Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction

James D. Roth and Keith A. Hobson

Abstract: The amount of isotopic fractionation (change in isotope ratios) between diet and animal tissues is generally poorly known and may be affected by trophic position. Diet–tissue fractionation of stable-carbon and -nitrogen isotopes was measured in several tissues of red foxes (*Vulpes vulpes*) raised on a commercial pellet feed. Stable carbon isotopic fractionation in red fox was positive for all tissues and was greatest in fur (2.6‰), intermediate in muscle (1.1‰), and least in liver and blood fractions (0.4–0.6‰). These carbon isotope fractionation values were greater than those previously measured for mammalian herbivores but were similar to values for marine mammals in most tissues. Little variation in stable nitrogen isotopic fractionation occurred among tissues, except in the blood fractions. Nitrogen isotopic fractionation was much higher in blood serum (4.2‰) than in liver, muscle, and fur (3.3–3.5‰). Cellular fractions of blood had the lowest fractionation values (2.6‰). There was a significant age effect in nitrogen- but not in carbon-isotopic fractionation. Subadult foxes (<1 year) were significantly enriched in ^{15}N compared with adult foxes for fur, muscle, and liver (no blood was collected from adults). The cause of this enrichment is unclear, but it may be related to the higher rate of protein synthesis and catabolism in growing animals. This study is the first to report isotopic fractionation values for a terrestrial mammalian carnivore. Such estimates are necessary to interpret stable-isotope patterns in wild carnivores.

Résumé : L'importance du fractionnement des isotopes (changement dans les rapports entre les isotopes) entre le régime alimentaire et les tissus est généralement mal connue et peut être affectée par la position trophique. Le fractionnement régime–tissus des isotopes de carbone stable et d'azote a été mesuré dans plusieurs tissus de Renards roux (*Vulpes vulpes*) nourris de boulettes commerciales. Le fractionnement des isotopes de carbone stable chez le renard était positif dans tous les tissus et c'est dans la fourrure qu'il était le plus élevé (2,6 ‰), il était intermédaire dans les muscles (1,1 ‰) et c'est dans le foie et dans le sang qu'il était le plus faible (0,4–0,6 ‰). Les valeurs du fractionnement des isotopes de carbone se sont avérées plus grandes que les autres valeurs mesurées chez des mammifères herbivores, mais semblables à celles des mammifères marins pour la plupart des tissus. Il y avait peu de variation dans le fractionnement des isotopes d'azote stable entre les tissus, sauf dans les fractions du sang. Le fractionnement de l'azote dans le sérum sanguin (4,2 ‰) était beaucoup plus élevé que dans le foie, les muscles ou la fourrure (3,3–3,5 ‰). Les fractions cellulaires du sang avaient le plus faible taux de fractionnement (2,6 ‰). L'âge avait un effet significatif sur le fractionnement de l'azote, mais pas sur celui du carbone. Les subadultes (<1 an) étaient significativement plus riches en ^{15}N que les adultes, dans la fourrure, les muscles et le foie (aucun prélèvement de sang n'a été fait chez les adultes). La cause de cet enrichissement n'est pas claire, mais il se peut qu'il soit attribuable au taux plus élevé de synthèse et de catabolisme des protéines chez les animaux en croissance. Cette étude est la première à donner des valeurs de fractionnement pour des mammifères terrestres carnivores. De telles estimations sont nécessaires à l'interprétation des patterns d'isotopes stables chez les carnivores sauvages.

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Introduction

The measurement of naturally occurring stable isotopes is becoming widespread as a tool for ecologists interested in community structure and ecosystem function. Stable-isotope ratios in animal tissues are related to those of their diet (DeNiro and Epstein 1978, 1981; Tieszen et al. 1983; Tieszen and Boutton 1989). In particular, the ratio of the stable isotopes of nitrogen ($^{15}N/^{14}N$) changes predictably with trophic level, mainly owing to the excretion of isotopically light nitrogen in urine (Steele and Daniel 1978; Peterson and Fry 1987). Thus, stable-nitrogen isotopes reflect the trophic

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position of an organism within a food web (Ambrose and DeNiro 1986; Hobson and Welch 1992). Stable carbon isotope ratios ($^{13}C/^{12}C$), on the other hand, change very little with trophic position and therefore reflect sources of primary productivity (Vogel 1978; Chisholm et al. 1982; Schoeninger and DeNiro 1984). In combination, measurements of these stable isotopes provide a powerful tool for understanding trophic relationships and tracing the flow of energy and nutrients. Because tissues reflect diet integrated over time, this technique reflects relatively long term dietary information compared with traditional methods of estimating diet (DeNiro and Epstein 1978; Tieszen et al. 1983; Hobson and Clark 1992*a*). Furthermore, stable-isotope measurements reflect assimilated foods, not merely what has been recently ingested, and avoid biases inherent in analyzing scats or stomachs containing items with different digestibilities (Lockie 1959).

The metabolic rates of different tissues within an organism determine the turnover rates of stable isotopes in tissues (Tieszen et al. 1983; Hobson and Clark 1992*a*). Therefore, dietary information over several time scales, from a few days to the total lifetime (Tieszen and Boutton 1989), can be obtained by measuring several tissues within an individual. For example, stable-isotope ratios of liver tissue may reflect diet assimilated over the previous week, whereas muscle tissue provides dietary information from the previous 1–2 months (Tieszen et al. 1983; Hobson and Clark 1992*a*). Stable isotopes of most elements in metabolically inactive tissues (e.g., fur, feathers, skin, and nails) do not turn over, and reflect the diet of individuals only during a limited period of growth. The ability to extract different kinds of information from a single individual is particularly useful for studies on animals that switch between alternative food sources or take advantage of some seasonally occurring resource (e.g., insect outbreaks or migratory prey or predators). However, tissue isotope ratios also vary within an individual raised on a constant diet, because isotopes fractionate differently between diet and various tissues (Tieszen et al. 1983). The mechanisms of isotopic fractionation (change in isotope ratios due to the different rates at which various isotopes undergo chemical reactions) between an animal's diet and its tissues are not well understood (van der Merwe 1982; Tieszen and Boutton 1989), but fractionation patterns must be known to interpret stable-isotope data (Gannes et al. 1997). These patterns can be documented in laboratory situations with animals raised on controlled diets and have been measured in rodents (DeNiro and Epstein 1978; Tieszen et al. 1983), aquatic invertebrates (Parker et al. 1989), birds (Hobson and Clark 1992*b*), and some marine mammals (Hobson et al. 1996). However, no studies have measured diet–tissue isotopic fractionation in a terrestrial carnivore.

Isotopic fractionation may be related to body size, differences in digestive physiology, or diet quality (Tieszen and Boutton 1989) and may also differ with trophic level (Krueger and Sullivan 1984; Lee-Thorp et al. 1989). The objective of this study was to measure diet–tissue isotopic fractionation in red foxes (*Vulpes vulpes*) raised on commercial pellet feed. Establishing the isotopic-fractionation factors in this carnivore raised on a known diet will provide important base-line information for interpreting stable-isotope patterns in wild carnivores.

Methods

In December 1997, we collected tissue samples from 20 red foxes (silver phase) raised on a commercial fur farm in southern Ontario. Ten individuals were subadult (about 8 months old) and 10 were adult (1–6 years old) at the time of sampling. All animals had been fed a commercial pellet food (Martin Fox Growth; ≥33% protein and ≥16% fat) from May, when pups were weaned, until pelting in early December. We collected five subsamples of pellets from three different batches purchased at different times from late 1997 to early 1998. Breeding adults had been fed a meat diet during reproduction (January–May), prior to the switch to the pellet diet.

Pelted carcasses were weighed to the nearest 0.1 kg. From each individual, we clipped a sample of the fur that remained around the feet after pelting and collected approximately 5 g of liver tissue and 5 g of muscle tissue (carcasses were frozen immediately after death and sampled 1 week later). We collected blood (5 mL) from a separate, additional group of five subadult foxes just prior to pelting; these samples were separated into serum and cellular fractions by centrifugation. Because the isotopic turnover rates in these tissues are fairly rapid in other species (Tieszen et al. 1983; Hobson and Clark 1993), we felt confident that these samples, obtained 7 months after the diet switch, reflected only the pellet diet. Fur grown during the autumn molt should reflect diet during that time. These animals were not food-limited, so it is unlikely that energy reserves would be used for new fur growth.

Fur samples were cleaned with soap and water to remove debris, rinsed with distilled water, dried at 90°C, and homogenized using scissors. Other samples were freeze-dried and then powdered with mortar and pestle. Lipids were removed from the muscle and pellet samples in a Soxhlet apparatus, with chloroform as solvent, for at least 8 h, since variations in lipid concentration can significantly influence stable carbon isotope ratio measurements (Rau et al. 1992). Stable-isotope ratios of carbon and nitrogen were measured on a continuous flow isotope-ratio mass spectrometer in the Stable Isotope Facilities at the Department of Soil Sciences, University of Saskatchewan. Stable-isotope signatures are expressed, most often, in delta (δ) notation, as parts per thousand (‰), as follows:

$$[1] \qquad \delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N and R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample and standard, respectively. The standards for ^{13}C and ^{15}N are Pee Dee Belemnite (PDB) and atmospheric N_2, respectively. Measurement accuracy was within 0.1‰ for carbon and 0.3‰ for nitrogen.

Data are expressed throughout as mean ± 1 SE. We tested for normality using the Shapiro–Wilk statistic. We used analysis of variance (Zar 1984) to assess differences in $\delta^{13}C$ and $\delta^{15}N$ among the three batches of pellet food. We compared age and tissue types using two-way ANOVA. When age differences were detected, we used separate ANOVAs on adult and subadult foxes to compare tissue types; otherwise, ages were pooled in a single one-way ANOVA. When significant differences were encountered, pairwise comparisons of batch or tissue type were made using Tukey's test (Zar 1984). A 0.05 probability of a type I error was considered significant.

Results

Each age class contained nine males and one female, and the female had the lightest mass in the age class. The body mass of subadult carcasses (5.84 ± 0.18 kg) was significantly greater than that of adult carcasses (5.20 ± 0.24 kg; $t = 2.16$, df = 18, $p = 0.044$).

Fig. 1. Stable carbon isotopic fractionation between diet (mean $\delta^{13}C = -18.7 \pm 0.1‰$) and various tissues of red foxes. Samples of serum and blood cells were from five subadult foxes and samples of liver, muscle, and fur were from equal numbers of adult ($n = 10$) and subadult ($n = 10$) foxes. Values are means; error bars reflect standard error. Actual mean $\delta^{13}C$ values for fur, muscle, liver, blood cells, and serum were –16.1, –17.6, –18.3,–18.0, and –18.1‰, respectively.

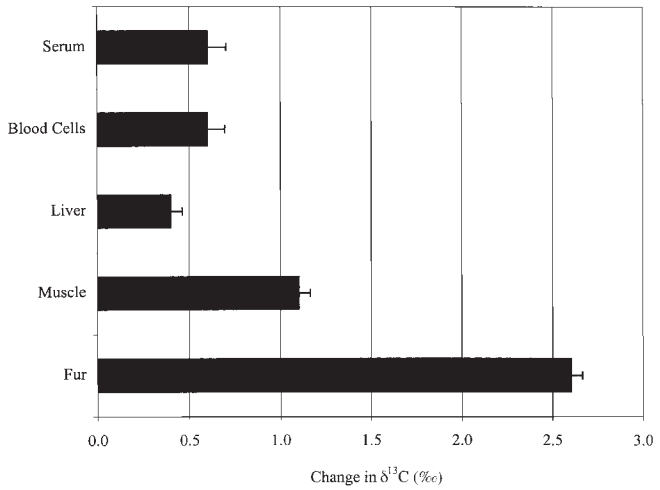
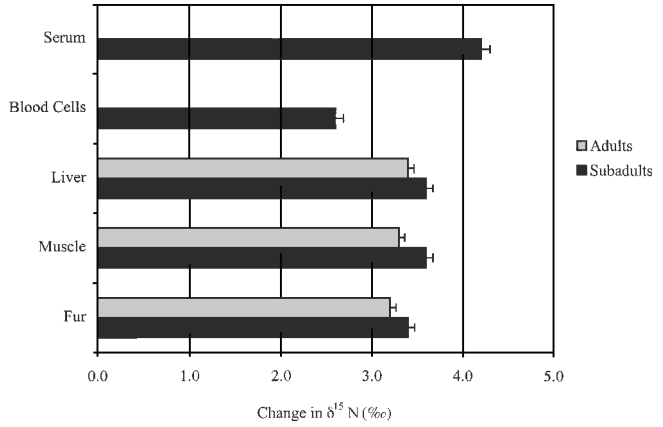


Fig. 2. Difference in stable nitrogen isotope ratios between diet (mean $\delta^{15}N = 4.9 \pm 0.1‰$) and red fox tissues of adult ($n = 10$) and subadult ($n = 10$) foxes (blood samples were from five additional subadult foxes). Age differences were all significant ($p < 0.02$). Values are means; error bars reflect standard error. Actual mean $\delta^{15}N$ values for fur, muscle, liver, blood cells, and serum in subadult foxes were 8.3, 8.5, 8.5, 7.5, and 9.1‰, respectively, and for fur, muscle, and liver in adult foxes were 8.1, 8.2, and 8.2‰, respectively.



The ^{15}N content did not differ among the three batches of pellet feed ($F_{[2,12]} = 2.09$, $p = 0.17$; overall $\delta^{15}N = 4.9 \pm 0.1‰$, $n = 15$). However, the ^{13}C content differed among the three batches ($\delta^{13}C = -18.1 \pm 0.2‰$, $-18.7 \pm 0.2‰$, and $-19.1 \pm 0.1‰$ for the three batches; $F_{[2,12]} = 9.42$, $p = 0.0035$), with batch 1 being significantly different from batches 2 and 3 (Tukey's HSD: $p = 0.045$ for batch 2 and 0.003 for batch 3). We assume these values are representative of the variability in the diet during the 7 months prior to sampling. Therefore, we used the overall average $\delta^{13}C$ ($-18.7 \pm 0.1‰$) to determine diet–tissue isotopic fractionation values.

Significant diet–tissue isotopic fractionation occurred in all tissues for both carbon (Fig. 1) and nitrogen (Fig. 2). There were no differences in ^{13}C between adult and subadult foxes for liver, fur, or muscle ($F_{[1,54]} = 2.16$, $p = 0.15$), so ages were pooled for further comparisons of carbon. The $\delta^{13}C$ values differed among tissues ($F_{[4,64]} = 537.2$, $p < 0.00001$), with fur having the greatest enrichment and differing significantly from all other tissues (Tukey's HSD for unequal n, $p = 0.0001$ for each comparison). Muscle was also significantly enriched over liver and both blood fractions ($p < 0.0008$ for each comparison). The stable carbon isotope fractionation of blood serum was similar to both liver ($p = 0.34$) and the cellular fraction of blood ($p = 0.98$), which were only marginally different from each other ($p = 0.06$).

Subadult foxes were enriched in ^{15}N compared with adults for the three tissues collected from both age groups ($F_{[1,54]} = 25.7$, $p < 0.0001$; Fig. 2), so ages were analyzed separately for nitrogen. Nitrogen isotopes differed among tissues for subadult foxes ($F_{[4,34]} = 35.7$, $p < 0.00001$). There was no difference in ^{15}N content among fur, muscle, and liver (Tukey's HSD for unequal n, $p > 0.4$ for each comparison), but blood serum had significantly greater enrichment than any other tissue ($p = 0.0001$ for each comparison) and blood cells had significantly less ($p \leq 0.01$ for each). Nitrogen isotopes did not differ among adult tissues ($F_{[2,27]} = 2.62$, $p = 0.09$), but no blood samples were obtained from adults.

Discussion

Isotopic fractionation in biochemical reactions occurs because similar molecules of slightly different masses react at different rates (Peterson and Fry 1987). Different metabolic pathways, therefore, will likely produce different isotopic fractionation values. For example, bacterially synthesized amino acids fractionate carbon by different amounts because of different biochemical synthetic pathways (Macko et al. 1987). Although herbivore diets usually contain adequate amounts of protein, the mixture of amino acids derived from vegetation probably differs from that required by the animal. Thus, herbivores probably synthesize a large proportion of their required amino acids by transamination of keto acids derived from the carbohydrate part of their diet (Krueger and Sullivan 1984). Carnivore diets, on the other hand, consist primarily of protein and lipid, and the amino acid requirements for growth would probably be satisfied directly from the diet, since the amino acid distribution in meat would be close to that required for growth. Therefore, the different enzymatic pathways between dietary uptake and tissue formation experienced by herbivores and carnivores are likely to result in different amounts of isotopic fractionation. Furthermore, different macronutrients within a bulk diet (proteins, lipids, and carbohydrates) may have different stable isotope values (DeNiro and Epstein 1978; van der Merwe 1982). Proteins are generally enriched in ^{13}C by about 4‰ over carbohydrates and by about 6‰ over lipids, so animals whose tissue proteins are derived solely from dietary protein might be expected to contain more ^{13}C. Our results are consistent with this prediction. Stable carbon isotopic fractionation values for red fox liver, muscle, and fur were higher than values found in mammalian herbivores (DeNiro and Epstein 1978;

Tieszen et al. 1983) but similar to values found in marine carnivores (Hobson et al. 1996).

The order of stable carbon isotopic fractionation values among red fox tissues was the same as the order found for harp seals (Hobson et al. 1996), gerbils (Tieszen et al. 1983), and mice (DeNiro and Epstein 1978), with metabolically inactive fur having the greatest carbon isotopic fractionation compared with diet, followed by muscle and, then, liver (Fig. 1). The only departure from this trend was that, in harp seals, the carbon isotopic fractionation of the cellular fraction of the blood was much greater (1.7‰) than that found here in red fox. Hobson et al. (1996) point out that differences in blood composition in diving mammals like seals may influence isotopic-fractionation values. In contrast, Hilderbrand et al. (1996) found no differences in stable carbon isotope values among tissues (except fat) for black bears, mice, and rabbits that had been fed a constant diet, although small sample sizes may have influenced these results.

Our results are consistent with those of previous studies of the distribution of stable-nitrogen isotopes in food webs, viz., that there is a fairly constant stepwise enrichment in ^{15}N of 3–4‰ between trophic levels (Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Hobson and Welch 1992). For muscle tissue, however, nitrogen-isotopic fractionation was greater in red foxes than in other organisms (DeNiro and Epstein 1981; Hobson and Clark 1992*b*; Hobson et al. 1996). This difference may be due, in part, to the diet used in this study. Fractionation factors may differ for conspecifics raised on different diets (DeNiro and Epstein 1978; Tieszen and Boutton 1989; Hobson and Clark 1992*b*). For example, Hobson and Clark (1992*b*) found that diet–tissue isotopic fractionation values for nitrogen were lower in American crows (*Corvus brachyrynchos*) raised on fish than in crows raised on a plant-based diet. Although the vegetative component of red fox diet can be fairly high in some populations (Scott 1943), in the wild, these animals usually feed predominantly on animal tissues, which contain mostly protein and fat. The pellet diet that these farmed foxes were fed contained up to 50% carbohydrate, which may have slightly elevated stable nitrogen isotopic fractionation. However, other tissues had stable nitrogen isotopic fractionation factors similar to those found in previous studies of other organisms, and the variability in $\delta^{15}N$ among all the tissues we sampled was even less than the variablility that has been measured elsewhere (DeNiro and Epstein 1981; Hobson and Clark 1992*b*; Hobson et al. 1996; but see Hilderbrand et al. 1996).

This study is the first to document an effect of age on diet–tissue fractionation of stable-nitrogen isotopes that could not be explained by differences in diet. Other investigators found no age effect in birds (Hobson and Clark 1992*b*), mussels (Minagawa and Wada 1984), or cattle (Sutoh et al. 1987). Some fish have shown an increase in $\delta^{15}N$ with body mass (or age), but this result was consistent with a dietary change (Rau et al. 1981; Hobson and Welch 1995). Likewise, seabird chicks and adults differed in $\delta^{15}N$ concentrations, but this difference was probably due to real dietary differences between the age groups (Hodum and Hobson 2000). There was a very weak correlation between age and $\delta^{15}N$ in African elephants (Tieszen et al. 1989), but this result may also be related to diet. Neonates feeding exclusively on mothers' milk are enriched in ^{15}N compared with maternal tissues (Fogel et al. 1989; Bocherens et al. 1995; Hobson and Sease 1998), an effect similar to a trophic-level effect.

It is unclear why isotopic fractionation values should be higher in younger animals than in adults with an identical diet. Nutritional stress can cause elevated levels of ^{15}N as animals begin to metabolize their own tissues (Hobson et al. 1993), but subadult foxes were actually heavier than adults. In birds, plasma concentrations of urea and uric acid are higher in growing chicks than in adults, which is probably related to higher rates of protein synthesis in growing animals (Featherston 1969; Wolf et al. 1985; Alonso et al. 1991). Since these waste products are typically depleted in ^{15}N compared with body proteins (Peterson and Fry 1987), a greater loss of isotopically light nitrogen may result in the heavier isotope being concentrated to a greater extent in growing tissues. Thus, it may be that, during an animal's growth phase, the higher rate of protein synthesis and catabolism causes faster isotopic turnover and magnifies the trophic effect, by causing an even greater loss of the isotopically light nitrogen in urine.

In the closely related Arctic fox (*Alopex lagopus*), there is an age-related trade-off between body condition and reproductive development (Hall 1989). Younger animals have the largest subcutaneous fat reserves, while the oldest animals have the heaviest reproductive organs. There is also a seasonal decline in fat reserves that corresponds to increased reproductive development in spring. Although we did not measure the body condition of the red foxes we sampled, the larger body mass of the subadult foxes may have been due to a similar trade-off, and different metabolic processes associated with energy storage and reproductive development may have contributed to the differential isotopic fractionation with age we detected. Clearly, further studies of the effect of age-related metabolic processes on fractionation factors are called for.

Naturally occurring stable isotope ratios can be a very powerful tool in studies of animal ecology, provided that the assumptions required for dietary reconstruction are validated by studies such as the one presented here (Gannes et al. 1997). This study is the first to measure diet–tissue isotopic fractionation in a terrestrial mammalian carnivore. The isotopic fractionation values for the fur and blood fractions are particularly valuable, as stable isotope ratios of these tissues provide a nondestructive method of dietary reconstruction (Hobson and Clark 1993).

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References

Alonso, J.C., Huecas, V., Alonso, J.A., Abelenda, M., Munoz-Pulido, R., and Puerta, M.L. 1991. Hematology and blood chem-

istry of adult white storks (*Ciconia ciconia*). Comp. Biochem. Physiol. A, **98**: 395–397.

Ambrose, S.H., and DeNiro, M.J. 1986. The isotopic ecology of East African mammals. Oecologia, **69**: 395–406.

Bocherens, H., Fogel, M., Tuross, N., and Zeder, M. 1995. Trophic structure and climatic information from isotopic signatures in pleistocene cave fauna of southern England. J. Archaeol. Sci. **22**: 327–340.

Chisholm, B.S., Nelson, D.E., and Schwarcz, H.P. 1982. Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science (Washington, D.C.), **216**: 1131–1132.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**: 495–506.

DeNiro, M.J., and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta, **45**: 341–351.

Featherston, W.R. 1969. Nitrogenous metabolites in the plasma of chicks adapted to high protein diets. Poult. Sci. **48**: 646–652.

Fogel, M.L., Tuross, N., and Owsley, D.W. 1989. Annual report of the director of the Geophysical Laboratory, Carnegie Institute, Washington, 1988–1989. Geophysical Laboratory, Washington, D.C.

Gannes, L.Z., O'Brien, D.M., and Martinez del Rio, C. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology, **78**: 1271–1276.

Hall, M.N. 1989. Parameters associated with cyclic populations of Arctic fox (*Alopex lagopus*) near Eskimo Point, Northwest Territories: morphometry, age, condition, seasonal and multiannual influences. M.Sc. thesis, Laurentian University, Sudbury, Ont.

Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K., and Servheen, C. 1996. Use of stable isotopes to determine diets of living and extinct bears. Can. J. Zool. **74**: 2080–2088.

Hobson, K.A., and Clark, R.G. 1992*a*. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor, **94**: 181–188.

Hobson, K.A., and Clark, R.G. 1992*b*. Assessing avian diets using stable isotopes II: factors influencing diet–tissue fractionation. Condor, **94**: 189–197.

Hobson, K.A., and Clark, R.G. 1993. Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk, **110**: 638–641.

Hobson, K.A., and Sease, J.L. 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using steller sea lions. Mar. Mamm Sci. **14**: 116–129.

Hobson, K.A., and Welch, H.E. 1992. Determination of trophic relationships within a high Arctic marine food web using delta-carbon-13 and delta-nitrogen-15 analysis. Mar. Ecol. Prog. Ser. **84**: 9–18.

Hobson, K.A., and Welch, H.E. 1995. Cannibalism and trophic structure in a high Arctic lake: insights from stable-isotope analysis. Can. J. Fish. Aquat. Sci. **52**: 1195–1201.

Hobson, K.A., Alisauskas, R.T., and Clark, R.G. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor, **95**: 388–394.

Hobson, K.A., Schell, D.M., Renouf, D., and Noseworthy, E. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can. J. Fish. Aquat. Sci. **53**: 528–533.

Hodum, P.J., and Hobson, K.A. 2000. Trophic relationships among Antarctic fulmarine petrels: insights into dietary overlap and chick provisioning strategies inferred from stable isotope analysis. Mar. Ecol. Prog. Ser. In press.

Krueger, H.W., and Sullivan, C.H. 1984. Models for carbon isotope fractionation between diet and bone. *In* Stable isotopes in nutrition. ACS Symp. Ser. No. 258. pp. 205–222.

Lee-Thorp, J.A., Sealy, J.C., and van der Merwe, N.J. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. J. Archaeol. Sci. **16**: 585–599.

Lockie, J.D. 1959. The estimation of the food of foxes. J. Wildl. Manage. **23**: 224–227.

Macko, S.A., Fogel, M.L., Hare, P.E., and Hoering, T.C. 1987. Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. Chem. Geol. **68**: 79–92.

Minagawa, M., and Wada, E. 1984. Step-wise 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.

Parker, P.L., Anderson, R.K., and Lawrence, A. 1989. A $\delta^{13}C$ and $\delta^{15}N$ tracer study of nutrition in aquaculture: *Penaeus vannamei* in a pond growout system. *In* Stable isotopes in ecological research. *Edited by* P.W. Rundel, J.R. Ehleringer, and K.A. Nagy. Springer–Verlag, Berlin. pp. 288–303.

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

Rau, G.H., Sweeney, R.E., Kaplan, I.R., Mearns, A.J., and Young, D.R. 1981. Differences in animal ^{13}C, ^{15}N and D abundance between a polluted and an unpolluted coastal site: likely indicators of sewage uptake by a marine food web benthos. Estuar. Coast. Shelf Sci. **13**: 701–707.

Rau, G.H., Ainley, D.G., Bengtson, J.L., Torres, J.J., and Hopkins, T.L. 1992. $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea birds, seals, and fish: implications for diet and trophic structure. Mar. Ecol. Prog. Ser. **84**: 1–8.

Schoeninger, M.J., and DeNiro, M.J. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim. Cosmochim. Acta, **48**: 625–639.

Scott, T.G. 1943. Some food coactions of the northern plains red fox. Ecol. Monogr. **13**: 427–473.

Steele, K.W., and Daniel, R.M.J. 1978. Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies. J. Agric. Sci. **90**: 7–9.

Sutoh, M., Koyama, T., and Yoneyama, T. 1987. Variations of natural ^{15}N abundances in the tissues and digesta of domestic animals. Radioisotopes, **36**: 74–77.

Tieszen, L.L., and Boutton, T.W. 1989. Stable carbon isotopes in terrestrial ecosystem research. *In* Stable isotopes in ecological research. *Edited by* P.W. Rundel, J.R. Ehleringer, and K.A. Nagy. Springer–Verlag, Berlin. pp. 167–195.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and Slade, N.A. 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. 1982. Carbon isotopes, photosynthesis, and archaeology. Am. Sci. **70**: 596–606.

Vogel, J.C. 1978. Isotopic assessment of the dietary habits of ungulates. S. Afr. J. Sci. **74**: 298–459.

Wolf, S.H., Shreiber, R.W., Kahana, L., and Torres, J.J. 1985. Seasonal, sexual, and age-related variation in the blood composition of the brown pelican (*Pelecanus occidentalis*). Comp. Biochem. Physiol. A, **82**: 837–846.

Zar, J.H. 1984. Biostatistical analysis. 2nd. ed. Prentice Hall, Englewood Cliffs, N.J.