STABLE ISOTOPES AND THE DETERMINATION OF AVIAN MIGRATORY CONNECTIVITY AND SEASONAL INTERACTIONS

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PERSPECTIVES IN ORNITHOLOGY

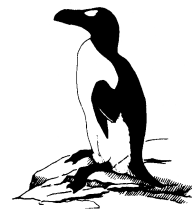# STABLE ISOTOPES AND THE DETERMINATION OF AVIAN MIGRATORY CONNECTIVITY AND SEASONAL INTERACTIONS

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For several years, researchers interested in factors that influence the fitness of migratory birds have realized that events occurring at times and locations outside the breeding season can influence ultimate fecundity and recruitment (reviewed by Webster and Marra 2005). Intuitively, this concept of seasonal interaction is appealing, because there is considerable literature showing that timing of arrival on the breeding grounds ultimately influences the quality of breeding habitat obtained by competing individuals, timing of clutch initiation, and reproductive success. Clearly, for migratory birds, spring arrival times are influenced by departure times from wintering areas and the biotic and abiotic conditions experienced *en route*. In turn, departure times from winter quarters are likely associated with premigratory physiological condition, which may be determined by winter habitat quality (e.g. Marra and Holmes 2001). Like the seasonal interaction hypothesis, migratory connectivity (or the degree to which individuals from a breeding population winter together) has recently received considerable attention, because it determines, to a large degree, opportunities for adaptation, speciation, and the extent to which migrants respond demographically to climate change and other mechanisms that alter habitat quality at various scales (Webster and Marra 2005). Whereas we usually are able to evaluate only annual survivorship (ϕ_{an}) of populations of birds breeding in the temperate zone, for migrants, this survivorship implicitly involves components related to other periods and locations:

$$\phi_{an} = S_b \times S_m \times S_w \times (1 - \mu)$$

where S_b, S_m, and S_w correspond to breeding, migratory, and wintering survivorship, respectively, and $(1 - \mu)$ is the probability that an individual returns to a breeding habitat where it is recorded. If we were able to link biotic and abiotic events determining survivorship at these various stages of the annual cycle of a migratory bird, the predictive power of our demographic models would be substantially enhanced (Sillett et al. 2000, Sillett and Holmes 2002, Dugger et al. 2004). Similarly, the study of natal and breeding dispersal in birds is a phenomenon that would benefit tremendously from establishing such spatial linkages (Hobson 2004).

Until the mid-1990s, our ability to make migratory connections and evaluate seasonal interactions was limited by conventional mark–recapture techniques applied to focal populations. Although this approach can be powerful, it is typically most effective when applied to

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large data sets, usually available only for game species (Hobson 2003). The recent development of stable-isotope analysis (SIA) techniques has changed that situation, because the SIA approach does not rely on mark–recapture but only on the single capture of individuals to infer breeding (or occasionally wintering) origin. This approach has been the subject of several recent reviews (Hobson 1999, 2003, 2005a; Rubenstein and Hobson 2004), and the interested reader is referred to these as well as to Kelly (2000) and Martínez del Rio and Wolf (1995) for more background on isotopic applications to migratory connectivity in particular, and more broadly to avian nutritional ecology. In addition to defining origins of individuals, the isotopic quantification of endogenous and exogenous contributions to eggs provided the first direct measurements of potential seasonal interactions involving females that travel to breed (Gauthier et al. 2003, Hobson 2005b). This is a very rapidly evolving field (Fig. 1) and has not been without its growing pains. Just how powerful is this approach and to what degree can it answer key questions associated with migratory connectivity and seasonal interactions?

Turnover, Discrimination, and the Search for Isoscapes

The principle behind the application of SIA of avian tissues to infer migratory connectivity is simply that isotopic patterns in nature can be variable; when a bird moves between one known "isotopic landscape" or "isoscape" (G. J. Bowen pers. comm.) and another, its tissues will retain isotopic information from the previous location for a period depending on the elemental turnover in that tissue. For feathers, isotopic information is usually "locked in," because feather keratin is metabolically inert following formation; so this material is particularly useful when molt chronology and general location (breeding vs. wintering grounds vs. *en route*) are unambiguous. Fortunately, birds are amenable to captive study where their diets can be switched isotopically, thus mimicking a locational shift between isoscapes. To date, such studies have been conducted on various captive birds including Japanese Quail (*Coturnix japonica*), Great Skua (*Stercorarius skua*), Dunlin (*Calidris alpina*), Canvasback (*Aythya valisineria*), Garden Warbler (*Sylvia borin*), and Yellow-rumped
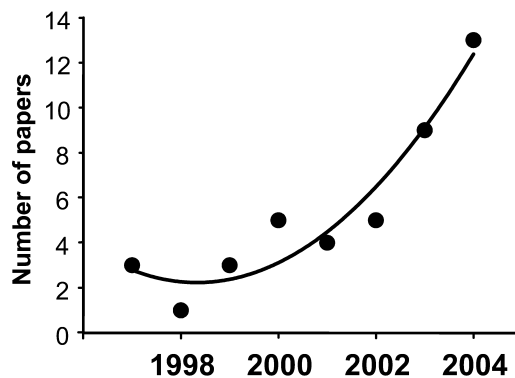

Fig. 1. Number of published studies focusing on the application of stable-isotope analyses to track movements in birds by year. Estimates were based on a review of the literature, including searches using the Web of Science database. Review papers are also included in the annual counts (from Mazerolle 2005; see also Martínez del Rio and Wolf 2004).

Warbler (*Dendroica coronata coronata*) (reviewed by Evans Ogden et al. [2004]). Although this is a diverse taxonomic group, the combined results suggest that elemental turnover rate is associated with metabolic rate and that this follows the expected allometric relationship to body size (K. A. Hobson unpubl. data). The problem, of course, is that such turnover rates usually apply to birds fed *ad libitum* and not forced to exercise; these experimental conditions in no way approximate migration. This was potentially a problem with the interpretation of the study by Marra et al. (1998) that showed a weak but significant relationship between arrival time and muscle $\delta^{13}C$ values of American Redstarts (*Setophaga ruticilla*) arriving at Hubbard Brook, New Hampshire. Marra et al. (1998) reasoned that the pattern of increasing $\delta^{13}C$ values with arrival time was consistent with later arrival of birds wintering in poorer, more xeric habitats and provided the first direct evidence of seasonal interactions in a migratory passerine. Recently, K. A. Hobson and E. Yohannes (unpubl. data) conducted a diet-switch experiment on Rose-coloured Starlings (*Sturnus roseus*) trained to use a wind tunnel. Interestingly, that study showed no difference in blood isotopic turnover rate between exercised (3–4 h per day flying) and nonexercised groups, which suggests that such activity levels do not result in increased blood-cell replacement. If migration does not involve significant protein catabolism and is

essentially fueled by fats stored after stopover, turnover rates for blood or muscle measured in captive, nonexercised, birds may well be appropriate for modeling the temporal window represented by tissue isotopic measurements (Hobson 2005a).

A potentially more serious problem facing investigators using isotopic measurements of avian tissues to model seasonal interactions is the establishment of appropriate isotopic discrimination factors linking dietary isotope values with avian tissue values. Again, these values have been defined empirically using captive birds (reviewed by Evans Ogden et al. 2004), but recently it has been shown that diet quality, expressed in terms of the total carbon-to-nitrogen ratio (C:N), can affect these discrimination factors (Pearson et al. 2003; but see Hobson and Bairlein 2004, Robbins et al. 2005). This is less of an issue for birds with consistently high-protein (i.e. high N) diets than for those that switch between insect (low C:N) and plant or fruit (high C:N) diets. If isotopic discrimination values can change depending on nutritional status, it may be difficult to tease out diet change from a physical change in location associated with a bird's movement to a new isoscape. Until further experimental research is conducted, establishing migratory connectivity for birds that switch diets seasonally may be problematic, at least for isotopes that are typically used for biome or habitat associations (e.g. $\delta^{15}N$ and $\delta^{13}C$) and for tissues that are metabolically active.

Describing isoscapes relevant to making migratory connections depends on the spatial scale of interest and the mechanisms driving isotopic heterogeneity in food webs. In terrestrial systems, variation in food-web $\delta^{13}C$ values are driven largely by differences among plant photosynthetic pathways (C3, C4, CAM), but several other processes can contribute to isotopic variation among plants. Plants show very similar C3-based $\delta^{13}C$ values in temperate areas of the continent. Hotter, more xeric areas can show more enriched values; this can result from plant water-use efficiency mechanisms that enrich $\delta^{13}C$ values of C3 plants toward more C4 values. These general patterns are well known and it is reasonably straightforward to trace birds that may have moved between C3- and C4- or CAM-dominated regions (e.g. Wolf and Martínez del Rio 2000).

Similarly, we also know that food-web $\delta^{15}N$ values are influenced by climate, degree of soil exposure, and more and more by anthropogenic input of nitrogen to the biosphere (Nadelhoffer and Fry 1994). For both $\delta^{15}N$ and $\delta^{13}C$ measurements, agriculture—involving the use of nitrogen-based fertilizers and the planting of both C3 and C4 crops—contributes to isotopic complexity at landscape scales and beyond. Indeed, a strong case can be made that $\delta^{15}N$ and $\delta^{13}C$ isoscapes in North America, and indeed throughout the world, are simply too complex to be used reliably to track migratory birds, and most researchers tend to shy away from interpreting these sorts of data. Koch et al. (1995) pointed out that the usefulness of the much-celebrated forensic technique of using SIAs of African elephant (*Loxodonta africana*) ivory to identify population of origin (Vogel et al. 1990, van der Merwe et al. 1990) was exaggerated, because of annual changes in climate that can cause concomitant changes in food-web $\delta^{15}N$ and $\delta^{13}C$ values in Africa. Although it is true that $\delta^{15}N$ and $\delta^{13}C$ isoscapes are necessarily problematic in wildlife tracking (e.g. Graves et al. 2002), there are several points that need to be considered in any critique.

Stable isotope measurements represent integrations over many events.—An isotopic measurement of an organism's tissue represents a time-integrated average of numerous individual feeding events and represents an equilibrium condition between tissue formation and replacement. For blood and muscle tissue, these processes represent periods of perhaps a month in a small passerine (i.e. a period arbitrarily representing 3–4 half-lives or 87–93% of "new" integrated dietary input). Depending on the species, this dietary integration likely represents several hundred feeding events. When we consider a slow-turnover tissue like bone collagen, the stable isotope value will represent many thousands of events. It is the nature of isotopic signal averaging that short-term or low-frequency isotopic variation in food webs is smoothed; so a single isotope value can be very powerful and less prone to stochastic events than generally realized. This is not to suggest that researchers should not be conscious of possible isotopic complexity, but rather that isotopic variation needs to be interpreted in terms of mass balance and the turnover rate of the tissue being examined.

Individual species' ecology, habitat requirements, and range distributions help to constrain isotopic options.—At each stage of the annual cycle, the range of isotopic heterogeneity to which a bird is exposed through local food webs will depend on constraints imposed by individual and species feeding ecology and the range or distribution of possible origins for the species. Birds with relatively narrow breeding ranges, such as the Kentucky Warbler (*Oporornis formosus*), will necessarily involve a narrower range of potential isoscapes than species like Tennessee Warbler (*Vermivora peregrina*) or Swainson's Thrush (*Catharus ustulatus*). The same argument applies to considerations of complexities associated with agricultural isotopic heterogeneity that a bird may be exposed to, a factor particularly relevant when using stable-carbon and stable-nitrogen isotopic measurements to delineate origins. Species that are almost exclusively dependent on prey derived from closed-canopy forest, such as the North American wood warblers (*Dendroica* spp.), will be less influenced than species more associated with agricultural landcapes, such as Red-winged Blackbird (*Agelaius phoeniceus*; Wassenaar and Hobson 2000). The value of the SIA approach will therefore depend on life-history traits of individual species or species groups, so that blanket statements about whether SIA "works" or does not are meaningless.

Large-scale plant carbon-13 isoscapes are becoming available.—Recent advances in remote sensing and geographic information systems (GIS) technology are making it possible to combine information layers to derive expected C3 and C4 plant distributions on the basis of averaged climate parameters and knowledge of plant physiology (Still et al. 2003). Additionally, spatially referenced data sets on agricultural crop plantings are also possible. Combined, these sorts of derivations will help to further constrain expected $\delta^{13}C$ isoscapes relevant to avian ecology. An example of where $\delta^{13}C$ patterns of food webs work at continental scales to define origins of migrant organisms was provided by Hobson et al. (1999a), who defined a combined deuterium (δD) and $\delta^{13}C$ isoscape for monarch butterflies (*Danaus plexippus*) raised on milkweed (*Asclepias* spp.) throughout their eastern range in North America. Surprisingly, that study showed impressive continental structure in both isotopes, which makes it possible to define natal origins of butterflies with greater resolution than could have been achieved with each isotope singly. Nonetheless, anthropogenic inputs to the isotopic reservoir available to birds can certainly be a cause for concern and will always be a factor to consider in most connectivity scenarios (Alexander et al. 1996).

DEUTERIUM TO THE RESCUE?

Although ecologists have only recently become aware of patterns of deuterium (δD) in precipitation on various continents and their significance in tracking migratory wildlife (Bowen et al. 2005), the North American growing-season average precipitation (δD_p) map is arguably the most well-known isoscape currently available. Without question, it is the most useful isotopic pattern available to studies of migratory connectivity for terrestrial species. The demonstration that feathers of birds (δD_f) have values that reflect the continental pattern of δD_p has provided us with a very powerful tool to interpret approximate origins of migrant birds in North America and elsewhere. However, the pattern of δD_p in North America usually results in strong latitudinal, but often weak longitudinal, inference. For species showing relatively narrow longitudinal distributions, this approach has been extremely powerful (Kelly et al. 2002, Rubenstein et al. 2002, Hobson et al. 2004a). The δD feather–precipitation isotopic relationship is remarkable, given the potential for annual variability in continental weather patterns; but as with all tools, there are certainly potential hazards in the application of this technique (Rubenstein and Hobson 2004). The following points will hopefully clear up some of these questions and provide direction for future research.

A multitude of averages: The nature of the International Atomic Energy Association database.—The pattern of deuterium concentration in precipitation across continents that forms the basis of most recent migratory connectivity studies is the four-decade-old Global Network of Isotopes in Precipitation (GNIP) data set produced by the International Atomic Energy Association (IAEA). This immense data-gathering exercise was initiated to help scientists understand the derivation of freshwater resources available throughout the world. Understandably, the nature of the data set is uneven, both in time and space; so the best

characterization of these continental patterns is derived from the long-term averaging of the isotopic data and depiction of patterns through spatial interpolation or kriging techniques. Various algorithms and refinements of the data interpretation are available (e.g. Hobson et al. 2004a, Bowen et al. 2005), and GIS tools now make it possible to depict, for example, average precipitation values corrected for elevation (e.g. Meehan et al. 2004). As with all areas, coverage for North America is uneven, especially for northern latitudes. High-relief regions are also difficult to map isotopically, and mountainous areas on the western side of the continent show high isotopic complexity compared with the Midwest (see also depictions for the European Alps and Andes of South America in Bowen et al. [2005]). In addition, coastal regions are susceptible to local influences and dry inland locations to synoptic weather events that can contribute to relatively high inter-year variance in δD_p (Hobson 2003). Relative humidity is another variable known to influence food-web δD values, and modifications to δD_p using such data are possible (Cormie et al. 1994). Given such scope for variability, the reasonably good relationship we see between δD_f and δD_p for passerines, including data sets involving specimens from museums and spanning a considerable time range (Hobson and Wassenaar 1997, Wassenaar and Hobson 2000, Kelly et al. 2002; see also Meehan et al. 2001, Lott and Smith 2006) is impressive, with variance accounted for typically being >80%. However, as recently reported by Lott and Smith (2006) for a large raptor feather (δD_f) data set in North America, there is evidence for regional differences in the intercept of the relationship between δD_f and δD_p. This suggests that the isotopic discrimination factor linking δD_f and δD_p, previously assumed to be about −25‰, at least for passerines (Wassenaar and Hobson 2000, Hobson et al. 2004a), may not be applicable to all areas of the continent or for all avian taxa. Smith and Dufty (2005) have also recently explored isotopic variation in feathers of raptors in detail and considered several sources of variation (see below).

Despite the tremendous power of the δD_f technique as compared with conventional approaches in delineating origins of migratory birds, there are two important cautions that researchers need to consider seriously when using this technique. The first is that we would rarely expect local δD_f values to match exactly the interpolated values provided by the various continental δD_f base maps. This is simply the statistical nature of our expectation to match a single year with a long-term (four-decade) average. Where possible, calibration of local-site δD_f values using locally grown feathers from species of interest is recommended (e.g. Hobson et al. 1999). Researchers should also resist the temptation to break down the IAEA data set into monthly averages for a given location (e.g. Smith and Dufty 2005), because we expect food webs to reflect seasonal patterns in δD_p rather than short-term variations. Considering laboratory issues and the nature of the IAEA data set, an average error on the order of 6‰ is not unreasonable and will undoubtedly be higher or lower, depending on year and location. Secondly, the isotopic base maps typically in use in North America and Europe are based on the long-term growing-season average δD_p values, and regression analyses performed to date have been between feathers produced by breeding birds and the δD_f value expected from locally breeding or molting birds. Researchers interested in defining origins of birds wintering on various continents should realize that such regressions have not yet been performed; ideally, we would like to obtain δD_f maps of expected wintering values (see Mazerolle et al. 2005). This would be feasible for large data sets available for North America and Europe, but again it is not clear how fast food webs respond isotopically to seasonal changes in δD_p.

Other concerns with deuterium.—Measurements of δD have provided the greatest advances in making migratory connections, but there are properties of hydrogen in food webs that undoubtedly contribute to the variance we observe. One issue is that hydrogen bonds formed with oxygen and nitrogen are relatively weak compared with those with carbon, and a certain percentage of hydrogen in any sample will exchange with ambient water vapor. This has prompted research into ways of calibrating lab measurements made at different locations where ambient water vapor δD can vary (Wassenaar and Hobson 2003). This is still a work in progress, but ultimately it should be possible for all labs to adopt a single method of measuring and reporting avian tissue δD values not influenced by ambient conditions. Currently, it is usually not possible to make direct

comparisons of data across labs. Although the extent of this calibration error is probably relatively small, it will change seasonally as ambient water-vapor δD changes. The lack of calibration is clearly limiting the potential for accumulating large comparable data sets through time. A related problem with δD measurements is that hydrogen from drinking water can exchange with all tissues (Hobson et al. 1999b). This is not a problem for species that derive the bulk of their water requirements through diet or for those whose drinking water and diet are driven primarily by the same source of precipitation. However, drinking water of very different isotopic composition than the precipitation-based food consumed will produce tissues with potentially confounding δD values. Evapotranspiration of body water, especially common in hot and arid regions, will result in the enrichment of tissue δD values in birds (McKechnie et al. 2004). If this is not corrected, enrichment of tissue δD values in this way tends to place birds at more southerly latitudes than their actual provenance, and we are already seeing feather δD data sets in which a small percentage of individuals cannot be reliably placed on δD base maps for North America because they are too positive (K. A. Hobson and L. I. Wassenaar unpubl. data). Species like accipiters that grow feathers during energetically demanding periods such as incubation and chick-rearing may have enriched δD_f values, because of evaporative loss of body water, compared with those species that molt after the breeding season (Meehan et al. 2003, Smith and Dufty 2005).

It must be stressed that the relationship between δD_p and δD_f holds for food webs that are driven by precipitation providing hydrogen to shallow-rooted plants, and the relationship is expected to break down if there is a significant groundwater contribution. In addition, in cases where birds derive nutrition from aquatic food webs, in turn driven by hydrogen from water reservoirs such as lakes and wetlands, weaker relationships between δD_p and δD_f are expected. Moreover, water bodies are prone to seasonal changes in δD values because of interactions between evaporation and precipitation (Gibson 2002). Fortunately, continent-wide patterns of δD values are available for surface waters (Coplen and Kendall 2000, Kendall and Coplen 2001) to assist in evaluating the reliability of using δD_f measurements for wetland-associated

species. Although there is generally an excellent correlation between δD of river waters and δD_p (Kendall and Coplen 2001) for relatively small- to medium-sized drainage catchments (<130,000 km²), the relationship does not hold for larger systems. Thus, it is unclear with what confidence the deuterium isotope approach can be used for wetland species using downstream sites on large drainages for food or drinking water or both.

Judging from analysis of museum specimens, there is good evidence that the pattern of δD_p for North America has remained reasonably robust for the past 100 years. However, current and pending climate change may alter the nature of these patterns toward new equilibria. More proximately, the database should also be examined for the effects of the El Niño and Southern Oscillation (ENSO) in North America and the effects of the North Altantic Oscillation (NAO) in Europe. Although generation of new deuterium-precipitation base maps to track climate change is not impossible, it represents a huge undertaking that will take several years to achieve reasonable statistical confidence. In the meantime, individual research projects that are able to ground-truth bird tissue δD values for known locations may be able to calibrate models on the basis of the long-term IAEA average. Here, it is recommended that researchers use feathers from juveniles or adults known to have synthesized tissues at a known site rather than from nestlings that may have different δD_f values because of different physiological processes influencing deuterium (Smith and Dufty 2005) or heat stress in the nest (McKechnie et al. 2004).

Statistical considerations and developments in data interpretation.—Associating a tissue stable-isotope value with geographic position is essentially a problem of assignment. In this respect, the stable-isotope field shares common analysis goals with the field of population genetics (Webster et al. 2002). As such, there is interest in using Bayesian statistical approaches and conditional probabilities because, in some applications to migratory connectivity, assignment relies on prior knowledge of the expected isotopic distribution in a given area of interest (Wunder et al. 2005). Rather than relying on simple correlations between latitudinal position and δD_f, Wunder et al. (2005) demonstrate the advantage of using probability-based models

for assignment using isotopic data. Such an approach is similar to the discriminant-function analyses adopted by Caccamise et al. (2000) in their three-isotope approach to evaluate origins of wintering Canada Geese (*Branta canadensis*) and the two-isotope study of Wassenaar and Hobson (2000) on Red-winged Blackbirds from western North America. Wunder et al. (2005) and Smith and Dufty (2005) have raised some valuable points that will improve the way in which we infer origins using isotopic data. However, the high variability in δD_f found by Wunder et al. (2005) for Mountain Plover (*Charadrius montanus*) chicks from single locations across their breeding range in western North America is perplexing, because such large isotopic variance has not been reported in other systems. Perhaps some geographic areas are just too isotopically complex (because of topographic relief or dynamic water sources?) to successfully apply the stable-isotope technique to infer origins of birds. Alternatively, physiological aspects of feather growth in precocial species adapted to high-altitude environments may involve complex reworking of precipitation-based hydrogen. Meehan et al. (2003) have similarly evoked physiological effects on hydrogen discrimination in adult versus nestling Cooper's Hawks (*Accipiter cooperii*; see also Duxbury et al. 2003, Smith and Dufty 2005).

Another recent area of development is the refinement of probability estimates of origins on the basis of additional information on relative abundance of birds across breeding or wintering grounds. Here, the North American Breeding Bird Survey (BBS) can potentially allow us to modify derived probability of origin based on regional differences in species abundance (Royle and Rubenstein 2004). Presumably, the same approach could be used to modify probability-of-origin estimates for hatch-year birds using the Monitoring Avian Productivity and Success (MAPS) data set. On the other hand, Royle and Rubenstein (2004) did not provide convincing evidence that their approach provided any particularly useful information on assignment for the Black-throated Blue Warbler (*D. caerulescens*), because they considered such broad regions of origin with relatively sparse data. For any given year of study, it may be more parsimonious to take the isotope data at face value and to describe potential origins using only GIS tools (Mazerolle et al. 2005,

Lott and Smith 2006). Today, GIS tools make such depictions relatively routine, and authors can describe proportions of population origin using quartiles or confidence intervals (Hobson et al. 1999, Mazerolle et al. 2005). Nonetheless, GIS pictures are simple portrayals of potential origins based on model assumptions, and the combined approaches of probabilistic models and GIS spatial analyst tools are encouraged.

A question that often arises pertains to the accuracy of placing individuals to various latitudes of origin using only feather δD values. Meehan et al. (2001) were the first to consider this rigorously. For their study on Cooper's Hawks migrating through the Florida Keys, they estimated that the technique had a resolving power of ~1.5° of latitude. This likely represents an optimal scenario and should probably be considered the best we can do with this single isotope in general applications. However, the error associated with latitude of origin in North America depends on region of interest (Wunder et al. 2005, Lott and Smith 2006) and ultimately on how close together the δD_p isoclines are. Broadly spaced isoclines result in larger distances of error compared with more closely spaced isoclines. Thus, latitudinal distance error will increase, for example, in northern areas of the North American continent.

Recommended Future Work

Extending isoscape coverage to the wintering grounds.—In North America, most migrants that winter south of the U.S.–Mexican border reside in Mexico. However, only two IAEA locations currently exist for that country. If a reliable deuterium base map could be constructed for Mexico and for other important wintering regions in Central America and the Caribbean, this would assist in the delineation of origin of winter-grown tissues (e.g. claws, some pre-alternate feathers grown in spring on wintering grounds). For small islands with considerable coastal influence, reliable δD_p maps will probably remain unavailable. For species that winter with resident conspecifics in Mexico (e.g. Loggerhead Shrike [*Lanius ludovicianus*]), the proportion of resident and migrant individuals could be quantified. Such a Mexican isotopic base map would also be of great benefit to researchers interested in dispersal of resident Mexican species within that country. A useful

deuterium isoscape for Mexico could be generated using a handful of well-placed IAEA-style sites or, more feasibly, derived from the isotopic measurement of feathers of resident species grown at known locations.

Combining isotopic base maps with additional layers of constraint appropriate for each species.—Although significant ambiguity exists for continental δD_p base maps, much of this can be reduced by imposing constraints of species range and habitat selection. This GIS exercise involves combining various spatially explicit data sets, but clearly, the extent of these non-isotopic data sets, both currently available and to be developed, has not been fully explored. Biome coverage maps are now available for most continents, as are layers that define urban regions, agricultural areas, and so on (see Acknowledgments). One can imagine delineating possible source of an area-sensitive forest passerine by combining the δD_f base maps with excluded nonforested regions or forests beneath a given threshold size or distance from roads or urban centers (e.g. Tankersley and Orvis 2003). The only restriction in the development of this field seems to be limitations associated with remote-sensing capabilities and the availability or cost of the spatially explicit digital databases.

Development and adoption of interlaboratory methods to calibrate feather deuterium measurements.—Some progress has been made using feather standards that have been steam equilibrated and measured for nonexchangeable hydrogen. However, a current concern is how individual-feather grain size from powdered samples can influence δD values (G. J. Bowen and L. I. Wassenaar pers. comm.). With the extremely small samples required for δD measurements (0.1–0.3 mg), sample inhomogeneity seems to be playing a more important role. There has yet to be a controlled study that contrasts preparation techniques and specifically examines the effect of using powdered versus whole-feather or subsamples from vane versus rachis, for example. Such investigations need to be accomplished using captive birds raised on constant isotopic diets and drinking-water to create feathers that are not affected by diet changes.

Exploring deuterium continental base maps for freshwater-associated species.—In the same manner that previous researchers have tested the relationship between δD_f and δD_p for terrestrial species, we should examine the relationship between δD_f of wetland-associated species and seasonally weighted average δD for surface waters. Currently, this would be most feasible for North America on the basis of the previous efforts of Kendall and Coplen (2000).

Quantifying continent-scale isotopic variance for isoscapes.—Ideally, a base map of variance in δD_p derived from the IAEA database would allow a more rigorous analysis of error associated with placement of individuals to origin. In this way, we could, to some degree, determine the confidence with which we assign individuals to origin using δD_f values. This is not a trivial task, given the nature of the IAEA data set, but we should soon see a spatially explicit variance base map for δD_p and $\delta^{18}O_p$ (G. J. Bowen pers. comm.). Similarly, spatial depictions of isotopic variance to be expected in avian tissues for other isotopes may also be feasible.

What physiological processes determine avian tissue isotope values and elemental turnover rates?—Fundamental to our interpretation of avian tissue stable-isotope values is our knowledge of the isotopic variance we might expect because of diet nutritional quality, diet C:N values, exercise, and age. Although comparative data sets from wild birds are useful (e.g. Smith and Dufty 2005), understanding isotopic variance within and between individuals requires controlled laboratory studies where diet (including drinking water in the case of δD), ambient conditions, and exercise can be strictly controlled. Such studies are urgently required to resolve perplexing δD data associated with raptors. The recent suggestion that δD values reflect trophic level (Birchall et al. 2005) is questionable but deserves controlled studies using birds. Also, more studies are needed that mimic migration using wind tunnels and explore the effect of dietary switching approximating such nutritional shifts in the wild.

What isotopic changes can we expect from mobilization of endogenous nutrient stores to eggs?— Our current assumption that patterns of isotopic discrimination associated with the mobilization of endogenous tissues are the same as those associated with an income carnivore model (Gauthier et al. 2003) have not been tested. Given the unlikelihood of birds in captivity cooperating by laying eggs under strict capital conditions, isotopic comparisons between endogenous tissue reserves (protein and lipids) and the same egg macromolecular components for species

that are strict capital breeders (some penguins and high-latitude geese?) are required. These data will help refine mixing models designed to quantify seasonal interactions at the level of direct nutrient transfer from wintering grounds or stopover sites to breeding grounds and so assist us in refining an important quantitative tool to assess seasonal interactions directly related to reproduction.

Bridging the gap between earth sciences and ornithology.—To date, isotopic forensic tools developed to track seasonal interactions and movements of birds, mammals, and insects have relied primarily on the elements C, N, O, H and S. Other elements are known to show isotopic patterns that can ultimately be linked to geological processes and, in some cases, to specific regions of continents and marine areas. Basic relationships between surficial geology and avian tissue isotope values for several elements, including those of Sr and Pb, are now needed (e.g. Beard and Johnson 2000, Outridge et al. 2003). The recent advent of inductively coupled plasma mass-spectrometric methods opens up a vast array of isotopic measurements for elements previously not available.

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