The use of stable-isotope ratios in ornithology

Tony (A. D.) Fox and Stuart Bearhop

ABSTRACT The use of mass spectrometry to analyse the stable-isotope ratios of bird tissues has become an important new tool for research ornithologists in the last 20 years. Because stable isotopes vary geographically and according to specific biological processes in the environment, they provide a unique forensic means of understanding more about avian biology and ecology than we can learn using conventional techniques alone. The stable-isotope ratios present in different tissues of birds reflect the ratios in the environment at the time those tissues were constructed. However, because of the rapid turnover of some tissues compared with others, an individual bird will bear within its body constituents a record of its present and past exposure to different isotopic environments. Because the stable-isotope ratios of specific elements vary geographically (e.g. hydrogen along oceanic to continental gradients) and between habitats (e.g. nitrogen and carbon in marine versus terrestrial ecosystems), they offer a unique means of studying the ways migratory birds move between different parts of the planet and of understanding the habitats they exploit. This review looks at some of the innovative ways in which the technique has been used in a variety of recent studies of birds. The technique is not restricted to breaking new ground in high science; in terms of understanding migration strategies, the importance of breeding, migratory and wintering habitat and the feeding ecology of birds, the study of stable-isotope ratios is becoming ever more important in supporting conservation actions.

It is lucky for us, and for contemporary ornithology, that many chemical elements come in more than one form. You might not think so at this point but, hopefully, no matter how boring you find sub-particulate chemistry, by the end of this article you will have been persuaded that this is indeed the case! Although geologists have studied stable-isotope ratios in materials for almost 50 years and their use, therefore, is not new, their application to the study of birds has taken off only in the last 10–20 years, as access to the instrumentation required for their analysis (mass spectrometers) has improved and analytical procedures have become cheaper and more efficient. As our understanding of the processes affecting the distribution and abundance of stable isotopes in nature increases, so does the number and range of potential applications. It therefore seems timely for a brief review of this rapidly evolving field of ornithology, to see how widely the techniques have been applied to date and to consider the potential for future applications.

The term ‘stable-isotope ratios’ sounds complex, but the concept is in fact relatively simple. In nature, each chemical element tends
to occur in one or more forms, called isotopes. These isotopes differ in the number of neutrons present in each atom; the more neutrons, the heavier the atom and the greater the atomic mass. Stable isotopes persist over prolonged periods of time – unlike radioactive ones, which are unstable and decay rapidly so that their ratios in the environment change more quickly over relatively short time periods. The important point here is that the ratios of many stable isotopes of common elements in the environment reflect well-defined natural processes which often differ in time and space. For example, hydrogen is an abundant element that has two stable isotopes, the normal ‘light’ form (conventionally denoted as \(^{1}\text{H}\), because it has one proton and no neutrons in its atom, so there is just one subatomic particle in the nucleus) and the ‘heavy’ form (also known as deuterium, and denoted \(^{2}\text{H}\) because it has two particles in its nucleus, one proton and one neutron). Deuterium is relatively rare in nature – sea water contains one deuterium atom to approximately 6,500 light atoms of hydrogen. Nevertheless, despite the fact that deuterium is always much the rarer member of the pair, the actual ratio of these two stable isotopes varies substantially around the globe, for instance with ambient temperature. It is this type of variation in stable-isotope ratios that offers such a remarkable range of possibilities, many of which are discussed below, which we can use to improve our understanding of the natural world around us.

The atomic mass of the hydrogen responsible for forming water makes a difference to its physical properties. ‘Heavy water’ is formed from oxygen combined with the isotope deuterium (atomic mass 2) and is denser than ‘light’ (ordinary) water, which is formed from oxygen and hydrogen (atomic mass 1). Heavy water tends to be precipitated out of clouds before light water, so as moist air travels inland, the amount of heavy water is increasingly depleted. This means that highly continental areas (such as the middle of the Russian Arctic) receive much lower levels of deuterium in precipitation compared with Europe’s western seaboard, which experiences a much higher relative proportion of heavy water in precipitation. A similar process happens with increasing altitude, meaning that in mountainous areas, we can also expect deuterium to be less abundant in precipitation than in comparable lowland areas.

All this gives us good reason to believe that

---

**Box 1. Measuring stable-isotope ratios using mass spectrometry**

Stable-isotope ratios are measured using a mass spectrometer. This equipment measures the ratio of the electric charge of a particle in relation to its mass. This is achieved thanks to Newton’s second law of motion, namely that the acceleration of a particle is inversely related to its mass. The mass spectrometer vapourises the sample and converts the constituent parts into ions, charged particles that are then accelerated from a chamber and concentrated into a narrow beam which is subjected to a strong magnetic field. Because each isotope atom has a unique combination of mass and charge when ionised, the magnetic field bends the stream of particles to a different degree dependent on these two characteristics. For instance, light hydrogen will be deflected less by the magnetic field than heavy hydrogen, so the stream of concentrated ions comprising a mixture of these two isotopes will separate in the chamber and arrive at two different points in the detection part of the apparatus, dependent on their mass. Detectors can be positioned in the detector chamber, according to the degree to which the ion stream of a specific element of specific mass and charge would be expected to be deflected. To determine the relative abundance of one isotope to another, the machine measures the relative charge arriving at the different positions expected for each isotope. For hydrogen and many common elements, this means collecting the electric current from two streams originating from the two different isotopic forms, but for some elements with more isotopes, more detectors are required (see below). Although the theory is straightforward, in reality the differences in mass between isotopes of the same element are typically incredibly small and the less abundant isotopes in a sample usually very rare, so modern mass spectrometers have to be extremely sensitive to obtain accurate measurements. The wider application of stable-isotope research in ornithology in recent years has undoubtedly been due to both the increased availability of such machines in universities and research establishments throughout the world and the technological developments allowing rapid throughput of large numbers of samples.
the stable-isotope ratios of hydrogen in rainwater (and hence in groundwater) show some predictable geographical patterns – but how does this affect birds? All biological activity relies on water, and living organisms not only take up water from their environment but are totally dependent on it for maintaining all of their cellular activity. Hence, the hydrogen stable-isotope ratio of the bodies of all living things will reflect that of the environment in which that organism grew up. For example, Bullfinches Pyrrhula pyrrhula in Britain (subspecies pileata) and Denmark (subspecies coccinea) have hydrogen stable isotopes in their feathers that are characteristic of the oceanic groundwaters of western Europe, whereas those of the large nominate subspecies pyrrhula, originating from northern Scandinavia and continental Russia, show more depleted levels of heavy hydrogen in their feathers (see fig. 1 and Newton et al. 2006). The stable-isotope ratio of oxygen shows similar patterns, so the ratio of $^{18}\text{O}/^{16}\text{O}$ (the two commonly occurring natural isotopes of oxygen) shows very strong gradients in groundwater that correlate with temperature and oceanic–continental and upland–lowland gradients similar to those of hydrogen.

**Fig. 1.** Mean (± standard errors) hydrogen stable-isotope determination ratios of feather samples taken from Bullfinches *Pyrrhula pyrrhula* caught in Nimtofte, eastern Denmark. Birds were either non-breeding migrants from Scandinavia and Russia (subspecies *pyrrhula*, shown by diamond symbols) or resident, locally bred birds (subspecies *coccinea*, shown by triangle symbols); filled symbols indicate females, open symbols indicate males, for both samples. The data are those presented in Newton et al. (2006). Given the hydrogen stable-isotope gradients in groundwater, the predicted range of values in feathers grown in Denmark and Scandinavia/Russia are shown as shaded areas for the two regions as indicated, based on contour maps in Hobson et al. (2004a) (see also fig. 3).
These geographical patterns of isotope ratios in rainfall and groundwater (and hence by inference in bird tissues) have been established for many years on a continental scale, both in North America (Hobson & Wassenaar 1997) and in Europe (Hobson et al. 2004b), and, recently, on a global scale (Bowen et al. 2005). As with all living organisms, when birds absorb chemicals from water and their food, their metabolism subtly changes the stable-isotope ratios so that, when converted into their own tissues, they are slightly different from those in the environment from which they were obtained. Thanks to field and laboratory studies, our knowledge of how the stable-isotope ratios of particular elements in bird diet change when they become incorporated into different avian tissues (so-called diet-tissue fractionation or enrichment rates; see Hobson & Clark 1992a,b, Mitzutani et al. 1992) is now increasing. What this means is that birds are effectively carrying around a record of where they have been and what they have been eating in the stable-isotope ratios of their tissues. And, if we know something about how these ratios vary across different areas and different food types, then, based on these tissue isotope ratios, we can make inferences about where birds have been and what they have been feeding on.

**Migratory patterns traced by feather stable-isotope ratios**

Now things start to get interesting. The situation is relatively simple if a bird is sedentary. Under these circumstances, the stable hydrogen (and oxygen) isotopes of the various organs and tissues of the bird (feathers, claws, blood, bones, fat, muscle, etc.) will all reflect the composition of the local precipitation in the areas where that bird was raised and lived its life. But what if that bird migrates from, say, the Russian Arctic (extreme continental environment) to western Europe (moist oceanic environment)? How will the stable isotopes in various bodily organs differ? To answer this question, it is important to realise that different body parts are subject to different rates of renewal, another process that will affect the stable-isotope composition or ‘signature’ of different tissues. In birds, the claws grow slowly and continuously, while feathers tend to be grown during relatively short and discrete periods. An important point about these two tissues is that they are inert, in the sense that they are metabolically inactive. Hence, the stable-isotope ratio of a feather will reflect the highly specific isotopic environment in which it was grown, and this information will remain ‘locked away’ in that feather until it is shed at the next moult. Some birds regularly replace different feather tracts at different stages of the annual cycle; and many do so in quite different areas along their migratory corridor. This means that the stable-isotope ratios of adjacent feather tracts on a bird may provide information on habitat selection and diet from multiple discrete periods in the past. Stable-isotope-ratio-analysis therefore provides a powerful tool than can (potentially) reveal the secrets of where a particular bird has been during a migratory episode. This is extremely useful in cases where, for example, it is known that certain feathers were grown in the natal areas while others were replaced on the wintering grounds – because if the isotopic environments of these two places are radically different, then, even with less than a milligram of feather material (see Box 1 and Wassenaar & Hobson 2006), it becomes possible to distinguish these differences in fragments of feathers.

We recently used this technique to show that a first-winter Baikal Teal *Anas formosa* from southeast Denmark in November 2005 was almost certainly a wild bird (fig. 2 and Fox et al. 2007). It had been shot mistakenly as a Eurasian Teal *A. crecca* and submitted as part of the Danish national scheme (organised by the National Environmental Research Institute) to sample the age and sex ratios of huntable species. In this case, it was clear that the wing feathers and some of the old, notched and worn tail feathers would have been grown on the site where the bird was hatched and reared, whereas adjacent new, unworn tail feathers had been grown in the previous few weeks. We were also able to find body feathers on the neck that showed signs of peripheral wear, in contrast with others that were clearly of more recent origin. We reasoned that if the bird was genuinely wild, the old feathers grown on the Siberian breeding grounds would show signs of being grown in a highly continental isotopic environment, namely with highly negative hydrogen and oxygen stable-isotope ratios (see fig. 3). In contrast, the newly grown feathers should reflect stable-isotope ratios more characteristic of the oceanic environment of western Europe. On the other hand, if the bird had been raised in captivity in Europe, it would be highly
unlikely that old and new feathers would show such a pattern, and more likely that both would reflect a western/oceanic hydrogen stable-isotope ratio, even accounting for possible variation caused by commercial feed provided in a captive environment. The mass spectrometer analysis confirmed that the old feathers showed highly depleted stable-isotope ratios of hydrogen and oxygen, quite unlike those of the new feathers, which matched those of Mallard A. platyrhynchos feathers from west European birds (fig. 2). The feathers grown before fledging matched the ratios expected based on the Eurasian isotopic contour maps of Bowen et al. (2005), strongly suggesting that the bird was indeed a genuine vagrant that had been raised in continental Siberia and migrated to Denmark in its first autumn.

In this case, we were fortunate to be able to test a well-founded hypothesis, comparing the results from mass spectrometry against our predictions to infer the route taken by this individual on autumn migration. The use of stable-isotope analysis is not an automatic means of proving vagrancy in all cases of rare birds, however, and a number of criteria need to be met before a potential case becomes suitable for application of the technique:

1. It must be possible to establish the bird’s age and the different feather tracts (and the state of these) that could potentially be used in any analysis.

2. Moult patterns and timing in the species concerned should be well described, so that the time of growth is known for specific feather tracts. This is necessary to pinpoint which feathers were grown when (and hence where) in the annual cycle.

3. It is important to be able to infer the potential route and timing of the migration that has taken the bird to the point of capture since the last moult of specific feathers.

4. There must be sufficient isotopic contrast between the environments in which the different feathers were grown, based on good available geographical knowledge (such as the maps of Bowen et al. 2006), because the technique is not suitable for pinpointing geographical origins with a high degree of accuracy.

Fig. 2. Plot of stable-isotope ratios of feathers from a Baikal Teal Anas formosa shot in southeast Denmark in November 2005 (open symbols). Feather samples come from: (1) worn neck feather; (2) secondaries; (3) old unmoulted tail feather; (4) primary upperwing-covert; and (5) secondary underwing-covert (all grown on the natal area). These can be compared with: (6) new body feather; and (7) newly grown tail feather adjacent to (3) (6 and 7 grown in the wintering area). The mean of samples from Mallards Anas platyrhynchos obtained in summer from western Europe is shown as a solid square ± standard deviations (from Hobson et al. 2004b). The approximate range of values for feathers regrown in western Europe and the breeding range of Baikal Teal (after Kear 2005) are shown as shaded blocks for the two elements concerned, based on the maps of Hobson et al. (2004a) and Bowen et al. (2005).
valuable to examine museum skins of species that are of similarly uncertain vagrancy status (e.g. Ruddy Shelduck *Tadorna ferruginea*), in cases where the above criteria are met, so that an objective assessment of the status of the individuals involved can be made.

**Using stable-isotope ratios of other organic elements for ornithological research**

The potential of the technique is by no means restricted to proving genuine vagrancy. There is an enormous range of possibilities to help us to understand linkages between different geographical areas used by migratory birds at different stages of their annual cycle. Although ringing recoveries provide much information and insight into migration patterns, in non-hunted species (especially passerines) recovery rates are typically minuscule; as a result, our knowledge is highly restricted and fragmented, and often biased according to the way birds with rings are recovered and reported by humans. Ringing recoveries of quarry species may often explain more about the distribution of hunting effort, for example, than that of the birds themselves! Analysis of stable-isotope ratios in avian tissues cannot offer pinpoint accuracy to the same degree as place of ringing and recovery, but it can provide excellent information on regional movements of individuals, allowing the identification of different migratory strategies and population structures within flyways.

It is not just hydrogen and oxygen that show geographical variation in isotopic ratio. For example, the stable-isotope ratios of carbon...
(\(^{13}\)C/\(^{12}\)C) and nitrogen (\(^{15}\)N/\(^{14}\)N) in zooplankton show a strong east–west gradient, with more enriched ratios of both elements in the Bering and Chukchi Seas relative to the arctic waters of eastern North America. Zooplankton support the entire food chain up to the level of top predators, such as King Eider Somateria spectabilis, and zooplankton carbon and nitrogen isotope ratios are transferred up the food chain in a predictable manner. King Eiders nesting in the central Canadian Arctic winter in both the Atlantic and the Pacific Oceans, and the very limited ringing recoveries available suggest that approximately half go in either direction. However, the situation is complicated by the fact that there is much higher hunting pressure in Greenland than elsewhere in the Arctic, so many more birds are recovered to the east than to the west. Mehl et al. (2004) used carbon and nitrogen stable isotopes in head feathers (which are known to be grown on the wintering grounds; Mehl et al. 2005) to show that, in contrast to the ring-recovery data, between two-thirds and three-quarters of birds in their sample had wintered in the west (Pacific) while only between a third and a quarter had wintered in west Greenland and the northwest Atlantic.

Stable-isotope analysis thus offers the potential of establishing a chemical link between breeding locations, migration stopover sites and wintering locations of individual birds. For example, by sampling the hydrogen stable-isotope ratios in the feathers of Wilson’s Warblers Wilsonia pusilla from museum skins (known to have been collected at specific breeding sites), it was possible to show that the technique was a good measure of breeding latitude. On this basis, monitoring of feathers sampled from autumn migrants in New Mexico showed that birds breeding farthest north migrated earliest in autumn (Kelly et al. 2002). The same authors gathered feather material from birds throughout the wintering areas, from Mexico down through Central America and were also able to demonstrate that birds breeding farthest north in North America wintered farthest south in winter. A classic example of leapfrog migration, this was completely unsuspected in this species and provides another example of a biologically significant result from stable-isotope analysis that was not apparent from limited ring-recovery data. A different migration strategy, that of ‘chain migration’, was discovered in a study of Sharp-shinned Hawks Accipiter striatus in North America (Smith et al. 2003). Hydrogen stable-isotope ratios were used to show that birds from lower latitudes passed through earlier and wintered farther south than those from higher latitudes.

Stable-isotope ratios can also be valuable in identifying previously unknown populations with distinct ranges. Using hydrogen stable-isotope ratios, Hobson et al. (2001) tried to link populations of Bicknell’s Thrush Catharus bicknelli breeding in northeast North America with known wintering grounds in the Dominican Republic. This they achieved very successfully but the study also identified a subpopulation of

First-winter male American Redstart Setophaga ruticilla, Connecticut, USA, September 2007. For many migratory species, the effort of arriving early on the breeding grounds is rewarded by having the best breeding territories to choose from. For American Redstarts wintering in Jamaica, stable-isotope work has linked winter habitat quality with arrival times; birds wintering in good habitats are in better condition for the return journey to North America, and leave earlier. Marra et al. (1998) showed that stable-isotope signatures of invertebrate prey differed between good and poor winter habitats, which in turn could be detected in the muscle tissue of birds arriving on the breeding grounds in spring (see pp. 122–123).
wintering birds with more depleted stable-isotope ratios than those measured in known breeding areas. This was unexpected and suggested that populations existed at higher elevations or latitudes than those previously known, prompting a search for the 'missing' thrush populations in southeastern parts of the boreal forests of Quebec. Using point counts and song playback in likely areas, two previously unknown nesting areas were eventually located. Analysis of feathers from these new populations produced values which corresponded with those of the more depleted ratio types found in the winter quarters (Hobson et al. 2004a), confirming the value of such forensic studies in extending our knowledge of avian distribution and dispersal patterns.

**Turnover rates of stable isotopes in different avian tissues and organs**

In contrast to inert feathers and claws, the body organs are maintained in an aqueous solution and supplied with energy and nutrients, derived partly from the bird’s own body stores, but predominantly from the environment in which the bird finds itself at a given point in time. Consequently, the stable-isotope ratios of blood respond more rapidly to the surrounding isotopic environment than those ‘locked up’ in feathers and claws. In fact, studies have shown that, even within the blood constituents, blood cells and plasma have different stable-isotope turnover rates: plasma generally replaces itself every 3–5 days whilst avian blood cells may have a half-life of up to four weeks (Hobson & Clark 1993; Hobson 2005). Hence, if the liquid and cellular fractions of a blood sample are separated, it is possible to compare isotopic dietary composition over the past few days (using plasma) with that of the past few weeks (based on the separated blood cells). Specific organs show other patterns over time, because they may be constructed and broken down at specific stages in the annual cycle when the bird is exposed to different environments with different patterns of isotopes. For example, breast muscles, typically built up during preparation for migration, will be formed using protein derived from food consumed at premigratory staging areas where such body changes take place. These staging areas may have very different stable-isotope signatures compared with both the wintering and the breeding grounds. Like the feathers, muscles may retain a record of a bird’s movement for particular periods of the life cycle. However, in contrast to feathers, muscle is replaced constantly and the original signal is gradually quenched over time. Other tissues, including bone collagen, may integrate stable-isotope ratios over longer periods of time, giving even more opportunities for their use in tracing the chemical history of a single individual.

**‘Capital’ versus ‘income’ breeders**

Such isotopic knowledge about specific organs is also important when it comes to understanding how females invest nutrients in their eggs, not least in deciding between the ‘capital’ (using body stores as the raw material for eggs)
versus ‘income’ (deriving nutrition for egg formation from diet) strategy (see Drent et al. 2006). This is particularly important for high Arctic breeders, such as geese, which have little time on the nesting grounds to accumulate the reserves of protein and fat required to form eggs. How important are the stores of nutrients and energy that these birds carry with them from the spring staging areas and even the wintering grounds, compared with those they can glean from the snow-covered tundra on their arrival? In situations where the isotopic signatures of wintering, spring staging and breeding areas differ (which, fortunately for us, is often the case), it is possible to gain some insights.

For example, based on the stable-isotope ratios of carbon (13C/12C) and nitrogen (15N/14N) in the adults and different parts of the eggs, it has been found that high Arctic Greater Snow Geese *Anser caerulescens atlanticus* derive more than two-thirds of the resources invested in their eggs from the tundra breeding areas, and that less than one-third is brought with them as body stores from elsewhere (Gauthier et al. 2003). In contrast, many high Arctic waders apparently do not mix nutrients stored in their body tissues from staging areas with those derived at the nesting grounds (Klaassen et al. 2001). In this case, Arctic invertebrates derived from tundra ecosystems have distinctly different stable-isotope ratios from those of the estuaries where the birds winter and stage in spring. Analysis of the eggs and down of hatchlings from ten different Arctic-breeding waders, including long-distance migrants such as Red Knot *Calidris canutus*, showed isotopic ratios characteristic of Arctic invertebrates rather than those of marine estuaries – showing that these Arctic waders are ‘income’ not ‘capital’ breeders as previously thought.

**You are what you eat – describing diet through the use of stable-isotope measurements**

Carbon and nitrogen are just as interesting when it comes to inferring what birds have been eating, as opposed to where they have been or where nutrient stores came from. Plants tend to contain less 13C than the atmosphere because the processes involved in carbon dioxide uptake tend to discriminate against 13C – the reason being that 13C is heavier than 12C (and is consequently slower to diffuse into leaves and cells) and forms slightly stronger chemical bonds than the lighter isotope. Almost all plants on the planet fall into two categories based on their exclusive use of one of the two contrasting ways of assimilating carbon dioxide into their cell metabolism. The vast majority (about 95% of known species) begin this process by forming pairs of three-carbon-atom molecules and are called C3 plants. The remaining 5% form four-carbon-atom molecules and are known as C4 plants; such plants tend to be adapted to warmer or more arid environments. Despite being in the minority, however, the C4 plants include some important crop species, notably some cereals and maize, which fractionate the two carbon isotopes differently from C3 plants. Plants can also differ in their stable-nitrogen-isotope signatures as a consequence of variation in a number of processes, with nitrogen fixation and soil chemistry being important drivers; and it is worth remembering that the latter is heavily influenced by agricultural activities such as fertiliser applications. All this means that there are different stable-isotope ratios of different elements in plant tissue, even when they grow in close proximity, which in turn means that we can differentiate the diets of, for example, Lesser Snow Geese *A. c. caerulescens* that feed on natural marsh vegetation, on farmed rice or on maize. The geese exploit three food sources with distinctively different carbon and nitrogen stable isotopes and if individuals specialise on any one food type, it shows up in their body tissues. By analysing stable-isotope ratios in individual geese, Alisauskas & Hobson (1993) showed that, when faced with a choice of winter feeding areas, individuals appeared to specialise on particular foods, a mechanism that appeared to define feeding subpopulations on the basis of feeding ecology and habitat choice.

The forensic knowledge locked in the feathers of an individual bird enables researchers to look back at precisely what that individual has been feeding on. As the Lesser Snow Goose example shows, this can highlight differences in feeding ecology, diet and habitat use between individuals. In that case, the same information could have been derived by collecting droppings from marked individuals, but that would be a laborious and in reality difficult project. For groups such as diving seabirds, however, it is generally impossible to get detailed information about what individuals
feed on and how they obtain their food. Using stable-isotope ratios in both feathers and blood, Bearhop et al. (2006) were able to show sex differences in the diets of Gentoo Penguin Pygoscelis papua, Kerguelen Shag Phalacrocorax verrucosus and South Georgian Shag P. atriceps georgianus, which, in the case of the last species, persisted over long time periods. These differences probably relate to differences in body size, the larger males being able to dive deeper than females. More intriguingly, there were strong relationships between feather and blood isotope ratios in the two shags, suggesting that individuals are highly specialised in terms of diets and that specialisation is maintained over long periods (because the feathers were grown in the non-breeding season and the blood was sampled during nesting). In other words, birds have individual ‘tastes’ and differ in what they eat, which is probably dependent upon the foods that a particular individual is adept at catching. Similar age- and sex-specific differences in diet have also been shown among Southern Giant Petrels Macronectes giganteus using similar techniques (Forero et al. 2005).

Another study of south Atlantic diving birds used stable isotopes in feathers to show that four different species of petrel from Kerguelen Island dispersed over a much wider range of habitats (coastal to oceanic waters from Antarctica to the tropics) compared with the same four species on South Georgia, which wintered mainly locally around the archipelago (Cherel et al. 2006). These studies demonstrate the potential of stable-isotope analysis of feather tissue for locating the moulting areas of seabirds that undergo complex migration patterns every year, especially in helping to investigate foraging ecology during the poorly known non-breeding period (Cherel et al. 2000).

Of course, if you eat junk food, it too will show up in your body! For this reason, stable-isotope ratios of scavengers (such as crows (Corvidae) and gulls (Laridae)) that use rubbish tips and other sources of human waste may be highly variable and extremely exotic, because they tend to eat anthropogenic material imported from around the world, all reflecting the varied isotopic environments in which they originated (Hobson et al. 2004b). On the other hand, these remarkable mixes of stable-isotope signatures can be useful in showing how important birds are in the nutrient cycles of urban landscapes. A study in Japan has shown that up
to 53% of the phosphorus and 27% of the total nitrogen input to evergreen forest fragments in urban landscapes came from the droppings of the large roosting aggregations of Jungle Crows *Corvus macrorhynchos* (Fujita & Koike 2007). Using stable-isotope analysis, they could show that the crows played an important role for the woodland by importing nutrients in their faeces which were derived from rubbish (from fish, livestock, and/or C4 plants such as corn) with high $^{13}$C and $^{15}$N ratios, gleaned from residential and business areas, which would not normally appear in such an ecosystem.

There are many other pitfalls and shortcomings when considering the use of stable isotopes in the tissues of birds to determine their prey, which confirms the need always to study both predator and prey to understand the processes involved. For example, freshwater birds may feed on fish that spend most of their life in the sea but which migrate up rivers temporarily – for example to spawn – and thus will have different signatures from birds feeding on local, non-migratory fish. However, an understanding of such processes will invariably enable the use of such anomalies to be used to advantage in furthering our understanding of avian diets.

### Understanding how factors operating throughout the annual cycle affect bird abundance (‘carry over’ effects)

We have already seen that the study of stable isotopes can be a powerful approach in understanding some of the more complex patterns in avian ecology. In particular, as in the ‘capital’ versus ‘income’ breeder debate, it helps us to connect different phases in the annual cycle and understand better how factors operating at one point in the cycle affect an individual at other times: ‘carry over’ effects.

It is well known that, for migratory birds, early arrival on the breeding grounds in good physical condition is an important prerequisite for successful reproduction. American Redstarts *Setophaga ruticilla* arrive at nesting areas over a period of about a month, with later arrivals often in poor condition. These late arrivals not only have diminished chances of finding a good territory but their poor condition is likely to affect survival too. Nothing was known about the causes of these differences until a stable-isotope study of their Jamaican winter quarters revealed that winter habitat quality determined the birds’ physical condition and departure date from the wintering grounds, and in turn their...
condition and arrival time in nesting areas (Marra et al. 1998). Birds wintering in Black Mangrove Avicennia germinans forest (the best quality habitat) began their spring migration earlier and in better condition than those wintering in secondary-growth scrub (a lower quality habitat). The two habitats were characterised by different amounts of C3 and C4 plants so that isotope signatures of invertebrate prey differed between mangrove and scrub; and this in turn could be detected in muscle tissue of birds arriving on the breeding grounds in spring. Similarly, studies of stable-isotope ratios in the claws of Black-throated Blue Warblers Dendroica caerulescens in the Bahamas during spring migration showed that birds wintering in better quality habitats were in better condition (with greater fat stores and larger pectoral muscles) than those in suboptimal scrub habitats (Bearhop et al. 2004). Again, good condition during spring migration is likely to translate into earlier arrival and/or better condition on return to the breeding grounds, which in turn has consequences for reproduction.

These two studies show that events in tropical wintering areas affect the condition of migratory songbirds during migration, their reproductive success on the breeding grounds and, potentially, survival. They also provide some evidence that winter habitats may be limiting in such species, forcing less fit individuals to exploit suboptimal habitats, in which they are less successful than fitter birds in better habitats. Both studies are examples of research that not only revealed a great deal about the ecology of bird populations, but provided vital information to support effective conservation, in this case, the importance of natural forest as wintering habitat for North American passerine migrants.

Another fascinating study that concerns ‘habitat matching’ between summer and winter involves Icelandic-nesting Black-tailed Godwits Limosa limosa islandica. Gunnarsson et al. (2005) used stable isotopes to determine the habitat quality used by marked individuals which could be followed to the wintering grounds. The analysis of $^{13}$C isotope ratios in feathers grown in late winter showed that those godwits which used estuarine sites in Europe at that time (the best overwintering habitats) tended to breed on the most productive sites in Iceland; in other words, individuals that occupy higher quality breeding sites also used better quality wintering sites. Since adult godwits are highly philopatric (returning to the same site year after year), the initial choice of winter habitats by juveniles (which are not accompanied by their parents from the breeding areas) may be crucial to the future survival, timing of migration and reproductive output of those individuals.

Using stable isotopes to unravel migration routes and identify winter quarters

The technique can also be extended to delineate migratory divides and provide indication of the potential wintering areas of some long-distance migrants. Willow Warblers Phylloscopus trochilus are particularly amenable to such studies since, unlike many other species, they undergo two complete flight-feather moults each year. Thus,
feathers collected early in the breeding season contain information on winter habitat selection. In Sweden there are two subspecies of Willow Warbler, with only marginally overlapping breeding ranges: P. t. trochilus breeds mostly at latitudes below 61°N whereas P. t. acredula tends to breed at latitudes above 63°N. Chamberlain et al. (2000) measured the 13C and 15N isotopes of feathers from the two subspecies, which they found to be isotopically distinct. They showed that the average 13C and 15N ratios in wing feathers (grown on the African wintering quarters) of acredula were significantly higher than those in wing feathers of trochilus. This confirmed the sparse ringing data, which hinted that the two subspecies occupy geographically (and isotopically) distinct wintering grounds in Africa or used different habitats (exploiting different diets) in the same area. A study of different breeding populations of Barn Swallows Hirundo rustica showed a similar pattern. Stable-isotope ratios in the feathers of birds breeding in Switzerland were significantly different from those in the feathers of birds that had bred in England (Evans et al. 2003). The 13C signatures of Swiss birds were significantly lower than those of English birds, but 15N signatures did not differ between the two populations. Here, the authors concluded that Swiss birds probably feed on prey in winter that are more reliant on C3 vegetation, from woodlands, than the prey of English birds, which are more reliant on C4 vegetation, from grasslands. In contrast, a study of a single population of Barn Swallows breeding in Denmark showed a very clear bimodal distribution of both carbon and nitrogen stable-isotope ratios that strongly suggested two discrete and different wintering areas for birds exploiting the same nesting area (Moller & Hobson 2004).

Atkinson et al. (2005) used 13C and 15N isotopes in flight feathers of Red Knots to identify at least three different discrete wintering areas used by birds caught on spring migration in Delaware Bay, northeast USA, en route to their high Arctic breeding areas. The data suggested that around 58% probably wintered in Bahia Lomas (in Chile), c. 30% in Florida, c. 6% in Rio Grande (Argentina), while the remaining 8% were not classifiable. This sort of approach has been used on a much wider spatial scale to determine the wintering localities of a range of wader species, based on flight-feather stable-isotope chemistry of birds captured on their breeding grounds (Farmer et al. 2004). In this case, a combination of different isotopes present in feathers grown in the winter quarters could be used with some success to identify where breeding birds wintered, even discriminating birds from two closely spaced wintering sites in Tierra del Fuego.

**Using sequences of feather regrowth to understand avian biology**

In the case of the Baikal Teal (see study mentioned above), and indeed virtually all wildfowl, the flight feathers are all grown simultaneously, and all will reflect the isotopic characteristics of the moult site. However, most other birds moult flight and body feathers sequentially, which means that adjacent feathers on a bird will reflect the particular chemical environments prevailing at the time of construction. Hence, Thompson & Furness (1995) were able to show that the diet of Fulmars Fulmaris glacialis...
changed during the period of sequential primary moult. A Fulmar’s innermost primaries are regrown at the end of the chick-rearing period, while the outer feathers are progressively replaced well into winter. Isotopic ratios showed that the birds were consuming prey from increasingly lower in the marine food chain as the primary moult progressed. In long-distance migrants, these differences provide clues about where specific feathers were grown, and help us to understand the often highly complex moult strategies adopted by some birds, such as Savi’s Warbler *Locustella luscinoides*, which may replace feathers twice in the annual cycle (Neto et al. 2006). Such knowledge of moult patterns is also essential for supporting effective conservation. Until recently, the sub-Saharan African wintering quarters of the Globally Threatened Aquatic Warbler *Acrocephalus paludicola* were completely unknown. Such a lack of information about the wintering grounds hampers conservation efforts, so Pain et al. (2004) looked at stable-isotope ratios in the flight feathers of Aquatic Warblers, which were known to be grown on the wintering grounds. Although the results were not sufficient to pinpoint the winter quarters accurately, they did find significant differences in $^{13}$C ratios, indicating that geographic segregation of populations also occurred in the winter quarters. Even more interestingly, they found that winter isotope signatures were correlated with breeding latitude and longitude, suggesting strong links between the breeding and wintering grounds and possibly some evidence of leapfrog migration. Subsequently, the discovery of a significant wintering population of Aquatic Warblers in northwest Senegal, perhaps holding up to a third of the world population of the species, owed much to the role of stable-isotope analysis in signposting likely regions of West Africa. The technique also confirms the importance of stopover sites, since studies of the stable-isotope ratios in the feathers of migrating passerines in sub-Saharan Africa show close similarity from year to year (Yohannes et al. 2007). This has enormous conservation implications, because it shows that these migrating birds feed on the same prey every season, emphasising that their site fidelity and habitat selection makes them highly vulnerable to habitat change and destruction in staging areas.

**Stable isotopes can reveal facets of the evolutionary process**

As well as revealing much about migration patterns, stable isotopes can also help to explain the evolution of the migratory process. Bearhop et al. (2005) used habitat-specific stable-isotope signatures to study Blackcaps *Sylvia atricapilla*. 

---

73. Fulmar *Fulmaris glacialis*, Westray Firth, Orkney, April 2004. The majority of birds moult their feathers sequentially and, for larger birds, the stable-isotope composition of particular feathers may show how diet changes as the moult progresses. Thompson & Furness (1995) mapped the changing diet of Fulmars, in which moult begins at the end of the breeding season and is often not completed until late winter.
During the last 50 years, Blackcaps have been increasingly wintering in Britain and northern Europe, and studies have shown that this new migratory behaviour has a genetic basis. Bearhop et al. (2005) showed that birds wintering in new areas (Britain & Ireland) could be distinguished from those using traditional wintering areas in Iberia and North Africa, based on stable-isotope signatures in their claws. Moreover, the study showed that these two groups of birds mated assortatively with respect to wintering area — in other words, they mated with birds from the same wintering areas far more often than would be expected by chance. They found evidence that this was probably because birds wintering farther north were more likely to arrive back at their breeding areas before those wintering farther south. This mechanism effectively keeps the two types separate, even though they overlap in their breeding ranges and habitat. Furthermore, birds wintering farther north also produced larger clutches and fledged more young. This clearly shows that birds adopting the ‘new’ winter strategy were far more successful at producing young than those retaining the ‘traditional’ strategy. This is striking evidence for the mechanism behind the rapid increase in the numbers of Blackcaps coming to British and Irish bird feeders in winter. These findings are not just important for our knowledge about Blackcaps, they also describe a fundamentally important process in the evolution of migratory divides, new migration routes, and wintering quarters. In particular, the results show that the timing of breeding is a way in which subpopulations of birds may become genetically isolated, even though they overlap in breeding range and habitat.

**Marine versus terrestrial stable-isotope ratios**

Another facet of using carbon and nitrogen stable-isotope ratios is that $^{13}C$ and $^{15}N$ tend to be much more abundant in marine ecosystems compared with freshwater or terrestrial systems, and their ratios in bird tissues can be used, therefore, to demonstrate to what degree individual birds forage in the two habitat types. This has great advantages for comparing longer-term differences in foraging strategies between both individuals and species. For example, Bearhop et al. (1999) used isotopic
profiles of feathers grown at different times in the annual cycle to investigate variability in the amount of marine protein in the diet of Great Cormorants *Phalacrocorax carbo* in England. Coastal-breeding birds were sampled at inland sites in winter. Stable-isotope analysis of the flight feathers, which are renewed after the breeding season, showed that most birds had been feeding exclusively on freshwater fish prior to being sampled. Coastal breeders had thus become freshwater specialists at inland sites during the non-breeding season – and do not commute between inland waters and the sea to feed in the winter. By using stable-isotope ratios in blood samples, it has also been possible to chart the shift in diet of wintering Brent Geese *Branta bernicla* from sea grasses such as *Zostera* in early winter to the point where they are feeding almost exclusively on terrestrial grasses in spring (Inger *et al.* 2006). A similar approach has been used to estimate the relative proportion of marine and terrestrial protein in the diets of gulls feeding on rubbish tips (Hobson 1987).

Even within the marine environment, it is possible to use $^{13}$C and $^{15}$N ratios in bird tissues to see at which trophic levels birds are feeding, because both elements show higher values at higher levels in the food chain. Stable carbon isotopes in the Pacific also reflect an inshore versus offshore gradient in prey that can help to identify where seabirds are feeding (Hobson *et al.* 1994). It is often important to know what seabirds are feeding on (zooplankton, crustaceans, small pelagic fish, etc.), but studies of diet based on stomach contents, direct observations or prey remains collected at breeding colonies give only a snapshot of diet over time and are often biased towards items that are resistant to digestion (Votier *et al.* 2001). Several studies have now shown that stable-isotope analyses confirm the trophic relationships of seabirds suggested by the results of conventional methods, and many have helped to explain how different species can co-exist by feeding on different prey (e.g. Hobson *et al.* 1994, Dahl *et al.* 2003, Forero *et al.* 2004).

**Applications using stable isotopes of other elements**

Yet other elements present in geological substrates characterise local isotopic ratios which influence those in the food chain and which can then be detected in a given organism. Some ele-
ments, such as sulphur, strontium and lead (all occur in four different stable isotopic forms), can be used because they show geographical differences linked to geological patterns or to pollution by humans. Sulphur can be useful where predators exploit a mixture of terrestrial and marine prey, because sulphur $^{34}$S ratios tend to be higher in marine systems than in terrestrial ones (Lott et al. 2003). Strontium has been shown to be useful in North America, for example, where high $^{87}$Sr ratios are typical of geologically old crystalline rocks of the Appalachian Mountains, in contrast to limestone bedrock elsewhere. Chamberlain et al. (1997) caught Black-throated Blue Warblers on their Caribbean wintering grounds and identified birds from different breeding areas based on strontium isotope ratios in tissues. Lead from earlier industrial sources (such as petrol additives or residues from mining or smelting) provides another potential source of information about spatial patterns. Pain et al. (2007) used lead isotope analysis to show that the most likely source of lead responsible for poisoning Red Kites Milvus milvus in England was ammunition used to kill animals on which the kites were scavenging.

**Some concluding thoughts**

This review has barely scratched the surface of the literature available on the use of stable-isotope analyses in current ornithology, but then the ornithological world has hardly begun to scratch the surface of the possibilities this technique opens up. The availability of stable-isotope-ratio measurement offers such a vast range of forensic possibilities for the study of birds that there is no doubt that its future application will further extend our knowledge in the coming years.

One particular field that offers exciting prospects is that of ornithological archaeology. In fact, one of the first ornithological applications of the technique was an investigation of the foraging habits of the Great Auk Pinguinus impennis based on isotopic signatures of bone collagen (Hobson & Montevecchi 1991). Stable-isotope analysis has also been applied to the ageing of seabird colonies (based on $^{13}$C and $^{15}$N influence in soils from marine habitats; Hawke 2004), as well as to examining changes in diet over extended timescales (e.g. Fulmars in the North Atlantic over a period of c. 90 years, where birds shifted from feeding on offal associated with whaling activities at the turn of the twentieth century to prey of lower trophic status in recent times; Thompson et al. 1995). In this sense, collections of museum specimens around the world assume an even greater importance when considering the many secrets that might be revealed by feather analysis. One such major project is already underway, analysing feathers of Slender-billed Curlew Numenius tenuirostris specimens, to try to establish former breeding and wintering areas more accurately and in turn better understand its present status and potential for recovery.

In a rapidly changing world, it is all the more important that we understand changes in habitat use and migratory behaviour that are occurring among...
birds in relation to major human developments and climate change. It is also increasingly important that we understand the importance of breeding, wintering and staging habitats in the annual cycle to particular bird populations. As shown in this article, stable-isotope studies can play a major role in providing new information, which can then play a direct role in effective conservation. It is crucial that research effort continues to support stable-isotope research, now and into the future.

Acknowledgments

We thank the many people who have enlightened us about the use of stable isotopes, most importantly Keith Hobson, who has been the major pioneer in this field and who has been gracious in his help, advice and support in using the technique. Thanks also go to the many authors and co-workers that have been responsible for applying the technique to the many different fields of ornithology. Grateful thanks also to Gabriel Bowen for supplying the global map of deuterium isotope ratios.

References


—, Piatt, J. F., & Pitocchelli, J. J. 1994. Using stable isotopes to...
Dr Tony (A. D.) Fox, Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute, University of Aarhus, Kåge, Grenåvej 14, DK-8410 Rønde, Denmark
Dr Stuart Bearhop, Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall TR10 9EZ

The use of stable-isotope ratios in ornithology


Dr Tony (A. D.) Fox, Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute, University of Aarhus, Kåge, Grenåvej 14, DK-8410 Rønde, Denmark
Dr Stuart Bearhop, Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall TR10 9EZ

Finally released from his duties as BBRC secretary during the interregnum between Mike Rogers and Nigel Hudson, Pete Fraser is once more turning his attention to the scarce migrants report. A report covering the years 2004–2006 is now in preparation. The production of this report depends in no small part upon the goodwill of the county and regional recorders who provide the raw data. In order to make the report as complete as possible, data for the relevant species, for the years 2004–2006 inclusive, would be gratefully received; these can be e-mailed (preferably as soon as possible) to statistician@bbrc.org.uk Alternatively, copies of all county or regional reports may be sent to: Pete Fraser, 2 The Parade, Truro, Cornwall TR1 1QE.

Information concerning records for the report for 2004–2006 can be found at www.scarce-migrants.org.uk