

# Establishing Migratory Connectivity in the Rufous Hummingbird (*Selasphorus rufus*) Using Plumage $\delta D$ and Chroma

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## Introduction and Aims

The Rufous hummingbird (*Selasphorus rufus* Gmelin, Trochilidae) undertakes the longest migration for its size of any bird. Breeding as far north as Southeast Alaska, it overwinters in Mexico and the Gulf Coast of the US<sup>1,3</sup>.

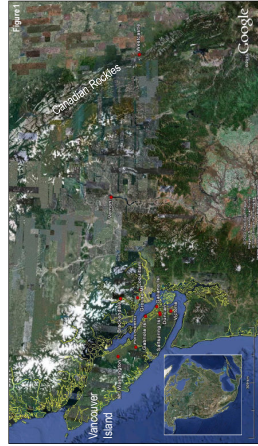
*S. rufus* is recognised as a *Species of Continental Importance* on the 'Partners in Flight' (PIF) watchlist<sup>4</sup>. In recent years, banders at a number of sites in British Columbia and the US Pacific Northwest have noticed a reduction in the numbers of *S. rufus* in the spring migration.

Migratory bird species require suitable habitat on both breeding and wintering grounds, as well as on the migration routes that link them. The first step in elucidating the possible causes of decline in *S. rufus*, therefore, is to investigate its degree of migratory connectivity<sup>5</sup>. We sought to address two questions:

1. Does *S. rufus* exhibit a high degree of migratory connectivity, or do those from one breeding area disperse and merge with others on different wintering grounds?
2. A relatively small but apparently growing population of *S. rufus* overwinters along the Gulf Coast of the US<sup>3</sup>. What is the breeding range of this population?

## Study Area

The study encompassed 12 *S. rufus* breeding / migration sites along an 800 km-wide East-West swathe in Western Canada: 10 in the Georgia Depression / Coast Mountains, one in the Southern Interior, and one in the Southern Rockies, East of the Continental Divide (Figure 1).



## Materials & Methods

*S. rufus* molt on the wintering sites<sup>2</sup>. In order to estimate the geographic extent of these sites<sup>2</sup>, we examined two endogenous markers<sup>6</sup> in newly-grown feathers (rectrices, R4) of after-hatch-year (AHY) *S. rufus* trapped during migration monitoring in the spring and summer of 2007: deuterium (D) and chroma. Deuterium in precipitation ( $\delta D_p$ ) is geographically predictable on a continental scale; since D is incorporated into feathers via diet, it is possible to estimate the geographic area in which the feather was grown by quantifying the D within the feather keratin ( $\delta D_f$ )<sup>7</sup>. Isotopic analyses were performed using continuous-flow isotope-ratio mass spectrometry (CF-IRMS)<sup>8</sup>.

## Materials & Methods (continued)

The rufous coloration from which *S. rufus* derives its name is due to carotenoid pigments. These cannot be synthesized by vertebrates, and must be sequestered instead via diet<sup>9-12</sup>. As a result, geospatial differences in carotenoid availability will result in corresponding differences in feather color between populations. Chroma (approximating to saturation) provides a proxy estimate of relative carotenoid concentration in plumage<sup>13</sup>. Reflectance measurements were taken with a spectroradiometer (USB2000, Ocean Optics), and chroma calculated as:

$$\text{Chroma} = \sqrt{(Q_y - Q_x)Q_z^2 + (Q_x - Q_y)Q_z^2}$$

Where  $Q_x$ ,  $Q_y$ ,  $Q_z$ , and  $Q_a$  are the summed reflectances of the red (625-700 nm), green (475-550 nm), yellow (650-625 nm), blue (400-475 nm) and total visible (400-700 nm) wavebands, respectively<sup>13</sup>.

Cluster analysis (Ward's, Euclidean Distance<sup>14</sup>) was then undertaken to differentiate between groups of individuals based on  $\delta D_f$  and chroma. The optimum number of groups was ascertained using root-mean-square standard deviation (RMSSTD) validity index plots<sup>14</sup>. We then used X<sup>2</sup>-tests to ascertain significance of assignment to cluster.

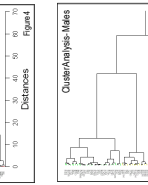
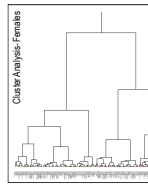
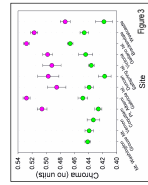
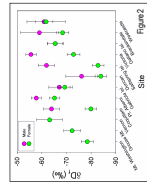
## Results

There were significant effects of Site and Sex on both  $\delta D_f$  and chroma ( $P < 0.05$ , ANOVA). There were also significant interactions between factors; as a result, further analyses were conducted separately by Sex.

For several sites at which both sexes were sampled, males showed significantly less negative  $\delta D_f$  values (Figure 2). Males also exhibited higher chroma values than females (Figure 3).

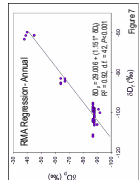
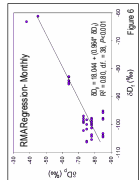
Cluster analyses based on  $\delta D_f$  and chroma produced four groups per sex (Figures 4 and 5).

To plot wintering range estimates from  $\delta D_f$  data directly onto published  $\delta D_p$  isoscapes, we used Reduced Major Axis (RMA) regression<sup>15</sup> to predict the  $\delta D_f$  values that would result in the observed  $\delta D_f$  values from feathers of known geographical origin. These included feathers from hatch-year (HY) birds sampled on the breeding range in Western Canada, as well as newly-molted feathers from both HY and AHY birds on the wintering ranges in Mexico and the Southwest US. Since we used two isoscapes, we employed two regressions. The first isotope covered North America including Mexico, and was based on the International Atomic Energy Agency (IAEA) Global Network of Isotopes in Precipitation (GNIP)<sup>16</sup>.

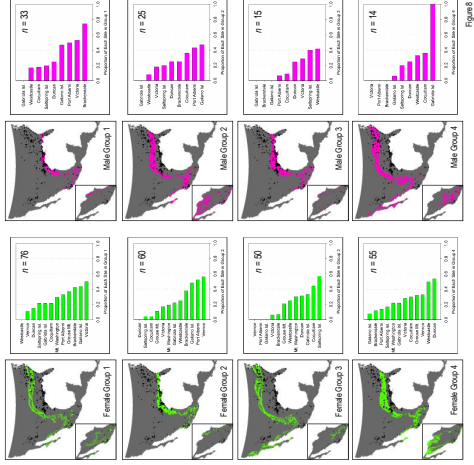


## Results (continued)

We estimated  $\delta D_p$  values corresponding to the collection date (month) of the *S. rufus* feathers of known geographic origin, using OIPC v.2.2 (Online Isotopes in Precipitation Calculator)<sup>15</sup>. The second isotope covered Mexico only<sup>16</sup>, although at higher sampling resolution (234 sampling sites vs. 2 for the IAEA-based isotope). This isotope employs temporally-integrated isotopic signatures derived from groundwater. Therefore, we used annual mean OIPC-derived  $\delta D_p$  values, rather than monthly values for each feather location, in the second RMA regression.



The RMA regressions were undertaken using the RMA V1.17 software package<sup>17</sup>. Finally, we plotted estimates of each group's wintering range by overlaying its RMA-derived  $\delta D_p$  values (95% C.I.s) onto both isoscapes. The RMA equations are presented in Figures 6 and 7. Estimates of wintering location (95% C.I.s) are presented for each group in Figure 8 (below). The large maps display the 95% C.I.s for each group for the months December-February inclusive, based on the relationship presented by the monthly RMA regression in Figure 6. The isotope on which they are overlaid is that of Bowen (2010; [waterslopes.org](http://waterslopes.org))<sup>7,18</sup>. The inset maps present the 95% C.I.s for each group on an annual basis for Mexico alone, based on the relationship presented by the annual RMA regression in Figure 7. The isotope is that of Wassenaar et al. (2009)<sup>16</sup>. In each map, the black dots represent reported sightings (2000-2010) of *S. rufus* for the months December-February inclusive, based on data collected by the Avian Knowledge Network (AKN) database<sup>19</sup>. To the right of each map, the proportion of birds from each site present in a given group is represented by a bar graph.



## Conclusions

1. Intersexual differences in  $\delta D_f$  (males > females) suggest a degree of allopatry on the wintering ranges. This hypothesis is supported by observational evidence from Mexico<sup>2</sup>. Other explanations include intersexual differences in discrimination against deuterium uptake (AD), earlier molting of males, and intersexual differences in foraging strategy on the wintering grounds resulting in differential access to 'recharge' water<sup>20,21</sup>. There is currently no supporting evidence for these alternative hypotheses.
2. The results also suggest that migratory connectivity in the populations of *S. rufus* investigated in this study is not particularly high; each of the groups in Figure 8 comprises individuals from several of the breeding sites.
3. The most likely candidates for populations of *S. rufus* overwintering on the Gulf Coast of the US are in Groups 2 and 4 for females, and Groups 1 and 3 for males.

## Acknowledgements

This work was generously supported financially by Royal Roads University, and by the US Forest Service, under the 'Wings Across the Americas' initiative. We are particularly indebted to the many people who helped with the collection of feathers: the project would not have been possible without their generous support. We thank: S. Acton, I. Bacon, C. Carrothers, S. Contreras-Martinez, N. Cox, D. Craig, C. Culp, J. Finlay, D. Gellately, P. Gregory, A. Hall, B. Hawkins, K. Hobson, M. Hoebel, A. Hurley, C. Hutchison, D. Jimoff, C. Lively, G. Loughridge, K. Low, A. Lynn, D. Lynn, S. Mademont, D. Manly, J. Moran, A. Nightingale, M. Noble, K. Poulton, S. Robbins, L. Rogers, J. Schondube, B. Sienieks, R. Teo, S. Van Wilgenburg, S. Walker, and G. West.

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