Thrush), during spring migration at three sites along the northern coast of the Gulf of Mexico (GOM). Several parameters are needed to estimate the strength of migratory connectivity while accounting for sampling uncertainty (Cohen et al. 2018, Hostetler and Hallworth 2017). These include, the location of where the animal was captured, the distance between the capture sites, the relative abundance within each capture site, potential target locations, the distance between the target locations and a measure of location uncertainty. To estimate the strength of migratory connectivity from stable hydrogen isotopes, we first sampled n (n = 1000) assignment locations for each individual (XY, geographic coordinates) from the probabilistic isotope assignment ($\delta^2$H) using a multinomial distribution following: $XY \sim \text{Mult}(n, \delta^2H)$. We used these assignment locations to assign individuals to target locations which we defined as polygons representing isotope bands equivalent to 12‰ (the standard deviation used to generate probabilistic isotope-assignments) originating from 25°N and continuing until 85°N. The 60° span (25°N-85°N) was chosen to ensure the entire distribution of all three species was included. For each species the target locations were clipped by the species distribution so that only those target locations that fell within the species distribution were used to estimate migratory connectivity (number of target locations: American Redstart: n = 11; Ovenbird: n = 11; Wood Thrush: n = 6; Fig. A1).

We extended the estMC function within the MigConnectivity package (Hostetler and Hallworth 2017) to estimate migratory connectivity strength (MC) from the generated random points. Similar to the approach for light-level geolocator data (Cohen et al. 2018), one can use a bootstrap, sampling with replacement from the animals with isotope data. In this case, location uncertainty is applied by also sampling from the generated random points for each sampled animal. We used 1000 bootstrap samples
to estimate MC for each of the three species. We used the number of captures per net hour as our measure of relative abundance (another input for MC) at each of the three capture locations within the Gulf of Mexico.

We determined the differences in the strength of migratory connectivity using a newly developed function called diffMC within the MigConnectivity package (Hostetler and Hallworth 2017). The diffMC function uses independent estimates of MC to test for differences in the strength of migratory connectivity for two or more species. It does this by sampling with replacement from each species’ own samples (bootstrap or otherwise) of MC, then taking the difference:

$$\Delta MC_{ij}^s = MC_i^{(s)} - MC_j^{(s)}$$

(A1)

where $\Delta MC_{ij}^s$ is the $s$th sampled difference in MC between species $i$ and $j$ and $MC_i^{(s)}$ is the $s$th sampled MC value for species $i$. The difference in migratory connectivity strength between the two species ($\Delta MC_{ij}$) can be estimated as the mean of the sampled differences:

$$\Delta \hat{MC}_{ij} = \frac{\sum_{s=1}^{S} \Delta MC_{ij}^s}{S}$$

(A2)

where $S$ is the number of samples drawn. Similarly, uncertainty (standard error and confidence intervals) in $\Delta MC_{ij}$ can be estimated:

$$\hat{SE}(\Delta MC_{ij}) = sd(\Delta MC_{ij}^*)$$

$$\left[ \Delta \hat{MC}_{ij,low(\%)} , \Delta \hat{MC}_{ij,high(\%)} \right] = \left[ \Delta MC_{ij}^{(\alpha/2)} , \Delta MC_{ij}^{(1-\alpha/2)} \right]$$

$$\left[ \Delta \hat{MC}_{ij,low(BC)} , \Delta \hat{MC}_{ij,high(BC)} \right] = \left[ \Delta MC_{ij}^{(\alpha_1)} , \Delta MC_{ij}^{(\alpha_2)} \right]$$

$$\alpha_1 = \Phi\left(2\hat{z}_o + \Phi^{-1}(\alpha/2)\right) \quad \alpha_2 = \Phi\left(2\hat{z}_o + \Phi^{-1}(1-\alpha/2)\right)$$

$$\hat{z}_o = \Phi^{-1}\left(\frac{\#\{ \Delta MC_{ij}^* < \Delta \hat{MC}_{ij} \}}{S}\right)$$

(A3)
where $\hat{\sigma}(\Delta MC_y)$ is the standard error in the difference estimated as the standard deviation of the samples, $\left[\hat{\Delta MC}_{ij,\text{low}(\%)}^{\text{low}(\%)}, \hat{\Delta MC}_{ij,\text{high}(\%)}^{\text{high}(\%)}\right]$ are simple $1-\alpha$ percentile-based confidence intervals, and

$\left[\hat{\Delta MC}_{ij,\text{low}(BC)}^{\text{low}(BC)}, \hat{\Delta MC}_{ij,\text{high}(BC)}^{\text{high}(BC)}\right]$ are bias-corrected confidence intervals. For this study, we drew 100,000 bootstrap samples of differences and present the bias-corrected confidence intervals.

**Migratory Connectivity Simulations**

We ran a series of simulations to test the accuracy of the newly devised function to estimates MC from stable-isotope assignments within the MigConnectivity R package (Hostetler and Hallworth 2017). We simulated MC using five different target location configurations (A: 3 latitudinal bands, B: 5 latitudinal bands, C: 10 latitudinal bands, D: bands representing the isotope assignment standard deviation (12 ‰) and E: bands representing 2 standard deviations (24 ‰) bands. In each simulation ($n = 1000$), we generated the ‘true’ breeding location for each of 300 individuals. The isotope values used to estimate MC were extracted from the underlying feather isoscape based on the simulated breeding coordinates.

We ran 1000 simulations where we estimated MC for each of the 5 different target location configurations. We used the three capture locations (see Study Sites) as our origin sites and assumed relative abundance was equivalent among the three origin populations. Simulations indicate that target locations generated using the standard deviation (12 ‰) used to make probabilistic isotope assignments provided the best estimates of MC. While simulations indicated a small bias in MC estimated from isotopes, coverage of the true value was high (98.5%; true MC included in the 95% credible interval; Table A1).

**References**

TABLE A1. Simulation results for estimates of migratory connectivity (MC) with five different configurations for assignments location. The five configurations included were 3° (A), 5° (B), and 10° (C) latitudinal bands, and bands derived from isotope units equivalent to 1 (12‰, D) and 2 (24‰, E) standard deviations used to make probabilistic isotope assignments. We also estimated the Mantel correlation (r_M) within our simulations. For MC and r_M, we report the realized mean (the true locations of the simulated birds) and the estimated value. The error is the difference between the estimated and the realized value and is reported along with the root mean square error (RMSE). The coverage is the proportion of simulations where the bias corrected 95% confidence interval overlaps MC realized.

<table>
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<th>Target Location Configuration</th>
<th>MC realized</th>
<th>MC estimate</th>
<th>MC error (RMSE)</th>
<th>MC coverage</th>
<th>r_M realized</th>
<th>r_M estimate</th>
<th>r_M error (RMSE)</th>
<th>r_M coverage</th>
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<td>-0.066 (0.068)</td>
<td>0.146</td>
<td>0.059</td>
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<td>0.156</td>
<td>0.057</td>
<td>0.044</td>
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<tr>
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<td>0.040</td>
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<td>0.003</td>
<td>0.062</td>
<td>0.047</td>
<td>-0.015 (0.019)</td>
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</tr>
<tr>
<td>D</td>
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<td>0.267</td>
<td>0.050</td>
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<tr>
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SUPPLEMENTAL FIGURES:

Figure A1. Target locations used to estimate the strength of migratory connectivity for three migratory songbirds captured during migration along the northern coast of the Gulf of Mexico. Target locations were defined using 12‰ stable-hydrogen isotope bands which is equivalent to the standard deviation used to create probabilistic assignments.