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TECHNIQUES FOR WILDLIFE NUTRITIONAL ECOLOGY

Frederick A. Servello, Eric C. Hellgren, and Scott R. McWilliams

INTRODUCTION

Like other aspects of wildlife science, the scope and sophistication of nutritional analyses have expanded significantly in recent years. For example, our expanding knowledge of the role of plant secondary metabolites as defenses against herbivory has greatly changed the concept of food quality for wild herbivores. Also, indirect assessments of diet and nutrition parameters using stable isotopes, urine metabolites, and fatty acid analyses are now relatively common. Further, there have been refinements or new applications of techniques to assess the ecological energetics and nutritional condition of animals in wild populations. Overall, our more comprehensive understanding of wildlife nutritional ecology has contributed greatly to efforts to understand foraging strategies of wildlife species and assess management and ecological questions via modeling.

Nutritional ecology is the study of the inter-relationships between food resources in the environment and consumptive use of these food resources by wild animals. Consumptive use includes how wild animals procure, digest, absorb, and metabolize available foods to satisfy their requirements for health, growth, reproduction, and activity. In this chapter, we review techniques for understanding the nutritional ecology of wildlife and assessing wildlife habitats and populations from a nutritional perspective. While the basic principles of nutritional ecology are applicable to all species, approaches and techniques used may vary greatly among taxonomic groups because of the nature of the foods eaten, species' behavior, or the feasibility of collecting data in particular environments. We have strived to include a wide range of vertebrate wildlife in our examples and literature sources, and to note the applicability of individual techniques for different taxa. This chapter also covers techniques for both captive wildlife studies and assessments of wild populations.

To understand the nutrition of wild species, a biologist must integrate information on an animal's consumptive use of foods with its requirements for maintenance and production. Interactions among nutritional variables are common with food consumption and nutrient requirements influenced by environmental and social factors. This increases the potential for a complex web of interactions. Therefore, interpreting nutritional data involves evaluating it in the larger context of a species' ecology. This higher level of synthesis, which often involves modeling, has the potential to reveal new insights about a species' nutritional ecology or change perspectives on the importance of individual nutritional factors. Thus, we have briefly reviewed goals and approaches for studying feeding strategies and simulation modeling of nutritional ecology.

NUTRITIONAL AND ENERGETIC REQUIREMENTS

Factors Affecting Energy and Nutrient Requirements

An important but complex endeavor in wildlife nutrition research is the estimation of energy and nutrient requirements of animals. Requirements vary with life functions (maintenance, growth, reproduction), season, and temperature, and also may be influenced significantly by physiological adaptations. For example, white-tailed deer (*Odocoileus virginianus*) reduce food and energy intake in winter as the result of seasonal fluctuations in hormone production (Silver et al. 1969, Thompson et al. 1973). In addition, nutrient or energy requirements for diets are sometimes interactive. For example, the protein: energy ratio is as important as the protein content of the diet in affecting performance in poultry (Scott et al. 1982), and may be the case for wild monogastric species. Rode and Robbins (2000) showed that maintenance energy requirements in 2 species of bears (Ursidae) decreased with increasing protein content (2.3 to 35%) of the diet. Increasing dietary fiber content also can reduce energy and protein digestibility and lead to decreased basal metabolism (Veloso and Bozinovic 1993).

Energy expended and nutrients used by an animal can be for different purposes. The maintenance energy requirement is the chemical energy required to meet the costs of basal metabolism, thermoregulation (in homeotherms), and activity (Robbins 1993). Basal metabolism, which is measured under conditions of rest, thermoneutrality, and a post-absorptive state, is the energy required to maintain basic life processes and cellular activity of an animal. Homeotherms have additional energy demands to thermoregulate or maintain their body temperature. Other activities necessary for survival of the animal, such as feeding, predator avoidance, social interactions, and migration also require energy expenditure simply for maintenance. Nutrient (e.g., N, minerals) requirements for maintenance are adequate amounts of nutrient intake to balance nutrient use in a nonreproductive, adult animal. Energetic and nutrient requirements for growth and reproduction are above and beyond maintenance requirements because extra energy and nutrients are required to produce new body tissue (growth) or to produce biomass in offspring (reproduction).

Techniques Used with Captive Wildlife

Energy requirements of captive animals can be estimated by measuring energy expenditure or by using feeding trials. Nutrient requirements usually are calculated through feeding trials. Nutrient or energy requirements have been quantified for relatively few wild species, and even less is known about suboptimal tolerances. Because diets of suboptimal quality are probably common in wild populations, understanding the effects of nutrient intake over a range of suboptimal levels is important.

Feeding Trials and Energy Requirements

A feeding trial approach involves placing an animal on diets of differing levels of digestible or metabolizable energy intake and measuring the energy level necessary to maintain body weight. The most common approach is to vary the amount of food offered to captive animals and to plot weight change versus energy intake. Regression analysis is used and the point on the regression line where body weight change equals 0 is taken as the energy requirement for maintenance (Ullrey et al. 1970). Variations on this method include altering the amount of food given to each animal until weight stabilizes (Keiver et al. 1984) or, taking as an estimate, the energy intake level for a time period when weight change was stable (<1–2%) (Case and Robel 1974, Williams and Kendeigh 1982). Maintenance requirements estimated in this way are greater than basal metabolic rate because the animal is thermoregulating and conducting some activities, but will generally be less than the maintenance requirements of a free-ranging animal (Robbins 1993). Caution is necessary if body mass is used as the only criterion for energy or nutrient balance because, as the composition of body tissue changes, the energy density of additional lost body mass changes. For example, the energy density of lipid tissue is about 8–9 times that of muscle.

Feeding Trials and Nutrient Requirements

Nutrient requirements, especially nitrogen (or protein), are measured primarily through feeding trials. The nitrogen requirement for maintenance is digestible nitrogen intake that produces tissue nitrogen balance (TNB) equal to 0. Tissue nitrogen balance (also called nitrogen retention) equals 0 when nitrogen intake is equal to excretion of endogenous and indigestible or nonmetabolizable nitrogen plus assimilated nitrogen for normal tissue replacement (e.g., hair replacement). Nitrogen is lost from the body as either endogenous urinary nitrogen (EUN) or metabolic fecal nitrogen (MFN). Endogenous urinary nitrogen is the excreted nitrogen resulting from normal metabolism and is a constant proportion of metabolic body mass (Mould and Robbins 1981*b*). It is normally expressed as a rate (mg/day or mg/kg$^{0.75}$/day). Metabolic body mass is body mass (in kg) raised to the 0.75 power. This conversion is based on the scaling relationship between body mass and metabolic rate in mammals. Metabolic fecal nitrogen consists of microbes, digestive enzymes, mucus, and gastrointestinal epithelial cells accumulated during digestion and is proportional to feed intake (Mould and Robbins 1981*b*). Except during periods of substantial adult tissue growth (e.g., molt), nitrogen costs for adult growth are small and generally ignored (Maynard et al. 1979). Metabolic fecal nitrogen and EUN can be measured in mammals, and their sum is a minimal estimate of maintenance nitrogen requirements (Mould and Robbins 1981*b*). Separate estimates of MFN and EUN cannot be readily obtained for birds because fecal matter and uric acid mix in the cloaca and are excreted together.

A commonly used alternative approach for estimating nutrient requirements is to relate nutrient intake to nutrient balance using a balance trial. Experimental diets containing varying levels of a particular nutrient (N, Na, Ca, K, P) are fed to captive animals to produce varying levels of nutrient intake. For nitrogen balance trials, experimental diets must contain sufficient energy to maintain energy balance; otherwise part of urinary nitrogen may result from tissue catabolism and inflate estimated nitrogen requirements (Maynard et al. 1979, Carl and Brown 1985). Food intake and feces and urine production are measured during a collection period. After the nutrient of interest in the food, feces, and urine is measured, balance is calculated as nutrient ingested (intake) – nutrient excreted (used) expressed in units of g or mg/metabolic body mass/day. Minimal nutrient requirements can be estimated as the x-intercept of the regression of nutrient balance versus nutrient intake (Felicetti et al. [2000] provide an example with nitrogen requirements in porcupines [*Erethizon dorsatum*]), much the same as it is with energy requirements.

The balance method to quantify nutritional intake and use is not new as it was initially used in animal science prior to 1860 (Schneider and Flatt 1975). Limitations of balance trials have been discussed by several authors (Hegsted 1976, Jeejeebhoy 1986, Young 1986). The major criticism is that balance studies typically produce high estimates of retention because of the difficulty in quantifying all excretory materials and, thus, requirements are underestimated (Hegsted 1976, Young 1986). For zero balance (intake = excretion) to represent a valid estimate of maintenance requirements, all dietary intake and excretory outgo must be measured. Another assumption for zero balance to accurately estimate the maintenance requirement is for zero balance to be associated with good health (Jeejeebhoy 1986). Those who have critically appraised the balance technique conclude that nutritional balance studies provide useful and important data on nutrient requirements and changes in nutrient metabolism under varying physiological states (Young 1986). The limitations of the technique should be recognized and further investigation into its utility is suggested (Young 1986, Murphy 1993). Once requirements are estimated, additional feeding trials should be conducted to test their validity.

A variant of the balance technique to measure nutrient requirements is to examine the relationship between nutrient intake and use and to identify the point at which the slope changes in this relationship. Raubenheimer and Simpson (1994) provided a general approach to the study of nutritional processes that involved discriminating the various compartments to which ingested nutrients are allocated. The most basic form of a nutrient budget for nutrient n over time t is

$$I_n = (R + D)_n ,$$

where I is nutrient intake, R is nutrient retained, and D is nutrient dissociated, or not retained, during the defined time period. Plots of these components, especially of intake on the x axis and use (D) on the y axis allow exploration of nutritional efficiencies (Fig. 1, line A represents intake = use). A key element of the model of Raubenheimer and Simpson (1994) is their expectation that efficiency of use of a nutrient should be maximal when intake is below requirements and that it should decrease thereafter (Fig. 1). The level of intake at which this change in slope occurs has been termed the nutrient target (Raubenheimer and Simpson 1994) or, alternatively, the requirement. Because of obligatory endogenous losses necessarily added to nutrients dissociated, line C (Fig. 1) is a closer representation of a mineral budget, with the point where intake equals use (IT2) being the predicted requirement. This model has strong deductive support because the notion of a physiological requirement implies that nutrient dissociation will increase once the intake requirement has been reached (Raubenheimer and Simpson 1994). It is axiomatic that nutrient intake and use remain in long-term balance at varying rates of intake (Guyton and Hall 1996) in adult, nonreproductive mammals. For example, net retention or depletion of sodium could result in severe physiological alterations, such as extracellular expansion or reduction, acid-base imbalance, and electrochemical abnormalities.

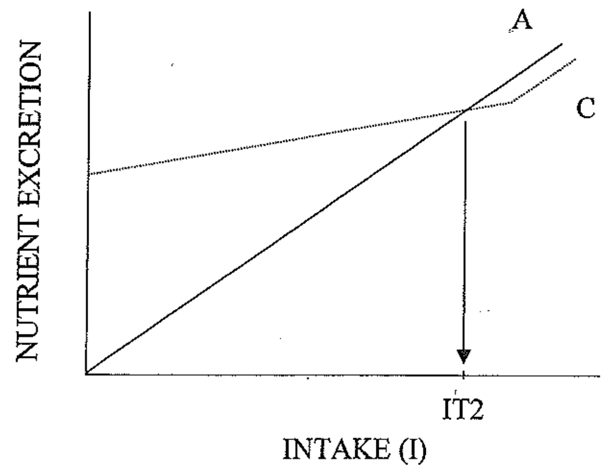


Fig. 1. Plot of nutrient intake vs. nutrient excretion, where line A is where intake = excretion, and line C represents a decrease in nutrient use efficiency with increases in intake (adapted from Raubenheimer and Simpson 1994).

Grasman and Hellgren (1993) estimated phosphorus requirements in white-tailed deer by modeling phosphorus excretion (y) as a function of phosphorus intake (x) using an exponential model ($y = ae^{bx}$). Requirements were assumed to be where intake equalled excretion, and these estimates were supported by data collected concomitantly on endocrine, physiologic, and performance (body mass, feed intake) responses to varying phosphorus intake. Estimates of nitrogen (Murphy 1993, Asleson et al. 1996) and sodium (Hellgren and Pitts 1997) requirements for maintenance were measured by similar approaches.

Calorimetry

Energy expenditure can be measured directly through heat production by an animal, a technique termed direct calorimetry. Although this method is technologically feasible, it has not been widely used for wildlife species

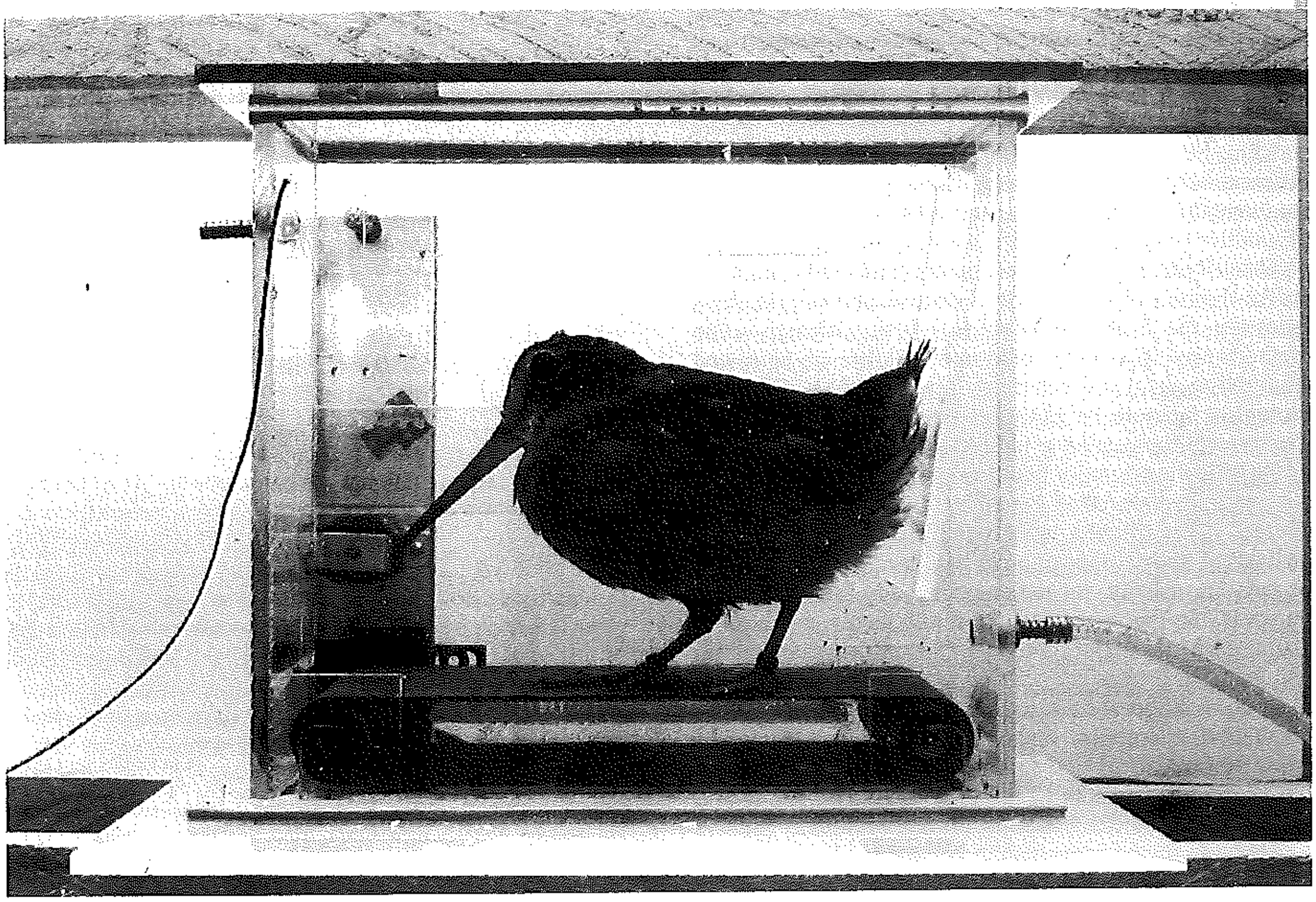


Fig. 2. Energy expenditure measured for American woodcock (*Scolopax minor*) walking on a treadmill in a respiration chamber (photograph by W. M. Vander Haegan).

because indirect measures are easier and more economical (Mautz 1978). Indirect calorimetry measures energy metabolism from estimates of O_2 consumption or CO_2 production. Sampling the volume and composition of expired air is required to estimate energy expenditure by this technique, which requires animals be confined to sealed chambers through which atmospheric air is pumped (Fig. 2), placing face masks on experimental animals that route expired air into sensors of sampling bags, or using tracheal fistulas (Mautz 1978, Robbins 1993). Chambers are usually used for small species (<1 kg) with masks primarily restricted to large mammals (Robbins 1993). The method is indirect because O_2 consumption/CO_2 production is equivalent to a given amount of energy expenditure depending on which substrate (fat, protein, or carbohydrate) is being metabolized. The energy equivalent of consumed O_2 is less variable than respired CO_2; therefore, measurement of O_2 consumption is preferred (Robbins 1993).

The difficulty of collecting gases expelled from an animal in a free-ranging state restricts measurement of energetic expenditure by indirect calorimetry to captive animals. However, in small vertebrates confined to sealed chambers that are sufficiently large to allow activity, indirect calorimetry may provide a reasonable estimate of free-ranging expenditures. In addition, energy expenditure involved in individual activities (e.g., standing, swimming, locomotion) can be estimated using indirect calorimetry in captive animals by measuring gas production/consumption during the target activity. These estimates can be combined with field data to create time-energy budgets.

Estimates of energetic expenditures are not equivalent to energetic requirements, at least if requirements are defined as maintaining body mass, maintaining a given body composition, or producing some level of performance (e.g., growth, reproductive rate). These estimates simply measure metabolism by animals during the time of measurement.

We have focused on explaining techniques for measuring energy requirements and metabolic rate of a focal species. A fundamental concept that has emerged from comparative studies of metabolic rate of a wide diversity of animals is that metabolic rate increases with body size in a predictable, quantitative manner (Kleiber 1932, Schmidt-Nielsen 1984). This allows researchers to predict the metabolic rate of any animal, even a species for which no empirical measurements of metabolic rate have been made, given only measurements of body size and published quantitative relationships. The study of how aspects of an animal's biology such as metabolic rate change with body size of the animal is known as allometrics (Schmidt-Nielsen 1984).

NUTRITIONAL CONDITION

A primary focus of wildlife management is to ensure that wildlife in a given area have the requisite food, water, and cover for adequate survival and reproduction. Evaluating whether habitat is satisfying the requirements of wildlife at some point in time often involves assessing the "condition" of wildlife. Because an animal's nutritional condition can affect its survival and reproduction, understanding how animal condition changes over time and in different habitats provides insights into population dynamics, competitive interactions, and other important aspects of wildlife ecology.

Quantifying the nutritional condition of a wild animal is unlike the task of most medical doctors and veterinarians assessing the health of people or pets, although some methods used to assess condition of wildlife are borrowed from the medical and veterinary sciences. When wildlife biologists measure the nutritional condition of an animal, they typically measure or indirectly estimate one or more of the following 5 major body components: water, fat, protein, carbohydrates, and minerals. In contrast, a medical doctor or veterinarian would rarely be interested in an analysis of body composition in part because most humans or pets are usually not deficient in body fat, protein, or minerals. In this section, we describe techniques used to estimate the 5 major body components of an animal, although we primarily focus on techniques used to estimate body fat because of its central importance in metabolism and energetics, and hence nutritional status, of wildlife. We also briefly discuss other techniques (e.g., blood and urine metabolites) used to assess the health and condition of wildlife.

Methods used for evaluating condition of wildlife can be categorized into 2 main groups based on whether the animal's condition is assessed while alive (nondestructive techniques) or after death (destructive techniques). Many traditional techniques used to evaluate nutritional condition of wildlife were based on analysis of hunter-killed animals. The literature on condition of game species based on carcass analysis has a long history and forms the foundation upon which many contemporary approaches for assessing nutritional condition of wildlife have been developed. Nondestructive techniques by definition provide an indirect estimate of body condition in that fat, protein, or some other body components are not directly measured in total as can be done with destructive techniques. "Condition indices" are an important subset of both nondestructive and destructive techniques. Such indices provide a measure that is correlated with some body component (e.g., a fat score of 1 on a 7-point scale) as opposed to providing a direct measure or estimate of the quantitative amount of the body component.

We discuss each major technique used to assess the nutritional condition of wildlife. For each technique, we describe the method and discuss its applicability for a diversity of wild vertebrates. We begin with a description of whole-animal body composition because it allows us to define the 5 major body components and describe how they are measured. Whole-animal body composition is the primary destructive technique used to assess body condition of wild animals. It is also necessary for assessing the accuracy and precision of any of the nondestructive techniques used to estimate or index body condition of live animals. Ideally, a measure, estimate or index of body condition is accurate and precise, but also relatively easy to obtain, objective, and able to detect changes in body condition of individuals of different age and gender, and at different seasons and locations (Harder and Kirkpatrick 1994).

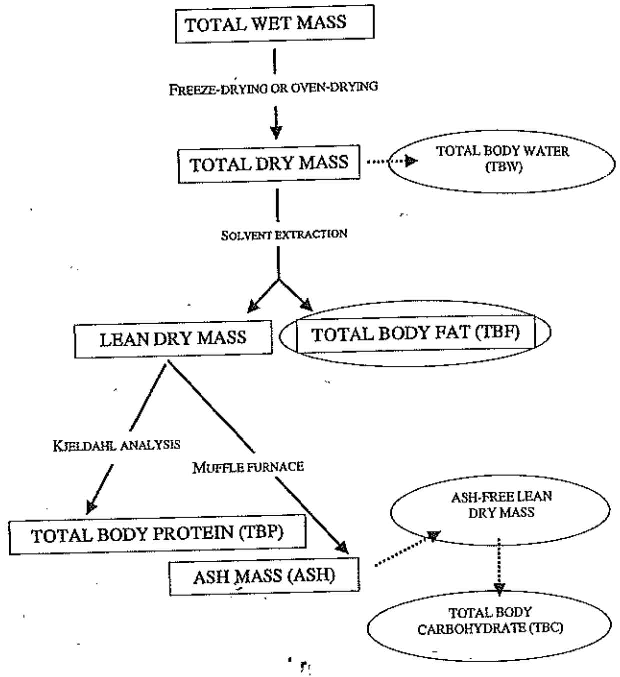


Fig. 3. Flow diagram of the stepwise procedure for conducting whole-body analysis to estimate the 5 major body components of an animal: water, fat, protein, carbohydrates, and minerals (or ash). Body components in boxes are directly measured whereas those in circles are calculated as the difference between 2 measured body components. For example, total body water (TBW) is estimated as the difference between total wet mass and total dry mass of the carcass. Note that total body fat (TBF) is measured directly when using a Goldfisch apparatus, but must be estimated by subtraction of lean dry mass from total dry mass if fat is extracted using the Soxhlet apparatus. Ash-free lean dry mass has been used as an index of TBP and is calculated as the difference between lean dry mass and ash mass. Total body carbohydrates (TBC) are commonly estimated as the difference between ash-free lean dry mass and total body protein. All measurements are typically expressed as dry mass (g dry) except for total wet mass (g wet).

Whole-animal Body Composition

In the discussion that follows, we assume the body composition analysis is conducted using the whole carcass, although subsampling or dividing the carcass may be necessary or more convenient for some studies. Methods of storage and preparation of the carcass prior to whole-body analysis are beyond the scope of this chapter and vary depending on the objectives of the study (Alisauskas and Ankney 1992, Reynolds and Kunz 2001).

The first step in whole-body analysis is drying the carcass by either freeze-drying or oven drying using a convection or vacuum oven (Fig. 3). The difference between total wet body mass and total dry body mass provides a direct measure of total water mass (or total body water, TBW). We recommend freeze-drying because it does not change tissue composition, although change in tissue composition associated with oven drying may be minimal for fat (Kerr et al. 1982). If oven drying is used, we recommend a drying temperature of 60–90 C. Overheating of tissue in ovens can cause volatilization and loss of organic matter (especially proteins), whereas slow drying of tissue at warm temperatures may allow decomposition of tissue.

After drying, the carcass is ground using a meat grinder or electric mill to homogenize the sample and to ensure complete extraction of nutrients. Total body fat is then measured by solvent extraction of lipids from the dried, ground carcass using a Soxhlet apparatus (Sawicka-Kapusta 1975), Goldfisch apparatus (Anonymous 1990), or ANKOM fat extractor. Petroleum ether or diethyl ether is the preferred solvent for most wildlife studies because these solvents extract neutral lipids, which are the primary form of storage energy in animals (Blem 1980, 1990). Chloroform and methanol extract other lipids (e.g., phospholipids which are primarily structural fats in animals) as well as non-lipids (Dobush et al. 1985), which results in an overestimate of total body fat. The difference between total dry body mass and the dried, extracted (fat-free dry or lean dry) mass provides a direct measure of total body fat (Fig. 3). Total body fat can also be expressed on a mass-dependent basis by dividing total body fat by body mass (either wet or dry) or by lean dry mass. Total body fat has been used as an index of general nutritional condition in small mammals (Fleharty et al. 1973), in a few studies of ungulates (Torbit et al. 1985), and commonly in studies of birds (Brown 1996).

For many wildlife studies, the whole-animal body composition analysis ends after fat extraction with estimates of total body water (TBW), total body fat (TBF), and lean dry mass (LDM) (Fig. 3). Lean dry mass has been used as an index for body protein in birds (e.g., Raveling 1979, Alisauskas and Ankney 1985) and mammals (e.g., Kiell and Millar 1980, Atkinson et al. 1996, Reynolds and Kunz 2000), although this can be inaccurate because lean dry mass is composed of 3 components: proteins, ash, and carbohydrates. However, carbohydrates are typically only a small proportion of an animal's mass. A more complete analysis of LDM is necessary if proteins and minerals in animals are dynamic such as in growing animals, reproducing females, or in migratory wildlife.

Ash content is measured by combusting lean dry samples in an ash-oven or muffle furnace at 500–550 C for at least 5 hours (Pierson and Stack 1988). After combustion, the remaining ash is composed of inorganic minerals. The difference between lean dry mass and ash mass is appropriately called ash-free lean dry mass (AFLDM) (Fig. 3) and is primarily composed of protein (91–94%, Reynolds and Kunz 2001) and carbohydrates. Generally, AFLDM provides a better estimate of body protein than LDM, although an accurate direct estimate of body protein requires separate analysis of either the lean dry mass or a subsample of the whole dry carcass. Total body protein (TBP) is usually estimated by directly measuring nitrogen content of the sample by the Kjeldhal procedure.

Body Mass and Structural Measures

Body Mass

Changes in body mass over time may indicate changes in nutritional condition of the animal. For example, state wildlife agencies often use dressed carcass weights of 1.5-year-old male deer taken during the autumn hunting season as a measure of deer condition and habitat quality. In this instance, body mass is a function of fat content and structural size of the animal, so that changes in body mass over several years indicate changes in condition of the deer herd. A younger age-class of male deer is used in part because body mass of older male deer changes dramatically during the rut period (Warren et al. 1981). Anderson et al. (1972) concluded that eviscerated carcass weight (whole body weight minus all fat within the body cavity and all viscera except the esophagus and trachea) was a good index of condition in female mule deer (*Odocoileus hemionus*) but not in males.

Biologists should be cautious about general use of body mass as a condition index. Body mass dynamics can be influenced by age, reproductive status, and other physiological factors that may complicate inferences about food resources or habitat (e.g., Servello and Kirkpatrick 1988). Although changes in body mass over time are often thought to indicate changes in fat content of wildlife, the relationship between body mass and actual fat mass is often weak (r^2 = 0.4–0.6; Bailey 1979, Whyte and Bolen 1984, Johnson et al. 1985). The use of body mass as an index of fat assumes there are no simultaneous changes in body components other than fat. This assumption is clearly violated for many species of birds and mammals because they change both their lean and fat components over time (van der Meer and Piersma 1994, Reynolds and Kunz 2000).

Structural Measures as Indices of Body Condition

Various structural measures have been used as indices of body condition and nutritional status of wildlife. For example, Klein (1964) used a ratio of femur length to hind foot length to compare long-term nutritional status of 2 populations of Sitka black-tailed deer (*O. h. sitkensis*). The index is based on the fact that growth of the bones in the hind foot is relatively more complete at birth than is the femur. Thus, this ratio in an adult deer can indicate the amount of skeletal growth occurring over the lifetime of an animal and, hence, the relative long-term nutritional regime.

Antler beam diameters have long been used as indices of nutritional status in cervids (e.g., Severinghaus et al. 1950, Riney 1955). Rasmussen (1985) suggested that antler beam diameter of yearling males was the most practical method for monitoring the health and vitality of deer populations. However, antler size of deer may be primarily influenced by nutritional status just prior to antler development and not necessarily of range conditions and nutritional status throughout the year (Ullrey 1982).

Condition Indices Based on Ratios of Body Mass to Size

A primary difficulty with using only changes in body mass or structural measures to indicate nutritional condition is that individual animals often differ in body size. Thus, bigger, heavier animals in one location may be in no better condition than smaller, lighter animals in another location. This has led many researchers to calculate a condition index that divides the body mass of an animal by some estimate of its size (usually a linear measure such as arm/wing, leg, or body length, but lean mass and fat-free body mass have also been used [Harder and Kirkpatrick 1994, Hayes and Shonkwiler 2001]). Such body condition indices are believed to be correlated with animal quality such as nutritional condition, fat content, or even

Darwinian fitness (Krebs and Singleton 1993, Brown 1996, Viggers et al. 1998). Unfortunately, few studies test the validity of a condition index and demonstrate the index actually indicates what the biologist thinks it indicates (e.g., fat mass, production of offspring, survival).

Although condition indices that use a ratio of body mass to body size are easy to compute and commonly used in wildlife studies, they may have serious flaws. Most importantly, they are dimensionless variables that are often difficult to interpret biologically (Alisauskas and Ankney 1992, Hayes and Shonkwiler 2001) and, in most cases, the relationship between body mass and size is not isometric as assumed (Packard and Boardman 1987, Packard and Boardman 1999). The common problem with such indices is they work only if body mass increases linearly with body size (slope = 1, intercept = 0) so that deviations of an individual from this overall pattern indicates differences in animal condition (Hayes and Shonkwiler 2001). If this assumption is true, the ratio of body mass: body size will not be correlated with body size. Typically, however, the condition index is correlated with body size, so that animals with different condition index values may have different nutritional condition or they may simply differ in size. In addition, ratio variables are plagued with numerous statistical and inferential problems (Jacob et al. 1996, Hayes and Shonkwiler 2001).

Unfortunately, more sophisticated statistical approaches for estimating body size (e.g., Principal Components Analysis of many structural measures) or the condition index (e.g., using residuals from a regression of body mass on body size) do not solve the problems of biological interpretation and allometry (Green 2001, Hayes and Shonkwiler 2001). Instead, the best approach is direct analysis of the data used to generate the condition indices combined with well-designed validation studies, rather than the continued use and faith in simple ratios of body mass and body size (Packard and Boardman 1999, Hayes and Shonkwiler 2001).

Growth Rate

One special case where body mass and structural size are often accurately used to indicate nutritional status of animals is when used for growing animals. Whereas differences in growth patterns between species can reveal evolutionary adaptations to ecological conditions (Derrickson 1992, Ricklefs and Starck 1998), variation in growth between individuals of the same species reflects in part variation in the nutritional environment during growth (Ricklefs et al. 1998, Schew and Ricklefs 1998). Thus, variation in growth rate of immature animals has been used to assess the adequacy of the nutritional environment for a wide range of wildlife including colonial nesting waterbirds (Nisbet et al. 1998, Golet et al. 2000), waterfowl (Sedinger 1992), landbirds (Schew and Ricklefs 1998), as well as many mammals (Crête and Huot 1993, Wigginton and Dobson 1999, Lesage et al. 2001).

Ideally, a complete growth curve (i.e., animal size as a function of time) for many individuals is measured so that the nonlinear pattern of growth can be statistically modeled and a few interpretable parameters of growth rate can then be estimated [e.g., asymptotic mass or size, growth rate(s) for specified time periods]. Ricklefs (1968, 1973) and Starck and Ricklefs (1998) presented a detailed discussion of the types of mathematical analysis, curve fitting, and statistical models used in describing animal growth. However, estimating growth rate of young animals in wild populations is complicated because it is often difficult to regularly capture and measure body mass and structural size of many individuals throughout growth without negatively affecting growth rate. More often, measurements of body mass or structural size are made a few times and growth rates are estimated for roughly the middle portion of the growth period when growth is linear (e.g., Emms and Verbeek 1991, Golet et al. 2000).

Fat Indices

Discrete Fat Depots

Subcutaneous, visible fat depots are commonly used as an index of fat reserves in live migratory birds. A qualitative index called a "fat score" is used to rank the amount of visible fat in the furcular region (claviculocoracoid area; Fig. 4) of songbirds (Helms and Drury 1960). Fat scoring can provide a reliable index of subcutaneous fat, although accurate use of this technique requires extensive training of observers (Krementz and Pendelton 1990). Similarly, the shape of the abdominal profile of geese (Anserinae) has been categorized into 4 fat-reserve classes (Owen 1981) and used to document changes in goose condition in relation to age, gender, and habitats used.

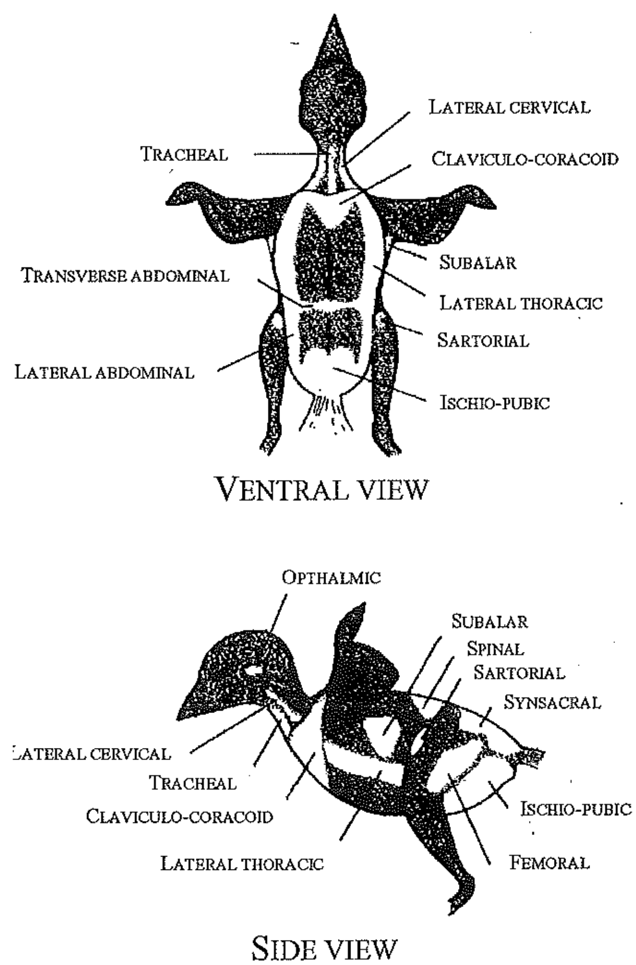


Fig. 4. Distribution of subcutaneous fat in the white-crowned sparrow (*Zonotrichia leucophrys*) (copied with permission from King and Farner 1965).

Discrete fat depots within dead birds and mammals have been used as an index of total body fat, specifically, and body condition, in general. Fat depots in certain locations within the animal may provide better indices of total body fat in part because the use and deposition of fat may occur in a relatively prescribed order. The following pattern of fat catabolism has been described for mammals (Riney 1955): subcutaneous fat over the rump and saddle is used first, then fat in the abdominal cavity, and finally bone marrow fat is used. The opposite sequence is followed during replenishment of fat stores. Although these patterns of use and deposition of certain fat stores appear to be generally true, there is considerable overlap in the timing of this mobilization and storage for a given fat depot (Ransom 1965). The pattern of fat use and deposition in birds is less clear and shows more interspecific variation than in mammals. For example, fat catabolism in Canada geese (*Branta canadensis*) initially involved the simultaneous use of both abdominal and subcutaneous fat, although the subcutaneous fat depots were the last to be used (Raveling 1979). Blem (1976) reported that birds deposit subcutaneous fat first followed by abdominal fat, whereas King (1967) observed the opposite pattern. Even if a certain pattern of fat catabolism and storage is evident in a given species, distinguishing between abdominal and subcutaneous is often difficult, especially in relatively fat animals. Thus, it is not surprising that the best predictors of total body fat in wild animals used the combined weight or a ranked index of all these fat depots (Ankney and MacInnes 1978, Cook et al. 2001).

Fat associated with specific internal organs has also been used as an index of body fat in birds and mammals. For example, gizzard fat has been reported to be a suitable index of body fat in ring-necked pheasants (*Phasianus colchicus*) (Dowell and Warren 1982), ruffed grouse (*Bonasa umbellus*) (Servello and Kirkpatrick 1987*a*), and northern bobwhite (*Colinus virginianus*) (Koerth and Guthery 1988). Similarly, kidney fat has been used as an indicator of whole body fat in white-tailed deer (Finger et al. 1981) and fisher (*Martes pennanti*) (Garant and Crête 1999), but it is more commonly used as a good indicator of abdominal fat reserves in many mammals, particularly ungulates (Smith 1970) and lagomorphs (Flux 1971, Jacobson et al. 1978) although not brush-tailed possum (*Trichosurus vulpecula*) (Bamford 1970). Riney (1955) described the standard method for measuring the kidney fat index (KFI; ratio of fat on kidney: kidney mass) in mammals (Fig. 5). Because kidney weight of ungulates changes seasonally (Batcheler and Clarke 1970, Dauphiné 1975), some control for effect of season and perhaps age is required if KFI is to be used to indicate seasonal changes in fat reserves (Van Vuren and Coblentz 1985).

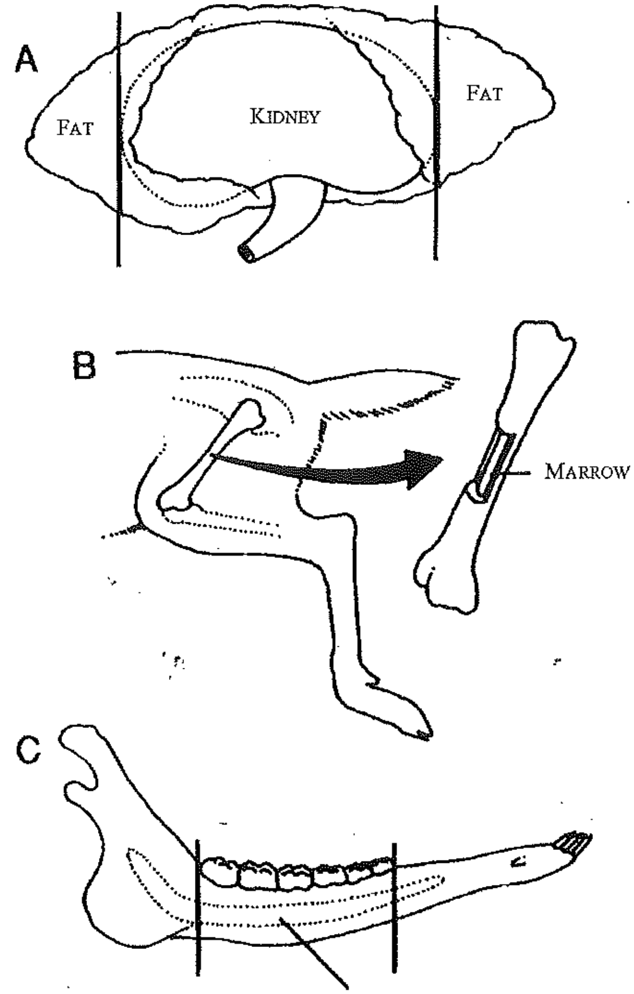


Fig. 5. The amount of fat surrounding the kidney (A) provides an index to visceral fat (adapted from Riney 1955). The amount of fat in the marrow of mammalian long bones such as the femur (B) (adapted from Cheatum 1949) or the mandible (C) (adapted from Nichols and Pelton 1974) provides a measure of energy in this depot of last resort. Fat (in A) or bone (in C) lateral to the vertical lines is trimmed and discarded.

Bone Marrow Fat

The amount of fat within certain bones of freshly killed wildlife has been commonly used as a condition index for larger mammals, especially Cervidae (Cheatum 1949, but see Cook et al. 2001). Bone marrow fat has also been effectively used as a condition index for smaller mammals [e.g., cottontails (*Sylvilagus* spp.) (Jacobson et al. 1978, Warren and Kirkpatrick 1978), brush-tailed possum (Bamford 1970)], and waterfowl (Raveling et al. 1978, Hutchinson and Owen 1984). In general, less bone marrow fat indicates poor condition and malnutrition of individuals because animals in good or excellent condition have similar, maximum amounts of marrow fat (Mech and DelGiudice 1985, Cook et al. 2001). Bone marrow fat in combination with the kidney fat index may provide an estimate of nutritional status of mammals over a wider range of conditions than either technique alone (Ransom 1965, Kie et al. 1983). We caution that analyzing these data using mean fat or index values is problematic because relationships to total body fat are not linear.

Measuring bone marrow fat involves choosing a specific bone(s) and then quantitatively measuring the amount of fat (Fig. 5). Traditionally, fat is measured in a bone marrow sample from the femur of birds and mammals or the mandibular (jaw) cavity of cervids (Harder and Kirkpatrick 1994). The amount of fat in the bone marrow sample is measured by oven drying (Neiland 1970) or solvent extraction (Verme and Holland 1973), and then expressed as the percentage of fat in the wet or dry mass of bone. Fat levels in bone marrow should be expressed on a dry-matter basis to avoid problems associated with dehydration of samples after collection and before oven drying (Harder and Kirkpatrick 1994).

Isotope Dilution of Body Water

Water within an animal is not evenly distributed among its tissues. For example, a gram of body fat in an animal contains almost no water whereas a gram of body protein contains on average about 70% water (Speakman et al. 2001). This uneven distribution of body water among body tissues means the fatter an animal becomes the lower the water content as a percentage of it's body mass. Thus, in principle, body fat can be estimated by measuring body mass and water content of any animal. Empirical studies of a variety of wild animals have demonstrated that total body water is positively related to fat-free body mass ($r^2 > 0.95$; Child and Marshall 1970, Campbell and Leatherland 1980) and negatively related to body fat ($r^2 > 0.95$; Bailey 1979, Johnson et al. 1985). Direct measurements of body water in dead individuals generally provide a reliable method for estimating total lean or fat mass of the animal (Gessaman 1998).

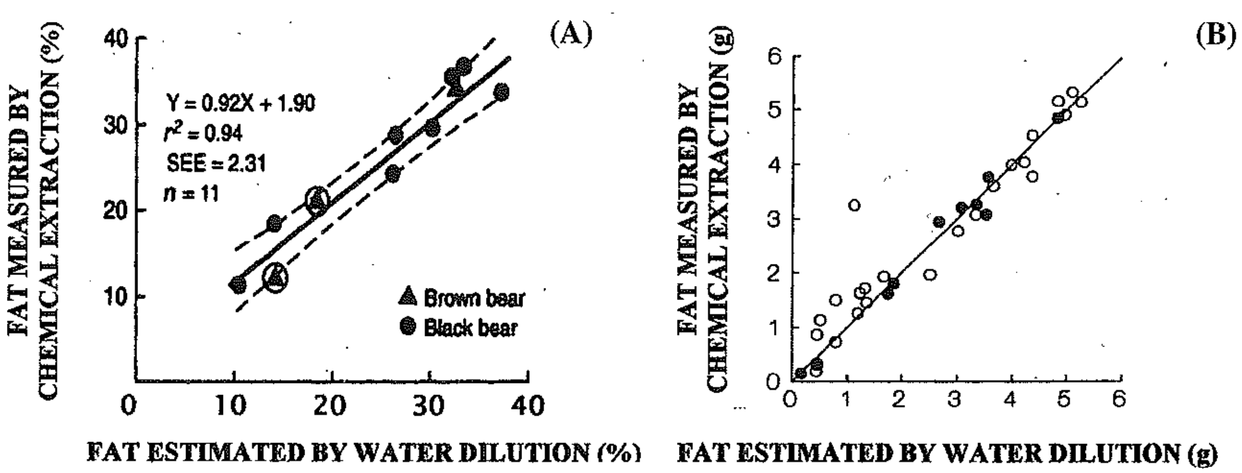
Recent advances in use and measurement of stable isotopes of water provide great promise for estimating body composition of live wild animals by nondestructively measuring their body mass and total body water (Speakman et al. 2001). Radioactive isotopes of water (most often tritium, ^3H_2O) have also been successfully used to estimate total lean and fat mass of wildlife species (Crum et al. 1985, Hildebrand et al. 1998). However, using radioactive compounds in the field requires additional safety precautions and permits that are often difficult to obtain. Stable isotopes of water (most often deuterium, ^2H_2O) require no such permits and can be used to conveniently and accurately estimate total body water of live wild animals.

The isotope-dilution technique involves injecting a known amount of deuterium (or other isotopically-labeled water) into the animal, allowing the isotope time to equilibrate with normal body water (ca. 30 min for small songbirds and mammals, as long as 3 hrs for bears), taking a blood sample, and then measuring the concentration of deuterium in the sample (Speakman et al. 2001) (Box 1). The accuracy and precision of this technique can be ascertained by conducting a calibration study in which estimates of body water using isotope dilution are directly related to measured lean and fat mass of individuals (e.g., Oftedal et al. 1996, Karasov and Pinshow 1998). The isotope dilu-

Box 1. Isotope-dilution method for estimating body composition of a live animal.

A known amount of isotopically labeled water (usually deuterium, ^2H, or tritium, ^3H, although ^{17}O and ^{18}O can be used) is administered orally or by injection into the animal. Administration of the labeled water is normally performed by intraperitoneal or intramuscular injection. Immediately after administration, the labeled water is localized near the site of injection and needs time to equilibrate with the entire body water pool of the animal. For animals provided no food or water, this equilibration period is only 15–30 minutes for small (<30 g) animals, between 30–60 minutes for larger animals such as quail and geese, and as long as 3–7 hours for bears and horses (Speakman et al. 2001). Provision of water and food during the equilibration period directly dilutes the body water pool, affects the isotope enrichment, and complicates estimation of body water pool size (Speakman et al. 2001). We suggest no food or water be provided to the animal during the relatively short equilibration period.

After the equilibration period, the body water pool is sampled most commonly by taking a blood sample, although urine, saliva, feces, and breath have also been used (Speakman et al. 2001). Blood samples (10–100 µL) from animals are typically collected in glass, flame-sealed capillary tubes and stored in the refrigerator. For studies that use deuterium or tritium-labeled water, blood samples are microdistilled under vacuum (Wood et al. 1975) prior to analysis. Tritium is analyzed by scintillation counting. Deuterium can be analyzed by infrared spectroscopy (Karasov et al. 1988) or isotope ratio mass spectrometry (Speakman et al. 2001). Estimates of body water using isotope dilution are used to predict body composition of the living animal as shown below for predicting fat mass of 2 species of bear (A) (from Hildebrand et al. 1998) and black-capped warblers (*Sylvia atricapilla*) (B) (from Karasov and Pinshow 1998).



tion technique has been used to predict lean and fat mass within 5 and 15%, respectively, of actual lean and fat mass in wildlife including bears, seals (Pinnipeda), and songbirds (Gessaman 1998, Speakman et al. 2001).

The primary advantage of isotope dilution for estimating lean and fat mass of wildlife is that it can be performed on a live animal that can then be released. Also, estimates of body composition are relatively accurate and precise, and can be used, in principle, on wildlife of any size and type. The primary disadvantages of this technique are that it is invasive (requiring blood sampling), requires some expertise to administer a quantitative amount of isotope in the field, and the laboratory analysis of the blood samples requires sophisticated equipment (e.g., infrared spectroscopy or isotope ratio mass spectrometry) although commercial laboratories conduct such analyses for a fee.

Electrical Conductivity and Bioelectrical Impedance

The conductivity of a live animal is directly related to the amount of dissolved electrolytes (primarily potassium, sodium, and chloride ions) in its tissues, with lean tissue containing the majority of body water and, thus, dissolved electrolytes. This means, in principle, that a measure of an animal's conductivity can be used to estimate amount of lean mass in the animal, as well as its' fat mass usually by subtraction of lean mass from total body mass.

Electrical conductivity can be measured between 2 discrete points on the surface of the animal [a technique known as bioelectrical impedance analysis (BIA)] or by placing the animal within a chamber and recording conductivity of the whole animal using a technique known as total body electrical conductivity (TOBEC®). Bioelectrical impedance analysis has been used primarily on humans (van Marken Lichtenbelt 2001) although it has been used for estimating body condition of large mammals such as elk (*Cervus elaphus*) (Cook et al. 2001) and bears (Farley and Robbins 1994, Hildebrand et al. 1998). Total body electrical conductivity has been used to estimate body composition of a diversity of wild animals including reptiles (Angilletta 1999), and many birds and mammals (e.g., Walsberg 1988, Zuercher et al. 1997, Scott et al. 2001).

Total body electrical conductivity instruments directly measure the energy absorbed by the animal while it is within the detection chamber. The instrument provides the user with a relative measure of the energy absorbed (an "E value") by the animal, which is proportional to the total body electrical conductivity of the animal. Estimation of lean or fat mass from the E value requires a calibration study. This involves recording the E value of a subset of live animals and directly measuring the body composition of these same animals using destructive methods. Regression models are then developed that relates TOBEC® E value to measured lean (or fat) mass for this subsample of animals. These models are used to predict lean (or fat) mass of individuals that were not used in the calibration study given their measured E value.

A number of factors will introduce substantial error in the TOBEC® measurement if they are not carefully controlled including size of the measurement chamber relative to size of the animal, positioning of the animal within the detection chamber, hydration state and body temperature of the animal, and fullness of the animal's digestive tract (Scott et al. 2001). With appropriate procedural controls, TOBEC® has been used to accurately predict lean mass (within 1–5% of actual lean mass) but not fat mass in a variety of wildlife species (Scott et al. 2001). Poor accuracy and precision of fat mass estimates occurs primarily because the absolute error (in grams of tissue) is the same for predicted lean and fat mass, and fat mass is a relatively small portion of whole body mass in animals.

Typically, species-specific calibrations have been used to estimate body composition using TOBEC® primarily because species often differ in size and shape which can greatly affect the accuracy and precision of the estimates (Scott et al. 1991, Asch and Roby 1995). Unfortunately, this limits use of TOBEC® to species for which a calibration study is feasible or has been done. Recent work with small migratory songbirds (10–30 g body mass) demonstrated that interspecific predictive models could be as accurate as intraspecific models (Whitman 2002), which may prove especially advantageous for studies of body composition of rare and endangered wildlife.

The primary advantages of TOBEC® for estimating lean mass of wildlife are that it is entirely noninvasive, measurements can be quickly made (<3 min), and the instrument is portable and easy to use in the field. In addition, TOBEC® is one of the few nondestructive techniques that can be used to measure short-term changes in body composition of the same individual(s) (e.g., Karasov and Pinshow 1998). The primary disadvantages of the technique are that it can only be used on certain wildlife (i.e., those that can fit into the detection chamber), separate validation studies must be conducted for each species or perhaps groups of species with similar body geometry, and it can reliably estimate only lean mass (not fat mass) of animals. Bioelectrical impedance analysis provides a potentially useful electrical conductivity technique for estimating body condition of wildlife that are too large to fit within TOBEC® detection chambers, although more validation studies are needed that evaluate accuracy and precision of BIA in field situations (e.g., Farley and Robbins 1994).

Ultrasound and DXA

Ultrasound is primarily used to estimate size of certain internal organs and fat depots rather than whole-animal body composition (Starck et al. 2001). Rump fat thickness measured using ultrasonography has been used to predict total body fat in ungulates (e.g., Stephenson et al. 2002), although such measurements become meaningless if animals have depleted these reserves (Cook et al. 2001). Baldassare et al. (1980) estimated fat depots in mallards (*Anas platyrhynchos*) using ultrasound, but could explain only 58, 65, and 59% of the variation in total body fat, subcutaneous fat, and omental fat, respectively, using this technique. Recent field-portable ultrasound models have been used to accurately measure the dynamics of pectoral muscle and stomach size in medium-sized shorebirds in relation to diet switching and migration chronology (Dietz et al. 1999). Starck et al. (2001) provide a discussion of the advantages and disadvantages of the technique and possible applications of ultrasound for studies of free-living wildlife.

Dual-energy X-ray absorptiometry (DXA) is a new technique (~10 years old) that is widely used for measuring bone density in humans. It has recently been adapted for measuring fat and bone-free lean mass in small (mouse- or sparrow-sized) animals using a "portable" 27 kg model and for dog-sized animals using larger models (Nagy 2001). The basic principle of the technique is that fat, bone-free lean tissue, and bone tissue attenuate photons produced by the instrument by different amounts; sophisticated software integrates this attenuation information from the scanned animal into an estimate of fat mass, bone-free lean mass, and bone mineral mass (and density). At present, scanning requires the animal be motionless (anaesthetized or dead); a scan requires 5–30+ minutes depending on resolution, and the instruments are relatively expensive (>$30,000 U.S.) (Nagy 2001). Recent validation studies have shown that DXA estimates of fat and lean mass were closely correlated ($r > 0.90$) with actual fat and lean mass measured by chemical extraction, and measurement precision was <10% (Nagy and Clair 2000). However, fat mass was consistently overestimated while lean mass was consistently underestimated (Nagy 2001). Application of this technique to most wildlife species awaits further technological innovations.

Blood and Urine Indices

Blood Indices

Blood characteristics have been used for decades as indicators of the nutritional status of wild animals. Reviews of their utility have been prepared by LeResche et al. (1974), Hanks (1981), and Franzmann (1985). LeResche et al. (1974) outlined steps required to identify relationships between nutritional status and blood characteristics. First, baseline values and ranges within boundary conditions need to be established for the species of interest. Next, major sources of variation (e.g., age, gender, season, time of day, handling procedures) that affect concentration of blood characteristics need to be identified. Finally, changes in blood values need to be tied to shifts in diet quality and animal condition when controlled for other sources of variation. Most research in this field has been conducted with large herbivores (Franzmann 1985) and carnivores (Franzmann and Schwartz 1988, Hellgren et al. 1989) in attempts to find indicators of the energetic and protein status of target species.

Blood urea nitrogen (BUN) is a key indicator of protein status in mammals. Urea, a nitrogenous compound, is a byproduct of protein metabolism and concentrations of BUN are positively related to protein intake when energy intake is above maintenance (reviewed by Harder and Kirkpatrick 1994). Blood urea nitrogen is relatively unaffected by stress of handling or by drug anesthesia. However, low-energy intake often leads to an increase in BUN concentrations as a result of protein catabolism to meet energetic requirements, and high-energy intake can lead to a depression of BUN concentrations due to more efficient use of protein (Harder and Kirkpatrick 1994). Hematological profiles (red blood cells, hemoglobin, hematocrit) also show promise as indicators of long-term protein and overall nutritional status (Franzmann 1985), although controlled experimental work tied to useful field applications are necessary to verify this promise.

Assessment of energy status or recent energy intake has been problematic. Several indices, including cholesterol, non-esterified fatty acids, and ketone bodies have been studied, but not found to be consistent or reliable indices across a range of energy intake levels (Franzmann 1985, Harder and Kirkpatrick 1994). Glucose concentrations are extremely sensitive to acute stress and virtually useless as a nutritional indicator. Triiodothyronine (T_3) was a consistent indicator of energy status in white-tailed deer in 60-day and 6-month feeding trials (Brown et al. 1995). Other researchers have reported similar findings with T_3 and thyroxine (T_4) (Bahnak et. al. 1981, DelGiudice et al. 1990, Watkins et al. 1991).

We reiterate the caution of Harder and Kirkpatrick (1994) regarding use of blood chemistries in nutritional research. All blood samples should be collected and handled as uniformly as possible. They should be taken at approximately the same time of day (due to diurnal rhythms in several physiological characteristics) and refrigerated as soon as possible after the sample is collected. If serum or plasma is collected for later analysis, samples should be centrifuged as soon as possible and frozen to prevent changes in blood values. Serum or plasma can be analyzed for individual characteristics with commercially available assay kits and basic laboratory equipment (e.g., spectrophotometry) or by commercial laboratories that use autoanalyzers to simultaneously analyze sample chemistries.

Managers considering use of blood variables to assess nutritional status should attempt to examine relationships between these variables and an independent measure of body composition, such as percent body fat. Gau and Case (1999) reported that individual parameters in blood were poorly related to percent body fat (estimated by bioelectrical impedance analysis) in grizzly bears (*Ursus arctos*) and advised against use of blood variables as indicators of nutritional condition. However, use of multivariate analyses, such as discriminant analysis, can be used to classify animals into broad nutritional groups. Hawley (1987) used blood variables to separate bison (*Bison bison*) into ration groups, and Brown et al. (1995) could discriminate among white-tailed deer on high- and low-protein or high- and low-energy diets. Blood chemistries are well suited for multivariate analyses because samples from individual animals are often analyzed for many variables.

Urine Indices

Nutritional indices based on analyses of urine collected in snow have been an expanding field of research in wildlife science. Urine indices have been tested and used primarily for ungulates in northern ecosystems, but there are some applications for carnivores. The primary advantage of urine indices is that large numbers of animals and multiple populations can be repeatedly sampled over the course of a winter in marked contrast to other techniques that typically are limited by the necessity to capture animals. Several urine metabolites have been evaluated as indicators of overall nutritional restriction, mobilization of energy reserves, digestible energy intake, food intake, or plant secondary compounds in diets. In all cases, metabolite concentrations are expressed as ratios with creatinine (C) to eliminate errors from dilution by snow. Creatinine is excreted at a relatively constant proportion to muscle mass (DelGiudice et al. 1995) and satisfactorily standardizes snow-diluted samples for all indices examined (DelGiudice et al. 1988, Saltz and Cook 1993, White et al. 1995).

Urea nitrogen (UN) has received the most in-depth evaluation. The UN:C ratio has a complex interpretation and is thought to be an index of overall nutritional restriction (DelGiudice 1995). The theoretical basis of the UN:C index for ungulates is that N content of diets is normally low in winter and N conservation by these species also keeps UN excretion low except when a combination of low food intake and reduced fat reserves causes substantial body protein catabolism and consequently elevated UN:C levels (DelGiudice 1995). DelGiudice et al. (1994) found a close relationship with the UN:C ratio and mass loss in captive deer, but relationships with fat reserves are less clear for wild deer apparently because the UN:C ratio is influenced by short-term food intake patterns (Parker et al. 1993*a*). Consequently, the UN:C ratio is interpreted as a generalized nutritional restriction index (DelGiudice 1995). Deer with ratios above 3.5 are considered in a state of serious nutritional restriction, and ratios of approximately 23 or more are indicative of animals approaching death from starvation (DelGiudice and Seal 1988, DelGiudice 1995). Controlled experiments with related species are limited, but similar relationships would be expected. It is recommended that data be reported as proportions of animals with ratios above 3.5 because proportions are simpler to interpret than mean values given the nonlinear relationship between nutritional restriction and UN:C (Ditchkoff and Servello 1999). Sample sizes of 20–35 per sampling period or unit are adequate for most applications, but sampling requirements are dependent on statistical approaches to be used (Ditchkoff and Servello 1999). Urea nitrogen:C in urine collected from snow also can be used as an index of fasting and recent feeding by gray wolves (*Canis lupus*). Wolves that had recently fed after fasting have sharply elevated UN:C ratios as a result of excretion of excess N from meat intake (DelGiudice et al. 1987*a*, Mech et al. 1987), but these authors caution that high UN:C also could result from wolves nearing death from starvation. Urea nitrogen:C likely is applicable to other carnivores in northern ecosystems and has been used to study the nutritional ecology of coyotes (*Canis latrans*) (Patterson et al. 2000); however, species-specific testing is recommended.

Allantoin (A) has good potential as a nutritional index for ungulates. Allantoin concentration in urine is associated with microbial biomass in the rumen and, with much of the energy and N absorption in ungulates a product of microbial fermentation, allantoin concentrations exhibit a positive relationship with intake of digestible energy (Vagnoni et al. 1996). Garrott et al. (1996) described A:C as an index of metabolizable energy intake. Field data from elk are consistent with expected winter patterns in food intake lending support to the value of this index (Garrott et al. 1996). A potential bias is that allantoin also may originate from endogenous sources as a result of loss of body mass (DelGiudice et al. 2000).

Cortisol:C has been evaluated as another general index of energetic status with ungulates (Saltz and White 1991, Saltz et al. 1992). Elevated cortisol:C indicates that energy reserves are being mobilized in response to an energy deficit, and Saltz et al. (1992) described the index as the rate of deterioration of animal condition. However, controlled studies on this index are limited and interpretation of field results may be complex (White et al. 1997).

Potassium:C is a potential index of food intake for ungulates (DelGiudice et al. 1987*b*). Potassium excretion is directly related to dietary intake, and K concentration in winter browses is relatively high (DelGiudice et al. 1987*b*, DelGiudice 1995). However, recent work has found that K:C may have a poor relationship with intake for deer eating mixed diets of browse (Servello and Schneider 2000), a potential problem for comparisons among populations. A second caution is that moribund deer may exhibit high excretion of K, another potential bias for this indicator (DelGiudice 1995). Nevertheless, K:C ratios in wild populations of deer have exhibited expected declining trends in winter (DelGiudice et al. 1989, Ditchkoff and Servello 2002). Potassium:C is recommended as supportive information to broader indexes of nutritional restriction (DelGiudice 1995).

Glucuronic acid (GA), a urine metabolite associated with excretion of secondary plant metabolites (McArthur et al. 1991), has been proposed as an indicator of diet quality for herbivores, i.e., dietary intake of anti-nutritional compounds (Servello and Schneider 2000). A problem with interpreting a GA:C index is that it also is affected by daily food intake because GA:C reflects total secondary plant metabolite intake. High GA:C values would indicate high intake of secondary metabolites, but low values could represent 1) low intake of food regardless of quality or 2) high intake of foods with low concentrations of secondary metabolites. A conservative approach of reporting the proportion of animals with high GA:C ratios (>6) is recommended (Servello and Schneider 2000).

A number of general recommendations have been made that apply to most or all urinary indices (Box 2). Finally,

Box 2. General recommendations for use of urine-based indices of nutritional status with ungulates.

1. Samples can be collected within 4 days after snowfall as long as ambient temperature remains below freezing (DelGiudice et al. 1988).
2. Sample sizes of 20–35 per sampling period are generally adequate, but the actual number required will depend on planned statistical approaches (Ditchkoff and Servello 1999, Pils et al. 1999).
3. Sampling schemes should be designed to avoid collecting >1 sample from an animal during a collection period.
4. There may be gender and age differences in urinary index data that may introduce biases in population-level results when examining temporal trends within or differences among populations (White et al. 1995, Pils et al. 1999).
5. Examine temporal trends in indices rather than rely on measurements for a single point in time (DelGiudice 1995).
6. Ancillary information on environmental factors or populations may be important for interpreting urinary index data (e.g., DelGiudice et al. 1989).
7. Use of multiple indices may strengthen conclusions because each measures a different aspect of nutritional status. Several indices can be measured in a single sample.

there has been a healthy discussion of the pros and cons of various urinary indices in the literature (e.g., Saltz et al. 1995) that is recommended reading for new users of these techniques.

Ptilochronology

Ptilochronology is a technique in which width of sequential dark and light bands on a bird's feather(s), called growth bars, are used to quantify daily growth rate of the feather. Feather growth rate is assumed to relate to nutritional status of the bird during feather growth (Grubb 1989). A standard protocol has been developed for measuring feather growth rate using growth bars of tail feathers (Grubb 1989). Because birds will replace tail feathers that are pulled out by predators or researchers, growth bars on an induced tail feather can be used to assess nutritional status of a bird during feather regrowth (~6 weeks) (Grubb 1995).

An early criticism of the technique was that feather growth rate seemed relatively insensitive to nutritional condition of the bird except when the bird was severely nutrient-limited (Murphy and King 1991, Murphy 1992). Recent studies have demonstrated that, at least in some songbird species, relatively mild nutrient-limitation can cause measurable declines in feather growth rate as estimated using growth bars (Grubb 1995, Jenkins et al. 2001). What remains to be demonstrated is whether the extent of under-nutrition consistently and quantitatively influences growth rates of feathers. Another difficulty with the technique is that growth bars are not sufficiently obvious in corresponding feathers of all individuals, for reasons yet unknown (Murphy 1992). In addition, feather growth bars are influenced by factors other than nutritional condition including gender and age of the bird, and season (e.g., Carrascal et al. 1998). Comparisons of feather growth bars in the original and induced feather on the same individual can help control for these factors, but requires assumptions about the nutritional status of the bird when the original feather was produced (Grubb 1992).

Ptilochronology appears to be a promising technique for nondestructively estimating nutritional status of birds during feather regrowth. However, widespread acceptance of the technique requires that we learn more about factors that influence feather growth rate and conditions that influence growth bars.

NUTRITIONAL ANALYSES OF FOODS

A common starting point for evaluating habitat from a nutritional perspective is to chemically or physically analyze available foods to assess their nutritional quality or value. The food constituents that might be measured include those that supply useful nutrients or energy, such as protein, fat, or soluble carbohydrates, and those that have negative effects on nutritional value, such as plant secondary metabolites. The number of nutritional variables that might be measured has increased greatly as we have learned more about the requirements of wildlife and factors that influence the nutritional value of foods. Broadly speaking, foods can be divided into 2 major fractions, or classes of chemically similar compounds. One is the easy to digest components such as protein, fats, sugars, and starches. These are sometimes categorized as soluble food components and are available to the animal via vertebrate enzyme and acid digestion. The complementary fraction is the variably digested and usually insoluble components, the fiber fraction in plants and bones, hair, feathers, etc. in carnivore prey. Some components of plants complicate the soluble versus insoluble (i.e., fiber) dichotomy. For example, some fiber compounds are soluble, and plant secondary metabolites, which may constitute significant proportions of plant biomass, may be misclassified as highly digestible fractions by some analyses. Although it is convenient to discuss food composition in terms of broad chemical fractions or classes of compound, more intensive analyses for individual nutrients or compounds may be desired.

Interpreting data on nutritional quality of foods can be complex.

1. *The importance of a particular nutrient or fraction differs among wildlife species.* For example, soluble fiber is undigestible for some species (e.g., bears, Pritchard and Robbins 1990), but is highly digestible by ruminant species. Or, 2 wildlife species may have different capabilities for reducing negative effects of plant secondary metabolites (McArthur et al. 1991), thereby mitigating the relative significance of that fraction. Unfortunately, despite improvements in techniques for measuring nutritional quality, there is still limited information on species-specific requirements and adaptations. Therefore, one must recognize that interpretation of nutritional quality of a given food is based on generally accepted relationships that have not been evaluated for all species or even all major species groups.
2. *The composition of a nutritional fraction is typically treated as if it is uniform even though it is not.* For example, 2 foods may have the same total fiber level suggesting equal nutritional value, but types of fiber and therefore digestibility, may differ substantially. Analogous problems occur with plant secondary metabolites because measured fractions have different arrays of compounds, each with unique nutritional effects.
3. *Analysis methods may not accurately measure a particular nutritional fraction in all foods.* Analysis methods theoretically divide food samples into fractions that have uniform chemical structure and nutritional characteristics. However, some analysis methods may fail in this regard, particularly when applied to plants with more complex chemistries than those used to develop the technique. For example, the sticky resins of conifer needles are not fats, but will be extracted as part of a fat analysis that uses organic solvents. One should be particularly careful when applying techniques to forages with unusual structure (e.g., ground lichens) that were not included in technique testing. Even given the above cautions, chemical analysis techniques provide a standardized and efficient approach for studying large numbers of foods. Methods for analyzing and quantifying nutritional value of wildlife foods have evolved greatly and, in the process, some methods have been discarded or changed as errors or problems were identified. While there is much useful information on nutritional value of wildlife foods from the 1940s to the present, data from early analyses should be re-evaluated based on our current knowledge of nutrition and method biases. For

example, the Proximate Analysis System (Scott et al. 1982), which was developed for agricultural crops, was used extensively in published reports on wildlife foods into the 1970–80s. Parts of this analysis system are valid and still used but some analyses have serious flaws.

Sample Collection and Preparation

Food samples collected for nutritional analyses need to be representative of those eaten by the wildlife species of interest. This can be a challenge because chemical composition of foods can be significantly affected by season, environmental conditions, plant size or growth form, and plant or animal part. For example, snowshoe hares (*Lepus americanus*) tend to avoid feeding on juvenile plants of paper birch (*Betula papyrifera*) because of higher secondary metabolite concentrations than in more mature stems (Reichardt et al. 1984). Similarly, decisions on plant parts to sample will be dependent on the wildlife species studied because the average bite size of herbivores may vary. For example, moose (*Alces alces*) browse twigs to a greater diameter-at-point-of-browsing than deer and, therefore, will eat twigs with lower average protein content because protein content of woody twigs increases from twig bases to tips (Moen 1985). For small species, like voles (*Microtus* spp.) that feed primarily on herbaceous vegetation, there may be even finer-scale selection for plant parts that may not be apparent to humans. Therefore, gross sampling of the above-ground vegetative parts of plants may not be representative of the foods actually eaten by small herbivores (Servello et al. 1984). Larger-scale sampling issues include seasonal and site-specific influences (e.g., shade vs. sun-lit areas) on the chemical composition of plants (Van Horne et al. 1988, Happe et al. 1990). For analyses of the prey of carnivores, decisions must be made about whether it is appropriate to include hair or feathers in samples. Overall, sampling designs for collecting foods for analyses must be based on knowledge of habitat selection, species of foods eaten, and foraging behavior of the wildlife species of interest. Study designs in Regelin et al. (1974), Schwartz et al. (1977), and Hobbs et al. (1983) are examples of intensive efforts to obtain representative samples. They attempted to duplicate food selection by observing tame animals as they fed and by simultaneously handpicking plants and plant parts at foraging sites.

The methods used to store and prepare samples for analyses can be critical for obtaining accurate results. Typically plant or prey samples need to be stored, dried, and ground or pulverized for analysis, although there are important exceptions with potential problems more common for plant tissue. Physical damage to leaves may affect measured concentrations of secondary plant metabolites and nutrients because compounds sequestered in vacuoles in leaves (Harborne 1991*b*) may interact with plant compounds when leaves are crushed (McLeod 1974, Swain 1979). Damaged leaves containing high concentrations of phenolics, a class of secondary plant metabolites, sometimes will develop a brown or black coloration indicating that oxidation of compounds has occurred (Ribereau-Gayon 1972). Crushing leaves also can cause release of volatile compounds (Mabry and Gill 1979).

Generally, collected plant samples should be kept cool after collection and processed as soon as possible. Even after plant leaves are picked, losses of sugars from respiration and enzymatic conversions of sugars to starches can occur (Smith 1973). These losses and changes can be reduced by cold storage. For accurate measurements of some nutritional variables, freezing is a necessity. For example, volatile terpene loss is reduced in collected samples by freezing immediately with dry ice or liquid nitrogen (Schwartz et al. 1980, Welch and McArthur 1981). Freezing fresh plant tissue is a common and useful method of long-term storage, but we caution that frozen-storage and subsequent thawing may cause changes in composition. It is more likely the freezing or thawing processes rather than storage causes the problems. For example, during the thawing process leaves with high concentrations of some secondary plant metabolites may develop a black coloration. The resulting new complexes of compounds may be mis-classified by some analyses (Mould and Robbins 1981*a*). Even dried plant samples will discolor over time at room temperature indicating that changes are occurring. Drying samples prior to freezing for storage should minimize problems. If samples will be dried by lypholization (i.e., freeze-drying) after frozen storage, the samples should be transferred to the lypholizer in a frozen state and not allowed to thaw.

Typically samples are dried for processing and analyses, but there are exceptions. Terpenoid fractions are extracted from fresh or fresh-frozen plant tissue because some terpene compounds are highly volatile (Mabry and Gill 1979, Personius et al. 1987). Extracts of fresh material can be used for analyses of plants that are highly affected by drying or storage methods but are awkward for analyzing large numbers of samples. If sample drying is required, it should be completed as soon as possible because chemical changes are less likely at low moisture levels. After drying, samples should be kept in individual airtight containers or a dessicator to prevent re-absorption of moisture from the air.

The choice of drying method may be critical. Oven drying can substantially alter measurements of chemical composition. Smith (1973) reported that oven-drying leaves below 50 C provides time for dry matter losses of nonstructural carbohydrates by respiration and enzymatic conversion of compounds. Drying above 80 C can result in thermochemical degradation, and drying above 50 C can cause nonenzymatic reactions among proteins and carbohydrates known as the browning or Maillard reaction. The latter causes analytical errors in fiber analyses (Van Soest 1965). Even at relatively low temperatures (40–60 C), oven drying can substantially decrease measured phenolic levels, a secondary plant metabolite, in plant tissue (Julkumen-Tiitto 1985, Servello et al. 1987, Nastis and Malechek 1988). Frozen storage and thawing before oven drying reduces phenolic concentrations more than oven-drying fresh material (Servello et al. 1987). Lyophilizing (freeze-drying) may be the mildest drying treatment because samples are held frozen during the drying process. It results in greater and likely more accurate values for some phenolic and fiber measurements (Servello et al. 1987, Nastis and Malechek 1988).

There is not one best drying method for plant samples. If extractions or analyses do not need to be done with fresh material, we recommend lyophilizing whenever possible, particularly when samples have been stored fresh-frozen.

If oven drying is the only practical option, we suggest drying at 40 C to minimize chemical changes. Most importantly, we suggest that researchers carefully review the potential effects of sample preparation on analytical methods used and foods being studied. We recommend lyophilizing for drying all animal tissue.

Most samples must be ground to a small particle size for analyses and for uniform sub-sampling. Generally, samples should be ground to pass a 0.5- or 1.0-mm screen, but larger tissues sizes may be acceptable for some analyses.

Food Quality Variables and Analytical Techniques

Water

Water content (%) is measured by weighing samples before and after oven drying at 100 C for 24 hours. We typically have little interest in the water content of foods *per se* although preformed water in food may be important for desert animals in meeting their water requirements. More commonly this procedure is used to measure the percent dry matter in samples that have been dried and ground for analysis. Percent dry matter is then used to express the data for other nutritional fractions on a dry-matter basis. Expressing all data on a dry matter basis allows comparisons among samples, nutritional fractions, and other studies.

Fat

Fats are the energy-rich tryglycerides and related compounds indicative of higher nutritional value. Crude fat content is measured by extracting a food sample with petroleum ether or diethyl ether, most commonly in a Soxhlet extraction device, which repeatedly washes the sample with ether (Maynard et al. 1979). The crude fat content is measured as percentage loss in dry weight of the sample after extraction. The estimate is referred to as crude fat because it measures all nonpolar compounds. It is also frequently referred to in the literature as the ether extract fraction, the name from the proximate analysis system. This method is generally satisfactory for measuring fat content in animal tissue. With plants, however, the crude fat estimate will include resins, waxes, and volatile oils and related compounds that have little or negative nutritional value. Seeds may be an exception in that they may contain significant concentrations of digestible oils. Fat data for plant foods should be interpreted with caution.

Protein

Protein provides building blocks in the form of amino acids and can be used for energy. Protein is generally highly digestible (>90%) by vertebrates (Robbins 1993). The crude protein content (%) of a food is estimated by measuring its nitrogen content and converting that to a crude estimate of protein based on the average proportion of nitrogen in protein. The commonly used Kjeldahl procedure for measuring nitrogen involves digesting the sample in H_2SO_4, neutralizing with NaOH, distilling the resulting ammonium, and titrating with acid (Church and Pond 1988). The estimated nitrogen content (%) expressed on a dry matter basis is then multiplied by 6.25 to calculate the crude protein content because, on average, protein contains 16% nitrogen (100/16 = 6.25). This fraction is referred to as crude protein because nitrogen occurs in compounds other than proteins. Crude protein may overestimate true protein content by as much as 22-52% in some plants (Sedinger 1984, Levey et al. 2000), but generally the nonprotein nitrogen fraction is considered small and ignored. Another caution in interpreting crude protein data is that some secondary plant metabolites may bind with plant proteins in an animal's digestive system, making a portion of the measured crude protein unavailable (Robbins et al. 1987*b*). The Kjeldahl procedure can be used with animal tissue (Fisher et al. 1992), but the protein content of animal tissue also can be measured indirectly using body composition analyses.

Fiber

In broad terms, the carbohydrate fraction of foods is comprised of soluble carbohydrates and fiber. Soluble carbohydrates are the completely digestible sugars and starches, whereas fiber is a complex of carbohydrates and other compounds that vary in digestibility. However, there is a soluble fiber fraction. Therefore, to measure fiber levels in wildlife foods and interpret this information, it is often important to know both the total fiber content and the relative amount of major fiber fractions. The detergent analysis system (Goering and Van Soest 1970) of measuring fiber fractions is recommended for herbivores capable of microbial digestion of fiber, whereas the total dietary fiber method (Prosky et al. 1984) is recommended for other species (Robbins 1993). Detergent analysis does not measure soluble fiber components, which is not a significant analytical error for species with microbial digestion, but may be important for other species especially if soluble fiber is a significant component of foods. In the detergent analysis system, a series of treatments is used to divide a food sample into various fractions. The principal division is to identify the proportions of highly digestible cell contents and variably digestible cell wall (i.e., fiber) fractions in a food sample using a detergent solution at neutral pH (Fig. 6). The division between cell contents and walls is not perfect; therefore, these fractions are more accurately referred to as neutral detergent solubles (NDS) and neutral

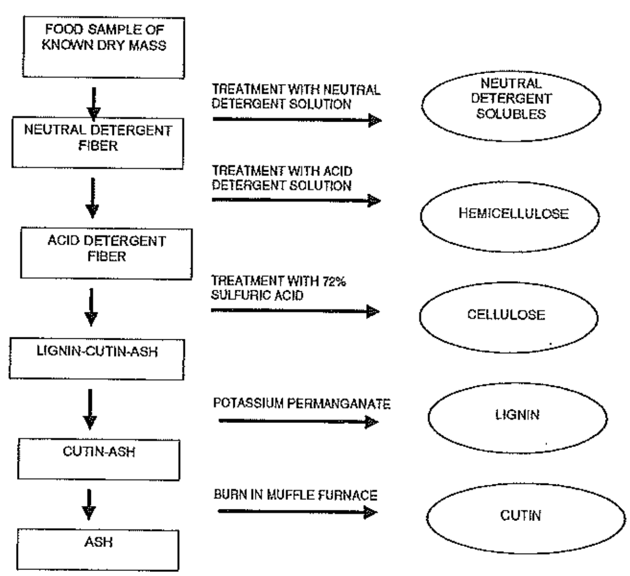


Fig. 6. Treatment steps and products from sequential detergent analysis of plant foods. Drying and weighing after each treatment gives the mass of each fraction remaining or removed. Rectangles are fractions remaining after analytical steps and ellipses are fractions removed.

detergent fiber (NDF) (Mould and Robbins 1981*a*). Theoretically, the magnitude of the soluble fraction is an indication of the proportion of readily digestible material in the food for both animals with simple stomachs and those that ferment foods. Additional steps are used to identify the composition of the insoluble fiber fraction. Treatment of the neutral detergent fiber with acid detergent theoretically removes hemicellulose and other minor compounds, which leaves a fraction called acid detergent fiber. It should be noted the hemicellulose fraction that is removed and measured by difference is poorly defined because it varies in composition and can be greatly influenced by relatively small analytical errors in the measurement of the larger NDF and ADF fractions. Acid detergent fiber is comprised of the major structural components of plants, cellulose and lignin, and is an important measure of forage quality. Subsequent treatment steps with either strong acids or oxidizers, depending on the method used, followed by burning in a furnace to measure ash content quantifies the proportions of cellulose and lignin in the food sample and ADF. Cutin, typically a small fraction, may be measured as part of the lignin fraction or may be measured separately. For mammalian herbivores, the amount of lignin-cutin in the ADF is the major factor affecting the digestibility of the total fiber fraction; digestibility of the ADF or total fiber fraction increases with a decreasing lignin content in the ADF (Mould and Robbins 1982).

Users should be aware of a number of modifications to the original system of detergent analyses and some potential biases. The original method used separate samples of foods for measurement of NDF and ADF. Currently, sequential measurement of NDF and ADF on the same sample is recommended (Mould and Robbins 1981*a*). It is also generally recommended that sodium sulfite not be used in the neutral detergent because it dissolved some lignin (Mould and Robbins (1981*a*), unless the forages contain tannins (Hanley et al. 1992). Also, treatment with amylase during neutral detergent extraction is recommended for foods with high soluble carbohydrate content to avoid overestimates of fiber concentrations (Robertson and Van Soest 1977).

The detergent analysis system was initially developed for analysis of agricultural crop species and analytical errors may occur when it is applied to the diverse array of plants eaten by wildlife. A common problem is that plant secondary metabolites will usually be extracted as part of the NDS. Therefore, the NDS fraction cannot be assumed to be highly digestible (or valuable) when plant secondary metabolites compose a substantial fraction of the dry matter in foods. Also, plants that do not primarily use a cellulose-lignin structure for physical support (e.g., ground lichens) may produce flawed detergent analysis data because relatively unique chemical components may be mis-classified.

The total dietary fiber method (Prosky et al. 1984) accounts for both insoluble and soluble fiber, such as pectins and gums, which may be undigestible by monogastric species (Robbins 1993). In this method, the food sample is first treated with an enzyme, and total dietary fiber is the percentage of the sample weight remaining after filtering and applying corrections for percent protein and percent ash in the sample. If the food has more than 5% fat, a preliminary fat extraction step is used.

Finally, there is a large body of published data on crude fiber concentrations in wildlife foods reported prior to 1980. Crude fiber is the fiber measurement from the outdated proximate analysis system (Scott et al. 1982), and is not appropriate for wildlife foods. Therefore, wildlife researchers should be cautious interpreting earlier published results.

Soluble Carbohydrates

It is not common to measure concentrations of sugars and starches in studies of wildlife foods for habitat evaluation. More typical is the measurement of neutral detergent solubles, which is the total fraction of highly digestible components (i.e., soluble carbohydrates, protein, fat). However, there are techniques for measuring soluble carbohydrates if desired (Smith 1973).

Ash

Ash is the mineral matter remaining after all water and organic matter are removed. Ash is measured by burning a sample in a muffle furnace at 500-600°C for at least 5 hours and weighing the residue. The ash content is expressed as a percentage of original dry matter. More detailed studies of specific minerals require further analysis of the ash content, usually by atomic absorption spectrophotometry.

Plant Secondary Metabolites

A number of types of plant secondary metabolites (PSM), also called allelochemicals, serve a role in defense against herbivory. Therefore, the concentrations of PSMs in wildlife foods are indicators of food quality. These metabolites are referred to as secondary because they do not have a primary role in plant growth or development (Gershenzon and Croteau 1991). Routine surveys of PSMs in wildlife foods are not commonly done as part of habitat assessments because laboratory analyses are complex. However, there is considerable effort to understand the significance of PSMs in foraging ecology of wild herbivores. This new information is greatly improving our understanding of habitat from a nutritional perspective. But, the widespread occurrence of PSMs greatly increases the complexity of habitat evaluation because 1) there are a large number of types of PSMs and specific compounds (Rosenthal and Berenbaum 1991), and the magnitude and types of negative effects on wildlife nutrition differ greatly among PSMs (Harborne 1991*a*); 2) herbivore species have varying adaptations, strategies, and capabilities for coping with intake of PSMs (Cork and Foley 1991, McArthur et al. 1991, Hagerman and Robbins 1993); and 3) PSM concentrations may be influenced by local environments (Bryant et al. 1991), plant growth stage (Connolly et al. 1980), browsing by herbivores (Bryant et al. 1992), and other factors (Jakubas et al. 1994). A comprehensive review of PSMs is beyond the scope of this chapter as is a review of analytical techniques because of the wide array of compounds and analyses. We recommend Palo and Robbins (1991) and Rosenthal and Berenbaum (1991) as excellent reviews on PSMs for wildlife biologists and suggest that anyone beginning work in this area undertake a detailed review of analytical techniques.

Phenolics are broadly categorized functionally and structurally as tannins or non-tannins. Tannins are large compounds (>500 molecular weight) that complex with and precipitate proteins in solution (Hagerman and Butler 1991). The smaller non-tannin phenolics include the common flavonoid pigments and other simple phenols (Harborne 1991*b*). Actual effects on herbivores may differ substantially among specific compounds within the above classes. Phenolic concentrations in plants vary seasonally, by stage of growth, and by plant part (Bryant 1981, Palo et al. 1985, Van Horne et al. 1988), and there is evidence that production of phenolic compounds by plants may be influenced by environmental conditions at a particular site and by browsing by herbivores (Bryant et al. 1991). Browsing damage also may influence plant growth rates and subsequent production of phenolic metabolites (Bryant et al. 1992). There are many studies of induction of PSM production, primarily by insects but also with vertebrate herbivores (Tallamy and Raupp 1991). All of these factors should be considered when designing field studies of PSM-wildlife interactions.

Tannin phenolics may reduce food intake, decrease protein digestibility, increase metabolic rates, alter digestive functions, alter acid-base balance with resulting effects on metabolic processes, or be toxic to wild herbivores (Buchsbaum et al. 1984, Lindroth and Batzli 1984, Smallwood and Peters 1986, Thomas et al. 1988, McArthur and Sanson 1993, Foley et al. 1995, Hewitt and Kirkpatrick 1997, Hewitt et al. 1999). Non-tannin phenolics, which may occur in relatively high concentrations in plants, also can reduce food intake (Lindroth and Batzli 1984) or can require significant energy expenditure for simply processing and eliminating these compounds after absorption (Lindroth and Batzli 1984). Actual effects on wildlife will depend not only on the nature of the specific tannin compounds, but also on adaptations of individual species for coping with phenolic ingestion. For example, some mammals have proline-rich saliva that reduces effects of tannin compounds by interfering with protein complexing (Foley and McArthur 1994). However, there is wide variation among species in capability to bind specific types of tannins, and these capabilities appear to be related to diet breadth (Hagerman and Robbins 1993). In addition, there is substantial variation among species in physiological strategies for detoxifying and excreting absorbed PSMs (McArthur et al. 1991, Robbins et al. 1991, Guglielmo et al. 1996). The metabolic oxidative system in animals includes several pathways for modifying or conjugating PSMs with other compounds to excrete them (McArthur et al. 1991). These physiological strategies may vary among species (Foley and McArthur 1994). Not only may these metabolic processes increase energy requirements (Guglielmo et al. 1996, Iason and Murray 1996), but also conjugation of large quantities of phenolics to other compounds for excretion may reduce pools of nutritionally valuable substrates (Illius and Jessop 1995). While there are general responses of herbivores to ingestion of phenolic compounds, the variety of compounds, effects, and adaptations suggest caution when evaluating the nutritional significance of PSM data for foods unless the ecology of the plant-herbivore interaction is well understood.

Phenolics are usually extracted from fresh or dried plant material with a polar solvent, usually methanol, acetone, ethanol, or ethyl acetate in an aqueous mixture (Martin and Martin 1982). Total phenolics are commonly assayed colorimetrically in plant extracts by the Folin-Ciocalteu procedure (Singleton and Rossi 1965). Total phenolic content provides a measure of a fraction of dry matter and gross energy in plants that is eaten, potentially absorbed and processed, and excreted without energetic gain (Foley 1992). However, total phenolic content has a poor relationship with protein-precipitating capability because it is a mixture of tannin and nontannin phenolics (Martin and Martin 1982). The total tannin fraction in plants is difficult to measure directly, and indirect measures that assay protein-binding capability appear to provide the most biologically meaningful indices of tannin effects because these methods mimic the reduction in protein digestion generally expected from tannins (Martin and Martin 1982, Mole and Waterman 1987). Precipitation of the protein, bovine serum albumin (BSA) (Hagerman and Butler 1978), has been used to quantify tannins in wildlife foods (e.g., McArthur et al. 1993). Hagerman (1987) also developed a relatively simple and inexpensive BSA precipitation method based on diffusion of tannin in a BSA-impregnated agar slab that is recommended for studies that require analyses of large numbers of samples (Hagerman and Butler 1991). These authors provide a good review of quantitative analyses for tannins. One should recognize that different protein-precipitating assays might not produce identical results (Martin and Martin 1982, Mole and Waterman 1987). In physiological studies of tannin fates in animals, different assays are used for measuring tannin concentrations or activity in biological samples such as saliva and urine (e.g., Austin et al. 1989, Hewitt and Kirkpatrick 1997).

Terpenoids are a large and abundant class of organic compounds in plants that are characterized by nonpolar carbon skeletons (Gershenszon and Croteau 1991). The major classes include the monoterpenoids, sesquiterpenoids, diterpenoids, and triterpenoids (Gershenszon and Croteau 1991) and are based on the number of 5-carbon units present (Harborne 1991*a*). Plant substances commonly referred to as essential oils, volatile oils, and resins contain terpenoid components (Nagy et al. 1964, Schwartz et al. 1980). Terpenes often function as feeding deterrents and the volatile nature of many makes them detectable by olfaction by mammalian herbivores (Epple et al. 1996). Therefore, food selection issues have been the focus of many wildlife studies on terpenes (Schwartz et al. 1980, Cluff et al. 1982, Personius et al. 1987, Duncan et al. 1994). Ingestion of terpenes also may decrease microbial digestion rates in herbivores (Schwartz et al. 1980, Cluff et al. 1982), alter acid-base homeostasis (Foley et al. 1995), can be toxic (Harborne 1991*a*), or increase water requirements (Dearing et al. 2002). The latter may be critical for species in arid environments (Dearing et al. 2002). As with phenolic compounds, absorbed terpenes may have to be metabolically modified for excretion (Dash 1988, McLean et al. 1993, Boyle et al. 2000) with a potential metabolic cost to the animal. These unusable compounds can represent a significant fraction of the gross energy in high-terpene forages (Foley and McArthur 1994). Detoxification of ingested terpenes by herbivores and its implications on feeding strategies is currently a major area of research in plant-herbivore interactions (e.g., Dearing et al. 2002). Because chromatography allows identification of many individual terpene compounds, these data are available in the literature for many plant species (e.g., Kamdem and Hanover 1993). However, interpretation of this detailed terpenoid information is difficult because of the paucity of parallel information on effects of individual compounds on wild herbivores. Analytical procedures are varied for this complex chemical class, but in wildlife studies terpenes are typically extracted by steam-distillation from fresh or fresh-frozen plant material because some compounds are highly volatile (Foley 1992, Personius et al. 1987), and quantification is done chromatographically (Gershenzon and Croteau 1991)

Gross Energy

The gross energy in a food is the total amount of energy released by complete oxidation and is expressed on a density basis as calories, kilocalories or joules per gram of food. The gross energy content of foods ranges from approximately 4 kcal/g for foods high in carbohydrates to about 9 kcal/g for foods high in fats (Church and Pond 1988:144). The proportion of useful energy for animals will vary among foods because some food constituents are more difficult to digest, and digestive capability varies among animal species. The gross energy content of food samples is routinely measured using adiabatic bomb calorimetry. Food samples are combusted in a high-pressure oxygen-filled chamber and released heat is measured in a surrounding water bath. Gessaman (1987) provided a good description of bomb calorimetry and sampling considerations.

MEASURING DIGESTION AND METABOLISM OF FOODS

Chemical or physical analyses of foods provide information on energy, nutrients, and anti-nutritional factors. Generally, one needs to measure digestion to understand the actual availability of nutrients and energy in a given food. Feeding trials of several types are widely used to measure amounts or proportions of nutrients and energy in foods that can be extracted by wildlife species. The general approach involves quantitatively measuring intake and excretion of dry matter, nutrients, or energy for a number of individual animals over several days. Feeding trials cannot be routinely done to survey food quality in habitats because this requires maintenance of experimental animals and laborious collections of test foods. A widely used alternative to feeding trials, particularly with ruminant species, is in-vitro digestion where food samples are subjected to a laboratory process that mimics the fermentation and digestive environment in the animal. This approach allows evaluation of a relatively large number of food samples, but has limitations. Considerable effort in the wildlife nutrition field has focused on understanding relationships between proportions of usable energy and nutrients measured in feeding trials, and food quality variables that can be measured via chemical analyses. These relationships allow indirect assessments of digestible energy and nutrients in foods, and provide a more time- and cost-efficient approach when large numbers of food samples must be measured for habitat evaluations.

The terminology used to describe digestion and metabolism of foods by animals is complex and not consistent. We use the term digestibility to refer to the proportion of dry matter or energy (kilocalories or joules) in the food eaten that is absorbed across the gut wall of the animal (i.e., not excreted in feces). Metabolizability refers to the proportion of dry matter or energy eaten that is retained for metabolic use by the animal (i.e., not excreted in feces, urine or as a gas). These variables are usually expressed more specifically as digestible dry matter (DDM), digestible energy (DE), or metabolizable energy (ME). When these variables are used to describe digestive or metabolic efficiency of an animal, it is more appropriate to express them on a proportion or percentage basis. In contrast, when these variables are used to describe quality of a food it is usually more appropriate to express them as a density function such as DE (kcal or joules) per gram of food dry matter. This accounts for foods that have varying gross energy or nutrient content. For example, 2 plant species eaten by a desert tortoise (*Gopherus agassizii*) may have the same proportions of digestible energy extracted, but one may have a considerably greater gross energy content. The food with the greater amount of gross energy will contribute more DE per gram eaten than that with a lower value.

The term, assimilation, is frequently used in the ecological literature, e.g., assimilated energy. Whether authors are using assimilation as a substitute for digestibility or metabolizability may not be clear, and one has to examine the methods and measurement units to identify the specific variable being reported. Also, only metabolizability is reported for birds because feces and urine cannot be easily separated for measurement, precluding calculations of digestibility. Digestion and metabolism values will sometimes be labeled as apparent or true values. Standard measurements from feeding trials produce "apparent" values because some fecal and urinary dry matter originates from the animal's tissues and not the test food. These endogenous losses include digestive enzymes, gastrointestinal epithelial cells, microbes, and excreted end products of metabolism (Maynard et al. 1979). True digestibility or metabolism values are calculated from the amounts of endogenous dry matter, energy, or nutrients in the excreta and then correcting the apparent values. However, when food intake is sufficiently high to maintain body mass, apparent and true values for waterfowl vary only by an average of 3 percentage units (Miller and Reinecke 1984).

Metabolizable energy values for foods are sometimes calculated as "nitrogen-corrected" because individual animals losing or gaining weight during a feeding trial will vary in the amount of endogenous urinary nitrogen in their excreta. Making this correction provides a more accurate measure of the rate of metabolism of the food. In practice though, nitrogen-corrected ME values usually differ little from uncorrected values (e.g., Beckerton and Middleton 1982, Sibbald and Morse 1982).

Feeding Trial Methods

The total collection feeding trial is the most common method for measuring digestive capability of animals, and digestibility and metabolizability of energy and nutrients in foods. The method for measuring true metabolizable energy in birds and techniques that use indigestible markers are variations of the total collection method, but unique enough for separate treatment.

Total Collection Method

The basic approach is to feed known amounts of a test

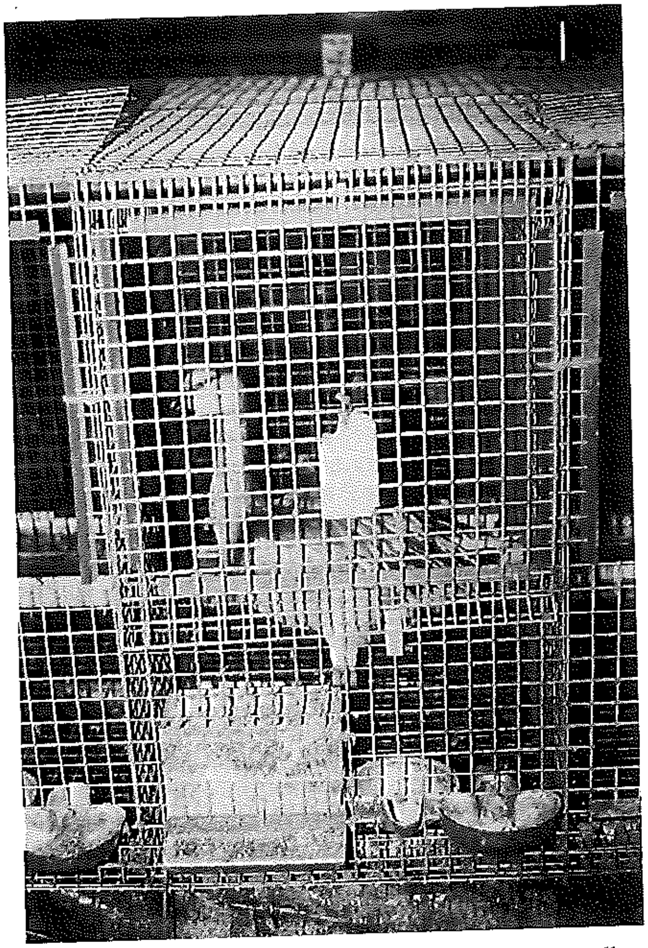


Fig. 7. Ten-week old snow goose (*Chen caerulescens*) in a total collection digestibility trial. Food intake and fecal production are quantitatively measured on a daily basis. Digestibility of the diet or components of the diet (e.g., fiber) are then estimated given measured intake and excretion rates.

food to a number of individual animals over a number of days. The food eaten is measured and the excreta produced is collected and weighed (Fig. 7). Hydrochloric acid or H_2SO_4 is added to urine collection bottles to prevent ammonia loss. Concentrations of energy or other nutrients in food consumed and excreta produced are used in calculations of digestibility or metabolizability. Typically an animal is fed a single food or diet, but there are techniques for measuring digestibility of single foods fed in mixed diets. Feeding trials are typically conducted in cages designed for separation and collection of food, feces, and urine. For species not using foregut fermentation, 3–6-day acclimation periods to test diets and 3–6-day collection periods are commonly used (e.g., Robel et al. 1979, Campbell and MacAuthur 1996), but longer periods may be more appropriate for some species. For ruminants and other foregut fomenters, 7–10-day acclimation periods and 7-day collection periods are commonly used. Mothershead et al. (1972) reported that a 10-day collection period was most accurate for white-tailed deer, but 7 days was not much of a disadvantage.

Total collection trials have been conducted with a wide array of wildlife species, and descriptions of digestion cages or crates, procedures, and additional helpful information for many species are available in methods and materials sections in published reports. Significant modifications of the basic approach are common with reptiles. It is a common practice to force-feed lizards and turtles when there is difficulty achieving satisfactory intake (Troyer 1984, Karasov et al. 1986*a*, Nagy and Medica 1986). Because of long food transit times in turtles, indigestible plastic markers have been used to demark a period of food intake and the resulting pulse of feces for measuring digestibility (Meienberger et al. 1993, Nagy et al. 1998). Reptiles must be allowed to bask or cage temperature must be regulated (Bjorndal 1991) because body temperature can influence digestive functions as well as metabolic rate and, therefore, food intake. For carnivores, it may be necessary to allow time for gut volume changes that may occur naturally in response to prey scarcity or abundance. For example, Harlow (1981) found that American badgers (*Taxidea taxus*) that had been fasted before the collection period had a slower food passage rate and metabolized 11% more of the energy in food than animals fed on a daily basis prior to the trial. Welch et al. (1997) used a long acclimation period with bears to ensure their digestive tracts had expanded to maximum capacity. Providing long acclimation periods or appropriate maintenance diets also may be important with herbivorous birds, such as grouse (Tetraoninae) and waterfowl (Anseriformes) as well as migratory landbirds, because their gizzards, intestines, or ceca change in size in response to diet (Moss 1973, McWilliams and Karasov 2001). The total collection method may be impractical for some species or situations, such as aquatic turtles (Bjorndal and Bolten 1993) and some seals (Goodman-Lowe et al. 1999).

Experimental animals must be acclimated or trained for handling and confinement in metabolism cages. In some cases, particularly with small species, wild-captured animals can be used if individuals will adapt to confinement. Experimental animals are frequently reared in captivity; however, even captive-raised animals must be acclimated to metabolic cages. For example, Mautz (1971) observed that food intake decreased with some captive-raised deer after confinement, and it required 9–12 days to return to pre-confinement levels. Training animals to accept confinement also lowers the risk of accidental injury to the animal and researchers. The number of animals used for an individual measurement of digestion or metabolism rates is variable, but 4–6 animals per trial is common. Mothershead et al. (1972) suggested that 5 deer per test diet were adequate for most digestion trials. If the animals are healthy and eating normally, standard errors for DDM, DE, and ME estimates are usually within acceptable limits with these sample sizes.

Maintaining regular and adequate food intake is important for accurate results. Low and variable food intake may increase data variability because relatively constant endogenous excretion increases error as food intake decreases (Miller and Reinecke 1984). Animals should be fed at the same time each day. Test diets are often fed *ad libitum,* but constant intake can be assured and selective feeding can be reduced by feeding at a slight reduction (e.g., 90% *ad libitum*). Chopping or pelleting plant material or homogenizing animal tissue often is used to prevent selective feeding; however, these treatments may influence digestion or feeding behavior in other ways (Petrie et al. 1997). In unusual circumstances, food might be force-fed to circumvent problems with low or variable intake and food selection such as with turtles (Meienberger et al. 1993). Access to grit may be necessary for herbivorous birds in metabolism trials, but studies have produced mixed conclusions (Robel and Bisset 1979, Petrie et al. 1997).

Digestion and metabolism rates are easily calculated (Box 3). Apparent dry matter digestibility is the proportion or percentage of food eaten on a dry basis that was not excreted in feces. The apparent digestibility of a specific food component (e.g., fiber, N) is calculated as the proportion of the nutrient consumed in the food that was digested. Similarly, apparent digestible energy (%) is calculated by measuring the gross energy in the food and feces and converting dry-matter mass to energy equivalents. To transform percent digestible energy values into energy densities (DE/g dry matter) for a food, the proportion of digestible energy is multiplied by the gross energy (kcal or joules/g) of the food. Apparent metabolizable energy is calculated by subtracting urinary and gaseous energy losses. The loss of gross energy as gas (primarily methane) production is sufficiently low that it is often ignored. Nitrogen-corrected metabolizable energy is calculated as: deviations from nitrogen balance (gains or losses of nitrogen) are corrected by adding to or subtracting from the excreta energy value the energy content of urinary nitrogen. Equivalents for these calculations are 8.22 kcal/g of nitrogen for birds (Scott et al. 1982:537) and 7.45 kcal/g (Maynard et al. 1979:196) for each gram of nitrogen retained or lost.

Some forages are too unpalatable or too low or high in nutritional quality to be fed singly in a total collection trial. An alternative approach is to mix test forages with a more palatable basal diet (often a commercial feed) and to measure the digestibility of both mixed and basal diets in total collection trials. The digestible dry matter of the test food can be calculated by difference from these data with the formula:

$$DDM_f = \frac{DDM_m - (DDM_b \times Proportion_b)}{Proportion_f},$$

where

DDM = digestible dry matter of foods or diets in percent,

Proportion = proportion of test food or basal diet in the mixed diet, and

f = test food,

b = basal diet, and

m= mixed diet.

It is assumed the basal diet has no effect on the digestibility of the test food, but associative effects can occur (Bjorndal 1991).

True Metabolizable Energy Method for Birds

Sibbald (1976, 1979) developed a method for measuring the true metabolizable energy (TME) in foods for poultry, which has been applied to wild avian species, primarily waterfowl (e.g., Petrie et al. 1998). The TME method is relatively fast and requires less of the test food than a conventional feeding trial. Birds are first fasted for 24–48 hours and then force-fed a small amount of food in a period of several minutes. Feces and urine are then collected in metabolism cages over the next 24-48 hours. Control birds are treated similarly except they are not fed; experimental birds can serve as their own controls in sequential trials. The excreta of control birds is collected and

Box 3. Equations for calculating dry matter digestibility (DDM), digestible energy (DE), digestibility of a specific nutrient, and metabolizable energy (ME) using data from digestion or metabolism trials. All equations calculate apparent values.

$$\text{DDM (\%)} = \frac{\text{Food intake}^{a} - \text{Fecal dry matter}^{a}}{\text{Food intake}} \times 100$$

$$\text{DE (\%)} = \frac{(\text{Food intake} \times \text{GE food}^{a}) - (\text{Fecal dry matter} \times \text{GE feces})}{(\text{Food intake} \times \text{GE food})} \times 100$$

Digestibility (%) of nutrient A

$$= \frac{(\text{Food intake} \times \%\text{ A in food}) - (\text{Fecal dry matter} \times \%\text{ A in feces})}{(\text{Food intake} \times \%\text{ A in food})} \times 100$$

$$\text{ME (\%)} = \frac{\text{GE intake} - (\text{Fecal GE} + \text{Urinary GE} + \text{Gaseous GE})^{c}}{\text{GE intake}} \times 100$$

[a] Food intake and fecal dry matter measured in grams or kilograms.

[b] Gross energy in kilocalories or kilojoules per gram dry matter.

[c] GE intake, fecal GE, etc. are calculated by multiplying dry matter eaten or excreted by the respective GE concentration. Gaseous GE loss is usually ignored.

weighed to estimate metabolic and endogenous dry matter and energy losses. The TME of the test food is calculated by subtracting the endogenous and metabolic energy losses of the control birds (C) from the fecal and urinary energy of the force-fed birds (F) as: TME (kcal or joules/g) = ([GE intake$_f$ – (EE$_f$ – EE$_c$)]/GE intake$_f$) × GE of test food, where GE intake$_f$ is the gross energy (kcal or joules) of the test food that was force-fed, EE$_f$ is the energy in the excreta from the fed bird (kcal or joules/g), and EE$_c$ is the energy in the excreta of the control bird (Guglielmo and Karasov 1993). This TME method has not been extensively tested against standard total collection trials with a variety of wild avian species.

Guglielmo and Karasov (1993) provide an alternative approach for estimating endogenous mass and energy losses in wild birds that does not require fasting (which is known to affect gut structure and function in birds, McWilliams and Karasov 2001). The method involves conducting a series of 3-day total collection feeding trials while feeding animals the food of interest over a range of fairly natural levels of food intake. Nitrogen-corrected endogenous mass and energy losses are then estimated using regression techniques.

Indicator Methods

For some wildlife species, measuring food intake and collecting all excreta for several days in conventional feeding trials may be impractical. This is the case for seals and turtles that periodically need to be in water. Indicator methods offer an alternative approach for measuring digestibility. An indigestible indicator is added to the test food and is measured in samples of food and feces during a subsequent feeding period. An accounting of total intake and excretion is not required. Percent digestible dry matter is calculated as:

$$\text{Digestible dry matter} = 1 - \frac{\%\ \text{indicator in food}}{\%\ \text{indicator in feces}} \times 100\%.$$

This method assumes the indicator is indigestible or is not changed in the digestive tract and that it mixes and moves uniformly with food. Chromic oxide has been used frequently (e.g., Goodman-Lowe et al. 1999), but other indicators have been used including uniquely-labeled radioactive or stable isotopes (Gasaway et al. 1976, Karasov et al. 1986*b*, McWilliams et al. 1999). Goodman-Lowe et al. (1999) provide a good example of the application of this technique with seals and describe calculations in detail.

In Vitro Methods

In vitro methods measure food digestibility by mimicking fermentation and digestive processes of ruminants and other foregut-fermenting species. In vitro digestion only requires small amounts of the test food samples and, therefore, is a more efficient method for evaluating large numbers of foods. The disadvantages of this technique are that animals must be available for the collection of rumen fluid, and a number of potential biases may influence results. The basic approach was developed by Tilley and Terry (1963) and involves inoculating a sample of food with rumen fluid and a buffer solution designed to simulate saliva. The fermenting sample is first maintained in a hot water bath for 48 hours. In a second stage, the fermented sample is treated with pepsin and mild acid. Modifications of this method include addition of a phosphate-carbonate buffer to reduce foaming and a reduced ratio of rumen fluid to buffer solution (Campa et al. 1984) or substituting the second stage of pepsin and mild acid with a neutral detergent extraction (Van Soest 1982).

Although the in vitro method is an efficient method for measuring digestibility, there are a number of potential sources of bias. There are conflicting reports on whether source of rumen fluid (e.g., donor species, wild vs. captive animals) can significantly influence digestibility estimates (Palmer et al. 1976, Welch et al. 1983, Campa et al. 1984, Jenks and Leslie 1988), but it seems clear the source can have an effect in some instances. Nastis and Malechek (1988) also found the donor animal's diet can have a substantial effect on estimates and that other additional bias may interact to produce highly variable results. Therefore, with wild herbivores, in vitro techniques are more appropriate for ranking food quality than predicting actual digestibility (Campa et al. 1984).

ASSESSING DIETS, DIET QUALITY, FOOD INTAKE, AND CARRYING CAPACITY

Ultimately the goal of wildlife nutrition research is to evaluate and understand the biochemical and biophysical relationships between animals and their environment, and how these relationships affect survival and reproduction of individuals and populations (Robbins 1993). We have largely discussed nutritional techniques and methods that occur in the laboratory or captive animal facilities in a reductionist, compartmentalized fashion (Parker et al. 1999). Several methods have been used to study the nutritional ecology of free-ranging animals and populations. These methods generally have been used to avoid assumptions, inadequacies, or difficulties of captive animal methods. For example, feeding trial data have limited value for estimating energy requirements of free-ranging animals. Also, analyzing or experimenting with all combinations of individual foods in the varied diets of some species can be impractical. Therefore, techniques that can provide information on natural diets selected by the animal are of value.

Indicators of Diet Composition

Stable Isotope Methods

The analysis of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) has been applied to a number of questions in ecology (Tieszen and Boutton 1989, Kelly 2000). In the area of nutritional ecology of wildlife, applications include using stable isotope ratios to reconstruct diets, assess physiological condition, and learn the fate of assimilated nutrients in individual animals (Gannes et al. 1997). Although stable isotope data cannot replace the detail provided by traditional dietary analyses, they have other advantages relative to understanding the trophic ecology of wild species.

Carbon, nitrogen, oxygen, and hydrogen, as well as other elements, have more than one naturally occurring isotope (Peterson and Fry 1987). Stable isotope data are expressed in the notation:

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

where R is the ratio of heavy to light isotopes (e.g., $^{13}C/^{12}C$) and δ is the isotope ratio of a sample relative to a standard in units of parts per thousand or per mil (‰) (Kelly 2000). Standards for carbon and nitrogen are Peedee Belemite limestone and atmospheric nitrogen, respectively. Most plant and animal tissues have a negative value of $\delta^{13}C$ and a positive value of $\delta^{15}N$, meaning a lower $^{13}C/^{12}C$ ratio and a higher $^{15}N/^{14}N$ ratio than the standards. Samples are analyzed after combustion using mass spectrometry. Several types of tissue can be used for isotopic analysis, including whole blood, plasma, liver, muscle, bone collagen, hair, and feathers.

The value of stable isotopes of carbon in animal ecology arises from the different isotope ratios of carbon fixed by terrestrial C_3, terrestrial C_4, and marine (C_3) plants. The photosynthetic pathways of the former 2 types of plants use different CO_2-fixing enzymes and lead to different $\delta^{13}C$ values (range of –10 to –14 ‰ vs. a range of –25 to –30 ‰). Marine plants have intermediate $\delta^{13}C$ values (–22 ‰) (Kelly 2000). Experimental studies in birds and mammals have demonstrated the carbon isotope ratio of animal tissues is a direct reflection of the carbon isotope ratio of plant tissues assimilated (not merely eaten) by that animal (DeNiro and Epstein 1978; Hobson and Clark 1992*a*, *b*). Therefore, animal tissue samples can be used to assess the relative importance of different groups of plants in dietary assimilation. For example, because warm-season grasses are typically C_4 plants, and forbs, shrubs, and cool-season grasses are C_3 plants, browsing and grazing herbivores can be distinguished in regions where C_4 grasslands dominate (Kelly 2000). In turn, carbon isotope values of tissues of carnivores are reflective of the carbon isotope ratios of their prey. Carbon-isotopic turnover also varies by tissue type, ranging from 2.6 days in liver to 173 days in bone collagen in Japanese quail (*Coturnix japonica*) (Hobson and Clark 1992*a*), allowing researchers to monitor temporal changes in the assimilated diet by measuring stable-isotope values in different tissues (Tieszen et al. 1983).

Nitrogen-isotope ratios of animal tissues are particularly important for supplementing food web and dietary studies. The review of Kelly (2000) summarized 3 conclusions about nitrogen-isotope studies: a 3–4 ‰ enrichment in $\delta^{15}N$ occurs with each trophic level; nitrogen-isotope ratios are useful for distinguishing among diets based on marine, terrestrial or nitrogen-fixing plants; and nutritional and water stress can cause variation in $\delta^{15}N$ levels.

Gannes et al. (1997) discussed several assumptions of the technique upon which inferences are based and recommended additional controlled experiments to understand the behavior of stable-isotope ratios. For example, using isotope data to identify the relative contribution of different food items to an animal's diet depends on the assumption that isotopic composition of the animal's tissues equals the weighted average of the isotopic components of the diet. However, this assumption usually fails because animals assimilate diet components with different efficiencies, animal tissues fractionate or change the isotope ratios from the ratios in the diet, and animals allocate diet nutrients differentially to specific tissues (Gannes et al. 1997). For example, Hobson and Clark (1992*b*) found that tissues in several bird species were enriched in $\delta^{13}C$ and $\delta^{15}N$ relative to their diet but this fractionation varied by species, diet, and tissue type. We recommend that researchers interested in using stable-isotope techniques consult with those already active in the field, refer to reviews of the subject to recognize the varied applications of these data to ecological questions (Peterson and Fry 1987, Tieszen and Boutton 1989, Kelly 2000), and realize the limitations and assumptions involved (Gannes et al. 1997).

Fatty Acid Analysis Methods

Fatty acid profiles of animal tissues provide an alternative, powerful approach for studying important aspects of the nutritional ecology of wildlife. These techniques take advantage of unique patterns of fatty acid composition in prey that reflect those of their local food webs and which are retained as they are transferred up the food chain by predation (Iverson 1993, Kelly 2000). For example, lipids in milk and storage tissue of marine mammals are characterized by an unusual array of fatty acids that are largely of ecological origin (Ratnayake et al. 1989, Smith et al. 1996) and which have been used to detect shifts in diet during breeding and nonbreeding periods (Iverson et al. 1997). Fatty acid profiles in milk of black bear (*Ursus americanus*) were derived mostly from endogenous fat stores, which presumably reflected the diet of the animals before the period of winter dormancy (Iverson and Oftedal 1992).

Assessing Diet Quality of Free-ranging Wildlife

Combining Food Habits and Diet Quality Data

A commonly used method for estimating quality of natural diets is to mathematically combine food habits data (percentages of foods in diets) with digestibility or nutrient data from nutritional analyses and feeding trials (e.g., Schwartz et al. 1977, Hobbs et al. 1982, Leslie and Starkey 1985). Composite diet quality for a given nutrient is calculated as:

$$\sum_{i}^{n} x_i y_i,$$

where x_i is the proportion of food item i in the diet and y_i represents the nutrient value of food item i. This method assumes hand-collected forages for analyses are representative of forages selected by the animal and food habits analyses are unbiased.

Equations for predicting digestible or metabolizable energy content and digestible protein of forages from chemical composition (Table 1) are available for white-tailed deer and elk (Mould and Robbins 1982; Robbins et al. 1987*a*, *b*), ruffed grouse (Servello et al. 1987*a*, *b*), black bear (Pritchard and Robbins 1989) and voles (*Microtus pinetorum*, *M. pennsylvanicus*) (Servello et al. 1983, MacPherson et al. 1985). With only a small amount of forage needed for chemical analyses (compared to that needed for feeding trials), estimates of DE and ME values can be obtained for a large number of forages and for specific plant parts in all seasons or under specific environmental conditions.

We caution that tests of these equations are necessary to validate their generality. Hanley et al. (1992) validated equations for predicting digestible protein and dry matter developed from feeding trials using white-tailed and mule deer by conducting digestion trials with an independent set of forages and black-tailed deer. Conversely, Guglielmo and Karasov (1995) found that equations predicting metabolizable energy from leaf and fruit foods of ruffed grouse

(Servello et al. 1987*b*) consistently overestimated metabolizability of winter grouse browse. The value of predictive regressions to refine evaluations of diet and habitat quality is unquestioned. However, the approach requires further assessment.

Analyses of Gastrointestinal Tract Contents

Analysis of gut contents from free-ranging animals is one step closer than forage analysis to measuring actual nutrient intake because foods and food parts actually selected by the animal can be analyzed. For example, Servello et al. (1984) and MacPherson et al. (1988) measured digestible energy in diets of wild voles using equations to predict digestible energy from a chemical analysis of stomach contents. Dietary ME for ruffed grouse can be predicted similarly from chemical analyses of crop contents (Servello and Kirkpatrick 1987*b*). Similar efforts were made to measure nutrient content in deer rumen contents (Kirkpatrick et al. 1969). These methods eliminate the bias between hand-picked samples and those selected by the animal. However, there is an assumption that the plant material is not substantially altered in the animal before collection by chemical or enzymatic reactions. This assumption does not appear to be a significant problem with voles (Servello et al. 1983), which are monogastric, or ruffed grouse (Servello and Kirkpatrick 1987*b*), which store food in the crop, but is likely a problem with ruminants. Another limitation is that destructive sampling is required to obtain the sample.

Indicator Techniques

Variations of the indicator techniques described for feeding trials have been applied to wild populations to measure digestibility of natural diets. Indicators must be naturally present in the food or prey, must not be digested or absorbed, and it must be possible to collect feces from wild individuals for analyses. For example, magnesium has been used with grouse (Moss 1973) and manganese has potential for use with northern fur seals (*Callorhinus ursinus*) (Fadely et al. 1990).

Fecal Indices of Diet Quality

Fecal nitrogen concentration has been proposed as an index of diet quality (Kie and Burton 1984, Leslie and Starkey 1985); however, there is considerable disagreement on its usefulness (Hobbs 1987, Leslie and Starkey 1987). Robbins et al. (1987*a*) reported high dietary tannin levels increased fecal nitrogen, which can lead to inaccurate conclusions. Recently, Barten et al. (2001) used fecal nitrogen as an index of dietary quality to test hypotheses about habitat-use tradeoffs by female caribou (*Rangifer tarandus*). Another proposed fecal index is DAPA, 2,6

Table 1. Equations for predicting the percent apparent digestible dry matter (DDM), digestible protein (DP; g/100g dry food), digestible energy (DE), metabolizable energy (ME), in diets from chemical analyses of foods.

Species	Foods	Equations[a]	Source
Elk	Forages, diets[b]	$DDM = 1.11\ NDS - 21.88 + NDF \dfrac{(176.92 - 40.50 \text{ Log } e^{x})}{100}$	Mould and Robbins 1982
White-tailed and mule deer[c]	Forages, diets	$DDM = [0.923e^{-0.0451x} - 0.03z](NDF) + [(-16.03 + 1.02\ NDS) - 2.8\ P]$ where: $P = -0.01 + (11.82\ BSA\ precipitation)$	Robbins et al. 1987*a*
		$DP = -3.87 + 0.928\ (\%\ crude\ protein) - 11.82\ BSA\ precipitation$	Robbins et al. 1987*b*
Ruffed grouse	Diets, foods, or crop[d]	$ME = 0.87\ (NDS - total\ phenolics) - 5.76$	Servello et al. 1987*b*
Black bear	Diets (plant foods)	$DP = -3.46 + 0.881\ (\%\ crude\ protein)$	
	Diets (whole animals)	$DP = -9.77 + 1.01\ (\%\ crude\ protein)$	
	Diets (meat and fish)	$DP = -3.82 + 1.01\ (\%\ crude\ protein)$	
	Diets	$DDM = 101.3 - 1.39\ (TDF)$	Pritchard and Robbins 1990
Pine vole	Diets, foods	$DDM = 1.18\ NDS - 19.42$ $DE = 1.12\ NDS - 14.31$	Servello et al. 1983
	Stomach contents	$DDM = 1.14\ AFNDS - 14.89$ $DE = 1.07\ AFNDS - 8.50$	
Meadow vole	Diets, foods	$DDM = 1.09\ NDS - 11.12$ $DE = 1.09\ NDS - 11.84$	MacPherson et al. 1985
	Stomach contents	$DDM = 1.08\ AFNDS - 1.30$ $DE = 1.07\ AFNDS - 1.60$	

[a] Chemical composition abbreviations: NDS = neutral detergent solubles; NDF = neutral detergent fiber; AFNDS = acid-insoluble, ash-free neutral detergent solubles; x = lignin and cutin content as a percent of the NDF; z = biogenic silica content of grasses; P = reduction in protein digestion; BSA = bovine serum albumin; TDF = total dietary fiber.

[b] Forages or diets low in tannin phenolic content.

[c] Also validated with black-tailed deer by Hanley et al. (1992).

[d] A modification of this equation is required for diets containing acorns.

diaminopinelic acid, which is found in cell walls of rumen bacteria. It is hypothesized that diet quality changes that alter rumen bacterial numbers will result in correlated changes in DAPA concentrations in ruminant feces (Kie and Burton 1984). Research has shown positive relationships among forage production, forage quality, other indices, and fecal DAPA (Kie and Burton 1984, Leslie et al. 1989, McCown et al. 1991). Under controlled, captive conditions, fecal DAPA was positively related to dietary energy, but negatively related to dietary protein in white-tailed deer (Brown et al. 1995).

Estimating Food Intake and Energetics of Free-ranging Wildlife

Field Techniques to Measure Energetics

Time–energy budget (TEB) and doubly-labeled water (DLW) methods are the 2 most commonly used techniques for estimating total daily energy expenditures of free-ranging animals. The TEB method has 2 parts: time spent in major activities or behaviors (e.g., foraging, resting) by the animal is quantified, and the activity data are converted to energetic equivalents from estimates of energy costs for each activity as measured in laboratory or controlled studies (Goldstein 1988, Karasov 1992, Speakman 1997). In some cases, estimates of the energy costs of activities are made using allometric models based on taxonomic groups (Weathers et al. 1984, Karasov 1992, Robbins 1993). Energy expenditure is calculated as:

$$E = \sum_{i=1}^{n} b_i \times t_i,$$

where E is energy expenditure (kilojoules/d), t_i is time (h/d) spent in activity i, and b_i is the energy equivalency (kilojoules/h) of activity i (McNab 2002). This method is most commonly used with birds because of the relative ease of collecting activity data (e.g., Ashkenazie and Safriel 1979, Stalmaster and Gessaman 1984, Morton et al. 1989). Problems include measuring energy equivalents of each activity, incorporating cost of thermoregulation into the equation (McKinney and McWilliams 2005), and large variances (because the variance is the sum of a series of terms; Travis 1982). Validation studies that compare estimates of daily energy expenditure (DEE) based on time-energy budgets with direct measures of DEE have shown that relatively minor errors in estimates of time and energy budgets can significantly affect estimates of DEE (Weathers et al. 1984).

Doubly-labeled water involves injection (labeling) of oxygen (oxygen-18) and hydrogen (tritium or deuterium) isotopes into an animal before its release and calculating the rate of CO_2 production (Fig. 8), which can be equated to metabolic rate, from the relative turnover rates of the isotopes measured upon recapture of the animal (Nagy 1980, Williams and Nagy 1984, Kam et al. 1987, Speakman 1997). Carbon dioxide production can be estimated from the difference in decay rates of the oxygen and hydrogen isotopes from the body over time because oxygen is lost as CO_2 and H_2O whereas hydrogen is only lost as H_2O (Fig. 9). Animals are usually sampled at least twice—once to obtain background measurements, inject the isotope, and obtain an initial equilibrated sample, and at least one other time to take the final sample to measure decay rates of the isotopes (Fig. 10). Webster and Weathers (1989) validated a single-sample DLW method for use with small or stress-sensitive species. Any biological sample containing body water can be used (Robbins 1993). Accuracy of the method was reported originally to be within 8-11% of validation methods (e.g., actual measurements of O_2 consumption or CO_2 production) (Nagy 1989), but with more recent refinements of the method, accuracy is within 2–4% (Nagy et al. 1999).

Fig. 8. Injection of deuterated water, a stable isotope of water, into the pectoral muscle of a Canada goose. After injection, individuals are allowed to rest undisturbed until the deuterated water has equilibrated with the animal's body water (ca. 60–90 min for a Canada goose).

Energy expenditure using the DLW method in free-ranging species is called field metabolic rate (Nagy 1987), which incorporates all metabolic costs incurred by the animal—basal metabolism, thermoregulation, activity, growth, etc. It does not, however, include the potential energy incorporated into new tissue produced during growth or reproduction. Its use has been limited in the past to smaller wildlife (Bryant et al. 1985, Tatner and Bryant 1986, Williams and Prints 1986, Gabrielsen et al. 1987) because of high costs of working with isotopes (Nagy 1989). However, better analytical methods now make possible its use on larger species (Nagy and Knight 1994,

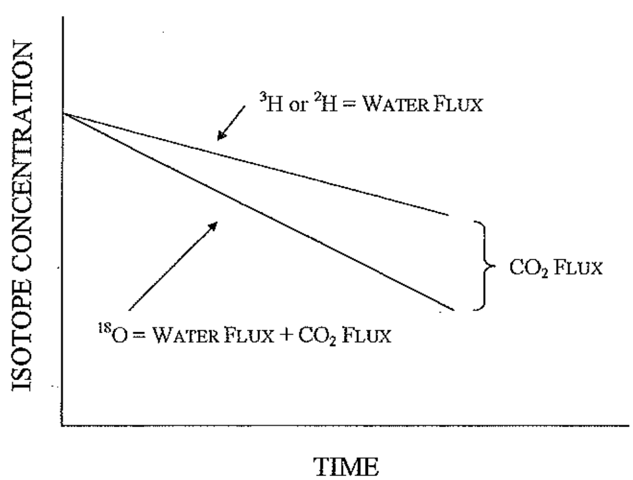


Fig. 9. The basis for use of doubly labeled water to measure CO_2 production and energy expenditure of wild animals (adapted from Robbins 1993).

Fig. 10. Blood sample (50–100 μL) of a Canada goose is drawn into a capillary tube after puncture of a leg vein with a 27-gauge needle. Blood is also commonly collected from the brachial vein on the wing of birds (not shown). Capillary tubes with blood samples can be temporarily sealed with clay (e.g., Critoseal) and permanently sealed and stored by flame sealing. Capillary tubes with blood should be refrigerated until laboratory analysis.

Williams et al. 2001). Nagy et al. (1999) provide the most complete review of field metabolic rates in mammals, birds, and reptiles.

Attempts have been made to examine energy expenditure by measuring heart rate (Holter et al. 1976, Wooley and Owen 1978, Mautz and Fair 1980, Kautz et al. 1981, Freddy 1984, Fancy and White 1985). Gessaman (1980) reported that heart rate satisfactorily measured energy expenditure of some American kestrels (*Falco sparverius*) he examined, but not of others. Seasonal variations also were a problem. Holter et al. (1976) observed that heart rate accounted for 78% of the variation observed in metabolic rates, whereas Mautz and Fair (1980) reported it ccounted for only 36% of the variance in energy expenditures. Thus, heart rate can be monitored remotely in free-ranging animals through telemetry, but variability in results restricts its application. A final method of examining energy requirements of animals is through use of stuffed mounts of a species with implanted heaters. The amount of heat required to maintain the mount at a certain temperature can be measured in different environmental settings or in different types of thermal cover. The heat required can be calibrated to the energy expenditure of a live animal in a laboratory thermal chamber by respiratory exchange. Estimation of incremental costs of thermoregulation under different conditions also can be made with this method. This method has been used for several wildlife species (Heller 1972, Thorkelson and Maxwell 1974, Bakken et al. 1983, Thompson and Fritzell 1988).

Techniques to Estimate Food Intake

In measuring energetic constraints on animal populations, food (or nutrient) intake may be the most useful parameter to measure because it represents the actual resource demand of animals on the environment (Nagy 1989). However, food intake is difficult to measure in the field. Most direct estimates of food intake have been made using tame, tractable animals. For example, esophageal fistulation has been used to measure food intake for grazing ungulates (Holleman et al. 1979, Wickstrom et al. 1984). A more complex method used by Renecker and Hudson (1985) involved clipping plant samples to simulate the diet of moose observed in an enclosure and measuring diet digestibility by the nylon-bag technique using fistulated animals. After total fecal collections from individual moose feeding in the enclosure were made for 24 hours, daily food intake was back-calculated. The bite-count method has been used frequently with captive ungulates to estimate food intake (Collins et al. 1978; Bengtson 1983; Wickstrom et al. 1984; Parker et al. 1993*b*, 1999). For this method, estimates are obtained for bite rate, simulated or actual bite weight, and total foraging time to calculate intake.

Isotope methods have also been used to estimate food intake. For example, sodium flux has been estimated by means of ^{22}Na turnover (Green 1978, Staaland et al. 1982, Gallagher et al. 1983, Green et al. 1984). If the sodium content of food items is known, food intake can be directly correlated with sodium turnover. Most of this work has been conducted with carnivores (DelGiudice et al. 1991) or nursing mammals (Green et al. 1997), which consume diets with fairly constant sodium content. DelGiudice et al. (1991) discussed several potential sources of discrepancies between sodium intake and turnover that could lead to under- or overestimation of sodium and overall food intake. For example, the exchangeable sodium pool may have a steadily increasing component from slow exchange of body fluid sodium with bone sodium. This would lead to overestimation of Na intake by ^{22}Na turnover (DelGiudice et al. 1991) until equilibrium is reached between these 2 pools (8 days in wolves). Bone sodium was believed to be a significant source of nonradioactive sodium diluting the exchangeable pool of sodium and leading to overestimates of sodium turnover in reindeer (Staaland et al. 1982). Gender differences in sodium metabolism associated with reproduction also affect the relationship between ^{22}Na turnover and sodium intake (DelGiudice et al. 1991). Finally, Alldredge et al. (1974) and Holleman et al. (1979) reported on use of natural fallout of radiocesium (cesium 137) to estimate intake for ungulates.

Food Availability and Nutritional Carrying Capacity

Food availability is often considered the most common limiting factor for wild animal populations. Boutin (1990) concluded from a review of population responses to food supplementation that in temperate environments, vertebrate populations are limited by food. However, it has proven difficult to integrate nutrient requirements of animals with available nutrient quality and quantity to estimate carrying capacity, and therefore assess the relationship of animal density to food availability. Data requirements for nutritional carrying capacity models are daunting. Necessary model parameters include individual animal requirements (e.g., daily energy expenditure or nitrogen requirements) and endogenous nutrient reserves, and biomass availability, production, and nutrient content of all food items consumed by the species in question. Because each of these parameters varies in space and time, it is easy to understand the difficulty in estimating nutritional carrying capacity.

Early models of nutritional carrying capacity assumed that food resources represented a single homogeneous quantity partitioned among animals according to their needs, with no or little adjustment for diet quality (Wallmo et al. 1977, Hobbs et al. 1982). The latter authors recognized that carrying-capacity estimates must account for biomass distribution of foods of different nutrient quality to properly reflect individual animal condition and population density. Hobbs and Swift (1985) subsequently developed a model that could predict the maximum number of animals that could obtain diets of a specified diet quality level (e.g., dietary protein, dietary digestible energy) or the maximum quality of diets obtained by a specified number of animals (Box 4). Hanley and Rogers (1989) provided an adjustment to the model that allowed for simultaneous consideration of digestible dry matter (a surrogate for digestible energy) and digestible protein. This model allowed managers to meet goals for individual animal condition and population density.

Use of the Hobbs and Swift (1985) model has been rare due to the large labor requirements to collect the data involved, and because the model is limited to ungulates (Ditchkoff and Servello 1998, DeYoung et al. 2000). However, nutritionally based carrying capacity models can be used for any species given that necessary data are collected (e.g., northern bobwhites; Guthery 1999). Changes in carrying capacity can be detected by sampling over time. For example, with the proper sampling design, managers can estimate changes in food supplies associated with management strategies and convert those to changes in estimated animal carrying capacity (Guthery 1999).

SYNTHESIS OF NUTRITIONAL INFORMATION

We have presented methods and techniques for measuring individual elements of the nutritional ecology of wildlife. Accurately interpreting data from analyses can be a challenge because individual nutritional factors are part

Box 4. Nutritionally based carrying capacity models that incorporate diet quality requirements: which data are needed and how can I use the model?

The model of Hobbs and Swift (1985) posed and attempted to answer the question "How much food is present in the environment that will allow a population of animals to obtain diets averaging a specific level of some nutrient?" Behind this question lay 2 other questions: 1) which nutrient, and 2) what is the nutrient level of interest? These latter 2 questions are specific to the objectives of the researcher or manager. In most cases, the nutrient would be the presumed limiting nutrient in the system or energy (e.g., digestible or metabolizable energy, nitrogen, phosphorus) and the specific level would be the concentration required for a particular nutritional state (e.g., maintenance, reproduction, growth). We refer the reader to Hobbs and Swift (1985) for a full description of the carrying capacity model that incorporates explicit nutritional constraints.

The model assumes a nutrient distribution relating forage quality to forage biomass (Fig. 11). Field data necessary to parameterize a discrete version (Fig. 11) of the continuous model are provided below.

1. A catalog of items in the diet of the study species. This list may include plant parts (leaves vs. stems) if animals distinguish these in their diet.
2. Biomass of each food item on the study area.
3. Nutrient content of each food item.

Hobbs and Swift (1985) provide an algorithm that allows one to calculate the amount of biomass available that has an average nutrient concentration equivalent to the management or research objective, given the data listed above. Once this biomass has been calculated, nutritional carrying capacity (animal-days/ha) is estimated by dividing this biomass (kg/ha) by average daily animal requirements (obtained from the literature or from separate experiments designed to measure daily nutrient requirements).

The model has been used in 2 recent papers to study the relative value of litterfall to white-tailed deer wintering in Maine (Ditchkoff and Servello 1998) and to estimate seasonal changes in nitrogen-related carrying capacity for desert mountain sheep (*Ovis canadensis nelsoni*) in Texas (DeYoung et al. 2000). The original paper (Hobbs and Swift 1985) illustrated how burned areas could support more mule deer and mountain sheep at a high nutritional plane than unburned areas. Prescribed fire produced greater amounts of higher-quality forage relative to controls. However, overall forage biomass decreased on burned sites, and traditional models estimating carrying capacity based on range forage supply would have concluded that burning was not an effective practice for habitat improvement.

Carrying capacity is a dynamic state and the model of Hobbs and Swift (1985) has the flexibility to track changes in carrying capacity, if the correct data can be collected. In addition, the model provides alternative estimates of carrying capacity based on the biology of the species under study. For example, estimates provided by DeYoung et al. (2000) showed how seasonal changes in forage availability and quality alter carrying capacity, especially at higher diet-quality levels. In spring, DeYoung et al. (2000) estimated that carrying capacity at a diet level of 1.5% nitrogen (approximately 9.4% crude protein and adequate for meeting lactation requirements) averaged 3.2 animals/km^2 across 3 mountain ranges. By winter, the ability of the habitat to support desert mountain sheep at this level averaged 0.1 animals/km^2 because of a decreasing supply of nutritious forage and a local drought. However, carrying capacity increased to 5.5 animals/km^2 in winter because lactational needs decreased after lambs weaned and the required dietary level of nitrogen was only 0.89% (approximately 5.6% crude protein) (DeYoung et al. 2000).

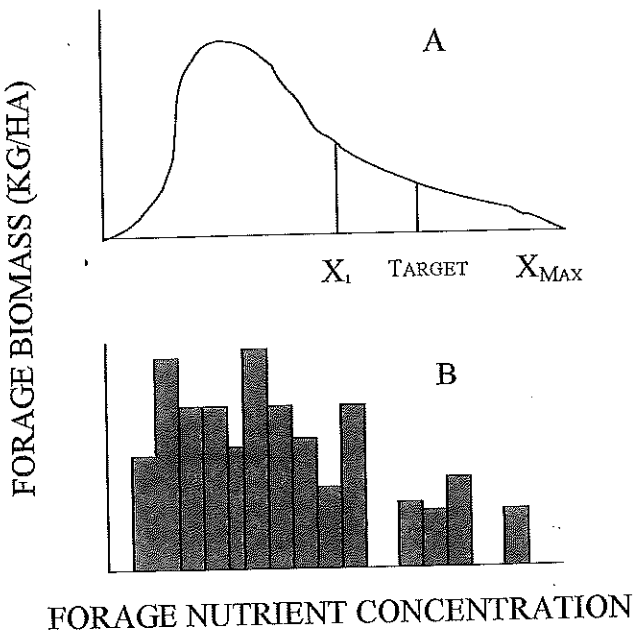


Fig. 11. Hypothetical continuous nutrient distribution (A) relating forage nutrient quality to forage biomass. "Target" represents the management objective for average nutrient level in the diet of the animal population, X_{MAX} represents the maximum concentration of nutrient found in any forage item, and X_1 represents the point at which the mean concentration of the biomass to the right of this point equals the target concentration (adapted from Hobbs and Swift 1985). Hypothetical discrete nutrient distribution (B) relating forage nutrient quality to forage biomass obtained from field data collection.

of larger physiological and behavioral processes. Some factors may have more or less importance when considered in this larger context, and some new insights may only be revealed from a more comprehensive analysis of overall ecology.

Foraging Strategies

The terms "foraging strategy" or "feeding strategy" are shorthand for the suite of behavioral, morphological, and physiological adaptations that allow an animal to consume and metabolize available energy and nutrients in its natural habitat. Study of wildlife feeding strategies shifts the focus from purely descriptive studies of diet and animal requirements to an analysis of how the selected diet of a certain species is influenced by, for example, distribution and abundance of potential foods, or costs of foraging including risk of predation and increased energy expenditure associated with chasing, handling, and digesting different prey (Schoener 1971, Stephens and Krebs 1986, Robbins 1993, Belovsky 1997). By placing the process of feeding and nutrition in an evolutionary context, study of feeding strategies relates the process of diet selection ultimately to an animal's fitness (Hughes 1990, Belovsky et al. 1999).

Feeding strategies have been studied in a wide range of wild animals including carnivores (MacCracken and Hansen 1987), primates (Chivers et al. 1984), rodents (Jenkins 1975), songbirds (Zach and Falls 1979, Krebs and Kacelnik 1991), waterfowl (Wood and Hand 1985, Tome 1988), and in many species of mammalian herbivores (Hanley and Hanley 1982, Hobbs et al. 1983, Krueger 1986, Belovsky and Schmitz 1994, Laca and Demment 1996). These studies used optimization models of food and habitat selection to compare predicted with actual preferences of wild animals (Pulliam 1989), relate diet selection to numerical and functional responses of prey and predator (Krebs et al. 1999, 2001), and incorporate survival probability and reproductive success into dynamic programming models of diet and habitat selection (Belovsky 1986, Mangel and Clark 1988). Studies of foraging strategies of wildlife have also focused on behavioral, morphological, and physiological characteristics that constrain diet and habitat selection (Demment and Van Soest 1985, Spalinger and Hobbs 1992, Illius and Gordon 1999, Dearing et al. 2000, Rode et al. 2001, Karasov and McWilliams 2004).

Despite many important studies, considerably more work is needed on feeding strategies of most wild species given their central importance in the ecology and management of wildlife. For example, wildlife biologists too often assume that an adequate quantity of food in the environment is sufficient for a given population regardless of the food's distribution and abundance. But, as observed by Robbins (1993) for elk in Yellowstone National Park, the quality of the food resource, its spatial distribution, and social interactions between and within wildlife species can reduce the density of usable food to below required levels. A more complete understanding of the feeding strategies of wildlife would greatly improve our assessments of the nutritional adequacy of natural habitats.

Modeling

Simulation modeling is used to identify the relative importance of nutrition variables and to gain new insights into interactions among factors that potentially affect nutritional ecology of a species. Models may address specific nutritional and physiological processes, such as the dynamics of a urinary index (Moen and DelGiudice 1997). We concentrate on more comprehensive modeling of nutritional ecology done for the purpose of evaluating habitats, populations, or management (e.g., Stalmaster 1983). A primary advantage of a modeling approach is that it may produce unforeseen information or hypotheses that are the product of the entire system in contrast to conclusions drawn from data on a few variables in field studies. Modeling also allows examination of questions that would otherwise be difficult using traditional experimental approaches. The common approach for evaluating relative importance of individual factors with a modeling study is with sensitivity analyses. In a sensitivity analysis, a single variable is changed a standard amount to identify effects on response variables in the model when all other variables are held constant. The relative sensitivity of model products to changes in each variable can be compared to gauge the relative importance of nutritional and related factors. Parallel fieldwork on variables can greatly strengthen modeling results and conclusions, and can be used to test *a priori* predictions from model simulations (e.g., Farmer and Wiens 1999).

Energetic models based on species autecology are the typical approach for linking nutrition with habitat or populations. This is because of the primary importance of energy acquisition for animals and that most nutrition and environmental parameters can be translated into energy

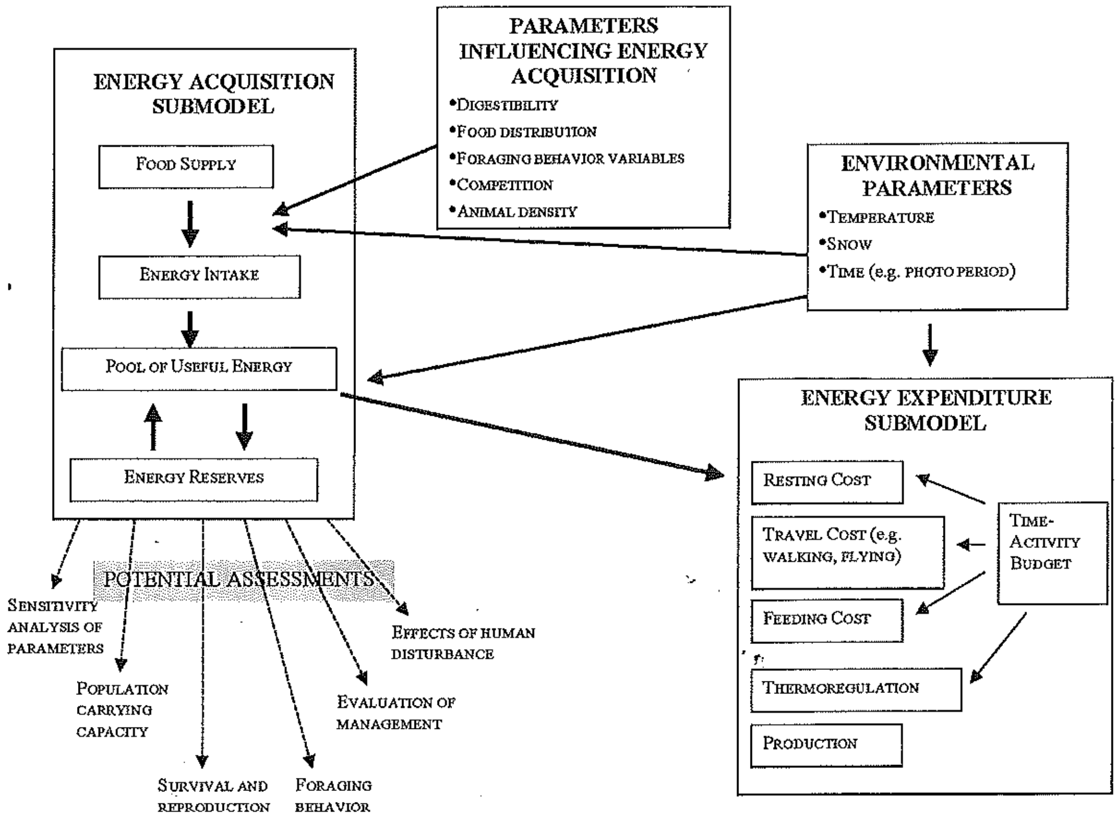


Fig. 12. Simplified diagram of an energetic simulation model for evaluating nutritional ecology of wildlife (adapted from Stalmaster [1983] and Hobbs [1989]). Small rectangles represent energy pools or expenditures. Thick solid arrows represent energy flows. Thin solid arrows represent variables that influence the system. Dashed arrows are indicators of potential applications of energetic modeling.

units. Most energetic simulation models have 2 basic elements (Fig. 12), calculation of energy acquisition and storage by individual animals from an available food supply, and calculation of energy expenditure by animals in response to basic metabolic requirements as well as activity patterns (e.g., Stalmaster 1983, Hobbs 1989). Classification of time spent in common activities (e.g., flying, walking, running, roosting) used in concert with average costs of activities allows evaluation of natural and human influences on energetic status (e.g., Stalmaster 1983). A variety of environmental and behavior parameters also may be incorporated into acquisition and expenditure submodels. Positive or negative energy balances of individual animals or populations are the basic metric for evaluating simulation results, but information generated on dynamics of other parameters may be just as valuable. For example, simulations of severe winter conditions may be examined to study the importance of traveling or thermoregulatory costs on energetics. There are a number of example conclusions possible from energetic modeling studies.

1. Use of stopover sites along migration routes for pectoral sandpipers (*Calidris melanotos*) is a function of the spacing and quality (for foraging) of the sites (Farmer and Wiens 1999).
2. Factors that influence energy acquisition by mule deer were generally more important than factors that influence energy expenditure (Hobbs 1989).
3. Browse digestibility affects body mass of moose more than other nutritional factors, including some forage distribution variables (Moen et al. 1997).
4. Salmon (*Oncorhynchus* spp.) carcass availability (including accessibility in river habitat) is as influential as salmon escapement past dams for predicting population sizes of wintering bald eagles (*Haliaeetus leucocephalus*) (Stalmaster 1983).

These examples represent information that would be difficult to generate from reductionist field studies. Thus, insights gained via nutritional ecology modeling add significantly to our knowledge of wildlife ecology and management.

SUMMARY

The wide array and improved techniques available today for nutritional ecology will help advance our understanding of wildlife-habitat relationships and improve investigations of habitat management issues. There is not a simple formula for nutritional ecology investigations with wild species because the nature of the foods eaten, species behavior, physiological adaptations to nutritional stress, life history strategies, and feasibility of collecting data in specific environments may vary greatly among species. Before undertaking nutritional ecology investigations, biologists should carefully consider the importance of nutrition and foraging variables in the ecology of the focal species, as well as practical issues for adequate data collection.

Nutritional ecology research frequently involves laboratory analyses of foods and studies with captive animals. While contributing important baseline information, we encourage biologists to also use parallel field studies to examine animal responses in natural settings or in wild populations. For complex questions, modeling may be required to better understand interactions of multiple variables including influences of behavioral and environmental factors.

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