Tracing diets and origins of migratory birds using stable isotope techniques

Keith A. Hobson

Abstract

Measurements of naturally occurring stable isotopes of several elements found in foodwebs are being used increasingly to address a number of ecological questions, particularly those involving nutrient flow and foodweb structure. However, only during the last decade has the potential of this technique been realized by avian ecologists, and there exists a multitude of potential applications in this field. In this paper, the uses of stable isotope applications to avian ecological studies are reviewed. In particular, emphasis is given to the use of $\delta^{15}N$ ($^{15}N/^{14}N$) measurements to delineate trophic position in marine systems and $\delta^{13}C$ ($^{13}C/^{12}C$) and ^{34}S ($^{34}S/^{32}S$) measurements to establish sources of nutrients in avian diets. Recent applications of the measurement of deuterium isotope ratios δD (D/H) in feathers to trace origins of migratory songbirds is also presented. When combined with DNA analyses, the stable isotope approach holds great promise for linking breeding and wintering areas of neotropical migrant birds and other migratory organisms.

Résumé

Les mesures de fréquences naturelles d'isotopes stables de plusieurs éléments que l'on retrouve dans les chaînes alimentaires sont de plus en plus utilisées pour répondre à plusieurs questions écologiques, tout particulièrement celles impliquant les flux de nutriments et la structure des chaînes alimentaires. Cependant, ce n'est qu'au cours de la dernière décennie que le potentiel de cette technique a été apprécié par les écologistes aviens. Dans cet article, l'utilisation de la technique des isotopes stables pour les études d'écologie avienne sont résumées. En particulier, l'emphase est mise sur l'utilisation des mesures de $\delta^{15}N$ ($^{15}N/^{14}N$) pour déterminer les positions trophiques dans les systèmes marins et de $\delta^{13}C$ ($^{13}C/^{12}C$) et $\delta^{34}S$ ($^{34}S/^{32}S$) pour déterminer les sources de nutriments dans le régime alimentaire des oiseaux. Des applications récentes des mesures de ratios d'isotope de Deutérium δD (D/H) dans les plumes pour retracer l'origine des oiseaux migrateurs sont aussi présentées. L'approche des isotopes stables, lorsque combinée avec des analyses d'ADN montre de grands potentiels pour relier les aires de nidification et d'hivernage des oiseaux migrateurs néotropicaux, et autres organismes migrateurs.

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Introduction

Conventional approaches to avian dietary studies have involved gut contents analysis, either by collecting birds, stomach flushing or the use of emetics (e.g., Laursen 1978; Harrison 1984; Duffy and Jackson 1986; Major 1990; Poulin et al. 1994), direct observation of foraging adults or, more typically, the prey they deliver to young, or the analysis of prey remains in the form of regurgitations, pellets, or faeces found at nest or roost sites (Davies 1977; Loiselle and Blake 1990). Dietary inferences have also been made from analyses of contaminant or radioactive tracers in foodwebs (Smith et al. 1979; Beviss-Challinor and Field 1982; Macko and Ostrom 1994). Each of these approaches may adequately describe diet, particularly over the short term. However, they all have

considerable drawbacks, at least when applied in isolation. Gut content analysis involves destructive sampling and typically provides information only for the individual's last few meals. More importantly, this technique may be biased against soft-bodied prey or those materials that are more readily digested (Duffy and Jackson 1986). While being non-destructive, similar drawbacks are apparent with stomach flushing or the use of emetics and the analysis of prey remains or regurgitated pellets or faeces. Moreover, these approaches at best provide only a picture of what was ingested and not what was assimilated into the bird's tissues. So, unless details of assimilation efficiency and how foods enter nutritional pathways are known, frequency of occurrence or biomass of prey remains in stomachs may often provide misleading information (Duffy and Jackson 1986). Finally, analysis of prey remains is a tedious technique that requires researchers to have a good taxonomic knowledge of organisms available to birds.

The problems outlined above with respect to the analysis of individual diets are compounded further when one attempts to consider dietary relationships among several taxa within a foodweb. The difficulties inherent in systems or foodweb studies have undoubtedly resulted in the lag in empirical evidence for foodweb theory (Paine 1988). Stable isotope analysis is based on the measurement of naturally occurring stable isotopes of several elements that occur in foodwebs. This technique offers a number of advantages over conventional dietary approaches. In particular, dietary information is based on: (1) nutrients assimilated into consumers, not just those ingested; and (2) a dietary integration over various temporal scales ranging from a few days to the lifetime of the individual depending on the tissue analyzed. In addition, the collection technique is simple and straightforward and the analysis relatively inexpensive.

Stable isotope applications to avian dietary studies fall into four general categories: (1) the determination of trophic level of individuals and trophic relationships within complex systems, including correlations between trophic level and bioaccumulation or depuration of contaminants; (2) the determination of nutrient source or location of feeding; (3) the evaluation of endogenous vs exogenous reserves to reproduction; and (4) the tracking of migration and the linkage of breeding and wintering grounds in migratory species. All of these applications rely on the simple fact that stable-isotope signatures present in foodwebs can act as natural markers to trace dietary origins of birds and other

consumers. Such tracing can involve the delineation of feeding relationships at a local scale or at the scale of thousands of kilometres in the case investigations of migration. Following a brief background on the theory and measurement of stable isotopes, each of the above broad applications will be described and discussed. It is not the intention of this paper to provide an exhaustive review of the field of ecological applications of stable isotopes since excellent reviews are provided elsewhere (e.g., Peterson and Fry 1987; Rundel et al. 1988; Lajtha and Michener 1994). Rather, an emphasis will be placed on existing and potential applications to avian ecological studies.

Stable isotope background

In nature, elements exist in both stable and nonstable (i.e., radioactive) forms. Elements common to biological systems typically occur in more than one stable form and these are referred to as stable isotopes of that element. Isotopes differ in the mass of the nucleus and depend on the number of neutrons present. Characteristically, the heavier isotopes of an element occur more rarely in nature than the lighter isotope. Those elements in nature of most interest in terms of stable isotope applications are carbon, nitrogen, oxygen, sulphur and hydrogen, with the overwhelming majority of work being focused on carbon and nitrogen (see Table 1).

Table 1. Average terrestrial abundances of the stable isotopes of elements of primary interest in avian dietary and tracing studies (from Ehleringer and Rundel 1988).

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

Isotopes behave differently in biogeochemical processes due to thermodynamic and kinetic

considerations. In general, differences in physical and chemical properties of isotopes are proportional to differences in their masses (Broecker and Oversley 1976). The net difference in isotope abundance resulting from these processes is known as isotopic fractionation. In nature, isotopic fractionation processes discriminate for or against an isotope of a particular element in the order of a few percent. Analytically, it is most convenient to measure these small absolute differences in isotopic composition by measuring isotopic differences between a sample and a known standard. Isotopic composition of a sample is usually expressed in delta (δ) notation as follows:

$$\delta X_{std} = (R_{sample}/R_{std} - 1) \times 1000$$

where δX_{std} is the isotope ratio in delta units relative to a standard, and R_{sample} and R_{std} are the absolute isotope ratios of the sample and standard, respectively. Delta values are expressed as parts per thousand ($^o/_{oo}$) or on a "per mil" basis. Currently, there are four international standards for the five principal light isotopes of interest in this paper. The PeeDee belemnite (PDB) standard is used for carbon, atmospheric air (AIR) for nitrogen, the Canyon Diablo meteorite (CD) for sulfur and the standard mean ocean water (SMOW) for hydrogen and oxygen. The arbitrary choice of these standards results in ^{13}C samples typically being negative (i.e. the standard is usually enriched in ^{13}C compared to the unknown) and ^{15}N samples being positive (atmospheric air is typically depleted in ^{15}N relative to the unknown). Stable hydrogen and oxygen isotope values are typically negative and stable sulfur isotope values can be both positive and negative. Below, I provide a brief background on each element of interest. Information on oxygen is not presented because, despite its potential use in avian ecological investigations, few researchers have conducted studies using this element (but see Schaffner and Swart 1991). In addition, hydrogen isotope analysis can often provide similar types of information in studies involving tracing of water.

Carbon

Carbon occurs in two stable forms as ^{13}C and ^{12}C. Carbon isotopic compositions of animals reflect those of the diet within about 1$^o/_{oo}$ (DeNiro and Epstein 1978; Peterson and Fry 1987). For this reason, stable-carbon isotope analysis is an ideal tool for tracing origins of nutrients in foodwebs since carbon may enter the base of foodwebs with characteristic isotopic signatures due to a variety of biogeochemical processes and these change little throughout the foodweb. The two main processes of interest in this paper are those resulting in differences in $\delta^{13}C$ values of terrestrial versus marine foodwebs and $\delta^{13}C$ values of plants with C-3 vs C-4 photosynthetic pathways.

Carbon in the atmosphere is depleted in ^{13}C relative to dissolved carbonate in the oceans by about 7$^o/_{oo}$ (Craig 1953; Chisholm et al. 1982).This difference arises from the fact that dissolved inorganic carbon in the oceans is derived ultimately from the atmosphere. Effectively, heavier carbon dioxide enters into bicarbonate ion exchange at the ocean/atmosphere interface more readily than does lighter carbon dioxide. In general then, marine foodwebs tend to be enriched in ^{13}C compared with terrestrial C-3 foodwebs (see below) and this difference has been used to trace the relative contributions of terrestrial versus marine protein in the diets of contemporary (e.g., Hobson 1986, 1990) and prehistoric consumers (e.g., Chisholm et al. 1982; Hobson and Collier 1984).

The stable-carbon isotope composition of terrestrial plants is influenced in part by photosynthetic pathway and much of the earlier work on stable carbon isotopes in nature was concerned with linking isotopic fractionation with plant metabolism (reviewed by Ehleringer and Rundel 1988). In temperate areas, most plants have a so-called C-3 photosynthetic pathway resulting in $\delta^{13}C$ values close to -27$^o/_{oo}$. Plants with a C-4 photosynthetic pathway, often growing in more xeric conditions, have more enriched $\delta^{13}C$ values ranging between -9 to -14$^o/_{oo}$. The relative contributions of C-3 versus C-4 plants to foodwebs have been investigated using stable-carbon isotope measurements. In particular, the introduction of corn, a C-4 plant, to an otherwise C-3 based foodweb has been traced in prehistoric agricultural systems (e.g., Vogel and van der Merwe 1977; Bender et al. 1981).

Nitrogen

There are two stable forms of nitrogen, ^{14}N and ^{15}N. In terrestrial systems, nitrogen enters foodwebs through symbiotic fixation or through direct conversion of atmospheric nitrogen within plants (reviewed by Nadelhoffer and Fry 1994). These processes typically lead to different isotopic signatures of plants adopting these strategies. In marine systems, inorganic nitrogen occurs as molecular nitrogen, ammonia, nitrate, nitrite and nitrous oxide. Relatively little is known about the isotopic fractionation effects occurring between these nitrogen pools and phytoplankton (Owens 1987; but see Wada and Hattori 1978).

As was found for stable-carbon isotopes, the stable nitrogen isotopic composition of animals is related ultimately to the isotopic compositions of their diets (DeNiro and Epstein 1981; Macko 1981; Minagawa and Wada 1984). Through processes associated primarily with differential excretion of ^{14}N, animals incorporate dietary ^{15}N into their tissues preferentially, an effect due to discrimination against the lighter isotope during protein amination and deamination (Gaebler et al. 1966; Macko et al. 1982; Minagawa and Wada 1984). A broad survey of field and laboratory data confirms that there is a significant linear relationship between $\delta^{15}N$ values of an organism and its diet (DeNiro and Epstein 1981; Owens 1987). This phenomenon forms the basis of using $\delta^{15}N$ measurements to infer trophic level.

Sulfur

Sulfur occurs in nature in four stable forms, ^{32}S, ^{33}S, ^{34}S and ^{36}S. Of most interest in biological systems is the relative abundance of ^{32}S and ^{34}S (depicted as $\delta^{34}S$). The large variability (of the order of 150‰) in sulfur isotope ratios in natural materials from light sulfides to heavy sulfates and the apparent lack of a trophic enrichment effect (see Krouse 1988; Hesslein et al. 1993) has led to the primary use of stable sulfur isotopes as natural tracers in foodweb studies. In particular, differences in $\delta^{34}S$ between terrestrial and marine biota makes this isotope extremely useful in tracing relative contributions of terrestrial and marine-derived nutrients. Compared with other elements, it was previously relatively difficult to perform routine sulfur isotope measurements on biological material. However, with the advent of continuous flow isotope ratio mass spectrometry (IRMS), sulfur isotope measurements have become more routine. The first application to avian studies was by Trust (1993).

Hydrogen

Hydrogen occurs in two stable forms, ^1H (usually denoted as H) and ^2H or deuterium (D). Isotopic fractionation effects tend to be far more pronounced in hydrogen because the relative mass difference between H and D is greater than other isotopic elements. Ecological studies using stable hydrogen isotopes are often related to the deuterium content of local water. Once water leaves the ocean, climate and geography influence the large-scale pattern of the D isotope. Important geological and climatic factors affecting natural distributions of D/H ratios in precipitation include latitude, altitude, season, and distance inland (Dansgaard 1964; White 1989). At low latitudes where

seasonality is not pronounced, and at mid-latitudes in summer, the amount of rainfall becomes important in determining δD of precipitation (Dansgaard 1964). Generally, δD in precipitation decreases in a north-westerly direction across North America (Cormie et al. 1994; Hobson and Wassenaar 1997; Figure 1). δD also decreases with increasing altitude, is higher in summer and lowest in winter at latitudes above 30° (Ziegler 1989) and decreases with heavy versus sparse precipitation (Dansgaard 1964).

As with stable-carbon isotopes, most work on isotopes of hydrogen in biological systems has been restricted to investigations of isotopic measurements of plants (e.g., Smith and Epstein 1970; Estep and Hoering 1980; Ziegler 1989). Schimmelmann and DeNiro (1986) investigated trophic enrichment of deuterium in an aquatic food chain, but isotopic investigations of higher-trophic level organisms are generally lacking. Recently, Cormie et al. (1994) found high correlation of bone collagen δD with growing season rain δD in modern deer bone. This study provided the inspiration for investigations by Hobson and Wassenaar (1997) to use δD measurements of feathers grown on breeding grounds to link breeding and wintering grounds of neotropical migratory birds (see below).

Captive studies

Until relatively recently, there were two major limitations to the application of stable isotope analyses in avian dietary studies. First, it was not well understood how stable isotopes fractionate or change once they are incorporated into tissues. Tieszen et al. (1983) conducted controlled laboratory studies on gerbils (*Meriones unguienlatus*), but it was not known to what extent their findings were applicable to studies of birds, especially since birds produce different nitrogenous waste products compared with mammals. Mizutani et al. (1991a) opportunistically examined the tissues and diet of a single cormorant (*Phalacrocorax carbo*) raised on a constant diet of mackerel (*Pneumatophorus japonicus*) and provided the first published estimates of carbon and nitrogen isotopic fractionation between diet and bird tissues. However, the investigation of a single piscivorous bird did not allow extrapolation to birds in general, particularly to non-piscivores.

A second limitation to the application of stable isotope analysis to avian studies was that precise turnover rates of isotopes in tissues of wild birds were poorly known. Although Tieszen et al. (1983) had investigated stable isotopic turnover rates in gerbils by

Figure 1. Patterns of D_w for North America. Circles indicate precipitation sites (updated from Hobson and Wassenaar 1997).
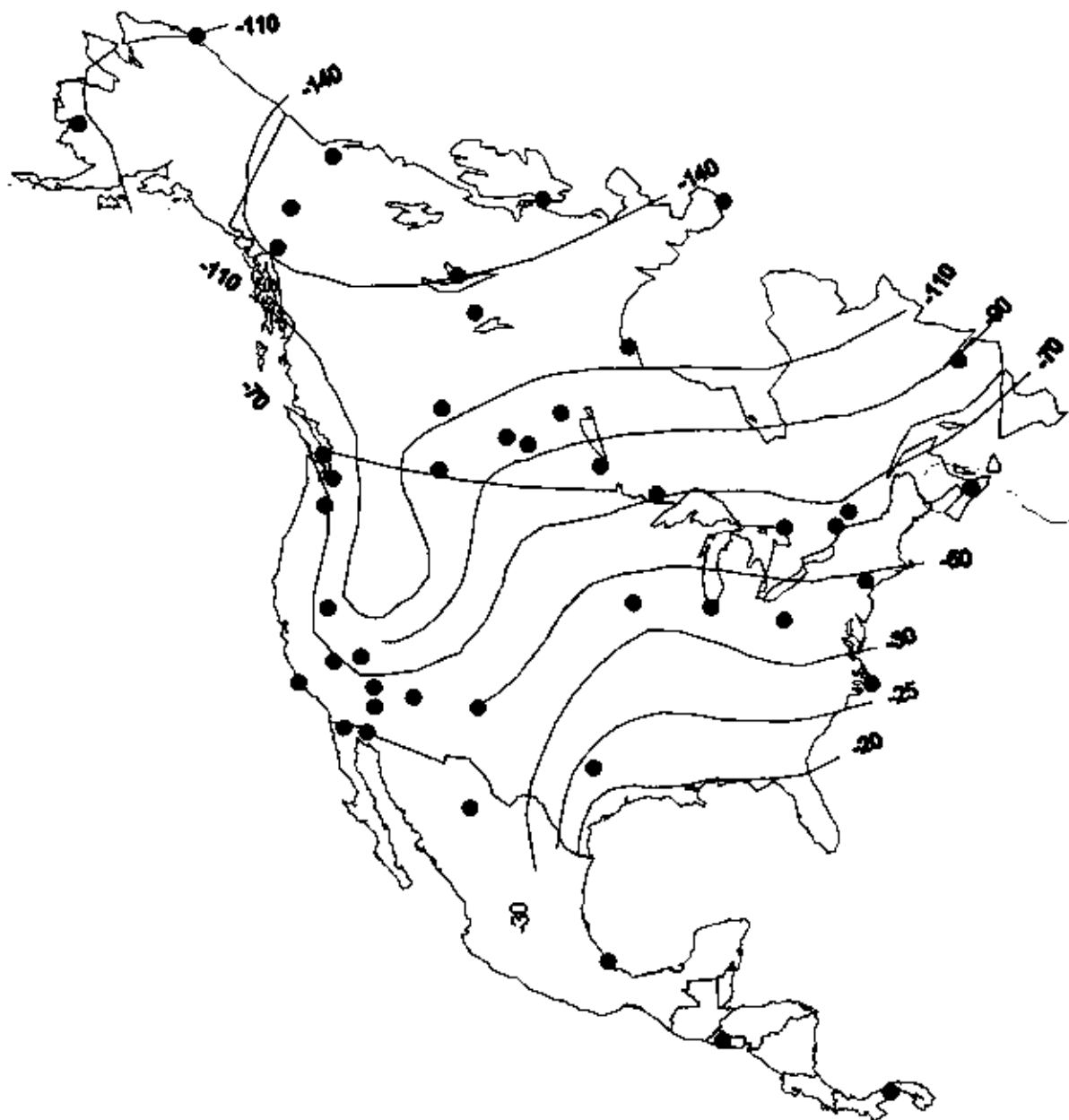
switching isotopic compositions of diets of laboratory animals under controlled conditions, again, it was not clear how reliably their results might apply to birds.

Isotopic turnover and fractionation of body tissues

The period over which tissue isotopic abundance will reflect the isotopic signature of a particular diet will depend, in part, on the isotopic turnover rate in that tissue. This rate is not to be confused with decay rates associated with radioisotopes. Rather, I refer here to elemental turnover in tissues, a process directly linked to the metabolic rate of that tissue. Tissues with rapid isotopic or elemental turnover will reflect recent diet whereas those with slow turnover will reflect longer-term dietary averages. The choice of tissue will

Table 2. Summary of diet-tissue fractionation values derived from captive studies of Hobson and Clark (1992b) and others. Sample sizes and food type in parentheses.

Tissue	Diet-tissue fractionation factor ($^o/_{oo}$)						
	Chicken (8) (TS)[1]	Quail (5) (TS)	Gull (14) (Perch)	Falcon (6) (Quail)	Crow (7) (Perch)	Piscivorous Birds (6 spp.)[2]	Cormorant (1) (Mackerel)[3]
Carbon							
Blood		1.2 ± 0.6	_0.3 ± 0.8	+0.2 ± 0.01			
Liver	+0.4 ± 0.2	+0.2 ± 0.6	-0.4 ± 1.0		3.7 ± 1.6		+1.3
Muscle	+0.3 ± 0.3	+1.1 ± 0.5	+0.3 ± 0.4		1.1 ± 1.0		+2.1
Collagen	+0.8 ± 1.2	+2.7 ± 0.4	+2.6 ± 1.1				+2.5
Feather	-0.4 ± 0.02	+1.4 ± 0.6	+0.2 ± 1.3	+2.1 ± 0.08		+2.9 to +3.8	+3.6
Nitrogen							
Blood		+2.2 ± 0.2	+3.1 ± 0.2	+3.3 ± 0.4			
Liver	+1.7 ± 0.1	+2.3 ± 0.2	+2.7 ± 0.1		2.9 ± 0.7		+2.3
Muscle	+0.2 ± 0.2	+1.0 ± 0.1	+1.4 ± 0.1		1.7 ± 1.0		+2.4
Collagen	+1.5 ± 0.1	+2.5 ± 0.4	+3.1 ± 0.2				+3.9
Feather	+1.1 ± 0.1	+1.6 ± 0.1	+3.0 ± 0.2	+2.7± 0.5		+3.7 to +5.3	+3.6

[1] TS refers to Turkey Starter commercial food mix.

[2] Mizutani et al. (1992).

[3] Mizutani et al. (1990).

depend, then, on the ecological question of interest; Tieszen et al. (1983) suggested that, by analyzing combinations of tissues, greater information concerning an animal's diet could be obtained. As a first step in establishing isotopic turnover rates applicable to birds, and isotopic fractionation factors between diet and a variety of avian tissues, Hobson and Clark (1992a,b) conducted several experiments using captive birds raised on isotopically known diets. They determined $\delta^{13}C$ and $\delta^{15}N$ diet-tissue fractionation factors for blood, liver, muscle, bone collagen and feathers of domestic chickens (*Gallus gallus*), Japanese Quail (*Coturnix japonica*) and Ring- billed Gulls (*Larus delawarensis*) and blood and feather samples of adult Peregrine Falcons (*Falco peregrinus*) raised on known isotopic diets (see Table 2).

Turnover rates of ^{13}C in tissues of fully grown Japanese Quail were established by switching the diet of an experimental group from a wheat-based (i.e. C-3) diet to a corn-based (i.e. C-4) diet and sampling tissues periodically for 212 days. An exponential model described patterns of isotopic turnover in all tissues. Turnover rates for quail tissues were ranked liver>blood>muscle>bone collagen (Table 3). Hobson

and Clark (1993) later expanded these results to include the potential isotopic assay of materials involving non-destructive sampling by determining the isotopic turnover rates of the cellular and plasma fractions of blood. By switching the diets of captive crows from C-3 to C-4 composition and sampling blood over a 45-day period, they demonstrated that cellular and plasma fractions had half lives of 29.8 and 2.9 days, respectively. This suggests that where birds can be captured alive, the isotopic analysis of blood fractions can provide the same dietary information as that based on muscle and liver.

Table 3. Elemental carbon turnover rates in tissues of captive-raised Japanese Quail.

Tissue	Half-Life (d)	Source
Blood - whole	11.4	Hobson and Clark (1992)
Blood - cellular	29.8	Hobson and Clark (1993)
Blood - plasma	2.9	Hobson and Clark (1993)
Muscle	12.4	Hobson and Clark (1992)
Liver	2.6	Hobson and Clark (1992)
Bone Collagen	173.3	Hobson and Clark (1992)

Isotopic turnover and fractionation related to egg production

Bird eggs are another potential source of material for isotopic investigation of avian diets because they are formed from nutrients that are derived ultimately from the diet of the laying female. Eggs are also usually readily available, either from the wild or through archived collections, and so are a convenient source of material for isotopic analysis. Another motivation for the isotopic investigation of eggs is that it can provide information on how quickly nutrients from the diet are incorporated into various egg components and the extent to which endogenous reserves are used in egg formation (Krapu 1981; Austin and Fredrickson 1987; Afton and Ankney 1991). If yolk is formed rapidly from dietary sources then the contribution of the new diet to the isotopic signal in the yolk should be proportional to the additional mass of yolk formed using the new diet.

Previous studies have shown that stable-carbon and oxygen isotope analysis of the organic matrix (C) or carbonate (C and O) component of eggshells can reveal dietary information from both archaeological and contemporary specimens (von Schirnding et al. 1982; Schaffner and Swart 1991). However, prior to Hobson (1995), only Trust (1993) had used other components of eggs such as the yolk and albumen fractions and virtually no information existed on how isotopic signatures change or fractionate between diet and the various components of the avian egg (but see von Schirnding et al. 1982; Schaffner and Swart 1991). Hobson (1995) investigated isotopic signatures of eggs laid by captive Japanese Quail and wild-strain Mallards (*Anas platyrhynchos*) raised on grain-based diets, and three species of falcon raised on quail, to determine possible influences of diet. That study of bird eggs indicated fairly uniform patterns of isotopic fractionation between diet and yolk, albumen, yolk lipid, shell membranes and shell carbonate. However, the magnitude of fractionation differed substantially between egg components, reflecting differences in the biochemical and metabolic processes involved in tissue synthesis (Tieszen and Boutton 1988; Nakamura et al. 1982; Krueger and Sullivan 1984). The magnitude of nitrogen isotope fractionation between diet and albumen, yolk and shell membrane were similar, being close to +3.4‰. This value is in agreement with the 3-5‰ ^{15}N trophic enrichment seen in terrestrial and marine food webs (Schoeninger and DeNiro 1984; Fry 1988; Hobson et al. 1994). Nitrogen in yolk, albumen and membranes occurs primarily as protein, and ^{15}N fractionation between diet and protein

generally occurs during processes of amino acid amination and transamination (Macko et al. 1982). As such, protein synthesis and its corresponding isotopic fractionation in egg components is similar to the synthesis of other proteins in the adult bird.

Shell carbonate is highly enriched in ^{13}C compared to diet for all species examined (von Schirnding et al. 1982; Schaffner and Swart 1991; Hobson 1995). Animal shell carbonates are typically enriched over substrates (Fritz and Poplawski 1974; Fry and Wainright 1991) and, in the case of birds, it is likely that major carbon isotopic fractionation occurs during the formation of carbonate ions by carbonic anhydrase of oviduct fluids (Simkiss and Tyler 1958; von Schirnding et al. 1982). However, falcons showed a consistently lower carbonate enrichment factor compared with quail and mallard fed plant-based diets. In their isotopic study of seabird eggshells, Schaffner and Swart (1991) also reported smaller differences between eggshell carbonate and diet for protein versus carbohydrate feeders. Similar differences in carbon isotopic fractionation have also been recorded between herbivore and carnivore bone apatite and collagen (Kreuger and Sullivan 1984) and, in this sense, carbonates in bone apatite are analogous to carbonates in the eggshell matrix.

In general, herbivore diets consist of relatively more carbohydrates and fewer proteins and lipids than carnivore diets. Carbohydrates in herbivore diets are allocated primarily to energy metabolism whereas protein is allocated mainly to growth and maintenance of tissues such as collagen. Bone apatite (and shell carbonate) is derived from blood bicarbonate which is in turn generated from the metabolism of energy substrates (DeNiro and Epstein 1978). Carnivores depend relatively more on lipids than on carbohydrates for their energy metabolism and since lipids are depleted in ^{13}C relative to proteins and carbohydrates, the carbon available for bone and shell formation in carnivores should be, on average, more depleted relative to diet than for herbivores. More recently, Ambrose and Norr (1993) and Tieszen and Fagre (1993) investigated the relationships of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate by manipulating the isotope composition of macronutrients in the diets of laboratory rats and mice. Both studies demonstrated that carbonates in bone apatite are derived from whole diet whereas bone collagen is derived primarily from dietary protein (see also Ambrose 1993). Dietary protein not used in tissue maintenance and growth is apparently used in energy metabolism. The above

discussion emphasizes that, in addition to analyzing whole diet, it is important to consider also fractionation patterns associated with dietary macromolecules such as carbohydrates, lipids, proteins and even individual amino acids (Tieszen and Boutton 1988; Macko et al. 1982).

Isotopic enrichment due to fasting or nutritional stress

During their study of isotopic fractionation between diet and tissues of various captive birds, Hobson and Clark (1992b) determined that American Crows *(Corvus brachyrhynchos)* raised on a plant-based diet did not grow as well and had tissues enriched in ^{15}N relative to diet, compared with birds raised on a high protein diet of perch. While it appeared as though nutritional stress caused differential enrichment in ^{15}N, interpretation of these results was confounded by the different diets. For this reason, Hobson et al. (1993) conducted another set of experiments on captive quail raised on a single diet (one batch of Turkey Starter) but subjected a treatment group to a ration that maintained their body mass while allowing the control group ad libitum access to food that supported normal growth. They found that the treatment group had enriched ^{15}N values of muscle, liver, bone collagen, feather and blood compared with the control group. This provided the first evidence that nutritional stress could cause isotopic enrichment in consumers' tissues.

To evaluate whether or not such enrichment occurs in nature, Hobson et al. (1993) also measured ^{15}N values in tissues of nesting Ross' Geese *(Chen rossii)* before and after their fast during incubation on their high arctic breeding grounds. As predicted, post incubation geese showed enriched tissue ^{15}N values compared with pre-incubating birds. Thus, substantial evidence of ^{15}N enrichment due to nutritional stress was established. However, the precise mechanisms for this enrichment effect are poorly understood.

Ambrose and DeNiro (1986) similarly observed that ^{15}N values of the bone collagen of drought-tolerant herbivores were higher than those of water-dependent species in East Africa and related this effect to physiological processes relating to mechanisms of water conservation. These authors noted that herbivorous mammals on diets high in protein have the capacity to excrete highly concentrated urine under conditions of heat and water stress and that this is accompanied by often spectacular increases in urea output. Urea is the major form of excreted nitrogen in mammals and is significantly depleted in ^{15}N relative to the diet (Steele and Daniel 1978). The excretion of

a more concentrated urine with a quantitative increase in the excretion of ^{15}N-depleted urea in water stressed mammals would result in higher ^{15}N/^{14}N ratios in the unexcreted nitrogen. Subsequent ^{15}N enrichment of the remaining body nitrogen incorporated into tissues is expected from considerations of isotopic mass balance (see also Ambrose and DeNiro 1987). Hobson et al. (1993) proposed an analogous mechanism to account for apparent enrichment of ^{15}N observed in fasting birds.

Nitrogen available for the synthesis of body proteins can be recycled from metabolic amino acid pools with inputs from assimilated foods and through protein breakdown or catabolism. A primary source of nitrogen isotopic fractionation is believed to occur during processes of deamination and transamination of amino acids (Gaebler et al. 1966; Macko et al. 1982; Minagawa and Wada 1984). In this way metabolized amino acids are enriched during anabolism (i.e. tissue synthesis), and nitrogenous waste products depleted in ^{15}N relative to diet (e.g., Steele and Daniel 1978). Under conditions of fasting and nutritional stress, a greater proportion of nitrogenous compounds available for protein synthesis are derived from catabolism (i.e. tissue breakdown) and, since this source of nitrogen has already been enriched in ^{15}N relative to diet, additional enrichment in the metabolic nitrogen pool must occur. One consequence of this process would be the eventual enrichment in ^{15}N of all body tissues relative to periods without stress (see also Swick and Benevenga 1977; Figure 2). The extent of δ^{15}N enrichment in tissues due to fasting and nutritional stress should be influenced by the isotopic turnover rates in those tissues. Metabolically active tissues (e.g., liver) are expected to show the effects of enrichment due to stress more readily than tissues with slower isotopic turnover (e.g., bone collagen in adult birds, Tieszen et al. 1983). Consistent with this suggestion, Hobson et al. (1993) found that adult female Ross' Geese showed a greater enrichment in liver δ^{15}N values compared with those of muscle. Experimental quail showed high δ^{15}N enrichment in both liver and bone collagen but these birds were still growing and bone isotope values were more dynamic than that expected for adult birds.

Stable isotope analysis of the tissues of wild animals and their prey to delineate diet or trophic relationships within communities offers numerous advantages over conventional approaches (e.g., Ambrose and DeNiro 1986; Peterson and Fry 1987). However, the reliability of the isotope approach depends directly on our understanding of processes

Figure 2. Hypothetical depiction of nitrogen enrichment due to nutritional stress. In situations where food intake diminishes and catabolism increases, the total body protein pool is expected to become enriched (providing excretion still occurs).
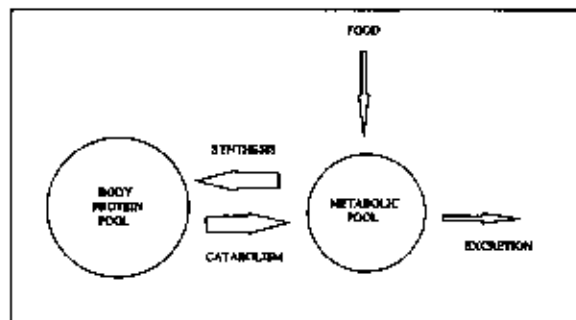
contributing to the abundance of stable isotopes in consumer tissues. Studies using ^{15}N analysis to infer diet or trophic position must take account of the nutritional history of the individuals whose tissues are being examined.

The effect of ^{15}N enrichment associated with changes in body composition is particularly relevant to isotopic studies of birds, especially the many species which lose body mass during egg-laying and incubation. Among waterfowl, for example, it was previously felt that most of this mass loss reflected gonadal regression and depletion of fat reserves (e.g., Hanson 1962; Harris 1970). However, breast, leg and gizzard muscles are important protein reserves during incubation in arctic-nesting Lesser Snow *(Chen caeruluscens)* and Ross' Geese (Ankney and MacInnis 1978). As noted by Ankney (1977), even in species in which the incubating female does not fast, considerable mass loss occurs (Weller 1957; Oring 1969; Anderson 1972). Penguins also fast during egg-laying (Richdale 1947) and several species of seabirds may undergo periods of food stress due to competition near colonies or crashes in prey stocks (e.g., Hunt et al. 1986; Erikstad 1990). In addition, nestling seabirds may experience protracted periods of reduced growth due to intermittent food provisioning (e.g., Ricklefs et al. 1980). Although there is tremendous potential for the application of stable-isotope analysis to avian dietary studies, researchers should consider possible ^{15}N enrichment of tissues in some individuals or species due to physiological effects rather than diet per se.

Foodweb source studies

The first applications of stable isotope analysis to

avian studies was to determine source of feeding. This approach is based on the fact that isotope ratios in consumers reflect those in their prey and, where two isotopically distinct sources of food are available, a simple linear mixing model can be constructed to predict the relative nutritional inputs from each source (Figure 3). The measurement of several stable isotopes in consumer and diet can allow better resolution of dietary contributions and may also allow the approximation of inputs from more than two isotopically distinct sources (e.g., Mariotti et al. 1984; Hobson et al. 1997).

Tietje and Teer (1988) examined the reliance of Northern Shovelers *(Anas clypeata)* on freshwater and saline habitats using $\delta^{13}C$ analyses of pectoral muscle tissues and concluded that these birds did not move between habitats during the midwinter period. Another, more recent, tracer application to waterfowl studies involved the analysis of stable carbon and sulfur isotopes in muscle, eggs, and prey tissues of Redhead Ducks *(Aythya americana)* on breeding and wintering grounds (Trust 1993). That study revealed differential dietary sources for carbon and sulfur on wintering grounds and generally alluded to birds reflecting the isotope ratios of foods available to them. Using stable carbon isotope analysis of bone collagen, Hobson (1986) provided a minimum estimate of the importance of terrestrial C-3 based garbage in the diets of Glaucous-winged Gulls *(Larus glaucescens)* wintering near Vancouver, British Columbia. While that study represented an advance over previous conventional dietary studies, it was complicated by the fact that terrestrial and marine isotopic endpoints were not well understood. Mizutani et al. (1990) later analyzed $\delta^{13}C$ values in feathers of Great Cormorants and delineated freshwater and marine protein inputs to the diets of birds having access to freshwater and marine biomes during the period of feather growth.

The first isotopic analysis of more than one tissue type to determine dietary inputs to birds over different time periods was provided by Hobson (1990) who used stable-carbon and nitrogen isotope analyses of muscle and bone collagen from Marbled Murrelets *(Brachyramphus marmoratus)* that had been collected in marine coastal areas and at a freshwater lake in British Columbia. The impetus behind that study was to determine if murrelets occurring on the lake actually foraged there to any significant extent. By creating a model depicting isotopic values expected from birds feeding exclusively in a freshwater C-3 biome compared with a coastal marine biome, Hobson (1990) was able to determine that individuals collected

Figure 3. Depiction of a simple linear mixing model for a bird feeding (a) exclusively at a marine dietary endpoint, (b) exclusively at a terrestrial dietary endpoint, and (c) at 50% marine and 50% terrestrial position.
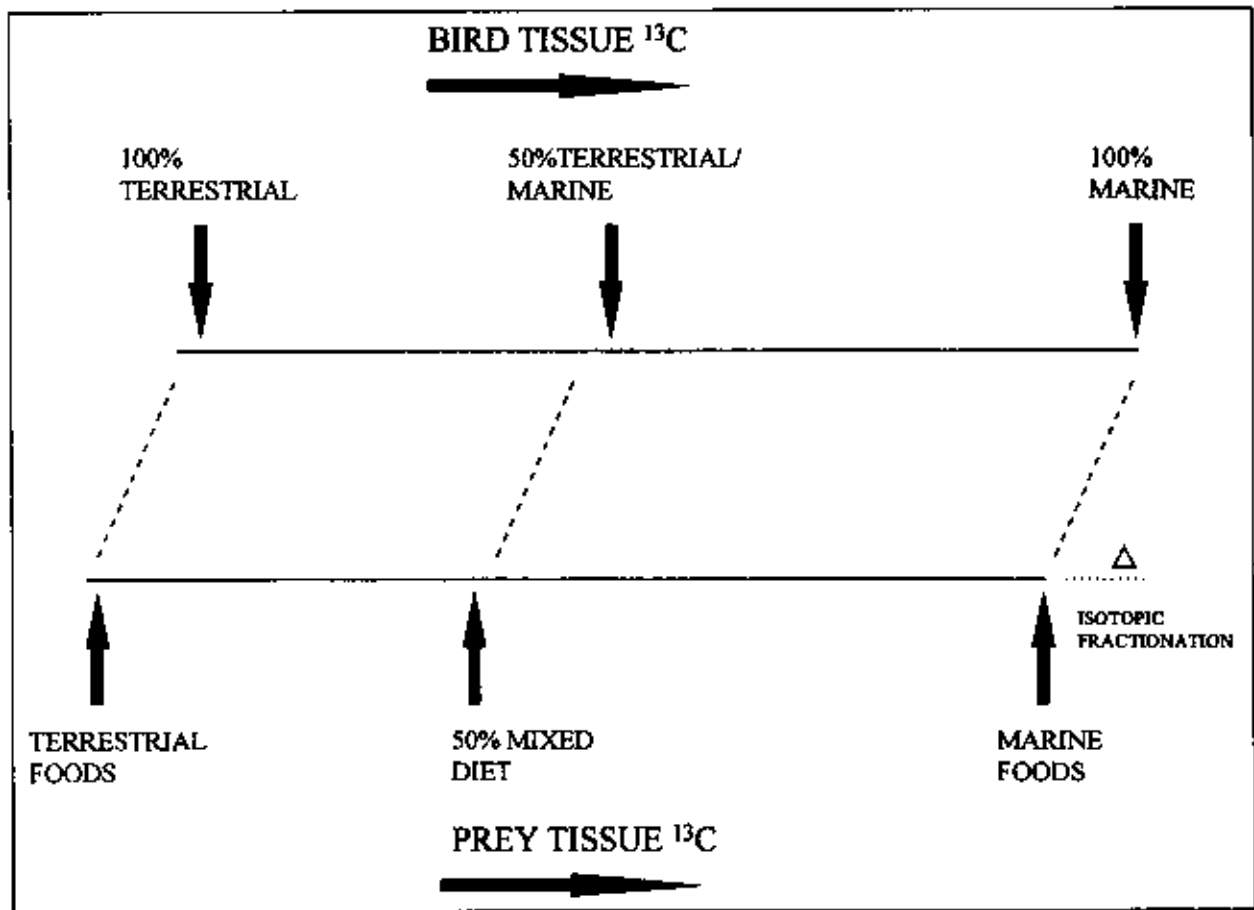
on the lake had short-term (i.e., based on muscle tissue) freshwater-derived protein inputs to their diets ranging from 50 to 100%, but had little evidence of long-term (i.e., based on bone collagen) reliance on freshwater for feeding. Hobson and Sealy (1991) conducted a similar isotopic study on Northern Saw-whet Owls (*Aegolius acadicus*) from the Queen Charlotte Islands, British Columbia. after observing that stomachs of several road-killed owls contained intertidal invertebrates. These authors wondered to what extent the *brooksii* subspecies of owls on the Queen Charlottes depended on this marine food source throughout the year. By conducting isotopic analyses of owl and terrestrial and marine prey tissues from the Queen Charlottes, and Northern Saw-whet and Boreal Owl *(A. funereus)* tissues from mainland populations with no access to marine foods, these authors were able to construct a two-source dietary input model for both muscle and collagen tissues of owls. The model based on muscle provided dietary estimates for a

period of about a month (see above) and revealed a dependence on marine amphipods ranging from zero to complete. However, the analysis of collagen from the same individuals showed no long-term dependence on amphipods. Thus, it was was concluded that the owls that foraged intertidally on amphipods were either opportunistic or depended upon this food source only for short periods during the nonbreeding season.

Alexander et al. (1996) recently applied $\delta^{13}C$ and $\delta^{15}N$ analyses of tissues and prey of Long-billed Dowitchers (*Limnodromus scolopaceus*), Stilt Sandpipers (*Calidris himantopus*), and Hudsonian (*Limosa haemastica*) and Marbled Godwits (*L. fedoa*) at an inland migratory stopover in Saskatchewan. Alexander et al. (1996) noted the broad isotopic range of dietary samples including highly enriched $\delta^{13}C$ values for sago pondweed tubers (*Potamogeton pectinatus*) and were able to demonstrate that godwits relied primarily on tubers, whereas the other species fed mostly on invertebrates. By analyzing a time series

of liver samples in birds throughout the stopover period, they were able to monitor dietary change through time for each species. However, the authors cautioned that researchers using stable isotopes to assess migratory shorebird diets should be aware of possible complications arising from isotopic variability within prey types, even over small geographic ranges. High isotopic variability at inland agro-wetland complexes may preclude reliable isotopic assessment of shorebird diets due to fertilizer input, ammonification (i.e., the enrichment of soil ^{15}N due to a loss of isotopically lighter ammonia through evaporation) and often pronounced chemical differences between wetlands.

Using stable isotopes to trace nutrients to reproduction

Other elements with stable-isotope values that are typically enriched in marine versus terrestrial foodwebs include sulfur, hydrogen and nitrogen (Fry and Sherr 1988; Michener and Schell 1994). The occurrence of isotopically distinct inputs to various systems also forms the basis for using stable-isotope measurements to trace sources of pollutants in foodwebs (reviewed by Macko and Ostrom 1994).

Dietary shifts in birds from terrestrial or freshwater C-3 to marine ecosystems (and vice versa) influence isotopic signatures in a variety of tissues (Hobson 1986, 1990; Mizutani et al. 1990; Thompson and Furness 1995). Bird eggs are particularly amenable to this type of analysis since nutrients required for egg production are derived ultimately from the diet of the laying female (von Schirnding et al. 1982; Schaffner and Swart 1991; Hobson 1995; Jarman et al. 1996). In their recent examination of seabird eggs and marine prey, Jarman et al. (1996) suggested that the combined use of contaminant and stable-isotope analyses of egg proteins and lipids might provide information on the allocation of endogenous versus exogenous lipid reserves to eggs. This, together with the isotopic investigations of Hobson (1995) using captive-raised birds, clearly suggests that stable isotopic analyses of various egg components can provide information on the source of nutrients in egg production.

Although birds might be expected typically to synthesize egg proteins directly from diet, systematic declines in somatic protein in response to protein demands during egg production have been demonstrated in wild waterfowl (reviewed by Alisauskas and Ankney 1992; see also Houston et al. 1995; Williams 1996). In such cases, the occurrence of

carbon or sulfur from proteins originating in body reserves that were in turn derived from areas separate from the breeding grounds is possible. Lipid nutrient storage prior to reproduction has been demonstrated in waterfowl (Alisauskas and Ankney 1992; but see Perrins 1996) and it was this component of eggs that we assumed would have the greatest likelihood of providing isotopic evidence for transfer between marine and freshwater systems by migrating birds.

Hobson et al. (1997) performed δ^{13}C and δ^{34}S analyses on eggs of two migratory species of fish-eating birds, Caspian Terns (*Sterna caspia*) and Double-crested Cormorants (*Phalacrocorax auritus*) from Lake Ontario, Canada, to examine the extent to which nutrient reserves acquired on marine wintering grounds are transferred to eggs laid on freshwater breeding grounds. In order to establish isotopic patterns typical of eggs of birds using marine and freshwater C-3 biomes, eggs of Herring Gulls (*Larus argentatus*), a year-round resident on the Great Lakes, and those of Caspian Terns and Herring Gulls, breeding respectively in the Gulf Coast of Texas and Atlantic coast of Canada, were analyzed isotopically. Individual egg components showed distinct isotope values that were similar for both migratory and non-migratory birds breeding on Lake Ontario and were significantly lighter than those from species breeding in a marine biome. Hence, there appears to be little evidence for significant nutrient transfer between the two biomes. The intermediate isotope values shown for egg components of Herring Gulls breeding on the Atlantic coast suggest nutrient input from terrestrial as well as marine sources. These results indicated the utility of stable-isotope analysis for tracing endogenous and exogenous contributions to reproduction in birds and further validated the use of migratory birds as indicators of breeding area contaminant levels and their effects on the Great Lakes.

Trophic relationships

Numerous studies have demonstrated a step-wise trophic enrichment of ^{15}N in marine foodwebs (reviewed by Michener and Schell 1994). However, it has only been relatively recently that this approach has been applied to studies of seabirds Hobson (1990) who measured archived tissues of Marbled Murrelets from coastal British Columbia. This study provided the impetus to expand isotopic investigations to whole seabird communities and Hobson and Welch (1992) then investigated stable-carbon and nitrogen isotope ratios in the high arctic foodweb of Lancaster Sound. That study, based on an analysis of particulate organic

matter (POM) through polar bears (*Ursus maritimus*), showed an overall trophic enrichment of 3.8‰ for ^{15}N and little or no trophic effect for ^{13}C. Within marine birds, Dovekie (*Alle alle*) and Common Eider (*Somateria mollissima*) were shown to occupy lower trophic levels than the more piscivorous Thick-billed Murre (*Uria lomvia*) and Black Guillemot (*Cepphus grylle*). The highest trophic level was determined for Glaucous Gull (*Larus hyperboreus*) that in turn fed to some degree on murre chicks. Hobson (1993) extended this work by modelling short-, intermediate, and long-term trophic positions of the Lancaster Sound seabird community by considering isotope ratios of liver, muscle, and bone collagen, respectively. Importantly, Hobson (1993) refined these models by using tissue-dependent isotopic fractionation factors determined from studies of captive Ring-billed Gulls (Hobson and Clark 1992b).

Following the initial work in Lancaster Sound, Hobson et al. (1994) then applied a similar approach to delineating a more complex seabird community involving 22 species in the Gulf of Alaska. Both the Lancaster Sound and Gulf of Alaska studies revealed that lower trophic-level organisms were more important to several seabirds than was previously recognized. In addition, these studies also clearly demonstrated that inshore or benthic feeding species were more enriched in ^{13}C than those species feeding offshore on more pelagic foodwebs (see also Hobson et al. 1995). The isotope approach proved valuable, then, in delineating both spatial and trophic aspects of seabird feeding relationships.

An interesting further application of trophic delineation of seabirds using stable-nitrogen isotope analyses was the isotopic investigation of the diet of the extinct Great Auk (*Pinguinus impennis*). Hobson and Montevecchi (1991) measured δ^{13}C and δ^{15}N values in bone collagen of adult and juvenile Great Auks excavated at Funk Island, Newfoundland, and determined that adult birds fed at a trophic continuum from crustaceans through piscivorous fish. The few juveniles measured occupied low trophic positions. These results led to the hypothesis that chicks may have been fed large quantities of euphausiids by regurgitation, suggesting further a greater evolutionary convergence between Great Auks and southern hemisphere penguins than was previously thought (Prince and Harris 1988).

Following the very clear demonstration of the value of an isotope approach to seabird studies (Hobson et al. 1994), other researchers have since applied this technique. Notably, Thompson et al.

(1995) investigated historical and contemporary diets of Northern Fulmars (*Fulmarus glacialis*) from two northeast Atlantic colonies by analyzing δ^{13}C and δ^{15}N values in archived feathers. These authors provided isotopic evidence for a change in trophic level following the end of whaling when offal apparently became less available (see also Thompson and Furness 1995). Minami et al. (1995) investigated stable-carbon and nitrogen isotope ratios in pectoral muscle of Sooty (*Puffinus griseus*) and Short-tailed (*P. tenuirostris*) Shearwaters during their northward migration in the North Pacific Ocean and provided evidence for changes in dietary preference during this period. That work was continued by Minami and Ogi (1997) who also used δ^{15}N values in Sooty Shearwaters to infer migratory pathway used by individuals to reach the North Pacific. Recently, Gould et al. (1997) investigated trophic relationships of albatrosses associated with squid driftnet fisheries in the North Pacific Ocean and provided evidence for differences in diet among species. Although not trophic determination studies *per se*, a few researchers have also used stable nitrogen isotope ratios of soils in order to delineate locations of historical seabird rookeries (Mizutani et al. 1986, 1991b,c; Mizutani and Wada 1988).

Tracking migratory birds

Most songbirds that breed in temperate forests of North America migrate annually to wintering sites in the southern United States or neotropics (Hagan and Johnston 1992; Finch and Stangel 1993). However, virtually no information exists on links between discrete breeding and wintering areas. This lack of information is of particular concern because neotropical migrant songbirds have shown dramatic declines in recent decades (Robbins et al. 1989; Hagan et al. 1992; Robbins et al. 1993; Askins et al. 1990; Sherry and Holmes 1993; Rappole and MacDonald 1994). Essential to the conservation of these songbirds is an ability to link breeding and wintering populations (Myers et al. 1987; Sherry and Holmes 1995; Chamberlain et al. 1997), especially since many species are philopatric to wintering as well as breeding sites (Greenwood and Harvey 1982; Rappole et al. 1983, 1992; Holmes and Sherry 1992). Such information would contribute to understanding factors influencing population regulation in these species and would be a powerful tool in their protection since conservation efforts could be matched at both ends of migration routes and at intermediate migratory stopover sites (Moore and Simons 1992). To date, it

has been difficult to trace migratory paths of neotropical songbird populations by marking with bands or transmitters, through the use of molecular techniques (Moore and Simmons 1992; Wenink et al. 1994) or through behavioural characters (e.g., DeWolfe and Baptista 1995). However, the recent measurement of naturally-occurring stable isotopes in animal tissues has been used to delineate geographically-distinct populations (van der Merwe et al. 1990; Vogel et al. 1990; Alisauskas and Hobson 1993) and this approach may also be suitable for the study of migrant songbirds (Chamberlain et al. 1997; Kelly and Finch 1998).

Stable-hydrogen isotope ratios (δD) in precipitation show continent-wide patterns (Sheppard et al. 1969; Taylor 1974) with a general trend of decreasing δD in precipitation from the Gulf of Mexico across the North American continent to higher latitudes in a northwesterly direction (reviewed by Cormie et al. 1994; Figure 1). Previous studies have shown that δD signatures in rainfall during the growing season are translated to plant biomass and higher trophic-level consumers (Shiegl and Vogel 1970; Estep and Dabrowski 1980; Yapp and Epstein 1982; Cormie et al. 1994). For birds, the non-exchangeable isotopic composition of feathers (i.e. the signature that remains fixed following feather formation) reflect diet only during the period of feather growth (e.g., Hobson and Clark 1992a). Since many species of migrant songbirds grow feathers on or close to their breeding grounds before migration, the δD values of the non- exchangeable hydrogen fraction of their feathers should be correlated with δD values for local growing season precipitation (δD_w). Preliminary evidence for the use of δD analysis of bird feathers to discern the origin of feather growth was provided by Chamberlain et al. (1997) in their study of Black-throated Blue Warblers (*Dendroica caerulescens*) breeding along a north-south gradient of the eastern seaboard of the United States. These authors demonstrated that deuterium values in whole feathers, including exchangeable and non-exchangeable components (δD_f), were correlated with those they expected from rainfall in areas where birds were sampled.

Hobson and Wassenaar (1997) expanded on these findings by investigating δD patterns in songbird feathers on a continental scale. Moreover, because there have been no experimental studies investigating relationships between δD_f and δD in diets of birds under controlled conditions, they conducted studies on captive birds to investigate patterns of isotopic fractionation in δD between whole diet and feathers. Hobson and Wassenaar (1997) examined the relationship between δD_f in wild passerines and δD_w from their breeding locations across North America in order to see if the hydrogen isotope technique could be applied broadly and across species of similar trophic level. At a wintering site in Guatemala, they also tested this technique by examining δD_f of wintering songbirds having both broad and narrow breeding distributions in North America. A strong positive correlation between δD_f and δD_w ($r^2=0.83$) was found. This indicates that songbird δD_f reflects δD_w in areas where feathers were grown and that the technique is largely independent of species occupying a particular trophic level. Hobson and Wassenaar (1997) observed also that δD_f for hatch-year Least Flycatchers (*Empidonax minimus*) were significantly lower than those of adults (i.e. -102.6±2.4 versus -66.1±5.4, n=7,8, t=5.81, p<0.001). This observation was consistent with the fact that adults of this species moult and grow new flight feathers *after* their migration to Central America where δD_w is heavier than at Canadian breeding sites. The results of δD_f analyses of wintering neotropical migrants in Guatemala illustrated the utility of this approach for linking breeding and wintering grounds.

A potential limitation to the general application of δD measurements to food web studies is the exchange of hydrogen between ambient environment and non-carbon bonded (e.g., O-H and N-H) hydrogen in organic matter (DeNiro and Epstein 1981; Schimmelmann 1991; Schimmelmann et al. 1993; Cormie et al. 1994). In various organic materials, hydrogen bound to oxygen and nitrogen is known to exchange with hydrogen in local water vapor, thereby presenting problems of interpretation and analysis. In the study of Hobson and Wassenaar (1997) and that of Chamberlain et al. (1997), δD_f measurements were made without correcting for possible isotopic exchange between feather samples and local water vapor. However, both studies demonstrate a strong relationship between δD_f and δD_w where feathers were grown and not where they were analyzed. Of the 40% of hydrogen occurring in keratin that is potentially exchangeable (i.e. that bonded to oxygen or nitrogen) only 15% or less appears to exchange with ambient water vapor, a process taking approximately 2 weeks (Chamberlain et al. 1997). Nonetheless, it will be necessary to address this issue, particularly in cases where bird feathers are retrieved from the wintering grounds for those species known to migrate between areas encompassing a broad gradient in δD_w. In such

cases, it will be necessary to equilibrate all feathers with water of known isotopic composition or extract only the non-exchangeable fraction of keratin prior to isotopic analysis (e.g., Schimmelman et al. 1993; Cormie et al. 1994; Chamberlain et al. 1997). Such procedures would also allow comparison of hydrogen isotope data for feathers between laboratories.

The utility of using δD_f measurements to link breeding and wintering sites of neotropical migrants will vary according to individual species' ecology and distribution, and the use of additional stable isotopes may also better segregate individuals or populations (van der Merwe et al. 1990; Vogel et al 1990; Alisauskas and Hobson 1993; Chamberlain et al. 1997). In addition, refinements to our knowledge of the distribution of stable hydrogen isotope ratios in rainfall and how these relate to local foodwebs involving migratory birds, will allow corrresponding improvements in our ability to link breeding and wintering sites. However, despite the need for further refinements, the large hydrogen isotopic gradient in rain across North America currently allows the ready discrimination of distinct breeding populations of songbirds and other organisms. This phenomenon provides a new means of associating birds on their wintering grounds to general areas in North America where their feathers were grown.

Future directions
The measurement of naturally-occurring stable isotopes of several elements to elucidate the ecology of individual species or communities of birds has undergone tremendous growth during the last decade and cover a broad spectrum of applications (Table 4). However, there are areas of isotopic research that should be encouraged in order for the field to progress. One area of immediate interest is the continuation of studies using captive birds raised on controlled diets in order to improve our understanding of isotopic

fractionation factors between diet and several tissues (see also Gannes et al. 1997). The earlier studies of Hobson and Clark (1992a,b) and Mizutani et al. (1990, 1992) must be considered preliminary. A better understanding of how fractionation factors are affected by diet is also required, and the hypotheses of Hobson (1995) regarding the influence of carnivory and herbivory on isotope fractionation involved in the synthesis of egg components need to be confirmed. Captive studies could also be used to better understand the behavior of hydrogen and sulfur isotopes in foodwebs involving birds. Both of these isotopes are currently much underused and our knowledge of factors influencing their fractionation and abundance in nature is rudimentary. Future applications of stable isotope analyses will also provide a direct means of quantifying the role of endogenous and exogenous reserves to reproduction in a wide variety of species and will likely put to rest a number of outstanding questions and controversies in this area of avian reproductive physiology and ecology.

To date, the vast majority of isotopic applications to foodweb studies have focused on bulk tissues such as muscle, liver, blood and feathers. However, with changing technology it is now possible to look at isotopic signatures of individual amino acids and fatty acids (e.g., Macko et al. 1983; Abrajano et al. 1994). Analysis of these components may allow a more refined means of tracing source of feeding in birds since it allows the tracing of those essential materials that are necessarily derived only from diet and not synthesized within the animal.

The recent development of the use of stable hydrogen isotope analysis to track migratory birds and other wildlife represents an extremely exciting area of research, particularly when integrated with other marker or tracer techniques such as DNA and trace element analysis. This approach may provide the only viable means of linking breeding and wintering areas

Table 4. Summary of the typical applications of stable isotope analyses to avian ecological studies.

Application	Typical Tissues	Isotope(s)	Approximate Cost/sample (1998$)
Source of feeding:			
C_3 versus C_4, CAM	muscle, blood, feather, eggs	^{13}C	$10-15
marine versus terrestrial/freshwater	muscle, blood, feather, eggs	$^{13}C, ^{34}S, ^{15}N, ^{87}Sr, ^{18}O$	$10-60
inshore versus offshore	muscle, blood, feather, eggs	$^{13}C, ^{34}S$	$10-60
Trophic level:	muscle, blood, feather, eggs	$^{15}N, ^{13}C$	$10-20
Natal origin and migration route:	keratin (feather, nail, bill)	D, $^{18}O?, ^{87}Sr, ^{13}C$	$100

for small migratory songbirds. An important avenue of research in this regard will be the development of more accurate isotopic maps of North America and an understanding of the scope of variablity in patterns of D/H in rainfall between years.

Finally, while I have espoused the virtues of using stable isotope analyses in ecological investigations involving birds, it is important to realize that this approach cannot, nor should it, replace more traditional methods. Instead, it is very clear that, for dietary studies, stable isotope analyses will rather *augment* those methods that provide more taxonomic information (see Sydeman et al. 1997). Nonetheless, the renewed interest in how birds fit into larger ecosystems and their possible roles as ecological indicators (e.g., Furness and Greenwood 1993), suggests that the stable isotope approach will provide new information that could not be obtained using any other techniques.

Acknowledgements

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