

STABLE ISOTOPES IN ANIMAL ECOLOGY: ASSUMPTIONS, CAVEATS, AND A CALL FOR MORE LABORATORY EXPERIMENTS

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Abstract. For decades, plant ecologists have used naturally occurring stable isotope ratios to disentangle ecological and physiological processes. The methodology can also become a very powerful tool in animal ecology. However, the application of the technique relies on assumptions that are not widely recognized and that have been rarely tested. The purpose of this communication is to identify these assumptions, to characterize the conditions in which they are not met, and to suggest the laboratory experiments that are needed to validate them. The ease with which isotopic data can be gathered and the growing popularity of the method are generating a large amount of data on the isotopic ecology of animals. The proper interpretation of these data demands that we identify the assumptions on which these inferences are based, and that we conduct comparative laboratory experiments to assess their validity.

Key words: *body condition; ¹³C; dietary reconstruction; ¹⁵N; stable isotopes; trophic levels.*

INTRODUCTION

The analysis of naturally occurring stable isotope ratios has been part of the toolbox of plant physiological ecologists for decades. Stable isotope ratios of carbon (¹³C/¹²C), nitrogen (¹⁵N/¹⁴N), and hydrogen (¹H/²H) are used routinely to assess photosynthetic mode (Ehleringer and Monson 1993), to measure water balance (Farquhar et al. 1989), and to trace a plant's nitrogen sources (Handley and Raven 1992). Stable isotope analyses also have the potential to become a powerful tool in animal physiological ecology. Stable isotope ratios in animal tissues can be used to reconstruct diets (Hobson and Clark 1992, Angerbjörn et al. 1994, Koch et al. 1995), to trace movements (Fry 1983, Schell et al. 1989, Fleming et al. 1993, Koch et al. 1995), to assess physiological condition (Hobson et al. 1993), and to determine the fate of assimilated nutrients within an animal (Tieszen et al. 1983, Tieszen and Fagre 1993). Animal physiological ecologists have begun to recognize the utility for their discipline of measuring the variation in naturally occurring stable isotopes, and we foresee an explosive increase in their application.

Although the measurement of stable isotope ratios has the potential of making important contributions to animal ecology, the interpretation of these ratios relies on assumptions that, we believe, are not widely recognized. The purpose of this commentary is to identify

these assumptions, to describe the conditions under which they are not met, and to suggest the laboratory experiments that are required to validate them. It is becoming progressively easier and less expensive to measure stable isotopes in biological tissues (Handley et al. 1991), and technological advances are making the method increasingly available to animal ecologists. Not recognizing the assumptions and limitations of the method can lead to the accumulation of a large body of phenomenological data that is difficult to interpret, or that is interpreted incorrectly.

We argue that solid progress in the use and interpretation of stable isotope data in animal ecology will be achieved only if the collection of field data is accompanied by laboratory experiments. These experiments must be designed to determine the limits of inferences that can be derived from descriptive field data. Results from these experiments will allow the design of sound, statistical methods of field survey that will take into account the sources of variation found in laboratory studies. Our commentary is not intended as a comprehensive review of the uses of stable isotopes in animal physiological ecology. Rather, we make selective use of examples to emphasize and illustrate our contentions. For conciseness, we focus our discussion on carbon and nitrogen.

Stable isotopes and the reconstruction of animal diets

The foods that animals eat often exhibit characteristic isotopic signatures. Plants with different modes of

photosynthesis exhibit contrasting $^{13}\text{C}/^{12}\text{C}$ ratios (Griffith 1991, 1992), and foods derived from marine sources often have different isotopic composition than foods derived from terrestrial and freshwater sources (Chisolm et al. 1982). These differences have been used to identify the relative contribution to an animal's diet of plants with different photosynthetic pathways (Boutton et al. 1980, 1983), and of foods with marine vs. terrestrial origins (Chisolm et al. 1983, Hobson 1987). Probably the best known example of an application of stable isotopes in diet reconstruction is the identification of the transition to a maize-based diet in Amerindian paleodiets (Vogel and Van der Merwe 1977, DeNiro 1987). *Zea mays* is a C_4 plant and, consequently, has a relatively high $\delta^{13}\text{C}$ (the normalized $^{13}\text{C}/^{12}\text{C}$ ratio of sample to standard in parts per thousand) Briefly, and a bit schematically, an upward shift in the $^{13}\text{C}/^{12}\text{C}$ ratios of human remains in the archaeological record in North America and Mesoamerica is often interpreted as being caused by the adoption of a maize diet. The difference in isotopic composition between C_4 grasses ($\delta^{13}\text{C}$ ranging from -20‰ to -9‰) and C_3 forbs ($\delta^{13}\text{C}$ ranging from -34‰ to -22‰) has also been used to discriminate grazers from browsers in contemporary African savannas (Ambrose and DeNiro 1986) and in the paleontological remains of herbivores (Koch et al. 1994).

Using the isotopic signal in an animal's tissues to determine the relative contribution of different food items to its diet relies on a very important assumption: that the isotopic composition of an animal's tissues equals the weighted average of the isotopic composition of the constituents of its diet. For example, for a herbivore consuming C_4 and C_3 plants,

$$\delta^{13}\text{C}(\text{animal tissue}) = p \cdot \delta^{13}\text{C}_{(\text{C}_4)} + (1 - p) \cdot \delta^{13}\text{C}_{(\text{C}_3)},$$

where p equals the fraction of C_4 grasses included in the diet, and $\delta^{13}\text{C}_{(\text{C}_4)}$ and $\delta^{13}\text{C}_{(\text{C}_3)}$ are the isotopic compositions of C_4 and C_3 plants, respectively. This assumption is rarely valid for three important reasons: (1) animals assimilate dietary components with varying efficiencies; (2) animal tissues fractionate the isotopes in their diet (change the isotopic ratios); and (3) animals allocate nutrients in their diet differentially to specific tissues. Physiological ecologists have long recognized the first problem in attempts to characterize foraging and nutrient intake, and for many dietary components, assimilation is known or can be measured (Karasov 1990).

Even when animals are fed isotopically homogeneous diets, the isotopic composition of their tissues can differ from that of their diet (Macko et al. 1982, 1986, Hobson 1995). For example, when lipid is synthesized from dietary carbohydrate, the isotopic composition of the synthesized lipid becomes relatively en-

riched in ^{12}C (DeNiro and Epstein 1977, Monson and Hayes 1982). Fat deposited directly from dietary lipid would not be expected to show the same fractionation. Bacterially synthesized amino acids show large variation in carbon isotope ratios, despite an isotopically uniform carbon source (Macko et al. 1987). Different biochemical synthetic pathways for amino acids, therefore, fractionate to varying extents. One can also observe this effect in animal tissues, in which the amino acid composition of a tissue can influence its carbon isotope value. For example, collagen contains 33% glycine, a relatively ^{13}C -enriched ($+8\text{‰}$) amino acid, and tends to be enriched relative to other tissues (Hare et al. 1991). Fractionation in tissues is not a fatal flaw for dietary reconstruction, provided that fractionation factors for different tissues are measured in the laboratory. Unfortunately, we have few data on the fractionation values for various tissues and tissue components in a more or less complete assemblage of species. Comparative data are needed because the assimilation and metabolic pathways that lead to fractionation differ among animals.

The interpretation of the isotopic composition of animal tissues is further complicated by a phenomenon that has been termed "isotopic routing" (Schwarcz 1991). The isotopes contained in different dietary components are not first well mixed and then allocated to different tissues or tissue components (Krueger and Sullivan 1984). Instead, they are routed differentially to specific tissues and body compartments (Tieszen and Fagre 1993). Consequently, tissues often do not reflect the isotopic composition of the bulk diet, but the isotopic composition of the nutrient component of the diet from which the tissue was synthesized. The composition of body protein in omnivores, for example, often reflects the isotopic composition of dietary protein (Ambrose and Norr 1993).

Anthropologists and paleontologists traditionally have used bone collagen, which is largely composed of protein, to analyze isotopic composition for dietary reconstruction. Recognition of the principle that protein isotopic composition often reflects that of dietary protein, and not that of bulk diet, has led many researchers to shift from collagen to the analysis of the carbonates contained in bone apatite (Tieszen and Fagre 1993). These carbonates are synthesized from circulating bicarbonate (LeGeros 1981); hence, they probably reflect the isotopic composition of the components of the diet that are catabolized (Ambrose and Norr 1993). Animals feeding on diets with low protein contents often reserve dietary protein for tissue maintenance rather than catabolizing it for energy ("protein sparing"; Castellini and Rea 1992). Consequently, apatite carbonates probably underestimate the contribution of dietary protein.

To illustrate the problems associated with isotopic routing, we propose a hypothetical, but plausible, example. Assume an omnivorous species such as a brown bear (*Ursus arctos*) in a coastal environment. The marine environment yields fish, which are a source of protein and lipids (Servheen 1987), whereas the terrestrial environment yields berries, which are a source of carbohydrate (Hamilton 1987). Because it is energetically more efficient to deposit lipids and to catabolize dietary carbohydrates directly (Blem 1976, Pond 1981), and because protein is routed into the protein component of tissues, the isotopic composition of the tissues of these bears will greatly underestimate the contribution of terrestrial sources to their diets.

In animals with foregut fermentation, rumen microbes metabolize dietary components to the common denominator of volatile fatty acids (Van Soest 1994). During protein synthesis, they "mix" the nitrogen of all dietary components, and even that of deaminated body protein through urea recycling, with the carbon skeletons of all other dietary components (Haupt and Haupt 1968, Macrae and Reeds 1980). Thus, in foregut-fermenting herbivores, the mixing action of microbes ameliorates the problems that isotopic routing poses to dietary reconstruction. In hindgut fermenters and omnivores with modest fermenting abilities, however, the degree to which the isotopic signatures of different dietary constituents mix depends on many factors, including the degree of fermentation, urea recycling, and nitrogen balance.

Urea recycling leads to the synthesis of amino acids from the nitrogen of dietary and tissue protein and the carbon skeletons of other dietary components (Haupt 1963). Therefore, urea recycling probably increases the isotopic mixing of protein and nonprotein dietary constituents. Animals feeding on high-protein diets probably use dietary protein exclusively for tissue synthesis and catabolize excess dietary protein, carbohydrates, and lipids (Haupt and Haupt 1971). Animals feeding on protein-deficient diets, in contrast, probably "spare protein" and utilize the nitrogen from deaminated tissue protein to synthesize new amino acids, using the carbon skeletons derived from dietary carbohydrates and lipids (Fisler et al. 1982). We therefore expect protein balance to have an important influence on the degree to which the isotopic signature of dietary protein is conserved in a consumer's tissue protein.

We need more experimental data to interpret the growing body of information on the isotopic composition of animal tissues, and to determine how this composition is related to the animal's dietary constituents. Laboratory research must ascertain the fate of nutrients labeled with contrasting isotopic signatures in animals exhibiting varying degrees of fermentative digestion, nitrogen budgets, and urea recycling. Without these

comparative experimental data, the interpretation of relationships between the isotopic composition of diets and of consumers gathered in the field will remain tenuous. An important side benefit of these experiments is that the data gathered will shed light on how animals allocate nutrients and nutrient components to different tissues, which is a long-standing question in ecological physiology (Sibly and Calow 1986).

Stable isotopes, trophic level, and body condition

Stable isotopes can be used to elucidate not only an animal's diet but also its trophic level and body condition. These latter two uses of stable isotopes are consequences of the same biochemical process. Protein in consumers has a higher $^{15}\text{N}/^{14}\text{N}$ ratio than dietary protein (Ambrose and DeNiro 1986). This difference appears to be due to preferential removal of "light" (^{14}N -containing) amine groups by the enzymes responsible for amino acid deamination and transamination (Macko et al. 1986, 1987). Excreted nitrogen contained in ammonia, urea, and uric acid is lighter than body and dietary protein (Steele and Daniel 1978). Animals in neutral nitrogen balance typically show fractionations ranging from +2‰ to 5‰ (DeNiro and Epstein 1981) between dietary nitrogen and tissue nitrogen. Because a consumer's nitrogen is heavier than its diet, nitrogen in the tissues of animals higher in the food chain tends to be heavier (i.e., to have more positive $\delta^{15}\text{N}$ values) than that of animals lower in the food chain (Minagawa and Wada 1984).

The tissues of starving animals show a progressive increase in the $^{15}\text{N}/^{14}\text{N}$ ratio as lean body mass decreases (Hobson et al. 1993). Because starving animals literally "live on their own meat" (Waterlow 1968), the mechanisms by which their tissues become enriched in ^{15}N are the same as those causing trophic-level nitrogen fractionation. The excreted "lighter" nitrogen is not replaced by dietary protein; therefore, the animal becomes progressively more ^{15}N enriched over the course of starvation. For this reason, changes in the nitrogen isotopic composition of animal tissues can be used as indicators of changes in body condition (Hobson et al. 1993).

Most current indicators of body condition in animals assume that body fat content is a good indicator of fitness and ignore lean-tissue mass (Robbins 1993). Although percentage body fat is probably an adequate index of body condition under some conditions (such as hibernation), it is clearly inadequate under others (Kirkpatrick 1980). Body lipids can also be difficult to estimate, except by using lethal methods (Robbins 1993). Nonlethal indicators of lean-tissue stores are needed to complement body condition index based on fat content (DelGiudice 1995, Grubb 1995). Because tissues in starving animals become enriched in ^{15}N , the

changes in $^{15}\text{N}/^{14}\text{N}$ ratios measured in urine, hair, and blood may be good indicators of lean-tissue losses (Hobson et al. 1993).

Nitrogen (^{15}N) enrichment in the tissues of animals losing mass depends on the intricacies of protein catabolism. The physiological events accompanying starvation at the whole-organism level are relatively well studied (Castellini and Rea 1992), but data on the events at the tissue level that are likely to influence ^{15}N enrichment and that permit about predictions the relative rates of enrichment of different tissues are scanty. Physiological ecologists can greatly contribute to progress in isotopic ecology by providing a quantitative description of the fates of amino acids in different tissues, by "calibrating" the isotopic enrichment of different tissues during starvation, and by providing the mechanisms that give rise to this enrichment (see Millward 1979, 1989). To our knowledge, no studies have calibrated the rate at which different tissues become enriched in ^{15}N as a function of their rates of mass loss, protein catabolism, and deamination (see Bond and Barrett 1993). These studies are essential if stable isotopes are to be used to assess body condition in both extinct and extant animals.

A call for laboratory experiments

The physical and biological processes that lead to contrasting distributions of naturally occurring stable isotopes can provide a valuable tool for animal physiological ecologists; however, their application and interpretation are not without challenges. The interpretation of stable isotope patterns in the field provides yet another example of how research in ecology and physiology are inextricably linked. Because the processes that generate stable isotope ratio patterns are ecological, physiological, and biochemical, understanding patterns at one level requires an awareness of processes in the others. Stable isotope ratios are correlated with dietary, trophic level, and body condition patterns. However, an isotope ratio difference between the protein carbon or nitrogen of two individuals does not necessarily indicate different diets, nor does it necessarily indicate trophic level or condition differences. Interpretations of such differences, particularly small ones, will take careful investigation and will benefit greatly from comparative information and the use of sound statistical techniques. A much deeper understanding of the processes that lead to fractionation in, and isotope routing to, different tissues is needed for their use in dietary reconstruction. Devising indices of body condition will require a better understanding of protein catabolism. Our enthusiasm for these techniques thus stems both from their power and from anticipation of the avenues of integrated research that their validation will open.

The ease with which stable isotope ratios can be measured, and the promise of the information that stable isotope data can provide, will probably lead to an explosion of their use by field ecologists and to an avalanche of data. The proper interpretation of the inferences that can be generated from these data demands that we conduct comparative laboratory experiments. These experiments will provide the firm foundation needed to set the limits to what can be deduced from stable isotope data gathered in the field.

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LITERATURE CITED

- Ambrose, S. H., and M. J. DeNiro. 1986. The isotopic ecology of East African mammals. *Oecologia* **69**:395–406.
- Ambrose, S. H., and L. Norr. 1993. Carbon isotopic evidence for routing of dietary protein to bone collagen, and whole diet to bone apatite carbonate: purified diet growth experiments. Pages 1–37 in J. Lambert and G. Grupe, editors. *Molecular archaeology of prehistoric human bone*. Springer-Verlag, Berlin, Germany.
- Angerbjörn, A., P. Hersteinsson, K. Lidén, and E. Nelson. 1994. Dietary variation in Arctic foxes (*Alopex lagopus*): an analysis of stable carbon isotopes. *Oecologia* **99**:226–232.
- Blem, C. R. 1976. Patterns of lipid storage and utilization in birds. *American Zoologist* **16**:671–684.
- Bond, J. S., and A. J. Barret. 1993. *Proteolysis and protein turnover*. Portland Press, Portland, Oregon, USA.
- Boutton, T. W., M. A. Arshad, and L. L. Tieszen. 1983. Stable isotope analysis of termite food habits in East African grasslands. *Oecologia* **59**:1–6.
- Boutton, T. W., B. N. Smith, and A. T. Harrison. 1980. Carbon isotope ratios and crop analyses of *Arphia* (Orthoptera: Acrididae) species in southern Wyoming grassland. *Oecologia* **45**:299–306.
- Castellini, M. A., and L. D. Rea. 1992. The biochemistry of natural fasting at its limits. *Experientia* **48**:575–582.
- 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.
- Chisholm, B. S., D. E. Nelson, and H. P. Schwarcz. 1983. Marine and terrestrial protein in prehistoric diets on the British Columbia coast. *Current Anthropology* **24**:396–398.
- DeGiudice, G. D. 1995. Assessing winter nutritional restriction of northern deer with urine in snow: considerations, potential, and limitation. *Wildlife Society Bulletin* **23**:687–693.
- DeNiro, M. J. 1987. Stable isotopes and archaeology. *American Scientist* **75**:182–191.
- DeNiro, M. J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**:261–263.
- DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the

- distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**:341–351.
- Ehleringer, J. R., and R. K. Monson. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* **24**:411–439.
- Farquhar, G. D., J. R. Ehleringer, and L. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Molecular Biology* **40**:503–537.
- Fisler, J. S., E. J. Drenick, D. E. Blumfeld, and M. E. Swendseid. 1982. Nitrogen economy during very low calorie and very low protein diets. *American Journal of Clinical Nutrition* **35**:471–486.
- Fleming, T. H., R. A. Nuñez, and L. S. Lobo-Sternberg. 1993. Seasonal changes in the diets of migrant and nonmigrant nectarivorous bats as revealed by carbon stable isotope analysis. *Oecologia* **94**:72–75.
- Fry, B. 1983. Natural stable carbon isotope tag traces Texas shrimp migration. *Fishery Bulletin* **79**:337–345.
- Griffith, H. 1991. Applications of stable isotope technology in physiological ecology. *Functional Ecology* **5**:254–269.
- . 1992. Carbon isotope discrimination and the integration of carbon assimilation pathways in terrestrial CAM plants. *Plant Cell and Environment* **15**:1051–1062.
- Grubb, T. C. 1995. On induced caching and induced constructions as unambiguous indices of body condition. Pages 98–110 in R. Yosef and F. E. Lorer, editors. *Shrikes of the world: biology and conservation*. Western Foundation of Vertebrate Zoology, Los Angeles, California, USA.
- Hamilton, A. N. 1987. Classification of coastal grizzly bear habitat for forestry interpretations and the role of food in habitat use by coastal grizzly bears. Thesis. University of British Columbia, Vancouver, British Columbia, Canada.
- Handley, L. L., and J. A. Raven. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell, and Environment* **15**:965–985.
- Handley, L. L., C. M. Scrimgeour, S. F. Thornton, and J. I. Sprent. 1991. Determination of the natural abundances of the stable isotopes of ^{15}N and ^{13}C by mass spectrometry: a simplified manual method for the preparation of N_2 and CO_2 . *Functional Ecology* **5**:119–124.
- Hare, P. E., M. L. Fogel, T. W. Stafford, Jr., A. D. Mitchell, and T. C. Hoering. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *Journal of Archaeological Science* **18**:277–292.
- Hobson, K. A. 1987. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. *Canadian Journal of Zoology* **65**:1210–1213.
- . 1995. Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. *Condor* **97**:752–762.
- Hobson, K. A., R. T. Alisauskas, and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. *Condor* **95**:388–394.
- Hobson, K. A., and R. G. Clark. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor* **94**:181–188.
- Haupt, T. R. 1963. Urea utilization by rabbits fed a low-protein ration. *American Journal of Physiology* **205**:1144–1150.
- Haupt, T. R., and K. A. Haupt. 1968. Nitrogen conservation by ponies fed a low-protein diet. *American Journal of Veterinarian Research* **32**:579–588.
- Haupt, T. R., and K. A. Haupt. 1971. Transfer of urea nitrogen across the rumen wall. *American Journal of Physiology* **214**:1296–1303.
- Karasov, W. H. 1990. Digestion in birds: chemical and physiological determinants, and ecological implications. Pages 391–415 in M. L. Morrison, C. J. Ralph, J. Verner, and J. R. Jehl, editors. *Avian foraging: theory, methodology, and applications*. Studies in Avian Biology Number 13. Lawrence, Kansas, USA.
- Kirkpatrick, R. L. 1980. Physiological indices in wildlife management. Pages 99–112 in S. D. Shennittz, editor. *Wildlife management techniques manual*. Wildlife Society, Washington, D.C., USA.
- Koch, P. L., M. L. Fogel, and N. Tuross. 1994. Tracing the diet of fossil animals using stable isotopes. Pages 63–92 in K. Lajtha and R. H. Michener, editors. *Stable isotopes in ecology and environmental science*. Blackwell Scientific Publications, New York, New York, USA.
- Koch, P. L., J. Heisinger, C. Moss, R. W. Carlson, M. L. Fogel, and A. K. Behrensmeier. 1995. Isotopic tracking of change in diet and habitat use of African elephants. *Science* **267**:1340–1343.
- Krueger, H. W., and C. H. Sullivan. 1984. Models for carbon isotope fractionation between diet and bone. Pages 205–220 in T. Turnland and P. E. Johnson, editors. *Stable isotopes in nutrition*. American Chemical Society, Symposium Series Number 258, Washington, D.C., USA.
- LeGeros, R. Z. 1981. Apatites in biological systems. Pages 1–45 in B. Pamplin, editor. *Inorganic biological crystal growth, progress in crystal growth, and characterization*. Volume 4. Pergamon Press, New York, New York, USA.
- Macko, S. A., M. L. Fogel-Estep, M. H. Engel, and P. E. Hare. 1986. Kinetic fractionation of nitrogen isotopes during amino acid transamination. *Geochimica et Cosmochimica Acta* **50**:2143–2146.
- Macko, S. A., M. L. Fogel-Estep, M. H. Engel, and P. E. Hare. 1987. Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chemical Geology* **65**:79–92.
- Macko, S. A., W. Y. Lee, and P. L. Parkere. 1982. Nitrogen and carbon fractionation by two species of marine amphipods: laboratory and field studies. *Journal of Experimental Marine Biology and Ecology* **63**:145–149.
- Macrae, J. C., and P. J. Reeds. 1980. Prediction of protein deposition in ruminants. Pages 225–249 in P. J. Buttery and D. B. Lindsay, editors. *Protein deposition in animals*. Butterworths, London, UK.
- Millward, D. J. 1979. Protein deficiency, starvation, and protein metabolism. *Proceedings of the Nutrition Society* **38**:77–88.
- . 1989. The nutritional regulation of muscle growth and protein turnover. *Aquaculture* **79**:1–28.
- Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relationship between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* **48**:1135–1140.
- Monson, K. D., and J. M. Hayes. 1982. Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. *Geochimica et Cosmochimica Acta* **46**:139–149.
- Pond, C. M. 1981. Storage. Pages 190–219 in C. R. Townsend and P. Calow, editors. *Physiological ecology: an evolutionary approach to resource use*. Blackwell Scientific Publications, London, UK.
- Robbins, C. T. 1993. *Wildlife feeding and nutrition*. Academic Press, San Diego, California, USA.
- Schell, D. M., S. M. Saupe, and N. Haubenstock. 1989. Nat-

- ural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat use. *Ecological Studies* **68**: 260–269.
- Szwarcz, H. P. 1991. Some theoretical aspects of isotope paleodiet studies. *Journal of Archeological Science* **18**: 261–275.
- Servheen, C. 1987. Grizzly bear compendium. National Wildlife Federation, Washington, D.C., USA.
- Sibly, R. M., and P. Calow. 1986. Physiological ecology of animals: an evolutionary approach. Blackwell Scientific Publications, Oxford, UK.
- Steele, K. W., and R. M. Daniel. 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. *Journal of Agricultural Science* **90**:7–9.
- Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implication for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* **57**:32–37.
- Tieszen, L. L., and T. Fagre. 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO_2 , bone collagen, bioapatite, and soft tissues. Pages 123–135 in J. Lambert and G. Grupe, editors. Molecular archaeology of prehistoric human bone. Springer-Verlag, Berlin, Germany.
- Van Soest, P. 1994. Nutritional ecology of ruminants. Cornell University Press, Ithaca, New York, USA.
- Vogel, J. C., and N. J. Van der Merwe. 1977. Isotopic evidence for early maize cultivation in New York State. *American Antiquity* **42**:238–242.
- Waterlow, J. C. 1968. The adaptation of protein metabolism to low protein intake. Pages 61–72 in R. A. McCance and E. M. Widdowson, editors. Calorie and protein deficiencies. Little, Brown and Company, Boston, Massachusetts, USA.