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EFFICACY OF STABLE ISOTOPE RATIOS IN ASSIGNING ENDANGERED MIGRANTS TO BREEDING AND WINTERING SITES

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Abstract. A primary constraint on effective conservation of migratory animals is our inability to track individuals through their annual cycle. One such animal is the endangered southwestern subspecies of the Willow Flycatcher, which is difficult to distinguish from conspecifics. Identifying wintering regions used by the endangered subspecies would be an important step in formulating an effective conservation strategy. Our objective was to use stable isotope ratios as a means of identifying wintering sites of Southwestern Willow Flycatchers. We analyzed stable isotope ratios of carbon, nitrogen, and hydrogen from feathers of breeding and wintering Willow Flycatchers. Based on winter samples, we document a positive trend in hydrogen isotope ratios across latitude. We also found that Willow Flycatchers use C_4 food webs south of 8° N latitude, but we found no evidence of use of C_4 food webs farther north. Nitrogen stable isotope ratios of feathers showed no discernable geographic variation. Discriminant function analyses, based on stable isotope ratios of wintering Willow Flycatchers, were only useful (>50% accurate) for assigning individuals to winter regions if the regions were large and the threshold probability for assignment was relatively high. When using these discriminant functions, most breeding samples of Southwestern Willow Flycatchers were assigned to two wintering regions: central Mexico and Ecuador. We think that assignment of Southwestern Willow Flycatchers to Ecuador is unrealistic. Given the large percentages of samples that could not be classified with certainty, we are not confident that these two regions are truly more likely to harbor wintering Southwestern Willow Flycatchers than other winter regions. We think our inconclusive results are due primarily to weak and nonlinear gradients in isotope ratios across the winter range of Willow Flycatchers.

Key words: $\delta^{13}C$; δD; $\delta^{15}N$; Empidonax traillii extimus; *migratory connectivity; stable isotope ratios; Willow Flycatcher.*

Introduction

Successful management of endangered species requires that factors limiting survivorship and fecundity be identified and ameliorated. Historically endangered Neotropical migratory birds have been managed primarily by trying to increase fecundity and survival in the North Temperate Zone. It is evident, however, that management focused solely on the months when birds are on the breeding grounds may not be sufficient to create positive abundance trajectories (e.g., Rappole et al. 2003, Hamel et al. 2004).

The Willow Flycatcher (*Empidonax trailii*; see Plate 1) is a widespread Neotropical migrant (Sedgwick 2000). Individuals in the southwestern portion of this species' range have long been recognized as a taxonomically distinct subspecies (*E. trailii extimus*; Unitt 1987, Browning 1993). The *extimus* subspecies has been protected under the Endangered Species Act since 1995 (USFWS 1995). Management efforts focused on the breeding populations have not produced a positive trend in abundance (Marshall and Stoleson 2000). Recent population analyses suggest that annual survivorship of the subspecies is similar to other Neotropical migrants, as well as other non-endangered Willow Flycatcher subspecies (Noon and Farnsworth 2000, Newell et al. 2005). Similarly, within breeding season survival is high (Stoleson et al. 2000, Newell et al. 2005).

Southwestern Willow Flycatchers spend up to eight months on winter territories from central Mexico through Ecuador. At two sites in Costa Rica, Koronkiewicz (2002) found high within-season and between-year survivorship, as well as very high site fidelity on winter territories. However, little is known about survivorship in other parts of the Willow Flycatcher's winter distribution. Similarly, extent of winter sympatry between *extimus* and other subspecies on the winter

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grounds is unknown, although investigation of museum specimens has suggested that the subspecies do not segregate on the winter grounds (Unitt 1997). Efforts to manage Southwestern Willow Flycatchers outside of the breeding season are hampered by these factors, and by the fact that it is difficult or impossible to separate individuals of the *extimus* subspecies from other Willow Flycatchers in the field (Hubbard 1999). These limitations have made it difficult to target regions within the Willow Flycatcher's winter range for research and management. We investigated whether these difficulties could be overcome through analysis of stable isotope ratios in feathers.

Stable isotopes have been used as a tool for understanding connections between breeding and wintering grounds (Kelly and Finch 1998, Webster et al. 2002, Rubenstein and Hobson 2004, Hobson 2005). Stable isotope ratios are commonly used as intrinsic markers of breeding origins in migratory birds (Kelly et al. 2002, Rubenstein and Hobson 2004, Hobson 2005, Kelly 2006). These studies also make it clear, however, that efficacy of the stable isotope approach varies depending on which isotopes are analyzed, where the source tissues were grown, the physiological condition of the sampled organism, and the underlying spatial gradients in isotopes of diet items and surface water that determine tissue isotope ratios.

We analyzed stable isotope ratios of carbon, nitrogen, and hydrogen in feather tissue to determine if these ratios could be used to link winter locations of Willow Flycatchers to breeding regions. This approach requires (1) knowing the location at which feathers were grown and (2) documenting variation in isotopic signatures across a geographic range (Kelly and Finch 1998). Among passerine birds it has been repeatedly demonstrated that the isotope ratios in feathers reflect those of the diet at the time of molt (e.g., Hobson et al. 1999). In contrast with most Neotropical–Nearctic migrant passerines, however, Willow Flycatchers grow their feathers on the winter ground (Pyle 1997) and therefore have isotopic signatures that reflect their diet at the wintering site. There are numerous recent studies that use a north-to-south gradient in hydrogen isotope ratios in the temperate zone (e.g., Kelly 2006). Scarcity of a precipitation sampling station in the winter range of the Willow Flycatcher make it unclear whether this approach will be useful for this species.

Our specific objectives were to (1) evaluate whether stable isotope ratios of feathers collected from Willow Flycatchers on their wintering grounds carry an identifiable geographic signature; and to (2) examine stable isotope ratios of feathers collected from Willow Flycatchers (after-hatch-year adults) on their breeding grounds to determine if breeding sites corresponded to areas of the winter grounds. We also discuss possible alternative methods for determining connectivity in this species, as well as general ways in which future studies could improve on our methods.

FIG. 1. Feather collection sites on the breeding grounds (black circles) and winter grounds (gray circles) of the Willow Flycatcher.

METHODS

Field methods

We collected Willow Flycatcher feathers on the wintering and breeding grounds from 1998 to 2004. Wintering sites included Mexico, Costa Rica, El Salvador, Panama, and Ecuador (Fig. 1). Breeding feathers were collected in New Mexico, Arizona, Utah, California, Oregon, Washington, Tennessee, and North Carolina (Fig. 1).

Identification of *Empidonax* flycatchers on the wintering grounds presents a challenge. However, Gorski (1969), Koronkiewicz and Sogge (2000), and Koronkiewicz et al. (2006) demonstrated that wintering Willow Flycatchers exhibit territorial responses and sing when solicited using tape playback of breeding ground vocalizations much as they do during breeding season surveys (Sogge et al. 1997). Because the Willow Flycatcher song is distinctive from all other flycatchers, we used a variety of broadcast vocalizations (Gorski 1969, Sogge et al. 1997, Koronkiewicz and Sogge 2000) to elicit song and verify species identification.

We captured Willow Flycatchers using mist nets. Tape playback was used to entice birds into the nets (Sogge et al. 2001). We monitored nets continually to minimize stress and chance of predation of birds captured. We banded birds with numbered U.S. Fish and Wildlife Service bands. We processed birds following guidelines in Ralph et al. (1995). We did not attempt any

subspecies determinations for birds captured during the winter. We collected contour (body) feathers or a primary feather from each adult Willow Flycatcher during the banding process. The feathers were placed in a macrofuge tube or small envelopes and transported to either Northern Arizona University (carbon and nitrogen) or the University of New Mexico (hydrogen) for isotope analysis. Different laboratories were used for different isotope analyses to save time and money. We are not aware of any published data that suggest that hydrogen isotope data analyzed in one laboratory are systematically biased relative to carbon and nitrogen data analyzed elsewhere.

Laboratory methods

Feather isotope analysis methods follow those of Kelly et al. (2002, 2005). For hydrogen stable isotope ratio analysis, 0.1–0.2 mg of feather was removed and wrapped in a silver capsule. Hydrogen isotope ratios were analyzed in August 2002, May 2005, and July 2005. Capsules were loaded into an autosampler and dropped into a high temperature reduction furnace (Finnigan TC/EA) interfaced through an open split (Finnigan MAT Conflo II, ThermoFinnigan, Bremen, Germany) with an isotope ratio mass spectrometer (Finnigan MAT Delta plus XL). The reduction furnace was used to pyrolize feathers samples at 1450°C.

Some of the hydrogen in feather keratin exchanges with atmospheric hydrogen (Chamberlain et al. 1997). Wassenaar and Hobson (2003) developed a method of estimating the δD values of the non-exchangeable fraction of feather keratin. Most of our data were collected prior to the availability of this equilibration method. Therefore our δD values are of total feather hydrogen rather than just the non-exchangeable fraction. Nonetheless, total hydrogen δD values of feathers have been useful in many studies (Chamberlain et al. 1997, Hobson and Wassenaar 1997, Kelly et al. 2002, 2005). All samples were air equilibrated for two weeks prior to sampling to minimize effects of differential exchange. While we would have ideally analyzed all of our samples on the same day, this level of synchrony was logistically impossible. Samples were analyzed when there was funding available to do so and we could arrange for time in the laboratory.

Carbon and nitrogen stable isotope ratios were also analyzed via continuous-flow isotope ratio mass spectrometry. For these analyses we encapsulated 0.1–0.2 mg of feather in tin and dropped these samples into a Carlo Erba elemental analyzer (800°C; Thermo Fisher, Rodano, Milan, Italy) interfaced with the isotope ratio mass spectrometer. For all analyses, we express the ratio of stable isotopes (H_2/H_1, C_{14}/C_{13}, N_{15}/N_{14}) in a sample as the parts per thousand (‰) deviation from a standard (SMOW for H; PDB for C, and AIR for N). We report deviation from these standards in delta notation (δD, $\delta^{13}C$, $\delta^{15}N$). The precision of the analyses for hydrogen was ±2.0‰, and ±0.2‰ for carbon and nitrogen.

Statistical analysis

Our analysis proceeded in three steps: (1) first we used hierarchical cluster analysis to group winter sampling sites together into regions based on geographic locations; (2) then we used discriminant function analysis to determine whether isotopic data were sufficient to correctly assign birds to their known winter sampling region; and (3) finally we used the discriminant functions developed in step 2 to examine the probability that birds sampled on the breeding ground resided in each winter region. By using discriminant analysis we make the assumption that we sampled the entire extant winter range of the Willow Flycatcher. We know this assumption to be false, but still think that our approach is useful for answering our question. In other cases where this assumption has been violated, this method has still produced high rates of successful classification (Caccamise et al. 2000, Kelly et al. 2005). Moreover, this assumption is not unique to our approach. Any attempt to classify birds to sites of origin must assume that the entire universe of origins has been sampled.

Cluster analysis of winter sites

We analyzed hydrogen, carbon, and nitrogen stable isotope ratios of feathers collected from 208 wintering Willow Flycatchers at 41 locations throughout Mexico, Central America, and Ecuador. Short distances separated many of these sites. Based on previous isotopic patterns and assignment tests (Kelly et al. 2002, 2005, Clegg et al. 2003), we knew that it was unlikely that stable isotope ratios alone could successfully assign a high percentage of birds to a single correct sampling site out of the 41 possibilities. Rather, we expect that stable isotope ratios might differ among regions of the winter range and that these regional differences might be useful in assigning birds to geographic regions. To explore the regional level relationship between stable isotope ratios of Willow Flycatcher's feathers and capture site, we used a hierarchical clustering algorithm to group the 41 sampling sites into five and ten regions based solely on the proximity of those sites to one another (i.e., latitude and longitude). The selection of five and ten clusters was arbitrary, but our limited exploration of analyses using other numbers of clusters suggests that there were no thresholds in correct classification rates as the number of clusters changed. Rather there was a gradual decline in correct classification rates as the number of clusters increased. We think that results from five and ten clusters are illustrative of these gradual changes. The clusters were formed using the agglomeration method based on squared Euclidean distances (SPSS 2002).

Assigning birds to winter regions

We divided our winter samples in half at random. We used half the data (training data, $n = 115$ birds) to build discriminant functions that maximized the ability to classify birds to the correct sample region. In the discriminant function analyses, region was the depen-

dent variable and stable isotope ratios (δD, $\delta^{13}C$, and $\delta^{15}N$) were the independent variables. The independent variable that minimized Wilks' lambda was entered into the model at each step. If that variable had an F value of ≥ 3.84, it was retained for that step. At each step those variables with an F value ≤ 2.71 were removed from the discriminant function model. Sample regions were defined by the previously described cluster analysis. We ran separate discriminant function analyses for both five and ten sample regions. We used the remaining half of the data (test data, $n = 93$) to get a realistic assessment of how much classification ability would be lost when the discriminant functions were confronted with a new data set.

These discriminant functions were built assuming equal prior probabilities. Some other applications of this method have argued that priors ought to be proportional to number of samples collected at a site (e.g., Royle and Rubenstein 2004). We do not think this is the best approach in our case for two reasons. First, proportional priors ought to be based on an estimate of bird density. The number of samples we collected at each site does not reflect bird density. Second, using proportional priors, by definition, increases the likelihood that a given unknown sample will be assigned to sites with high priors (i.e., where density is high). Overall, this approach would increase the correct classification rate of the analysis. The correct classification rate at sites where few samples are collected, however, would decrease. If Southwestern Willow Flycatchers winter allopatrically from other Willow Flycatchers, we would expect densities within their winter range to be relatively low (i.e., sites with low priors). Given that we are interested in correctly classifying birds to these sites, it seems unwise to use proportional priors in this case.

We also evaluated performance of discriminant functions while varying the threshold probability used to allow the functions to classify an individual to a region. That is, we only assigned a sample to the most likely region if it had a probability that was more than the threshold probability. We used threshold probabilities of 0.20, 0.33, and 0.50. When analyzing classifications for five regions, only the 0.33 and 0.50 classification thresholds differ from a threshold of 0.0 (i.e., 1/5 regions = 0.2).

Predicting the wintering sites of breeding Southwestern Willow Flycatchers

We analyzed feathers collected from 99 breeding Willow Flycatchers at 16 locations throughout the United States. These samples represent the *brewsteri*, *adastus*, *traillii*, and *extimus* subspecies (Fig. 1). To determine where birds of the *extimus* subspecies were likely to spend the winter, we used discriminant functions derived from winter training data to assign samples collected on the breeding ground to the most likely wintering site. Because the *extimus* subspecies is of most interest, we restrict our analysis to these southwestern birds.

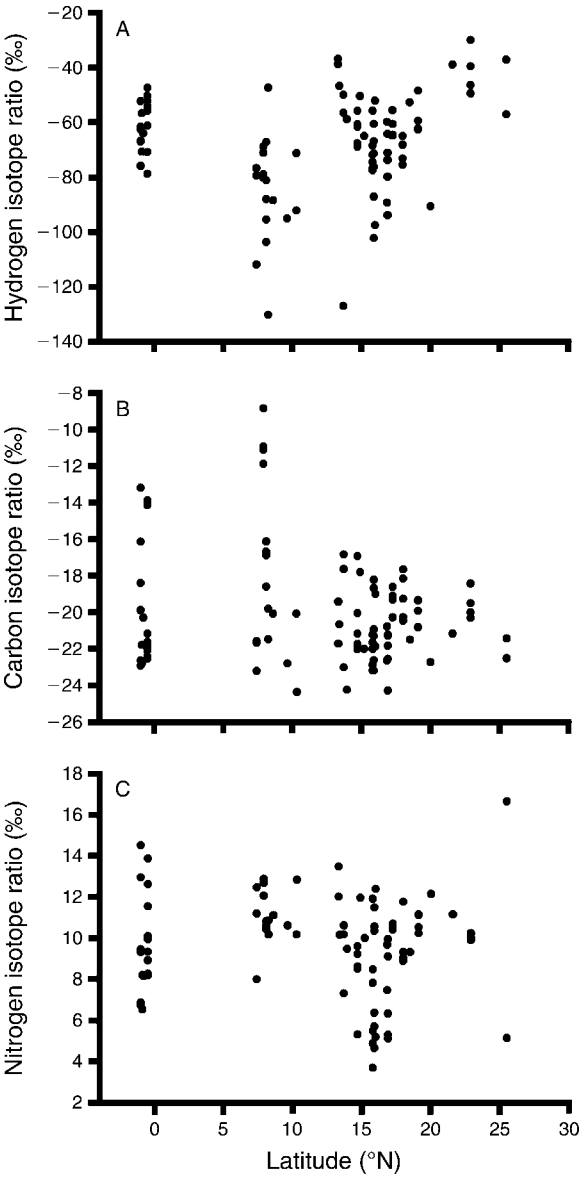

FIG. 2. Relationships among stable isotope ratios of (A) hydrogen, (B) carbon, and (C) nitrogen and latitude of sites where feathers of Willow Flycatcher were collected. Isotope ratios are relative to air for nitrogen, Pee Dee Belemnite (PDB) for carbon, and standard mean ocean water (SMOW) for hydrogen.

RESULTS

Willow Flycatcher feather isotope ratios and geographic location

There were broadscale relationships among stable isotope ratios of Willow Flycatcher feathers and geographic location of the collection sites (Fig. 2). There was a decline in the hydrogen isotope ratio of feathers

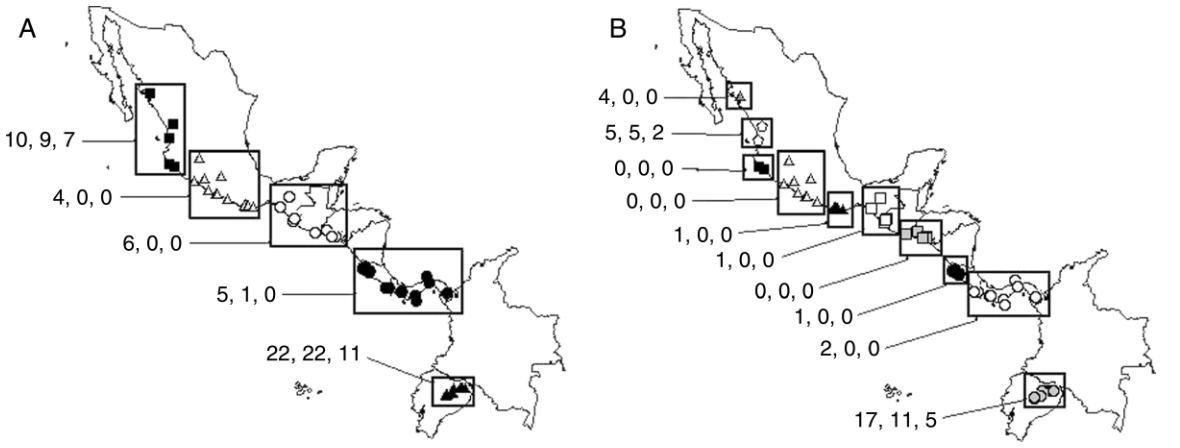

FIG. 3. (A) Five and (B) 10 regions sampled for wintering Willow Flycatchers, determined through a hierarchical cluster analysis. In each case, different symbols identify sites assigned to different regions. Numbers to the left of each region indicate the number of breeding samples of Southwestern Willow Flycatchers that were assigned to that region using threshold probabilities of 0.20, 0.33, and 0.50, respectively.

from central Mexico through Central America. In particular from the equator northward there was a strong correlation between H isotope ratio and latitude ($r = 0.51$, $P < 0.001$). It is important to note that this trend is opposite that found in the temperate zone (i.e., less enriched further north). This relationship did not hold south of the equator, in Ecuador. The values from Ecuador's samples are also interesting because they contridict expectations based on precipitation isotope maps (Bowen and Revenaugh 2003).

Second, there was a weak negative association between carbon stable isotope ratios and latitude ($r = -0.18$, $P < 0.005$) driven by an absence of any C_4 signal north of 8° north latitudes ($\delta^{13}C > -17‰$). There were substantial differences in nitrogen stable isotope ratios among sites, but there was no general association with sampling latitudes.

Clustering and assigning winter samples

We used hierarchical clustering to aggregate samples into five and ten regions that were spatially continuous (Fig. 3). We used these two aggregations to assign Willow Flycatchers to winter regions using discriminant analysis. The best classification rates were achieved when only nitrogen and hydrogen isotope ratios were used (Table 1). Because carbon and hydrogen isotope ratios were significantly correlated ($r = -0.2$, $P < 0.005$), the addition of carbon isotope ratio data did not improve the model regardless of whether assignments were made to five or ten regions. The discriminant function analysis using test data was able to correctly classify >90% (5 regions) or 60% (10 regions) of the samples when the threshold value was high (0.50; Table 2). However, very few samples had classification probabilities that were >0.50; that is, most samples were unclassified when the threshold was high. When data were analyzed without a classification threshold, discriminant functions were only able to correctly classify between 40% and 50% of samples. Correct classifications were not evenly distributed across the geographic range of samples. Rather, some samples and regions had distinct isotopic signatures that allowed more reliable classification (Table 2).

Predicting wintering sites of breeding birds

We compared nitrogen and hydrogen stable isotope ratios of breeding feather samples among the *extimus*, *brewsteri*, *adastus*, and *traillii* subspecies (Fig. 4). It was evident that, while there was substantial overlap among these samples, the southwestern (*extimus*, −56‰ + 18‰, $n = 47$) and west coast (*brewsteri*, −59‰ ± 10‰, mean ± SD, $n = 16$), subspecies tended to be less enriched for hydrogen than those of the interior west (*adastus*, −40‰ ± 7‰, $n = 19$) and eastern (*traillii*, −44‰ ± 9‰, $n = 17$; $F_{3,95} = 9.4$, $P < 0.001$). Moreover only four of 65 *extimus* birds had $\delta^{13}C$ values that were >−17‰, indicating that few were utilizing C_4 dominated habitats during molt. In addition it is interesting to note that the mean values for all subspecies are enriched relative to the values found in the center of the Willow Flycatchers winter range. Between 8° N and 18° N latitude there are many birds with H isotope ratios <−80‰, which is outside of the standard deviation of all but one of the sampled breeding populations.

TABLE 1. Loading of the two independent variables (δD and $\delta^{15}N$) used to create discriminant functions to classify Willow Flycatchers to regions of the winter range.

Variable	Function 1	Function 2	Function 1	Function 2
δD	−1.035	0.397	1.033	0.389
$\delta^{15}N$	−0.805	0.762	−0.791	0.771

Notes: We also used $\delta^{13}C$ values in the analyses, but they were not used in creating the discriminant functions because they did not improve classification rates. Separate analyses were run for ten and five regions. Regions were delineated via hierarchical cluster analysis (Fig. 3).

TABLE 2. Number of wintering Willow Flycatchers correctly classified to regions using discriminant function analyses (with sample size in parentheses).

	Training, by threshold value			Test, by threshold value		
Region	0.20	0.33	0.50	0.20	0.33	0.50
Analyses with 5 regions						
1	33 (39)	32 (33)	24 (24)	22 (27)	20 (20)	17 (17)
2	4 (19)	0 (10)	0 (3)	0 (11)	0 (6)	0 (0)
3	2 (18)	0 (5)	0 (1)	2 (17)	0 (0)	0 (1)
4	14 (28)	8 (11)	2 (4)	6 (28)	4 (10)	2 (2)
5	7 (11)	7 (8)	4 (4)	2 (10)	1 (3)	1 (1)
Total	60 (115)	47 (67)	31 (36)	32 (93)	25 (45)	20 (21)
Analyses with 10 regions						
1	7 (16)	1 (9)	1 (6)	1 (12)	0 (10)	0 (4)
2	10 (16)	10 (11)	5 (5)	6 (7)	6 (6)	3 (3)
3	0 (4)	0 (1)	0 (1)	0 (4)	0 (0)	0 (0)
4	0 (7)	0 (1)	0 (0)	0 (8)	0 (0)	0 (0)
5	0 (4)	0 (2)	0 (0)	0 (1)	0 (0)	0 (0)
6	6 (8)	5 (5)	0 (0)	5 (7)	1 (1)	0 (0)
7	0 (4)	0 (2)	0 (1)	0 (7)	0 (0)	0 (0)
8	0 (1)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)
9	1 (2)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
10	3 (4)	2 (2)	1 (1)	0 (2)	0 (0)	0 (0)
Total	27 (66)	18 (33)	6 (14)	12 (33)	7 (17)	3 (7)

Notes: Analyses used either five or ten regions as described in Fig. 1. Sample sizes (N) are the number of samples from that region for which probability of assignment to at least one region exceeded the threshold value (0.20, 0.33, 0.50).

To examine these patterns quantitatively, we used the discriminant functions built with the winter training data to assign breeding ground *extimus* samples to the most likely winter region based on feather stable isotope data. These analyses assigned most birds to two regions, Mexico and Ecuador (Fig. 3). This assignment seems to be heavily influenced by H isotope ratios, which were most enriched in the Mexico and Ecuador samples. We do not think these assignment results accurately reflect the entire winter range of the Southwestern Willow Flycatchers.

DISCUSSION

Stable isotopes as indicators of migratory connectivity in Willow Flycatchers

Stable isotope ratios of feathers collected from wintering Willow Flycatchers revealed that there was an enrichment in hydrogen isotope ratio with latitude north of the equator (Fig. 2). This pattern did not hold in Ecuador. Based on maps of isotope ratios in precipitation we expected these Ecuadorian birds to have more depleted H isotope ratios (Bowen and Revenaugh 2003). In contrast, these birds were considerably more enriched than expected. It would be interesting to know if the explanation for this anomaly has to do with the timing or location of molt among these birds relative to other Willow Flycatchers.

We only found Willow Flycatchers that relied on C_4 food webs south of 8°N latitude (Fig. 2). However, this pattern may reflect bias in the sites sampled across the range as we made no effort to stratify the sampling sites by habitat type with respect to latitude. Alternatively the pattern may suggest that birds are using both C_3 and C_4

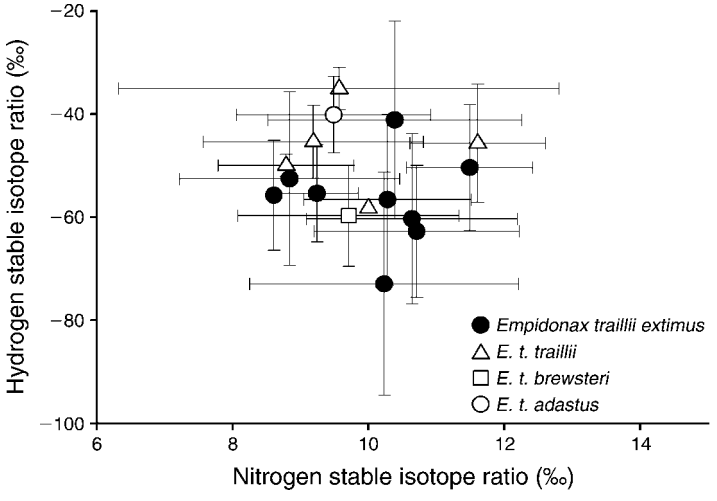

FIG. 4. Nitrogen and hydrogen stable isotope ratios (mean ± SD) of feathers collected from breeding Willow Flycatchers. Sample locations are depicted in Fig. 1. Isotope ratios are relative to air for nitrogen and standard mean ocean water (SMOW) for hydrogen.

PLATE 1. The Willow Flycatcher is a migrant whose subspecies are difficult to distinguish, particularly outside of the breeding season. The southwestern subspecies is protected in the United States by the endangered species act. Identifying areas used by this subspecies during migration and winter is a primary conservation objective. Photo credit: Bob Steele.

carbon sources below 8° latitude and only C_3 sources north of 8° north latitude.

The pattern in hydrogen isotope ratios was useful in assigning a few wintering Willow Flycatchers to the regions in which they were captured using discriminant analysis (Table 2). However, the discriminant functions were only able to correctly classify between 40% and 50% of samples. Much of this difficulty relates to the anomalously enriched values of feathers from Ecuador. While these rates are better than that expected at random (10–20%), they are unlikely to be sufficient to direct an extensive management effort.

On the breeding grounds there were clearly subspecies level differences in the H stable isotope ratios. This difference suggests that, on average, individuals of the west coast (*brewsteri*) and southwestern (*extimus*) subspecies were wintering in different locations from individuals of interior western (*adastus)* and eastern (*traillii)* subspecies. It would be useful to know whether these differences persist among breeding populations of *adastus*, and *traillii* that are closer, geographically, to breeding populations of *extimus*. It is possible that these differences are part of a more gradual change in isotope ratios across the breeding range. For example, if Willow Flycatchers exhibit any of the common forms of either chain or leapfrog migration, we would expect these types of geographic associations among breeding and wintering birds (Smith et al. 2003). This finer scale geographic information might also start to define whether and how breeding and winter regions are linked.

When discriminant functions based on winter samples were applied to breeding samples, the most common

prediction, regarding Southwestern Willow Flycatchers was that they winter in either central Mexico or Ecuador. While these areas had H isotope ratios that matched those of breeding Southwestern Willow Flycatchers, it seems unlikely that Willow Flycatcher winter distribution occurs only in two geographically disparate regions. We think it is more likely that Southwestern Willow Flycatchers might winter over a broad range but that isotope signatures were only distinct enough to allow classification at the edges of the winter distribution. Also in comparing the distribution of breeding and winter samples it is clear that many birds in the center of the winter range have H isotope ratios that were less enriched than any of the breeding population samples (Figs. 2 and 4). Therefore it would have been surprising if the discriminant function analysis assigned breeding birds to these regions. It is unclear where these deuterium-depleted birds from the center of the wintering range breed, but they seem unlikely to be part of the *extimus* subspecies.

Because the isotopic gradients across the winter range of Willow Flycatchers were weak and *extimus* is relatively rare, it was not possible to use isotope ratios to identify boundaries of the winter range of this subspecies. It is possible that there is no segregation of subspecies on the wintering grounds (Unitt 1997). However, this pattern would be unusual among species of passerine for which these patterns have been documented. Although for some species, mixing of subspecies during winter has been reported (Kelly and Hutto 2005). It is also possible that segregation occurs by habitat or elevation, which our samples were inadequate to detect.

Remedies and suggestions for future research

Other studies have shown that power to correctly classify birds to sites of origin can be greatly enhanced through use of other molecular and biogeochemical markers (Szep et al. 2003, Dockx et al. 2004). In particular, if a robust phylogeography for Willow Flycatchers could be developed, integration of genetic and stable isotope data would probably improve classification rates (e.g., Kelly et al. 2005). However, extensive genetic work with Willow Flycatchers has not yet revealed strong phylogeographic patterns.

There have been enormous advances in our understanding of potential and limitations of stable isotope approaches since our data collection began in 1998. A more rigorous stratified random study design in which each potential habitat was sampled over the identical time window with a single year would undoubtedly produce less noisy data. Collection of only first primary feathers and analysis of those feathers in a single session would also undoubtedly produce cleaner data. Corrections of H isotope ratios for exchangeable hydrogen would also be beneficial. In order to execute this study design in this region, considerable funding and lead time would be necessary, as well as permits to access public and private lands and the collection and export of body tissues for stable isotope analysis. These obstacles are significant and the shortcoming of data collected over a protracted period as they were in this study must be weighed against the opportunity costs of waiting until (or if) sufficient funding is available to implement a more rigorous design.

To more efficiently manage and conserve Southwestern Willow Flycatchers in their wintering habitat, we recommend the following: (1) continue to pursue a robust phylogeography using more sensitive genetic techniques, such as microsatellite markers. Once developed this information can be combined with data from stable isotope ratios and other intrinsic markers to improve subspecies and geographic classification of Willow Flycatchers on their wintering grounds; and (2) collect feather samples from a more geographically diverse set of Willow Flycatchers for use in stable isotope analysis.

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