The Condor 94:189–197

ASSESSING AVIAN DIETS USING STABLE ISOTOPES II: FACTORS INFLUENCING DIET-TISSUE FRACTIONATION[1]

KEITH A. HOBSON
Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

ROBERT G. CLARK
Canadian Wildlife Service, Prairie and Northern Wildlife Centre, 115 Perimeter Road, Saskatoon, Saskatchewan S7N 0X4, Canada and
Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

Abstract. Studies using stable-isotope analysis to infer diet require a knowledge of how stable-isotope ratios in consumer tissues are related to dietary values. We 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 for blood and feather samples of adult Peregrine Falcons (*Falco peregrinus*) raised on known isotopic diets. In most cases tissues were enriched in $\delta^{13}C$ and $\delta^{15}N$ compared to diet. However, fractionation values differed among species, diets, and tissue types and this variation must be considered in isotope dietary studies. We found little evidence that isotopic fractionation factors are influenced by age in adult birds and provide fractionation factors appropriate for granivores feeding in a C-3 biome and for higher trophic-level piscivores and carnivores. American Crows (*Corvus brachyrhynchos*) raised on a perch diet grew faster and showed lower nitrogen diet-tissue fractionation values than crows raised on a plant-based diet. We suggest that nutritional stress caused substantial increases in diet-tissue fractionation values due either to: (1) mobilization and redeposition of proteins elsewhere in the body; or (2) amino acid composition changes in tissues.

Key words: Stable isotopes; isotopic fractionation; diet; carbon-13; nitrogen-15; Gallus gallus; Coturnix japonica; Larus delawarensis; Corvus brachyrhynchos; Falco peregrinus.

INTRODUCTION

Stable carbon and nitrogen isotope analyses of plant and animal tissues are now recognized as powerful tools for investigating dietary patterns and trophic relationships within past and present ecosystems (reviewed by DeNiro 1987, Peterson and Fry 1987, Rundel et al. 1989). This technique is particularly suited to determining the relative contributions of two isotopically distinct sources of organic matter to the diets of consumers (e.g., Chisholm et al. 1982) and yields time-integrated information of assimilated not just ingested foods (Tieszen et al. 1983). However, despite the potential of the stable-isotope technique to avian ecological studies, it has only recently been applied to studies of birds. For example, using measurements of stable-carbon isotope concentrations, Hobson (1987) investigated the dependence of Glaucous-winged Gulls (*Larus glaucescens*) on foods derived from garbage dumps. Similarly, relative contributions of freshwater-derived proteins in the diets of marine birds (Marbled Murrelets, *Brachyramphus marmoratus,* Hobson 1990; and cormorants, Mizutani et al. 1990) have been established isotopically (see also Schaffner and Swart 1991, Hobson and Sealy 1991). More recently, Hobson (1990, 1991) estimated trophic relationships of seabirds using stable-nitrogen isotope analysis of various tissues.

A critical assumption implicit in the application of the isotope technique is that stable-isotope concentrations of consumer diets can be related to those of consumer tissues in a predictable fashion. The stable-carbon isotopic composition of the whole body of animals appears to be enriched in ^{13}C relative to diet by about 1‰ (DeNiro and Epstein 1978, Fry et al. 1984). Corresponding enrichment values for ^{15}N appear more variable (Owens 1987) but are, on average, about 3‰ (Minagawa and Wada 1984, Wada et al. 1987, Fry 1988).

In most dietary investigations using stable isotopes, it is impractical to analyze whole bodies of consumers. Moreover, it is often desirable to

[1] Received 1 August 1991. Accepted 25 October 1991.

analyze various tissues separately since both short- and long-term dietary information may be obtained by analyzing tissues with fast and slow isotopic turnover-rates, respectively (Tieszen et al. 1983, Hobson and Clark 1992), and isotopic analysis of several tissues (e.g., blood, feathers) may be accomplished without killing birds.

Isotopic concentrations of various biochemical components of foods fractionate or change when incorporated into consumer tissues and the direction and magnitude of this change depends upon the tissue (Tieszen et al. 1983, Mizutani et al. 1991). Here, we use the term "fractionation" to describe changes in the isotopic signal between diet and consumer tissues but recognize that this effect is due to both selective biochemical assimilation of dietary components with varying isotopic signatures (see Tieszen and Boutton 1989) and by isotopic discrimination. In order to relate isotopic concentrations in a given tissue to those in a bird's diet, it is necessary, then, to establish isotopic fractionation values for each tissue type. With this information it is possible to predict dietary isotope values according to the relationship:

$$D_t = D_d + \Delta_{dt}$$

where D_t is the isotopic concentration in the tissue, D_d the isotopic concentration of the diet, and Δ_{dt} the isotopic fractionation factor between diet and tissue. Using laboratory-raised mice or gerbils, DeNiro and Epstein (1978) and Tieszen et al. (1983) established the relationship between $\delta^{13}C$ values of tissues and those of diets. More recent investigations using $\delta^{15}N$ values of animal tissues indicate also an enrichment of this isotope in various tissues compared to diet (DeNiro and Epstein 1981, Minagawa and Wada 1984).

There is almost no information available on isotopic fractionation factors between diet and avian tissues (but see Bender et al. 1981; Mizutani et al. 1990, 1991) nor it is clear to what extent isotopic fractionation values established for laboratory-raised mammals are applicable to wild birds. Variation due to diet, age, metabolism, nutritional stress and differences among individuals have been suggested but it is presently unknown to what extent these factors may complicate dietary studies using these isotopes (Owens 1987, Tieszen and Boutton 1989). This is of particular concern to ornithologists investigating dietary habits of wild birds using isotopic measurements.

We investigated stable-carbon and nitrogen isotopic fractionation values between diet and avian tissues for both domestic and captive-reared wild birds. We undertook this study to determine how these fractionation factors vary (1) among species; (2) in a piscivorous, carnivorous, and granivorous species; and (3) in response to nutritional stress.

METHODS

Our study was composed of three main parts. First, we raised domestic chickens (*Gallus gallus*) and Japanese Quail (*Coturnix japonica*) on commercial grain-based diets in order to determine how diet-tissue fractionation values may differ between species. Second, we raised Ring-billed Gulls (*Larus delawarensis*) on a fish diet and obtained blood and feather samples from captive Peregrine Falcons (*Falco peregrinus*) raised on quail in order to establish fractionation values applicable to dietary studies of piscivorous and carnivorous birds. Finally, American Crows (*Corvus brachyrhynchos*) raised on diets causing normal and slow growth patterns were sampled opportunistically in order to investigate the effects of nutritional stress on isotopic enrichment patterns. In each case, birds were raised until the end of their growth phase before their tissues were analyzed isotopically. For this reason, ages of species examined differed slightly.

CHICKENS AND QUAIL

Eight chickens and five quail were each taken from captive stock that had been raised for several generations on commercial turkey starter. Experimental birds were each raised from hatch on known, homogenized batches of turkey starter for six weeks before being sacrificed for isotopic analysis. Birds were fed *ad libitum* and kept at 25°C under a 12 hr photoperiod. Samples of quail and chicken diets were sampled isotopically throughout the experiment (Table 1).

GULLS AND FALCONS

Fourteen Ring-billed Gull chicks taken (under permit) from a colony at Reed Lake, Saskatchewan, approximately two days after hatch in June 1989, were brought into captivity and raised on a diet of perch (*Perca flavescens*) with vitamin supplements. Perch of a uniform size class were obtained from Smutz Lake, Saskatchewan, during May–July 1989, gutted and ground to a uni-

TABLE 1. Mean (± SD) stable-carbon and nitrogen isotope values and percent nitrogen content for diets used in this study. Samples were taken from single homogenized batches over the course of the study.

Species	Diet	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	% N
Chicken	Turkey starter (batch I)	5	−19.9 ± 0.2	4.7 ± 0.1	4.1 ± 0.6
Quail	Turkey starter (batch II)	5	−24.3 ± 0.1	5.5 ± 0.2	4.0 ± 0.4
Gulls, Crows	Perch	7	−24.3 ± 0.2	14.2 ± 0.2	11.8 ± 0.3
Crows	Grain-based mix	6	−20.3 ± 0.2	6.2 ± 0.3	8.3 ± 0.7

form consistency using a Hobart Commercial grinder and then kept frozen.

Blood and feather samples from three male and four female adult Peregrine Falcons raised from hatch for six to 14 years on an exclusive diet of quail were obtained for isotopic analysis. Quail fed to falcons were raised from hatch for six weeks on a commercial feed mixture (turkey starter) and were obtained from a single colony. Falcons ate primarily pectoral muscle from quail carcasses but some vicera were also consumed.

CROWS

Ten nestling American Crows, 10–15 days old, were taken from four nests near St. Denis, Saskatchewan (see Ignatiuk and Clark 1991), during May–June 1989 and assigned, at random, to either a plant or perch diet (described above). The plant-based diet consisted of a single homogenized batch composed of ground wheat, ground soybean, bone meal, minerals, canola oil and gelatin. We felt that genetic variation in growth would be reduced by placing at least one sibling per family in each diet group. Crows were fed to repletion every 1–2 hr during daylight. They were weighed every morning until 25 days old and then every 2–3 days thereafter. Crows were always weighed (using a Pesola scale to the nearest gram) before the first feeding to ensure that their gastrointestinal tracts were empty. Because crows were not raised from hatch on known diets we used them opportunistically only to examine the effects of nutritional stress on isotopic fractionation patterns. We did not consider it appropriate to use them for estimates of isotopic fractionation factors *per se* since we could not control for early nestling diet.

Gulls and crows were maintained in captivity in a ventilated, climate-controlled room (18–20°C), equipped with full-spectrum lighting. Both species were raised up to 60 days before being sacrificed for isotopic analysis.

ISOTOPIC ANALYSIS

Avian tissues and dietary samples were stored frozen, later freeze-dried and then powdered. Collagen was extracted from bones as a gelatin using the method of Longin (1971) as modified by Chisholm et al. (1983). Feathers were cleaned of surface contaminants using ether in an ultrasonic cleaner, rinsed with clean ether and then air dried. Lipids were removed from muscle, collagen and dietary samples using a Soxhlet apparatus with chloroform solvent for at least 8 hours. Samples for $\delta^{13}C$ analysis were loaded into Vycor tubes with powdered CuO and silver wire and combusted at 850°C for 4 hr. Nitrogen samples were converted to ammonia by Kjeldahl reaction and then to N_2 gas using LiBrOH (Porter and O'Dean 1977).

Carbon dioxide and nitrogen gas were analyzed using a Micromass 602E mass spectrometer. Stable isotope concentrations were expressed in δ notation according to the following:

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

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. $R_{standard}$ for ^{13}C and ^{15}N are the PDB standard and atmospheric nitrogen (AIR), respectively. Based on several hundred replications, standard deviations for carbon (lentil) and nitrogen (glycine) internal standards were 0.1‰ and 0.3‰, respectively. For nitrogen, identical standard deviations (i.e., 0.3‰) were found for pea and wheat grain standards and for a bone collagen standard (SFU-SIS-B01).

STATISTICAL ANALYSES

To test for the effects of diet on growth (body mass) of juvenile crows, we used analysis of covariance (ANCOVA), adjusting for possible effects of family, age and the interaction between diet and family; for these analyses, significance was based on Type III sums of squares. Tests for differences among tissues for each species, or dif-

TABLE 2. Carbon (C) and nitrogen (N) diet-tissue fractionation factors (mean ± SD) for captive-raised birds, and the results of ANOVA tests for significant differences among species' means. Results of Tukey's multiple comparison test are indicated by superscripts where means having at least one superscript in common are not significantly different ($P > 0.05$). The first superscript refers to species comparisons (horizontal). The second superscript refers to tissue comparisons (vertical).

Tissue		Diet-tissue fractionation factor (‰)			Species ANOVA[1]	
		Chicken	Quail	Gull	*F*	*P*
Carbon						
Blood		–	$+1.2 \pm 0.6^{a,a}$	$-0.3 \pm 0.8^{b,a}$	12.6	**
Liver		$+0.4 \pm 0.2^{a,a}$	$+0.2 \pm 0.6^{a,a}$	$-0.4 \pm 1.0^{a,a}$	2.6	ns
Muscle		$+0.3 \pm 0.3^{a,a}$	$+1.1 \pm 0.5^{b,a}$	$+0.3 \pm 0.4^{a,a}$	7.0	**
Collagen		$+0.8 \pm 1.2^{a,a}$	$+2.7 \pm 0.4^{b,b}$	$+2.6 \pm 1.1^{b,b}$	8.1	**
Feather		$-0.4 \pm 0.02^{a,a}$	$+1.4 \pm 0.6^{a,a}$	$+0.2 \pm 1.3^{a,a}$	1.4	ns
Tissue ANOVA	*F*	0.7	11.9	17.2		
	P	ns	***	***		
Nitrogen						
Blood		–	$+2.2 \pm 0.2^{a,a}$	$+3.1 \pm 0.2^{b,a}$	7.1	*
Liver		$+1.7 \pm 0.1^{a,a}$	$+2.3 \pm 0.2^{b,a}$	$+2.7 \pm 0.1^{b,a}$	12.3	***
Muscle		$+0.2 \pm 0.2^{a,b}$	$+1.0 \pm 0.1^{b,b}$	$+1.4 \pm 0.1^{b,b}$	14.0	***
Collagen		$+1.5 \pm 0.1^{a,a}$	$+2.5 \pm 0.4^{b,a}$	$+3.1 \pm 0.2^{b,a}$	12.9	***
Feather		$+1.1 \pm 0.1^{a,a}$	$+1.6 \pm 0.1^{a,a}$	$+3.0 \pm 0.2^{b,a}$	26.7	***
Tissue ANOVA	*F*	26.5	5.8	16.1		
	P	***	**	***		

[1] ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

ferences among species for each tissue, were performed using one-way (factor) analysis of variance (ANOVA). Tukey's multiple comparison procedure was used to test for differences between specific tissues or species. All analyses were performed on the Statistical Analysis System (SAS Inst. 1985).

RESULTS

Relative to the dietary values (Table 1), tissues of captive-raised chickens, quail and gulls were generally more enriched in ^{13}C and ^{15}N (Table 2). In all cases, $\delta^{13}C$ values for collagen were more enriched than other tissues. Diet-tissue fractionation factors differed significantly among species (Table 2). For quail and chickens, carbon and nitrogen fractionation values differed for all tissues except carbon values for liver and feathers, and nitrogen values for feathers.

Since falcons ate primarily quail pectoral muscle tissue, we used only isotope values for this tissue when approximating diet-tissue fractionation. Diet-tissue fractionation values for adult falcon blood and feather samples were, respectively, $+0.2 \pm 0.01$ and $+2.1 \pm 0.08$‰ for carbon, and $+3.3 \pm 0.4$ and $+2.7 \pm 0.5$‰ for nitrogen. We found no evidence that fractionation values were influenced by age in falcons (carbon: diet-feather Pearson's $r = 0.48$, $P > 0.3$, diet-blood $r = 0.24$, $P > 0.6$; nitrogen: diet-feather $r = 0.31$, $P > 0.3$, diet-blood $r = -0.18$, $P > 0.7$, $n = 6$ in all cases).

The effect of diet on body mass of growing crows was significant ($F = 169.0$, $P < 0.001$; Fig. 1), when adjusted for age ($F = 616.7$, $P < 0.001$), family ($F = 19.9$, $P < 0.001$), and the interaction between diet and family ($F = 14.4$, $P < 0.0001$). Interaction between diet and family indicated that the influence of diet on growth varied among families. For example, crows from one family (Fig. 1a) showed similar patterns of mass change, whereas, in the other families (Figs. 1b–d), crows eating the plant diet initially lost much more mass than their siblings on fish. The significance of this ANCOVA model ($F = 125.6$, df = 8, 175, $r^2 = 0.852$, $P < 0.001$) was not improved (<0.1 increase in r^2) with the inclusion in the model of the measurement of chick body mass at the beginning of the test. However, initial body mass was significant ($F = 117.1$, df = 9, 174, $P < 0.001$, $r^2 = 0.858$). Generally, younger or smaller crows (e.g., Fig. 1b) appeared to be more seriously affected by the switch to a plant diet. Crows raised on fish had growth curves that were similar to those raised in the wild (Ignatiuk and Clark 1991).

Nitrogen diet-tissue fractionation values were significantly lower for crows raised on perch than

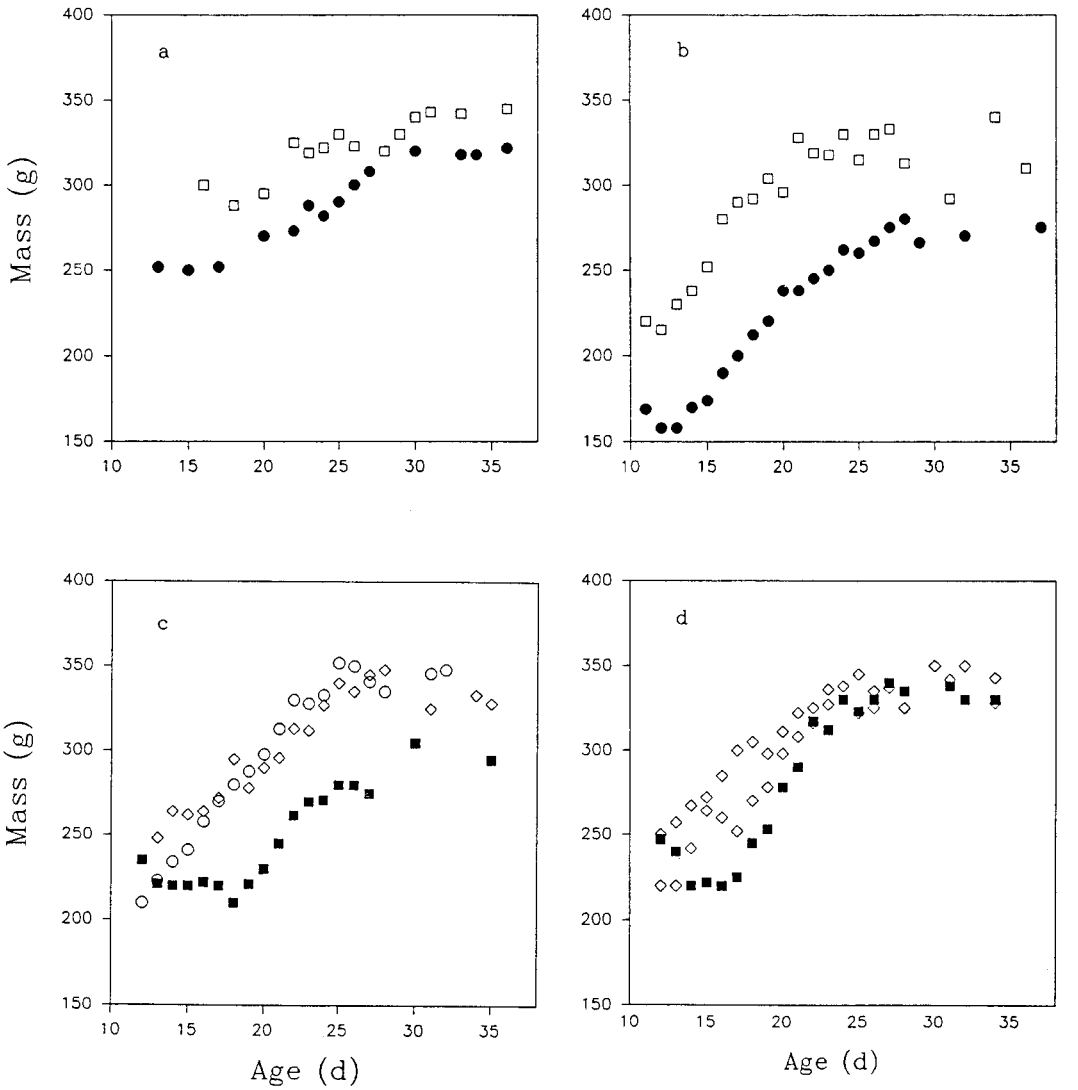

FIGURE 1. The relationship of body mass with age for four families (a–d) of American crows used in this study (males ○, females □, unknown ◇). Open symbols indicate birds raised on perch diets, solid symbols indicate birds raised on grain-based diet.

for those raised on the plant-based diet for all tissues (Fig. 2, Student's t-test, $t > 8.7$, $P < 0.05$ for all comparisons) except blood ($t = 2.1$, $P = 0.21$). No consistent pattern was observed for differences in carbon fractionation values (Fig. 3, $t < 4.6$, $P > 0.05$ for all comparisons).

DISCUSSION

COMPARISONS WITH PREVIOUS STUDIES

Few controlled laboratory studies using animals raised on known isotopic diets have been performed and so there are few data with which to compare our results. The most comprehensive studies conducted to date are by DeNiro and Epstein (1978, 1981) who established carbon and nitrogen isotopic diet-tissue fractionation factors for mice and insects. These authors determined that two species of insects fed the same diet had whole body $\delta^{15}N$ values that differed by as much as 3.2‰. With the exception of crows, we did not raise the same species on different diets or each species on the same diet in order to unequivocally establish the effect of diet or species on isotopic fractionation values. Nevertheless, our results suggest that diet and perhaps species

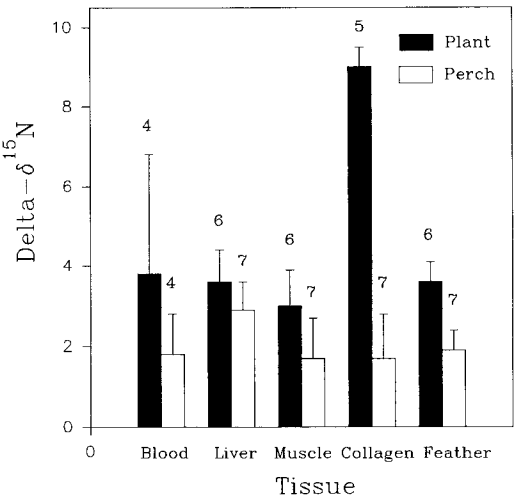

FIGURE 2. Nitrogen diet-tissue fractionation values for crows raised on perch (open bars) and plant-based (solid bars) diets. Vertical lines indicate SD and numbers above bars sample sizes.

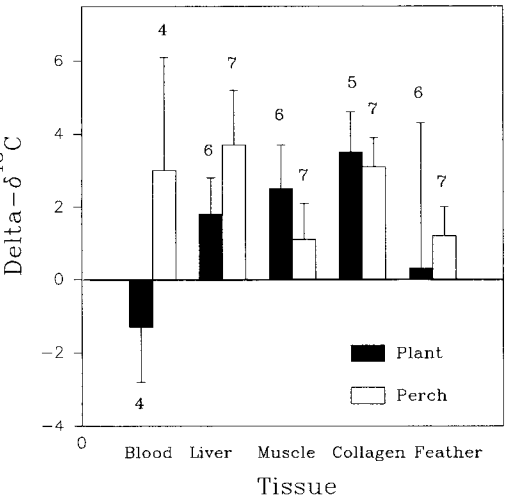

FIGURE 3. Carbon diet-tissue fractionation values for crows raised on perch (open bars) and plant-based (solid bars) diet. Vertical lines indicate SD and numbers above bars sample sizes.

influence diet-tissue isotopic fractionation values in growing birds.

Chickens and quail fed plant-based diets generally differed in both carbon and nitrogen diet-tissue fractionation values, with chickens generally showing the least isotopic enrichment in each case. Stable-carbon isotope values for plants differ according to the photosynthetic pathway involved (i.e., C-3, C-4 and CAM, see Peterson and Fry 1987). Although the chicken and quail diets were similar in their total nitrogen and ^{15}N content, the chicken diet was more enriched in ^{13}C compared to the quail diet perhaps due to the presence of C-4 plant-based proteins (e.g., corn). Other researchers have suggested that diet-tissue isotopic fractionation factors may differ for C-3 and C-4 plant-based diets (Bender et al. 1981, Sullivan and Krueger 1981). DeNiro and Epstein (1978, 1981) similarly found that fractionation values change depending on the type of diet involved. Alternatively, extensive artificial selection in *Gallus* for growth and stress tolerance may have resulted in different isotopic fractionation values and broader $\delta^{13}C$ distributions in tissues compared to the other species examined.

Due to the importance of bone collagen in palaeodietary reconstructions, several investigators have estimated the fractionation factor between diet and this tissue. Based largely on studies of mammals, estimates range from 3.9 to 5.3‰ for carbon (DeNiro and Epstein 1978, 1981; van der Merwe and Vogel 1978; Chisholm 1986) and from 2.4 to 4.0‰ for nitrogen (DeNiro and Epstein 1981, Schwarcz et al. 1985). Our study using birds shows lower diet-collagen fractionation values for carbon but comparable values for nitrogen. The extent of enrichment of body $\delta^{15}N$ values in animals may be due, in part, to the type of excretory nitrogen formed (Owens 1987). Birds differ from most mammals in that they produce uric acid rather than urea. This may result in diet-tissue fractionation values that differ from those of mammals (but see Minegawa and Wada 1984). While this does not appear to be the case for collagen, more comparative studies are required for other tissues.

An important assumption in isotopic studies designed to establish diet-tissue fractionation factors is that fractionation values obtained for young lab animals are close to those of wild adults. To our knowledge, Mizutani et al. (1991) are the only other researchers who have analyzed a piscivorous bird raised on a known diet. They measured isotopically the tissues of a single zoo-raised adult cormorant (*Phalacrocorax carbo*) and found that, compared to diet, feather, bone, muscle and liver $\delta^{15}N$ values were enriched by 3.6, 3.9, 2.4, and 2.3‰ and $\delta^{13}N$ values were enriched by 3.6, 2.5, 2.1, and 1.3‰, respectively. Mizutani et al.'s (1991) $\delta^{15}N$ results are comparable to our average values for gulls raised on a fish diet but, with the exception of collagen values, their $\delta^{13}C$ enrich-

ment values do not agree with our findings for young gulls. It is possible that this discrepancy is due to the fact that Mizutani et al. (1991) apparently did not remove lipids from their diet or cormorant tissue samples prior to isotopic analyses. Lipids are isotopically lighter in ^{13}C compared to whole body or protein values (DeNiro and Epstein 1978, Tieszen et al. 1983) and differential concentrations of lipids in various tissues can complicate isotopic investigations. For these reasons, we routinely remove lipids from our samples. However, the differential presence of lipids could not account for discrepancies in feather carbon isotope values since feathers are composed primarily of keratin.

As with all previous controlled laboratory investigations designed to establish diet-tissue fractionation values, with the exception of falcons, we raised birds through the growth phase only. Nonetheless, young gulls showed nitrogen fractionation values similar to those found by Mizutani et al. (1991) for adult cormorant tissues and these values were within 1 standard deviation with blood and feather values established for adult falcons. This suggests that birds feeding exclusively on animal protein may show similar nitrogen isotopic fractionation values for tissues.

For nitrogen, our results do not suggest that fractionation values differ substantially between adult and young birds raised on fish or meat diets. However, without further investigation, we are unable to determine if age effects or lipid content of tissues were responsible for the large differences between our carbon results for young gulls and those of the adult cormorant studied by Mizutani et al. (1991). Minagawa and Wada (1984) demonstrated that age had no effect on isotopic fractionation in the soft tissues of mussels and Sutoh et al. (1987) determined that age did not influence ^{15}N enrichments in the liver and kidney of cattle. Rau et al. (1981) showed a positive correlation of $\delta^{15}N$ and body mass for wild Dover Sole (*Microstomus pacificus*) but this may have been due to real dietary change with age. We found that, at least for adult falcons, age did not influence stable-carbon or nitrogen isotopic fractionation values between diet and blood or feathers.

EFFECTS OF NUTRITIONAL STRESS

Crows that lost body mass and grew relatively poorly on a plant diet showed tissue $\delta^{15}N$ values that were highly enriched compared to birds that underwent normal growth on a fish diet. We suggest that these differences are due to the effects of nutritional stress and not simply diet. Our confidence in this hypothesis has been strengthened recently by the results of a study of quail raised on the same diet but with different levels of nutritional stress during growth (Hobson and Clark, unpubl. data). During periods of nutritional stress proteins may be mobilized from tissues for use elsewhere in the body (e.g., Swick and Benevenga 1977). This process of mobilization and deposition of proteins may result in additional isotopic fractionation favoring the heavier isotope of nitrogen. Such an effect would be consistent with our observation that bone collagen underwent the greatest isotopic enrichment if bone growth was favored at the expense of other tissues such as pectoral muscle. This appeared to be the case in our study since crows showing decreases in mass showed no decrease in growth of other (bone) measurements (Hobson and Clark, unpubl. data). Nitrogen-15 enrichment has similarly been found in the bone collagen of mammals that experience water stress and it is suspected that this is the result of the retention of enriched, unexcreted nitrogen that is available for the synthesis of this protein (Heaton et al. 1986, Ambrose and DeNiro 1987). Crows fed the plant diet during growth may have experienced some water stress but, since similar ^{15}N enrichment effects were observed in nutritionally stressed quail that had unlimited access to water (Hobson and Clark, unpubl. data), water stress alone cannot explain our findings for crows.

An alternative explanation for the observed ^{15}N enrichment in the tissues of stressed crows involves possible changes in the amino acid compositions of crow tissues. Since differential hydrolysis of amino acids occurs during periods of stress (Felig 1975), the amino acid profiles of the tissues of stressed birds may differ from those of unstressed birds. This might result in further differences in the stable-isotope concentrations of tissues between these groups because individual amino acids differ in their stable isotopic signatures (Macko et al. 1983).

CAPTIVE VS. FIELD STUDIES

Relative to diet, $\delta^{13}C$ values of the tissues of captive birds showed much higher variability than $\delta^{15}N$ values (Table 2). We have no explanation for this, particularly since we removed lipids from all samples prior to isotopic analysis and because

diet and isotopic analysis techniques were relatively minor sources of variance. In contrast to our laboratory study, isotopic investigations of wild animals have often shown narrower isotopic distributions despite the potential for variation in diets among individuals. Hobson and Schwarcz (1985) measured bone collagen $\delta^{13}C$ values in populations of voles (*Microtus townsendii*, n = 22) and mule deer (*Odocoileus hemionus hemionus*, n = 55) and found a range of 1.8‰ for both species (SD = 0.5–0.6‰). Fry and Parker (1979) found narrow ranges in muscle $\delta^{13}C$ for Vermillion Snappers (*Rhomboplites aurorubens*, n = 41, range = 0.7‰) and Red Snappers (*Lutjanus campechanus*, n = 54, range = 1.4‰) collected from the Gulf of Mexico. Clearly, more field and laboratory investigations are required to elucidate sources of variation in tissue isotope composition.

The high variability in $\delta^{13}C$ of gull tissues indicates that diets would have to differ by about 1.4‰ in order to resolve this difference using collagen (with n = 14, α = 0.05 and β = 0.10). In contrast, the measurement of $\delta^{15}N$ values in the same sample of gull collagen would allow dietary differences to be detected at the 0.4‰ level. At the moment, a reasonable approximation of the SD of the distributions of isotope concentrations in wild populations feeding on the same diet is about 0.5‰ for both isotopes. Thus, a wild population with intrinsic isotopic standard deviation of the order of 0.5‰ for a given tissue would require sampling a minimum of 10 individuals from each population to detect a 1‰ difference in diet.

FUTURE STUDY

We have demonstrated that isotopic fractionation values established previously for mammals are not necessarily applicable to avian dietary studies. Furthermore, at least for juvenile birds, diet-tissue isotopic fractionation values differ amongst avian tissues and may be influenced further by diet, stress, and possibly by species. Researchers using measurements of stable isotopes in food webs to gain quantitative estimates of contributions from isotopically distinct sources to the diets of birds must take this variation into account. We suggest that the values we obtained for quail and gulls might be useful in studies of birds with terrestrial C-3 granivorous and fish or meat diets, respectively.

It is clear that further studies are required to elucidate the range of isotopic fractionation factors occurring between diet and avian tissues. In particular, in cases where it can be demonstrated that birds have been raised on an isotopically constant diet, the isotopic analyses of tissues of birds raised in captivity for several years would be extremely valuable. Such studies are required to see if fractionation values determined for juvenile birds are applicable to adults. Another useful approach to establishing tissue fractionation values in adult birds would be to measure isotope concentrations in those wild species whose diet remains isotopically constant over time. In this regard, tissues of non-migratory dietary specialists and their prey would be particularly valuable.

ACKNOWLEDGMENTS

We are grateful to T. Clark, R. Dawson, C. Fehr, D. Meger and K. Warner for their help in caring for captive gulls and crows, to M. Gloutney, M. Ramsay, D. Smith, J. Virgl and Fisheries Branch, Saskatchewan Parks and Renewable Resources, for providing perch, and particularly to P. Stevens for technical advice on the composition of bird diets. We also thank P. Stevens and L. Oliphant for supplying chickens and quail. This manuscript benefited greatly from the critical reviews of B. Fry and L. L. Tieszen. Our study was financed by the Canadian Wildlife Service and Natural Science and Engineering Research Council (NSERC) Operating Grants to R. G. Clark (OPG0042640) and M. A. Ramsay (OGP0036712). Personal support to K. A. Hobson was provided by an NSERC postgraduate scholarship and a University of Saskatchewan Graduate Fellowship. Our research protocol was authorized by the Canadian Council on Animal Care.

LITERATURE CITED

AMBROSE, S. H., AND M. J. DENIRO. 1987. Bone nitrogen isotope composition and climate. Nature 325:201.

BENDER, M. M., D. A. BAERREIS, AND R. L. STEVENTON. 1981. Further light on carbon isotopes and Hopewell agriculture. American Antiquity 46:346–353.

CHISHOLM, B. S. 1986. Reconstruction of prehistoric diet in British Columbia using stable-isotope analysis. Ph.D.diss., Simon Fraser Univ., Burnaby, BC.

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

CHISHOLM, B. S., D. E. NELSON, AND H. P. SCHWARCZ. 1982. Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216:1131–1132.

DENIRO, M. J. 1987. Stable isotopy and archaeology. Amer. Sci. 75:182–191.

DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. 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.

Felig, P. 1975. Amino acid metabolism in man. Ann. Rev. Biochem. 44:933–955.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol. Oceanogr. 33:1182–1190.

Fry, B., R. K. Anderson, L. Entzeroth, J. L. Bird, and P. L. Parker. 1984. ^{13}C enrichment and oceanic food web structure in the northwest Gulf of Mexico. Contrib. Marine Sci. 27:49–63.

Heaton, T.H.E., J. C. Vogel, G. Von La Chevallerie, and G. Collett. 1986. Climatic influence on the isotopic composition of bone collagen. Nature 322:822–823.

Hobson, K. A. 1987. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diet. Can. J. Zool. 65:1210–1213.

Hobson, K. A. 1990. Stable isotope analysis of Marbled Murrelets: evidence for freshwater feeding and determining of trophic level. Condor 92:897–903.

Hobson, K. A. 1991. Stable-isotopic determinations of the trophic relationships of seabirds: preliminary investigations of alcids from coastal British Columbia. Can. Wildl. Serv. Spec. Publ., Ottawa.

Hobson, K. A., and R. G. Clark. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:179–186.

Hobson, K. A., and H. P. Schwarcz. 1985. Variation in $\delta^{13}C$ values in bone collagen for two wild herbivore populations: implications for palaeodiet studies. J. Archaol. Sci. 13:101–106.

Hobson, K. A., and S. G. Sealy. 1991. Marine protein contributions to the diet of Northern Sawwhet Owls on the Queen Charlotte Islands, British Columbia: a stable-isotope approach. Auk 108: 437–440.

Ignatiuk, J. B., and R. G. Clark. 1991. Breeding biology of American crows in Saskatchewan parkland habitat. Can. J. Zool. 69:168–175.

Longin, R. 1971. A new method of collagen extraction for radiocarbon dating. Nature 230:241–242.

Macko, S. A., M. F. Estep, P. E. Hare, and T. C. Hoering. 1983. Stable nitrogen and carbon isotopic composition of individual amino acids isolated from cultured microorganisms. Carnegie Inst. Washington Yearbook 82:404–410.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta 48:1135–1140.

Mizutani, H., M. Fukuda, Y. Kabaya, and E. Wada. 1990. Carbon isotope ratio 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.

Owens, N.J.P. 1987. Natural variations in ^{15}N in the marine environment. Adv. Mar. Biol. 24:389–451.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18: 293–320.

Porter, L. K., and W. A. O'Dean. 1977. Apparatus for preparing nitrogen from ammonium chloride for nitrogen-15 determinations. Anal. Chem. 45: 514–516.

Rundel, P. W., J. R. Ehleringer, and K. A. Nagy. 1989. Stable isotopes in ecological research. Springer-Verlag, Berlin.

SAS Institute, Inc. 1985. SAS user's guide: statistics. SAS Institute, 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.

Schwarcz, H. P., J. Melbye, M. A. Katzenberg, and M. Knyf. 1985. Stable isotopes in human skeletons of southern Ontario: reconstructuring paleodiet. J. Archaeol. Sci. 12:187–206.

Sullivan, C. H., and H. W. Krueger. 1981. Carbon isotope analysis of separate chemical phases in modern and fossil bone. Nature 292:333–335.

Sutoh, M., T. Koyama, and T. Yoneyama. 1987. Variations of natural nitrogen-15 abundances in the tissues and digesta of domestic animals. Radioisotopes 36:74–77.

Swick, R. W., and N. J. Benevenga. 1977. Labile protein reserves and protein turnover. J. Dairy Sci. 60:505–515.

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.], 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.

van der Merwe, N. J., and J. C. Vogel. 1978. ^{13}C content of human collagen as a measure of prehistoric diet in Woodland North America. Nature 276:815–816.

Wada, E., M. Terazaki, Y. Kabaya, and T. Nemoto. 1987. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res. 34:829–841.