

From birds to butterflies: animal movement patterns and stable isotopes

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Establishing patterns of movement of wild animals is crucial for our understanding of their ecology, life history and behavior, and is a prerequisite for their effective conservation. Advances in the use of stable isotope markers make it possible to track a diversity of animal species in a variety of habitats. This approach is revolutionizing the way in which we make connections between phases of the annual cycle of migratory animals. However, researchers must exercise care in their application of isotopic methods. Here, we review stable isotope patterns in nature and discuss recent tracking applications in a range of taxa. To aid in the interpretation and design of effective and insightful isotope movement studies, we discuss a series of key issues and assumptions. This exciting field will advance rapidly if researchers consider these aspects of study design and interpretation carefully.

Unraveling patterns of movement at various scales is essential for our understanding of the ecology, life history, behavior and conservation of most animals. However, this requires individuals or populations to be tracked on a seasonal or annual basis [1], a difficult task if the animal is small, the distances covered are vast or the habitat is remote.

Tracking animal movements can be done either directly, by following individuals over time and space using some form of extrinsic marker, or indirectly, by inferring their origins using natural, intrinsic biological or biogeochemical markers (i.e. forming links between populations or seasons) (Box 1). Biogeochemical markers are particularly useful for studying animal movements because they do not require marking or recapturing individuals and they provide time-integrated information that can be linked directly to geographical regions. The most commonly used

Box 1. Using markers to track animal movements

Extrinsic markers

Remote-sensing techniques (e.g. radio transmitters or satellite technology) and individual tags (e.g. leg rings, neck collars or plumage dyes) are the most common types of extrinsic marker used to track animal movement patterns. Radio telemetry works well for large animals and those that move shorter distances, but not as well for smaller, long-distance migrants. Although advances in such techniques (e.g. satellite technology) offer new promise and do not rely on recapturing or re-sighting individuals, size and cost are still prohibitive [1]. Tagging requires initial capture, and then recapturing or re-sighting specific individuals, and so often suffers from the 'needle-in-a-haystack' effect. For example, intensive ringing efforts have provided good data for many larger migratory birds, such as waterfowl and shorebirds [1], but not for many of the smaller, threatened songbirds [29,52]. Extrinsic markers can also affect behavior, and although they supply excellent data about movements of a few individuals, such information might not be typical of entire populations.

Biological markers (intrinsic)

Biological markers (e.g. morphological, behavioral and genetic variation) rely less on capturing specific individuals and more on locating members of the same population or those from a similar geographical area [1,23,54]. However, migratory animals and species that move or

disperse vast distances tend to show few differences (i.e. low intra-specific variation in genetic structure or trait diversity) among populations [1], and such markers might only work over geographical scales of thousands of kilometers [23,54].

Biogeochemical markers (intrinsic)

Biogeochemical markers (e.g. trace element concentrations and stable isotopes) have been used to infer geographical origins and to differentiate among populations of animals, and recent developments in analytical techniques now enable routine measurement of many elements and their isotopes [52,55]. Stable isotope measurements in animal tissues reflect those isotope values in food webs, and so provide information about diet and location of feeding. Tissue isotope signatures in animals caught at one location can be compared with those of individuals caught at another to provide information about population-level movements between geographically separated areas. That is, because stable isotopes indirectly provide information about animal movements (as opposed to directly in the case of using extrinsic markers), isotope signatures from many individuals caught in one area or population are needed, and thus provide movement patterns relevant to populations, rather than individuals. Moreover, stable isotope methods do not rely on recapture or re-sighting previously captured individuals.

Glossary

Isotope discrimination: net difference in isotope abundance caused by variable behavior of isotopes of any given element in biogeochemical processes owing to thermodynamic and kinetic considerations relating to nuclear mass differences.

Migratory connectivity: links between breeding and non-breeding populations. Migratory connectivity differs from migration (i.e. the regular seasonal movement of animals from one place to another, often from a breeding site to a non-breeding site and back) in that it refers to direct connections, and the strength of those connections, between seasonal locations or populations. Migratory connectivity can vary in strength, ranging along a continuum of weak to strong: strong connectivity occurs when most individuals from one breeding population move to the same wintering location, whereas weak connectivity occurs when individuals from a single breeding population migrate to several different wintering locations [1].

Trophic enrichment: the difference in isotope ratios between an animal and its putative diet. Heavier isotopes of any given element increase in abundance compared with lighter isotopes through the process of isotope discrimination.

biogeochemical markers are naturally occurring stable isotopes of key elements. Here, we provide an overview of applicable natural stable isotope patterns in the environment and explore recent tracking applications in a broad diversity of taxa. We emphasize key issues and assumptions that are needed to interpret and design effective isotope movement studies. Our goal is not to review all previous isotope movement studies, but to synthesize recent, relevant studies and provide a framework for how to expand this field. The adoption of well designed isotope movement studies will provide new information about factors that have the most influence on demographic rates in animal populations, insights into the evolution of migration across several taxa and crucial information for the conservation of threatened migratory species.

Stable isotopes and their abundance in nature

Stable isotopes are naturally occurring stable forms of elements with differing nuclear masses, which confer disparate physical properties that cause such isotopes to behave differently in biogeochemical processes. They are measured by a mass spectrometer as isotopic differences relative to international standards and reported as ratios in delta (δ) units as parts per thousand (‰). Stable isotopes are incorporated directly from diet into animal tissues with varying degrees of TROPHIC ENRICHMENT (see Glossary). For any element, isotope abundance varies naturally in the environment because of a range of biological and biogeochemical processes, and can be further influenced by human practices (Table 1).

Isotopes that are useful in animal movement studies can be divided into two categories based on their elemental atomic masses: those of light (e.g. carbon, nitrogen, sulfur, hydrogen and oxygen) and heavy (e.g. strontium and lead) elements. The abundance of light isotopes tends to be influenced by both biological and biogeochemical processes. For instance, stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values are influenced directly by biochemical processes during fixation in plants, but whereas $\delta^{13}\text{C}$ values in animal tissues accurately reflect those of their diet, $\delta^{15}\text{N}$ values in animal tissues show considerable enrichment and are affected by water and nutritional stress [2,3]. Stable sulfur ($\delta^{34}\text{S}$) isotope values vary widely

in both terrestrial and marine environments, and accurately reflect sources of nutrients in food webs [4]. Hydrogen (δD) and oxygen ($\delta^{18}\text{O}$) isotope ratios in animal tissue accurately reflect those in lakes, rivers, oceans and meteoric waters (i.e. groundwater recently originating from the atmosphere) [5].

The abundance of heavy isotopes tends to be influenced by biogeochemical processes. The stable isotopes of strontium ($\delta^{87}\text{Sr}$), a non-nutrient element that has similar chemical properties to calcium, often replace calcium in bones during nutrient uptake and can thus be used to trace the movement of minerals from soil through the food web [6]. Other heavy isotopes that are useful in animal movement studies are those of lead ($\delta^{206,207,208}\text{Pb}$), which vary naturally in soils and bedrock and as a result of anthropogenic inputs [7], and are taken up incidentally in some tissues.

Animal movement: characterizing populations and forming links

The goal of most isotope movement studies is to examine MIGRATORY CONNECTIVITY. Unraveling migratory connectivity using stable isotopes involves: (i) choosing a tissue representing the appropriate temporal period of integration of geographical information (Box 2); (ii) isotopically characterizing and differentiating among populations of interest; and (iii) linking populations between seasons by inferring geographical origins based on isotopic similarity. Because distinct isotopic landscapes occur in nature, it is possible to exploit these patterns to monitor movements of individuals traveling among them. This requires no other intervention from the researcher in the form of mark and recapture, but it does require a clear understanding of these isotopic patterns in nature.

Characterizing and differentiating among populations

The first step in tracking animal movements is to characterize and then differentiate among populations. Doing this using extrinsic markers generally involves marking a few individuals in a population, which, ultimately, will be the only animals that actually provide information about movement patterns. By contrast, using stable isotopes to characterize populations involves examining the isotope signatures of a few individuals that are representative of the entire population. Once the isotope signature of the population is known, all of the individuals from that population can be used to provide information about movement. Stable isotopes measured from a diversity of tissues have been used to identify and distinguish among individuals from different populations or habitats in a variety of animal taxa [8]. Here, we highlight some of the tissues and isotopes used in studies of mammals, birds and fish:

Mammals. Some of the earliest isotope movement studies were conducted with the use of marine mammals, and primarily examined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in both metabolically active and inert tissues to assign individuals to different foraging locations [8–10]. Similarly, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{87}\text{Sr}$ values in elephant tusks were used to classify individuals to different areas [8]. More recent work suggests that tropical bat dependence on fruit- versus

Table 1. Processes that influence stable isotope abundance and natural environmental isotope patterns

Stable isotope	Processes that influence isotope abundance		Natural environmental patterns (i.e. no anthropogenic influences)			Refs
	Biological and/or Biogeochemical	Anthropogenic	Terrestrial	Marine	Terrestrial versus marine	
Carbon $\delta^{13}\text{C}$ $^{13}\text{C}:^{12}\text{C}$	Vary in plant tissue with: • Isotopic fractionation during photosynthesis in C_3 , C_4 and CAM spp. ^a • Ambient conditions that limit enzymatic reactions during photosynthesis or alter stomatal opening	Agricultural crops (i.e. C_4 -based) in natural ecosystems (i.e. C_3 -based) Atmospheric or aquatic point-source pollution	Decrease with increasing latitude ^{b,c} Increase with increasing altitude ^b Mesic (i.e. dry) habitats more enriched compared to xeric (i.e. wet) habitats in C_3 -based systems ^d	Decrease with increasing latitude ^e Northern oceans more enriched compared to southern oceans ^e Benthic more enriched compared to pelagic ^e	Marine more enriched compared to terrestrial ^f	[2,3,8]
Hydrogen δD $^2\text{H}:^1\text{H}$ Oxygen $\delta^{18}\text{O}$ $^{18}\text{O}:^{16}\text{O}$	Vary in meteoric waters with: • Precipitation patterns • Temperature • Elevation • Relative humidity	Irrigation with ground water Creation of water impoundments that influence local climate patterns Anthropogenically induced climate change	Decrease with increasing latitude ^g Decrease with increasing altitude ^g Highest in summer and lowest in winter above 30° latitude ^g Decrease moving inland ^g	No patterns	Marine more enriched compared to terrestrial ^h	[3,5,8]
Lead $\delta^{206,207,208}\text{Pb}$ $^{208}, ^{207}\text{Pb}:^{206}\text{Pb}$	Vary in plant tissue with type of geological substrate	Point-source pollution Airborne pollution from fossil fuels	Older soils more enriched compared to younger soils ⁱ	No patterns	No patterns	[7]
Nitrogen $\delta^{15}\text{N}$ $^{15}\text{N}:^{14}\text{N}$	Vary in plant tissue according to how plants fix N: • Symbiotic fixation • Direct conversion of atmospheric N	Agricultural origin (e.g. fertilizers) Land-use practices that result in ammonification or the differential loss of ^{14}N	Xeric (i.e. wet) habitats more enriched compared to mesic (i.e. dry) habitats ^j	Northern oceans more enriched compared to southern oceans ^k	Marine more enriched compared to terrestrial ^l	[2,48,66]
Strontium $\delta^{87}\text{Sr}$ $^{87}\text{Sr}:^{86}\text{Sr}$	Vary in plant tissue with: • Age and type of geological substrate • Atmospheric deposition from natural sources	Nuclear fallout Airborne pollution from fossil fuels Land-use practices that expose bedrock or other sources	Calcium-rich soils more enriched compared to calcium-poor soils ^m Older soils more enriched compared to younger soils ⁱ	No patterns	No patterns	[6]
Sulfur $\delta^{34}\text{S}$ $^{34}\text{S}:^{32}\text{S}$	Vary in nature with: • Distribution of light and heavy sulfides in bedrock • Quality of plant growing conditions (i.e. aerobic versus anaerobic) • Atmospheric deposition from natural sources	Point-source pollution Airborne pollution from fossil fuels	No patterns	Estuarine and marsh more enriched compared to marine ⁿ Benthic (i.e. inshore) more enriched compared to pelagic (i.e. offshore) ⁿ	Marine more enriched compared to terrestrial ^o	[4,48,67]

^aPlants use one of three different types of photosynthetic pathway [C_3 , C_4 or crassulacean acid metabolism (CAM)] that utilize different CO_2 -fixing enzymes and result in varying ranges of $\delta^{13}\text{C}$ values [2,3].

^bOwing to temperature differences [2,3].

^cOwing to differences in abundance of C_4 plants [2].

^dOwing to difference in water use efficiency [3].

^eOwing to temperature differences, surface-water CO_2 concentrations and differences in plankton biosynthesis or metabolism [2].

^fOwing to bicarbonate as a carbon source and slower diffusion of CO_2 in marine environment [2].

^gOwing to rainfall patterns and temperature differences [3,5].

^hOwing to evaporation of seawater and subsequent condensation of cloud moisture over land [3].

ⁱOwing to the age of bedrock (and thus decay rate of isotope) [7].

^jOwing to water and nutrient stress [2].

^kReasons are unclear [2].

^lOwing to inorganic nitrogen being an important contributing factor in marine environment [48].

^mOwing to the abundance of calcium (and thus strontium) in bedrock [6].

ⁿOwing to anoxic conditions [67].

^oOwing to bacterial sulfate reduction in marine environment [67].

Box 2. Stable isotopes in different tissues

Stable isotope ratios are incorporated directly from the diet into animal tissues, but the degree of ISOTOPE DISCRIMINATION (see Glossary) varies according to dietary elemental composition [46] and the residence time of elements in tissues depends on metabolic turnover rates [56]. Many recent experimental studies have determined residence times of some isotopes in a variety of different tissues [8,46,56–58]. For example, turnover rates vary among species owing to the effect of body size on overall metabolic rate [59] and isotope ratios might vary predictably with age [43], but more experimental work on residence times in different tissues is needed for all taxa [41] in a variety of controlled and wild settings [52].

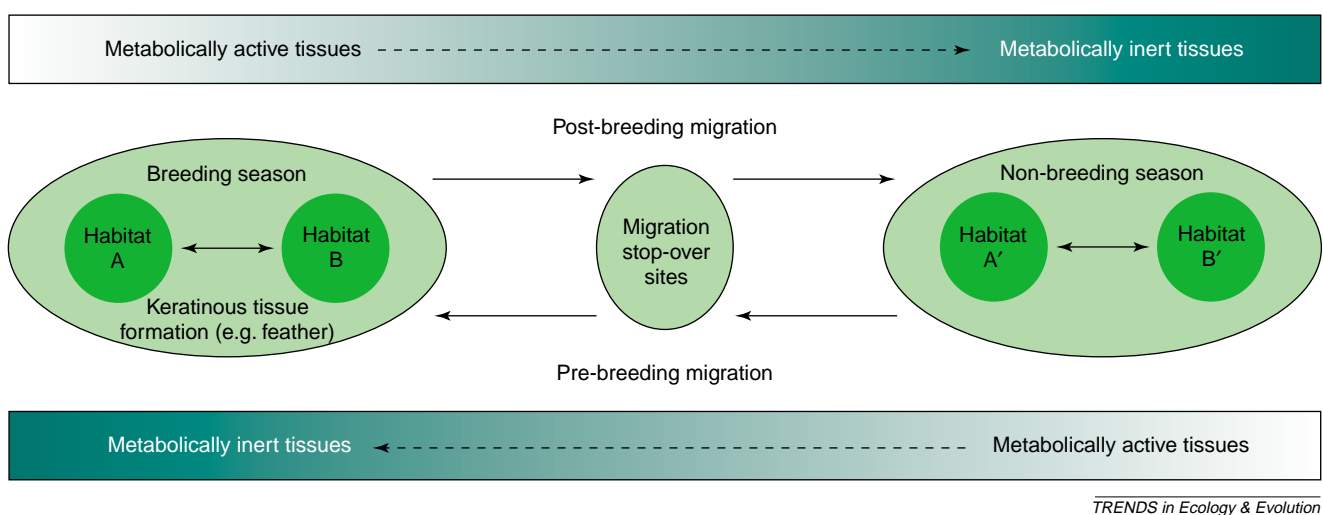
Most isotope movement studies designed to track migrants (Figure 1) have used metabolically inert tissues for which growth periods are well defined (e.g. feathers and claws of migratory birds, hair and claws of mammals and wing membranes of insects) [14,58]. More proximate spatial information can be gleaned from metabolically active tissues that might help to identify newly arriving individuals versus residents [60], or correspond to a temporal window of interest. Choosing several tissues with different periods of temporal integration of geographic information might provide movement history of individuals spanning the entire annual cycle [61].

Metabolically inert tissues

Metabolically inert tissues can be used to study seasonal movement patterns because isotope ratios reflect food-web conditions at the time of tissue growth and remain unchanged despite animal movements [8]. Most metabolically inert tissues of interest in tracking animal movements are keratin based (e.g. baleen, bill, claw, feather, hair, horn or nail) and, although some of these tissues can continue to grow over an extended period, stable isotope ratios are locked into the keratin structure during growth [8]. Hydrogen isotopes are the exception; some non-carbon-bound hydrogen exchanges with hydrogen in ambient water vapor [20], but this can be corrected for through equilibration techniques [62].

Metabolically active tissues

Some metabolically active tissues rapidly turnover elements in a matter of hours or days (e.g. blood plasma or liver), whereas others can take up to several weeks (e.g. muscle or whole blood) or even several months or years (e.g. bone collagen) [20,46,56–58]. Residence times of carbon, nitrogen, and sulfur isotopes in a variety of tissues are known [8,46,56–58], but further work is needed to establish patterns in these and other isotopes, particularly in wild individuals [8].



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Figure 1. Choosing appropriate tissues for isotope movement studies of birds. Studies of avian movement patterns can be designed to examine both long-term movements between seasons, as well as short-term movements between habitats within a season. Tissues can be aligned along a continuum of metabolic turnover rates, and thus used to study movements over various timescales. Choosing appropriate tissues will ultimately depend on ease and utility of sampling and type of question being asked. Tissues with rapid turnover rates (e.g. blood plasma or liver) can be used to study primarily short-term movement patterns, whereas tissues with slower turnover rates (e.g. muscle or whole blood) can be used to examine longer-term movements. Metabolically inert tissues (e.g. keratinous tissues) are best for studying movements between seasons.

insect-based diets, determined primarily using $\delta^{15}\text{N}$ analyses [11], could provide insights into bat movements based on seasonal fruiting chronologies [12]. In addition, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of keratin in ungulate hooves, which have been used in retrospective dietary studies, show potential for studies of ungulate migration [13].

Birds. Early work with birds showed that individuals from distinct habitat types and populations could be distinguished by measuring $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in metabolically active tissues (e.g. muscle and blood) and, more recently, by analyzing δD , $\delta^{87}\text{Sr}$ and $\delta^{34}\text{S}$ values in metabolically inert tissues (e.g. bone, feather and claw) [8,14].

Fish. Studies of fish have generally examined isotopes in the metabolically inert fraction of otoliths (ear bones), which show annual growth rings that reflect isotope ratios (e.g. oxygen, carbon and strontium) in the surrounding

water and can be used to classify individuals to different habitat (e.g. coastal marine, estuarine, river or lake) [15]. Recent analysis of the organic fraction of salmon otoliths (e.g. $\delta^{34}\text{S}$) has provided increased spatial resolution of fish origins, and can ultimately be used to trace locations to the embryonic stage [16]. In addition, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses of metabolically active tissues (e.g. eggs, muscle and adipose fin), which, unlike otoliths, do not require destructive sampling, can also be informative for movement studies [17].

Linking populations between seasons to infer geographical origins

Establishing the breeding origins or the long-distance movement patterns of an individual or population between seasons is the ultimate goal of most animal movement

studies. With extrinsic markers, this involves following only marked individuals between periods of the annual cycle. With stable isotope markers, however, once a few populations have been characterized isotopically in one stage of the annual cycle (e.g. breeding season), randomly chosen individuals can be collected at another stage (e.g. non-breeding season) and their origins can be inferred. Here, we highlight studies of mammals, reptiles, birds, fish and insects that have linked populations between seasons:

Mammals and reptiles. Most work on mammal and reptile movements using stable isotopes has been done in the marine environment. For example, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses of baleen in whales [8] and of a variety of tissues in pinnipeds [9,10] established long-distance movement patterns among different foraging locations. Similarly, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of eggs of loggerhead turtles *Caretta caretta* were related to foraging areas of the female before migration to nesting beaches [18].

Birds. Birds have received particular attention for isotope movement studies because they are relatively easy to capture and non-destructively sample (e.g. feathers). Links between breeding and wintering populations of migrant warblers [19–24] and other passerines [25–27] have been established. Some studies have provided information about population mixing on the wintering grounds [22,25] and differential migration patterns by breeding location [22], whereas others have explored the timing of migration as birds passed through North American ringing stations to reveal migratory patterns of different breeding populations and age classes [21,28,29].

Stable isotopes have been used to link populations of threatened avian species between their breeding and wintering grounds. A study of δD values in the endangered Bicknell's thrush *Catharus bicknelli* revealed an unknown breeding population in northeastern North America [25]. $\delta^{13}\text{C}$ and δD analyses of black-throated blue warblers *Dendroica caerulescens* suggested that heavy deforestation on the Caribbean island of Hispaniola could have contributed to population declines in parts of the southern North American breeding range of this species [22].

Recent work has examined the relationship between population dynamics in breeding and non-breeding seasons. Tissue $\delta^{13}\text{C}$ analysis in American redstarts *Setophaga ruticilla* determined that winter habitat quality directly affected physical condition and departure times of the birds, which, in turn, affected breeding arrival times, condition and reproductive success [30,31].

Fish. Although less work has been done with fish, some very informative studies have examined isotopes in both metabolically active and inert tissues to look at migration and dispersal patterns. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ measurements of the inorganic fraction of otoliths established migration patterns in many marine and anadromous fish [15] and, along with a variety of trace elements, showed low natal dispersal in weakfish *Cynoscion regalis* from the east coast of the USA [32]. $\delta^{87}\text{Sr}$ values in otoliths of Atlantic salmon *Salmo salar* in eastern North America reflected watershed geology of local rearing streams [33] and, when combined with $\delta^{15}\text{N}$ values, which varied owing to differences in agricultural sources, it was possible to give individual

stocking streams unique isotopic identities [34]. Finally, muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were used to explore migrations of landlocked gobies *Rhinogobius* sp. between a Japanese lake and its river tributaries [35,36], and revealed ontogenetic movements among mangrove, seagrass and reef habitats in tropical reef fish [37].

Insects. Some of the most powerful isotope movement studies have been done with insects, because it is possible to conduct controlled manipulations, as well as sample large numbers of wild individuals. Combined controlled experiments and field sampling using $\delta^{13}\text{C}$ and δD analyses of wing membranes in monarch butterflies *Danaus plexippus* revealed that the 13 known Mexican wintering colonies consisted mainly of butterflies from the midwestern USA [38,39]. Recently, this work has been expanded to show that North American monarchs also disperse to Cuba [40].

Considerations for designing and interpreting isotope movement studies

There are several issues and assumptions relating to the biology and life history of a migratory species, as well as to the biogeochemistry and ecology of the ecosystems involved, that must be recognized [41]. These factors contribute variance to isotope measurements of individuals and populations. Recognizing these considerations can help minimize natural sources of variation that are inherent to all isotope movement studies.

Biology and life history

Although it is implicitly assumed that all individuals in a sampling location have similar tissue isotope ratios, this might not be the case if there are differences in diet, foraging location or metabolism among individuals. For example, red-winged blackbirds *Aegialius phoenicius*, which feed in a range of local habitats from natural wetlands to farmlands, showed high variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that corresponded to foraging location [42]. Although there is no relationship between body size and $\delta^{15}\text{N}$ values [2], young might show variation in $\delta^{15}\text{N}$ values if they feed on a different diet (e.g. more protein rich), if they feed at a different trophic level [43] or if their metabolism differs from that of adults [41,44].

Understanding tissue growth patterns (Box 2) is essential for interpreting isotope movement studies. For example, although it is generally assumed that most songbirds in North America molt at their breeding sites, δD analysis of Swainson's thrush *Catharus ustulatus* revealed that flight feathers were molted during migration when the birds were south of their breeding grounds [29]. It is also important to consider molt chronology and duration of feather growth relative to location [45], as well as to understand the nutritional pathways that are associated with tissue growth and how isotope ratios vary in different types of tissue or parts of the same tissue [41,46,47].

Biogeochemistry and ecology

Geographical and inter annual climatic variation can affect isotope movement studies. In spite of a reasonable knowledge of worldwide average δD and $\delta^{18}\text{O}$ patterns

Box 3. What we have learned and future directions

Lessons learned

δD values are most useful for studying avian migration in Neotropical (North–South America) migrants breeding in North America [20–22,25–29,42] because they show high latitudinal geographical structure. $\delta^{13}C$ values have proved effective for studying breeding origins in some Neotropical species [20,22], but not others [25,27,29]. By contrast, $\delta^{13}C$ and $\delta^{15}N$ values have proved informative for Palaearctic (Eurasia–Africa) migrants (because of the east–west moisture gradient across sub-Saharan Africa) [19,24,63]. Although δD values have not proved useful for those Palaearctic migrants that molt in Africa [24,63], latitudinal δD patterns in western Europe might aid in studying the movements of species that molt in European breeding grounds [52].

Areas of focus

δD is a powerful indicator of geographical location and merits further attention. Studies must evaluate more precisely the discrimination between growing season average precipitation δD and feather δD . Currently, our best estimate is -25% [27], but this should be corroborated for several species and based on food-web δD values at the same locations where feathers are grown. Further studies of physiological mechanisms influencing feather δD values are also needed, given recent evidence that young and adult birds from the

same site might differ [44]. Future continent-wide maps of feather δD values must take into account altitudinal effects and emphasize local or regional departures from the long-term feather δD contour base maps.

New techniques

Many isotope techniques and promising elements have been underutilized for animal movement studies. Recent advances in continuous-flow isotope-ratio mass spectrometry (CFIRMS) now enable rapid and routine assays of several elements. For example, sulfur, which can be measured routinely using CFIRMS, can typically be associated with a few amino acids that are common in feathers and can greatly improve differentiation among individuals or populations [64]. Moreover, analyzing stable isotopes in specific compounds (e.g. amino acids) using gas chromatography and combustion-isotope ratio mass spectrometry might eventually improve the resolution of isotope patterns once details of biochemical pathways are established [65]. Heavy isotopes, such as strontium and lead, have also been underutilized, and extraction of such isotopes from keratin-based tissues will pave the way for their use in more ecological studies. New advances in inductively coupled plasma mass spectrometry enable the routine measurement of numerous heavier isotopes, leading to a true multivariate approach to isotopic patterns in nature that could be linked to trace element profiles [55].

in meteoric waters [5], in areas or times with low rainfall, or in areas that are subject to synoptic (i.e. single or stochastic) rainfall events, deviations from these averages are still poorly understood [48]. Moreover, differences in bedrock composition and heterogeneity of soils on a microhabitat scale might affect $\delta^{15}N$ and $\delta^{87}Sr$ values [6,33]. A study of black-throated blue warblers illustrated how weather patterns originating in the Atlantic Ocean affected feather δD values in coastal populations [20]. Year-to-year variation in rainfall could also indirectly affect isotope movement studies [47] by influencing relative food abundance and diet selection.

Altitude influences isotope patterns and could potentially impact movement studies. Altitudinal patterns in $\delta^{13}C$ and δD , but not $\delta^{15}N$, have been shown in eight species of Andean hummingbirds along an elevational gradient [49]. Recent work with black-throated blue warblers suggested that $\delta^{13}C$ values varied as much over a local 750-m elevational range as they did over the entire breeding range of the species [50]. However, latitudinal isotope patterns were still stronger than any influence of altitude [22]. This highlights the point that within-population variance in isotope signatures can often be as high as among-population variance. Using isotopes with high geographic structure, multiple isotopic systems and analysis methods that take these variances into account will help in assigning origins [51].

Future directions

The application of stable isotopes to animal movement studies is expanding rapidly and there are many new and exciting directions in which this field could proceed (Box 3). Preliminary work has shown promise in using stable isotopes to examine natal dispersal patterns [25,32,50], population mixing and segregation on wintering grounds [22,25] and links between breeding and non breeding demography, particularly related to physical condition and fitness [30,31].

With the potential for these new studies comes the continued need for more controlled work [41,52] to better understand many of the biological, ecological and biogeochemical factors that influence isotope ratios. For example, we have a poor understanding of the nutritional strategies used by animals for tissue synthesis, how these approaches change with life-history stage and, ultimately, how such processes influence stable isotope tracing methods. Moreover, because new evidence suggests that trophic enrichment can be influenced by elemental composition of diets [46], we must carefully consider the consequences of animals switching between high carbohydrate and high protein foods [45]. To improve the resolution of isotope movement studies, we must apply new isotope techniques (Box 3), use more powerful statistical procedures modeled after movement studies using other types of markers [8,48,51] and conduct studies that combine different types of techniques and markers [23,40,53]. As improved genetic tools, more advanced remote sensing technology and powerful new isotope techniques become less time consuming, cheaper and require smaller sample sizes, combining multiple approaches will enable us to unravel complex movement patterns in a diversity of species living in a variety of habitats.

Acknowledgements

We thank the participants of the Connectivity of Migratory Birds workshop (held at the Smithsonian Environmental Research Center, Maryland, 2000 and funded by the National Science Foundation) for stimulating many of these ideas. We thank C.P. Chamberlain for comments about the article. D.R.R. acknowledges the Howard Hughes Medical Institute for continued support.

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