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ASSESSING AVIAN DIETS USING STABLE ISOTOPES I: TURNOVER OF ^{13}C IN TISSUES[1]

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Abstract. Studies of birds that use stable isotopes as dietary tracers require estimates of how quickly stable isotopes in tissues are replaced by isotopes derived from the diet. However, isotopic turnover rates in animals in general, and birds in particular, are poorly understood. We established the turnover rates of ^{13}C in tissues of grown Japanese Quail (*Coturnix japonica*) by switching the diet of an experimental group from a wheat-based (C_3) diet to a corn-based (C_4) diet and sampled 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 with the half life of carbon ranging from 2.6 days in liver to 173.3 days in bone collagen. A similar diet-switch experiment was conducted on captive American Crows (*Corvus brachyrhynchos*) and feather samples were assayed isotopically. Stable isotope values of crow feathers reflected diet during periods of growth. We suggest that stable isotope analysis could be used to determine relative contributions of endogenous and exogenous nutrient sources for feather growth and egg production in captive and wild birds.

Key words: *Japanese Quail;* Coturnix japonica; *American Crow;* Corvus brachyrhynchos; *isotopic turnover; carbon; stable-isotope analysis; diet determinations.*

INTRODUCTION

Stable-carbon and nitrogen isotope analysis is being used increasingly as a tool to delineate dietary patterns in terrestrial and marine ecosystems (reviewed by Peterson and Fry 1987, Rundel et al. 1989) because stable-isotopic compositions of consumer tissues can often be related predictably to stable-isotopic compositions of diet (DeNiro and Epstein 1978, 1981). In dietary studies of birds, stable-carbon isotope analysis has been used to determine relative contributions of marine and terrestrial foods to the diets of various species since foods within these biomes often differ isotopically (Hobson 1986, 1990, Mizutani et al. 1990, Hobson and Sealy 1991). More recently, Hobson (1990a, 1990b) determined that stable-nitrogen isotope analysis can be used to establish trophic relationships among seabirds (see also Hobson and Montevecchi 1991; Schaffner and Swart 1991). C_3 and C_4 plants have characteristic carbon-isotope signatures due to their different photosynthetic pathways, and so other potential applications of the stable-carbon isotope technique include the determination of relative contributions of C_3 and C_4 plant-based proteins to avian diets in areas where these two plant types coexist (see Schirnding et al. 1982, reviewed by Tieszen and Boutton 1989). Naturally occurring stable isotopes have also been used to establish sources of organic matter in the diets of organisms using estuaries or coastal marshes (reviewed by Fry and Sherr 1989) and so might be appropriate for dietary studies of migratory or other wetland species.

The stable-isotope technique is useful in situations where two isotopically distinct dietary sources are available to consumers. In such cases, isotopic analysis of tissues provides quantitative information on the relative contributions of each source to the diet. The isotope approach typically cannot replace conventional techniques where detailed (i.e., taxonomic) dietary information is needed, particularly when several dietary options are available. However, important advantages to using naturally occurring stable isotopes as dietary indicators over conventional techniques include i) isotopic dietary estimates are based on assimilated and not just ingested foods and ii)

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comparatively long-term dietary information can be obtained.

The period over which tissue isotopic concentrations will reflect the isotopic signature of a particular diet will depend, in part, on the isotopic turnover rate in that tissue. Tissues with rapid isotopic turnover will reflect recent diet whereas those with slow turnover will reflect longer-term dietary averages. The choice of tissue type for isotopic analysis will 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 might be obtained. By measuring the isotopic composition of both pectoral muscle tissue and bone collagen from Northern Saw-whet Owls (*Aegolius acadicus*) on the Queen Charlotte Islands, British Columbia, Hobson and Sealy (1991), for example, established the *seasonal* importance of marine amphipods in this species' diet.

Currently, there are two major limitations to the application of stable-isotope analysis in avian dietary studies. First, it is not well understood how stable isotopes fractionate or change once they are incorporated into tissues (but see Mizutani et al. 1991). Knowledge of diet-tissue fractionation factors is critical to studies concerned with predicting isotopic compositions of diets from isotopic compositions of consumer tissues. A second limitation is that precise turnover rates of isotopes in tissues of wild birds are poorly known. To date, only Tieszen et al. (1983) have investigated stable-isotopic turnover rates in mammals by switching isotopic compositions of diets of laboratory animals under controlled conditions. Their study was conducted on gerbils (*Meriones unguienlatus*) and it is not known to what extent their findings are applicable to studies of birds.

As a first step in establishing isotopic turnover rates in various tissues of birds, we conducted controlled laboratory tests using captive Japanese Quail (*Coturnix japonica*) and American Crows (*Corvus brachyrhynchos*). By switching the diets of an experimental group of grown quail to one consisting of a different stable-carbon isotopic signature, we established isotopic replacement curves for blood, liver, muscle and bone collagen. A similar procedure was followed for crows but here we evaluated non-destructive sampling assays using feathers only. To our knowledge this is the first attempt to establish turnover rates of carbon in avian tissues by switching the stable-carbon isotope composition of diets.

METHODS

TURNOVER IN QUAIL

Our experimental approach followed that of Tieszen et al. (1983), who switched gerbils between corn (C_4)- and wheat (C_3)-based diets. Corn is substantially more enriched in ^{13}C compared to wheat and so can be used as a convenient tracer in experiments involving diet switching. In previous studies, we established that nutritional stress can cause changes in the stable-isotope values of tissues (Hobson and Clark 1992). Rather than switching quail between pure corn and pure wheat diets, a procedure which would ensure a major isotopic signal change but might result in complications due to nutritional stress, we decided to ensure that experimental and control diets were very similar nutritionally. A special batch of homogenized commercial grower was formulated in which corn replaced wheat in a proportion ensuring no change in crude protein (23%), fat (5%) and fiber (5%). For this reason, the stable-carbon isotopic composition of control and experimental feeds differed by only 4‰, considerably less than was the case in the study of Tieszen et al. (1983) but of sufficient magnitude to trace isotopic turnover in tissues. Several isotopic measurements of experimental and control feeds were made throughout the study.

Quail were obtained from a colony in which birds were fed the commercial wheat-based grower (i.e., control diet) for several generations. After hatching, thirty-six birds were raised on the wheat-based diet for eight weeks until completion of growth (Blem 1978) and then split randomly into a control group ($n = 6$) and an experimental group ($n = 30$). We used quail that had achieved adult size in order to ensure that changes in tissue isotope values reflected true carbon turnover and not a combination of growth and turnover. Control birds were maintained on the original wheat-based diet and three of these birds were sacrificed for isotopic analysis at the start of the experiment (day 0) and three at the end (day 212). Experimental birds were switched to a corn-based diet on day 0 and three birds were randomly chosen and sacrificed (using Halothane overdose) for isotopic analysis on days 2, 4, 6, 10, 17, 35, 84, 121, and 212. Before sacrificing birds, approximately 1 ml of blood

was obtained from the brachial vein. Pectoral muscle, liver and humerus were removed from carcasses and stored frozen. Collagen was removed from bones as a gelatin according to the method described in Chisholm et al. (1983). Muscle and liver were thawed, rinsed in distilled water and, together with collagen samples, were freeze dried, powdered, and lipids removed using a Soxhlet apparatus with chloroform as the solvent. Because carcasses were not drained of blood, it is possible that some blood remained in muscle and liver samples. However, we expect that this residue would be extremely small compared to the bulk of the tissue being measured isotopically.

CROW FEATHERS AS DIETARY INDICATORS

Nine yearling American Crows, raised in captivity from nestlings, were held in a large outdoor aviary, provided with shelter and natural perches, and given access to a mixture of dog food, barley and duck grower, as well as water, *ad libitum*. When they had replaced their second primary, we changed their diet to the corn-based mixture, measured the length of new, growing feathers, and weighed the crows (nearest 5 g) using a Pesola scale. With two crows we used tail feathers since their primaries had been too damaged by abrasion.

After two weeks we measured the length of feathers that had been measured previously and recorded body mass. At that time, we changed their diet to one composed primarily of wheat, and held them on this diet for three weeks. We then measured feather lengths and body mass and clipped portions of feathers that had been grown on either the corn- or wheat-based diets. We are confident that the crows ate the prescribed diets. First, we noted that the food provided was eaten, rather than scattered throughout the pen. Second, the change in body mass of crows was either negligible or positive when fed both corn ($\bar{x} \pm$ SD = 14 ± 10 g, range = 0–33 g, n = 9) and wheat (18 ± 16 g, range = −4 to 36 g) based diets. Third, we found no evidence of irregular feather growth in these crows. The daily growth of primary feathers averaged 7.8 ± 1.8 mm (range = 6–11, n = 7).

ISOTOPIC ANALYSIS

Samples for isotopic measurement were loaded into pyrex tubes with 1 g of wire-form CuO, evacuated, flame sealed and then combusted to CO_2 at 550°C for 6 hr. We established previously that, for $\delta^{13}C$ analysis, this technique gives comparable results to that using combustion in Vycor tubes at 850°C and our cryogenic distillation technique resulted in no analytical problems with high sulfur samples (Swerhone et al. 1991). Stable-carbon isotope concentrations were made on cryogenically distilled CO_2 using a VG-SIRA 12 mass spectrometer and values expressed in δ notation as parts per thousand (‰) relative to the Peedee Belemnite (PDB) standard as follows:

$$\delta^{13}C = [(R_{sample}/R_{standard} - 1)] \times 1{,}000$$

where $R = {}^{13}C/{}^{12}C$. Based on several hundred replications, the standard deviation for a graphite internal standard was 0.1‰.

RESULTS

QUAIL

Details of the isotopic compositions of quail and crow diets are given in Table 1. Stable-carbon isotope values of tissues from the experimental group of quail shifted toward more positive values over the course of our experiment (Table 2). This shift was consistent with the incorporation in tissues of isotopically enriched carbon from the corn-based diet. For all tissues, the stable-carbon isotope values for the quail control group did not appear to change over the course of the experiment (Table 2). Relative to other tissues, the enriched bone collagen $\delta^{13}C$ values observed before the diet switch were not apparent at the end of the experiment (Kruskal-Wallace test, H = 10.6, P = 0.3). This was a consequence of the much longer half-life of carbon in bone collagen relative to other tissues.

Patterns of carbon turnover in quail tissues resembled exponential models (Fig. 1) and so, for each tissue, we fitted our data to equations of the form $y = a + be^{ct}$ using PROC NLIN of the Statistical Analysis System (SAS Institute 1985). Here, y represents the $\delta^{13}C$ value of the tissue in question, a and b are parameters determined by initial and asymptotic conditions, c is the turnover rate of carbon in the tissue, and t is time (d) since the diet switch. This exponential equation provided a good fit for all of our data (Fig. 1). The $\delta^{13}C$ values for liver, blood and muscle, but not for bone collagen, reached asymptotic values during our experiment. We calculated half-lives of tissue carbon by solving the equation half-life = ln(0.5)/c. The null hypothesis that there is a single population underlying

TABLE 1. Stable-carbon isotope values ($\delta^{13}C_{PDB}$, mean ± SD ‰) of diets used in quail and crow captive-rearing experiments.

Study	Wheat-based	(*n*)	Corn-based	(*n*)	Mean difference
Quail	−24.1 ± 0.6	3	−19.5 ± 0.6	3	4.6
Crow	−23.7 ± 0.4	3	−20.1 ± 0.5	4	3.6

all tissue exponential equations was rejected ($F_{12,91} = 16.8$, $P < 0.0001$) using the method described by Zar (1984:347) for multiple regression equations. In addition, blood and muscle tissues that gave similar isotopic turnover rates were nonetheless significantly different ($F_{4,53} = 4.5$, $P < 0.01$).

CROWS

Portions of crow feathers grown during the period when birds were fed a wheat-based diet ($\bar{x}$ ± SD = −19.3 ± 0.7‰, $n = 9$) were depleted in ^{13}C relative to those portions of feathers grown on a corn-based diet (−16.6 ± 0.7‰, $n = 8$; Student's t test, $t = 4.00$, $P < 0.01$). The mean isotopic difference between feather $\delta^{13}C$ values and those of the diet during which that portion of the feather was grown were +3.5 and +4.4‰ for corn and wheat diets, respectively.

DISCUSSION

ISOTOPIC TURNOVER IN AVIAN TISSUES

The isotopic measurement of whole tissues yields an integration of the isotopic signatures of various components making up those tissues. Each of these components (e.g., proteins) typically have characteristic turnover rates within each tissue resulting in the presence of "pools" of carbon with differing turnover rates. Thus, the turnover rates we have established experimentally for grown quail represent integrations of the values for the various components of those tissues. Turnover values we have established for whole blood may not, for example, be applicable to those of separate blood fractions such as plasma or haemoglobin (Waterlow et al. 1978).

The turnover rates of dietary carbon varied widely amongst the quail tissues examined. This confirms that dietary information based on different periods of assimilation may be obtained by measuring stable-isotope concentrations in various tissues (Tieszen et al. 1983). Half-lives of carbon ranged from 2.6 days in liver to 173.3 days in bone collagen. Although they did not measure bone collagen, Tieszen et al. (1983) similarly found that half-lives of dietary carbon in gerbils ranged from 6.4 days for liver to 47.5 days for hair, and demonstrated that carbon turnover rate was correlated linearly with the metabolic rate of tissues.

The high metabolic rate of liver (Schoenheimer 1949, Waterlow et al. 1978) results in the rapid turnover of dietary carbon in that tissue and our results suggest that, for birds, isotopic analyses of this tissue can provide very short-term dietary information. In contrast, turnover rates of carbon in bone collagen of adult birds are extremely slow (see also Stenhouse and Baxter 1979) and isotopic measurements of collagen may yield dietary information based on a lifetime integration. However, collagen values of young birds are expected to reflect primarily diets during the growth phase of the bone and so, in cases where the age of the bird is unknown, care must be taken in the interpretation of bone iso-

TABLE 2. Stable-carbon isotope values ($\delta^{13}C_{PDB}$, mean ± SD ‰, $n = 3$ in all cases) for various tissues of grown quail raised on control (wheat-based) and experimental (corn-based) diets. Time is measured from the start of the diet switch.

Time (days)	Blood	Liver	Muscle	Collagen
Control				
0	−23.4 ± 0.1	−23.7 ± 0.4	−23.2 ± 0.4	−21.2 ± 0.2
212	−23.6 ± 0.2	−23.2 ± 0.3	−23.5 ± 0.4	−21.1 ± 0.3
Experimental				
212	−20.4 ± 0.3	−20.5 ± 0.2	−19.5 ± 0.4	−19.8 ± 0.3

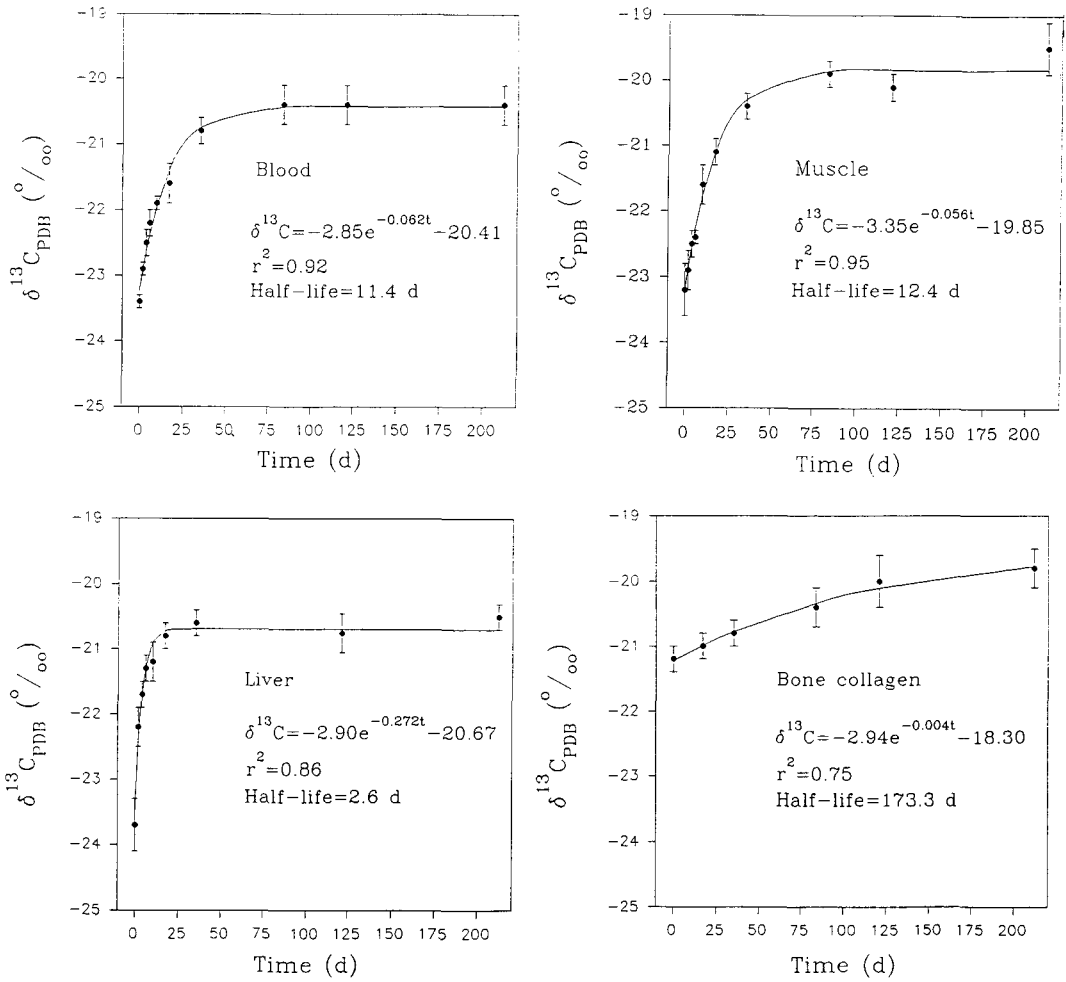

FIGURE 1. Stable-carbon isotope exponential models for quail tissues. Data are means (closed circles) ± SD (vertical lines) and sample sizes are $n = 3$ for each point.

topic data (see Hobson and Montevecchi 1991). In the present study, after 173 days on the new diet, half of the quail collagen still reflected diet during the growth phase of the first 56 days of life.

Japanese quail have high energy requirements. Blem (1978) pointed out that estimates of resting metabolism and adult existence energy levels in this species are higher than values predicted from standard metabolism equations for non-passerines (e.g., Freeman 1967, Lasiewski and Dawson 1967, Aschoff and Pohl 1970, Kendeigh 1970). It is possible, then, that the turnover rates calculated for various quail tissues may be faster than for other birds with lower basal metabolism. On the other hand, our laboratory birds were confined to pens and were not exercised extensively. In wild birds, field metabolic rates are often considerably higher than basal metabolic rates (Nagy 1987) and so it is possible that faster turnover rates occur in wild birds than those calculated for captive quail. Thus, our study provides a useful first approximation to stable isotopic turnover rates in avian tissues but further studies are required to determine how variations in metabolic rate (e.g., due to body size, developmental stage or activity) influence isotopic turnover in tissues. We also note that it would be useful, in future studies of this nature, to increase sample sizes of birds analyzed at each stage of diet switch experiments. Larger sample sizes would help to more accurately delineate patterns

of turnover, as well as increase statistical power when testing differences among tissues.

The isotopic half-life of 12.4 days found for pectoral muscle is considerably lower than the value of 27.6 days found by Tieszen et al. (1983) for gerbil muscle and strengthens Hobson and Sealy's (1991) suggestion that carbon-isotopic turnover in avian muscle tissue is likely faster than that found for gerbils. We found similar turnover rates of carbon in quail blood and pectoral muscle. This suggests that when birds can be captured alive, stable-isotopic studies of avian diet need not necessarily involve the killing of birds. Analyses of separate fractions of blood such as serum might allow short-term dietary resolution approaching that of liver. Another advantage of using blood is that it would allow multiple sampling of the *same* individual and so creates the possibility of monitoring dietary changes of known individuals through time.

When dietary carbon is incorporated into a particular tissue, a characteristic fractionation or change in the isotope value relative to diet is expected. In crows, we found that the mean fractionation factor between diet and feathers ranged from +3.5‰ for the corn diet to +4.4‰ for the wheat diet. Both values are close to the diet-feather fractionation factor of +4.0‰ found by Mizutani et al. (1990) for a captive cormorant fed fish. We determined also that, for captive crows, the isotopic compositions of feathers track isotopic changes in diet while they are being grown (after growth, feathers are essentially inert structures with no further isotopic exchange with body constituents). This is based on our observation that the difference (2.7‰) between mean isotopic values for feathers grown on control (wheat) and experimental (corn) diets was in the same direction and of similar magnitude to the mean isotopic differences between these diets (3.6‰).

IMPLICATIONS FOR STUDIES OF NUTRIENT RESERVE DYNAMICS FOR MOLT AND REPRODUCTION

In birds, molt and reproduction are processes often requiring major energy demands (e.g., King 1974) and it is not clear to what extent these demands are met by relative contributions from endogenous reserves and diet (Hanson 1962, Krapu 1981, Austin and Fredrickson 1987, Afton and Ankney 1991). To date, studies concerned with nutrient dynamics during molt or reproduction have necessarily relied on associations between the dynamics of tissue mass loss and stage of molt or reproduction, or on evidence for general catabolism or mobilization of nutrients during these processes. A more desirable approach would be to establish *directly* the relative contributions of nutrients from endogenous and dietary sources to the composition of feathers and eggs. If dietary carbon, either in the form of proteins or lipids, differed isotopically from an endogenous source (e.g., pectoral muscle or adipose tissue), and if turnover rates of isotopes in endogenous sources were slow relative to periods of feather or egg production, then it should be possible to determine the relative contributions from either of these sources. For captive birds, such an isotopic difference between endogenous and dietary carbon could be obtained by switching diets at the time of feather or egg synthesis. If eggs or feathers showed a strong isotopic shift in the direction of the isotopic value of the new diet, then this would indicate a strong dietary component to feather or egg synthesis. A similar experimental approach was used successfully by Wilson et al. (1988) to determine the amount of dietary and endogenous reserves used by cows in milk synthesis (see also Metges et al. 1990).

Wild birds that undergo a molt migration or change diet during molt could potentially switch to a new isotopic diet during feather growth. Similarly, birds arriving on the breeding grounds with endogenous reserves for egg laying should lay eggs with isotopic signatures representing diet obtained elsewhere. Birds that winter in marine environments, or in agricultural areas where corn is an important component in the diet, and breed in terrestrial C_3 ecosystems would, for example, encounter foods that differ substantially in their δ^{13} values (e.g., Hobson and Sealy 1991; Schaffner and Swart 1991; see also Mizutani et al. 1990; Alisauskas et al. 1988; Alisauskas and Hobson, unpubl. data).

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LITERATURE CITED

AFTON, A. D., AND C. D. ANKNEY. 1991. Nutrient-reserve dynamics of breeding Lesser Scaup: a test of competing hypotheses. Condor 93:89–97.

ALISAUSKAS, R. T., C. D. ANKNEY, AND E. E. KLAAS. 1988. Winter diets and nutrition of midcontinental Lesser Snow Geese. J. Wildl. Manage. 52: 403–414.

ASCHOFF, J., AND H. POHL. 1970. Der ruheumsatz von vogeln als funktion der tageszeit und der korpergrosse. J. Ornithol. 111:38–47.

AUSTIN, J. E., AND L. H. FREDRICKSON. 1987. Body and organ mass and body composition of post-breeding female Lesser Scaup. Auk 104:694–699.

BLEM, C. R. 1978. The energetics of young Japanese Quail, *Coturnix coturnix japonica*. Comp. Biochem. Physiol. 59:219–223.

CHISHOLM, B. S., D. E. NELSON, K. A. HOBSON, H. P. SCHWARCZ, AND M. KNYF. 1983. Carbon isotope measurement techniques for bone collagen: notes for the archaeologist. J. Archaeol. Sci. 10:355–360.

DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmichim. Acta 42:495–506.

DENIRO, M. J., AND S. EPSTEIN. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45:341–351.

FREEMAN, B. M. 1967. Oxygen consumption by the Japanese Quail, *Coturnix coturnix japonica*. Br. Poult. Sci. 8:147–152.

FRY, B., AND E. B. SHERR. 1989. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems, p. 196–229. *In* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy [eds.], Stable isotopes in ecological research. Springer-Verlag, Berlin.

HANSON, H. C. 1962. The dynamics of condition factors in Canada Geese and their relation to seasonal stresses. Arctic Inst. North Amer. Tech. Pap. No. 12.

HOBSON, K. A. 1986. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. Can. J. Zool. 65:1210–1213.

HOBSON, K. A. 1990a. Stable isotopic determinations of the trophic relationships of seabirds: preliminary investigations of alcids from coastal British Columbia. *In* W. A. Montevecchi and A. J. Gaston [eds.], Studies of high-latitude seabirds 1. Behavioural, energetic, and oceanographic aspects of seabird feeding ecology. Can. Wildl. Serv. Occ. Pap. No. 68.

HOBSON, K. A. 1990b. Stable isotope analysis of marbled murrelets: evidence for freshwater feeding and determination of trophic level. Condor 92:897–903.

HOBSON, KEITH A., AND ROBERT G. CLARK. 1992. Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94:189–197.

HOBSON, K. A., AND W. A. MONTEVECCHI. 1991. Stable isotopic determination of the trophic relationships of Great Auks. Oecologia, 87:528–531.

HOBSON, K. A., AND S. G. SEALY. 1991. Marine protein contributions to the diets of Northern Sawwhet Owls on the Queen Charlotte Islands: a stable-isotope approach. Auk 108:437–440.

KENDEIGH, S. C. 1970. Energy requirements for existence in relation to size of bird. Condor 72:60–65.

KING, J. R. 1974. Seasonal allocation of time and energy resources in birds, p. 4–70. *In* R. A. Paynter, Jr. [ed.], Avian energetics. Nutall Ornithol. Club, Cambridge, MA.

KRAPU, G. L. 1981. The role of nutrient reserves in Mallard reproduction. Auk 98:29–38.

LASIEWSKI, R. C., AND W. R. DAWSON. 1967. A re-examination of the relation between standard metabolic rate and body weight in birds. Condor 69: 13–23.

METGES, C., K. KEMPE, AND H. L. SCHMIDT. 1990. Dependence of the carbon-isotope contents of breath carbon dioxide, milk erum and rumen fermentation products on the $\delta^{13}C$ value of food in dairy cows. Br. J. Nutr. 63:187–196.

MIZUTANI, H., M. FUKADA, Y. KUBAYA, AND E. WADA. 1990. Stable-carbon isotope ratios of feathers reveals feeding behavior of cormorants. Auk 107: 400–403.

MIZUTANI, H., Y. KABAYA, AND E. WADA. 1991. Nitrogen and carbon isotope compositions relate linearly in cormorant tissues and its diet. Isotopenpraxis 4:166–168.

NAGY, K. A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. Ecol. Monogr. 57:111–128.

PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18: 293–320.

RUNDEL, P. W., J. R. EHLERINGER, JR., AND K. NAGY [eds.]. 1989. Stable isotopes in ecological research. Springer-Verlag, Berlin.

SAS INSTITUTE, INC. 1985. User's guide version 5 ed. SAS Inst., Inc., Cary, NC.

SCHAFFNER, F. C., AND P. K. SWART. 1991. Influence of diet and environmental water on the carbon and oxygen isotopic signatures of seabird eggshell carbonate. Bull. Mar. Sci. 48:23–38.

SCHIRNDING, Y. V., N. J. VAN DER MERWE, AND J. C. VOGEL. 1982. Influence of diet and age on carbon isotope ratios in ostrich eggshell. Archaeometry 24:3–20.

SCHOENHEIMER, R. 1949. The dynamic state of body constituents. Harvard Univ. Press, Cambridge, MA.

STENHOUSE, M. J., AND M. S. BAXTER. 1979. The uptake of bomb ^{14}C in humans, p. 324–341. *In* R.

Burger and H. E. Seuss [eds.], Radiocarbon dating. Proc. 9th Int. Conf. on Radiocarbon Dating (1976). Univ. Calif. Press, Berkeley.

SWERHONE, G.D.W., K. A. HOBSON, C. VAN KESSEL, AND T. W. BOUTTON. 1991. An economical method for preparation of plant and animal tissue for $\delta^{13}C$ analysis. Comm. Soil Sci. Plant Anal. 22: 177–190.

TIESZEN, L. L. AND T. W. BOUTTON. 1989. Stable carbon isotopes in terrestrial ecosystem research, p. 167–195. *In* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy [eds.], Stable isotopes in ecological research. Springer-Verlag, Berlin.

TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57: 32–37.

WATERLOW, J. C., P. J. GARLICK, AND D. J. MILLWARD. 1978. Protein turnover in mammalian tissues and in the whole body. North Holland Publ., New York.

WILSON, G. F., D.D.S. MACKENZIE AND I. M. BROOKES. 1988. Importance of body tissues as sources of nutrients for milk synthesis in the cow, using ^{13}C as a marker. Br. J. Nutr. 60:605–617.

ZAR, J. H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Englewood Cliffs, NJ.