CHAPTER 3

Applying Isotopic Methods to Tracking Animal Movements

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Contents

I. INTRODUCTION

Stable isotope methods have proven to be a boon to ecologists in the last decades as witnessed by the veritable explosion of papers dealing with a diversity of taxa ranging from invertebrates to polar bears. This follows on from a well-established tradition of development and adoption of stable isotope techniques seen in other disciplines such as the earth sciences, plant physiology, and anthropology. The vast majority of the more recent contributions have focused on dietary reconstructions or trophic interactions because stable isotope measurements can provide information on sources of nutrients to food webs and trophic level according to the perhaps overused maxim that "you are what you eat plus a few parts per mil." More recently, attention has turned to applying stable isotope measurements as

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forensic tracers of origin for migratory or dispersing individual organisms. This development relies on three basic isotopic principles:

1. Consumer (animals including humans) stable isotope values reflect those of the food web they are in equilibrium with. Should food webs used by a migratory organism differ isotopically and spatially, then stable isotope values in the consumer can provide unambiguous information on previous consumer locations.
2. The time period over which this spatial information is retained will depend on the tissue chosen. For metabolically active tissues, this represents a moving window of forensic information. For metabolically inactive tissues, spatial information will be locked in indefinitely but will only reflect position for the short period of integration reflecting the growth of that tissue.
3. Mechanisms related to dietary transfer of isotopic signals to consumer tissues including isotopic discrimination, exercise, and metabolic routing are known and accounted for.

In practice, it is rare that all three principles will be satisfied or known with sufficient confidence! However, depending on the organism, much of this uncertainty can be constrained and, as we shall see, useful inferences can be made with respect to previous provenance of individuals based on isotopic measurements of their tissues. The careful blend of knowledge of the life history of the organism of interest, knowledge of likely isotopic landscapes or "isoscapes" experienced by that organism, and the physiological parameters that can influence isotopic inferences makes up the *art* of using stable isotopes to track migratory organisms.

II. ISOTOPIC DISCRIMINATION

The "you are what you eat plus a few parts per mil" maxim is formalized in the equation:

$$\delta C_t = \delta d + \Delta_{dt}$$

where δC_t is the measured stable isotope value measured of a tissue in the consumer, δd is the stable isotope value of the diet, and Δ_{dt} is the diet-tissue isotope discrimination factor (Chapter 2). We now know that this discrimination factor is a great oversimplification and that it does not necessarily take into account metabolic routing of specific macronutrients such as proteins, lipids, and carbohydrates. Recent research has also determined that the diet-tissue discrimination factors are influenced by the quality of the diets and so are likely not static for most wild animal populations. Because our ability to place an organism in a particular isoscape is sensitive to our knowledge of the true discrimination factors associated with an organism or dietary regime of interest, researchers should bracket their estimates based on an honest assessment of how well they know such factors. At most, this will require dietary experiments with the organism of interest, or, at a minimum, a sensitivity analysis to determine the effect of varying discrimination values on the outcome of GIS models or other methods used to "place" an organism.

A. Nitrogen Isotopes

Stable nitrogen isotopes in the tissues of consumers really represent a means of tracing protein pathways derived from diet because this element is largely absent in lipids and carbohydrates. For essential amino acids, nitrogen will largely be incorporated with little isotopic discrimination into the protein pool of the consumer. Nonessential amino acids typically involve more opportunities for isotopic discrimination during protein synthesis and so the net discrimination we see for $\delta^{15}N$

measurements in consumers will reflect the degree to which the diet meets the amino acid requirement of the consumer (Robbins *et al.* 2005).

In general, poorer quality diets will likely result in greater overall diet-tissue discrimination for ^{15}N than high-quality diets. An important currency in experiments designed to establish tissue-specific $\delta^{15}N$ values in migratory animals is the C:N ratio of the diet and this ratio alone may provide a useful indicator of diet quality and the ultimate discrimination factor to apply in natural situations. Isotopic discrimination associated with $\delta^{15}N$ will also depend on the means of voiding nitrogenous waste. Here, a major difference is found between aquatic invertebrates that void nitrogen via ammonia compared to terrestrial vertebrates (Post 2002). There is also evidence that ungulates adapted to arid conditions are able to conserve water by recycling urea that ultimately influences whole body tissue $\delta^{15}N$ values (Ambrose and DeNiro 1986, Sealy *et al.* 1987). Hobson *et al.* (1993) also determined that birds that fast and undergo significant protein catabolism during incubation, like geese breeding at high latitudes, also experience an increase in body $\delta^{15}N$ values.

Knowledge of these sorts of physiological processes is necessary, when using tissue $\delta^{15}N$ values of migratory organisms to infer origins. The current consensus is that researchers should strive to use the most parsimonious value associated with their specific organism of interest. The review of isotopic discrimination in $\delta^{15}N$ across several taxa by Vanderklift and Ponsard (2003) identified mode of excretion and environment (marine, freshwater aquatic, terrestrial) as important factors (see also Post 2002).

B. Carbon Isotopes

Unlike nitrogen and sulfur, carbon is present in all three dietary macromolecules (protein, fat, carbohydrates) and so $\delta^{13}C$ measurements of consumer tissues will reflect these various sources. The more varied sources of carbon to consumer tissues undoubtedly contribute to more variable diet-tissue $\delta^{13}C$ discrimination factors compared with those found for most of the other light elements. However, in many cases, lipids in diets are transferred directly with little isotopic modification to lipids in the consumer. Carbohydrates are often burned directly for energy production, producing CO_2 as the only carbon byproduct, and hence $\delta^{13}C$ values in breath CO_2 can be used as a means of directly tracing origins of carbohydrates in diet. Unfortunately, we have little idea of the appropriate carbon isotopic discrimination factors that currently apply between dietary substrates and breath CO_2 in animals (Podlesak *et al.* 2005). Carbon isotope values of proteins can theoretically originate from all three dietary macromolecules but is more likely to be associated with dietary proteins, especially for carnivores. In general, we expect lower diet-tissue isotopic discrimination factors for $\delta^{13}C$ compared with $\delta^{15}N$.

C. Sulfur Isotopes

Sulfur in consumer tissues is derived from the sulfur-bearing amino acids (*e.g.*, cystein, methionine) and so $\delta^{34}S$ measurements are closely linked to dietary protein pathways. Unlike the other light isotopes, we expect little S isotopic discrimination between diets and consumer tissues again because of little opportunity for the essential amino acids to be isotopically modified in consumers. As a result, $\delta^{34}S$ measurements make for a useful direct tracer in food web and migration studies (Krouse *et al.* 1991, Hebert and Wassenaar 2005).

D. Hydrogen and Oxygen Isotopes

As emphasized below and in Chapter 4, hydrogen is a particularly useful element for tracking migratory wildlife. However, this element presents a number of challenges in terms of understanding how the δD measurements of consumer tissue relate to hydrogen sources that, in most terrestrial systems, is driven by the global water cycle, driving primary production. Like carbon, hydrogen occurs in all three dietary macromolecules and so recognition of metabolic routing is important. However, the most interesting challenge is the fact that a portion of the hydrogen in any tissue exchanges with body water, a component which is presumably more labile than dietary derived hydrogen. Drinking water as well as diet thus constitutes a source of hydrogen in animals. Using a controlled laboratory study, Hobson *et al.* (1999a) maintained quail (*Coturnix japonica*) on a single diet but exposed groups to drinking water of vastly different δD value. They found that hydrogen from drinking water accounted for about 20% of the total hydrogen in various tissues. Interestingly, this was the case for lipids with no exchangeable hydrogen bonds indicating that body water can exchange with hydrogen in precursor molecules involved in lipid synthesis. The overall diet-tissue discrimination factor for hydrogen is complicated to the extent that one ideally could apply the δD values of both diet and drinking water and knowledge of how these are partitioned for various tissues. Such details do not exist for most animal systems and so overall diet-tissue discrimination factors are currently estimated using more phenomenological approaches as discussed below.

The use of δ^{18}O measurements to track wildlife is in its infancy because of previous technological constraints of routinely measuring oxygen isotopes in animal tissues. That situation has now changed due to online pyrolytic techniques (Chapter 2). In many systems, there is a tight coupling between δ^{18}O and δD values because of the meteoric relationship described by Bowen and West (Chapter 4). Thus, in many cases, no additional information will be derived from performing δ^{18}O measurements in addition to δD measurements on the same tissue. As well, δD values typically span a much larger range than δ^{18}O measurements in terrestrial food webs and so can potentially provide greater resolution with respect to source discrimination and an overall better signal-to-noise ratio. Oxygen occurs in proteins but not in lipids or carbohydrates. However, sources of oxygen include drinking water and air and thus, like hydrogen, it is difficult to predict isotopic discrimination factors associated with each contribution and “working values” will need to be derived largely from future examination of wild and captive animals.

III. TISSUE AND ISOTOPIC TURNOVER: THE MOVING WINDOW

That stable isotope values in consumer tissues reflect an integration of feeding events over various time periods has been known and experimentally demonstrated for decades. Tieszen *et al.* (1983) were the first researchers to conduct “diet switch” experiments whereby captive animals were allowed to reach equilibrium under one dietary regime before being switched to an isotopically different diet (Figure 3.1). Tissues were sampled following the diet switch and the uptake of the new isotopic dietary signal monitored. This approach has now been used by several researchers examining various species and most have fit an exponential uptake curve to describe the pattern of isotopic change in tissues.

$$D(t) = a + b\exp(-ct)$$

where $D(t)$ is the stable isotope value of the tissue at time t, a is the asymptotic tissue value, b is the absolute change in tissue isotope value between initial and asymptotic conditions, and c is a rate constant defining tissue turnover. For cases where researchers wish to consider effects of growth (k) as well as metabolic turnover (m), the overall rate constant c can be expressed as ($k + m$). This approach has worked well to provide estimates of elemental turnover rates in various tissues of birds, fish, and mammals (*e.g.*, Hesslein *et al.* 1993, Bosley *et al.* 2002, Dalerum and Angerbjörn 2005). One potential disadvantage of these previous studies is that they were necessarily based on sedentary, nonexercised individuals in laboratory settings. Can the elemental turnover rates obtained from such studies be

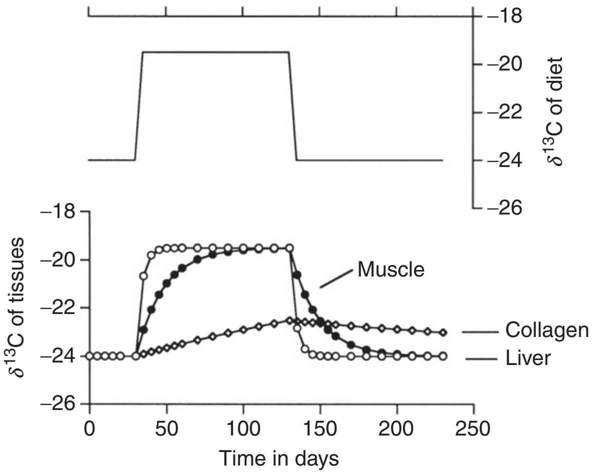

FIGURE 3.1 Conceptual depiction of the way in which different tissues will respond to an isotopic diet switch. We expect close coupling between the diet isotope trajectory and fast turnover tissues like liver or blood plasma. Much slower response is expected for slow turnover tissues like bone collagen. Based on the captive Japanese quail (*Coturnix japonica*) study of Hobson and Clark (1992).

directly applied to migrating individuals that, in the case of birds, can undergo many hours of sustained flight? Might we not expect more rapid elemental turnover in tissues of exercising versus sedentary organisms? This is still not clear but Hobson and Yohannes (2007) used Rosy Starlings (*Sturnus roseus*) trained to fly in a wind tunnel to provide a first approximation of this effect for the cellular fraction of blood. They performed a C3 to C4 diet switch on birds that flew several hours per day and contrasted the isotopic turnover rates with those of a control group, which was not exercised. Interestingly, they found no difference between the two groups. This suggests that erythrocyte production was unaffected by exercise at least to the level sustained in this experiment. More studies are needed and the use of wind tunnels is clearly the best way to explore turnover rates in migratory birds and insects. However, these results are encouraging and suggest that turnover rates established to date may be appropriate for most isotopic studies on migratory organisms. The other good news is that elemental turnover rates appear to follow expectations based on allometry (Figure 3.2). It is possible, then, to estimate turnover rates for various tissues based on the body mass of the organism of interest even though that species has not been tested experimentally (see also Carleton and Martínez del Rio 2005).

Recently, others have investigated a new way of analyzing and interpreting uptake curves based on isotopic dietary switch experiments (Cerling *et al.* 2007). Instead of fitting exponential equations to these curves to estimate turnover rates, they applied a technique established by radiochemists to estimate relative contributions of different radionuclide decay curves to a single (measured) decay function. This approach involves the determination of reaction progress variables that are derived from a process that involves linearizing the decay curves. Interestingly, such an approach has suggested that instead of representing a single elemental pool that changes according to the exponential function used previously, there is evidence that these curves can represent more than one source pool with each having a different elemental turnover rate. Perhaps the best current interpretation of this phenomenon is that essential amino acids are transferred relatively quickly from diet to tissues whereas nonessential amino acids are manufactured from dietary components and so represent a lag time prior to incorporation into consumer tissues. This new way of looking at elemental turnover in animals shows potential for promoting our understanding of how elements from diet and body stores are ultimately routed to consumer tissues and how these can differ temporally in terms of dietary integration.

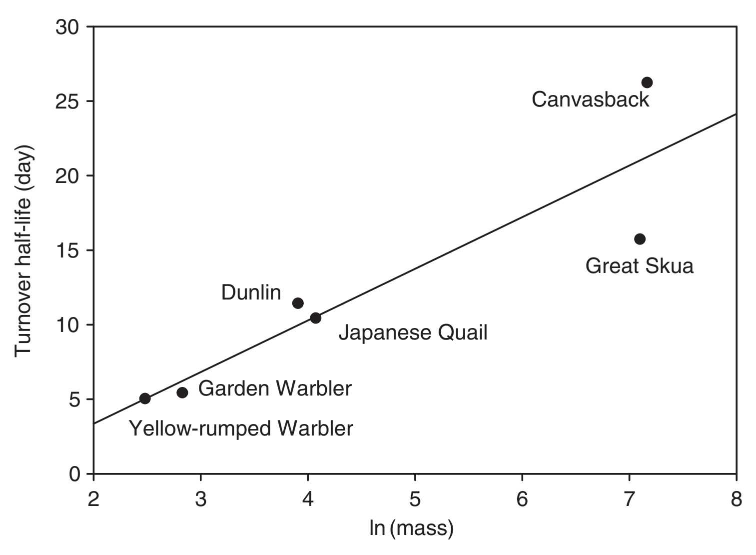

FIGURE 3.2 Relationship between turnover half-life (days) for whole blood as determined by a switch in δ^{13}C values of food of captive birds and the natural logarithm of body mass (grams). Based on studies by Hobson and Clark (1992), Haramis *et al.* (2001), Bearhop *et al.* (2002), Hobson and Bairlein (2003), Pearson *et al.* (2003), and Evans-Ogden *et al.* (2004). Figure shows the predominantly allometric relationship between elemental turnover rate and body size.

However, this important development in the way we consider turnover experiments in future by no means negates the results of earlier work using the more conventional approach and the *net* elemental turnover measured by fitting the exponential function still provides a phenomenological estimate of the time period a given isotopic measurement of an organism represents.

Once we have decided on a realistic estimate of the half-life of an element in the tissues of a migratory organism, we need to decide on a convention that best quantifies the time period represented by the isotopic measurement of that tissue. Most authors have considered that a tissue realistically represents about three half-lives or the time required for 87.5% of the original signal to be replaced by a new signal. Put another way, we should at least be able to detect the 12.5% of the original material remaining by our isotopic measurement. While this is a rule-of-thumb, our true ability to resolve between an original tissue signal and the asymptotic signal reached at a new location will depend upon the isotopic separation between these two signals and the nature of the distributions of these signals for populations of individuals in dietary equilibrium at each of these locations. The greater the isotopic difference between initial and final conditions (*i.e.*, the greater the value *b*), and the smaller the variance associated with each equilibrium condition, the more capable we will be of detecting isotopic information from a previous location (Figure 3.3).

As a hypothetical example, imagine a situation where the mean δ^{13}C value of a population at equilibrium with the diet at the new location (*i.e.*, *a*) can be statistically resolved at a minimum difference of 1 per mil from that of the more recently arrived population (such resolution will of course depend on the variance associated with each mean population isotope value, the nature of those distributions, and overall measurement error). If the population means were separated by only 4 per mil initially, then we would only detect a signal representing two half-lives of a given tissue. If, however, the populations were initially separated by 6 per mil, then we could detect a difference between these populations for three half-lives of that tissue. This example illustrates why researchers should strive to estimate the magnitude of these model parameters and the isotopic variance associated with them when trying to define the time periods over which their isotopic measurements correspond for migrating individuals or populations.

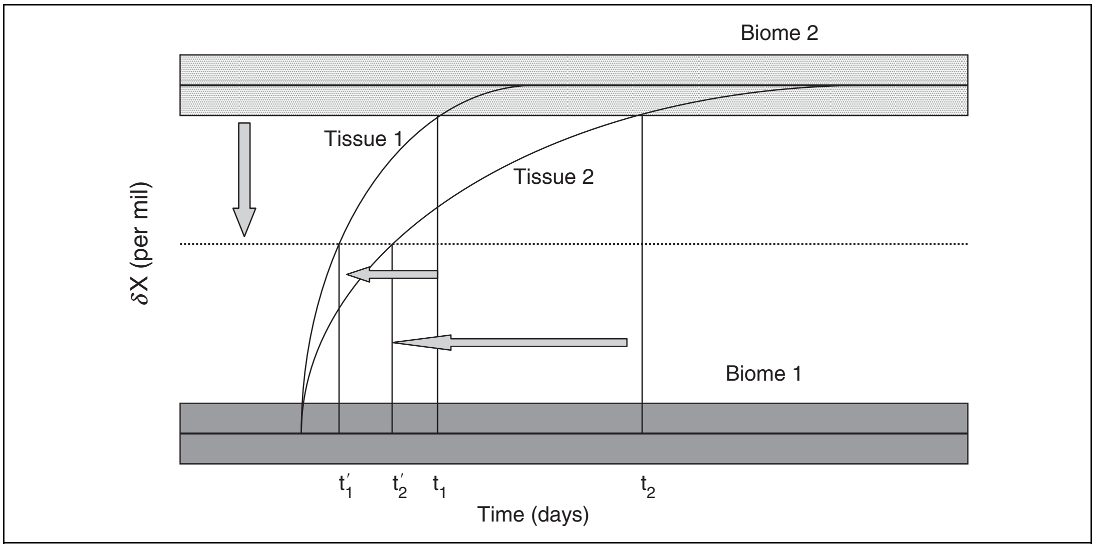

FIGURE 3.3 When an organism moves from one isotopic biome to another, our ability to detect the original biome signal will depend on the tissue we choose and the magnitude of the isotopic separation between the two biomes. Here, tissue 1 has a faster elemental turnover rate than tissue 2. The bands about each biome indicate the isotopic resolution or measurement error corresponding to organisms in that biome. The scenario of reducing the isotopic distance is demonstrated with the arrows. Here, we can see that the time over which we can detect the original biome signal is reduced (primed notation).

Phillips and Eldridge (2006) explored the utility of using more than one tissue for isotopic assay in order to detect the actual time an individual organism has spent in a new environment. Such information is less useful for estimating *where* an organism came from but can provide insights into the value of the migratory stopover environment. This approach is based on the contrast between tissues with different turnover rates, typically a fast turnover tissue like liver, plasma, or breath CO_2 and a slower turnover tissue like muscle or the cellular fraction of blood. The model assumes that the researcher knows the initial and asymptotic tissue isotope values, the measured isotope value of tissues at some time after arrival, and the necessary rate constants associated with the tissues used. The model does a good job of estimating the time since diet shift and the magnitude of the isotopic difference between initial and asymptotic conditions except in circumstances where the elapsed time was only a small fraction of half-life of the slower turnover tissue or when the diet shifts were small (*i.e.*, less than 10 times the measurement error).

As with all models, we are forced to make a number of assumptions to have any hope of achieving a solution. In the case of discerning dietary history or previous geographical provenance of a migratory organism, we typically make the assumption that the organism was in full equilibrium with its previous diet upon arrival. This will depend on the tissue used, and so there would be a much higher likelihood of equilibrium conditions reached for shorter turnover tissues. Use of intermediate- or long-turnover tissues may be less useful for a number of migrants that move quickly among different stopover locations. As we shall see later in examples of detecting dispersal in organisms, there are some circumstances where we are only interested in knowing that the arrival signature is *different* from the local food web signature and are less concerned that it can be associated with a particular location. There will also be error associated with estimates of rate constants ($k + m$) and estimates of these errors are typically poorly known for most wild species. Finally, all current models assume a direct dietary source of nutrients to tissues and do not consider situations where organisms may be metabolizing stored nutrients for maintenance.

IV. APPLICATIONS OF STABLE ISOTOPES TO MIGRATORY MOVEMENT

The recent development of stable isotope methods to tracking migratory wildlife has already provided a rich literature to illustrate the breadth of applications using the light isotopes of C, N, H, O, and S. (reviewed in Hobson 1999a, Rubenstein and Hobson 2004, Hobson 2005a). In general, these applications can be split into (1) inferences of animal origins based on biome markers, typically using isotopes of C, N, and S and (2) those using continental-scale δD isoscapes. The use of δD measurements has brought with it immense opportunities but also challenges as we attempt to fill in the information gaps associated with this complicated element and so hydrogen will be discussed separately.

A. Migratory Movement Among Isoscapes

As it was recognized that stable isotope measurements can provide information on habitats or biomes, it was not long before researchers began to explore the use of stable isotopes to infer origins of migratory animals moving among such isoscapes. While the focus of this volume is terrestrial ecosystems, some of the earliest isotopic investigations revealed distinct differences between marine and terrestrial food webs with marine organisms typically having more positive δ^{13}C, δ^{15}N, δ^{34}S, δD, and δ^{18}O values compared with their terrestrial counterparts (Hobson 1999a). As many migratory organisms use both terrestrial and marine biomes throughout their annual cycles, these marine versus terrestrial isotopic differences have become extremely useful (*e.g.*, Atkinson *et al.* 2005). Plant physiologists have also pioneered the use of stable isotope measurements to discern C3, C4, and CAM-based photosynthetic pathways using both δ^{13}C and δD measurements and have more recently investigated the effect of water-use efficiency mechanisms in C3 plants that generally leads to an enrichment of plant tissue ^{13}C.

One of the earliest applications of stable isotope methods to investigate animal spatial movement was made by Killingly (1980) who inferred the temperature of water during calcite formation of barnacles attached to the skin of California Gray Whales (*Eschrichtius robustus*) using δ^{18}O measurements and by Killingly and Lutcavage (1983) who examined δ^{18}O and δ^{13}C measurements in barnacles on loggerhead turtles (*Carettaq caretta*). That work has since been followed by a number of studies on the inorganic fraction of otoliths in freshwater and marine fish (*e.g.*, Meyer-Rochow *et al.* 1992, Kennedy *et al.* 1997). Other excellent examples of using marine isoscapes to infer spatial movements of marine mammals have been provided by isotopic analyses of the baleen plates of the western Arctic population of bowhead whales (*Balaena mysticetus*) migrating annually between the Beaufort and Bering seas (Schell *et al.* 1989) and southern right whales (*Eubalaena australis*) that annually cross the Southern ocean convergence, a zone of dramatic changes in food web δ^{13}C and δ^{15}N (Best and Schell 1996). Currently, there is great interest in establishing marine isoscapes that can be used to help track the movements of marine animals (Gómez-Diaz and González-Solis 2007, Hobson 2007).

Within terrestrial systems, some of the earliest applications of isotopic measurements to tracking origins of animals were conducted in Africa. That continent has attracted a number of isotopic studies over the years due to its importance archaeologically and as a center of current animal diversity and conservation concern. Terrestrial food webs in Africa also include varied C3- and C4-dominated isoscapes and, as discussed later, a very diverse and dynamic δD isoscape. Two simultaneous yet independent studies used stable isotope measurements of elephant (*Loxodonta africana*) ivory and bone collagen to infer origins of that material as a forensic tool to counter the illegal ivory trade (Van der Merwe *et al.* 1990, Vogel *et al.* 1990). Elephants feeding primarily on grasses sample a C4 food web and so have more positive δ^{13}C values compared to those feeding in woodlands on C3 browse. Elephants feeding in more arid areas may also have higher δ^{15}N values than those in more mesic habitats (Heaton 1987). Combined with assays of Pb and Sr isotopes, these studies showed strong segregation among several African elephant populations and underlined the forensic utility of stable isotopes to infer origins of several taxa. Unfortunately, some of the early enthusiasm was later tempered by the observation of strong year-to-year variations in food web $\delta^{15}N$ values within the Amboseli National Park presumably due to climatic variation (Koch *et al.* 1995). This illustrates the need to know the natural range of variation in stable isotope patterns spatially and temporally when evaluating the accuracy of the technique when inferring animal origins.

Fortunately, many terrestrial systems are less dynamic isotopically and strong and consistent isotopic differences are maintained over decadal or longer time frames. This is especially the case with the use of $\delta^{13}C$ measurements to track the use by animals of C3, C4, and CAM food webs. A very interesting application of $\delta^{13}C$ measurements to investigate mechanisms affecting the phenology of animal migration was that of Flemming *et al.* (1993) who showed that the nectarivorous bat *Leptonycteris curasoae* switched from C3 flowering plants during the winter to CAM flowering columnar cacti as it migrated north in the spring. The bat tissue isotopic data revealed how the species has adapted to the phenology of CAM "nectar corridors" during their northward migration. Hobson (1999b) showed that migrant insectivorous songbirds known to originate in boreal forest of North America had consistently lower feather $\delta^{15}N$ values than those species from more southern agricultural zones. Similarly, Hebert and Wassenaar (2001) were able to use $\delta^{15}N$ measurements to segregate waterfowl originating in agricultural zones that are typically enriched in ^{15}N compared to more natural temperate regions (see also Hobson *et al.* 2005).

A seminal study by Wolf and Martinez del Rio (2000) on the isotopic ecology of two species of doves using a desert region in the southwestern United States provides an excellent example of how tissue δD and $\delta^{13}C$ measurements can provide strong inferences on the diets, and hence feeding locations, of volant animals. The saguaro cactus (*Carnegiea gigantea*) represents a valuable food and water resource to animals in arid environments. This CAM resource is imbedded in a C3 plant biome and so fruit consumption can be traced by $\delta^{13}C$ measurements. However, water derived from CAM plants is also highly enriched in deuterium compared with surface waters and this provided evidence that while the two species consumed food from the saguaro, they differed in their dependence of this plant for drinking water. This topic will be revisited later in relation to factors affecting the use of deuterium basemaps for tracking migratory animals.

Other studies have exploited the strong C4 $\delta^{13}C$ signal of agricultural corn to infer the origins of migratory herbivorous birds. Alisauskas *et al.* (1998) were able to assign newly arriving lesser snow geese (*Chen caerulescens*) to a migratory staging area in the Missouri Valley, United States, because local birds subsisted largely on corn agriculture whereas immigrants arrived from distinct C3 habitats or non corn-growing regions to the south. In their study of feathers of red-winged blackbirds (*Agelaius phoeniceus*) across a latitudinal gradient in North America, Wassenaar and Hobson (2000) found that birds formed feathers from pure C3 to pure C4 food webs. Similar results were found for loggerhead shrikes (*Lanius ludovicianus*) on that continent (Hobson and Wassenaar 2001). Both of these examples indicate the way in which corn and other agricultural C4 crops imbedded in a C3 landscape can provide information on origins of migrant animals. However, $\delta^{13}C$ isoscapes in North America or other areas with intense agricultural production that have a mix of C3 and C4 plants will be necessarily complicated to model.

A study that clearly had profound influence on the field of identifying seasonal interactions or carry over effects from one season to another was that of Marra *et al.* (1998). Those authors examined the effect on tissue $\delta^{13}C$ values of American Redstarts (*Setophaga ruticilla*) occupying habitat of different quality on the Jamaica wintering grounds. Likely due to the effect of water-use efficiency in C3 plants, food webs in wet mangrove forest were more depleted in ^{13}C than those in xeric scrub habitat and such habitat markers were passed on to redstarts inhabiting those habitats. Long-term studies on this wintering population of redstarts suggested that those birds occupying the better (moister) habitat were the first to reach a body condition that allowed them to migrate back to the United States and

Canada to breed. Thus, the prediction was that early spring arriving male birds in New Hampshire would have muscle tissue relatively depleted in ^{13}C compared to later arriving birds. This was indeed the pattern that was found.

Why was this paper so well received (including the cover illustration of migrating redstarts on the cover of Science!)? Well, for years it has been known that early arriving birds on the breeding grounds tended to have greater reproductive success than those arriving later or initiating clutches later. It was long assumed that such effects on fitness were influenced entirely by factors on the breeding grounds. The Marra *et al.* (1998) paper clearly showed there was a direct link between conditions experienced by individuals on the wintering grounds and their ultimate fitness and renewed interest in the concept of migratory connectivity and seasonal interactions.

This work was followed up by an interesting modeling exercise by Norris *et al.* (2004a) who, using path analysis, were able to theoretically link tissue δ^{13}C values to number of offspring produced! Of course, this exciting work rests entirely on our ability to detect a wintering ground isotopic signal in newly arriving birds on the spring breeding grounds. Some have expressed concern that the use of whole blood or muscle tissue in small, migrating passerines is very risky because these birds may take as much as 2–3 weeks to reach their breeding destinations, and elemental turnover rates for these tissues are really quite rapid in these small creatures. Indeed, although statistically significant, the relationship between muscle δ^{13}C value and arrival date on the breeding grounds in the Marra *et al.* (1998) study was rather weak. Nonetheless, follow-up investigations have confirmed this winter ground signal using much larger sample sizes of arriving redstarts (Norris *et al.* 2005). Bearhop *et al.* (2003) also demonstrated the utility of using stable isotope measurements of slow-growing claw material in birds and intercepted Black-throated Blue Warblers (*Dendroica caerulescens*) during migration in the Bahamas Bearhop *et al.* (2004). Similar to the Marra *et al.* (1998) study, they found that birds in better body condition were from more mesic (moist) versus xeric (dry) habitats as shown by their claw δ^{13}C values.

Chamberlain *et al.* (2000) investigated δ^{13}C and δ^{15}N values in feathers of Willow Warblers (*Phylloscopus trochilus*) located along a breeding latitudinal gradient in Scandinavia. Two subspecies are segregated along this gradient that differ in morphology and assumed African wintering grounds based on a handful of band returns. Unlike most Nearctic migratory birds, Palearctic species typically replace flight feathers on their wintering ground in Africa and so it was possible to infer aspects of the wintering habitats using isotopic analyses of these feathers. It was found that the southern *trochilis* subspecies had feathers more indicative of the mesic habitats of sub-Saharan West Africa than the northern *acredula* subspecies with supposed wintering areas in Central, East, or South Africa. These results agreed generally with the limited band return data and with isotopic analysis of molting birds from known capture sites in Africa (Bensch *et al.* 2006). However, the follow-up study using a larger dataset by Bensch *et al.* (2006) found higher δ^{13}C values in general than those found by Chamberlain *et al.* (2000). This discrepancy is somewhat reminiscent of the forensic analysis of African ivory and the warning by Koch *et al.* (1995) that δ^{13}C and δ^{15}N isoscapes in some regions of Africa may be highly variable among years.

Because one of the principles in applying stable isotope methods to studies of animal migration is that we have direct or good knowledge of the isoscapes encountered by any organism of interest, it may come as a surprise to some readers that very useful information on the structure of migratory populations can be obtained even when this is not the case (Hobson 2005b). Barn swallows (*Hirundo rustica*) breeding in Denmark are known to winter in South Africa but more refined information on connectivity is not available. Møller and Hobson (2004) investigated δD, δ^{13}C, and δ^{15}N values in African-grown feathers of Danish swallows and discovered that the distribution of δ^{13}C and δ^{15}N values was bimodal with a rare group (6% of the population) having very different values compared to the main group. These researchers concluded that this represented different winter habitats or regions occupied in Africa by the two groups. They investigated other data obtained for these birds from the breeding ground and found that the "rarer" group had chicks of larger mass and tarsus than those of the common group and that their chicks also had a much reduced T-cell immune response, indicating their immune systems were less challenged. The current hypothesis is that this rare group in Denmark originated from breeding populations within Scandinavia. So, while no one would advocate a "shotgun" isotopic approach to the analysis of tissues from migrant animals, the structure of those distributions can also provide valuable information that can assist in the development of new testable hypotheses. The deliberate analysis of the isotopic structure of breeding populations of animals can also be used as a means of detecting immigrants into those populations. Hobson *et al.* (2004a) used this approach to investigate minimum estimates of dispersal into breeding populations of American Redstarts and Ovenbirds (*Seiurus aurocapillus*), using δD measurements of feathers.

Møller *et al.* (2006) continued their isotopic analysis of barn swallows in Europe by examining those populations occurring within and outside the zone of influence of the Chernobyl nuclear accident and for samples obtained before and after the incident. They determined that correlation between δ^{13}C and δ^{15}N values in winter-grown feathers differed significantly between regions for females but not for males. This was interpreted as indicating that birds from the non-Chernobyl region were composed of females from a smaller recruitment area. The reasoning was that individuals with greater dispersal distance molted their feathers across a larger wintering area, thus increasing the probability of higher isotopic variance. Weak positive δ^{13}C and δ^{15}N correlations within "isotopic populations" seem to be common and the decoupling of this relationship might be a good indicator of mixed populations. This area of research requires much more study. The intriguing nature of the Chernobyl study is that stable isotope methods might well provide insight into the occurrence of source and sink breeding populations of migratory animals, an aspect of populations that is often extremely difficult to evaluate by other means.

The movement of animals between marine, estuarine, and terrestrial or freshwater habitats holds great potential for inferring their past habitat use and potential migratory origins. Tietje and Teer (1988) were among the first to use stable isotope methods to investigate how wintering Northern Shoveler (*Anas clypeata*) ducks use coastal and inland freshwater wetlands and were able to demonstrate sedentary behavior among late wintering individuals. Other studies have primarily used δ^{13}C measurements to infer movement of piscivorous birds between marine and freshwater habitats (Mizutani *et al.* 1990, Bearhop *et al.* 1999), and Hobson (1987) even used this approach to infer use of garbage dumps by coastal wintering gulls near an urban center.

While not terrestrial, migratory movements of fish with an anadromous life stage present an isotopic opportunity and, as indicated, fish have the added advantage of carrying an isotopic record in their otoliths and scales (Nelson *et al.* 1989, Trueman and Moore 2007) and soft tissues (Hobson *et al.* 2007a). Kennedy *et al.* (1997) and Harrington *et al.* (1998) have nicely demonstrated how stable isotopes of several elements can be used on the organic and inorganic fractions of otoliths to identify natal streams of Atlantic Salmon (*Salmo salar*) intercepted as adults at sea. Essentially, the suite of δ^{13}C, δ^{15}N, and δ^{87}Sr measurements formed unique combinations of values reflecting the geological substrate and land-use practices surrounding drainage basins of key salmon-producing streams.

Even within entirely freshwater habitats in the terrestrial environment, there clearly is substantial isotopic variability that can be used to examine movements of fish and Hesslein *et al.* (1991) used δ^{13}C, δ^{15}N, and δ^{34}S measurements of muscle in broad whitefish (*Coregonus nasus*) and lake whitefish (*Coregonus clupiformis*) in two freshwater regions of the Mackenzie Delta in Northern Canada to infer their movements. Recently, there has been great interest in using fish tissue δD values because stream and river inputs to lake systems can have very different water δD values (Doucett *et al.* 2007). Thus, freshwater systems have great potential for a multi-isotope approach to trace migrations and movements of aquatic species. They also have the added advantage of being reasonably tightly constrained spatially and it should be possible to literally create multi-isotopic basemaps of the major aquatic space used by migrant fish.

B. Traveling to Breed: The Isotopic Tracing of Nutrients to Reproduction

A major impetus for studying migration is the concept that migration involves trade-offs between other life-history demands including reproduction, molt, and so on. The degree to which migratory animals carry with them nutrients that were acquired elsewhere for the production of young, the so-called *capital* (vs *income*) strategy is intrinsically linked to the costs and benefits of migration (Drent 2007). Although not an application of stable isotopes to infer origins of individuals or populations *per se*, the recent development of isotopic tools to trace origins of nutrients to reproduction is an important development that is of interest here (Hobson 2006).

As we have seen, a necessary principle to the application of isotopic models to infer migratory origins is the establishment of isotopic discrimination factors that link substrates to products. Here, the discrimination factors between endogenous (obtained locally) and exogenous (stored) sources of nutrients and eggs were needed to investigate such strategies in birds. Hobson (1995) raised Japanese quail (*C. japonica*) on a plant-based diet and peregrine falcons (*Falco peregrinus*) on a quail diet and then related dietary lipid and lipid-free δ^{13}C and δ^{15}N values in diet to corresponding isotope values in egg yolk lipid, lipid-free yolk, albumin, and shell carbonate. The derived isotopic discrimination factors between diet and egg components differed between the herbivore and carnivore diets reflecting, in part, differential macromolecular routing of lipids, carbohydrates, and proteins. An unexpected benefit of considering the carnivore income breeding model was the fact that it provided the first estimate of isotopic discrimination factors that were likely applicable to the capital breeding strategy. The reasoning here is simply that conversion of dietary protein (muscle) and lipids to eggs during an income process of egg formation should be kinetically and thus isotopically similar to the process of the production of eggs from endogenous muscle and lipid stores. Gauthier *et al.* (2003) were the first to apply this reasoning to generate the first fully quantitative estimate of the role of endogenous and exogenous nutrients to reproduction in Greater Snow Geese (*Chen caerulescens atlantica*).

The first study to use stable isotope measurements of egg components to infer origins of nutrients was Trust (1993) who examined δ^{13}C and δ^{34}S values in eggs of redhead ducks (*Aythya americana*) breeding in Manitoba, Canada. Hobson *et al.* (1997) measured isotopically eggs of three colonial waterbirds breeding on Lake Ontario, Canada, in order to determine if there was evidence for the transfer of marine nutrients acquired on the wintering grounds to eggs laid on the freshwater breeding grounds. That study lead to a more ambitious examination of five species of gulls, four species of terns, and one jaeger breeding on Great Slave Lake, Canada (Hobson *et al.* 2000a). As indicated by δ^{13}C and δ^{15}N measurements of tissues, birds arrived with largely marine-derived endogenous nutrients but only two species (*Larus argentatus*, *L. canus*) showed a strong indication of transferring marine-derived lipids to eggs and two species (*Sterna caspia*, *S. hirundo*) showed evidence of marine-derived protein contributions to eggs.

An interesting development in using stable isotopes to examine nutrient transfer to eggs was provided by the first use of hydrogen isotope (δD) measurements. Hobson *et al.* (2004b) examined δD and δ^{13}C values of endogenous reserves (muscle and fat) and egg components in redhead ducks breeding in Manitoba, Canada. δD measurements provide the advantage that not only is there a very large isotopic difference between terrestrial and marine food webs but that even among terrestrial food webs, deuterium can be a powerful indicator of latitude in North America (Chapter 4). The study showed striking patterns of isotopic change in endogenous tissues throughout the season for both isotopes but there was little evidence for any endogenous nutrient inputs to eggs for this species.

In a similar study using both δ^{13}C and δ^{15}N analyses of females and their eggs, Hobson *et al.* (2005) examined Barrow's Goldeneye (*Bucephala islandica*) breeding in central British Columbia, Canada. Although there was overall little evidence for a capital breeding strategy in goldeneyes, compared with later-laid eggs, the first-laid egg showed the greatest probability of including endogenously derived nutrients of both protein and lipid. Klaassen *et al.* (2001) provided a creative application of stable isotope analyses to nutrient allocations among Arctic waders by measuring $\delta^{13}C$ values in natal down of newly hatched individuals together with those values of feathers from adults growing feathers on the marine wintering grounds and juveniles later growing feathers on the breeding grounds. The authors reasoned that if eggs contained marine-derived endogenous nutrients, natal down should reflect that input and would thus be more enriched in ^{13}C compared to juvenile feathers that were presumably based entirely on local foods. Contrary to the inference of Klaassen *et al.* (2001), in their isotopic study of Red Knot (*Calidris canutus islandica*) and Ruddy Turnstone (*Arenaria interpres interpres*) breeding in the high Arctic, Morrison and Hobson (2004) found evidence for endogenous nutrient allocation to early laid eggs.

To date, the most quantitative attempt to ascertain the extent of endogenous and exogenous nutrient allocations to eggs has been conducted by Gauthier *et al.* (2003). That study used isotopic discrimination factors corresponding to capital versus income models in multisource isotope mixing models for protein and lipid contributions to eggs of Greater Snow Geese. Because the two groups of local foods available to geese, graminoids and forbs, were isotopically different, these authors used a three-input (*i.e.*, gramminoid, forb, endogenous tissue), two-isotope mixing model that accounted for differences in the [C] and [N] values of the foods.

C. The Deuterium Breakthrough

The success of the applications discussed so far depend to a large degree on how well we know the ecology of the species of interest (*e.g.*, does it use marine habitats for a portion of its annual cycle?, does it make use of corn or other isotopically distinguishable agricultural crops?) or on how well constrained or how well we know the isoscapes through which the animal moves or has access to. On a case-by-case basis, the success of a stable isotope approach could range between failures (*i.e.*, no *a priori* knowledge of the isotopic options available for inference) to providing unequivocal evidence of previous origins. The demonstration that deuterium and oxygen isotopes occur at continent-scale, and predictable patterns of abundance in precipitation and that those patterns could be passed on to animals growing tissues at those locations, provided a major breakthrough because this was the first example of a relatively robust isotopic basemap for use in animal migration studies.

As developed by Bowen and West (Chapter 4), deuterium values in precipitation in North America show a continent-wide pattern with a general gradient of relatively enriched values in the southeast to more depleted values in the northwest (Sheppard *et al.* 1969, Taylor 1974). Previous studies had also established strong correlations between growing season average δD values in precipitation and those in plant biomass (Yapp and Epstein 1982). However, it was the work of Cormie *et al.* (1994) on deer bone collagen that clearly demonstrated that such patterns were also passed on to organisms at higher trophic levels and that work inspired Chamberlain *et al.* (1997) and Hobson and Wassenaar (1997) to examine how well such precipitation isotope values were passed on to birds growing feathers at known locations at a continental scale across North America. The advantage to using birds was that their molt chronologies are relatively well known and most migrants breeding in the United States and Canada molt flight feathers prior to their southward migration. The ability to capture an individual on the wintering grounds and use its feather to predict an approximate latitude of breeding origin was indeed a major breakthrough and the strong correlation ($r^2 = 0.89$) measured by Hobson and Wassenaar (1997) between feather δD and the mean growing season average precipitation δD for forest songbirds across the central part of the North American continent was almost too good to be true. Fortunately, that large-scale spatial pattern has since been confirmed by several other researchers on various avian species (Table 3.1) even though some intriguing variation in this relationship has emerged (Chapter 5).

The first comprehensive application of the use of δD measurements in the study of migratory animals came fairly early with the investigation of the isotopic structure of Monarch butterflies (*Danaus plexippus*) wintering in Mexico. This example is worth considering in some detail because it

TABLE 3.1 Relationship between stable hydrogen isotope ratios of mean precipitation (δD_p) and the δD values of collagen, feather, hair, keratin, or chitin assumed to have been produced from those sources

Species	Equation	r^2	Model	Source
Birds				
6 species of North American songbird	$\delta D = -31 + 0.9\delta D_p$	0.83	H	Hobson and Wassenaar (1997)
6 species of North American songbird	$\delta D = -25 + 0.9\delta D_p$	0.88	B	Clark *et al.* (2006)
6 species of North American songbird	$\delta D = -19.4 + 1.07\delta D_p$	0.86	B	Bowen *et al.* (2005)
Black-throated Blue Warbler	$\delta D = -51 + 0.5\delta D_p$	0.86	CH	Chamberlain *et al.* (1997)
Red-winged blackbird	$\delta D = -27 + 1.1\delta D_p$	0.83	H	Wassenaar and Hobson (2000)
Bicknell's Thrush	$\delta D = -26 + 0.7\delta D_p$	0.48	H	Hobson *et al.* (2001)
Wilson's Warbler	$\delta D = -51.7 + 0.4\delta D_p$	0.36	B	Kelly (2000)
Wilson's Warbler	$\delta D = +\,14.47 + 1.41\delta D_p$	0.91	M	Paxton *et al.* (2007)
Wilson's Warbler	$\delta D = -21 + 0.7\delta D_p$	0.48	M	Meehan *et al.* (2004)
Mountain Plover	$\delta D = +\,17.4 + 1.26\delta D_p$	0.36	B	Wunder (2007)
23 species of European birds	$\delta D = -7.8 + 1.27\delta D_p$	0.65	B	Hobson *et al.* (2004d)
23 species of European birds	$\delta D = -22.3 + 0.77\delta D_p$	0.85	B	Bowen *et al.* (2005)
Cooper's hawk	$\delta D = -34 + 1.0\delta D_p$	0.83	H	Meehan *et al.* (2001)
Inland generalist raptors	$\delta D = -40 + 0.62\delta D_p$	0.59	H	Lott *et al.* (2003)
Inland bird-eating raptor	$\delta D = -44.2 + 0.54\delta D_p$	0.37	H	Lott *et al.* (2003)
Coastal generalist raptors	$\delta D = -38.8 + 0.55\delta D_p$	0.19	H	Lott *et al.* (2003)
Coastal bird-eating raptors	$\delta D = -104.7 - 0.59\delta D_p$	0.12	H	Lott *et al.* (2003)
Non-coastal bird-eating raptors	$\delta D = -41.1 + 0.58\delta D_p$	0.46	H	Lott *et al.* (2003)
9 species of raptors	$\delta D = -52.2 + 0.28\delta D_p$	0.09	H	Lott *et al.* (2003)
9 species of diurnal raptors	$\delta D = -37 + 0.6\delta D_p$	0.51	M	Meehan *et al.* (2004)
Raptors in South Carolina	$\delta D = -25 + 0.7\delta D_p$	0.18	M	Meehan *et al.* (2004)
Flammulated owl	$\delta D = -8 + 0.9\delta D_p$	0.66	M	Meehan *et al.* (2004)
12 species of raptors	$\delta D = -5.6 + 0.91\delta D_p$	0.62	M	Lott and Smith (2006)
Scaup	$\delta D = -27.8 + 0.95D_p$	0.64	B	Clark *et al.* (2006)
Mallards and Northern Pintail	$\delta D = -57 + 0.835D_p$	0.56	M	Hebert and Wassenaar (2005)
Other animals				
Deer collagen	$\delta D = 4 + 1.02\delta D_p$	0.94	C	Cormie *et al.* (1994)
Hoary bat	$\delta D = -25 + 0.8\delta D_p$	0.60	M	Cryan *et al.* (2004)
Monarch butterfly	$\delta D = -79 + 0.62\delta D_p$	0.69	H	Hobson *et al.* (1999b)
Beetle (chitin)	$\delta D = 33.2 + 1.60\delta D_p$	0.74	B	Gröcke *et al.* (2006a)

Model refers to basemap developed by Bowen *et al.* (2005): B; Meehan *et al.* (2004): M; the original or updated dataset used in Hobson and Wassenaar (1997): H; the dataset derived by Cormie *et al.* (1994): C, and the dataset derived by Chamberlain *et al.* (1997): CH.

still represents a good template for stable isotope tracking of migratory wildlife. The eastern population of the Monarch Butterfly in North America overwinter in about 13 known roost sites in the high-altitude Oyamel Fir (*Abies religiosa*) forests of central Michoacan and Mexico states. In spring, only gravid females migrate north, reaching Texas where they lay eggs on milkweed (*Asclepias* species) plants. The new generation emerging travels further north to repeat the process at higher latitudes. Finally, in one of the most spectacular migrations of any animal, in late summer only monarchs 4–6 generations removed form those ancestors that migrated northward from Mexico the previous spring then return to the *same* roost sites that they have never seen before.

Hobson *et al.* (1999b) first created an isotopic basemap corresponding to butterflies produced throughout their breeding range during the summer of 1996. An isotopic basement is composed of isotope measurements made on individuals from known locations that sufficiently spans the entire breeding range. This feat was accomplished through the aid of the nonprofit Monarchwatch organization (monarchwatch.org), who were able to solicit volunteers and educators from 86 locations across the monarch breeding range to successfully raised 4–12 butterflies on milkweed grown locally. Only milkweed watered by natural rainfall was used. From that sample, butterflies from 33 sites were selected for δD and δ^{13}C analyses of wing tissue performed to produce a year-specific basemap depicting isotopic patterns for C + H isotopes. In addition to the wild-reared group of monarchs, the relationship between δD and δ^{13}C of milkweed tissue and butterfly keratin and between wings and the water used to raise milkweed was investigated under controlled laboratory conditions using three batches of known δD water. Those captive studies showed extremely tight ($r^2 = 0.99$) relationships in each case demonstrating how insect wing keratin δD is derived exclusively from water available to plants with most of the isotopic discrimination occurring between water and plants (see also Ostrom *et al.* 1997). Wassenaar and Hobson (1998) then applied this basemap to portray origins of monarchs who were produced during 1996 and later collected from all known winter roost sites in Mexico that winter. That resulted in the insight that the winter roost sites were panmictic, made up of butterflies from all over the breeding range, and most importantly, revealed that half the population was produced largely in Kansas, Nebraska, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, and Ohio corresponding to the corn, soybean, and dairy producing region of the midwest. Thus, while conservation of this species was previously focused almost entirely on the precarious winter roosts in Mexico, these isotope studies pointed to the possibility that prime monarch breeding habitat was concentrated in areas of intense agricultural production in the United States where milkweed was controlled and where genetically modified corn was being used that produced BtK, a bacterium that targets Lepidoptera (Losey *et al.* 1999).

Here we revisit the original monarch dataset using modern GIS tools (see Chapter 4) compared to the original hand-kriged and interpolated results (Figure 3.4). Overall, we obtained a very similar finding to the original conclusions, but derived a more detailed picture of potential origins of monarchs that shows a somewhat wider potential band of origins (Figure 3.3). In this exercise, origins were assigned using georeferenced raster basemaps of expected δD and δ^{13}C surfaces interpolated from the original captive rearing work. Based on δD and δ^{13}C values from the wintering colonies in Mexico, 50% tolerance limits (TLs) at a 95% confidence level were calculated for each isotope separately (Walpole and Meyers 1993). Because assignment of origins is sensitive to the accuracy of the underlying surfaces, and/or individual variability at similar breeding locations, we calculated standard deviation of δD and δ^{13}C among individuals grown at the same location within the same rearing sites ($n = 140$ individuals at 33 sites). Average within-site standard deviation across all sites was 4.5‰ for δD and 0.3‰ for δ^{13}C values; these values were then used to extend the portrayed 50% TLs (*i.e.*, sensitivity limits) by subtracting these values from the lower TL and adding them to the upper TL. Origins were then portrayed for each isotope separately by reclassifying cells within the expected δD and δ^{13}C surfaces to values of 50% if they fell within the calculated TLs, and no data otherwise, using a spatial analyst reclassify operation within ArcGIS v 9.1 (ESRI, Redlands, California). In order to further constrain origins, the raster surfaces portraying 50% TLs (including sensitivity limits) for each isotope

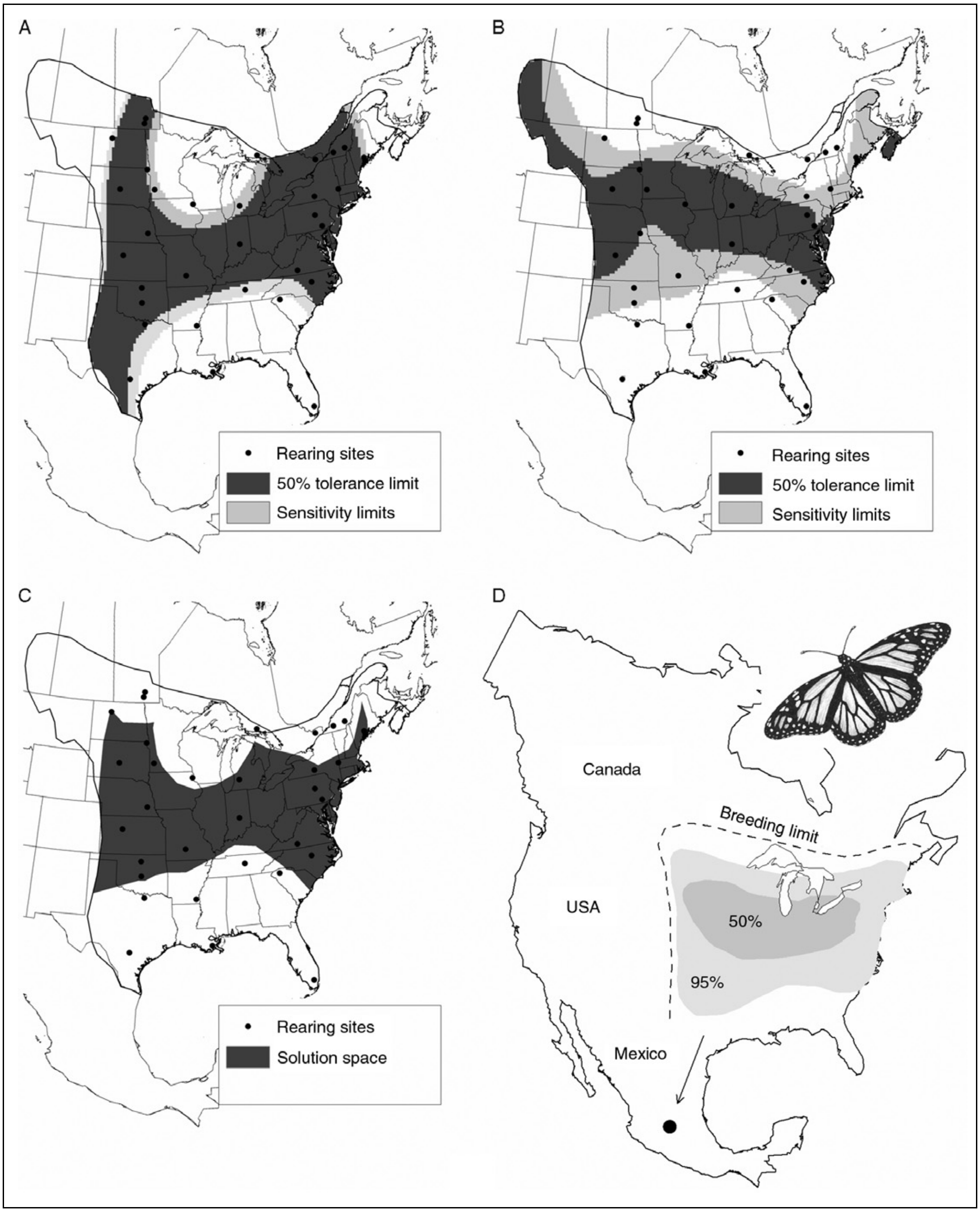

FIGURE 3.4 GIS-based natal origins of ~50% of Monarch butterflies overwintering population in Mexico using two stable isotope markers; (A) natal origins based on raster cells from an interpolated surface of δ^{13}C falling within the 50% tolerance limits ($\pm$0.29‰ (SD) to account for interindividual variability at sites); (B) natal origins estimated based on raster cells from an interpolated surface of δD falling within the 50% tolerance limits [$\pm$4.5‰ (SD)]; (C) the dual-isotope constrained estimate of natal origin for ~50% of the population using a GIS overlay of tolerance limits for both δ^{13}C and δD and incorporating both isotope's sensitivity limits. The results of panel C are therefore directly comparable to that of panel D; (D) the original hand-krigged and interpolated data of Wassenaar and Hobson (1998), using the original basemap calibration data of Hobson *et al.* 1999b.

were converted to vector format, and an ArcGIS intersect operation used to select only those portions of the two surfaces that overlapped. The way in which origins of populations are depicted using GIS tools is a work in progress but this exercise clearly shows the potential of this approach. Wunder and Norris (Chapter 5) further elaborate on Bayesian approaches that could be applied to deciphering origins of monarchs using our dataset.

The monarch isotope work continued several years later with an investigation into the source of butterflies occurring in Cuba in the autumn. There is a resident population of monarchs in Cuba, but researchers had noticed a seasonal increase in abundance. Stable isotope analyses were combined with cardenolide fingerprinting (related to the species of milkweed used and its geographical distribution) of monarchs and revealed that there was an influx of migrant monarchs to Cuba in November and that these individuals originated from southeastern Canada to the eastern United States (Dockx *et al.* 2004). So, it appears as though some monarchs make a “mistake” during their southward migration to Mexico and end up in Cuba. Recently, the origin of a single monarch that was found in Britain where they are considered an accidental vagrant was investigated. The mean wing keratin of this individual provided by Clive Farrell was −45 ± 2.5‰, clearly indicating that the butterfly was well outside the range of North American monarchs and was most likely from breeding populations off West Africa.

Notwithstanding the very clear conservation implications of the monarch isotope study, what made this such a powerful example of how to apply stable isotope methods to illuminate animal migration? Well, first, the monarch presents a very tight coupling between the animal of interest and its single genus of host plant. That clearly removed a source of variation in the isotope data for any regional population of monarchs. Second, the authors created a reference basemap using two stable isotopes that provided greater power to resolve origins of monarchs than could be obtained from either isotope used singly. Finally, the isotopic basemap applied exactly to the year of production of the cohort sampled on the wintering grounds and so removed variance associated with year effects or the use of long-term average precipitation datasets. In short, it was an organismal and year-specific basemap. Unfortunately, there will be very few opportunities for most of the other migratory animals of interest where we can control sources of variance and most studies will be forced to rely much more on inference based on long-term datasets such as the Global Network of Isotopes in Precipitation (GNIP). Having said that, a number of very important insights into origins of migratory animals have already been made using inferences based just on the pattern of deuterium in rainfall in North America and other continents, using the map lookup approach (Chapter 5).

Several recent applications using δD measurements have involved migratory birds in North America and have had a strong conservation motivation. Populations of loggerhead shrike have declined in North America and Hobson and Wassenaar (2001) and Perez and Hobson (2007) investigated the structure of wintering populations in the southern United States and northcentral Mexico. They were able to show that both Mexico and Florida were important wintering areas for northern breeding populations of this species. Because previous few band returns over the last 50 years pointed only to Texas, this work quickly identified new areas of potential concern. The Bicknell’s Thrush (*Catharus bickneli*) currently breeds in precariously small island habitats in the eastern United States and winters in the Caribbean. Based on a sample of birds wintering in the Dominican Republic, Hobson *et al.* (2004c) determined that a significant proportion of the population bred further north, likely in southern Quebec. Follow-up surveys indeed confirmed this. A similar study using both δD and δ^{13}C measurements of Black-throated Blue Warblers (*D. caerulescens*) on the wintering grounds revealed an important split in origins with those birds breeding in the south wintering in the eastern Antilles and those breeding in the north wintering in the west (Figure 3.3; Rubenstein *et al.* 2002). That southern populations seem to be declining compared with the northern populations suggests that deforestation patterns on the wintering grounds may be a factor. Other important advances, especially with migrant songbirds, have involved the delineation of catchment areas of constant-effort migration monitoring stations for a variety of species (Wassenaar and Hobson 2001, Mazerolle *et al.* 2005, Dunn *et al.* 2006).

By defining the approximate origins of birds caught during migration, analysis of population trends from these stations will be enhanced.

Another aspect of wildlife management concerns an understanding of where within the range of a species most of the young are produced. This is especially important for highly managed game species that are harvested during their fall migration and on the wintering grounds. Hobson *et al.* (2006) defined the origins of harvested Sandhill Cranes (*Grus canadensis*) through the central Flyway of North America using δD measurements of feathers and claws and identified the Hudson Bay lowlands and southern boreal forest as regions of highest productivity. Similarly, Hobson *et al.* (in review) examined origins of Lesser Scaup (*Aythya affinis*) taken by hunters during their autumn migration and compared isotopically inferred origins to *a priori* knowledge of the breeding densities of birds throughout most of their range. That exercise showed that more birds than expected were harvested from the central boreal and parkland region whereas fewer birds than expected were taken from the northern boreal. Such work has suggested that either there is a disproportional take of birds from the center of the species range and/or that the northern wetlands are producing fewer birds. The important point with both of these studies is that this type of information could not be obtained so readily using previous conventional mark-recapture techniques.

Other applications of the deuterium basemap for North America have been attempts to evaluate population structure and connectivity of migratory songbirds. By sampling birds across the wintering grounds, preliminary pictures are now emerging for Yellow Warbler (*Dendroica petechia*; Boulet *et al.* 2006), American Redstart (Norris *et al.* 2006), Henslow's Sparrow (Ibargüen 2004), loggerhead shrike (Chabot *et al.*, unpublished data), and Mountain Plover (Wunder 2007). These studies are primarily limited by the amount and geographical extent of winter captures. Kelly *et al.* (2002) also nicely demonstrated a "leapfrog" migration system in western populations of Wilson's Warbler (*Wilsonia pusilla*; Figure 3.5).

An interesting aspect of the behavior of δD is that deuterium in precipitation tends to rain out more at lower elevations than at higher elevations. This is a well-known phenomenon that results in an altitudinal "depletion" in δD from −1 to −4‰ per 100 m rise in elevation, depending on the gradient and temperature change (Clark and Fritz 1997). Similarly, the demands of plant adaptations to harsher

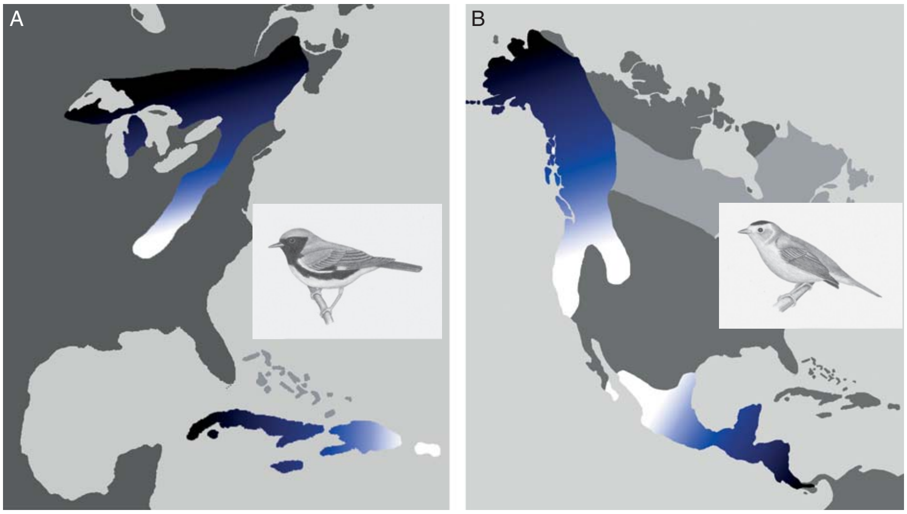

FIGURE 3.5 Migratory connectivity determined using stable isotope analyses of feathers based on the results of (A) Rubenstein *et al.* (2002) for the Black-throated Blue Warbler (*Dendroica caerulescens*) and (B) the leapfrog migration pattern of the Wilson's Warbler (*Wilsonia pusilla*) discovered by Kelly *et al.* (2002).

growing conditions at higher altitudes tend to result in plants with higher δ^{13}C values at higher elevations. As there are several species that perform altitudinal migrations, especially in the tropics, it was of interest to see if tissues grown along an altitudinal gradient reflected such patterns. Hobson *et al.* (2003) examined δD and δ^{13}C values of feathers of hummingbirds inhabiting the Ecuadorean Andes and found good agreement between actual and predicted feather δD based entirely on a global model (see Chapter 4). As expected, feather δ^{13}C values increased with altitude. Thus, for any given species, it should be possible to estimate approximate elevations at which feathers and other tissues were grown. By examining different tissues with different windows of isotopic integration, the possibility exists to infer previous altitudinal movements.

To date, primarily applications to the North American continent have been presented. That is understandable since the first studies using animal δD measurements were conducted there and the pattern of δD in precipitation follows a strong gradient with latitude. More recently, applications have been developed in Europe and Africa. The first depiction of the European growing-season basemap was presented by Hobson (2003) and since then more sophisticated treatments have been presented by Bowen *et al.* (2005). A first test of the relationship between feathers and predicted precipitation δD in Europe was presented in Hobson *et al.* (2004d) and that study revealed little difference between the relationships derived using growing season or annual precipitation δD. In addition, a poorer relationship was found with these regressions compared to similar work conducted in North America. Nevertheless, some valuable work has already been published based on the European picture.

Bearhop *et al.* (2005) used feather and claw δD measurements to distinguish between those Blackcaps (*Sylvia atricapilla*) breeding in Germany that had wintered in the United Kingdom and those that had wintered in Spain. The overwintering of this species in the United Kingdom is a relatively recent phenomenon encouraged by changes in climate and the provisioning of overwintering birds by feeders. The consequences of this “new” wintering population was that compared with the more traditional Spanish wintering birds, those wintering in Britain arrived in Germany earlier and tended to mate with others from the same wintering grounds. This “assortative mating” provides the conditions for speciation and was elegantly quantified using only δD measurements. Other investigations in Europe have inferred the origins of irruptive species to western Europe like Bullfinches (Newton *et al.* 2006) and, more recently, Crossbills (*Loxia curvirostra*; Marquiss *et al.* in press). The crossbill study was able to contrast origins of individuals from several eruptions to Scotland over a 100-year period using feathers from museum specimens. More recently, Lormee, Hobson, and Wassneaar (unpublished data) are investigating the origins of hunter-killed wood pigeons (*Columba palumbus*) in France using feather δD measurements. That study is designed to establish important baseline information to help manage the hunt of this species in Europe. Researchers at the University of Lund are also using δD measurements to investigate migration in two species of butterfly (*Vanessa atalanta*) occurring in Europe (O. Brattström and S. Åkesson, unpublished data).

The deuterium basemap for Africa shows rather dramatic changes seasonally. However, an interesting and potentially very useful feature is the more depleted values in the southern part of the continent and the extremely enriched region in the northeast, centered on Sudan and Ethiopia. In their investigation into potential wintering sites of the endangered aquatic warbler, Pain *et al.* (2004) did not find δD measurements to be particularly useful and instead advocated the use of δ^{15}N and δ^{13}C measurements to define wintering areas in Africa. On the other hand, Yohannes *et al.* (2005, 2007) has investigated δD together with δ^{15}N and δ^{13}C measurements in feathers of several migrant passerines moving through East Africa where some of them stop to molt en route to more southern wintering areas. In these studies, δD measurements were useful in segregating among locally molting birds and those that delayed their molt until reaching their final (southern) destinations. One of the challenges to applying the δD approach in Africa will be to determine which rainfall matters in terms of regional and local food webs and it may well be that depictions of mean annual precipitation δD isoscapes are less useful than those based on more seasonal periods.

While the vast majority of studies tracking migrant animals using the deuterium approach have been on birds and insects, Cryan *et al.* (2004) applied this technique to migratory bats and produced a regression between altitude-corrected mean growing season precipitation δD and δD of hair from known site of capture where bats presumably grew their hair. That study was based on museum specimens taken over a 97-year period but there was no evidence of effect of time on the regression. The reasonably good regression ($r^2 = 0.6$) suggests that while mammal hair is composed of α-keratin and bird feathers are made up of β-keratin, such structural differences have little effect on discrimination during hydrogen uptake ultimately from precipitation. Cryan noted that other sources of variation in the dataset may have been because of effects of heat stress (McKechnie *et al.* 2004), relative humidity (Cormie *et al.* 1994), sources of water to food webs other than precipitation, and possibly northward postmolt movements of bats away from their molt sites where they were subsequently captured.

D. The Deuterium Challenge

Despite the tremendous advance provided by the deuterium basemaps now available for several continents, there are a number of issues related to this isotope that need to be resolved before the field can advance further. Some of these issues undoubtedly relate to the fact that CFIRMS techniques have only recently become available for δD and to date, no official organic standards are available to help coordinate measurements and quality control among laboratories. While these issues can largely be resolved using comparative equilibration techniques discussed in Chapter 2, there are still laboratories that are not following this protocol. It must be stressed that reproducibility can be achieved routinely within ±2‰ for keratin material like feathers and hair. Also, by using reliable analytical techniques, researchers will not need to rely on running all of their samples in a narrow window of time to avoid effects of ambient water vapor in the laboratory and their measurements will be comparable among all time periods and all laboratories participating in this approach regardless of location. Such an approach would reduce the necessity of applying dubious "correction factors" among locations where samples were measured (*e.g.*, Norris *et al.* 2006).

Beyond issues related to measurement error, there is the issue of the appropriate precipitation to feather (or other keratin) δD discrimination factor that should applied to our data in order to create expected keratin δD surfaces or basemaps (for those cases where a species-specific basemap like that of Lott and Smith (2006) does not exist). For songbirds, a value of −25‰ has been suggested (Hobson 2005a). That value was based on measurements of ovenbird (*S. aurocapillus*) feathers grown by adult birds that returned to a study site over several years and insights provided by measurements of captive red-winged blackbirds. That value also seemed appropriate from a regression of feather δD against growing-season average precipitation δD for birds sampled across a large gradient in latitude in North America (Clark *et al.* 2006). Recently, Langin *et al.* (2007) provided additional support for this discrimination factor based on δD measurements of adult American Redstart feathers of known origin. However, such a discrimination factor is almost certainly not applicable to all birds in all situations (Table 3.1) and Wunder and Norris (Chapter 5) discuss in more detail the sources of variance in these regression relationships, including slope and y-intercept. In their analysis of songbird feathers from Europe, Hobson *et al.* (2004c) derived a different relationship between feathers and growing-season precipitation δD, albeit based on birds where origin of feather growth was only assumed and not proven. Also, in their extensive survey of raptors across North America, Lott and Smith (2006) determined that their estimate of precipitation to feather δD discrimination value was generally greater in magnitude than that derived for songbirds and showed evidence of geographical variation. Areas of North America showing the greatest departure (*i.e.*, the highest residuals for the regression between measured feather δD and precipitation δD) of raptor feather δD values from expected were along the Pacific coast of the continent and in the arid southwest region of the United States. Lott and Smith speculated that the coastal effect may have been due to departures of the predicted precipitation δD because of dynamic weather effects, not modeled well in this region. They also suggested that the deuterium enrichment in feathers found for the arid southwest region may have been due to the incorporation of CAM plants into the local food webs used by raptors. Another explanation is that this region of high residuals corresponds very well to that influenced by the North American monsoon, an area of northwestern Mexico and the southwest United States that often experiences significant late-summer rains, and it is quite possible that these differentially drive local food webs used by molting raptors and other species. This underlines the importance of considering regional "problematic" areas when using isoscape basemaps (Chapter 4) and also points to regions of North America that undoubtedly require more research and ground truthing.

In some ways it is not at all surprising that estimated tissue to precipitation discrimination factors for δD will vary and that the relationships measured so far rarely have a slope of unity. First, hydrogen is an element that will exchange with weak O–H or N–H bonds and this can take place with drinking water and overall body water (Hobson *et al.* 1999a). Second, we know that body δD values can increase as a result of heat stress (McKechnie *et al.* 2004) and presumably as a function of work or high metabolism that results in increased body evapotranspiration. Powell and Hobson (2006) found that Wood Thrush (*Hylocichla mustelina*) growing feathers in Georgia had higher feather δD values than expected from the feather δD basemap and speculated that heat stress during molt may have been a factor. Third, while we assume that trophic δD discrimination effects are minor and that most of the precipitation to tissue discrimination occurs between precipitation and plants, this has not yet been investigated experimentally (Birchall *et al.* 2005). Notably, it is indeed possible that feathers of birds grown in the nest may differ from those of the adults feeding them due to differences in metabolism, drinking water, thermal regime, and diet. Langin *et al.* (2007) found evidence that feathers of young redstarts indeed differed from those of adults.

An impressive number of studies have examined the relationship between animal tissue δD and the predicted precipitation δD values corresponding to real or assumed origins (Table 3.1). The amount of variance explained by these regressions range from 36% to 91% for cases not influenced by possible marine sources of hydrogen (Lott *et al.* 2003). However, it is also clear that there is considerable variation in the nature of these regressions even within groups like songbirds and for similar tissues (*i.e.*, feathers). Unfortunately, these relationships encompass all sources of error and it is currently not clear what is driving such differences. Several studies differ in the lab protocols used and the way in which they have dealt with equilibration through the use of various keratin standards (Chapter 2). It is also possible that the models used to estimate the expected mean growing-season average precipitation δD influence the results even though they are typically based on the same GNIP dataset. The range in regression relationships seen for three independent studies of the Wilson's Warbler in the western region of its range in North America is particularly interesting. Again, this topic receives more in-depth treatment in Chapter 5.

Another concern among would-be users of the deuterium basemaps for the various continents is the variability inherent in using a 40+-year average IAEA GNIP dataset for any given year where organisms are sampled (Chapter 4). As demonstrated with the monarch study, the only sure way to avoid this sort of variance is to create a basemap for the year of interest but this is clearly beyond the scope of most researchers for most organisms. Second, if a study site is close to one of the IAEA GNIP sampling stations, then it is possible for the researcher to potentially derive a year-specific tissue value for the site of interest but this would only provide local information most appropriate for discerning local from immigrant individuals (Hobson 2005a). More realistically, if researchers can obtain animal tissues from known individuals grown in the year of interest that could act as a reasonable proxy for local integrated isotope values.

The following chapters will address more specifically the nature of the available online products now available for researchers to estimate mean annual or mean growing-season precipitation δD values for their sites. Those products use an average monthly precipitation surface to weight contributions to long-term annual or growing-season precipitation δD. While this is entirely appropriate and these products have been of immense assistance, researchers need to be aware that changes in long-term weather patterns can result in departures of real average precipitation δD from those predicted from the model, a concern in these times of potentially rapid climate variation. Areas such as Saskatoon, Canada with its own GNIP sampling site, that experienced drought conditions in the last decade have shown differences between measured and assumed growing-season precipitation δD as much as 20–30‰.

Another issue involves our general poor understanding of which rainfall matters. The good relationship obtained between feather δD and mean annual growing season δD in North America (Hobson and Wassenaar 1997) was for *forest* birds distributed through the central region of the continent. Closed-canopy forest with shallow root systems may well integrate food web δD available to birds and other animals over such long time periods. However, is this the case for more pulsed ecosystems like grasslands, or deserts? In arid areas, rainfall driving local food webs can occur in just a month or two and different seasonal rains can drive different components of the food web as mentioned above with the North American monsoonal rains in southeast United States and northwest Mexico (Ehleringer *et al.* 1991). In other riparian systems, snowmelt may have the greatest influence on local food web δD. In other systems where animals may be influenced by aquatic emergent insects, tissues grown later in the season may be more enriched than those grown earlier if evapotranspiration enriches aquatic food web δD over the season. Much to our surprise, waterfowl feather δD values follow closely the expected growing-season average value for at least the temperate region of North America (Clark *et al.* 2006), despite the potential for this enrichment effect. All of these sources of variation require careful consideration.

There have been some interesting papers published over the last decade that have called into question the general applicability of using feather δD measurements to infer origins of migratory birds and it is worth considering a few of these carefully here. Wunder *et al.* (2005) provided a very useful contribution in their study of Mountain Plover (*Charadrius montanus*) chicks across their breeding range in western North America. They found that δD, δ^{13}C, and δ^{15}N measurements were poor predictors of latitude using probability-based models (see also Chapter 5). Of particular note was the high range of chick δD values for a given site. One can only speculate on the factors responsible for this. As noted above, growing chicks may be under a very different thermoregulatory and metabolic regime compared with adults and this may have consequences for their body δD values. In this regard, we may also find differences between chicks of precocial versus altricial species but that has not been investigated. Diet and the source of water to local food webs may have been highly variable in these habitats and range from snowmelt to precipitation. Of note also in this study (although not related necessarily to within-site isotopic variance) is the fact that latitude per se may not be the best metric to use in a part of the continent where isotopic δD contours are convoluted due to altitudinal effects. However, regressions involving feather δD versus predicted mean growing season δD for both the Mountain Plover dataset and the Wilson Warbler (*W. pusilla*) dataset of Kelly *et al.* (2002) actually show lower r^2 values compared with those using latitude (Wunder 2007, J. Kelly, pers. comm.).

Another paper using δD together with δ^{13}C and δ^{15}N, analyses involving shorebirds was that of Rocque *et al.* (2006) who examined feathers of American and Pacific Golden Plovers (*Pluvialis dominica* and *P. fulva*) and the Northern Wheatear (*Oenanthe oenanthe*). All species bred in Alaska where they were sampled and wintered in South America, the Pacific islands and Asia, and Africa, respectively. The authors expected winter-grown feathers to be much more enriched in deuterium compared with those grown in Alaska based on generalized patterns of δD in precipitation throughout the globe (Bowen *et al.* 2005). The authors also felt there was enough *a priori* information to expect that the isotope approach would successfully discriminate these birds to known groups based on their continent of molt. Wheatears behaved as expected with Alaska-grown feathers agreeing closely with that expected from the deuterium feather basemap. However, winter-grown wheatear feathers were not enriched in deuterium as expected and in fact were not different form the Alaska feathers. Variation among feathers of *P. dominica* was large, and those of *P. fulva*, much more enriched than expected. Wintering ground feathers of the plovers were more enriched than the breeding grounds but were also highly variable. The authors claim to be confident that the feathers were grown where they assumed and so what to make of these data? Like the Wunder *et al.* (2005) results, shorebirds seemed to show unexpectedly high isotopic variation within a single (100 km) location in Alaska. As nonstop migrants, often traveling thousands of kilometers in a single flight, these birds are uniquely adapted physiologically to turning themselves from feeding machines to flying machines with truly amazing adaptations of shifting tissue resources (including digestive tracts) (Piersma *et al.* 1999, Battley *et al.* 2000). Perhaps such adaptations influence variation in feather δD values. Shorebirds may also sample a broad range of terrestrially linked food items that show microgeographic isotopic variance. Clearly, we need to know more about this group using captive experiments. As for the Wheatears, is it possible they grew those winter feathers on the breeding grounds? If not, then is it also possible that they grew feathers in Africa based on high-lipid (and thus with depleted δD values) diets in anticipation of a nonstop trans-Atlantic flight? Finally, with the complex array of isoscapes available to birds on wintering grounds at *continental* scales, especially those for shorebirds that use everything from marine to high altitude terrestrial biomes and agricultural areas, is this study really a test of the stable isotope approach to migration studies? Alexander *et al.* (1996) found large isotopic (δ^{13}C and δ^{15}N) variability within a single stopover site used by migrating shorebirds in Saskatchewan, Canada.

In addition to shorebirds, another "problematic" group of birds from a deuterium isotope perspective are the raptors. Despite some early encouraging results from Meehan *et al.* (2001) in depicting origins of migrating Cooper's hawks (*Accipiter cooperii*) through southern Florida, more recent studies have pointed to some unexpected results. As mentioned, Lott and Smith (2006) have generated a raptor feather basemap for North America based on collections of feathers at nests and on museum samples. That product is certainly the most extensive available for any animal group and points to likely differences between passerines and raptors in the precipitation to feather δD discrimination factor. Meehan *et al.* (2003) in their contrast of nestling and adult Cooper's hawk feathers grown at known sites found a good agreement with expected for nestlings but a poorer fit for provisioning adults. While this may have been related to dietary differences, others have suggested that adults molt during the brood rearing phase and so may become more enriched in deuterium than expected due to this extra work phase (Smith and Dufty 2005). Most birds separate molt and regrowth of feathers from other energetically taxing periods like breeding and migration.

The potential linkage between feather δD values and factors related to workload and physiology is a fascinating one. In their much celebrated study on American Redstarts (resulting in an unprecedented *second* cover illustration of redstarts for Science!), Norris *et al.* (2004b) concluded that males investing in reproduction late in the breeding cycle faced a trade-off in subsequent timing of molt and migration and were forced to molt tail feathers during migration. Thus, individuals whose breeding schedule was known the year before and who tended to breed late had tail feathers more enriched in deuterium suggesting molt south of their breeding grounds. In addition, tail feathers corresponding to these more southern locations had fewer carotenoids present, an independent measure of stress during feather growth (Norris *et al.* 2007). The reason this paper was so important is the fact that it provided one of the first independent measures of a trade-off between reproduction and other life-history traits faced by a migratory organism. However, there are other possible interpretations that now require further investigation. First, birds were forced to breed late due to experimental manipulations and so these data were not based on entirely natural circumstances. Second and most important, the physiological stress encountered by late breeding, after several previous nesting attempts, may have simply elevated the body δD values such that feathers molted on the breeding ground were correspondingly enriched in deuterium compared with birds less taxed (see Norris *et al.* 2007). Perhaps, redstarts were "changed into raptors" in this respect.

In another study on redstarts at the same study site, Langin *et al.* (2007) examined the range of variation in redstart feather δD values for birds known to have attempted breeding at the site the year before and over a 4-year period. Those authors cautioned that the *range* in values they measured should be a warning to researchers interested in using the δD approach to depict origins of birds in

North America. However, their study stands out as a shining example of an almost exact fit between the mean feather isotope value of their entire population over 4 years and the *expected* value based on the application of a −25‰ discrimination factor between mean growing season precipitation δD for their site and feather δD. Moreover, of the 42 individuals sampled, half were within 2‰ and 80% were within 6‰ of the mean. This now begs the question of what was driving the few outliers and how should we deal with these sorts of questions. According to the paper by Norris *et al.* (2004b), some of those outliers could have been birds that molted further south of this breeding site. On the other hand, Langin *et al.* (2007) measured a large range in insect prey δD values at this riparian site, so it is equally possible that food δD may change seasonally here and birds growing feathers late sample a different isotopic food web than those growing feathers earlier. All of which is to say that we often require a "reality check" when considering the constraints we should place on our isotopic interpretations, especially when we do not know well what may really be going on in nature. Langin *et al.* (2007) placed a great deal of emphasis on the *range* of feather δD values they measured rather than emphasizing the tight nature of the distribution. This pertains to the risk of incorrectly assigning locations to *individuals* (see also Wunder *et al.* 2005, Rocque *et al.* 2006, Wunder 2007, Chapter 5). Another approach, where appropriate, is to consider defining origins of *populations*. It is precisely that approach that has been taken by authors who have used GIS tools to depict 50% or 75% TLs of origins of avian populations or other means of delineating origins of portions of the population (Wassenaar and Hobson 1998, DeLong *et al.* 2005, Hobson *et al.* 2006, 2007b, Lott and Smith 2006, Figure 3.2).

The paper by Norris *et al.* (2004b) underlines the need to know what life history factors can contribute to interpretation of tissue stable isotope values. For birds, molt patterns are reasonably well known for most species. However, stable isotope measurements themselves have provided important qualifiers. The molt of flight feathers of northern populations of the loggerhead shrike are essentially bimodal with inner primaries, secondaries, and tail feathers usually being molted on the breeding grounds but other feathers being grown on the wintering grounds following a suspension in molt, a pattern discovered accidentally using stable isotope measurements (Perez and Hobson 2006). Other birds undergo prealternate molt of some body feathers on the wintering grounds prior to migration allowing us to investigate aspects of winter origins or habitat use (Mehl *et al.* 2005, Mazerolle *et al.* 2005). Unfortunately, information on the reliability or extent of prealternate molt or on the extent of delayed molt in migrating birds is often not available (Hobson *et al.* 2000b). Another alternative is to use claws that are continuously growing. Birds captured soon after their arrival on the breeding grounds should have claws that have retained information from the wintering grounds (Bearhop *et al.* 2005, Mazerolle and Hobson 2005). While we need more controlled studies to establish growth rates of claws for a variety of species, contrasting stable isotope values of claws against a metabolically active tissue like blood can in fact provide insight into periods where these tissues "agree" isotopically.

V. SUMMARY

This chapter started with the admission that situations where all three principles of isotopic tracking of migratory animals are met will be rare. The degree to which researchers are successful in applying isotopic methods will depend very much on the organism of interest, its geographical range, and ecophysiology. Such applications also fundamentally depend on how well we know the nature and behavior of the appropriate isoscapes. The most elegant applications will usually be situations where alternative isoscapes are very different and species experience simple isotopically dichotomous situations during their travels. Here, the long-standing success in using stable carbon isotope analyses to delineate C3 versus C4 or CAM food webs or the use of stable hydrogen isotope analyses to further separate C4 and CAM pathways provide distinct advantages. Terrestrial organisms that also spend part of their lives in marine or estuarine situations definitely lend themselves to isotopic tracking using several elements. Altitudinal migrants constrained by latitude and longitude also represent a useful application of δD and δ^{18}O measurements providing the movement represents several hundred meters. We will have more trouble in cases where underlying isotopic gradients are less distinct or where alternative origins overlap isotopically.

The application of deuterium measurements in animal tissues to place them on continental basemaps undoubtedly has provided the single greatest impetus in this field of isotopic tracking. Again, the success of this approach will depend very much on which part of the basemap we are dealing with. Distinguishing between arctic and prairie origins of migratory birds in North America or between those from Scandinavia or Spain in Europe will be relatively straightforward. We are faced with more of a challenge in distinguishing between birds or other animals originating across latitudinal bands on both continents or from regions that are more spatially restricted (*e.g.*, Szymanski *et al.* 2006). So, while this chapter has shown that the isotope approach has provided an extremely exciting and powerful boon to researchers and conservationists, it is not a "silver bullet" to be applied without full recognition of the limitations. How then might the field proceed from here? The next two chapters will deal explicitly with the very dynamic fields of isoscape mapping and statistical inference as they relate to placing organisms to origin and it is these areas that very much deal with key quantitative constraints we face. We now realize that the application of a single precipitation to tissue δD discrimination factor across diverse species or particular geographic regions will inherently involve error and sensitivity analyses should be part of our approach in future studies (*e.g.*, Figure 3.4; Hobson *et al.* 2007b). In addition, we should realize the risks in placing all of our eggs in one isotopic basket. For example, several researchers have used three or four isotopes (δD, δ^{34}S, δ^{13}C, δ^{15}N, δ^{87}Sr) in a multivariate approach to examine origins of migratory populations (Caccamise *et al.* 2000, Hebert and Wassenaar 2005, Yohannes *et al.* 2007) but more isotopes is not necessarily a guarantee of greater spatial resolution. The particularly elegant study of migratory raptors by Lott *et al.* (2003) is also instructive. Those authors nicely separated coastal migratory raptors having access to marine-based resources from inland species using δ^{34}S measurements of feathers. This approach will theoretically allow the subsequent identification of those migrant birds where δD measurements could be used to approximate origins using the appropriate terrestrial δD isoscape (Table 3.1, and Figure 3.6).

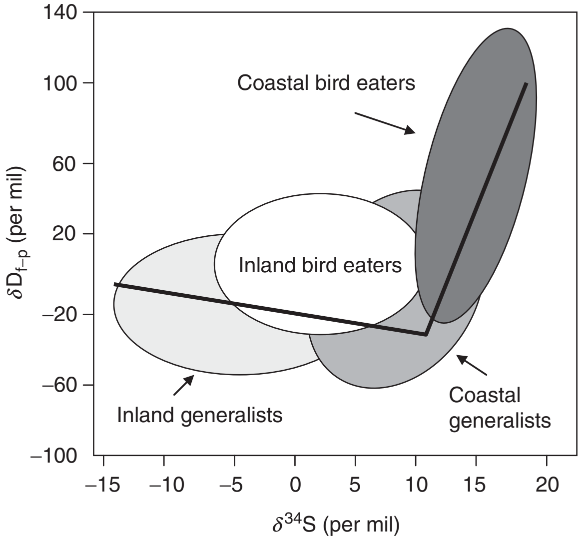

FIGURE 3.6 The relationship between the difference between feather δD and predicted precipitation δD (δD_{f-p}) and feather δ^{34}S for nine species of raptors breeding in North America (from Lott *et al.* 2003). This figure illustrates the way in which birds having access to marine protein can be distinguished by their enriched δD and δ^{34}S values.

By this point, the reader should be encouraged by the breadth of past isotopic applications to tracking migratory animals and realize the tremendous scope for future developments. The need for caution and consideration of the numerous assumptions involved will be sobering (Chapter 5). Nonetheless, more and more we are coming to terms with the nature of isotopic variance in the natural world and Kelly *et al.* (Chapter 6) provide direction on the path ahead.

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VII. REFERENCES

Alexander, S. A., K. A. Hobson, C. L. Gratto-Trevor, and A. W. Diamond. 1996. Conventional and isotopic determinations of shorebird diets at an inland stopover: The importance of invertebrates and *Potamogeton pectinatus* tubers. *Canadian Journal of Zoology* **74:**1057–1068.

Alisauskas, R. T., E. E. Klaas, K. A. Hobson, and C. D. Ankney. 1998. Stable-carbon isotopes support use of adventitious color to discern winter origins of lesser snow geese. *Journal of Field Ornithology* **69:**262–268.

Ambrose, S. H., and M. J. DeNiro. 1986. The isotopic ecology of East African mammals. *Oecologia* **69:**395–406.

Atkinson, P. W., A. J. Baker, R. M. Bevan, N. A. Clark, K. B. Cole, P. M. Gonzalez, J. Newton, L. J. Niles, and R. A. Robinson. 2005. Unravelling the migration and moult strategies of long-distance migrant using stable isotopes: Red Knot *Calidris canutus* movements in the Americas. *Ibis* **147:**738–749.

Battley, P. F., T. Piersma, M. W. Dietz, S. Tang, A. Dekinga, and K. Hulsman. 2000. Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proceedings of the Royal society of London, Series B* **267:**191–195.

Bearhop, S., D. R. Thompson, S. Waldron, I. C. Russell, G. Alexander, and R. W. Furness. 1999. Stable isotopes indicate the extent of freshwater feeding by cormorants *Phalacrocorax carbo* from inland fisheries in England. *Journal of Applied Ecology* **36:**75–84.

Bearhop, S., S. Waldron, S. C. Votier, and R. W. Furness. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* **75:**451–458.

Bearhop, S., R. W. Furness, G. M. Hilton, S. C. Votier, and S. Waldron. 2003. A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. *Functional Ecology* **17:**270–275.

Bearhop, S., G. M. Hilton, S. C. Votier, and S. Waldron. 2004. Stable isotope ratios indicate that body condition in migrating passerines is influenced by winter habitat. *Proceedings of the Royal Society of London (Series B)* **271:**215–218.

Bearhop, S., W. Fiedler, R. W. Furness, S. C. Votier, S. Newton, J. Waldron, G. Bowen, P. Berthold, and K. Farnsworth. 2005. Assortative mating as a mechanism for rapid evolution of a migratory divide. *Science* **310:**502–504.

Bensch, S., G. Bengtsson, and S. Åkesson. 2006. Patterns of stable isotope signatures in willow warbler *Phylloscopus trochilus* feathers collected in Africa. *Journal of Avian Biology* **37:**323–330.

Best, P. B., and D. M. Schell. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. *Marine Biology* **124:**483–494.

Birchall, J., T. C. O'Connel, T. H. E. Heaton, and R. E. M. Hedges. 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. *Journal of Animal Ecology* **74:**877–881.

Bosley, K. L., D. A. Witting, R. C. Chambers, and S. C. Wainright. 2002. Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Psuedopleuronectes americana* with stable isotopes. *Marine Ecology Progress Series* **236:**233–240.

Boulet, M., H. L. Gibbs, and K. A. Hobson. 2006. Integrated analysis of genetic, stable isotope, and banding data reveal migratory connectivity and flyways in the northern Yellow Warbler (*Dendroica petechia*; *Aestiva* group). *Ornithological Monographs* **61:**29–78.

Bowen, G. J., L. I. Wassenaar, and K. A. Hobson. 2005. Application of stable hydrogen and oxygen isotopes to wildlife forensic investigations at global scales. *Oecologia* **143:**337–348.

Caccamise, D. F., L. M. Reed, P. M. Casteli, S. Wainsight, and T. C. Nichols. 2000. Distinguishing migratory and resident Canada geese using stable isotope analysis. *Journal of Wildlife Management* **64:**1084–1091.

Carleton, S. A., and C. Martínez del Rio. 2005. The effect of cold-induced increased metabolic rate on the ratio of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). *Oecologia* **144:**226–232.

Cerling, T. E., G. Bowen, J. R. Ehleringer, and M. Sponheimer. 2007. The reaction progress variable and isotope turnover in biological systems. Pages 163–171 *in* T. E. Dawson and R. T. W. Siegwolf (Eds.) *Stable Isotopes as Indicators of Ecological Change*. Academic Press, London.

Chamberlain, C. P., J. D. Blum, R. T. Holmes, X. Feng, T. W. Sherry, and G. R. Graves. 1997. The use of isotope tracers for identifying populations of migratory birds. *Oecologia* **109:**132–141.

Chamberlain, C. P., S. Bensch, X. Feng, S. Akesson, and T. Andersson. 2000. Stable isotopes examined across a migratory divide in Scandinavian willow Warblers (*Phylloscopus trochilus trochilus* and *Phylloscopus trochilus acredula*) reflect their African winter quarters. *Proceedings of the Royal Society (Series B)* **267:**43–48.

Clark, I. D., and P. Fritz. 1997. *Environmental Isotopes in Hydrogeology*. Lewis Publishers, New York.

Clark, R. G., K. A. Hobson, and L. I. Wassenaar. 2006. Geographic variation in the isotopic (δD, δ^{13}C, δ^{15}N, δ^{34}S) composition of feathers and claws from lesser scaup and northern pintail: Implications for studies of migratory connectivity. *Canadian Journal of Zoology* **84:**1395–1401.

Cormie, A. B., H. P. Schawarcz, and J. Gray. 1994. Relationship between the hydrogen and oxygen isotopes of deer bone and their use in the estimation of relative humidity. *Geochimica et Cosmochimica Acta* **60:**4161–4166.

Cryan, P. M., M. A. Bogan, R. O. Rye, G. P. Landis, and C. L. Kester. 2004. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *Journal of Mammalogy* **85:**995–1001.

Dalerum, F., and A. Angerbjörn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* **144:**647–658.

DeLong, J. P., T. D. Meehan, and R. B. Smith. 2005. Investigating fall movements of hatch-year Flammulated Owls (*Ottus flammeolus*) in central New Mexico using stable hydrogen isotopes. *Journal of Raptor Research* **39:**19–25.

Dockx, C., L. P. Brower, L. I. Wassenaar, and K. A. Hobson. 2004. Do North American monarch butterflies migrate to Cuba? Insights from combined isotope and chemical tracer techniques. *Ecological Applications* **14:**1106–1114.

Doucett, R. R., J. C. Marks, D. W. Blinn, M. Caron, and B. A. Hungate. 2007. Measuring terrestrial subsidies to aquatic foodwebs using stable-isotopes of hydrogen. *Ecology* **88:**1587–1592.

Drent, R. D. 2007. The timing of bird's breeding seasons: The Perrin's hypothesis revisited especially for migrants. *Ardea* **94:**305–322.

Dunn, E. H., K. A. Hobson, L. I. Wassenaar, D. Hussell, and M. L. Allen. 2006. Identification of summer origins of songbirds migrating through southern Canada in Autumn. *Avian Conservation and Ecology* **1:**4 http://www.ace-eco.org/vol1/iss2/art4/.

Ehleringer, J. R., S. L. Phillips, W. S. F. Schuster, and D. R. Sandquist. 1991. Differential utilization of summer rains by desert plants. *Oecologia* **88:**430–434.

Evans-Ogden, L. J., K. A. Hobson, and D. B. Lank. 2004. Blood isotopic (δ^{13}C and δ^{15}N) turnover and diet-tissue fractionation factors in captive Dunlin. *Auk* **121:**170–177.

Flemming, T. H., R. A. Nunez, and L. S. L. Sternberg. 1993. Seasonal changes in the diets of migrant and non-migrant necatrivorous bats as revealed by carbon stable isotope analysis. *Oecologia* **94:**72–75.

Gauthier, G., J. Bêty, and K. A. Hobson. 2003. Are greater snow geese capital breeders? New evidence from a stable isotope model. *Ecology* **84:**3250–3264.

Gómez-Diaz, E., and J. González-Solis. 2007. Geographic assignment of seabirds to their origin: Combining morphologic, genetic, and biogeochemical analyses. *Ecological Applications* **17:**1484–1498.

Gröcke, D. R., A. Schimmelmann, S. Elias, and R. F. Miller. 2006. Stable-hydrogen isotope ratios in beetle chitin: Prelimiary European data and reinterpretation of North American data. *Quaternary Science Reviews* **25:**1850–1864.

Haramis, G. M., D. G. Jorde, S. A. Macko, and J. L. Walker. 2001. Stable isotope analysis of canvasback winter diet in Upper Chesapeake Bay. *Auk* **118:**1008–1017.

Harrington, R. R., B. P. Kennedy, C. P. Chamberlain, J. D. Blum, and C. L. Folt. 1998. ^{15}N enrichment in agricultural catchments: Field patterns and applications to tracking Atlantic salmon (*Salmo salar*). *Chemical Geology* **147:**281–294.

Heaton, T. H. E. 1987. The ^{15}N/^{14}N ratios of plants in South Africa and Namibia: Relationship to climate and coastal/saline environments. *Oecologia* **74:**236–246.

Hebert, C., and L. I. Wassenaar. 2001. Stable nitrogen isotopes in waterfowl feathers reflect agricultural land use in western Canada. *Environmental Science and Technology* **35:**3482–3487.

Hebert, C., and L. I. Wassenaar. 2005. Feather stable isotopes in western North American waterfowl: Spatial patterns, underlying factors, and management implications. *Wildlife Society Bulletin* **33:**92–102.

Hesslein, R. H., M. J. Capel, D. E. Fox, and K. A. Hallard. 1991. Stable isotopes of sulphur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. *Canadian Journal of Fisheries and Aquatic Science* **48:**2258–2265.

Hesslein, R. H., K. A. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by δ^{34}S, δ^{13}C, and δ^{15}N. *Canadian Journal of Fisheries and Aquatic Science* **50:**2071–2076.

Hobson, K. A. 1987. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. *Canadian Journal of Zoology* **65:**1210–1213.

Hobson, K. A. 1995. Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: Patterns of isotopic fractionation and turnover. *Condor* **97:**752–762.

Hobson, K. A. 1999a. Tracing origins and migration of wildlife using stable isotopes: A review. *Oecologia* **120:**314–326.

Hobson, K. A. 1999b. Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: Implications for evaluating trophic relationships and breeding origins. *Condor* **101:**799–805.

Hobson, K. A. 2003. Making migratory connections with stable isotopes. Pages 379–391 *in* P. Berthold, E. Gwinner, and E. Sonnenschein (Eds.) *Avian Migration*. Springer-Verlag, Berlin Heidelberg, New York.

Hobson, K. A. 2005a. Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. *Auk* **122:**1037–1048.

Hobson, K. A. 2005b. Using stable isotopes to trace long-distance dispersal in birds and other taxa. *Diversity and Distributions* **11:**157–164.

Hobson, K. A. 2006. Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* **94:**359–369.

Hobson, K. A. 2007. An isotopic exploration of the potential of avian tissues to track changes in terrestrial and marine ecosystems. Pages 129–144 *in* T. E. Dawson and R. T. W. Siegwolf (Eds.) *Stable Isotopes as Indicators of Ecological Change.* Academic Press, London.

Hobson, K. A., and E. Yohannes. 2007. Establishing elemental turnover in exercising birds using a wind tunnel: Implications for stable isotope tracking of migrants. *Canadian Journal of Zoology* **85:**703–708.

Hobson, K. A., and F. Bairlein. 2003. Isotopic discrimination and turnover in captive Garden Warblers (*Sylvia borin*): Implications for delineating dietary and migratory associations in wild passerines. *Canadian Journal of Zoology* **81:**1630–1635.

Hobson, K. A., and L. I. Wassenaar. 1997. Linking breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* **109:**142–148.

Hobson, K. A., and L. I. Wassenaar. 2001. A stable isotope approach to delineating population structure in migratory wildlife in North America: An example using the loggerhead shrike. *Ecological Applications* **11:**1545–1553.

Hobson, K. A., and R. W. Clark. 1992. Assessing avian diets using stable isotopes. I: Turnover of carbon-13 in tissues. *Condor* **94:**181–188.

Hobson, K. A., R. T. Alisauskas, and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analysis of diet. *Condor* **95:**388–394.

Hobson, K. A., K. D. Hughes, and P. J. Ewins. 1997. Using stable-isotope analysis to identify endogenous and exogenous sources of nutrients in eggs of migratory birds: Applications to Great Lakes contaminants research. *Auk* **114:**467–478.

Hobson, K. A., L. Atwell, and L. I. Wassenaar. 1999a. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Science* **96:**8003–8006.

Hobson, K. A., L. I. Wassenaar, and O. R. Taylor. 1999b. Stable isotopes (δD and δ^{13}C) are geographic indicators of natal origins of Monarch butterflies in eastern North America. *Oecologia* **120:**397–404.

Hobson, K. A., J. Sirois, and M. L. Gloutney. 2000a. Tracing nutrient allocations to reproduction using stable-isotopes: A preliminary investigation using the colonial waterbirds of Great Slave Lake. *Auk* **117:**760–774.

Hobson, K. A., R. B. Brua, W. L. Hohman, and L. I. Wassenaar. 2000b. Low frequency of "double molt" of remiges in ruddy ducks revealed by stable isotopes: Implications for tracking migratory waterfowl. *Auk* **117:**129–135.

Hobson, K. A., K. P. McFarland, L. I. Wassenaar, C. C. Rimmer, and J. E. Goetz. 2001. Linking breeding and wintering grounds of Bicknell's thrushes using stable isotope analyses of feathers. *Auk* **118:**16–23.

Hobson, K. A., L. I. Wassenaar, B. Milá, I. Lovette, C. Dingle, and T. B. Smith. 2003. Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. *Oecologia* **136:**302–308.

Hobson, K. A., L. I. Wassenaar, and E. Bayne. 2004a. Using isotopic variance to detect long-distance dispersal and philopatry in birds: An example with ovenbirds and American redstarts. *Condor* **106:**732–743.

Hobson, K. A., L. Atwell, L. I. Wassenaar, and T. Yerkes. 2004b. Estimating endogenous nutrient allocations to reproduction in redhead ducks: A dual isotope approach using δD and δ^{13}C measurements of female and egg tissues. *Functional Ecology* **18:**737–745.

Hobson, K. A., Y. Aubry, and L. I. Wassenaar. 2004c. Migratory connectivity in Bicknell's Thrush: Locating the missing populations using hydrogen isotopes. *Condor* **106:**905–909.

Hobson, K. A., G. Bowen, L. I. Wassenaar, Y. Ferrand, and H. Lormee. 2004d. Using stable hydrogen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia* **141:**477–488.

Hobson, K. A., J. E. Thompson, M. Evans, and S. Boyd. 2005. Tracing nutrient allocation to reproduction in Barrow's Goldeneye. *Journal of Wildlife Management* **69:**1221–1228.

Hobson, K. A., S. Van Wilgenburg, L. I. Wassenaar, H. Hands, W. Johnson, M. O'Melia, and P. Taylor. 2006. Using stable-hydrogen isotopes to delineate origins of Sandhill Cranes harvested in the Central Flyway of North America. *Waterbirds* **29:**137–147.

Hobson, K. A., R. J. F. Smith, and P. Sorensen. 2007a. Applications of stable isotope analysis to tracing nutrient sources to Hawaiian gobioid fish and other stream organisms. *Bishop Museum Bulletin in Cultural and Environmental Studies* **3:**99–111.

Hobson, K. A., S. Van Wilgenburg, L. I. Wassenaar, F. Moore, and J. Farrington. 2007b. Estimating origins of three species of Neotropical migrants at a Gulf coast stopover site: Combining stable isotope and GIS tools. *Condor* **109:**256–267.

Hobson, K. A., R. G. Clark, L. I. Wassenaar, and S. Van Wilgenburg. Natal origins of harvested Lesser Scaup (*Athya affinis*) in North America: Making connections to source populations with deuterium analyses of feathers. *Ecological Applications*, in review.

Ibargüen, S. B. 2004. *Population Connectivity: Combining Methods for Estimating Avian Dispersal and Migratory Linkages.* Ph.D. dissertation, Ohio State University. [Online.] Available at http://www.ohiolink.edu.proxy.lib.ohio-state.edu/etd/view.cgi?acc_num=osu1079979416.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* **78:**1–27.

Kelly, J. F., V. Atudorei, Z. D. Sharp, and D. M. Finch. 2002. Insights into Wilson's Warbler migration from analyses of hydrogen stable-isotope ratios. *Oecologia* **130:**216–221.

Kennedy, B. P., C. L. Folt, J. D. Blum, and C. P. Chamberlain. 1997. Natural isotope markers in salmon. *Nature* **387:**766.

Killingly, J. S. 1980. Migrations of California gray whales tracked by oxyen-18 variations in their epizoic barnacles. *Science* **207:**759–760.

Killingly, J. S., and M. Lutcavage. 1983. Loggerhead turtle movements reconstructed from 18O and 13C profiles from commensal barnacle shells. *Estuarine and Coastal Shelf Science* **16:**345–349.

Klaassen, M., A. Lindstrom, H. Meltofte, and T. Piersma. 2001. Arctic waders are not capital breeders. *Nature* **413:**794.

Koch, P. L., J. Heisinger, C. Moss, R. W. Carlson, M. L. Fogel, and A. K. Behrensmeyer. 1995. Isotopic tracking of change in diet and habitat use in African elephants. *Science* **267:**1340–1343.

Krouse, H. R., J. W. B. Stewart, and V. A. Grinenko. 1991. Pedospehere and biospehere. Pages 267–306 *in* H. R. Krouse and V. A. Grinenko (Eds.) *Stable Isotopes: Natural and Anthropogenic Sulphur in the Environment.* John Wiley and Sons, Toronto.

Langin, K. M., M. W. Reudink, P. R. Marra, D. R. Norris, T. K. Kyser, and L. M. Ratcliffe. 2007. Hydrogen isotopic variation in migratory bird tissues of known origin: Implications for geographic assignment. *Oecologia* **152:**449–457.

Losey, J. E., L. S. Rayor, and M. E. Carter. 1999. Transgenic pollen harms monarch larvae. *Nature* **399:**214–214.

Lott, C. A., and J. P. Smith. 2006. A GIS approach to estimating the origins of migratory raptors in North America using hydrogen stable isotope ratios in feathers. *Auk* **118:**16–23.

Lott, C. A., T. D. Meehan, and J. A. Heath. 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: Influence of marine prey base. *Oecologia* **134:**505–510.

Marra, P. P., K. A. Hobson, and R. T. Holmes. 1998. Linking winter and summer events in a migratory bird using stable carbon isotopes. *Science* **282:**1884–1886.

Marquiss, M., K. A. Hobson, and I. Newton, Stable isotope evidence for regionalised provenances of Common Crossbill *Loxia curvirostra* invasions into Western Europe. *Journal of Avian Biology*, in press.

Mazerolle, D., and K. A. Hobson. 2005. Estimating origins of short-distance migrant songbirds in North America: Contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *Condor* **107:**280–288.

Mazerolle, D., K. A. Hobson, and L. I. Wassenaar. 2005. Stable isotope and band-encounter analyses delineate migratory patterns and catchment areas of white-throated sparrows at a migration monitoring station. *Oecologia* **144:**541–549.

McKechnie, A. E., B. O. Wolf, and C. Martinez del Rio. 2004. Deuterium stable isotope ratios as tracers of water resource use: An experimental test with rock dove. *Oecologia* **140:**191–200.

Meehan, T. D., C. A. Lott, Z. D. Sharp, R. B. Smith, R. N. Rosenfield, A. C. Stewart, and R. K. Murphy. 2001. Using hydrogen isotope geochemistry to estimate the natal latitudes of immature cooper's hawks migrating through the Florida Keys. *Condor* **103:**11–20.

Meehan, T. D., R. N. Rosenfield, V. N. Atudorei, J. Bielefeldt, L. J. Rosenfield, A. C. Stewart, W. E. Stout, and M. A. Bozek. 2003. Variation in hydrogen stable-isotope ratios between adult and nestling Cooper's hawk. *Condor* **105:**567–572.

Meehan, T. D., J. T. Giermakowski, and P. M. Cryan. 2004. A GIS-based model of stable hydrogen isotope ratios in North American growing-season precipitation for use in animal movement studies. *Isotopes in Environmental and Health Studies* **40:**291–300.

Mehl, K. R., R. T. Alisauskas, K. A. Hobson, and F. R. Merkel. 2005. Linking breeding and wintering grounds of king eiders: Making use of polar isotopic gradients. *Journal of Wildlife Management* **69:**1297–1304.

Meyer-Rochow, V. B., I. Cook, and H. Hendy. 1992. How to obtain clues from the otoliths of an adult fish about the aquatic environment it has been in as a larvae. *Comparative Biochemistry and Physiology* **103A:**333–335.

Mizutani, H., M. Fukuda, Y. Kabaya, and E. Wada. 1990. Carbon isotope ratio of feathers reveals feeding behavior of cormorants. *Auk* **107:**400–403.

Møller, A. P., and K. A. Hobson. 2004. Heterogeneity in stable isotope profiles predicts coexistence of two populations of barn swallows *Hirundo rustica* differing in morphology and reproductive performance. *Proceedings of the Royal Society of London* **271:**1355–1362.

Møller, A. P., K. A. Hobson, T. A. Mousseau, and A. M. Peklo. 2006. Chernobyl as a population sink for barn swallows: Tracking dispersal using stable isotope profiles. *Ecological Applications* **16:**1696–1705.

Morrison, R. I. G., and K. A. Hobson. 2004. Use of body stores in shorebirds after arrival on High Arctic breeding grounds. *Auk* **121:**333–344.

Nelson, C. S., T. G. Northcote, and C. H. Hendy. 1989. Potential use of oxygen and carbon isotopic composition of otoliths to identify migratory and non-migratory stocks of the New Zealand common smelt: A pilot study. *New Zealand Journal of Marine and Freshwater Research* **23:**337–344.

Newton, I., K. A. Hobson, A. D. Fox, and M. Marquiss. 2006. An investigation into the provenance of Northern Bullfinches *Pyrrhula p. pyrrhula* found in winter in Scotland and Denmark. *Journal of Avian Biology* **37:**431–435.

Norris, D. R., P. R. Marra, T. K. Kyser, T. W. Sherry, and L. M. Ratcliffe. 2004a. Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society of London (Series B)* **271:**59–64.

Norris, D. R., P. R. Marra, R. Montgomerie, T. K. Kyser, and L. M. Ratcliffe. 2004b. Reproductive effort, molting latitude, and feather color in a migratory songbird. *Science* **306:**2249–2250.

Norris, D. R., P. R. Marra, T. K. Kyser, and L. M. Ratcliffe. 2005. Tracking habitat use in a long distance migratory bird, the American Redstart *Setophaga ruticilla*, using stable isotope ratios in cellular blood. *Journal of Avian Biology* **36:**164–170.

Norris, D. R., P. R. Marra, G. J. Bowen, L. M. Ratcliffe, J. A. Royle, and T. K. Kyser. 2006. Migratory connectivity of a widely distributed songbird, the American Redstart (*Setophaga ruticilla*). *Ornithological Monographs* **61:**14–28.

Norris, D. R., P. R. Marra, K. T. Kyser, L. M. Ratcliffe, and R. Montgomerie. 2007. Continent wide variation in feather color of a migratory songbird in relation to body condition and molting locality. *Biology Letters* **3:**16–19.

Ostrom, P. H., M. Colunga-Garcia, and S. H. Gage. 1997. Establishing pathways of energy flow for insect predators using stable isotope ratios: Field and laboratory evidence. *Oecologia* **109:**108–113.

Pain, D. J., R. E. Green, B. Giessing, A. Kozulin, A. Poluda, U. Ottosson, M. Flade, and G. M. Hilton. 2004. Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler *Acrocephalus paludicola*. *Oecologia* **138:**168–174.

Paxton, K. L., C. Van Riper III, T. C. Theimer, and E. H. Paxton. 2007. Spatial and temporal migration patterns of Wilson's Warbler (*Wilsonia pusilla*) in the southwest as revealed by stable isotopes. *Auk* **124:**162–175.

Pearson, S. F., D. J. Levey, C. H. Greenberg, and C. Martinez del Rio. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* **135:**516–523.

Perez, G., and K. A. Hobson. 2006. Isotopic evaluation of interrupted molt of northern breeding populations of the loggerhead shrike. *Condor* **108:**877–886.

Perez, G., and K. A. Hobson. 2007. Feather deuterium measurements reveal origins of migratory western Loggerhead Shrikes (*Lanius ludovicianus excubitorides*) wintering in Mexico. *Diversity and Distributions* **13:**166–171.

Phillips, D. L., and P. M. Eldridge. 2006. Estimating the timing of diet shifts using stable isotopes. *Oecologia* **147:**195–203.

Piersma, T., G. A. Gudmundsson, and K. Lilliendahl. 1999. Rapid changes in the size of different functional organ and muscle groups during refuelling and a long-distance migrating shorebird. *Physiological and Biochemical Zoology* **72:**405–415.

Podlesak, D. W., S. R. McWilliams, and K. A. Hatch. 2005. Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. *Oecologia* **142:**501–510.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83:**703–718.

Powell, L. A., and K. A. Hobson. 2006. Enriched feather hydrogen isotope values for Wood Thrushes sampled in Georgia, USA, during the breeding season: Implications for quantifying dispersal. *Canadian Journal of Zoology* **84:**1331–1338.

Robbins, C. T., L. A. Felicetti, and M. Sponheimer. 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* **144:**534–540.

Rocque, D. A., M. Ben-David, R. P. Barry, and K. Winker. 2006. Assigning birds to wintering and breeding grounds using stable isotopes: Lessons from two feather generations among three intercontinental migrants. *Journal of Ornithology* **147:**395–404.

Rubenstein, D. R., and K. A. Hobson. 2004. From birds to butterflies: Animal movement patterns and stable isotopes. *Trends in Ecology and Evolution* **19:**256–263.

Rubenstein, D. R., C. P. Chamberlain, R. T. Holmes, M. P. Ayres, J. R. Waldbauer, G. R. Graves, and N. C. Tuross. 2002. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science* **295:**1062–1065.

Schell, D. M., S. M. Saupe, and N. Haubenstock. 1989. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by δ^{13}C techniques. *Marine Biology* **103:**433–443.

Sealy, J. C., N. J. van der Merwe, J. A. Lee Thorp, and J. L. Lanham. 1987. Nitrogen isotopic ecology in southern Africa: Implications for environmental and dietary tracing. *Geochimica et Cosmochimica Acta* **51:**2707–2717.

Sheppard, S. M. F., R. L. Neilsen, and H. P. Taylor. 1969. Oxygen and hydrogen isotope ratios of clay minerals from porphyry copper deposits. *Economic Geology* **64:**755–777.

Smith, A. D., and A. M. Dufty. 2005. Variation in the stable-hydrogen isotope composition of Northern Goshawk feathers: Relevance to the study of migratory origins. *Condor* **107:**547–558.

Szymanski, M., A. Afton, and K. A. Hobson. 2006. Use of stable isotope methodology to determine natal origin of hatch-year mallards shot during fall in Minnesota. *Journal of Wildlife Management* **71:**1317–1324.

Taylor, H. P., Jr. 1974. An application of oxygen and hydrogen isotope studies to problems of hydrothermal alteration and ore deposition. *Economic Geology* **69:**843–883.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for δ^{13}C analysis of diet. *Oecologia* **57:**32–37.

Tietje, W. D., and J. G. Teer. 1988. Winter body condition of Northern Shovelers on freshwater and saline habitats. Pages 353–377 *in* D. J. Batt, R. H. Chaebreck, L. H. Fredrickson, and D. G. Raveling (Eds.) *Waterfowl in Winter.* University of Minnesota Press, Minneapolis.

Trueman, C. N., and A. Moore. 2007. Use of the stable isotope composition of fish scales for monitoring aquatic ecosystems. Pages 145–161 *in* T. E. Dawson and R. T. W. Siegwolf (Eds.) *Stable Isotopes as Indicators of Ecological Change.* Academic Press, London.

Trust, B. 1993. *Stable Carbon and Sulfur Isotopic Ratios in Migrating Redhead Ducks (*Aythya americana*) and Their Breeding and Wintering Habitats.* Ph D thesis, University of Texas, Austin.

Vanderklift, M. A., and S. Ponsard. 2003. Sources of variation in consumer-diet δ^{15}N enrichment: A meta analysis. *Oecologia* **136:**169–182.

Van der Merwe, N. J., J. A. Lee Thorp, J. F. Thackeray, A. Hall-Martin, F. J. Kruger, H. Coertzees, R. H. V. Bell, and M. Lindeque. 1990. Source-area determination of elephant ivory by isotopic analysis. *Nature* **346:**744–746.

Vogel, J. C., B. Eglington, and J. M. Auret. 1990. Isotopic fingerprints in elephant bone and ivory. *Nature* **346:**747–749.

Walpole, R. E., and R. H. Meyers. 1993. *Probability and Statistics for Engineers and Scientists.* 5th edn. Prentice Hall, Englewood Cliffs, New Jersey.

Wassenaar, L., and K. A. Hobson. 1998. Natal origins of migratory Monarch butterflies at wintering colonies in Mexico: New isotopic evidence. *Proceedings of the National Academy of Sciences* **95:**15436–15439.

Wassenaar, L. I., and K. A. Hobson. 2000. Stable-carbon and hydrogen isotope ratios reveal breeding origins of red-winged blackbirds. *Ecological Applications* **10:**911–916.

Wassenaar, L. I., and K. A. Hobson. 2001. A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* **35:**1845–1850.

Wolf, B., and C. Martinez del Rio. 2000. Use of saguaro fruit by white-winged doves: Isotopic evidence of a tight ecological association. *Oecologia* **124:**536–543.

Wunder, M. B. 2007. *Geographic Structure and Dynamics in Mountain Plover.* Ph.D. dissertation. Colorado State University, Fort Collins, Colorado.

Wunder, M. B., C. L. Kester, F. L. Knopf, and R. O. Rye. 2005. A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia* **144:**607–617.

Yapp, C. J., and S. Epstein. 1982. A re-examination of cellulose carbon-bound hydrogen D measurements and some factors affecting plant-water D/H relationships. *Geochimica et Cosmochimica Acta* **46:**955–965.

Yohannes, E., K. A. Hobson, D. Pearson, L. I. Wassenaar, and H. Biebach. 2005. Stable isotope analyses of feathers help identify autumn stopover sites of three long-distance migrants in northeastern Africa. *Journal of Avian Biology* **36:**235–241.

Yohannes, E., K. A. Hobson, and D. J. Pearson. 2007. Feather stable-isotope profiles reveal stopover habitat selection and site fidelity in nine migratory species moving through sub-Saharan Africa. *Journal of Avian Biology* **38:**347–355.