

IMPROVED ESTIMATES OF CERTAINTY IN STABLE-ISOTOPE-BASED METHODS FOR TRACKING MIGRATORY ANIMALS

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Abstract. The use of stable-hydrogen isotopes (δD) has become a common tool for estimating geographic patterns of movement in migratory animals. This method relies on broad and relatively predictable geographic patterning in δD values of precipitation, but these patterns are not estimated without error. In addition, δD measurements are relatively imprecise, particularly for organic tissue. Most models for estimating geographic locations have ignored these sources of error. Common modeling approaches include regression, range-matching, and likelihood-based assignment tests (including discriminant analysis). Here, we show the benefits of a simple stochastic extension to likelihood-based assignment tests that incorporates two estimable sources of error and describe the resulting influence on the certainty of assigning breeding origins for wintering American Redstarts (*Setophaga ruticilla*), a small Nearctic-Neotropical migratory bird. Through simulation, we incorporated both spatial interpolation error associated with models of δD in precipitation and analytical error associated with the measurement of δD in tissue samples. In general, assignments that did not include these sources of error fell within the ranges of the stochastic results, but the difference in proportion of birds assigned to any one breeding region varied by as much as 54%. To explore how the distribution of assignments generated from error models influenced the application of these results, we developed a simple model of winter habitat loss. We removed the proportion of Redstarts wintering at a particular site from the global population and then used the isotope-based assignments to predict the resulting population declines for each breeding region. This gave distributions of change in population sizes, some of which included no change or even a population increase. The sources of error we modeled may challenge the degree of certainty in the use of stable-isotope-based data on connectivity to predict population dynamics of migratory animals. We suggest that stronger inference will result from incorporating these sources of error into future studies that use δD or other stable isotopes to infer the geographic origin of individuals.

Key words: American Redstarts; assignment; likelihood; migratory connectivity; stable-hydrogen isotopes; uncertainty.

INTRODUCTION

Stable isotope analysis has become one of the most widely used and cost-effective tools for tracking seasonal movements of migratory animals (Hobson 1999, 2005, Webster et al. 2002, Rubenstein and Hobson 2004). Given the extensive field efforts associated with more traditional band-recovery approaches used to address range-wide movements, stable isotopes can provide substantially more information per unit effort, largely because each encountered animal is already effectively “marked” by the local environmental resources used to form tissues. Geographic variation in stable isotope compositions of organic tissues, such as feathers, blood, or muscle can arise through various biological, climatic, or geological processes (Kelly 2000, Rubenstein and Hobson 2004, Bowen et al. 2005). Because animal tissues

reflect the chemical properties of local food webs and because keratin is metabolically inert once formed, keratin from individuals can be sampled in one season to estimate the geographic origin in which it was formed during a previous season (e.g., Chamberlain et al. 1997, Hobson and Wassenaar 1997).

In spite of the rapid increase in the number of applied studies that use stable isotopes to track long-distance movements of animals, many of the underlying assumptions are only now being rigorously tested. For example, recent studies analyzing the isotopic compositions of known-origin birds have revealed that the spatial accuracy of assignments based on the use of some elements, such as hydrogen (deuterium : protium; δD), is probably lower than previously assumed, at least for some systems (Wunder et al. 2005, Rocque et al. 2006, Langin et al. 2007). Recent studies have also provided evidence that diet-tissue discrimination factors may not be uniform across a species range (Lott and Smith 2006), or among age classes within a population (Meehan et al. 2003).

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Common statistical methods for estimating the origin of individuals using stable isotopes include simple regression (Kelly et al. 2002, Rubenstein et al. 2002), regression trees (Hebert and Wassenaar 2005), range matching (Boulet et al. 2006), and assignment methods based on likelihoods or probabilities (Royle and Rubenstein 2004, Wunder et al. 2005, Norris et al. 2006). Range matching and assignment methods have an advantage over regression models in that the specific geographic origin of animals can be directly estimated. Here, we examine how two known sources of variation in δD influence assignment probabilities and the practical application of assignment results.

The first source of variation we call spatial interpolation error, which is the error that arises when data from points are interpolated over a predefined area. Previous studies have relied on either expected δD values from precipitation-based models (and have assumed a constant discrimination factor between precipitation and animals) or have constructed spatial interpolations directly from isotope values in sampled the tissue of the target species (e.g., Lott and Smith 2006). Unless every "pixel" within a species range is sampled, both types of studies are subject to this same source of error. Therefore, whether one is using tissues from species or values from the environment (e.g., precipitation in the case of δD), geographic regions will be characterized by mean values from a spatially interpolated map and the results will be subject to random process error associated with the spatial interpolation that could translate to variation in assignment probabilities.

The second source of variation we refer to as analytical (or measurement) error and is generated during the analysis of samples for stable isotope compositions (typically with continuous flow isotope ratio mass spectrometry [CF-IRMS]). Because it is not possible to count the number of atoms of each isotope in a sample gas and because conditions vary within the laboratory and within the CF-IRMS apparatus, the isotopic values depend on run-time calibration curves. Thus, the output values are actually expected (mean) values and repeated measurements for the same sample are rarely the same. Analytical error occurs in all elements but is particularly large for δD values. Typical analytical error values reported for δD range from 1‰ to 5‰ (SD) and may therefore contribute significant uncertainty, especially when considering regions or populations with mean values that differ by an amount within this scale (e.g., 4–20‰).

In this paper, we explore how these two sources of error affect analysis of δD data and conclusions about migratory connectivity in American Redstarts (*Setophaga ruticilla*), a long-distance migratory songbird that winters in the Caribbean and Central America and breeds in North America. Norris et al. (2006) analyzed δD values in feathers of birds sampled throughout the wintering range. Because Redstarts molt on or near the breeding grounds, δD values from wintering birds were

used to estimate breeding origin. The breeding range was divided into five breeding regions and a likelihood-based assignment test was used to determine a birds' most likely breeding origin (described in more detail in *Methods*). Our two goals are to (1) examine the degree to which assignment probabilities change when both spatial interpolation and measurement error are incorporated into the assignment test, and (2) use these sources of error to generate a distribution of possible assignments for a given individual bird instead of using a single "most likely" value. To demonstrate the utility and implications of the second goal, we apply these assignment distributions to a simple model of habitat loss allowing us to generate a range of expected values for population declines.

METHODS

The data set and presentation of results

We examined how two potential sources of error could influence the assignment of birds using a stable isotope data set from Norris et al. (2006) on American Redstarts. To describe patterns of connectivity between the wintering and breeding grounds, they analyzed δD values from 188 birds captured at 12 sites across the tropical wintering range. Because feathers were molted on or near the breeding grounds, δD values from wintering birds were used to estimate (assign) breeding origin. A priori, the Redstart breeding range was divided into five regions and a mean and standard deviation of δD for each region was calculated using values from a precipitation-based interpolation model developed by Bowen and Revenaugh (2003). For each individual sampled on the wintering grounds Norris et al. (2006) then calculated probabilities of growing feathers in each of the five breeding regions based on a normal likelihood function. In addition to regional δD values extrapolated from precipitation maps, Norris et al. (2006) used Bayes' rule to incorporate a prior probability of breeding abundance using estimates from interpolated Breeding Bird Survey data (Sauer et al. 2004). The breeding region with the highest posterior probability was considered to be the region where the individual bred the previous season (referred to here as the "assignment"). For purposes here, we used a flat (uniform) prior probability both for the original data set from Norris et al. (2006) and for our simulation models because we were interested in the influence of random error due solely to δD . However, our use of a flat prior does not mean that we think relative breeding abundance does not provide useful information for calculating probabilities of origin; we encourage the use of all types of prior information in the development and refinement of models used for migratory connectivity studies.

Using the δD data set on American Redstarts, we modeled (1) the effects of spatial interpolation error derived from precipitation-based maps of expected δD values, (2) the effects of analytical error derived from measurements of δD , and (3) the effects from both

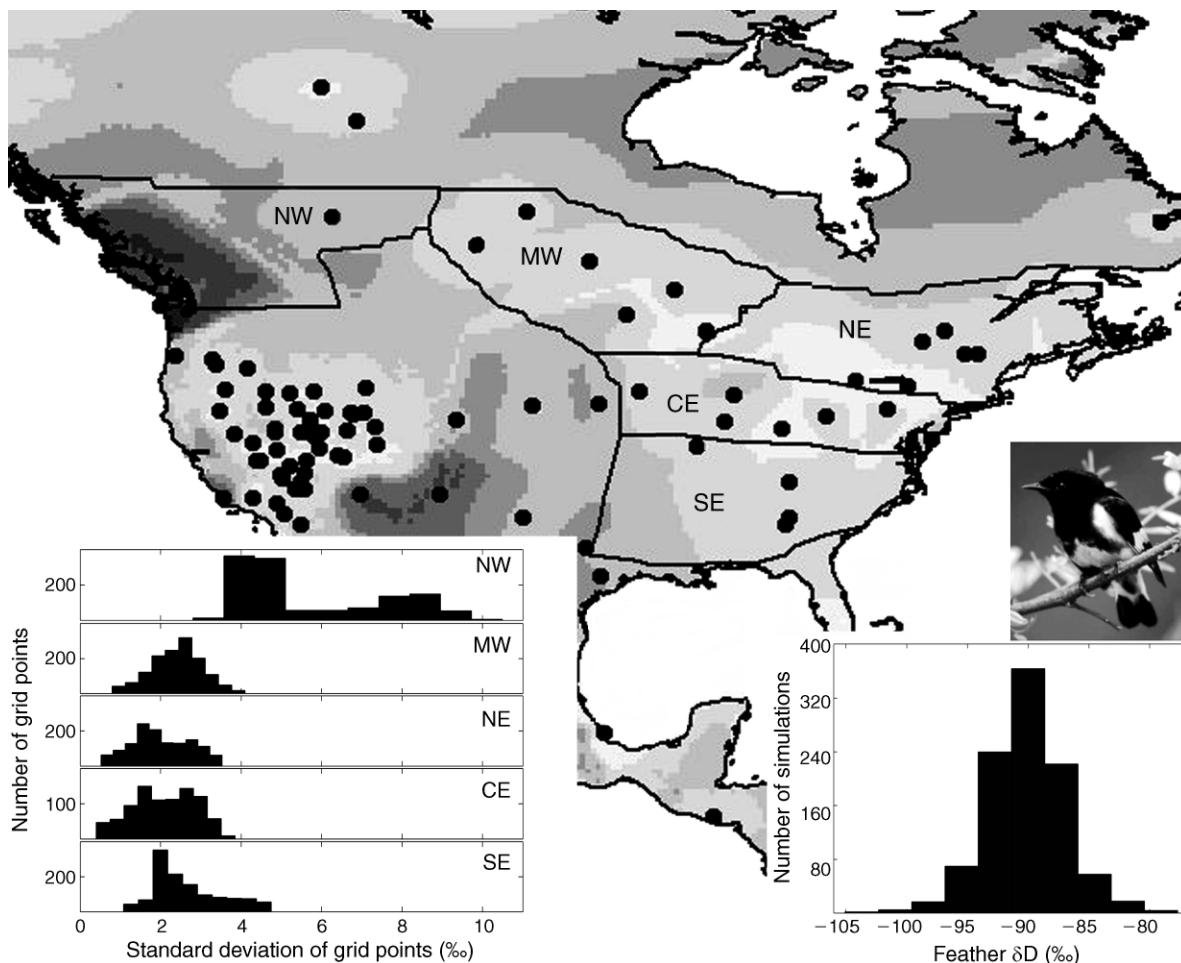


FIG. 1. Map of the study area. Outlined areas are predefined breeding regions from Norris et al. (2006); see Table 1. The gray-scale surface shows the spatial distribution for the degree of interpolation uncertainty (interpolation error) in the expected δD (stable-hydrogen isotope; deuterium : protium) values for precipitation; higher standard deviation values are darker in color, and the dots show locations of data stations (after Bowen and Revenaugh 2003). Histograms in the lower left show the frequency distributions of interpolation-associated standard deviations for the grid points in each of the five breeding regions. The histogram on the right shows the expected variation around the measured δD for an individual bird (the analytical error).

sources of error combined. For each of these scenarios, we quantified the range of probable outcomes (i.e., distribution of assignments to each of the five breeding regions derived from simulations) and compared this to the original outcome (i.e., the single assignment to the most likely breeding origin). To compare these two results, we present assignments as either pooled within each of the 12 wintering sites or pooled over the entire data set (all wintering sites). Last, to explore how these potential sources of uncertainty could influence the interpretation and application of results, we developed a model simulating habitat loss on the wintering grounds. This model allowed us to compare how a range of possible assignments to breeding regions can be used to describe the degree of certainty in detecting changes in population abundance on the breeding grounds. In the following subsections, we describe the sources of error and the habitat loss model in more detail.

Spatial interpolation error

To estimate the mean and standard deviation of δD values for each breeding region, Norris et al. (2006) used rescaled δD values from a spatial interpolation model of growing-season precipitation with a 20'(minute)-grid map resolution (Bowen and Revenaugh 2003). However, because of the limited spatial distribution of survey stations in North America, the precision of the δD estimate for each grid point in that model depends on the proximity to a station where measurements were made and on the degree to which measured values vary among data stations (Fig. 1). In turn, the distribution of δD values used to describe any predefined region depends on the number of stations and variance among stations encompassed by the region. To quantify the degree of certainty in the modeled δD values, Bowen and Ravenaugh (2003) jackknifed the data that were used to generate maps for mean annual δD in

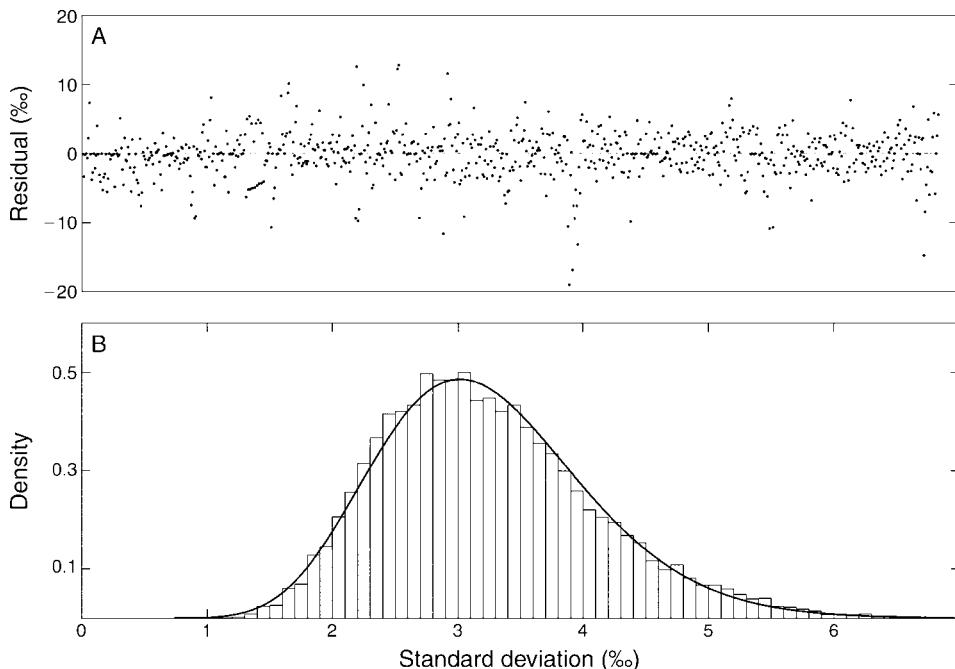


FIG. 2. Analytical error distributions. (A) The residuals from per-run calibrations for the measurements of two keratin standards over the course of a year at the USGS stable isotope lab in Denver, Colorado, USA. (B) Histogram and fitted gamma distribution for standard deviations from 10000 data sets simulated using the residuals in panel A. See *Methods* for more detail.

precipitation (called the “MAD grid”). Briefly, they simulated ~ 5000 sets of model parameters, randomly omitting data from one of the original data stations each time. Using these parameters sets and their joint probability distributions, they computed weighted standard deviations for each grid point (called the “SD grid”).

We used both the MAD and SD grids to quantify the effect of spatial interpolation error on the individual-level assignment of wintering birds to each of the predefined breeding regions. There were 1261 grid points in the northwest (NW) breeding region, 1388 in the midwest (MW) region, 1324 in the northeast (NE) region, 904 in the central-east (CE) region, and 1224 in the southeast (SE) region. We simulated 1000 sets of δD values for each grid point in each of the five breeding regions using independent random draws from a normal distribution with mean given by the value in the MAD grid and the standard deviation given by the SD grid. We then rescaled the δD values for precipitation to expected values for keratin using the rescaling for mean annual δD given in Bowen et al. (2005). For each of these 1000 simulated data sets, we replicated the likelihood-based assignment process for each of the 188 wintering birds. This gave us a distribution of 1000 assignments for each bird.

Analytical error

Analysis of δD values in organic tissue typically produces an analytical error between 1‰ and 5‰. To

quantify the effects of this error type on individual-level assignments, we estimated the distribution of standard deviations associated with the measurement of δD from continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a year-long series of measurements for two in-house keratin lab standards from the USGS Stable Isotope Laboratory in Denver, Colorado, USA. The standards were two cryogenically ground and homogenized batches of black bear (*Ursus americanus*) hair, one from a bear collected in Louisiana (LA), and the other from a bear collected in Alaska (AK). The accepted δD value for the LA standard ($-78.1\text{‰} \pm 3.0\text{‰}$) was based on 30 analyses conducted in labs in Denver, Colorado; Flagstaff, Arizona, USA; and Saskatoon, Saskatchewan, Canada. The accepted δD value ($-171.5\text{‰} \pm 3.3\text{‰}$) for the AK standard was based on 29 analyses conducted as part of the same effort in the same three labs. These values were calibrated to the VSMOW-SLAP scale via the CFS-CHS-BWBII scaling described in Wassenaar and Hobson (2003). Between two and five replicates of each standard were distributed throughout each of 150 auto-run batches for sample calibration purposes over the course of a year at the Denver lab. The auto-run calibrations were simple regressions of expected (accepted) values on the measured values of the standards. These calibration curves were fitted independently for each auto-run. The residuals from these regressions (782 observations, Fig. 2A) provided a distribution of analytical errors.

We then simulated 10 000 data sets by randomly sampling 16 values from the set of 782 residuals (16 is the number of unknown samples generally included in one auto-run at the Denver lab). For each set of 16 random samples, we computed the mean and standard deviation. In order to increase processing speed for the simulations, we fitted a gamma distribution to the set of 10 000 standard deviations (Fig. 2B); we chose a gamma distribution because values in this distribution are, by definition, always positive (as is the case with SD values), because of the relative flexibility (shape and scale) of the distribution, and because it allowed the use of a normal-gamma mixture model for the simulations. For each of the 1000 simulations, we drew an independent random sample from a normal distribution centered on the original measured δD value for each of the 188 birds with standard deviation given by an independent random sample from the gamma distribution described above. Each set of 188 samples was then subjected to the same likelihood-based assignment test as the original data, resulting in a distribution of 1000 assignments for each bird.

Simulation model of habitat loss

To explore a practical implication of incorporating these two sources of error, we developed a simple model of habitat loss for the wintering grounds. Our goal was to examine how a distribution of possible assignments to the breeding grounds generated for each individual could influence the confidence in predicting regional population declines.

We set the global population of Redstarts (n) to 1000, and let n_i be the population size for each breeding region (where $i = 1..5$). We assumed that the size of n_i was proportional to the winter sampling; each n_i was determined by the proportional number of wintering birds assigned to each breeding region from each wintering site; the number of birds wintering at each site was proportional to the number sampled there. The initial breeding region population sizes without modeling any source of error were $n_1 = 122$ (for the NW region), $n_2 = 229$ (for the MW region), $n_3 = 410$ (for the NE region), $n_4 = 138$ (for the CE region), $n_5 = 101$ (for the SE region), and the initial numbers of birds wintering at each site were as follows: Bahamas = 90, Belize = 53, Bermuda = 53, Cuba = 69, Dominican Republic = 59, eastern Mexico = 144, Florida = 96, Jamaica = 106, Panama = 53, Puerto Rico = 112, Trinidad-Tobago = 80, western Mexico = 85.

Next, we simulated habitat loss at each wintering site in turn and assumed that all birds at the wintering site that lost habitat perished. We then calculated the proportional reduction in n_i 's based on the original assignment outcomes and compared this with the range of declines in n_i 's based on the distribution of assignment outcomes generated from our models in the 1000 simulations. The proportional reductions in n_i for the simulations were relative to the respective model used.

For example, when the habitat for eastern Mexico was lost, the proportional reduction in n_1 in the analytical error model showed the number of birds that wintered in eastern Mexico and that were assigned to the NW region relative to the overall number of birds that were assigned to the NW region for each given simulation in the analytical error model. In other words, the original number of birds assigned to various breeding regions was not the same for every simulation; the percentage reduction in population size was therefore calculated on a per-simulation basis.

RESULTS

Effects of interpolation and analytical error on the assignment of birds

Using δD values from the MAD grid and the original δD values from the 188 Redstarts (Norris et al. 2006), 23 individuals were assigned to the NW region, 43 to the MW region, 77 to the NE region, 26 to the CE region, and 19 to the SE region (Fig. 3). In general, we found that these outcomes were likely to occur within the range of outcomes from our simulation models that incorporated analytical error, but not always likely to occur from our simulation models that incorporated spatial interpolation error. Fig. 3 summarizes the distribution of assignments from simulation models that incorporated spatial interpolation error, analytical error, and both types of error and compares these distributions to the original assignments without considering these error types. In each of the 1000 simulations, the spatial interpolation error model predicted fewer assignments to the NW region and more assignments to the MW region. The NW region was subject to the largest degree of spatial interpolation error (Fig. 1), and consequently the intersection between the probability distributions used for making assignments to the NW and MW regions (referred to here as the assignment boundary) shifted by the greatest amount (Fig. 4). However, in all cases, analytical error contributed more to the variation in assignments than did error from the spatial interpolation of precipitation values. Variance in assignments due to analytical error also appeared to be more generally consistent with the original assignments because the variation always encompassed the original outcome, even though it was not always centered on the original outcome. This suggests that if the only source of error were from spatial interpolation of precipitation values, the outcome observed in Norris et al. (2006) would be unlikely. In contrast, if analytical error were the dominant stochastic source, the observed results would be much more likely. The proportion of birds assigned to any one breeding region differed by as much as 54% when both error types were considered simultaneously. However, the average standard deviation in the difference of assignment proportions was 8%, and differences typically ranged to about 20% (Table 1).

The effects of spatial interpolation error on individual assignments depended on the value of δD for individual

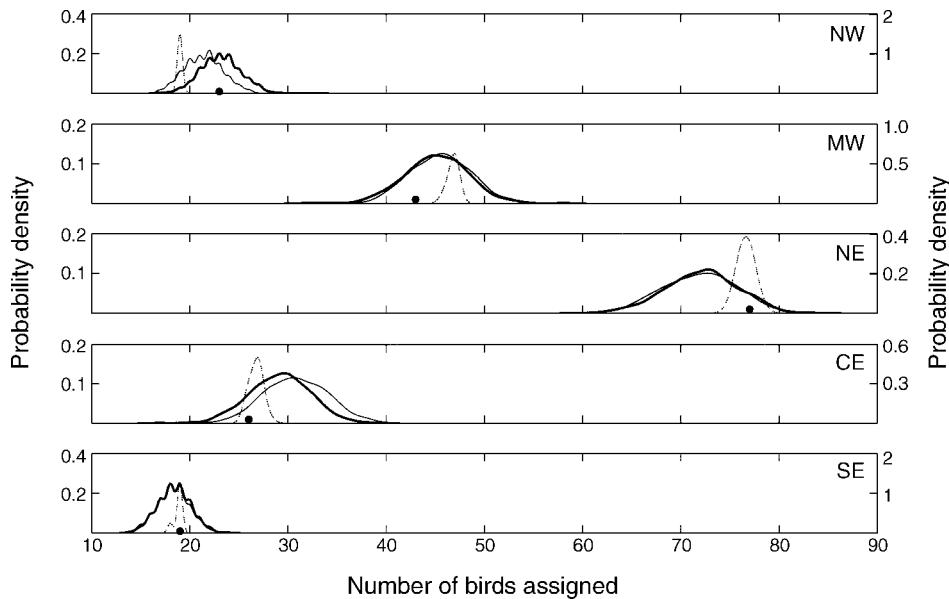


FIG. 3. Numbers of wintering American Redstarts (out of 188) assigned to each of the five predefined breeding regions (NW, MW, NE, CE, SE) shown in Fig. 1. Solid dots show the values of the original outcome that does not incorporate error. Curves with dashed lines show the probability densities based on the interpolation error simulations; scaling is shown on the right-hand y-axis. Thick solid-line curves show the probability densities from the analytical error simulations and are scaled by the left-hand y-axis. Thin solid lines are probability densities for simulations that considered both error types (scaled by the left-hand y-axis).

birds relative to the distribution of δD values of the regions. Error from spatial interpolation changed assignments only for those birds whose δD values were near the original assignment boundaries between any

two regions. Interestingly, we identified at least one bird near the assignment boundaries between each of the five breeding regions. There were five such birds in the sample of 188 Redstarts; the remaining 183 individuals

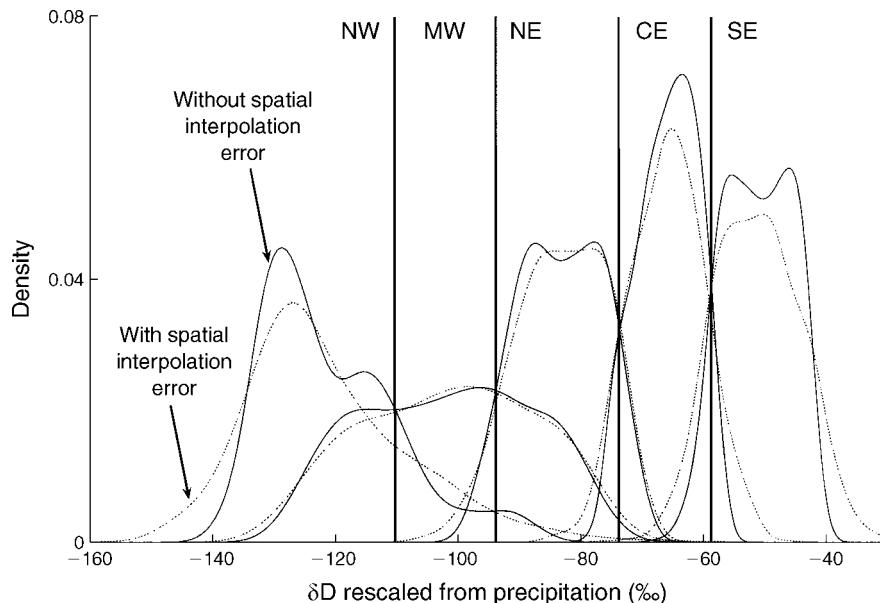


FIG. 4. Probability distributions, δD -based, used to assign individuals to each of the five breeding regions. Solid curves show the densities based on the values in the MAD grid (mean annual δD ; see *Methods*). Dashed lines show the results from one of the 1000 simulations that incorporated interpolation error using the SD grid (standard deviations; see *Methods*). Vertical lines are drawn at the assignment boundaries for the original outcome; the region to which individuals would be assigned is labeled across the top of the figure. Assignment boundaries would shift to where the dashed lines intersect for the single simulation result illustrated here.

TABLE 1. Percentage differences between the original outcome and the three simulation models in the proportional assignment of American Redstarts to each of five breeding regions.

Error type	NW		MW		NE		CE		SE	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Interpolation	-17 ± 0.4	-17, -13	9 ± 1	5, 9	-0.5 ± 0.8	-3, 1	3 ± 2	0, 8	-1 ± 2	-5, 0
Analytical	1 ± 9	-26, 43	5 ± 7	-26, 35	-6 ± 5	-22, 9	12 ± 12	-35, 50	-3 ± 9	-26, 26
Combined	-7 ± 9	-30, 22	6 ± 7	-21, 28	-7 ± 5	-22, 9	19 ± 12	-15, 54	-4 ± 9	-26, 21

Notes: All units are percentages and represent the distribution of differences between the percentage of all 188 Redstarts assigned to a given breeding region in the original outcome and the percentage assigned to that same region in each of the 1000 simulations. Breeding regions (shown in Fig. 1) are northwest (NW), midwest (MW), northeast (NE), central-east (CE), and southeast (SE).

were assigned to the same region in every one of the interpolation error simulations. In contrast, analytical error (by definition of how the error was incorporated in the simulation models) affected δD values for each bird rather than the regional assignment boundaries. Thus, on an individual basis, there were far more changes in the region to which an individual was assigned. In the analytical error simulation models, only 11 of the 188 Redstarts were assigned to the same region in all 1000 simulations. These 11 birds were individuals that had δD values near the extreme ends of the range; six were always assigned to the NW region (original δD range [-133‰ to -124‰]), and the other five were always assigned to the SE region (original δD range [-46‰ to -32‰]).

Overall, individual level assignments for the analytical error models remained relatively consistent. Including the 11 birds previously mentioned, 130 of the 188 birds (72%) were assigned to the same region in >800 of the 1000 simulations. The results for the model that included both types of error mimicked closely those for the analytical-only model; there were also 130 of the 188 birds assigned to the same region in >800 of the 1000 simulations. However, they were not the same 130 individuals identified in the analytical simulation models; six individuals were assigned to the same region >80% of the time in the analytical error simulation model, but <80% of the time in the simulations that modeled both analytical and spatial interpolation errors, and vice versa.

For illustrative purposes, we also show results of the error simulation for a single wintering site, eastern Mexico, because this site had the largest number of birds sampled during winter and was the only site that had at least one bird assigned to each breeding region (Fig. 5). The spatial interpolation error had no effect on the assignments of any individual wintering in eastern Mexico; all 27 Redstarts wintering there were assigned to the same region in each of the 1000 simulations. However, the δD value for one of the birds wintering in eastern Mexico was near the assignment boundary between the NW and MW regions, which resulted in the spatial interpolation models consistently assigning one less bird to the NW and one more to the MW than in the original outcome. In contrast, incorporating

analytical error into the δD values of each bird resulted in assigning 23 of the 27 birds (85%) to more than one region over the 1000 simulations. Four of these 23 birds were alternately assigned to one of three different regions (NW, MW, or NE) over the course of the simulations. Two individuals were assigned to either the NW or MW regions at about a 50:50 split, and another was assigned to the CE and SE regions with a split of about 50:50. Despite this potential for large variation in individual assignments, 24 of the 27 birds (89%) were assigned to the same breeding region in >800 of the 1000 simulations. The full matrix of individual assignments for each simulation set and the figures showing the per-simulation distributions of assignments for each wintering site are presented in the Appendix.

Effects of error on predicting population declines

We simulated habitat loss at each of the wintering sites and assumed that all birds at that site perished after the habitat was removed. For illustrative purposes, we again focus on eastern Mexico. Using the original assignments (i.e., without considering any source of error), the population of the NW region (n_1) was reduced by 60%, the MW region (n_2) by 23%, the NE region (n_3) by 1%, the CE region (n_4) by 4%, and the SE region (n_5) by 5%.

The distribution of reduction in n_i 's from the spatial interpolation error model encapsulated the original outcome for all but one region (NW; Fig. 6). All original outcomes were contained within the distributions for the simulations that included analytical error, as well as the simulations that included both error types. Based on the analytical error models, the relative percent reduction in population size ranged about 25% in the case the NW breeding region, 15% for the MW region, and was bimodal for the NE, CE and SE regions. The bimodality of these distributions indicated that some of the birds wintering in eastern Mexico had δD values within the range of analytical error from the assignment boundaries between regions. More importantly, it suggests that there was more than one "most likely" outcome in terms of potential reduction in population size. Specifically, results for the NE and CE regions suggest that a population change of ≥ 0 was reasonably probable.

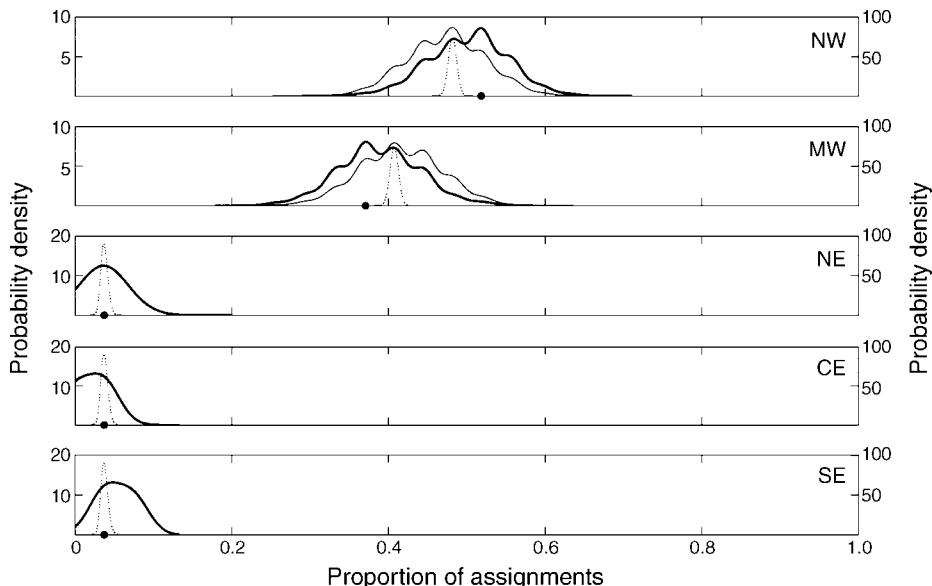


FIG. 5. Proportions of American Redstarts wintering in eastern Mexico that were assigned to each of the five breeding regions. Solid dots and line types are the same as in Fig. 3.

DISCUSSION

Our results illustrate the potential for relatively large amounts of uncertainty in the use of δD to assign individual migratory animals to particular geographic regions (Table 1). Because most studies in this field have been observational (i.e., unreplicated experiments), it is important to gain a clear understanding of how the associated assumptions affect the degree of certainty in

the conclusions of such research. Assuming that the single observed outcome represents the average behavior of a random process is profoundly different than assuming that the outcome could be anywhere within the range of likely outcomes from the same process. For example, even though birds were assigned to the same predefined breeding region in most of the simulations that included both error types, 178 of the 188 individuals

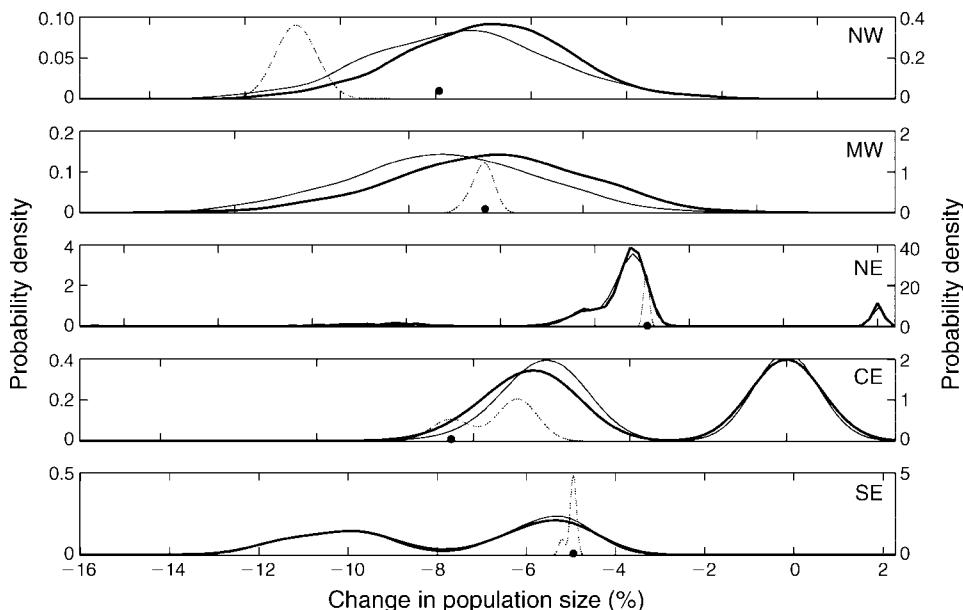


FIG. 6. Distributions of the change in population size of American Redstarts for each of the five breeding regions after loss of habitat in eastern Mexico (assuming that all the birds wintering in eastern Mexico perished; see *Methods*). Solid dots and line types are the same as in Fig. 3. Units are in percentages and refer to the number of birds that wintered in eastern Mexico and that were assigned to the NW region relative to the overall number of birds that were assigned to the NW region for each given simulation.

(95%) were alternately assigned to more than one different breeding region. Moreover, 63 birds (34% of all individuals) were alternately assigned to one of three different breeding regions within those simulations. Given the relatively broad geographic ranges of the predefined breeding regions, this was somewhat unexpected. However, the simple explanation is illustrated in Fig. 4; despite the wide geographic ranges of the breeding regions, the ranges of δD used to define (and therefore to discriminate between) breeding regions were relatively narrow, especially when considering the magnitude of the analytical uncertainty associated with δD measurements.

Although it is common practice to report some asymptotic form of analytical precision for stable isotope measurements (often as $\pm SD$ or $\pm SE$), we are aware of no studies that have directly considered or estimated the effects of analytical error on their results. Whereas it is likely that the actual δD value for tissue from an individual bird will fall within two standard deviations of the observed measurement (e.g., centered within a range of $\sim 12\%$ for most instrumentation; the asymptotic assumption), it remains possible that the observed measurement is greater than 15% different from the true value, the stochastic reality. Fig. 2 shows that it is possible that measurements of a highly homogenized standard keratin material can be off by nearly 20% (implying a range of 40%; Fig. 2A), despite the fact that the observed asymptotic standard deviation is 3.23% (Fig. 2B). Unfortunately, in the case of making a single measurement for an unknown sample (which is the most common approach) there is no way to directly measure the accuracy of that particular measurement. Because of this, incorporating a full distribution of possible assignments for each individual directly into data analysis is more informative than using the original single assignment. We suspect that doing so would affect the conclusions of many studies published thus far.

The analytical error simulations resulted in a wider distribution of assignments for an individual than did the spatial interpolation error simulations. This may be in part because the predefined breeding regions each contained a relatively large number of grid points (Fig. 1), thus reducing the estimated variance associated with the population of estimated precipitation values. This in turn dampens the effect of even relatively large spatial interpolation errors associated with any grid point. Error from spatial interpolation is likely to have a bigger impact on studies that encompass either smaller assignment regions or assignment regions for which there is relatively greater spatial interpolation uncertainty (e.g., fewer sampled locations or more complex topography).

Using predefined breeding regions can be an effective method to assign animals of unknown origin using δD but regions must be defined such that it is possible to overcome the relatively high variability introduced from both the spatial modeling process and natural hetero-

geneity in the landscape. It is important to realize that the geographic size of any region is not necessarily directly related to the isotopic variability (e.g., see Fig. 4); geographic context is the more defining feature. A key advantage to the use of predefined breeding regions over range-matching or simple regression approaches is that it does not rely on a single δD value for a given location. The process of using modeled precipitation values for a given region to define assignment probability distributions for each region effectively divides a gradient of potential δD values into bins defined by assignment boundaries (Fig. 4). Whereas this is an efficient means for assignment, it masks the degree of assignment uncertainty that can be seen when the probability densities for each of the potential regions of origin are plotted together (Fig. 4). That is, in some areas along the gradient of potential δD values (e.g., the one-dimensional isotope topology), the difference in density values between two or more regions can be small. This is of course exaggerated near the assignment boundaries between two regions. For example, a bird with a δD value between -80% and -100% may have originated from the NW, MW, or NE regions and a bird with a δD value between -50% and -70% could have originated from the MW, NE, or CE regions (Fig. 4). Moreover, if we accept that analytical uncertainty generates a range of values approximately 12% wide for a given measurement on an individual within unknown origin, we see that the ranges of δD for the MW and CE regions are on this same order of magnitude. That is, any assignment to either the MW or the CE region is likely tenuous.

Our results also demonstrate that using a range of possible assignment values for a given individual can have important consequences on how these results are interpreted. Patterns of migratory connectivity will be important for predicting changes in population size of migratory animals (Webster and Marra 2005). Here, we coupled a simple model of habitat loss on the wintering grounds with the distributions of assignments from our simulations to predict changes in population size on the breeding grounds. We show that incorporating a distribution of assignment values leads to a wide range of potential population declines (e.g., Fig. 6). Although the predicted reduction in regional populations based on the original assignments fell within the range of possible outcomes from the simulation models, in some cases, the range of possible outcomes included zero. Some simulations also predicted regional population increases despite the fact that the only numeric manipulation in our simulations was removal of birds. Regional increases occurred under the following two conditions: (1) individuals wintering at non-affected sites were assigned to different breeding regions in the two comparative iterations and (2) few or no Redstarts from the affected winter site were assigned to those breeding regions. These results suggest that ignoring both analytical and interpolation error may lead to erroneous conclusions

about population dynamics of migratory species. Estimating probability densities of assignments thus allows the incorporation of stochastic effects into more complex population models, which in turn leads to more robust inference about potential population dynamics.

There are, of course, other estimable sources of error that we did not consider here, but that are likely to affect conclusions from isotope-based assignments. Two known sources of error we did not consider include (1) temporal variation in the δD values in precipitation at any grid point (Farmer et al. 2002), and (2) variation in the discrimination factor between precipitation and animal tissue (Lott and Smith 2006). Both of these sources error will likely contribute additional uncertainty in assignments in animals of unknown origin and warrant investigation using an approach similar to what we have presented here.

In summary, certainty associated with likelihood-based assignment methods will depend on the level of understanding of any process that generates variance in either the measured δD value for an individual or in the values used to characterize a given region (a precipitation-based model in this example, but also extends to feather-based examples), especially for δD values near assignment boundaries. Likewise, any individual with a δD at an extreme end of the gradient will necessarily be assigned to the region at that end of the gradient, even though the individual may have actually derived from another, uncharacterized or unrecognized region. This means that the method is sensitive to how particular regions are predefined. Because of this sensitivity, considering distributions of individual-level assignments will lead to more robust inference than will the use of single assignments to the "most likely" region.

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APPENDIX

Original and simulated regional assignments for individual American Redstarts (*Setophaga ruticilla*) based on the observed value of $\delta^2\text{H}$ for the sampled feather and varied model assumptions (*Ecological Archives* A018-015-A1).