# CHAPTER 5

# Analysis and Design for Isotope-Based Studies of Migratory Animals

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## I. INTRODUCTION

One of the major advantages of using stable isotopes as intrinsic markers is that migratory individuals only have to be captured once to estimate a geographic move. However, the problem of geographic *assignments* (Table 5.1) to individuals of unknown origin also presents some significant quantitative

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**TABLE 5.1** Glossary of key terms associated with tracking animals using stable isotopes

| Term | Definitions in the context of isotope-based assignments |
|---|---|
| Assignment method | A way to place animals of unknown origin in a specific location during a previous period of the migratory cycle |
| Assign-time calibration | The use of known-location tissues to relate isotope values to geography and environmental material |
| Asymptotic standard deviation | The standard deviation that applies to very large sample sizes. This is not the value that applies to a single IRMS analysis run. It would apply if we were able to repeat our samples runs many times. |
| Bayes' Rule | A formal rule for inverting conditional probabilities: $P(A\|B) = P(B\|A) * P(A)/P(B)$ |
| Deterministic | Refers to an outcome that is not influenced by chance events. The structure of the process is known and well-defined. |
| Isoline | A line across space that represents equal isotopic values |
| Isoscape or base map | A map of isotopic values used to assign the locations of animals of unknown origin |
| Likelihood | The hypothetical probability an event in the past would have resulted in a particular outcome |
| Mean field | The asymptotic result for a stochastic process; the set of average values for a model |
| Monte Carlo integration | A simulation-based method for approximating a probability distribution function |
| Probability | The chance that a specified event will occur in the future |
| Run-time calibration | The use of known-value material (standards) to relate IRMS measurements to true values. The term run-time indicates that an independent calibration is made for each carosel of samples run using continuous flow IRMS |
| Stochastic | Refers to an outcome that depends on chance, a random event |
| Spatial interpolation | A method to generate predicated values for all points in space from a finite number of data points |

and statistical challenges. This chapter reviews and discusses quantitative approaches for making inferences about the geographic history of migratory animals using stable isotope values measured in sampled tissues. The most commonly used tissues include feathers, claws, fur, blood, muscle, or bone (see Chapter 2). Here, we describe the nature of stable isotope data and its direct relevance to estimating the origin of individuals, discuss some common assumptions, and show how the basic approach to assignment can be treated as a *calibration* problem. We then review and analyze modeling approaches that have been used to date (Table 5.2), briefly discuss potential for future extensions and improvements, and conclude with a discussion of sampling design considerations. Our goal in this chapter is to provide professionals and graduate students a comprehensive introduction to methods that have been used to assign individuals of unknown origin using stable isotopes.

## II. CALIBRATION AND THE ASSIGNMENT PROBLEM

An essential premise in using stable isotopes to track the movement of migratory animals is that detectable and predictable patterns (or at least differences) exist in the spatial distributions of stable isotopes in the environment from where migratory organisms obtained their diet (Chapter 3). For example, as noted in earlier chapters, there are relatively strong, well-known, and globally predictable geographic gradients in the hydrogen ($\delta$D) and oxygen ($\delta^{18}$O) isotope compositions of meteoric water (Dansgaard 1964). Geographic gradients in other isotopes are less well known. Stable strontium isotopes ($\delta^{87}$Sr) vary with the age and type of bedrock (Chapter 4). Spatial distribution models of

$\delta^{13}$C depend strongly on modeled distributions of C3 and C4 plants as well as models of plant respiration and photosynthesis and potentially of agricultural crops. We are unaware of any biogeophysical model or theory for describing expected spatial distribution of $\delta^{15}$N for any organisms' tissues.

When geographic patterns are detected for stable isotopes, they are really inferred from a finite number of sampling points across the landscape. Although patterns in $\delta$D of feathers have been modeled as linear functions of latitude and longitude (Kelly *et al.* 2002, Rubenstein *et al.* 2002), a *spatially interpolated model* (Table 5.1) is necessary to derive a continuous surface over which animals can be assigned to specific locations. For example, $\delta$D values in precipitation have been empirically modeled for North America (Hobson and Wassenaar 1997, Meehan *et al.* 2004) and worldwide (Bowen *et al.* 2005). The generation of these models produces *isoscapes* (Table 5.1) in which individuals of unknown origin can be assigned (Chapter 4).

The key assumption, however, is that patterns in stable isotopes derived from environmental or low trophic-level sources are faithfully maintained or translated through food webs. In other words, the *isotopic discrimination* (Table 5.1) between the stable isotopes in dietary sources and the tissue in the species of interest is both predictable and constant in time and space (Chapter 3). Thus, an animal incorporates isotopic values that are assumed to be representative of the location at which the tissue was grown. When the animal moves elsewhere, it can then be sampled to infer its previous geographic location. Quantitatively, then, in order to infer the geographic location most likely associated with measured stable isotope values, the most critical part of the approach is to calibrate the geographic model using tissues from animals of known origin. When the model is in explicit geographic map form, the resultant calibrated maps are often referred to as isotopic base maps.

Just as all isotope-ratio mass spectrometry data depend on *run-time calibrations* (Table 5.1) with universally accepted reference materials, or calibrated standards, so too do models for assigning individuals to locations depend critically on *assign-time calibrations* (Table 5.1) using "standards" (tissues of known geographic origin). The accuracy of isotope ratio mass spectrometer (IRMS) measurements is best when using standards with chemical compositions similar to that of the unknown samples (*e.g.*, using organic keratin standards to calibrate organic keratin samples; Wassenaar and Hobson 2002). In the same way, the accuracy of geographic model calibrations is improved by using "standards" with similar attributes as the samples for which we want to assign geographic provenance. This means that geographic assignment models perform best when calibrated using standards from the same species as the unknown samples, and that bracket the range of isotope values and all other covariates in both time and space from which the samples are drawn.

Ideally then, the calibration dataset would include samples obtained from across all potential areas in which the migratory species of interest could have originated. However, sampling tissues from all potential places of origin is not only costly, but very often logistically difficult or impossible for areas well beyond the reach of monitoring and sampling networks. Most studies have instead simply used previously estimated $\delta$D isoscapes, with the assumption that the predicted gradients in $\delta$D of precipitation are directly transferred to bird feathers (*e.g.*, Wassenaar and Hobson 2001, Norris *et al.* 2004, Bearhop *et al.* 2005, Mazerolle and Hobson 2005, Mazerolle *et al.* 2005, Boulet *et al.* 2006, Hobson *et al.* 2006, Norris *et al.* 2006, Perez and Hobson 2006, Hobson *et al.* 2007, Mazerolle and Hobson 2007). In mathematical terms, this assumption implies a slope of one and a fixed intercept (usually assumed to range from −20‰ to −25‰) for the regression of the isotope values in feathers on those for precipitation (Table 5.1, Chapter 3). The derivation of the intercept is typically done either by: (1) adopting a discrimination factor estimated from another species in a previous study (most common) or (2) estimating a discrimination factor for the species of interest but only from a single or few location(s). However, there have been relatively few studies that have addressed the assumption of a constant discrimination rate across space and between species (Lott *et al.* 2003).

Alternatively, isoscapes can be derived directly from tissues sampled from the species of interest over the time period of interest (Hobson *et al.* 1999). In such cases, the calibration is incorporated directly into the spatial interpolation. Once developed, an isoscape is a static surface that is generally then

**TABLE 5.2** Description of methods for assigning animals of unknown origin using stable isotopes

| Level of complexity | Model type | Description | Advantages | Disadvantages | Incorporates sources of error | Key references |
|---|---|---|---|---|---|---|
| Low | Map lookup | Isotope value of animal assigned to area based on isolines or isoscapes on a map | Easy to implement | Not rooted in probability, does not incorporate spatial variability of isotopes or other known source of error | No | Chamberlain *et al.* (1997), Hobson and Wassenaar (1997), Meehan *et al.* (2001), Bearhop *et al.* (2005), Lott and Smith (2006), Hobson *et al.* (2007), Paxton *et al.* (2007) |
|  | Linear regression | Origin inferred based on a regression of isotopes on latitude and/or longitude | Easy to implement | Does not incorporate spatial variability of isotopes within regions or other error sources | No | Kelly *et al.* (2002), Rubenstein *et al.* (2002) |
|  | Classification trees | Origin inferred based on series of hierarchical, discrimination-based decision rules | Can be applied to multiple isotopes, does not require distributional assumptions, accommodates both continuous and categorical predictors | Does not incorporate most error, no *a priori* hierarchy for multiple isotopes, does not provide degree of certainty for branching decisions | No | Hebert and Wassenaar (2005a,b) |
|  | Likelihood-based assignments | Origin inferred from probability density functions for isotope values from given regions | Can be applied to multiple isotopes, provides probability of assignment for given individual, easy to implement | Does not incorporate most error, simply assigns region for animal based on highest likelihood value, requires sampling all potential regions of origin | Some | Royle and Rubenstein (2004), Kelly *et al.* (2005), Wunder *et al.* (2005), Norris *et al.* (2006) |

| | | | | | | |
|---|---|---|---|---|---|---|
| ↓ | Likelihood with priors | Same as above but adds prior information (isotope or other sources using Bayes’ Rule) | Can be applied to multiple isotopes can utilize non-isotopic information (see Table 5.3) | Does not incorporate most error, simply assigns region for animal based on highest posterior probability, requires samplings all potential of origin | Some | Royle and Rubenstein (2004), Wunder *et al.* (2005), Norris *et al.* (2006) |
| | Stochastic extension of likelihood | Same as above but adds known sources of error associated with isotope data | Can be applied to multiple isotopes, incorporates multiple sources of error, provides a range of possible assignments for a given animal | Computationally intensive | Yes | Wunder and Norris (2008) |
| High | Probability surfaces | Models stochastic error process over mean field surface | As above, incorporates known variance sources is applied as a continuous probability surface over space | Computationally intensive | Yes | Wunder (2007) |

treated as a *deterministic* (Table 5.1) process to describe the spatial pattern in the isotope of interest. As we discuss below, all these calibrated models represent only the first step in the modeling process for assigning provenance to migratory animals. Ultimately, we would like to get beyond these simple cartographic exercises to models that provide surfaces for describing geographically explicit probabilities of origin for individual animals.

Recent studies have demonstrated that there can be substantial variation in isotope values (especially $\delta$D) in feathers from a group of individuals sampled at a given site, or even within a single tissue type (Chapter 2). Isotopic differences between age groups represent one of the best-documented examples (Duxbury *et al.* 2003, Meehan *et al.* 2003, Smith and Dufty 2005, Langin *et al.* 2007, Wunder 2007). Lott *et al.* (2003) demonstrated that there are geographic differences in the relationship between $\delta$D in feathers and precipitation, and Wunder *et al.* (2005) documented within-location differences among years for $\delta$D, $\delta^{13}$C, and $\delta^{15}$N in mountain plovers (*Charadrius montanus*). Langin *et al.* (2007) showed systematic differences in $\delta$D between blood and feathers between known-origin American redstart (*Setophaga ruticilla*) adults and nestlings. These findings emphasize the need for more studies aimed at understanding the mechanisms that drive differences in isotopic discrimination factors over space, between species, and across life histories.

Hobson (2005) noted the utility of developing isoscapes that are derived directly from the tissues of interest (*e.g.*, bird feathers), but only one such effort has been undertaken to date (Hobson *et al.* 1999). Such endeavors require broad spatial sampling for the species (and tissue) of interest and require that multiple individuals be sampled at each location to estimate within-site variation. Another need for localized calibration arises because broad geographic patterns most likely change over time (*e.g.*, they vary seasonally, annually, by decade, by century). Thus, it is best to calibrate models with known origin tissues collected from a representative time period of interest. For example, assigning wintering migratory birds to breeding origins would be optimized by sampling known-origin tissues on the breeding grounds in the previous year. And because the relationship between broad geospatial patterns likely varies with species, age of and condition of individuals as well as with a host of environmental factors, calibrations with known origin tissues should ideally cover the expected range for all of these covariates. In other words, the most robust models for estimating the origin of migratory animals are those that are calibrated as specifically as possible, both in space and over an appropriate time frame.

We recognize that in most cases, developing species- and tissue-specific isoscapes may be unrealistic due to both logistical difficulties (*e.g.*, inaccessible areas) and financial constraints. There is, therefore, a critical need to test general hypotheses that attempt to explain observed differences in isotope discrimination factors between and within species, between different tissues, and across space. Testing should include a combination of *in situ* studies of wild animals and controlled laboratory studies, ideally in a back-and-forth way that promotes understanding of mechanistic factors in natural settings. Only by advancing understanding in this way we will be able to derive some general rules about variation in isotope discrimination and be able to identify optimal sampling schemes for generating more robust isoscapes that can be used to track the movement of many migratory species.

## III. CHARACTERISTICS OF ISOTOPE DATA FOR USE IN STUDYING MIGRATORY ANIMALS

Measurements of stable isotope values using IRMS are reported relative to a known internationally accepted reference and use the typical "$\delta$" notion (Chapter 2). The $\delta$-values are ratios of ratios that can range from –1000‰ to positive infinity. The $\delta$-values are calculated using a calibration that usually consists of a two-point (or a few-point) linear regression using reference materials (standards) that (should) span the natural isotopic range of the unknown samples. The full range of possible values is never covered by any natural dataset, and the range of interest is almost always narrow enough that a careful linear calibration is an excellent approximation (Gröning 2004). The important point to recognize is that the $\delta$-value of a sample is not an exact value, but is derived from a linear regression of results from primary reference materials (standards).

Jardine and Cunjak (2005) remind us that IRMS measurement of bulk unknown samples (of variable mass and isotopic heterogeneity) can not be 100% accurate and are affected by inherent variability in the preparative procedures. Although the IRMS apparatus itself is capable of very fine precision (*e.g.*, calculating ratios to the sixth decimal place), the magnitude of the $\delta$ differences we may want to detect in the environment can be on the same order as the precision of the instrumentation. This point should not be overlooked by those wanting to use stable isotopes in ecology. It is not reasonable, for example, to claim that a difference of 10‰ between two populations is significant if the analytical error associated with the particular isotope measurements is ±3‰. This is because the ANOVA or other similar tests for significant differences between means assume that the δ-values are *exactly* known. However, the δ-values are each only known within ±3‰, which means that it is not unreasonable to expect to observe two different measurements for the same sample (not two samples from the same population) that are 6‰ apart. More importantly, this suggests the utility of using *stochastic* (Table 5.1) approaches to analyze conclusions about stable isotope data.

## IV. STATISTICAL ASSUMPTIONS FOR USING ISOTOPE DATA TO INFER ORIGIN

### A. Statistical Independence

The assumption of statistical independence requires samples be drawn at random from the at-large target population of interest. In linear models that relate stable isotope values to latitude, for example, clustered sampling designs (*e.g.*, many individuals from a few locations) illustrate a classic violation of this assumption. For example, suppose that feathers from 140 birds were sampled for $\delta$D. Suppose that those 140 birds were taken from only 10 sites that vary in latitude. In this case, there are not really 138 degrees of freedom for fitting a linear model (140 minus 1 for the slope and 1 for the intercept); there are only 8 degrees of freedom. In this example, site is the more appropriate sample unit because we are trying to relate stable isotope values to geographic variables, and each of the 14 birds sampled from a single site shares the same value for the response variable (latitude). The samples for each site are better treated as replicates than as independent samples. Statistically, this problem can be overcome by including a random effects term (site) in the linear model, or considering a repeated measures modeling framework.

### B. Identically Distributed (Process Homogeneity)

Implicit in all stable isotope studies that seek provenance of migratory animals is the assumption that all individuals in the population derived from a particular location are subjected to the same processes that generate variance in tissue isotope values. For example, we assume that all individuals of the target species respond in the same way to environmental stresses, foraging at roughly the same position in relatively similar food webs, and developing tissues at roughly the same rate. This strongest assumption that a site (characterized by the measurement of multiple individuals) is isotopically homogeneous will almost never be met.

Isotopic variation among individuals stems from differences in what they consume, when they consume it, and under what conditions they develop tissues, all of which potentially contain useful information. The second source of stable isotopic variability comes from isotopic heterogeneity within an individual animal. Organic tissues within an animal may develop and senesce at different rates. As such, this too can provide useful information about changes in geography, environmental conditions, diet, or life history trade-offs. The third source of variance is IRMS measurement error derived from run-time calibrations, and it is a simple matter to measure the calibration residuals to quantify analytical error. Although the error is usually not the same for each analysis run, the *asymptotic standard deviation* (Table 5.1) of these residuals is generally all that is reported. As more is learned about systematic deviations from this assumption of process homogeneity, relevant covariates can be added to adjust models.

## V. POPULATION-LEVEL VERSUS INDIVIDUAL-LEVEL GEOGRAPHIC ASSIGNMENTS

Are we interested in the distribution of geographic origins estimated from δ-values for individuals in a sample population of migratory animals, or are we interested in the geographic origin associated with the average δ-value for a population of migratory animals? These subtly different questions have traditionally been treated in different ways. However, we argue that population-level questions about the origin of migratory animals are best treated by compiling individual-level assignments rather than summarizing δ-values from a given sample of individuals (*e.g.*, using the average δ-value). This is because we are not really interested in δ-values themselves but rather in a transformation of those values to some geographic location(s). Because there is not a perfect 1:1 transformation from δ-value to geographic coordinates, it is important to transform the δ-value for each individual data point into a geographic value *before* determining the population-level characteristics of the distribution of geographies. Otherwise, we may potentially lose very valuable information associated with sources of "error," or variation that may be systematic, and therefore informative.

Assigning individuals to geographic origins one at a time and exploring properties of the resultant geographic distribution is more flexible and natural than using the average of the δ-values to determine the average geographic origin for a sample population for two important reasons: (1) it does not force the data to follow the normal distribution (or any other assumed distribution) and (2) it does not provide an overly optimistic sense of precision by (a) ignoring among- or within-individual variance in the transformation to geography and (b) ignoring measurement error. More importantly, the goal of many, if not most studies of migratory animals, is to determine if there is any geographic structure in the sampled population. In other words, the goal is to describe the distribution of assignments for the sampled population. It is therefore counterproductive to fit an assumed distribution (structure) for the population prior to doing the assignment analysis.

First, describing a population by calculating the arithmetic average (mean) and standard deviation for the isotopic data implicitly assumes normality. The mean ($\mu$) and standard deviation ($\sigma$) are the two parameters that fully specify the normal distribution; $\mu$ is the location and $\sigma$ is the scale. However, if the sample population is actually a mix of two distinct populations, it should theoretically follow a bimodal distribution. Using $\mu$ and $\sigma$ to describe the distribution will put most of the probability mass in a location between the two modes, where few data actually exist. If we leave the distribution alone as bimodal, the bulk of the probability mass will be defined by two disjunct ranges.

As an example, let us consider a case where we wish to determine the North American breeding origins for a population of migratory songbirds wintering in the Caribbean. To do this, we might calculate a mean and standard deviation of $\delta$D values from feathers of birds at the wintering population. Because these feathers were grown on the breeding grounds, we can then infer the origin of this population based on a $\delta$D isoscape derived for North America. To do this, we could calculate the mean and standard deviation of the δD values for the entire sample population and then shade the area of the δD isoscape that is centered on the mean and extends to either side by one standard deviation or some other scaling amount. Most of the shaded area of our map might, for example, be somewhere around New Brunswick and Maine. However, on closer inspection, the δD values from individuals sampled at the wintering population indicate an apparent bimodal distribution. If we were to assign individuals to breeding origins first, we would see that the population actually originated from one of two places: either Kentucky or Newfoundland but nowhere in between. Thus, it is important to first assign individuals to places of origin and then summarize this information based on the actual distribution of values that is generated by the population rather than assume a normal distribution and assign the population based on a mean and standard deviation.

The second reason for first transforming the δ-values to geographic values for each individual is less obvious. The δ-values in the same tissue from different individuals from the same population from the same single location are never identical. Repeated samples from the same individual are not even expected to be identical. Because of this, the transformation from δ-values to geographic location is not done with complete certainty. By assigning individuals to geographic locations first, we propagate the uncertainty associated with the transformation and provide a less biased answer about the geographic structure of the sample population.

For these two primary reasons, we suggest that the more conservative and potentially more informative approach is to consider the assignment of origins (transformation of δ-values to geographic locations) for each individual and then use the resulting distribution of geographic origins to address population-level questions. In Section VI, we show that only some of the assignment methods used to date can accommodate this perspective.

## VI. MODELING APPROACHES

### A. Map Lookup

The map-lookup approach is very straightforward. What we call map lookup simply involves defining geographic gradients (base maps) of isotope values, measuring an individual of unknown origin, and then assigning it to the area of the mapped gradient that corresponds to its isotope value. Typically, this approach capitalizes on the spatial patterns generated from indirect sources of information (*i.e.*, rainfall, primary producers) that are used to generate isoscapes.

The studies that originally jumpstarted the use of stable isotopes to study migratory movements (Chamberlain *et al.* 1997, Hobson and Wassenaar 1997) used the map lookup approach to illustrate the utility of δD for determining the provenance of migratory birds and it is still among the most commonly employed (Wassenaar and Hobson 2001, Norris *et al.* 2004, Bearhop *et al.* 2005, Mazerolle and Hobson 2005, Mazerolle *et al.* 2005, Boulet *et al.* 2006, Hobson *et al.* 2006, Lott and Smith 2006, Hobson *et al.* 2007, Paxton *et al.* 2007). The appeal of this approach is that it is easy to understand and apply because assignments of unknown animals require no additional computation.

Map lookup approaches treat the output from spatial interpolation models (isoscapes) as a known pattern-generating process. In other words, the baseline assumption is that the modeled pattern for, say, δD in precipitation effectively mimics the "truth." The task for any particular study then becomes to simply calibrate the map. In the case of δD, this calibration is an estimate of the isotope discrimination factor between rainfall and a tissue in the species of interest.

In practice, this approach has been limited to δD, and most studies focus on birds, usually sampling keratin in feathers or claws. For this reason, we limit our discussion here to studies of migratory birds using δD in keratin. The best performance for the map lookup approach will be obtained when researchers are calibrating isoscapes (base maps) with known origin tissues that are the same tissue type from the same species, age class, and habitats as the samples of interest; samples that were collected from across the full extent of the geographic range and that were collected during the same years as the samples obtained from migratory individuals of unknown origin. In the vast majority of cases, however, these data are simply not available. If researchers cannot provide study- or species-specific calibrations, the next best performance model will be obtained from published calibrations that correspond most closely to the study species and geographic range. Obtaining specific calibrations, however, is not only a recommended approach for the map lookup method but for all methods used for assignments.

There are some broad consistencies in the observed relationship between $\delta$D in precipitation and that of feather keratin within some geographic regions and among some species groups that can be used as crude calibration guidelines, but we note that published results vary quite a bit (see Table 3.1 in Chapter 3). For example, Hobson and Wassenaar (1997) estimated a single calibration curve for a collection of six species of songbirds that breed in the forests of eastern North America as $y = x - 34‰$, where $y$ is $\delta$D for feathers and $x$ is modeled $\delta$D for precipitation; this finding suggests a 1:1 transformation of isoscape precipitation values to expected feather values (*i.e.*, slope = 1). Hobson *et al.* (2001) estimated the same calibration curve for a different passerine of the eastern North American forest, the Bicknell's Thrush (*Catharus bicknelli*), as $y = 0.68x - 26.1‰$. The slope of the model (0.68) implies more than 30% expansion of modeled precipitation values compared with feather values. This suggests broad within-site variation relative to modeled precipitation values. Meehan *et al.* (2004) offer four calibrations for relating feathers to an elevation-corrected isoscape they present. Slopes range from 0.6 to 0.9, including a calibration of $y = 0.7x - 21‰$ for Wilson's warbler, the same species for which Paxton *et al.* (2007) later estimated $y = 1.4x + 14.467‰$ using the same isoscape but a sample collected at a different time and from different locations. In the first case, there is a 30% expansion of modeled $\delta$D in precipitation relative to feathers. In the second case, there is a 40% compression. This strongly suggests that it is unreliable to use a single calibration function for the Meehan *et al.* (2004) isoscape and that *case*-specific calibration will produce the most robust models.

North American Accipiter hawks have been well studied: Meehan *et al.* (2001) estimated a calibration between Cooper's hawk (*Accipiter cooperi*) feathers and the Hobson and Wassenaar (1997) isoscape as $y = x - 34‰$, implying the same 1:1 mapping as for some eastern North American songbirds. Using the same isoscape, Lott *et al.* (2003) estimated six different calibrations for a collection of nine species of diurnal North American raptors. The six groupings were based on diet and whether the species foraged in coastal areas. The associated calibration slopes ranged from −0.59 to 0.62, interestingly ranging from negative to positive associations between feathers and model output. Bowen *et al.* (2005) offer a different global isoscape along with one calibration specific to North American songbirds ($y = 1.07x - 19.4‰$) and one to European songbirds ($y = 0.85x - 22.3‰$).

Once a calibrated base map is obtained, the question then shifts to "How should we assign our $\delta$D values to regions on the map?" There are two distinct ways to represent geographic assignments using the map lookup approach. The first and most common way is to arbitrarily divide the isoscape using discrete *isolines* (Table 5.1), ranges, or "bins" (*e.g.*, Hobson and Wassenaar 1997, Boulet *et al.* 2006, Paxton *et al.* 2007). Animals are then assigned to the line or into the bin that contains the isotope value measured in its tissue. Using this approach, geographic origin for a sampled population can be represented as a histogram showing the number of individuals assigned to each bin. The second approach determines a range of values that "buffer" the value of interest that can be the δ-value for an individual (*e.g.*, Lott and Smith 2006) or the mean δ-value for a population (*e.g.*, Hobson *et al.* 2007). The next step then finds all cells in the isoscape that fall within that range. The buffer extent is usually related to the estimated measurement error or some similar standard deviation-like scaling. We suggest that researchers look to their calibration regressions for guidance in determining the buffer extent. For example, Meehan *et al.* (2001) regressed feather $\delta$D against precipitation $\delta$D and estimated the 95% confidence interval for *mean* values at ±3‰, implying a range of 6‰ for *population* means; the 95% *prediction* interval, however, was ±16‰, implying a range of 32‰ for *individual* $\delta$D values.

Hobson *et al.* (2007) used the map lookup method to determine general provenance for sampled populations of three species of birds passing through a migration-monitoring station. Here, the approach was not to look at the collective result of assigning birds to the map individually as in Lott and Smith (2006). Rather, they treated the groups of birds of each species as distinct populations, all of which were assumed to be normally distributed in $\delta$D. For each species, they then defined a range of $\delta$D values that would theoretically encompass 50% and 75% of a future sample dataset. These ranges were referred to as "tolerance limits." Tolerance limits are calculated as the mean ± a scaled standard deviation. The standard deviation was scaled by the $z$ score and the $\chi^2$ value associated with the specific sample size (with $\alpha = 0.05$). It is important to realize that these ranges were defined from the sample of $\delta$D values, independent of any geographic information. They assumed the Meehan *et al.* (2004) isoscape could be adjusted by a fixed offset of −25‰ for all species. All cells in the isoscape that were within the ranges defined above were assigned a value of "1" and all others were assigned a "0."

Tolerance limits were developed for engineering applications to determine the reliability of industrial production processes. In such applications, there is no interest in the structure of the sample population, only in the consistency of the production process; the engineers usually want to know how precise their processes need to be in order to still perform the task for which they were designed. In contrast, the question of interest in most studies of connectivity in migratory animals is related to characterizing the distribution of sampled population of unknown origin. For this reason, calculating the range of $\delta$D around the sample mean and then looking up that range on a base map is less informative for studying the geographic structure of the sample than would be the case if birds were looked up individually first.

Lott and Smith (2006) used map lookup for assigning individuals by buffering the $\delta$D value for each individual bird by ±8‰, and then looking up that range on an isoscape. As with all map lookup approaches, cells with values that fell within the range were assigned a value of "1" and cells that fell outside that range were given a value of "0." Thus, their approach did not make discrete categories *a priori* from the continuous isoscape as in other studies. Rather, the isoscape was made discrete by transforming it into a map of ones and zeros for each individual and then summing over these maps. This geographically indexed frequency distribution was then used to describe the structure of the distribution of probable origins for migrants.

In all cases mentioned above, the measured $\delta$D values for feathers were mapped directly to an isoscape (using Meehan *et al.* 2004 for all 2007 studies and Hobson and Wassenaar 1997 for all previous studies). Geographic regions for assignment are always characterized as being isotopically exact. In other words, the map lookup approach always results in a map of zeros (not the area of origin) and ones (the area of origin). The map lookup approach, therefore, does not allow direct probabilistic statements about provenance. Because of this, the approach tends to downplay the effects of various sources of variability and error. For example, any predefined region of potential origin will encompass a range of possible values, no matter if that region is circumscribed by isolines in an isoscape (*e.g.*, Hobson and Wassenaar 1997) or by knowledge of the breeding range (*e.g.*, Norris *et al.* 2006). As mentioned above, there are also known sources of error associated with isotope measurements themselves and with the isotopic discrimination between environmental samples and tissues. Only by formally quantifying all these sources of variation can we make statements about the probability that an individual derived from one region versus another. For these reasons, we feel that the field needs to move beyond a map lookup approach and we advocate more advanced methods for estimating the origin of migratory animals.

## B. Regression

This is the most simplistic statistical approach for determining the origin of migratory animals. Here, the calibration between tissue values and geography is direct. There is no intermediate model based on precipitation. This method makes the simple assumption of a direct linear relationship between latitude and/or longitude and stable isotope values observed in tissues. The idea is to simply fit a regression line, invert it, and use isotope values measured in feathers to predict the mean latitude or longitude associated with that value. It has not been widely used, primarily because there does not appear to be widespread evidence for a monotonic relationship between latitude/longitude and naturally occurring distributions of isotopes. Because of this, we do not advocate using regression models for estimating the geographic origin of migratory animals. However, regression models can be helpful for exploratory work so long as appropriate caveats are discussed. For example, direct low-order relationships between isotope values and latitude (or altitude) might allow crude differentiation for relative latitudes or altitudes of origin. However, to draw a firm conclusion about such a pattern, it is important to be able to rule out alternative mechanisms for the observed structure, such as within-location differences in diet, age, species, metabolism.

At least three studies relied primarily on regression for data analysis. Kelly *et al.* (2002) related latitude and $\delta$D in feathers of Wilson's warblers using linear regression. They used this relationship as indirect evidence for leapfrog migration in the species. Smith *et al.* (2003) used quadratic regression of $\delta$D in feathers on capture date during migration to infer that sharp-shinned hawk (*Accipiter striatus*) employ a chain migration pattern. Rubenstein *et al.* (2002) went a step further and used linear regression to predict the latitude and longitude of wintering black-throated blue warblers (*Dendroica caerulescens*) by considering latitude and longitude as response variables that depended on $\delta$D and $\delta^{13}$C values in feathers as predictor variables. This is really the only study that has used regression to directly infer latitude. It is worth noting that their model for determining the latitude of origin for a feather ($\text{latitude} = -0.097\delta D_f - 1.95\delta^{13}C_f - 12.55$) explained less than half (44%) of the variation in the data, and that the interval for predicting the origin of an individual was as wide as the sampled latitudinal range!

As intuitive as this method might appear to be, we do not advocate this approach except for the most basic exploratory or pilot studies, primarily because inverting the relationship between latitude/longitude and isotope values is not valid. For example, variation in $\delta$D is derived from changes in temperature (realized through variation in latitude), distance from an ocean (longitude), and elevation (Dansgaard 1964). The inverse is not true: variation in geographic location is not derived from variation in $\delta$D. Inverting a linear relationship like this is statistically invalid because the error term is generally in the $y$-direction and no error can exist in the $x$ values. When the response variable ($y$) is latitude, the only way to generate variation in that direction is by sampling different latitudes and within-site variation is error expressed in the $x$-direction. This problem can be fixed by adding a random effects term for sample location. However, such a solution renders the regression model useless for determining the origin of unknown samples because the sample location then needs to be known *a priori* (Wunder *et al.* 2005).

As we also noted for the map lookup approach, regression models are unable to incorporate multiple known sources error associated with using stable isotopes for estimating the origin of animals. Thus, regression models do not provide a probability of origin for a given area but rather a single deterministic assignment based on the regression line. Given that there are known inherent sources of variability that are difficult to accommodate in regression and map lookup approaches, we describe in the following sections how estimation of origin can be treated as a more formal probabilistic problem.

## C. Assignment Methods

Probabilistic assignment methods include a broad range of computational approaches, but all feature a discrete (categorical) response variable. Assignment methods consist of predefining all possible geographic locations of origin, then calibrating an assignment model by characterizing each of those locations with the distribution of stable isotope data. Ideally the characterization is derived from isotopic measurements for individuals known to have grown tissues at those locations, and all possible regions of origin are sampled. An alternative approach is to characterize those predefined regions in terms of the distributions of isotope values taken from calibrated isoscapes. Once a calibrated assignment model is obtained, stable isotope measurements from individuals of unknown origin are used to determine the most likely region of origin. We describe three general forms of assignment methods: (1) classification trees, (2) likelihood-based methods with a uniform prior distribution, and (3) likelihood methods with structured prior distributions, and we discuss the utility of extending these models into a stochastic framework.

## 1. Classification Trees

A classification tree is a derived hierarchy of decision rules for assigning novel data to one of two or more classes. Each decision rule provides a fork or "branching event" in the flow toward assigning a data point to one of the predefined classes (regions). Classification trees do not require distributional assumptions and can combine discrete and continuous covariates. They rely on clustering algorithms or similar recursive computations. Thus, classification trees will be especially useful for future exploratory work and pilot studies, especially as more isotopes and trace elements are used because they accommodate not only multimodal distributions of isotopes but also work fine with any combination of continuous or discrete predictor variables.

Hebert and Wassenaar (2005a,b) employed this approach to assign mallards (*Anas platyrhynchos*) and northern pintails (*Anas acuta*) to one of four predefined geographic regions in North America using the values of $\delta$D, $\delta^{34}$S, $\delta^{13}$C, and $\delta^{15}$N in feathers. Their application used univariate thresholds for decision branching. They tested the robustness of their model by assigning known-origin data not used to generate the classification tree. They did not, however, explore the robustness of the approach to how the potential geographic origins were predefined. As with all assignment methods discussed here, the efficacy of the approach will depend strongly on how the potential target geographic regions are defined.

The branching thresholds in classification trees can be fixed points along univariate gradients as in Hebert and Wassenaar (2005a,b) or they can be linear combinations of the predictor variables, as in discriminant function analysis. Rule sets derived from classification trees provide no specific information about how close the call is for a given split. In other words, it is very difficult to quantify the relative strength favoring one branch versus another. There are a host of software programs that employ various algorithms to ensure optimization of the trade-off between number of branching splits and predictive accuracy, but optimizations are just that—optimal solutions given the information at hand. If the likelihood for one region is nearly identical to that for another, there will still always be an optimal solution for differentiating between them, but this is not necessarily saying we have a great deal of confidence in doing so. As with any statistical modeling approach, this is just another way of saying that the performance of classification trees is limited by the quality and quantity of data.

## 2. Likelihood-Based Methods

One form of likelihood-based methods is discriminant analysis that can be used to classify a sample into one of two or more classes (regions). It has been used by several isotope-based studies to estimate the provenance of animals (Caccamise *et al.* 2000, Wassenaar and Hobson 2000, Farmer *et al.* 2004, Kelly *et al.* 2005, Rocque *et al.* 2006, Szymanski *et al.* 2007). Parameters for region-specific likelihood functions (always some form of the normal distribution) are then estimated from isotope data collected in each predefined region. The likelihood functions for each region are then evaluated for the isotope value measured for an individual of unknown origin, and the individual is then simply assigned to the region with the highest-valued likelihood (or probability). One advantage of likelihood-based methods is that they consider not only the mean isotope value of each region but also some measure of the variability.

Likelihoods can easily be converted to probabilities using *Bayes' Rule* (Table 5.1), which is a formal way to invert conditional probabilities. Bayes' Rule is simply an algebraic manipulation of an equality that springs from the definition of conditional probability. The probability of two events both happening, $P(\text{A and B})$ is the same as the probability of one event happening $P(\text{A})$, joined with the probability of the second event happening, given that the first event occurred, $P(\text{B}|\text{A})$. The vertical line means "given," or "conditioned on." In other words, $P(\text{A and B}) = P(\text{A}) * P(\text{B}|\text{A})$.

Because it does not matter which event occurs first, we can also write $P(\text{A and B}) = P(\text{B}) * P(\text{A}|\text{B})$. Recognizing that these two expressions are equal, we can write $P(\text{B}) * P(\text{A}|\text{B}) = P(\text{A}) * P(\text{B}|\text{A})$, rearrange and get $P(\text{B}|\text{A}) = P(\text{A}|\text{B}) * P(\text{B})/P(\text{A})$, which is Bayes' Rule. In this expression, $P(\text{B}|\text{A})$ is often referred to as the posterior probability of B given A, or the probability of some model parameters or hypotheses, given data. $P(\text{A}|\text{B})$ is the likelihood function, or the probability of the observed data given some model parameters or hypotheses. $P(\text{B})$ is referred to as the prior or marginal probability for B, or the probability of some model parameter without knowledge from data, and $P(\text{A})$ is also a marginal probability that serves as a normalizing constant. $P(\text{A})$ captures all possible outcomes for A, regardless of B. Because we have A conditioned on B, we sum or integrate over $P(\text{A}|\text{B}) * P(\text{B})$. That is, we integrate over all possible outcomes where A occurs.

The use of probabilities using Bayes' Rule makes the interpretation of results much more straightforward than with likelihoods alone because we can talk directly about the probability of model parameters or hypotheses, rather than indirectly discussing the probability of observing the same data in a hypothetical replicate sampling event. In fact, discriminant analysis can be thought of as a special case of Bayesian analysis where the sampling probability distribution (the likelihood function) is normal, and the prior probability distribution is uniform over the candidate regions. Discriminant analysis is, therefore, naturally extended into a more Bayesian approach by giving structure to the prior probability distribution.

As an example, Royle and Rubenstein (2004) assumed normal distributions for the stable isotope values in feathers collected from each of three broad regions and argue that relative abundance is a good proxy for the prior probability that an individual of unknown origin came from any of those three regions. In other words, they point out that in the absence of any isotopic information, the probability of an individual originating from any given region is proportional to the relative abundance of animals from that region. Norris *et al.* (2006) also used relative abundance for the prior probability distribution, but they differed from Royle and Rubenstein (2004) by using precipitation-based isoscape values to define the sampling probability distributions for each region. In cases where relative population abundance estimates are not available, the number of individuals sampled from each potential area of origin can be used as a proxy for the prior probability (Wunder *et al.* 2005). In Table 5.3, we have outlined information sources in addition to relative abundance that could be integrated with isotopic data as prior probabilities in assignment tests. However, we caution against relying too strongly on such distributions unless researchers are confident that their sampling effort was even across all regions of potential origin; no study has thoroughly investigated the robustness of these likelihood-based methods to the way in which target regions are geographically defined.

Assuming normal distributions for the likelihood function is by no means a requirement. Given the relatively sparse nature of the data in these studies (as with many migratory studies), the assumption of normality was used as a way to marginalize the influence of outliers in the data and to simplify computations. If more data were available, fully Bayesian methods could have been applied to fuller effect. Full Bayes circumvents the need for any strong distributional assumptions, and can therefore also simultaneously accommodate both discrete and continuously distributed covariates.

For example, consider a simple case where stable isotope data are expected to follow a multimodal distribution: a predefined location may include a mix of C3 and C4 plants (and therefore a mix of $\delta^{13}$C values). Suppose that the population of interest included two groups—one that foraged predominantly in C3-based food webs and one that foraged mostly in C4-based food webs. The distribution of $\delta^{13}$C for that population would look bimodal. Calibrating an assignment model using a normal distribution would result in low assignment probabilities for individuals from either of the two groups that are actually known to be from that location because the probability density function of $\delta^{13}$C was described

**TABLE 5.3** Types of nonisotopic information that can be used as priori probabilities for assigning animals of unknown origin

| **Type of information** | **Source of information** | **Relevance to assigning birds using Baysian inference** |
|---|---|---|
| Relative abundance | Large-scale surveys such as the Breeding Bird Survey or field data | Used because an individual of unknown origin already has a probability of originating from a given region based on relative abundance |
| Return rates | Field data on frequency of return in a population from one year to next | Could be used when estimating the natal origin of juveniles; probability of returning to natal grounds may also be related to distance |
| Mark-recaptures | Field data and large-scale coordinated efforts (*e.g.*, Bird Banding Laboratory) | Could be used if there is prior information on patterns of connectivity based on band recaptures; may not be useful if there are only a few recaptures |
| Migration distance | Previous studies on survival in relation to migration distance | Could be used as prior information if survival rates are known to vary with distance from potential regions of origin |
| Distribution range | Natural history | Constrains possible geographic origins to regions within known range |
| Phenotype | Museum skins, field data | Individuals with different phenotypes may be known beforehand to originate from specific regions; one caveat is that this only provides information on possible natal origins, does not accommodate possibility that an individual may have dispersed to an alternate location later in life |
| Genetics | mtDNA, AFLP, microsatellite | Same as above (including caveat) but for genetic composition |
| Isotopes | Light or heavy isotopes that vary geographically | May be particularly useful for studies that are updating or improving previous work |
| Trace elements | Chemical analysis of organic tissues | Same as above |

as a normal (unimodal) distribution. Fully Bayesian approaches or classification trees would perform better in such situations. Classification trees, however, remain less useful for directly determining the relative probability of taking one branch versus the other. In contrast, a fully Bayesian analysis provides direct probability estimates and therefore also provides insight about the relative strength of assigning an individual to one region versus another. However, none of these assignment methods provide much insight into the mechanistic underpinnings of the functional relationship between isotopes and geography.

Partly in response to this problem, we recently used a simulation framework to explore the reliability of asymptotic results from Bayesian assignments, given stochastic fluctuations associated with IRMS measurements and with the process of spatially interpolating sparse data on $\delta$D in precipitation (Wunder and Norris 2008). There, we reevaluated a study by Norris *et al.* (2006) that calculated the mean and standard deviation of expected $\delta$D values for five predefined breeding regions. The $\delta$D values were extracted from spatially interpolated precipitation maps (Bowen *et al.* 2005) and standard deviation values ranged from 5‰ to 13.6‰. In this study, we considered not only the variation in these expected values from the interpolated precipitation-based $\delta$D isoscape but also the uncertainty associated with measuring $\delta$D in feathers and the uncertainty associated with the spatial interpolation itself. The latter portion of uncertainty captures the variation associated with how far a geographic point is away from an actual data sampling station; the further away a point is, the higher variability there will be in interpolating the $\delta$D value. Regions with high variability in any of the distributions of predicted $\delta$D likely reflected higher amounts of topographic relief and temperature variation over its range. Regions with high spatial interpolation uncertainty were associated with similar physiographic features, and also reflected the relative distance from a true data station where $\delta$D in precipitation was directly measured. Thus, the overall confidence of assignments to these regions was much lower.

This stochastic modeling extension was useful for exploring the relative sensitivity of conclusions to the assumptions that isoscapes are perfectly predicted and that $\delta$D in feathers is perfectly measured. It represents a preliminary, if not also rudimentary, approach toward studying the state of our understanding of the relationship between $\delta$D in feathers and geography. This method can be extended to include other factors known to influence the certainty of our modeling efforts. For example, we might consider the effects of reasonable stochastic fluctuations in the functional relationship between $\delta$D in precipitation and that in feathers (variable isotopic discrimination).

## D. Probability Surfaces

Probability surfaces describe the probability that any point in space is the true origin of an individual for which stable isotope values are measured. In this way, probability surfaces provide a continuous response variable (typically represented as pixels/raster format on a map) constrained to values between zero and one. Because these are spatially continuous models, they rely on model output from spatially interpreted data. The nature of that model output determines the type of calibration that must be performed before proceeding to transform them into a spatially indexed probability distribution function. For example, if feather isotopes are to be compared against a precipitation-based isoscape, that isoscape must first be calibrated to reflect the isotope discrimination between feathers and precipitation. Alternatively, if the baseline process is an isoscape derived directly from the δ-values in feathers collected from across the spatial range of interest, then no further calibration is required—the calibration in such a case is the spatial interpolation itself.

The first crude effort to generate probability surfaces was recently attempted for determining the breeding origins of wintering mountain plovers (Wunder 2007). The basic algorithm used was to first calibrate the $\delta$D isoscape from Bowen *et al.* (2005) with feathers of known origin separately for both adult recaptures and fledglings. Next, a stochastic model was derived from three dependent (nested) variance-generating processes: (1) analytical error, estimated as described by Wunder and Norris (2008), (2) within-individual variance, differences within and among feathers estimated from values documented by Wassenaar and Hobson (2006); and (3) within-location variance, estimated from 112 values from the published literature and data collected for mountain plovers. These variance-generating processes are nested, in that a typical sampling process continues as follows: individual birds are sampled from a given location, which can be isotopically characterized by a probability distribution. That is, we first randomly sample individuals from the same location, each with some alternate mean δ-value. Then, we randomly sample a feather from each individual and we can then model among-feather variance. Finally, we measure the δ-value for the sampled feather, and we know this measurement is also estimated with error. The posterior distribution associated with the combination of these processes can be estimated using *Monte Carlo integration* (Table 5.1). Once this hierarchical model is parameterized, $\delta$D in feathers that were collected from birds on the wintering grounds (but grown during the breeding season) were used to evaluate the probability density function for each grid point in the calibrated isoscape. The result was a set of geographic maps showing the spatial distribution of the gradient in probability of origin for each individual.

In contrast to the binary response maps (ones and zeros) produced using map lookups by Lott and Smith (2006) and Hobson *et al.* (2007), probability surfaces are spatially explicit maps with values spanning the gradient from zero to one. Thus, they disclose the full range of possibilities based on estimable sources of error. For example, directly comparing the relative probability of any two points can be simply expressed in terms of odds ratios. This allows a great deal of flexibility for approaching problems from the full range of risk levels. There is no need to arbitrarily set threshold levels for the probability of making type I or type II errors. In probability surfaces, the probability space is geographically indexed, and not constrained to sample data as it is for the map lookup approaches discussed in Section VI.A that used tolerance limits.

This modeling approach is also useful in that it makes all sources of variance very transparent, providing insight into the most useful places for strategic experimentation. It is a simple matter via simulation to explore such questions about the relative gains for increased precision in any of the modeled variance-generating processes by iterating through the full parameter space for each process, while holding the others constant. This modeling approach holds great potential for providing back-and-forth dialogue between experimental and applied researchers that is necessary to advance isotope-based methods for determining geographic origin.

This use of probability surfaces is still relatively new and there is much room for further refinement. The approach is accessible to a wide range of study problems, but is currently most effectively done for migratory birds. Estimates of each source of variation need not be obtained for each specific study. Stochastic characterizations can be based on values in the literature. For example, Wunder (2007) used 112 estimates of within-population standard deviation, mostly from the literature, but only had 6 estimates for modeling the within-individual standard deviation, all from the literature on migratory birds. The primary disadvantage is that the procedure is relatively more computational intensive than either the map lookup method or likelihood-based assignments, but we believe that this approach provides the most robust models for estimating the origin of migratory animals over a continuous space.

## VII. SAMPLING CONSIDERATIONS

As emphasized in this chapter, the most robust study designs will carefully consider the general idea of calibration, from IRMS calibration all the way up to assign-time calibrations of modeled spatial patterns at the continental or global scales. In the case of discrete assignment methods, each potential site or region of origin should ideally be isotopically calibrated with tissue known to have derived from each region. In the case of a more continuous analytical approach based on gradients, the gradients need to be calibrated using tissues of known origin from across the full extent of the gradient range. In other words, because of a relative lack of mechanistic understanding about when and where to expect strong deviations from otherwise smoothed patterns, the most robust approach is to determine these patterns empirically.

Equally important in all empirical characterizations of the relationship between stable isotopes and geography is the specification of all known sources of variance. We have seen that measurement error, within-individual variance, within-location variance, among-location variance, among-age class variance, and among-species variances all affect the relationship between isotopes and geography. Thus, sampling designs will do well to consider designs meant to isolate the effects of these factors.

## VIII. SPATIAL CONSIDERATIONS

Calibration samples should ideally span the entire targeted geographic range of interest. That is, if the study seeks to infer the wintering grounds for a population of breeding birds, then the researchers should be sure to sample newly developed tissue from birds captured at the full suite of potential wintering locations across the wintering range. Alternatively, if using an isoscape, at a minimum, the extreme ends of the gradient for the isotope of interest need to be sampled. That is, suppose that the range of predicted δD values for the known wintering range of a species spans 100‰. In this case, at a minimum, the researchers would need to collect freshly developed tissue from the regions that represent the endpoints of that 100‰ range in order to develop a two-point (linear) calibration. Of course, a more ideal design would sample from far more than two locations and would explore the prospects of both linear and nonlinear calibrations.

## IX. TEMPORAL CONSIDERATIONS

Distributions of stable isotope values for any given location may vary over time (over the course of a season or over multiple years). This aspect is less studied (Farmer *et al.* 2002), but the general hypotheses for this variation are quite logical. Some seasons and years provide more or less available energy than others. It is this flux in available energy that yields temporal differences in the discriminatory incorporation of heavy isotopes into various compounds and tissues. For many of the tissues of interest in the application of isotope ecology to migration, there is a relatively wide temporal window over which fluctuation is integrated. For example, it takes from 10 to 30 days to synthesize most avian feathers. Or waterfowl may be breeding in wetlands that dry up over the summer.

## X. RELATIONSHIP BETWEEN SAMPLING AND PREDICTIVE RESOLUTION

This is an issue of error propagation and model choice. Predictive resolution and the necessary sampling resolution depend critically on model choice. Predictive resolution for models that use calibrated isoscapes or other interpolated surfaces is defined by the resolution of the spatial interpolation. Models in the family of discrete-response assignment methods allow the researcher to determine the predictive resolution to some degree.

For hydrogen isotopes, the best resolution that can be expected from any model using the δD isoscape of Bowen *et al.* (2005) is 0.33 degrees (www.waterisotopes.org). The δD isoscape of Meehan *et al.* (2004) (http://entomology.wisc.edu/∼tdmeehan/ddp.html) is a δD grid with a 1-km resolution. Both model surfaces were generated from the same isotope training dataset for δD in precipitation (as provided by Global Network for Isotopes in Precipitation). The differences and subsequent relative utility of each of these isoscapes relative to various case studies have not yet been explored. Potentially, the δD isoscape of Meehan *et al.* (2004) provides a model of the pattern in particular (at fine local scales), but not in general, and Bowen *et al.* (2005) provides the opposite. If this is the case, then the Meehan isoscape will require localized calibration for nearly every study, whereas the Bowen isoscape may not.

With all discrete assignment models, the researcher predefines the potential target regions, and therefore also the predictive resolution of origin *a priori*. However, there are as yet undefined thresholds of resolution beyond which we will not be able to isotopically differentiate among regions. Although it is possible to increase the resolution at which regions can be differentiated by increasing the number of samples and the number of markers (isotopes, trace elements, etc.), such an approach will always require exhaustive sampling. This so-called shotgun approach may work for one set of sample data, but when it fails to generate similar results for another independent data set, the researcher is left wondering why. Experiments that effectively isolate mechanisms responsible for generating variance are especially useful for refining models that relate isotopes in tissue to geography.

## XI. RELATIVE POPULATION ABUNDANCE CONSIDERATIONS

One of the primary goals in assigning migratory animals to geographic location using stable isotopes is to estimate overall patterns of connectivity between two or more periods of their annual cycle. Understanding these overall patterns will be important for predicting changes in population size and developing optimal conservation plans (Martin *et al.* 2007, Chapter 1). Here, estimates of relative population abundance during all periods of the annual cycle will play a critical role in our ability to link these patterns with predictive population models.

Consider a situation in which individuals are sampled for stable isotopes on the wintering grounds to infer their breeding origins. Twenty individuals are sampled at each of 15 sites throughout the wintering range. Assignment tests are then conducted on each individual and summarized for each site (see Section V above) to infer overall patterns of connectivity among wintering sites and breeding locations. However, it is more difficult to gain information about relative abundance if the sample size is fixed, rather than the sample effort. By fixing sampling effort (*e.g.*, same number of mist net hours at each site), it is easier to justify the assumption that sample size reflects relative abundance. If wintering sites are not sampled in proportion to their relative abundance, then inference from the modeled connectivity patterns may be somewhat misleading because it will overrepresent some sites and underrepresent others. Thus, one must be cautious when using these assignments to model the effects of habitat loss (Wunder and Norris 2008) or to determine how conservation plans in one season will influence population size the following season (Martin *et al.* 2007).

Information on relative population abundance from the period where animals are being assigned is also important. First, it can provide an indication of whether we have obtained a representative sample of individuals from the previous season. Following our example above, if we had perfect sampling coverage of the wintering grounds, then the proportion of birds assigned to each breeding region should match the relative abundance. If it does not then we can conclude one of two things: (1) we may have missed important areas in which the animals reside during the winter or (2) our sampling scheme may not have reflected the relative abundance on the wintering grounds. Second, as we have mentioned above (Section VI.C; Table 5.3), relative abundance can be used as a prior probability of origin in likelihood-based assignment tests (Royle and Rubenstein 2004). As long as prior probabilities of relative abundance do not dominate the generation of posterior probabilities (*i.e.*, when there are few differences in the isotopic data between potential regions of origin), then relative abundance can compliment isotopic information in a useful way. For example, when assigning animals to predefined regions, there can be individuals whose isotopic values effectively “ride the fence” between two or more regions. In this case, the incorporation of relative abundance acts as the tie breaker. It will assign that individual into whichever of the two regions has the highest estimated abundance.

## XII. CONCLUSION

Stable isotopes offer a powerful new tool for tracking the movement of migratory animals. The landmark pioneering studies that used stable isotopes to track patterns of migration primarily relied on simple calibration rules and the map lookup approach (Chamberlain *et al.* 1997, Hobson and Wassenaar 1997). Over a decade later, major advances have been made in terms of both calibration and assignment models. In this chapter, we have provided a comprehensive overview of the methods that have been used to estimate the origin of migratory animals using stable isotopes. In doing so, we have attempted to expose the major assumptions associated with each model, show how sources of error can be better and more directly incorporated into these models, and emphasize the importance of understanding the mechanisms that drive these sources of isotopic variation. Our hope is that future research will adopt and further refine these approaches to derive robust models that can be successfully used to track migratory animals throughout their annual cycle.

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