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## COMMENTARY

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### USE OF STABLE ISOTOPES OF HYDROGEN TO PREDICT NATAL ORIGINS OF JUVENILE MERLINS AND NORTHERN HARRIERS MIGRATING THROUGH THE FLORIDA KEYS

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**Abstract.** Stable isotopes of hydrogen have been used as a tool to determine migratory connectivity, or to link breeding and wintering grounds. Because isotopes serve as intrinsic markers, eliminating the need for birds to be relocated after an initial marking, they hold the potential to be an extremely useful tool. From 1998 to 2003 we gathered feathers from juvenile Merlins (*Falco columbarius*) and Northern Harriers (*Circus cyaneus*) during their fall migration in the Florida Keys and analyzed them isotopically in an attempt to determine their natal origins. Our results failed to reveal the natal origins of at least one of these two birds of prey.

**Key words:** *Circus cyaneus*, *Falco columbarius*, *hydrogen stable isotopes*, *Merlin*, *Northern Harrier*, *raptor migration*.

#### Uso de Isótopos Estables de Hidrógeno para Predecir el Origen Natal de Juveniles de *Falco columbarius* y *Circus cyaneus* Migrando a través de los Cayos de Florida

**Resumen.** Se han usado isótopos estables de hidrógeno como una herramienta para determinar la conectividad migratoria o para vincular los sitios de cría e invernada. Debido a que los isótopos sirven como marcadores intrínsecos, eliminando la necesidad de relocalizar a las aves luego de un marcado inicial, mantienen el potencial de ser una herramienta extremadamente útil. Desde 1998 a 2003, juntamos plumas de juveniles de *Falco columbarius* y *Circus cyaneus* durante sus migraciones otoñales en los cayos de Florida y analizamos sus niveles isotópicos para intentar determinar sus orígenes natales. Nuestros resultados no permitieron revelar los orígenes natales de al menos una de estas dos aves de presa.

## INTRODUCTION

### STABLE ISOTOPES IN MIGRATION RESEARCH

Stable isotopes have the potential to identify the natal grounds of migratory birds, to determine the extent that populations of breeding birds mix during migration or winter, and to monitor population trends. A number of studies have used ratios of stable isotopes of hydrogen ( $\delta D$ ) to investigate unanswered questions of animal migration (Lott et al. 2003, Smith et al. 2003, Hobson et al. 2004, DeLong et al. 2005). Values  $\delta D$  in feathers ( $\delta D_f$ ) correlate with variation in natural isotopes in the environment (Chamberlain et al. 1997), so isotopic signatures from feathers provide intrinsic markers allowing researchers to track the origin of migratory populations.

Base maps of isotope contours have been developed in an attempt to identify origins of individuals moving away from their natal site. Maps generated by GIS (geographic information system) reflect well-known hydrologic processes (Craig 1961, Rozanski et al. 1993) that result in predictable geographic patterns of  $\delta D$  in precipitation ( $\delta D_p$ ) (Meehan et al. 2004, Bowen et al. 2005). By determining the relationship between  $\delta D_p$  and  $\delta D_f$ , or the discrimination factor, these mapped values of  $\delta D_p$  should accurately predict the natal origins of migrating juveniles (DeLong et al. 2005, Lott and Smith 2006). Newer GIS base maps allow for the generation of a  $\delta D_p$  value for any location via interpolation. Recent expansions of isotope maps allow for the creation of base maps that are specific

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to user-selected time periods, thus allowing for the creation of project-specific maps (Bowen et al. 2013).

#### RAPTOR MIGRATION IN THE FLORIDA KEYS

During fall migration in North America, millions of raptors journey south to their wintering grounds, often concentrating along major topographical features such as mountain ridges to take advantage of updrafts (Heintzelman 1986, Kerlinger 1989) and coastlines to avoid open water (Heintzelman 1975). The tendency to fly over land when possible funnels many of these birds into the Florida Keys, a major migration corridor in fall, as it is the last peninsula of land they encounter on their journey south. Over 15 000 migrating raptors are counted in the Florida Keys each fall (Lott 2006), though little is known regarding the origins of these birds.

We attempted to assess the natal origins of Merlins (*Falco columbarius*) and Northern Harriers (*Circus cyaneus*) migrating through the Florida Keys.

#### METHODS

The banding station, a research site formerly operated by HawkWatch International as part of a long-term monitoring program (see Lott 2006 for full description), is midway on the island chain of the Florida Keys on Long Point Key in Curry Hammock State Park. This location is ideal, as it is near Long Key Channel, a 3.7-km stretch of open water, where raptors are concentrated (Hoffman and Darrow 1992), facilitating their capture. Raptors were trapped between mid-September and mid-November from 1998 to 2003 with mist nets, bow nets, and dho-gazas. Rock Pigeons (*Columba livia*), Ringed Turtle-Doves (*Streptopelia risoria*), and Java Sparrows (*Padda oryzivora*) were manipulated from a camouflaged blind to catch the attention of passing birds and lure them into nets.

Upon capture, raptors were taken inside the blind and morphological characteristics were recorded. Prior to their release, two to three breast feathers were pulled from juveniles and stored in an envelope. Because we only used feathers from first-year migratory birds (identified by their plumage), we feel confident that these feathers were grown on the natal grounds. Both Merlins and Northern Harriers retain their juvenile plumages until the spring of their second year (Warkentin et al. 2005, Smith et al. 2011), so the collected feathers should preserve the isotopic signature of the birds' natal area.

We prepared 180 feathers (120 from Merlins, 60 from the Northern Harriers) at the University of Arkansas Stable Isotope Laboratory. They were cleaned in a 2:1 chloroform/methanol solvent to remove any surface oils and air dried in a fume hood for 48 hr. We cut approximately 2–4 mm from the tip of each feather (including shaft and vane) and trimmed it to 350  $\mu\text{g}$  ( $\pm 10 \mu\text{g}$ ). We then packaged the samples into 3.5-  $\times$  5-mm silver capsules (Costech Analytical Technologies, Inc., Valencia, CA) and sent them to the stable-isotope laboratory at the National Water Research Institute in Saskatoon, Saskatchewan, Canada, for analysis.

Samples were analyzed via online continuous-flow isotope-ratio mass spectrometry (CF-IRMS) by protocols described in Wassenaar and Hobson (2002). The sample was converted into a gaseous form by combustion in an elemental analyzer, and the relative amounts of the different isotopic forms in the sample were measured. We report the results in  $\delta\text{D}$  notation ( $\delta\text{D sample} = [({}^2\text{H}/{}^1\text{H sample})/({}^2\text{H}/{}^1\text{H standard}) - 1] \times 1000$ ) (Peterson and Fry 1987), or the deviation of the ratio of deuterium ( ${}^2\text{H}$ ) to protium ( ${}^1\text{H}$ ) from the same ratio in an international standard (standard mean ocean water: v-SMOW; Faure 1986). Deviations of isotope ratios are recorded in parts per thousand (‰).

We used IsoMAP ([www.isomap.org](http://www.isomap.org)) as the source of our deuterium base map. IsoMAP allows the user to restrict the base map to specific times (years and months within years) and geographic areas of interest so that the user can create study-specific base maps. The user also has the option, data permitting, of including environmental predictor variables to predict isotope values. We include the years 1998 to 2003 in our model. We also included elevation as a predictive variable but because data were limited could not include any other predictor variables. We plotted the birds'  $\delta\text{D}_f$  values on the deuterium base map derived from IsoMAP (Lehnen 2013) and overlaid the breeding ranges of the Northern Harrier and Merlin (Ridgely et al. 2003) on the map for each species to geographically restrict sources of potential migrants to locations that are biologically plausible.

#### ANALYSES

We constructed a histogram of relative frequency depicting the percent of migrants whose range of values of  $\delta\text{D}_f$  included each value. (We divided the range of mapped isotope values into bins in increments of 1‰, then determined the frequencies by the number of ranges that intersected each bin.) For example, 30 Northern Harriers (50%;  $n = 60$ ) had ranges of estimated values of  $\delta\text{D}_f$  that included  $-74\text{‰}$ . We omitted from the graph all values of  $\delta\text{D}_f$  that included less than 5% of the sample of the two species.

#### RESULTS

Results were inconsistent with the species' established life histories, providing little to no insight into the origins of these migratory birds, particularly the Merlin. Values of  $\delta\text{D}_f$  for the Merlin ( $n = 120$ ) ranged from  $-125.02\text{‰}$  to  $22.14\text{‰}$ . Although results indicate that some birds may have originated within their known breeding range, most values implied an origin south of where Merlins are known to breed (Fig. 1).

For the Northern Harrier values of  $\delta\text{D}_f$  ( $n = 60$ ) ranged from  $-135.58\text{‰}$  to  $-13.69\text{‰}$ . A large portion of predicted values fell within the species' known breeding range (Fig. 2). However, as the eastern portion of that range narrows, predicted values begin to stray outside the range into the southeastern United States.

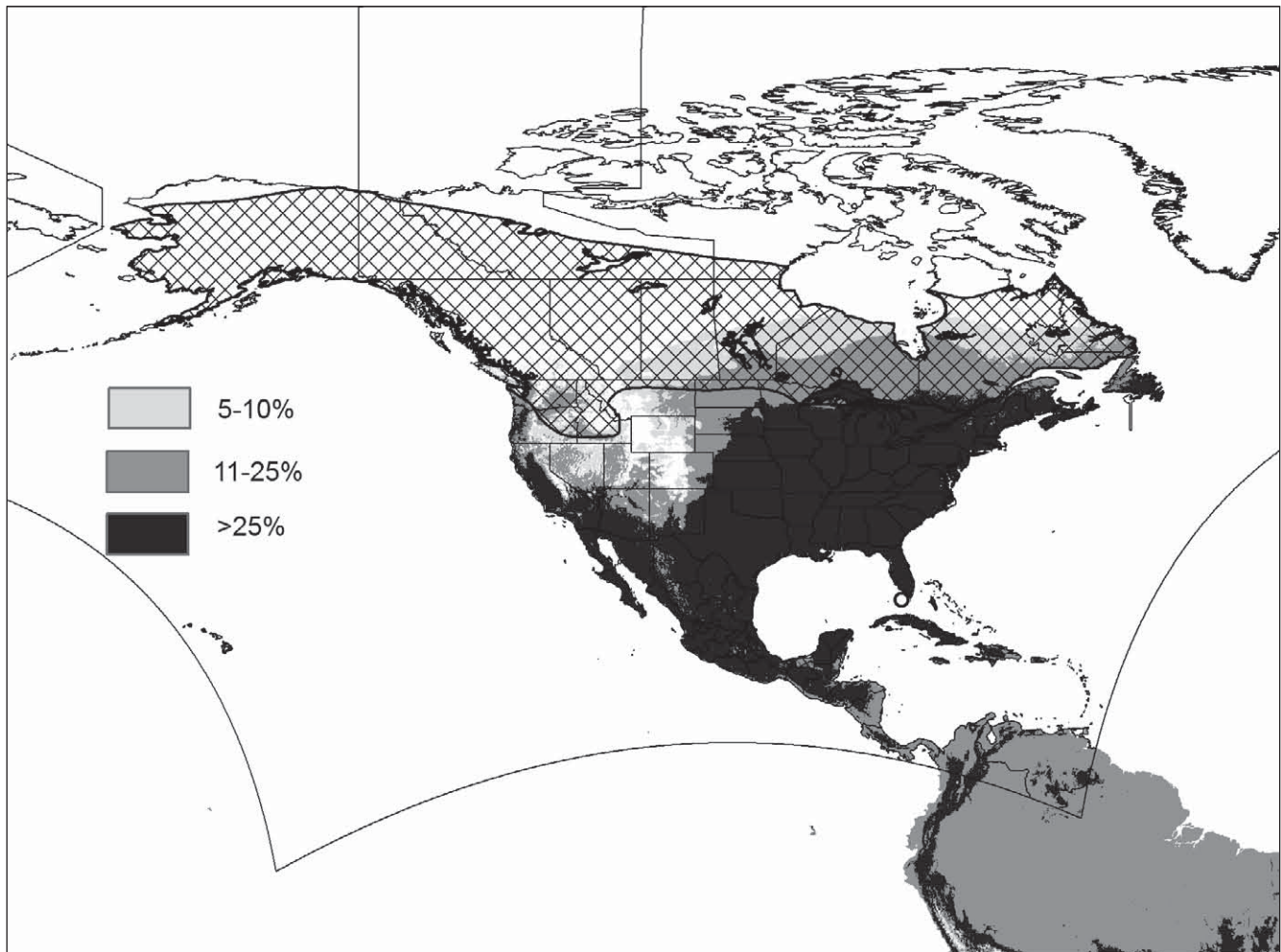


FIGURE 1. Relative frequencies of the predicted origins of 120 Merlins sampled in fall migration in Florida (location denoted by circle) plotted on the base map. Cross-hatched area, the Merlin's breeding range (Ridgely et al. 2003). Values of  $\delta D_f$  that constitute 5% to 10% of the sample are depicted in light gray, those that constitute 11% to 24% of the sample in medium gray, and those that constitute 25% or more of the sample in dark gray.

## DISCUSSION

Our modeling of values of hydrogen isotopes from feathers of the Merlin and possibly the Northern Harrier did not place birds reliably within the breeding ranges of these two species of migratory raptors. The model's predictions on the basis of feather values fell nearly entirely outside of the Merlin's known breeding range. Our model suggests that juvenile Merlins grew their feathers much farther south of anywhere the species has ever been documented to breed. Our model's results for the Northern Harrier are more feasible, but erroneous results with one species, coupled with isotope data implying that a moderate portion of harriers migrating through the Florida Keys originated south of that species' known breeding range, led us to question the validity of the harrier model as well.

It is peculiar that Merlin values plotted on the map are shifted systematically south. In a related study, modeling of results from feathers of Sharp-shinned Hawks (*Accipiter striatus*), Cooper's Hawks (*A. cooperii*), and Northern Goshawks (*A. gentilis*) captured at Hawk Mountain, Pennsylvania, predicted origins far south of those species' breeding ranges (L. Goodrich, pers. comm.).

In conclusion, our models of stable isotopes of hydrogen did not suffice to specify the natal origins of raptors migrating through the Florida Keys. In the future, better models and additional geographic data ought to be combined with data on hydrogen isotopes if a researcher attempts to discern where migrating raptors were hatched. To strengthen resolution, additional isotopes should be paired with hydrogen. If possible, isotope data should be corroborated with banding data or visual observations, though realistically this is rarely possible.



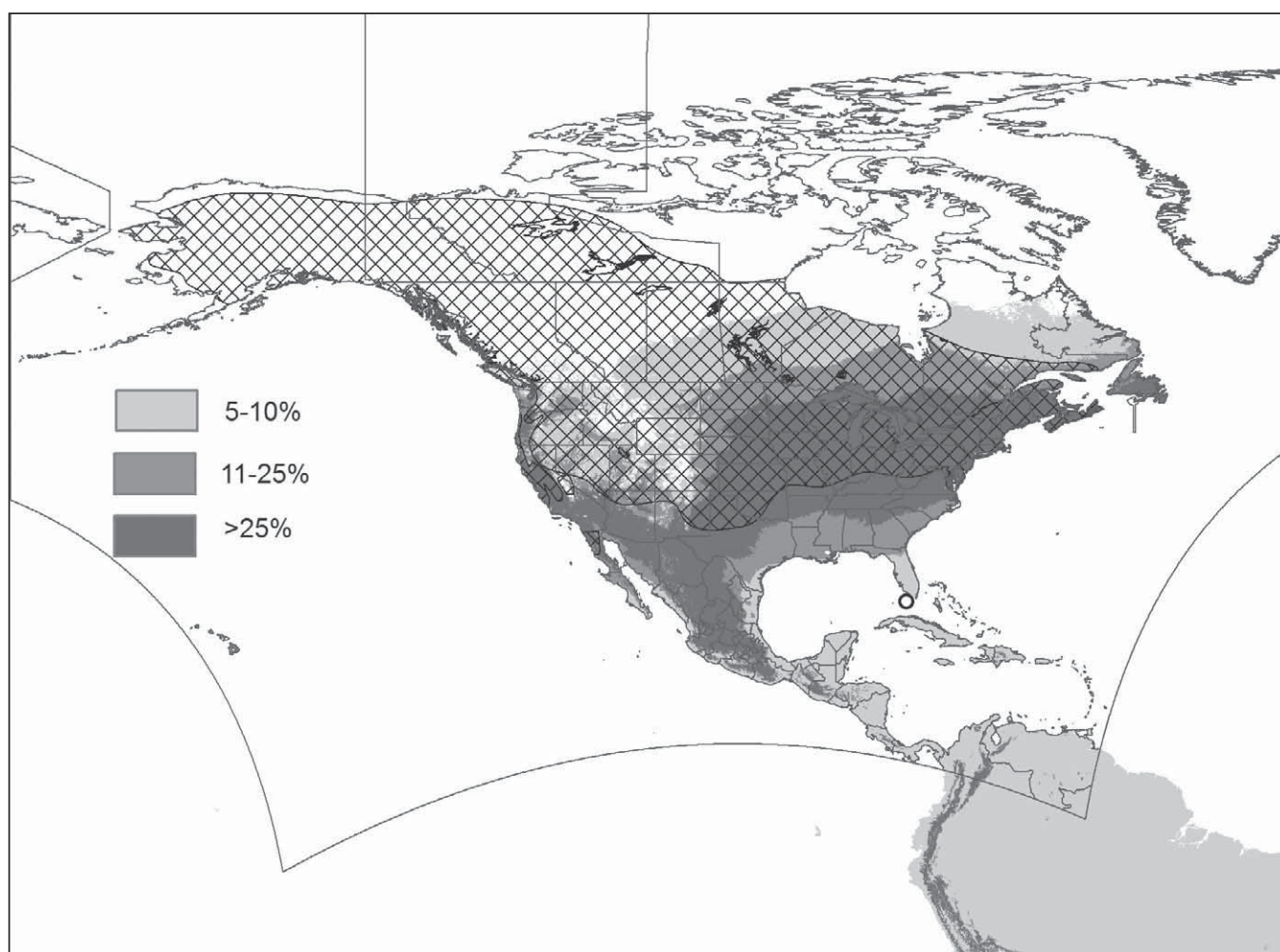


FIGURE 2. Relative frequencies of the predicted origins of 60 Northern Harriers sampled in fall migration in Florida (location denoted by circle) plotted on the base map. Cross-hatched area, the Northern Harrier's breeding range (Ridgely et al. 2003). Values of  $\delta D_f$  that constitute 5% to 10% of the sample are depicted in light gray, those that constitute 11% to 24% of the sample in medium gray, and those that constitute 25% or more of the sample in dark gray.

Greenberg et al. (2007) was the first study that used stable isotopes to predict the locality of a species and then verified its existence at that location. Data on movements provided by satellite telemetry could also be used to develop models that predict locations from isotope data, akin to assignment tests used widely in population genetics (e.g., Piry et al. 2004). One possible way to improve future studies like this is to combine stable-isotope markers with genetic markers; Chabot et al. (2012) found that by using genetic markers in combination with isotopic markers they achieved a three- to fivefold reduction in the size of the potential area of origin. Whenever possible, feathers of known spatiotemporal origin should also be included in analyses.

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