Chapter 2
Animal Migration Tracking Methods

2.1 Extrinsic Markers

The migration of birds has been studied for many years relying mostly on extrinsic passive markers attached to individual animals at the point of capture, with the expectation that a proportion of the marked individuals will then be identified in another location at a different point in time. Over the past 100 years, the most widespread approach has been through the application of markers such as leg bands, neck collars, or dyes. Many millions of birds have been tagged in this way but although this method has provided insights into migration, for the vast majority of bird species examined the recovery rate is low. An alternative to these simple devices is to use miniature transmitting devices – radio transmitters, radar and satellite tracking – that serve as active markers and are small enough (<0.5 g) to be attached to even small birds or mammals. The location of the marked animal can be inferred by tracing the individual using a receiver, or by triangulation using several receivers. Since the devices are miniaturized their range and battery life are restricted and they can provide information over only a few kilometers. Radar technology has also made useful contributions to studies on migration since it can provide information on animal movements over considerable distances, but since radar installations are fixed it is not possible to trace movements over the whole spectrum of migration routes used by birds. The most significant advances in tracking migratory animals have come from the use of satellite transmitters that allow highly accurate positioning of individual animals (Hiroyoshi and Pierre 2005; Whitworth et al. 2007; http://www.fao.org/avianflu/en/wildlife/sat_telemetry.htm). Much of the globe is covered by satellites so that animals can be monitored over thousands of kilometers. The technique can only be used on relatively large animals as the weight of the smallest transmitters is approximately 10 g, restricting their use to an animal weighing about 250 g, thereby excluding 80 % of the world's birds and 70 % of the mammals. With the exception of satellite transmitters all extrinsic markers require that individuals be recaptured, re-sighted or move within a detector's range at some time after initial

The original version of this chapter was revised: The chapter was inadvertently published with materials that were reproduced or modified without permission for the table headings in Tables 2.1–2.3 and 2.5 which has been updated now and also fix the citation for author Wunder and Norris (2008a and 2008b) in Table 2.5 and (2008b) on page 31. The correction to this chapter is available at https://doi.org/10.1007/978-3-319-28298-5_4

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marking. The probability of recapture depends on the number of observers, the regions and habitats and the chances of success are low. In addition, extrinsic methods tend to be biased towards regions with a high likelihood of mark-recapture (Hobson et al. 2004). A fundamental flaw in the use of an extrinsic marker is that it provides information only on the marked individuals. Geolocators and satellite tracking rely on small sample sizes and the devices may affect the behavior of the marked bird (Stutchbury et al. 2009). Extrapolating the findings from one individual to a whole population depends on how representative the marked individuals are. A single recovery or satellite track may not reveal what the population is doing.

2.2 Intrinsic Markers

The major advantage of an intrinsic marker is that it is not necessary to capture and mark an individual in advance, and every capture provides information on the origin of that animal. There is therefore, less bias than with extrinsic markers, where the location of the origin is dependent on accessibility; for example, remote areas might not be easily included in any migration study. Man-made contaminants such as dioxins and methyl mercury or other heavy metals could provide a means of inferring migratory origins as exposure to them varies throughout the world. Exposure of migratory animals to parasites or pathogens also varies geographically and could be used for determining movements but little research has been conducted as to how such markers might assist in deciphering migration patterns. In a study of Sharp-shinned Hawks (*Accipiter striatus*) in New Mexico, stable-hydrogen isotope ratios of feathers were estimated and blood was collected to quantify the prevalence and intensity of haematozoan infection in the birds (Smith et al. 2004). Twenty four percent of the birds were infected with *Leucocytozoon toddi*, 37 % with *Haemoproteus elani*, and 5 % with *H. janovyi*. This was the first documented occurrence of *H. janovyi* in North America in these hawks and the stable-hydrogen isotope analyses indicated that only birds originating from south-western North America harboured *H. janovyi* which could be of significance in regard to biogeography of that parasite species. The isotope studies also showed that birds infected with *Haemoproteus spp* were more widespread geographically than those with *Leucocytozoon* species. Another type of intrinsic markers that could potentially track animal movement geographically is the trace elements. Animals acquire quite distinctive chemical profiles relative to a particular geographical location and retain that profile when they move to another area. Trace element profiles have been used to determine if populations of breeding birds originate from different winter locations (Sze´p et al. 2008). However, there is little information on how trace elements vary over the globe, so it is of only limited potential. Stable isotope tools to trace animal migration have been used extensively in ecological studies but have not yet been utilized in work on the dissemination of transboundary animal diseases. There is potential for greater understanding the epidemiology of HPAI in relation to the migration of wild waterfowl by using stable isotope studies to identify the origins and stopover points of birds suspected of carrying HPAI. A summary of the advantages and disadvantages of extrinsic and intrinsic methods is given in Tables 1.1 and 2.1.

Table 2.1 Extrinsic markers for tracking animal migration. Reproduced with Permission from Hobson and Norris (2008)

Technique	Advantages	Disadvantages
Extrinsic	1. Can apply to a broad range of animals	1. Requires capture/recapture
	2. High spatial resolution	2. Biased towards initial captured population
Phenotypic variation	1. Inexpensive	1. Low resolution
	2. Can be used for historical specimen	2. Not applicable to all species
Banding/Marking	1. Inexpensive	1. Low recovery rates <0.5 %
	2. Gives information on start and end of migration	2. Data must be acquired over many years
		3. Only few banding stations across the world
		4. Marking and recovery are biased towards the banding station location
Radio-transmitter	1. Gives precise locations	1. Low range
	2. Gives precise trajectory	2. Expensive
		3. Transmitters might affect behaviour
Satellite transmitters	1. Precise animal trajectory	1. Expensive
		2. Only for use on large animals
		3. Transmitters might affect behaviour
International Cooperation for Animal Research Using Space ICARUS (www.IcarusInitiative.org)	1. Transmitter allows many different species to be tracked	1. High start-up investment
	2. Can track individuals over the globe	2. Technology not proven
Satellite tracking to determine the spread the spread of infectious diseases	3. Inexpensive after start-up costs	3. Transmitters might affect behaviour
Passive radar	1. Coverage over large geographical region	1. Coverage only from existing stations or portable instruments
	2. Inexpensive	2. Poor ability to determine species and individual movements
	3. Individuals need not be captured	
Transponders	1. Small size	1. Requires external activation
		2. Low range
		3. Coverage only form existing stations or portable instruments

(continued)

Table 2.1 (continued)

Technique	Advantages	Disadvantages
Geolocation tags	1. Produces animal trajectory	1. Individuals must be captured to download data
	2. Low weight	2. Accuracy relative to satellite tags low
	3. Inexpensive relative to satellite tags	

Studies of avian migration and population structure take advantage of geographical variation in stable isotope ratios, in particular the ratio of the two stable isotopes of hydrogen in precipitation. In North America, for instance, the ratio of growing season precipitation decreases from −20‰ VSMOW (Vienna Standard Mean Ocean Water) in Florida to −140‰ VSMOW in north-western Canada (McKechnie 2004). Because the deuterium of precipitation is incorporated into avian tissues via food webs and feathers are metabolically inert once grown, the deuterium values of feathers reflect the latitude at which they were grown. In species that moult prior to migration, feather deuterium can be used to identify the latitudinal origin of individuals, and hence link breeding and wintering grounds. Isotopic data can also be combined with information on capture dates or ring recovery. Stable isotope studies require a detailed knowledge of a species' moult schedule, and the assumption that the isotope signature of feathers reflects that of food webs in the region where the feather is grown must be verified. The technique has the best potential where there is a good spatial variation in the environmental locations in which the particular bird species is found.

The basic requirements in the use of stable isotopes are that:

- The characteristics of the environment through which the animal of interest moves are known. The term used to describe the mapping of large-scale and spatiotemporal distributions of stable isotope ratios in the environment and animals is an isoscape or isotopic landscape (Bowen and West 2008).
- Isotopic values in animal tissues can be discriminated from baseline isoscape values,
- The time period for acquisition into a particular animal tissue is understood. For instance, turnover of isotope will be greater in metabolically active tissues than in tissues with a slow, or no, turnover e.g. feathers (Table 2.2).

Just a handful of these stable isotopes are of any practical interest to studies on animal migration. These are the "light isotopes" of H, O, C, N, and S that are present in all components of the biosphere (plants and animals), the hydrosphere (water) and the atmosphere (gaseous O_2, N_2 and H_2O). These five elements comprise the bulk of all animal tissues (Table 2.3). These elements and their isotopes circulate in the biosphere to produce characteristic isotope distributions globally. Large pools of these elements provide stability in the overall isotope circulation e.g. in the ocean, for hydrogen and oxygen isotopes, inorganic carbon pool in the ocean for carbon

Table 2.2 Intrinsic markers for tracking animal migration stable isotopes. Reproduced with Permission from Hobson and Norris (2008)

Technique	Advantages	Disadvantages
Intrinsic	1. Not biased to initial capture population	1. Biased to final capture population
	2. Less labour intensive than most extrinsic methods	2. Lower resolution than extrinsic methods
Contaminants	1. Potentially high spatial resolution	1. Lack of distribution maps
		2. Transport of contaminants could give unreliable geographical signal
Parasites/Genetics	1. Several possible markers	1. Species specific
		2. Low resolution
Trace elements	1. Measurement of a large number of elements	1. Lack of distribution maps
	2. Potentially high spatial resolution	2. Expensive
		3. Requires more sample tissue
		4. Requires tissue that is metabolically inactive after growth
		5. Spatial resolution could be too high
		6. Some elements may be integrated into inactive tissue after growth is complete
Stable isotopes	1. Inexpensive	1. Low resolution
	2. Not species- or taxon-specific	2. No base maps
	3. Several isotopes can be combined to increase spatial resolution	3. Ideal tissue is metabolically inactive after growth
		4. Turnover rate of elements in active tissue is unknown
		5. Interpretation may be complicated by animals' physiology

isotopes, and sulphate in the sea for sulphur isotopes and the atmospheric reservoir for nitrogen. The presence of the stable isotopes varies widely in nature. All of the light isotopes have a common or abundant form, e.g. ^{1}H; 99.985 % and a more rare "heavier form", ^{2}H; 0.015 % (Table 2.3). The abundance ratios of these isotopes vary because of physical and chemical processes they undergo in nature and it is these variations that enable the use of stable isotopes in tracing migration. As it is difficult to measure the precise concentrations of the isotopes in samples, measuring the relative differences in isotopic ratios between a sample and a reference by means of a mass spectrometer is the way in which data are acquired. Since gas source isotope ratio mass spectrometers (IRMS) are used to measure light isotopes it is not possible to measure isotope ratios directly from a tissue sample – feather, blood, muscle, claw, hair – instead, the sample must first be combusted to form a gaseous analyte in an elemental analyzer, a gas chromatograph or a laser and the resulting gas can then be used to measure the isotopic ratios relative to a calibrated reference gas of

Table 2.3 Dry weight % abundance of light stable isotope ratios of interest in determining migratory connectivity in tissues. Reproduced with Permission from Wassenaar (2008)

Element	Weight (%)	Isotope ratios	δ Range (0/00)	Mass required (mg)
Carbon	30–40	$^{13}C/^{12}C$	05 to −65	0.2–1.5
Oxygen	27–40	$^{18}O/^{19}O$	+10 to +30	0.2–00.5
Nitrogen	12–19	$^{15}N/^{14}N$	−2 to +25	0.5–1.5
Hydrogen	6–8	$^{2}H/^{1}H$	−250 to +90	0.1–0.4
Sulphur	5–20	$^{34}S/^{32}S$	−20 to +30	1–2

Table 2.4 Average terrestrial abundances of the stable isotopes of major elements of interest in ecological studies the carbon cycle

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulphur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{36}S	0.014

the same type. Development of IRMS equipment has led to automatic sample processing to obtain multiple isotope assays from a single sample, on decreasing sample size and improving throughput rates. IRMS can measure isotopic ratio differences to the sixth decimal place or ±0.01 % (Table 2.4).

The carbon cycle involves active exchanges of CO_2 among the atmosphere, terrestrial ecosystems and the surface ocean. The ^{13}C value of atmospheric CO_2 is decreasing in response to inputs of ^{13}C depleted CO_2 from fossil fuel plus biomass burning and decomposition. Over the past 100 years the decrease may have been almost 1‰, from about −7‰ to −8‰.

Depending on how plants' photosynthesis process is materialized, they are classified in two large groups, C3 and C4, with very different values for $\delta^{13}C$. In the C3 group, the first photosynthesized organic compound has 3 atoms of carbon while in group C4, there are 4. Most plants (85 %) (E.g. trees and crops) follow the C3 photosynthesis pathway and have lower values of $\delta^{13}C$, between −22‰ and −30‰. The remaining 15 % of the plants are of type C4. The majority are tropical herbs and have high values of $\delta^{13}C$, between −10‰ and −14‰ (http://homepage.mac.com/uriarte/carbon13.html).

Carbon uptake by the dominant C3 plants on land involves a net fractionation of about 20‰ between the atmospheric CO_2 and plant biomass (−28‰). Carbon uptake by C4 plants, mainly tropical and salt grasses, involves a small net fraction-

ation of about 5‰. Soil organic matter globally contains several-fold more carbon than either the atmosphere or living plant biomass and is similar or slightly enriched in ^{13}C in comparison with the dominant vegetation. The exchange of CO_2 between the atmosphere and the surface of the ocean involves an equilibrium chemical fractionation between atmospheric CO_2 (−8‰) and the total CO_2 in surface ocean water.

2.2.1 The Nitrogen Cycle

Most nitrogen in the biosphere is present as N_2 gas in the atmosphere. This massive reservoir is well mixed with an isotope composition that is essentially constant at 0‰. Nitrogen in most other parts of the biosphere also has an isotope composition near the 0‰ value, from −10 to +10‰, primarily because the rate of nitrogen supply often limits reactions such as plant growth and bacterial mineralization. Under these conditions all available nitrogen can be consumed, without regard to isotope content and with no overall isotope fractionation. Thus, slow rates of N supply and limiting amounts of substrate N are often important for understanding nitrogen isotope distributions. Some cumulative and large fractionations do occur in the nitrogen cycle. Lakes appear more variable in isotope composition than the large world ocean. Large isotope contrasts might be expected between lakes in which primary production is limited by N (little fractionation by phytoplankton) versus P (abundant N → large possible fractionations during N uptake by phytoplankton). Where phytoplankton have different ^{15}N values than terrestrial vegetation, the nitrogen isotopes may function as source markers for autochthonous and allochthonous organic matter.

2.2.2 The Sulphur Cycle

Sulphate in the ocean is a large well-mixed sulphur reservoir whose isotope composition is 21‰ heavier than primordial sulphur in the earth and solar system at large. Fixation of sulphate by phytoplankton occurs with a small isotope effect, but sulphate reduction in marine sediment occurs with a large effect of 30–70‰. Over geological time, and partially in response to global-scale fluctuations in sulphate reduction activities, the ^{34}S values of oceanic sulphate have varied from about +10 to +33‰. Uplift and preservation of marine sedimentary sulphides and sulphate-containing evaporates on land have produced a patchwork of sulphur in terrestrial environments, each with different ^{34}S values for bedrock sulphur. Thus, large ^{34}S ranges must be assigned in general sulphur cycle diagrams. In spite of this, continental vegetation seems to average near +2 to +6‰ over large areas and is quite distinct from the +∼17 to +21‰ values of marine plankton and seaweeds. The stable isotope composition of sulphur entering the atmosphere can also be quite variable.

2.2.3 The Oxygen Cycle

There are three oxygen isotopes that act as tracers when the many common oxygen-containing molecules circulate in the biosphere. The water cycle controls much of the oxygen dynamics and oxygen isotope dynamics. Evaporation and condensation result in predictable variations in isotope compositions of water that are now routinely tracked at regional and global levels (http://isohis.iaea.org/userupdate/waterloo/index.html, http://www.waterisotopes.org/ and http://ecophys.biology.utah.edu/labfolks/gbowen/pages/Isomaps.html#IAEA).

Oxygen isotope studies with animals have focused on determining which local sources of water are used. The degree to which food influences ^{18}O variations in animals has not been determined fully.

2.2.4 The Hydrogen Cycle

Much of the hydrogen cycle involves water, with various processes in the water cycle leading to characteristic, large-scale geographic patterns of hydrogen isotopes in water. Ocean water is the main reservoir of hydrogen in the biosphere and the standard reference material "standard mean ocean water" for hydrogen isotope measurement. The isotope composition of ocean water is a good starting point for following isotope dynamics in the hydrological cycle. The transitions between liquid water and water vapour during evaporation and condensation involve kinetic and equilibrium reactions with isotope fractionation. Water vapour evaporating from the sea has ^{2}H values of −10 to −20‰, and as this process reverses during condensation and formation of rain and snow, this trend towards lower atmospheric ^{2}H values is amplified. As water vapour moves inland and up mountains, it progressively loses moisture and ^{2}H values decline further. These processes can be amplified yet again in colder regions where low temperatures promote stronger fractionations between vapour and condensate. A combination of high elevation and low temperature can result in ^{2}H values of −200 to −400‰ for water in high-elevation glaciers and for snow in Polar Regions. In less dramatic examples, large rivers fed by snowmelt that have continental origins can have much lower ^{2}H values than coastal marine waters. This makes ^{2}H source signals valuable tracers in coastal estuaries and floodplains linked to these rivers. Isotope hydrology studies often consider the water isotopes (hydrogen and oxygen isotopes) as markers for water sources and water circulation. Global-scale maps and animations of hydrogen and oxygen isotope variations in water are available on the Web (http://isohis.iaea.org/userupdate/waterloo/index.html or www.waterisotopes.org).

Analytical advances are making it easier to investigate the origins and cycling of hydrogen bound in organic matter. About 10–20 % of hydrogen in organic materials is exchangeable with water vapour present in normal laboratory air, but this exchange effect is understood and can be corrected for during routine analysis.

Hydrogen in animal tissues can be divided into three main pools: hydrogen derived from dietary sources, hydrogen from drinking water, and exchangeable hydrogen. For example, studies of quail fed deuterium-enriched showed that 10 % of all hydrogen was exchangeable, 30 % came from ingested water, and the remaining 60 % came from food. The overall finding for animals is that ^{2}H values are primarily controlled by diet, which in turn is strongly correlated with ^{2}H values of local water.

The reasons why hydrogen isotope tracers work well for studies of long distance migration depends on the fundamental chemistry of isotope fractionation. The water cycle of evaporation from oceans and precipitation inland involves isotope fractionations that leave behind the light isotopes. During chemical equilibrium fractionation, the vapour phase is enriched in the light isotopes, leaving the liquid phase heavier by difference or mass balance. The isotopically heavier liquid phase deposits as rain or snow, so that residual cloud-borne water moving inland or upwards is isotopically lighter and has lower ^{2}H values. Larger fractionations accompanying cold conditions in Polar Regions magnify some of these patterns, creating low ^{2}H values nearer the poles. This leads to continental-level patterns in the isotope compositions of water, in effect a giant isotope map created by the water cycle. Bird migrations take place across this chemical landscape, with birds at high latitudes having low ^{2}H values and birds near the equator having high ^{2}H values. The isotopes in the water provide a basic signal that first labels plants during photosynthesis and carbohydrate metabolism, then leads to general labelling of the local food web. The end result is that organic matter in materials such as bird feathers will have ^{2}H values that reflect the local water ^{2}H values. Migrating birds typically moult and form new feathers at the end of the summer, and feathers retain that late-summer isotope chemistry until the next year's moult.

2.3 The Stable Isotopes of Water on a Spatial Scale

As surface ocean waters evaporate into the atmosphere, the clouds formed are isotopically depleted in ^{2}H and ^{18}O relative to the ocean, resulting in an air mass that is similarly depleted relative to the ocean. In turn, as moisture is condensed from clouds during precipitation events, that water is isotopically enriched in ^{2}H and ^{18}O relative to the cloud, leaving the residual cloud mass isotopically depleted in ^{2}H and ^{18}O relative to the original cloud mass. The process of differential isotope depletion during precipitation results in a predictable pattern of depleted isotope ratios of precipitation as cloud masses move inland. Since the hydrogen and oxygen in precipitation become the primary source of H and O atoms incorporated into carbohydrates, proteins and lipids during microbe, plant and animal growth, these isotopes have the potential to carry a geographically based piece of information that are useful in migration studies.

The International Atomic Energy Agency (IAEA) and the World Meteorological Organization (WMO) has been conducting a worldwide survey of oxygen and hydrogen isotope content in precipitation. The objective is to collect basic data on

isotope content of precipitation on a global scale to determine temporal and spatial variations of environmental isotopes in precipitation to provide isotope data for the use of environmental isotopes in hydrological investigations. To this primary objective two other objectives have been added, providing input data to verify and further improve atmospheric circulation models, and to study of climate change. Since 1961, more than 800 meteorological stations in 101 countries have been collecting monthly precipitation samples for the Global Network of Isotopes in Precipitation – GNIP, IAEA 2006. Precipitation samples are collected in cooperation with WMO, national meteorological services and national authorities. The samples are analyzed in the IAEA's Isotope Hydrology Laboratory in Vienna, and in cooperating laboratories. Each contributing laboratory is responsible for the accuracy and precision of its own analyses. The GNIP homepage can be accessed at: – http://www-naweb.iaea.org/napc/ih/index.html. GNIP data may be used freely, provided the source is cited as follows: – IAEA/WMO (2006), Global Network of Isotopes in Precipitation, The GNIP Database, (http://www.iaea.org/water). From these and other observations, both temporal and spatial patterns emerged that established the basis for a global representation of the distribution of isotopes in water on a global basis. Analyses of the spatial distributions of hydrogen isotopes of waters across North America and Europe reveal substantial variations in stable isotope ratios, making it possible to distinguish precipitation in many geographical locations on the basis of their ^{2}H and ^{18}O values. There are no unique stable isotope ratio values for waters in a specific geographic location, but rather gradients or bands of different isotope ratio values allowing one to distinguish between locations if they were sufficiently far apart from each other. Bowen and colleagues extrapolated from this location-specific data of stable isotopes in water to construct spatial maps of the predicted isotopic composition of water throughout the world so that it is possible to estimate reliably the globally averaged ^{2}H and ^{18}O values of precipitation for different latitudes and longitudes using a calculator available at http://waterisotopes.org.

There are a number of programs available online that can be used to estimate the mean annual and monthly H and O isotope composition of precipitation in different locations. They are intended to facilitate the use of stable isotope data, amongst other uses, in ecological studies, and to standardize the interpolation of precipitation stable isotope data. The program for calculating isotopes – The Online Isotopes in Precipitation Calculator, OPIC – is based on the work of Bowen and Wilkinson (2002), Bowen and Revenaugh (2003) and Bowen et al. (2005). These papers provide a description of the methods. The data that were used to develop the database used by the OIPC are derived primarily from the International Atomic Energy Association/(WMO) Global Network for Isotopes in Precipitation (GNIP). To use the database it is only necessary to input basic information on location and elevation to acquire ^{2}H and ^{18}O values for the specified location. The program is periodically updated as new data, including that provided from sources other than the Global Network for Isotopes in Precipitation, are added to the database. The OPIC can be accessed from the website managed by Purdue University, West Lafayette, Indiana, USA, (http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/oipc.html). When the database is used in publications, reference should be made to the data-

base, The Online Isotopes in Precipitation Calculator, version XX (http://www.waterisotopes.org) and to publications by Bowen and Revenaugh (2003) and Bowen et al. (2005).

Another program that allows the user to download their own isoscape data is isoMAP, also based at Purdue University, that allows spatial analysis, modeling and prediction of stable isotope ratio variation in the natural environment It comprises web-based GIS and software tools that enable users to develop, and implement models for isotope distributions. This program can be accessed from http://isomap.rcac.purdue.edu:8080/gridsphere/gridsphere.

2.4 Deriving Isoscapes in the Absence of GNIP Data

Numerous studies have shown that precipitation isoscapes drive δD and $\delta^{18}O$ patterns in surface waters and in terrestrial food webs. While the GNIP dataset has provided the fundamental spatiotemporal foundation at a global geospatial scale, the GNIP stations are often spatially deficient for many regions of the planet that are of interest to ecologists. In North America, for example, Mexico has only two GNIP stations, which results in a spatial deficiency of water isotope data for that country. This spatial deficiency is of concern to researchers interested in large scale Mexican isotope hydrology, and to scientists interested in tracking migratory species to and from Mexico by using stable isotopes. Overcoming this GNIP spatial limitation would require adding spatially dense, long-term precipitation isotope collection stations across Mexico in order to obtain sufficient isotopic coverage of this topographically diverse country. This option is not viable for logistical and cost reasons, and so other approaches are needed to fill crucial information gaps. A possible approach is the application of the generalized globally predictive models for precipitation isoscapes (www.waterisotopes.org). However, these global models also lack sufficient or detailed on-the ground validation to determine how close they match or digress from reality at regional or country-wide scales (Bowen and West 2008).

In order to overcome this problem Hobson et al. (2009) developed a predictive general linear model (GLM) for hydrogen and oxygen isotopic spatial patterns in Mexican groundwater and then compared the results to a validation subset of field data, as well external data reported in the literature. The GLM used elevation, latitude, drainage basin (Atlantic vs. Pacific), and rainfall as the most relevant predictive variables. The GLM explained 81 % of the overall isotopic variance observed in groundwater, 68 % of the variance within this validation subset, and 77 % of the variance in the external data set. This predictive GLM is sufficiently accurate to allow for future ecological, hydrological and forensic isoscape applications in Mexico, and may be an approach that is applicable to other countries and regions where GNIP stations are lacking. They hypothesized that the stable isotopic composition of shallow phreatic groundwater in Mexico might serve as a useful proxy for integrating longer term (e.g. 5–10 year) precipitation infiltration inputs (Clark and

Fritz 1997). Groundwater infiltration is well-known to be a multi-year integrator of seasonally weighted rainfall events (Clark and Fritz 1997; Darling and Bath 1988; Rozanski 1985), and hence its isotopic composition closely reflects that of seasonally weighted long-term average inputs even in some of the most arid regions of the world (IAEA 2007). Groundwater was sampled from 234 sites at ~50 km latitudinal spacing to obtain high spatial resolution and country-wide coverage for the construction of a groundwater isoscape. Shallow groundwater infiltration in Mexico appeared largely unaffected by evaporation and reflected seasonally weighted precipitation inputs.

2.5 Use of Stable Isotopes for Migration Studies

The stable isotopes of H, O, N, C and S are routinely studied in bird migration because they are recorders of the dietary sources of the birds and these sources can be interpolated or specifically linked to ground proven or large-scale patterns in the global landscape and water environments (Hobson and Wassenaar 1997). Depending on whether the tissue of the migrating animal is biochemically fixed (feather, hair) or dynamic (blood, muscle) these stable isotopes provide fundamental information about where the animal has been and what it has been eating. The technology is relatively recent in inception and although it has been widely used approaches to its use are evolving, techniques are improving and methods of interpretation are being updated constantly. Stable isotopes as intrinsic markers do not require a recapture of the same animal to obtain data since the required spatial information is retained in the animals' tissues as a result of its stay in a particular habitat. Stable isotopes depend on several key requisites in order to function as intrinsic markers. Firstly, the animal must acquire a stable isotope into its tissues; since the light stable isotopes are key building blocks in animal tissues this is readily achieved. Secondly, the migrating animal must move between different environments and retain in its tissues measureable isotopic differences that can be linked to diet at previous or current locations. This is easily met by species that migrate seasonally across distinct isotopic landscapes such as between higher northern latitudes and lower latitudes in the southern hemisphere. It also requires that isotopic discrimination between the different seasonal landscapes and the tissues being measured is consistent or well-known, or has been empirically measured. Such conditions may not always be met with and provide considerable challenge to the interpretation of results. Any migration study related to HPAI will need to be conducted collaboratively between virologists, biologists and ecologists with experience in bird migration and scientists with access to a stable isotope laboratory facility. Stable isotope analysis is a costly procedure and it is essential that appropriate links are made that will ensure the right approaches to determine migratory connectivity are made and that a well-designed project that will answer the questions on virus dissemination and wild bird migration.

The IRMS used most frequently for SIA is the continuous flow isotope ratio mass spectrometer (CF-IRMS). These machines enable relatively low cost analysis, a high degree of automation and a high throughput. A technique known as comparative equilibration using CF-IRMS is used for δD analysis for migration research. This approach allows comparative results between different laboratories, ease of use and rapid, automated sample throughput of samples (Wassenaar and Hobson 2003). It requires the inclusion of pre-calibrated keratin working standards along with the unknown tissue samples. The hydrogen isotope exchange between ambient moisture in the air and the keratin standard and the samples is identical. The comparatively equilibrated samples and standards are then isolated from the atmosphere and analyzed together in a single session. Measurement of stable hydrogen in tissues is performed on H_2 derived from high-temperature flash pyrolysis. Pure H_2 is used as the sample analysis gas and the isotopic reference gas. A high temperature Elemental Analyzer and autosampler pyrolyzes samples to H_2 gas. The pyrolysis column consists of a ceramic tube filled with glassy carbon chips held at 1250–1350 °C, followed by molecular sieve gas chromatography column at 80–100 °C. The IRMS makes measurements in the following way: gases enter a white-hot region where electrons are boiled off and the sample gases are ionized. These ionized molecules have a positive charge and they are passed through a magnetic field that separates them according to their atomic mass and isotopes with the resulting ion beams focused into collectors for counting. Computers then calculate the final isotope values. The stable isotope ratios are then reported as a series of delta (δ) notations of positive or negative numbers expressed as parts per thousand (‰) relative differences to an international standard. All δD results are expressed in units per mil (‰) relative to the Vienna Standard Mean Ocean Water using the previously calibrated keratin standard.

One of the most important practical issues in using δD measurements to track large-scale terrestrial animal movement is the problem of hydrogen exchange in organic samples It is well established that δD measurements of feathers (and other organic materials) are problematical compared to $\delta^{13}C$ and $\delta^{15}N$ analyses due to the problem of uncontrolled hydrogen isotopic exchange between "exchangeable" organic hydrogen in the feathers and isotopically variable ambient moisture in the laboratory environment. If left uncorrected, δD measurements of the total hydrogen in an identical feather will yield different results at analytical laboratories located at different geographic locations, as well as over time due to geographical and seasonal changes in the hydrogen isotopic composition of ambient moisture. This would make it impossible to compare δD results between different laboratories. Wassenaar and Hobson (2003) showed how keratin δD analyses by CF-IRMS technology and the use of keratin standards and comparative equilibration can be used to provide cost effective and rapid δD analyses for migration research to provide cost effective and comparable δD results on feathers and other keratin-based samples among different laboratories. Three in-house keratin standards were prepared from chicken feather cow hoof and bowhead whale baleen. Each keratin type came from a single geographic location. All of them were solvent cleaned, dried, cryogenically ground, and homogenized. They were then analyzed 6 times each using the offline equilibration dual-inlet method described by Wassenaar and Hobson (2000).

The target weight of feather (and in-house keratin standard) required for routine online hydrogen isotope analysis by CF-IRMS in our laboratory was 350 ± 10 μg. Feather samples were cut from the same position on each feather and weighed using a microbalance, and transferred into 4.0 mm 3.2 mm isotope grade silver capsules. Capsules containing the keratin standard powder or feather were folded into tiny balls. All weights were recorded and the samples were stored in 96-well ELISA plates, loosely covered with the lid. Samples and keratin standards were then allowed to "air equilibrate" on the shelf with ambient lab air moisture at room temperature for >96 h prior to stable hydrogen isotope analysis. The hydrogen exchange process could be sped up by holding the samples and working standards in an oven at higher temperatures since showed that complete exchange with moisture occurs in less than 30 min at temperatures above 100 °C. For practical purposes, however, a 96-h equilibration at room temperature was deemed sufficient and easier, and could be easily implemented as a standard laboratory analysis procedure. Following 96-h comparative equilibration, all samples and standards were immediately loaded into the auto sampler carousel of the CF-IRMS and analyzed for δD.

The uncorrected hydrogen isotope values of the keratin standards from each CF-IRMS auto run were subjected to a least squares regression to derive a correction formula to be applied to all the keratin standards and feather samples within that auto run. A typical run included an in-house keratin standard at the beginning, 2 keratin standards after every 10–12 unknowns, and 2 or 3 keratin standards at the end of the auto run. The authors recommend that researchers performing δD analyses adopt this protocol in order to provide findings that are comparable among laboratories. They also suggest that the development and distribution of feather and keratin working standards that have a wide range of δD can be done on an individual basis, or preferably, developed and distributed as a future collaborative effort among avian researchers and stable isotope laboratories that provide feather δD analyses. Since CF-IRMS enables δD analyses to be conducted on small amounts of tissue samples it is possible intra-sample variance in the hydrogen isotopic due to any biological heterogeneities could exceed interpretations of environmental isotope. To help resolve this, feathers were obtained from captive birds to examine isotopic variance expected due to sample size, location, and heterogeneity factors, and from selected wild birds to examine isotopic variance due to these and to additional dietary or location changes during feather growth (Wassenaar and Hobson 2006). Captive bird feathers were sub-sampled along the vane on either side of a single feather at masses of 0.25, 0.35, 0.45, 0.6, 1.0 and 2.0 mg, and along the rachis. The results showed consistency of feather δD measurements across a wide range of sample masses. Within-feather δD isotopic variance for captive and some wild birds was as low as ±3‰ for vane material, which corresponds to a geospatial resolution of about 1° of latitude in central North America. Intra-sample variance for the rachis was ±5‰, with lower δD values for both wild and captive birds. Nevertheless, the authors recommend researchers assess the degree of intra- and

inter-sample hydrogen isotopic variation in the selected tissue growth period for the species of interest before geospatial interpretations of origin are attempted.

2.6 Approaches for Determining Migratory Connectivity

Patterns in stable isotopes derived from the environment are translated into food webs and the discrimination between stable isotopes and dietary sources and the feather is predictable and constant in time and space. The isotopic values of an animal are assumed to be representative of the location at which the feathers was grown and when the bird moves elsewhere it can be sampled to infer its previous geographic location. In order to infer the location most likely associated with the feather isotopic values it is necessary to calibrate the geographic model using tissues from birds of known origin. The accuracy of the IRMS measurements is best when using standards with chemical composition similar to the unknown samples (e.g. keratin standards to calibrate feather samples). Also, for geographical assignment it is best that the standards have similar attributes, i.e. the same species and isotopic values as the unknown samples. A calibration dataset should include samples from all areas from which the migrant of interest could have originated. However, sampling tissues from all potential places of origin is costly and logistically difficult if not impossible if the areas are remote and beyond the reach of sampling and collection networks.

The simplest method of assigning a bird to an isoscape is to define the geographic gradients based on maps of isotopic values, measure an individual and then assign it to the area that corresponds to the isotope values obtained. The spatial patterns are generated from indirect sources of information such as rainfall. The earliest studies on migration utilized this technique (Hobson and Wassenaar 1997) to use δD to study migratory birds and it is still in common use (Hobson et al. 2006, 2007). The method is simple to understand and apply and no additional computation is required to place birds in their geographical locations. More advanced methods for determining the origin of migratory animals require some degree of statistical manipulation. Assignment methods require the definition of all possible locations of origin, then characterizing these locations with the distribution of stable isotope data. The characterizing should, if possible, be derived from measurements of birds known to have grown tissues at those locations and all possible regions of origin are sampled. Once a calibrated assignment model is obtained, stable isotope measurements from individuals of unknown origin are used to determine their origins.

Although migratory individuals have only to be captured once in order to determine their migratory movements the assignment of their origin is challenging and it is necessary to make inferences about the history of an individual using the stable isotope measurement. Table 2.5 details some of the different modelling approaches that have been applied to estimate origin when using stable isotopes.

Table 2.5 Methods for assigning animals of unknown origin using stable isotopes. Reproduced with Permission from Wunder and Norris (2008a)

Level of complexity	Model type	Description	Advantages	Disadvantages	Incorporates sources of error	References
Low	Map lookup	Isotope value of animal assigned to area based on isoscapes on map	Easy to implement	Does not incorporate spatial variability of isotopes	No	Hobson et al. (2007)
	Linear regression	Origin inferred on regression of isotopes on latitude and/or longitude	Easy to implement	Does not incorporate spatial variability of isotopes within regions	No	Kelly et al. (2002)
	Classification trees	Origin inferred on basis of hierarchical, discrimination based decision rules	Can be applied to multiple isotopes. Does not require distributional assumptions	Does not incorporate most error, no a priori hierarchy for multiple isotopes does not provide degree of certainty for branching decisions	No	Hebert and Wassenaar (2005)
	Likelihood-based assignment	Origin inferred from probability density functions for isotope values from given regions	Can be applied to multiple isotopes. Provides probability of assignment for a given individual, easy to implement	Does not incorporate most error, simply assigns regions for individual based on highest likelihood value; requires sampling all potential regions of origin	Some	Kelly et al. (2005)

Level of complexity	Model type	Description	Advantages	Disadvantages	Incorporates sources of error	References
	Likelihood with priors	Same as above but adds prior information on isoscapes using Bayes' Rule	Can be applied to multiple isotopes and can utilize non-isotopic information	Does not incorporate most error. Simply assigns region for individual based on highest posterior probability; requires sampling all potential regions of origin	Some	Norris et al. (2006)
	Stochastic extension of likelihood	Same as above but adds sources of error associated with isotope data	Can be applied to multiple isotopes, incorporates multiple sources of error and provides a range of assignments for a given individual	Requires intensive computing	Yes	Wunder and Norris (2008b)
High	Probability Surfaces	Models stochastic error process over mean field surface	As above, incorporates known variance sources	Requires intensive computing	Yes	Wunder (2010)

2.6.1 Classification Trees to Predict Origins

Ecological data are often complex, unbalanced, and contain missing values. Relationships between variables may be strongly nonlinear and involve high-order interactions. The commonly used exploratory and statistical modelling techniques often fail to find meaningful ecological patterns from such data. Classification trees are statistical techniques ideally suited for exploring and modelling such data. They have a number of advantages over discriminant function analysis and linear regression, both of which are often used in stable isotope assignment studies. Classification trees represent a statistical technique well suited for modelling ecological data since the model development is hierarchical and based on logical if-then conditions and are both nonparametric and nonlinear (De'ath and Fabricius 2000). Hebert and Wassenaar (2005) used the classification tree approach to determine if ducks originating from different geographic areas had unambiguous multi-isotopic signatures. They used a multi-stable isotope analysis ($\delta^{34}S$, $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$) of secondary feathers from wild duck (*Anas platyrhynchos*) and northern pintail (*A. acuta*) ducklings from 52 sites in western North America. Ducklings from Alaska, northern Canada, the Prairies and California could be distinguished based upon their feather isotope values. Classification trees were developed by repeatedly splitting the data via algorithms that partition the data into mutually exclusive groups (De'ath and Fabricius 2000). Geographic patterns in feather isotopes were related to natural gradients produced by biogeochemical cycles and anthropogenic factors such as agrochemical usage.

2.6.2 Likelihood-Based Methods to Predict Origins

Discriminant analysis can be used to classify a sample into two or more classes (regions) and has been employed in determining the origin of birds. Parameters for region-specific likelihood functions are estimated from isotope data collected in each predefined region. The likelihood functions for each region are then evaluated for the isotope value measured for an individual of unknown origin and that individual is placed in the region with the highest-valued likelihood (or probability).

In studies on golden plovers (Prosser et al. 2006) interpolated maps were used of annual average δD values collected at stations across the wintering grounds of each of two species of plover to generate a priori predictions for changes in δD in feathers between breeding and wintering grounds (IAEA 2001; Bowen et al. 2005). Because there was no information on the specific wintering location of each bird, the expected range of δD feather values was evaluated from these maps (Bowen et al. 2005). The annual averages of deuterium in the precipitation across wintering areas were used to predict feather δD values. To assign individuals to group of origin, the principle

of "assignment with exclusion," was used, a method adopted from population genetics that uses an exclusion criterion to reject unlikely sources. The cross-validation procedure in discriminant analyses as an assignment method was used to classify feathers into known group of origin based on the three isotopic values. The limited number of sample contributed to low power of some assignments, but power was increased by using three isotope values for each sample.

2.6.3 Migration Studies Using Stable Isotopes

The first premise for using stable isotopes is that there are differences in the spatial distribution of the isotopes from where the migrants obtained their diet. The stable isotopes δD and $\delta^{18}O$ have the most predictable geographic gradients, but gradients for other light isotopes are less well known. Geographic patterns of stable isotopes are inferred from a number of sampling points across the landscape. The δD values in precipitation have been modelled for North America (Hobson and Wassenaar 1997) where the relationship between δD in feathers and growing season precipitation was examined for neotropical migrant songbirds breeding over a continent-wide isotopic gradient. The δD values were determined on feathers of 140 individuals of 6 species of wild insectivorous forest songbirds (*Setophaga ruticilla, Empidonax minimus, Vermivora peregrinus, Catharus ustulatus, Seiurus aurocapillus, Hylocichla mustelina*) taken from 14 breeding locations across North America. The δD of feathers was strongly correlated with the δD of growing season precipitation at breeding sites across North America. As feather hydrogen is metabolically inert after growth, this relationship was then used to assess the breeding origins of wintering migrants. Deuterium values of feathers from 64 individuals representing 5 species of migrants (*Helmitheros vermivorus, Wilsonia citrina, Hylocichla mustelina, Dumetella carolinensis, Seirus aurocapillus*) at a wintering site in Guatemala were consistent with those predicted from the known breeding ranges of these species. Bowen et al. (2005) described a method for interpolation of precipitation isotope values to create global base maps of growing-season (GS) and mean annual (MA) δD and $\delta^{18}O$. The use of these maps for forensic application was demonstrated using previously published isotope data for bird feathers in North America and Europe. The precipitation maps show that the greatest potential for applying both hydrogen and oxygen isotope forensics exists in mid- to high-latitude continental regions, where strong spatial isotope gradients exist. They showed that feather δD/precipitation δD relationships have significant predictive power both in North America and Europe, and zones of confidence for the assignment of origin could be described using these predictive relationships. These maps are available in GIS format at http://www.waterisotopes.org.

2.6.4 Determining Migratory Connectivity for Waterfowl in Asia

Although Asia is a prime focus for HPAI, and despite considerable interest in conservation and disease surveillance issues involving waterbirds in that region investigations on migratory connectivity in wild birds that might be implicated in the spread of the disease are few (Chang et al. 2008; Pérez et al. 2010). The great cormorant (*Phalacrocorax carbo sinensis*) is a potential carrier of HPAI to Taiwan and to better understand the migration of this bird, SIA studies were conducted on several populations from different breeding sites and overwintering grounds (Chang et al. 2008). Rather than capturing and removing feathers from live adult birds, newly dropped feathers found lying on the ground including primary, secondary and tail feathers at collected from three breeding sites in China (Qinghai Lake), and Russia (Ussuri River, and Chita Peninsula) and overwintering grounds in Taiwan (Kinmen Lake). All the collected feathers were newly dropped because these feathers were neither weathered, nor damaged, nor covered by a layer of dirt or dust. They were collected from different individuals by collecting them under widely separated nests at breeding sites and under separated roosting trees. Although δD values of feathers have typically been compared with the δD of precipitation to infer migration, the distributions of δD and $\delta^{18}O$ in precipitation across Asia are not as precisely known as in North America and Europe as there are fewer data collection sites and the frequency of data collection is more sporadic in Asia. This makes it more difficult to infer the origins of birds by comparing the δD values of precipitation and of feathers in Asia alone hence, a direct comparison of $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and δD values of feathers collected at both wintering and breeding sites was used to infer breeding locations of *P. c. sinensis* wintering at Kinmen. Analysis showed that it was unlikely that *P.c.sinensis* wintering at Kinmen came from the breeding sites in China and Russia.

Analysis of the data collected from the *P.c.sinensis* wintering at Kinmen, produced nine clusters plus two outliers and approximately 65 % of the birds overwintering at Kinmen come from four breeding populations. Using $\delta^{13}C$, δD, $\delta^{15}N$ or $\delta^{18}O$ values, excluded the possibility that cormorants wintering at Kinmen came from breeding populations at Qinghai Lake, Ussuri River or the Chita Peninsula. The general locations of possible breeding sites were based on the comparisons of feather δD values with the mean δD of precipitation during the breeding season according to the best available δD isocline maps of Asia, from which the data are spatially and temporally discontinuous. This comparison suggested that approximately 50 % of the *P.c.sinensis* wintering at Kinmen come from five breeding populations from the region around Lake Baikal or an area encompassing the Amur, Khabarovsk and Primorsky regions of Russia. The study showed that SIA has the potential to provide migratory information at the population level, to uncover the possible existence of previously unknown breeding populations, and to make predict migratory and possible future transmission pathways of HPAI. Moreover, combining multivariate analyses of stable isotopes with analyses of the phylogenic trees

of birds and HPAI may provide an opportunity to better understanding the interaction of bird migrations and the spread of HPAI.

In another study (Pérez et al. 2010) feather sample were collected from Barheaded Geese (*Anser indicus*), Whooper Swans (*Cygnus cygnus*), Mongolian Gulls (*Larus vegae mongolicus*), Curlew Sandpipers (*Calidris ferruginea*) and Pacific Golden Plover (*Pluvialis fulva*) at seven sites in northern Mongolia. After cleaning to remove oils, stable-hydrogen isotope (δD) analysis of one feather per individual was conducted at the stable isotope facility at the National Hydrology Research Centre, Saskatoon, Saskatchewan, Canada. Isotope analyses followed the comparative equilibration technique described by Wassenaar and Hobson (2003). Deuterium isotope ratios were expressed in δ notation in parts per thousand (‰) relative to the Vienna Standard Mean Ocean Water Standard Light Antarctic Precipitation (VSMOW-SLAP) scale. Measurement precision based on replicate measurements of within-run keratin standards was estimated to be of the order of ±2‰. Estimates of continental deuterium patterns in mean growing-season precipitation (δDp) for Asia were derived from Bowen 2009 (www.waterisotopes.org). Continental δDp data show an expected general decrease in deuterium abundance in precipitation with increasing latitude and altitude in Asia and form the basis for predicting the expected abundance of deuterium in feathers (δDf) from the average δDp (Hobson and Wassenaar 1997; Hobson 2008). Three major feather isotopic source areas in Asia were identified: Region A – northern Asia (<−138‰), B – catchment area (−138 to −104‰) and C – southern Asia (>−104‰) were delineated. The isotopic interval of 34‰ presented for Region B corresponded to latitudes ranging from approximately northern China to mid-Russia, and Regions A and C were north and south of those latitudes, respectively. These arbitrary cut-off values in δDf were chosen because they bracketed Region B between the lowest and highest 95 % CI out of the expected mean annual δDp values for the latitudes, longitudes and altitudes of the sampling sites. Once more precise information on the relationship between δDp and δDf can be established for Asia, more refined assignment will be possible (Hobson et al. 2009). Feathers from adult Pacific Golden Plover δDf had values of −33‰, clearly indicated that these feathers were moulted in southernmost Asian latitudes, suggesting that the feathers were probably grown on or near the wintering grounds prior to spring migration. Locally grown feathers from adult Barheaded Geese and Whooper Swans had more depleted δDf values than any other group because they live in a complex of lakes and ponds fed by high elevation runoff, and the waters are more depleted in deuterium than adjacent areas. Curlew Sandpiper had δDf values consistent with Region B indicating isotopic equilibrium with diet on their sampling area. The study demonstrated that stable isotopes could be used to improve knowledge of migratory and moulting patterns of wild waterbirds in Asia in a rapid and cost effective manner. However, additional studies are required since the δDp isoscape for Asia is so poorly described. Ground-truthing the relationship between δDf and δDp over a large geographic gradient in Asia will provide more information to derive appropriate isotopic calibrations (Wunder and Norris 2008b; Wunder 2010).

Arrival time on breeding or non-breeding areas is likely to be of interest in epidemiological studies exploring dissemination of HPAI by migratory birds. Precisely assessing the arrival time of individuals can be difficult, but by measuring carbon stable isotope turnover in avian blood it is possible to estimate arrival time for birds switching from one habitat to another (Oppel and Powell 2010). Stable carbon isotope ratios ($\delta^{13}C$) in blood assimilate to a new equilibrium following a diet switch according to an exponential decay function. Stable carbon isotope ratios in tissues reflect the isotope ratios of food sources (Hobson and Clark 1992). When migratory birds switch habitat their new diet will have a different isotopic signature if the two habitats are isotopically distinct (Peterson and Fry 1987). This switch to a new diet causes the isotope ratios in blood to change gradually over time until they reflect the isotope ratio of the new diet (Hobson and Clark 1992; Evans Ogden et al. 2004; Morrison and Hobson 2004). The rate of change in $\delta^{13}C$ is tissue dependent. For example, blood plasma generally assimilates to the stable isotope ratio of a new diet within a few days (Hobson and Clark 1992), whereas whole blood or the cellular fraction of blood (red blood cells, RBC) turn over within several weeks (Bearhop et al. 2002; Evans Ogden et al. 2004; Morrison and Hobson 2004). Several experimental studies have determined that isotopic turnover in blood closely follows an exponential decay function (Evans Ogden et al. 2004; Carleton and Martinez del Rio 2005). This relationship can be used to determine the time an animal switched diets if the isotope ratios of the old and the new diet are known.

A study of the arrival time of Eider Ducks (*Somateria spectabilis*) at breeding grounds (Oppel and Powell 2010) was estimated using only a single tissue (Phillips and Eldridge 2006). Since the turnover rate of stable carbon is not known in many bird species a mass dependent turnover rate constant was utilized in calculating the arrival time (Carleton and Martinez del Rio 2005). Data from whole blood and RBC measured in captive experiments were used to validate the approach of estimating the time since a diet switch. In each experiment, birds were switched from one isotopically distinct diet to another differing by at least 3 % in $\delta^{13}C$ (Hobson and Clark 1992, 1993; Bearhop et al. 2002; Evans Ogden et al. 2004). This provided data to develop a formula that could be used to determine arrival time. If the isotope signatures of a tissue from both the old and new environment are known and birds are captured at an unknown time after arrival, then blood $\delta^{13}C$ can be used to determine the time since elapsed since arrival. The isotopic signature of each diet can be characterized by sampling blood from birds that have been feeding in either environment for several weeks, or by using tissues that are metabolically inert after growth. Feathers sampled from the bird may be useful to determine $\delta^{13}C$ of the previous environment if the moulting strategy of the bird is sufficiently known and certain feathers are always grown in the previous environment. Plasma or RBC sampled from a bird captured at a later stage can be used to determine new $\delta^{13}C$ once birds have reached equilibrium with the new diet (Morrison and Hobson 2004). For breeding grounds, eggshell membranes collected from old nests provides a simple way to characterize the diet of birds (Oppel and Powell 2010).

Stable isotopes may also provide clues to help locate nonbreeding populations of birds that have unknown winter ranges. For instance, no records exist for the Coastal Plain Swamp Sparrow (*Melospiza georgiana nigrescens*) from October to late April because of the difficulty in tracking individuals between seasons. Stable isotope analyses of C, N, and H were used to predict where moult occurs and then those areas were then searched for individuals of this species (Greenberg et al. 2007). Feathers were clipped from the crown and rump and lower back of the birds. Analysis of the crown feathers was used to predict the δD value at the unknown site of winter migration. Selection of the sites was based on the estimates of isotopes in precipitation reported by Bowen and colleagues (www.waterisotopes.org). The $\delta^{13}C$ and $\delta^{15}N$ of rump feathers were consistent with the Sparrow moulting in more saline marshes. The values for the same isotopes from crown feathers revealed that winter moult in the Sparrow probably occurred in similar coastal brackish habitats. The δD of crown feathers indicated that pre breeding molt occurred at latitudes between South Carolina and Virginia. A subsequent search of this region located specimens of the birds, all of which were found in North Carolina or southeastern Virginia. The Sparrows were found predominantly in brackish marshes similar to their breeding habitat. On the basis of these observations, it appears that Coastal Plain Swamp Sparrows undergo a short southerly migration to a coastal region with substantially warmer winter conditions. This study was the first to make a specific geographic prediction based on stable-isotope analysis and to test the prediction in the field.

Many birds that pass through Africa on their migration cross the Sahara or Arabian deserts without delay, then stopover in northern tropical areas for several months before resuming their flights to the south. During the stopover they complete a partial or complete moult (Yohannes et al. 2007). Stable nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and hydrogen (δD) isotope profiles in feathers of nine migratory bird species trapped in Kenya were examined to test the extent to which they were segregated, geographically or by habitat, during their earlier autumn migration stopover in northeast Africa. The aim was to determine if isotopic differences between species varied between years, and whether the isotope profiles of individual species appeared to be consistent. The relationship between mean feather $\delta^{13}C$, $\delta^{15}N$ and δD assorted the migrants into several clustered groups. Similar feather isotope values among successive years revealed that each species tended to return to the same or similar stopover areas and selected habitat and diet that generated similar isotopic signatures. However, the stopover sites that the birds used were not identified.