Methods of
Energy Budget Analysis

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1. INTRODUCTION

Energy is the currency of living organisms, yet remarkably little is known about how free-ranging animals allocate energy in the course of a day or over longer periods. How animals apportion time and energy ultimately influences their survival and reproductive success. These and other questions are central themes in ecology that are embodied in the concepts of optimal foraging theory, reproductive effort (including parental investment theory), and life history strategies.

The study of energy metabolism and energy allocation by bats is in its infancy (Kunz, 1980). Preliminary estimates of daily energy expenditure (DEE) in free-ranging bats have involved constructing time-activity budgets from field observations and assigning assumed energy costs to each activity (e.g., Howell, 1979; Kunz, 1980; Burnett and August, 1981), estimating daily food intake (Kunz, 1974; Anthony and Kunz, 1977; Morrison, 1978; Kunz, 1980; Funakoshi and Uchida, 1980), and by assessing changes in body composition and energy content (Chapter 23). Doubly labeled water (DLW) has only recently been used to estimate energy expenditure in free-ranging bats (Helversen and Reyer, 1984; Bell et al., 1986).

There are advantages and disadvantages of using one or another method. The time-activity budget method is an intuitively attractive approach to assess individual variation in daily energy expenditure. However, it is often difficult to quantify the amount of time an animal spends in various activities and to apply accurate estimates of metabolism to each activity. This method assumes that costs are additive and they do not substitute (e.g., activity heat for thermoregulatory
heat). Field measurements of food ingested can provide a direct estimate of daily energy intake, but this requires knowledge of field diets and measurements of the digestibility of different foods. Moreover, rapid food transit time in bats (e.g., Buchler, 1975; Tedman and Hall, 1985) can lead to underestimates of digestible energy. Laboratory measurements of body composition and energy density of bats are valuable for assessing the allocation of energy to fetal and suckling growth and to seasonal changes in adult growth and fat storage, but these only contribute to a partial assessment of energy investment. The DLW method provides a direct measure of field metabolic rate (FMR) and offers a powerful approach for answering ecological questions related to energy expenditure of individuals. With this method, however, there are several unverified assumptions and potential errors (Lifson and McClintock, 1966; Nagy, 1980; Nagy and Costa, 1980). Energy expenditure and allocation by free-ranging animals can best be understood when several approaches are used concurrently.

The purpose of this chapter is to review methods of energy budget analysis appropriate for the study of free-ranging bats. These include doubly labeled water, food consumption, and time budgets. The schematic diagram of an energy budget (Fig. 1) illustrates major compartments for bats and other small mammals. The analysis of these and other compartments require different methods, equipment, and investment in time and effort. Other methods needed for energy budget analysis, including observational techniques, radiotelemetry, respirometry, body composition and calorimetry, and microclimate analysis, only briefly discussed here, are treated elsewhere in this volume.

2. DOUBLY LABELED WATER METHOD

2.1. Rationale

The DLW method works by measuring CO₂ production via the differential washout rates of injected isotopes of hydrogen and oxygen. This method, developed over 30 years ago, is based on the observation that the oxygen of respiratory CO₂ is in isotopic equilibrium with the oxygen of body water (Lifson et al., 1949). Most of the hydrogen isotope (deuterium, ²H, or tritium, ³H) is lost from the body as water, whereas the oxygen isotope (¹⁸O) is lost both as water and as respiratory CO₂ (Lifson and McClintock, 1966; Nagy, 1980). This makes it possible to determine CO₂ production by labeling both types of atoms of the body water in an animal and monitoring the relative turnover of the two isotopes. CO₂ production can then be converted to units of energy metabolism (field metabolic rate) using an appropriate equation and corrections made for diet composition and digestibility (see Nagy, 1980).

2.2. Field Metabolic Rate (FMR)

Doubly labeled water measurements of FMR integrate all metabolic costs, including basal metabolism, thermoregulation, metabolism of food (specific dynamic action), activity, and reproduction. Several authors have used the term “average daily metabolic rate” (ADMR) in studies using DLW in free-ranging animals (e.g., Hails and Bryant, 1979; Turner, 1983; Westerterp and Bryant, 1984), but we suggest that this practice be discontinued. ADMR as originally conceived (see Grodzinska and Gorecki, 1967), was based on metabolic measurements taken on animals confined to large chambers at temperatures similar to the natural environment.

2.3. Assumptions and Potential Errors

Assumptions and potential errors inherent in the DLW method have been reviewed (Lifson and McClintock, 1966; Mullen, 1973; Hails, 1977; Hails and Bryant, 1979; Nagy, 1980; Nagy and Costa, 1980). The major assumptions are: 1) body water volume remains constant during the measurement period, 2) rates of water flux and CO₂ production are constant through time, 3) isotopes label only the H₂O and CO₂ in the body, 4) isotopes leave the
body only as H₂O and CO₂, 5) specific activities of the isotopes in H₂O and CO₂ leaving the body are the same as in body water, and 6) labeled or unlabeled water or CO₂ in the environment does not enter the animal via respiratory or skin surfaces.

The magnitude of errors associated with the above assumptions is small in most situations, and can be avoided by careful choice of equations, animals, and sampling times (Nagy, 1980). Under some circumstances the errors may be unacceptably high. For example, if labeled animals breathe air containing unusually high concentrations (>1%) of unlabeled CO₂, this CO₂ may equilibrate with body fluids and be measured by the isotopes. Thus, DLW values may be falsely high because they include exogenous as well as endogenous sources of CO₂.

Potential errors particular to communal bats include high humidity and high levels of unlabeled CO₂ found in natural roosts, such as reported in some caves and mines (see Chapter 29 and Kunz, 1982). Theoretically, these conditions could introduce large errors in the calculations of CO₂ production and water flux (Lifson and McClintock, 1966; Nagy, 1980). Although ³H turnover gave an accurate estimate of water flux for pocket gophers confined to simulated burrow condi-
Doubly Labeled Water Method

1. LABEL WITH ISOTOPES  
2. EQUILIBRATION  
3. FIRST BLOOD SAMPLE  
4. FREE-RANGING  
5. SECOND BLOOD SAMPLE

Figure 2. Field protocol for doubly labeled water studies on free-ranging bats: 1) intraperitoneal injection, 2) equilibration, 3) initial blood sample, 4) free-ranging activity, and 5) final blood sample (see text for details).

...tions with 75% relative humidity (RH) (Gettinger, 1983), the RH of some bat roosts may be as high as 100% (Kunz, 1982). Studies are encouraged to determine the effects of high CO₂ levels and high humidity on results using the DLW method on bats that are normally exposed to these conditions.

2.4. Practical Considerations

There are several practical considerations when using DLW. These concern the choice of the study organism, field protocol (Fig. 2), isotope costs, and isotope analyses (see Mullen, 1973; Nagy, 1980; Nagy and Costa, 1980; Bryant and Westerterp, 1980; Nagy, 1983).

2.4.1. Choice of Organism

Aside from the biological significance of the results, the most important criterion in selecting bats for DLW studies is recapturability of the animals. Accessibility, high site fidelity, and high tolerance to disturbance all increase capturability and recapturability. Species that roost in protected shelters (Kunz, 1982) and form highly structured social groups (Bradbury, 1977) offer the best choices of animals for study. Their roost locations are predictable and adequate sample sizes can usually be obtained. Roost fidelity and recapture success are often linked to an animal's reproductive condition and social behavior. Lactating females are more likely to be recaptured at a maternity roost than are pregnant and non-reproductive females. Also, harem males are more likely to be recaptured than harem females, and harem members are more likely to be recaptured than members of non-harem (bachelor) groups (T. H. Kunz, unpubl.).

Flightless young are especially suitable subjects for study (e.g., Kunz and Anthony, 1982; Burnett and Kunz, 1982). High recapture success offers the potential for quantifying FMR and milk energy intake during the suckling period (Kunz and Nagy, unpubl.).
Although nursing females may transport neonates to alternate roosts following disturbance, they are less likely to move older sucklings, especially as they approach weaning size.

2.4.2. Capture and Blood Sampling Time

The time of day that bats are captured, injected, and bled can affect the amount of disturbance and bleeding success, and ultimately the error associated with calculating FMR. A consistent capture and processing time insures comparable values of body mass and minimizes overall disturbance to the colony. For best results adults and flying young should be captured in their roosts between 1100 h and 1300 h for initial processing. By 1100 h bats have voided most, if not all, of their feces from a previous meal, thus their body masses should have stabilized. Moreover, returning bats to their roost site immediately following treatment allows labeled individuals to reestablish social contacts with other members of the colony. This protocol allows enough time to process bats and return them for settling before the onset of nightly departure. State of hydration may affect the success of bleeding. Because adult bats, especially lactating females, are maximally dehydrated at the end of the day-roosting period, attempts to obtain blood samples at this time are less likely to be successful.

Flightless young are best captured and processed as soon as possible following the nightly departure of adults (e.g., Kunz, 1973; Kunz and Anthony, 1982). This protocol not only eliminates direct disturbance to roosting adults but also minimizes disturbance to the young. Dehydration is less likely to be a problem for sucklings at this time, particularly if they have recently nursed from their mother. Moreover, captures at this time eliminate the need to remove a suckling from its mother, and it is easier to locate marked individuals that might otherwise be difficult to find when adult bats are present. Young bats should be captured and processed as soon as possible after the departure of adults, since very young bats may enter torpor, making it difficult, if not impossible, to draw a sufficient sample of blood.

2.4.3. Injection Procedures

Detailed procedures for preparing and calibrating injection solutions of $^3$H$^1$H$^{18}$O are given in Nagy (1983). We prefer an injection solution consisting of 25 mCi/ml enrichment of $^3$H$^1$H$^2$O and a 90-99 atom % enrichment of $^2$H$^1$H$^1$O. After this tritium enrichment is mixed with the $^2$H$^1$H$^{18}$O enriched water, the tritium dose is about 1 mCi/kg body mass. Using this level of enrichment, injection volumes consist of about 3 ml/kg of animal. This volume is small enough so as not to overly increase the animal’s total body water (TBW). A 30 µl sample of each injection solution should be flame-sealed in a microcapillary tube (Section 2.4.8) to be used in the preparation of standards (Nagy, 1983). Procedures for using deuterium instead of tritium are discussed by Mullen (1973) and Hails (1977). The amount of deuterium administered will depend on the sensitivity and accuracy of the spectrometer being used for deuterium analysis. Selected commercially available sources of isotopes for DLW studies are given in Nagy (1983).

In the field, a calibrated glass syringe (e.g., Hamilton microliter syringe) is filled to the desired volume, and constant pressure is applied to the plunger during injection. We pull the skin of the abdomen to the side before injection to reduce leakage when the needle is withdrawn. Special care should be taken to avoid contamination of the syringe with blood or dirt.

Subcutaneous, intramuscular, and intraperitoneal injections have been used on bats, but we prefer the intraperitoneal route. Intravenous injections should be avoided and may prove fatal to small animals, and subcutaneous injections are slow to equilibrate. The size of syringe that is used depends upon the enrichment of the injection solution, the mass of the bat, and the volume of the injection solution to be used. Accurate, careful,
and reproducible injections can be made by using the same syringe and same volume of injection solution for each animal in a given experiment. The person who does the field injections also should calibrate the syringe volume in the laboratory, using distilled water (mass = volume) instead of the heavier injection solution.

2.4.4. Effects on Normal Behavior

The effects of isotope labeling on normal behavior (apart from disturbances caused by capture, handling, weighing, etc.) have not been investigated. Suter and Rawson (1968) found that the circadian rhythm of Peromyscus leucopus was temporarily altered when given drinking water containing highly-enriched deuterium oxide ($^2$HOO), although it has generally been assumed that such effects are minimal (but see Sutton and Nielson, 1974). Our observations indicate that some bats abandon roost sites after being captured, injected, and bled, but this response is influenced by such factors as species, sex, age, reproductive condition, and/or social behavior (see Bell et al., 1986). The DLW protocol most certainly involves some disturbance to animals, but this method is probably less subject to investigator bias and disturbance than some field estimates of time-activity budgets and related laboratory measurements of metabolism (see Congdon et al., 1982).

2.4.5. Measuring Body Mass

Precise and accurate measurements of body mass are extremely important in making calculations of FMR and water flux. Body mass should be taken as near as possible to the time of injection. Mass measurements taken at other times may cause errors in calculated TBW values, hence errors in FMR. Body mass should also be determined at the time of final bleeding. For bats having a body mass less than 100 g, we suggest that it be recorded to the nearest 0.01 g. This can be accomplished using a portable field balance (e.g., Ohaus, model 7401-02 or Torsion Balance, model DLM-2). Hand-held spring scales (e.g., Pesola) are less precise and often yield inconsistent and unreliable results. For bats weighing over 100 g, body mass measurements with a precision of 0.1 g are usually adequate.

2.4.6. Isotope Equilibration

To determine the amount of time required for isotope equilibration, individuals are injected with $^3$HOO, and serial blood samples are taken at regular intervals (e.g., 15 min) following injection. When several bats are being tested, we suggest they be housed individually to minimize the time needed to search for and process each animal and to avoid isotope exchange between animals. An injection solution consisting of $^3$HOO is used instead of one containing $^3$H and $^{18}$O since only one isotope (the least expensive) is needed. We micro-distill blood samples and determine the specific activity of $^3$H with a liquid scintillation counter (Section 2.6). The specific activity (CPM) of $^3$H is plotted against time following injection, and equilibration time is determined as the beginning of the asymptote on the plot (Fig. 3).

Results from our analysis (Kunz and Nagy, unpubl.) indicate that $^3$HOO equilibra-
BLEEDING PROCEDURES

Figure 4. Suggested procedures for bleeding bats in doubly labeled water studies: 1) venous puncture of cardiac vein in propatagium and 2) venous puncture of major vein in interfemoral membrane.

tion occurs within 30-45 min following intraperitoneal injection in adult *Myotis lucifugus* (7-10 g), *Eptesicus fuscus* (15-18 g), *Carollia perspicillata* (18-22 g), and *Phyllostomus hastatus* (70-90 g). This time is consistent with results from adult birds having a similar body mass (e.g., Hails and Bryant, 1979; Williams and Nagy, 1984) but is shorter than reported for similar size rodents (e.g., Mullen, 1971).

Time required for isotope equilibration may vary with body mass, route of injection, metabolic rate, and state of hydration. Isotopes injected into small animals (<5 g) are likely to equilibrate sooner than in larger individuals. If the initial blood sample is taken before isotopes have equilibrated, TBW will be underestimated because of falsely low isotope measurements. If isotopes reach equilibrium before the first blood sample is taken, some of the $^{3}$H$^{18}$O will be lost via normal routes of water loss, and input of unlabeled water from energy metabolism and ambient water vapor may combine to slowly lower the isotope concentration. This will lead to an overestimate of TBW. Because isotope equilibration takes longer when animals are dehydrated (Richmond et al., 1962), equilibration experiments should be conducted at the same time of day as the experiments to determine FMR and water flux in the field (Section 2.4.5).

2.4.7. Blood Sampling Procedures

Several procedures for bleeding bats have been described, including cardiac puncture (LaMotte, 1958), rupture of the infraorbital sinus (Baer, 1966), puncture of wing veins (Baer and McClean, 1972; Keegan, 1979; Gustafson and Demassa, 1985), puncture of the jugular vein (Baer and McClean, 1972), puncture of veins in the interfemoral membrane (Black and Wiederhielm, 1976), and the use of starved hemophagous bugs (Helversen and Reyer, 1984). The procedures described by Keegan (1979) and Gustafson and Demassa (1985) are valuable modifications for bleeding bats, but they may prove to be unnecessarily cumbersome for taking blood samples in the field.

Depending upon the species and size (age) of the animal, we have had good success bleeding bats using two relatively simple procedures (Fig. 4). We use 25-gauge needles to puncture veins in the interfemoral membrane or in the wings. Small surgical needles are less effective and lances should be avoided. For most small vespertilionid bats (young and
Temporary Seal

Flame Seal

Figure 5. Procedures for temporarily and permanently sealing glass microcapillary tubes: (A) Critocaps should be inserted firmly into both ends of the tube. (B) Flame seals can be successfully made by holding the capillary tube horizontally, but at an angle to the flame, so that the opening of the tube is directed away from the source of the flame. The capillary tube should be rolled between the thumb and forefinger until a small bead forms at the tip of the tube. Repeat procedure at other end.

adults ranging from 2-15 g), we have successfully taken blood from veins that run semi-parallel to the tail in the interfemoral membrane. In the field we accomplish this by first transilluminating the interfemoral membrane with a small incandescent light source (e.g., MineSpot Cap Lamp or Koehler Wheat Lamp; see Chapter 1). We then usually puncture only one vein in the first bleeding session, saving the other vein for when the bat is recaptured. The lamp allows one to easily see the vein, but it also produces a small amount of heat that stimulates venous dilation and blood flow. Rhythmic flexing of the leg on the side where blood is being taken often promotes the flow of blood. In larger species (>15 g), and those lacking an interfemoral membrane, we have successfully taken blood from the cardiac vein located near the leading edge of the propatagium. Attempts to draw blood from other veins in the wings should be avoided because of the risks of causing a hematoma (blood blister).

When the animal has been inactive or torpid, blood is commonly shunted away from the wing membrane. Blood flow can be stimulated by placing the bat’s body against a hot water bottle or other heat source. During the equilibration and processing periods we have successfully kept bats warm by allowing them to form small clusters (5-6 individuals) in small wooden roosts (see Chapter 1). Bats held in this manner usually remain warm, equilibrate sooner, and are thus easier to bleed than if housed alone or in cloth holding bags. Extremely small bats can be warmed by holding them in cupped, bare hands. Light etherization may be used to promote vasodilation and blood flow to peripheral veins before attempts are made to bleed bats (see Gustafson and Demassa, 1985).

Blood samples are routinely collected in 70 µl heparinized, glass microcapillary tubes. A minimum of 35 µl (activation analysis) or 10 µl (mass analysis) of blood is needed for routine analysis in DLW studies. If possible, 45 µl (activation analysis) should be collected in each of two tubes so that one can be used as a backup sample (Nagy, 1983). These volumes of blood can be drawn successfully from most full-grown bats, but one must be willing to accept smaller volumes from neonates and other immature bats. Holding the capillary tube at a slightly downward angle from the drop of blood should promote adequate filling.

The flow of blood from a punctured vein of a bat usually ceases soon after a sample has been taken; however, some animals may bleed profusely. Slight upstream pressure from the fingers, combined with blowing air on the wound, is often sufficient to stop the bleeding. If the animal continues to bleed, an application of powdered “Gelfoam” (sterile gelatin powder) at the place of bleeding may stop the flow of blood. Application of ice to the wound
may also reduce or stop the flow of blood in profuse bleeders. It is important to note that excessive bleeders may loose as much as 3-5% of TBW in a few minutes and this can lead to serious errors when calculating FMR and water flux.

When animals are very small and the taking of an initial blood sample would be harmful or might interfere with normal behavior, this step can be eliminated and isotope activity (from initial samples) can be estimated rather than measured (Nagy, 1983; Nagy et al., 1984a). In this procedure, animals are released immediately after being marked, injected, and weighed. A small sample of bats of comparable age, sex, and reproductive condition are weighed and injected, and blood is taken after the period of equilibration. These animals can then be killed and used to verify TBW and to quantify body composition. Ricklefs and Williams (1984) proposed a field protocol for eliminating initial blood samples and the potentially traumatic equilibration period by estimating initial isotope ratios (not absolute concentrations) from other animals that were injected and sampled initially. This procedure is suitable for determining FMR, but it cannot be used for determining water flux because the latter requires knowledge of absolute \(^{3}H\) activity after equilibration.

2.4.8. Sample Preparation

Capillary tubes containing blood samples should be sealed temporarily or permanently in the field (Fig. 5), labeled, and refrigerated to prevent samples from spoiling. Decayed samples usually produce contaminated water upon distillation, and this will cause errors in activation analysis. Blood should be centered in the microcapillary tube before sealing both ends. Critocaps provide temporary seals and are suitable when field conditions make flame-sealing hazardous or difficult. Critocaps should be removed and the ends of the tubes flame-sealed before transporting samples overland or by air. Flame seals are permanent, prevent loss of samples by evaporation, isotopic fractionation during evaporation, and dilution of samples by instillation of water from ambient humidity. Flame-sealed microcapillary tubes containing blood should not be frozen as this may cause the tubes to fracture when the water in blood expands (Nagy, 1983).

Lack of suitable field-refrigeration may pose problems until samples can be transported to the laboratory. We have successfully used blocks of ice or sealed, gel coolants (available in many sporting goods stores) to keep samples cold in the field. Gel coolants have an advantage in that they can be frozen in advance and kept at cold temperatures in small picnic coolers for up to 48 h in the field. They also can be used many times and without causing an accumulation of water in a cooler from melted ice. When it becomes necessary to keep samples cold for longer periods without access to ice or refrigeration, we have used a supply of instant catalytic coolants (available at many sporting goods stores) which, when activated and kept insulated, will keep samples cool for up to 24 h. Sealed blood samples should be distilled following the procedures described in Wood et al. (1975) and Nagy (1983). Undistilled, flame-sealed blood samples can be stored safely under refrigeration for several months.

2.4.9. Costs

Oxygen-18 labeled water is the most expensive of the isotopes involved in DLW studies. At current (1986) prices, this isotope costs about U.S. $180/kg of animal body mass to be injected, for studies involving \(^{18}O\) measurement by activation analysis or conventional mass spectrometry. Tritium and deuterium are much less expensive. For a bat weighing 20 g, total costs for isotopes are about $200/kg ($4 per bat). Analysis of isotopes in blood samples costs about $0.75 per sample for tritium and about $14 per sample for \(^{18}O\) using proton activation analysis (Nagy, 1983). Deuterium analysis is best done using isotope-ratio mass spectrometry, but the cost is high (about $45/sample). Isotope-ratio mass spec-
trometry can also be used to measure $^{18}$O in samples, but this method is expensive (about $40/sample). Because isotope-ratio mass spectrometric analysis is more accurate than proton activation analysis, animals can be injected with one-tenth, or less, of the $^{18}$O needed for proton activation analysis, thus reducing isotope costs. The total cost of a project depends mainly on the body mass of the animal to be studied, and is about the same with either $^{18}$O analysis method when individual animals weigh 1 to 5 kg. For smaller animals, the activation analysis method is less expensive overall, but for larger animals the mass spectrometer method is less costly.

2.5. Validation Experiments

The DLW method for measuring CO$_2$ production has been validated for several groups of vertebrates, including reptiles (e.g., Congdon et al., 1978; Nagy, 1980), rodents (Mullen, 1970; Gettinger, 1983), and birds (e.g., Utter and LeFebvre, 1971; Hails, 1977; Hails and Bryant, 1979; Williams and Nagy, 1984). Results from these and other studies indicate errors averaging less than 10% (also see Weathers et al., 1984).

A simple and reliable method of validation is to simultaneously measure CO$_2$ production from isotope turnover and from gravimetric measurements (e.g., Kendeigh, 1939; Williams and Nagy, 1984) or gas analysis using a CO$_2$ analyzer (e.g., Hails and Bryant, 1979). For active bats, we have successfully used a gravimetric approach by confining individuals to small metal or plexiglass metabolic chambers, which include a hollowed-out wood block (a simulated roost). Validation methods based on food consumption in the laboratory (e.g., Karasov, 1981; Gettinger, 1983) are less suitable for bats because of the difficulties in getting captive bats to eat and maintain a stable body mass, and associated problems with the quantitative collection of urine and feces.

Gravimetric measurement of CO$_2$ production can be accomplished by determining the mass increase of a CO$_2$ absorbent (NaOH-coated silica) after excurrent air from a metabolic chamber has passed through a tube of anhydrous CaSO$_4$ (Kendeigh, 1939). Williams and Nagy (1984) found that Ascarite (a CO$_2$ absorbent) gave off water after it had been used for a while. Thus, the mass of Ascarite did not increase in proportion to the CO$_2$ absorbed. This problem can be minimized by adding a small band of H$_2$O absorbent (anhydrous CaSO$_4$) at the end of the column of CO$_2$ absorbent.

2.6. Isotopes and Isotope Analysis

Several isotopes and analytical methods can be used in DLW studies, and the choices among these can affect costs and convenience. The hydrogen isotope can be either tritium or deuterium. Tritiated water is inexpensive both to purchase and to analyze. Liquid scintillation vials and chemical cost about $0.75 per sample (Nagy, 1975, 1983) and liquid scintillation counters are available at many institutions. Scores of tritium samples can usually be analyzed in one day. However, a drawback of using tritiated water is that its radioactivity requires users to obtain permits for its use in the field and laboratory.

For tritium analysis, 5 or 10 μl aliquants of water distilled from blood samples are placed in 10 ml of scintillation fluid for counting. A common scintillation cocktail consists of 3.6 g 2,5-diphenyloxazole (PPO), 250 ml Triton X-100, and 1 liter scintillation-grade toluene (Nagy, 1983). Plasma, urine, or saliva samples may be pipetted without distilling them first, but the results will contain larger errors due to variable quenching and variations in dry matter content between samples (which reduces the amount of water per unit volume of sample). In general, these methodological errors translate into large errors in calculated FMR, thus distillation is recommended (Nagy, 1980; Nagy and Costa, 1980).

Deuterium is a stable (non-radioactive) isotope of hydrogen, consequently no permits
are needed for its use. Deuterated water also is inexpensive to purchase, but analysis of this isotope may be costly and time-consuming. Higher concentrations of $^2$H (above about 0.1%) can be measured directly in water distilled from biological samples by means of infrared absorbence (Zweens et al., 1980). This requires an infrared spectrophotometer with dual, temperature-controlled sample chambers. Conventional mass spectrometers can also be used to measure deuterium at higher concentrations (Mullen, 1973), and isotope-ratio mass spectrometers, being much more sensitive, can measure deuterium at low concentrations (Schoeller et al., 1980; Matthews and Bier, 1983). For mass spectrometry, it is necessary to convert the hydrogen in samples into hydrogen gas for measurement of the mass 3 to mass 2 ratio. This requires a high vacuum line, and is relatively more time consuming.

There are two isotopes of oxygen, $^{17}$O and $^{18}$O, that can be used in DLW studies. Oxygen-17 is very expensive, but its natural abundance is so low that only small enrichments, and hence small doses are required. Unfortunately, no procedures for measuring $^{17}$O in biological samples have been developed, although mass-spectrometry should work. Oxygen-18 is a stable isotope, as is $^{17}$O, and $^{18}$O has been the isotope used in most DLW studies to date. Like deuterium, $^{18}$O can be used at both high and low concentrations. At higher levels (0.05 atom % excess and above), proton activation analysis (Wood et al., 1975) provides relatively inexpensive (ca. $15 per sample) measurements, and large numbers of samples (40 per day) can be processed routinely. In this procedure, blood samples are first distilled, then 8 μl aliquants (in triplicate) are sealed in glass microcapillary tubes, bombarded with protons which convert $^{18}$O to gamma-emitting (radioactive) $^{18}$F, and counted in a gamma spectrometer.

Oxygen-18 can also be analyzed by mass spectrometry. This analysis requires one to 5 ml of blood, but techniques requiring smaller volumes of sample are being developed in several laboratories. The oxygen in water is transferred to CO$_2$, either by isotopic exchange with CO$_2$ added to the container holding the sample (Schoeller et al., 1980), or by reacting the sample with guanidine hydrochloride (Mullen, 1973) or with BrF$_5$ and carbon (Hails, 1977) to produce CO$_2$ from water. For samples containing higher $^{18}$O enrichments (0.05 to 1.8 atom % excess), the ratio of mass 44 (CO$_2$) to mass 46 (CO$_{18}$O) can be measured with a conventional mass spectrometer (Mullen, 1973). Lower $^{18}$O enrichments (0.002 to 0.02 atom % excess) can be measured using the newer, isotope-ratio mass spectrometers (Schoeller et al., 1980; Matthews and Bier, 1983). This procedure reduces costs for $^{18}$O purchase, because much lower doses are required to obtain measurable enrichments, yet analytical costs are higher.

3. FOOD CONSUMPTION

3.1. Gravimetric Methods

Methods for estimating food consumption of bats based on gravimetric measurements were summarized by Kunz (1980). Methods appropriate for energy budget analysis of free-ranging bats include those involving mass increments from feeding, recording the volume (or size) and number of prey items eaten, or measuring the amount of feces produced from food consumed. To insure that total nightly consumption can be determined, the number and duration of feeding bouts should be known. Knowledge of water content, energy density, and the assimilation efficiencies of different foods are needed to convert prey or fecal mass to metabolizable energy.

3.1.1. Ingesta

One approach that has been used with moderate success for some species is to weigh individuals before and after each feeding period (Wimsatt, 1969; Kunz, 1974; Anthony and Kunz, 1977; Funakoshi and Uchida, 1975,
Ideally, it would be valuable to weigh the same individuals before and after each feeding period, but recapture success of marked individuals on the same night is often extremely poor. This method of estimating nightly food consumption is usually made by comparing average mass increments from samples of the same cohorts (e.g., age, sex, reproductive condition, etc.) for a given species. A major assumption is that the bats captured after having fed have the same mean mass (without food) as those actually weighed at the time of emergence (Wimsatt, 1969). This assumption is likely to be violated especially during pregnancy when females (carrying embryos) exhibit a wide range of developmental stages, and pregnant females in the pre- and post-feeding samples may differ in their average stages of pregnancy (Kunz, 1980).

If bats have two feeding periods, the prefeeding mass for the second period can be determined by weighing bats upon emergence from their night roost. Alternatively, this prefeeding mass can be estimated by subtracting the amount of feces and water lost during the night-roosting period from the body mass of bats as they enter the night roost (e.g., Anthony et al., 1981). The mass increment from the second feeding period (using the actual or adjusted mass at the end of the night-roosting period) is added to the increment from the first feeding period to estimate total nightly wet mass consumption. This procedure assumes that body mass losses due to evaporation and urination during the foraging period are balanced by water intake, or can be corrected for by measuring mass losses of bats that did not feed (i.e., caged animals over the same period).

Mean or individual mass increments from each feeding period can be converted to dry mass equivalents and multiplied by the energy density (kJ/g) of the food consumed. Energy equivalents of field diets or stomach contents are determined by calorimetry. Energy density for some insect prey and stomach contents of Myotis lucifugus are summarized in Chapter 12. Metabolizable energy is determined as the product of the dry mass energy equivalent and the percent assimilation efficiency. When possible, it is advisable to determine energy density of prey items that were captured at the same time and place where bats were observed feeding.

Direct observations of bats that transport food items to feeding roosts can sometimes yield data on amounts of food eaten (e.g., Morrison, 1978; Vehrencamp et al., 1977; Bell, 1982), but caution should be exercised in determining what is actually consumed. Some items transported to feeding roosts may be partly culled before being eaten (see Chapter 11). In some instances only the juices of fruits and leaves are ingested and pulp is rejected as fibrous pellets (Gardner, 1977). In other situations only parts of insects are ingested and wings and head capsules are culled by the bats (Chapter 11). Nutrient analysis (Chapter 15) and energy density should be determined only for those parts or items of food known to be ingested.

Some potential errors in estimating energy intake can be avoided by feeding bats in captive situations. Thomas (1984) calculated the amount of fruit eaten as the difference between the mass of fruit offered at the beginning of an ad libitum feeding period and the rejected fruit at the end of a designated feeding period (after correcting for the amount of water that evaporated from the fruit). It should be emphasized, however, that some bats in captivity may overeat (see Chapter 16). Moreover, energy expended on activity in captive situations is likely to be lower than under free-ranging conditions. However, thermoregulatory costs may be higher in captivity unless bats are maintained at temperatures near thermoneutrality (Chapter 20).

### 3.1.2. Egesta

Daily metabolizable energy of individual bats can be estimated from the nightly production of feces. By knowing the dry mass and energy density of feces produced on a given night, and correcting this for assimilation
efficiency of field diets, one can estimate the amount of metabolizable energy available to a bat (e.g., Kunz, 1980; Kurta, 1986). This approach has also been used to estimate energy intake in brooding House Martins (e.g., Bryant and Westerterp, 1983).

Collecting feces from insectivorous bats in the field may be accomplished by capturing individuals as they return from feeding and holding them individually in separate containers until all feces have been voided (e.g., Anthony and Kunz, 1977; Anthony et al., 1981; Swift et al., 1985; Kurta, 1986). The number of separate feeding periods can be determined from direct observations (Chapter 4), trapping (Chapter 1), or by monitoring the activity of bats with radio-transmitters (Chapters 4 and 7, and Section 4.3). To ensure that all feces are voided from a given feeding period, individual bats should be held in containers until the normal departure time on the following evening. If one or more feeding periods have been documented, feces should be collected from two different cohorts, one captured upon return from the first feeding period and the other captured after the second feeding period. The sum of the total dry mass is used to establish nightly energy intake. For example, if a bat produced a total of 0.20 g of dry feces and the assimilation efficiency was 0.88, the total dry mass of food would be 1.66 g or \([1/(1 - 0.88)] \times 0.20\). Assuming that the energy density of feces was 19.25 kJ/day, the nightly ingested energy available to this bat would be \((1.66 \times 19.25 = 31.95\,\text{kJ})\).

There are advantages and disadvantages in using this method to estimate daily energy intake. An important advantage is that energy intake may be estimated for individual bats. A disadvantage, as with the ingesta method, is that it may yield less than complete estimates of energy intake (see Kurta, 1986). This would occur if bats are captured before a feeding period has been completed or captured after some of the digested meal has already passed as feces. The former situation is avoided if bats return to their roost (and are captured) immediately after having fed.

Owing to high rates of food passage in some species (e.g., Buchler, 1975; Kunz, 1980; Funakoshi and Uchida, 1980), some fecal loss can be expected during feeding flights and before bats return to a roost. Kunz (1980) estimated that nightly fecal loss by insectivorous bats in flight was approximately 5% of total daily fecal production for species that return to a night roost after an early feeding bout. It may be higher for other species that feed continuously or intermittently throughout the night and do not use communal night roosts. The egesta method is not suitable for most frugivorous, nectarivorous, and sanguivorous bats because there is little or no easily “identifiable” fraction of digested material in the feces as there is with insectivorous bats. Rapid food passage time and fecal loss also is higher in frugivorous and nectarivorous species (Klitz, 1965; Wolton et al., 1982), making it difficult to collect feces from a single feeding period.

3.2. Isotope Dilution

Isotope dilution methods hold considerable promise for estimating food intake in bats. When an animal is labeled with an isotope \(^2\text{H}, \text{H}, \text{or} \quad ^{22}\text{Na}\) its specific activity will decline exponentially with time owing to the respiratory and/or excretory loss of isotopes, and the simultaneous dilution of isotopes through the gain of unlabeled water or mineral from food, drink, and metabolic water production. The rate of decline of specific activity of an isotope can be related to food intake (e.g., Nagy, 1975; Green, 1978). Isotope dilution can also be used to estimate daily milk input to suckling young (see Section 3.3.1).

3.2.1. Estimates from \(^3\text{H}\) Turnover

Because the DLW water method allows the determination of gross water flux by measuring the turnover of \(^3\text{H}\text{HO}\) (Section 2.1), it may be used to determine the daily food intake (e.g., Nagy, 1975; Shoemaker et al., 1976; Helversen and Reyer, 1984; Bell et al.,
This method is valid if the source of ingested water comes only from the food eaten (no drinking of water), or if the rate of consumption of free-standing water is known. In these cases, the portion of total water influx due to dietary water (preformed and metabolically formed) can be determined. Helversen and Reyer (1984) used this method to estimate nectar intake by *Anoura caudifer*. Other bats that obtain water from their food (e.g., frugivorous, sanguivorous, and some insectivorous species) should be suitable subjects for estimating food intake using this method. Bell et al. (1986) estimated nightly food intake in the desert bat, *Macrotus californicus*, which gains its water from the insects it eats and from metabolic water.

If the water content of the food is known, one can determine how much dry mass of food is needed to equal the measured amount of dietary water gain (equation 2 of Nagy, 1975). The metabolic water yield of the food can be determined by knowing the chemical composition and metabolizability of the ingested food. Alternatively, metabolic water production can be estimated from DLW measurements of energy metabolism in the field.

### 3.2.2. Estimates from Doubly Labeled Water (*^3^H^2^H^1^H^2^O*)

The rate of food consumption required to provide the metabolizable energy for an animal can be calculated from its FMR (Section 2.4) and knowledge of its diet. This approach requires an assumption that the animal eats no more and no less food than it needs to satisfy metabolic expenditures (i.e., it was in energy balance, or steady-state). Rates of CO₂ production are then converted to energy units based on chemical composition of the diet (Nagy, 1983).

### 3.2.3. Sodium Dilution

A technique described by Green (1978) to estimate food intake in the dingo (*Canis familiaris dingo*) is based on the turnover of ²²Na. This approach has not been tried but should work with bats. An important assumption is that there is no major source of Na intake (e.g., salt water) during the experiment. This technique is based on observations that an animal has two Na compartments, a non-exchangeable compartment (metabolically inert) and an exchangeable compartment containing about 75% Na. An animal is given an injection containing 20 μCi ²²NaCl and the isotope is allowed to equilibrate. Weighing, injection, bleeding, equilibration, blood sampling, and sample preparation procedures are as described above (Section 2.4). The specific activity of ²²Na is determined using a liquid scintillation counter. The ²²Na turnover rates and rates of food intake are calculated using equations in Green (1978). Knowledge of Na content (mg/kg) in the diet is required to calculate food intake. Sodium content of food items can be determined by using atomic absorption spectrophotometry (see Green and Eberhard, 1984).

### 3.3. Milk Yield and Energy Intake by Sucklings

Several methods have been used to quantify milk production in wild and domesticated mammals. These include mass differences of sucklings before and after feeding, timed milking, isotope dilution, and isotope transfer (reviewed in Oftedal, 1984a). For bats and most other small, free-ranging mammals, the first two methods are impractical. Isotope dilution and transfer methods (e.g., Macfarlane et al., 1969; Green and Newgrain, 1979) have been used successfully to estimate milk yield by lactating females and milk intake of sucklings in a variety of marsupial and placental mammals (Oftedal, 1984a, 1986). These methods hold considerable promise for studies on free-ranging bats (Kunz and Nagy, unpubl.).

#### 3.3.1. Tritium Dilution

This method requires that milk is the only source of exogenous water to sucklings. Although this method is limited to sucklings
before they begin to take solid food, it can yield valuable information on maternal investment and energy intake during the early postnatal growth period. Tritium is injected into suckling bats and milk consumption is measured from the exponential decline of isotopes in the body fluid of the suckling. The use of $^3$H/HO is an improvement over using $^3$HOO, because TBW can be estimated from the $^3$HO dilution space instead of $^3$H (see Nagy, 1980). This makes it possible to determine FMR as well as water flux of the suckling.

Sources of error associated with estimating milk intake from $^3$HOO dilution in suckling mammals were reviewed by Ofstedal (1984a, 1986). These include: 1) incorporation of isotopes into non-exchangeable hydrogen sites in newly synthesized tissue, 2) reduction of isotope concentration due to increasing size of the body water pool (Dove and Freer, 1979), 3) recycling of isotopes from suckling to mother (via maternal ingestion of urine and feces of sucklings) and from the mother back to the suckling by uptake of the isotope in milk (Baverstock and Green, 1975), and 4) entry of water into the body water pool from sources other than milk (e.g., ingested whole food and metabolized fat). Because young bats grow rapidly, the first source of error could be large (Lewis and Phillips, 1972). Error 2 can be eliminated by use of appropriate flux equations (Nagy and Costa, 1980). Equations to correct for error 3 have been suggested (Baverstock and Green, 1975; Dove and Freer, 1979).

A protocol to control for isotope recycling using one pup from a litter as an unlabeled control was suggested by Baverstock and Green (1975). This would work for bats with litters of two or more, but most species only produce a single offspring. In situations where suckling young begin to feed on solid food (or regurgitated blood) before they are weaned (e.g., Wilkinson, 1985), alternative isotope transfer methods may be more appropriate (Holleman et al., 1975). One such method involves the use of two isotopes (tritium and deuterium); first by measuring the transfer of tritium from milk of the mother to the suckling and then by determining the turnover of deuterium oxide ($^2$HOO) in the suckling (Holleman et al., 1975; Wright and Wolff, 1976). Other methods, involving the combined use of $^3$HOO and $^3$HOO (or some other isotope) in which both mother and suckling are labeled, are difficult to use with free-ranging bats because they require the simultaneous capture and recapture of the mother and sucklings. Under most circumstances capture and recapture of the mother and suckling together is extremely difficult in communal roosts, especially as the suckling matures and spends less time attached to its mother.

### 3.3.2. Sodium Dilution

Green and Newgrain (1979) described a method that relates the rate of $^{22}$Na turnover in sucklings with milk consumption. Animals are injected with $^{22}$NaCl ($5 \mu$Ci $^{22}$Na is diluted in 100 $\mu$l NaCl solution). Animals are bled and the samples are processed as described above (Section 2.4). Sodium-22 activity is determined with a liquid scintillation counter, and total Na content of blood is determined with an atomic absorption spectrometer.

The major advantage of using $^{22}$Na for estimating milk intake is that it avoids potential errors associated with pulmocutaneous exchange between litter mates and the mother (Friedman and Bruno, 1976). Use of $^{22}$Na instead of $^3$HOO also avoids the problem of the isotope being incorporated into milk solids during milk synthesis. Moreover, considerably less $^{22}$Na is recycled to the suckling via milk secretion (Green and Newgrain, 1979) as compared to $^3$H when $^3$HOO is used (Baverstock and Green, 1975).

### 3.3.3. Milk Energy Output

If age-specific milk intake (Section 3.3) and energy density of milk are known (e.g., Kunz et al., 1983), total milk energy output of lactating females can then be calculated for different stages of lactation. This latter value can be used as a measure of maternal investment (e.g., Trivers, 1972) and reproductive effort
(e.g., Williams, 1966). It should be noted that energy estimates will be in error if the analyzed milk samples are not representative. Data that do not distinguish stages of lactation are not sufficiently accurate for energy calculations (Ofstedal, 1984b).

3.3.4. Predictions from Allometric Equations

When it is impractical to estimate milk energy input-output using isotope turnover, an estimate may be derived for peak lactation using an appropriate allometric equation. Prediction of milk energy yield derived from metabolic mass \((m^{0.83})\) of sucklings in kilograms (Ofstedal, 1984a) appears to be an improvement over most previously published equations (e.g., Brody, 1945; Blaxter, 1961; Linzell, 1972; Hanwell and Peaker, 1977). One should be reminded, however, of the potential errors in allometric equations derived from the analysis of different taxa (see Harvey and Mace, 1982; Huesner, 1982).

4. TIME-BUDGET METHOD

4.1. Rationale and General Procedures

The time-budget method for estimating energy expenditure in animals is derived from observed daily activity budgets and averages of laboratory-derived estimates of energy expenditure for various activities. Time budgets are often difficult to estimate accurately, and many are commonly subdivided into only crude categories. Recent efforts to examine the reliability and sensitivity of time budgets in relation to variation in their major components (Travis, 1982; Weathers et al., 1984) have shown that time-budget estimates can vary markedly, depending on the model chosen and the kind of data that are used. Timed signals are best analyzed using an instantaneous time-sampling method (e.g., Altmann, 1974; Jacobsen and Wiggins, 1982).

The time-budget method has become increasingly used in the study of birds (e.g., King, 1974; Kendeigh et al., 1977; Walsberg, 1983) and several species of medium to large diurnal mammals but there has been few such studies on small mammals. The secretive and nocturnal behavior of most small mammals has generally hampered time-budget studies on this group. Notwithstanding, many bat species are ideal subjects for time-budget analyses. Their synchronous nightly departure and return behavior (reviewed in Erkert, 1982), combined with communal roosting behavior (in several species), potentially allows the establishment of time budgets from direct observations (Kunz, 1980). In some situations it is necessary to determine time budgets from a composite assessment of colony behavior (e.g., Burnett and August, 1981), including mean emergence and return times (Kunz, 1980), and the amount of time that day and night roosts are occupied (Anthony et al., 1981; Barclay, 1982). Moreover, observations of individually marked bats are possible using night vision devices, light tags (Chapter 4), and radiotelemetry (Chapter 7).

4.2. Roosting Activity

Time budgets of roosting bats may include time allocated to different behaviors, including grooming, crawling, resting, and intra-specific interactions (e.g., Burnett and August, 1981), as well as account for the energetic consequences of clustering and torpor (e.g., Trune and Slobodchikoff, 1976; Kunz, 1980; Kurta, 1986). Ideally, a roosting time-budget should integrate animal characteristics with radiative and convective properties of the environment (see Chapter 19). Meteorological sensors (including taxidermic mounts) can be used to measure the \(T_c\) (operative temperature) of natural roosts. For bats
that roost in protected shelters (e.g., caves, tree cavities, buildings), time-budget models that include heat-transfer parameters can be simplified by omitting measurements of forced convection and direct solar radiation. Estimates of roosting costs require laboratory-derived measurements of metabolic expenditure for each activity, but few such measurements have been made for bats. Exceptions include costs of thermoregulation at different temperatures and energy conserved by clustering. Metabolic costs for roost activities such as grooming and crawling continue to be based on unverified estimates from other taxa (Kunz, 1980).

4.3. Foraging Activity

Most energy budget studies of bats (e.g., Kunz, 1980; Burnett and August, 1981; Helversen and Reyer, 1984; Kurt et al., 1987) have used Thomas’ (1975) allometric equation 36 to estimate flight costs. Despite the attractiveness of this equation, caution is urged in applying it uncritically to estimate flight costs in species having different foraging strategies. At best Thomas’ equation predicts minimum power required for level flight; it does not include flight costs associated with aerial insect pursuit, hovering, obstacle avoidance, and other activities associated with different modes of flight. Moreover, extrapolation of estimates to animals having a body mass less than empirically measured estimates (<60 g) is questionable.

The development of miniature (<1 g) radio-transmitters (Chapter 7) has made it possible to quantify the amount of time individuals of some species spend on the wing. Nightly time budgets based on signals from radio-tagged bats can be quantified using one of several methods. Continuous records from one individual can yield the most accurate time budgets, but this approach is extremely time-intensive and yields only a minimum amount of data. Automated data logging of signals over short intervals (less than one min) can essentially provide a continuous record for several individuals. Cooper and Charles-Dominique (1985) described a microcomputer data acquisition system for use in monitoring the flight activity of radio-tagged bats. This system allows data collection in the field with a portable cassette tape recorder and can be easily adapted to ordinary radio-tracking equipment.

With the possible exception of aerial insectivores, foraging time should not be interpreted as “flight time.” Even some insectivorous species pause intermittently to ingest prey in the course of their nightly foraging activity (Kunz, 1982), and some species are known to capture their prey on the ground (Chapters 11 and 12). Moreover, the foraging activity of some insectivorous, frugivorous, carnivorous, and sanguivorous bats may involve little actual time in flight, except for commutes between roosts and feeding areas.

A procedure that we have used for estimating foraging costs combines time-budget data from radio-tagged bats and estimates of FMR from DLW. Foraging costs are determined by regressing FMR of radio-tagged bats against time spent in flight. This approach also has been used to estimate flight costs for birds (Hails, 1980; Turner, 1983; Flint and Nagy, 1984b) and swimming costs for penguins (Nagy et al., 1984).

Foraging costs may also be estimated by subtracting roosting time-energy budgets from daily energy expenditure (Section 3.1). This indirect method should be suitable for species whose roosting time and energy budgets can be determined from direct observation (Burnett and August, 1981), where roosting metabolism can be measured (Stack, 1985; Bell et al., 1986; Kurt, 1986) and where the species are too small to carry radio-transmitters. This approach has been used successfully in studies of aerial feeding swallows and martins (Turner, 1983). It may underestimate foraging requirements of lactating females, however, since it does not take into account energy requirements associated with milk energy output.
5. RATES AND EFFICIENCIES OF ENERGY TRANSFER

5.1. Integration of Complete and Partial Energy Budgets

Rates and efficiencies of energy transfer derived from complete and partial energy budgets are useful for describing daily and seasonal patterns of energy expenditure (see Brody, 1945). More importantly they can be used to test predictions from life-history theory (e.g., optimal foraging, reproductive effort, and parental investment). Integration of partial and complete energy budgets are made possible when a combination of time-budget, body composition, food consumption, and isotope methods are used. Even when energy investment is measured in terms of biomass, data on transfer efficiencies and costs of producing these tissues are often difficult to obtain. In calculating energy transfer efficiencies it is important to specify what category of efficiency (gross or net) and what category of food (gross, digestible, or metabolizable) is being used.

5.2. Feeding Rates and Foraging Efficiencies

Feeding rates can be estimated as the ratio of FMR to the metabolizable energy content of the diet (Section 3.2.2). These rates can be converted from units of dry mass to units of wet mass using appropriate conversion factors for different food items in field diets. Feeding rates calculated in this manner are those needed to meet maintenance costs and do not include the allocation to, or metabolism, of stored energy reserves.

If the total energy expenditure of an animal is known and the assumption is made that an individual is in energy balance (metabolizable energy = expended energy), foraging efficiency needed to achieve a balanced energy budget can be calculated as the ratio of metabolizable energy gained while foraging to the energy expended while foraging (Wolf et al., 1975; Nagy and Shoemaker, 1984). Foraging efficiency calculated in this manner indicates how much more metabolizable energy is acquired from foraging than is expended in the process of food acquisition. Foraging expenditures can be determined as described in Section 4.3. Metabolizable energy gained while foraging can be determined by using results from DLW (Section 2), nightly food intake (Section 3), or time-budget methods (Section 4).

5.3. Parental Investment

An important part of the daily energy budget of an animal is the amount of energy allocated to reproduction. Some of this energy is expended on courtship and mating but, for female bats, most is spent on the actual production of young and on parental care. The concepts of parental investment and reproductive effort have been of considerable theoretical interest, but empirical data from free-ranging animals needed to test predictions lag far behind the theory. Methods described here and elsewhere in this volume can help to bridge this gap.

5.3.1. Fetal Production

The net cost of fetal production can be estimated from sequential measurements of energy density of embryo(s) during the pregnancy period. The cost of gestation theoretically can be determined by subtracting the daily energy expenditure of non-reproductive females from that of pregnant females at different times during pregnancy. However, because most females at maternity roosts are pregnant, opportunities to measure daily energy expenditure in non-reproductive individuals are rare. In temperate regions pregnant females also replace tissue depleted during hibernation; thus, a complete analysis of energy partitioning during pregnancy requires body composition analysis of females and their embryo(s) (Stack, 1983; Pierson et al., in prep.).
5.3.2. Lactation Costs

The most complete and direct analysis of energy expenditure during lactation can be derived from data on FMR and milk energy output of lactating females. However, attempts to compare the costs of lactating females with non-reproductive females are plagued by the same problem noted for pregnant females. At best, estimates of daily energy expenditure for lactating females can be compared with those for pregnant females. Available estimates based on metabolizable energy during pregnancy and lactation for *Myotis lucifugus* indicate that energy expenditure during lactation increases by 20-50% (Anthony and Kunz, 1977; Kurta et al., 1987).

5.3.3. Gross Efficiency of Milk Production

The efficiency of converting energy of food consumed to the milk energy produced by lactating females can be estimated as the ratio of milk energy yield (kJ/day) to the total food intake (kJ/day). Milk energy yield from females is assumed to equal the milk energy intake of sucklings (Section 3.3). Estimates of gross efficiency (including maintenance costs) of milk production have not been determined for bats. For dairy cows and goats (Brody, 1945), laboratory rats (Romero et al., 1976), and shrews (Dryden and Anderson, 1978), gross efficiency of milk production is on the order of 33% (range 28 to 40%). If similar values are found for bats this would suggest that approximately one-third of the energy consumed by a lactating female should be recovered in milk. The net efficiency of milk production in domestic animals (expressed as a percentage of metabolizable energy, excluding maintenance costs) is nearly double that of gross efficiency (Brody, 1945).

5.3.4. Efficiency of Growth

The literature on growth efficiency is confusing and ambiguous. In part, this reflects the fact that categories of dietary energy (gross, digestible, or metabolizable) are not always specified, the stage of growth may not be defined, or because appropriate conversion factors have not been used. It is also confusing unless a distinction is made between trophic level production (growth) efficiencies and individual growth efficiencies.

We follow Brody (1945) in referring to gross growth efficiency as the term for individuals converting metabolizable energy (ME) to new tissue during postnatal growth (G), expressed as the ratio G/ME. This is comparable to the term energetic growth efficiency as expressed by Ricklefs (1983). Thus, for suckling bats and other mammals, gross growth efficiency is expressed as the ratio of age-specific energy density of sucklings to the metabolizable energy of ingested milk. Net growth efficiency is expressed as the ratio of G/(ME - M), where G and ME are defined above and M is maintenance energy. Because measurement of maintenance costs in growing animals is problematical (see Millward and Garlick, 1976), estimates of net growth efficiency in free-ranging bats (and other mammals) remain elusive.

During postnatal growth, gross (energetic) growth efficiency decreases with increasing age because the amount of energy allocated to new tissue decreases in comparison to the size of the maintenance expenditure component. The greatest growth efficiencies have been observed shortly after birth, followed by a steady decrease as more energy is allocated to heat production (Brody, 1945).

No estimates of gross or net growth efficiencies have been reported for bats. Gross growth efficiencies reported for domestic mammals average 0.35 during early postnatal growth (Brody, 1945). Estimates of gross growth efficiency range from 0.12 to 0.37 for five species of captive rodents [reported as net growth (production) efficiency by McClure and Randolph, 1980]. Net growth efficiency as determined for several species of captive-reared mammals ranges from 0.59 to 0.78 (Millard and Garlick, 1976).
5.4. Energetics of Fat and Protein Deposition

The energy cost of fat and protein deposition is the increment of dietary energy (here expressed as metabolizable energy) required to promote an increment in fat or protein (Puller and Webster, 1977). Most bats deposit fat when energy input exceeds demand (e.g., during postnatal growth and in preparation for migration and/or hibernation) and mobilize it when energy demand exceeds energy input (e.g., at weaning and during hibernation). The most dramatic fluctuations in fat deposition occur in hibernating species (McNab, 1974), although tropical species also undergo seasonal changes in body fat (McNab, 1976) associated with reproductive events and periodic food shortages. The most rapid rates of protein deposition occur in the pre- and postnatal growth periods.

5.4.1. Fat Deposition

There are no published cost estimates of fat deposition for bats. Rates of fat deposition in bats may be estimated from data derived from in vitro or in vivo methods of body composition analysis (Chapter 23). Attempts to estimate the costs of fat deposition in bats are complicated by factors such as molt, migration, courtship, and daily torpor, each of which may occur simultaneously with fat deposition. Investigations of food consumption, body composition analysis, and energy metabolism offer challenging opportunities to partition the costs of fat deposition and its mobilization in bats. Reported values for growing rats (1.4 kJ of metabolizable energy per kJ of fat deposited) may be used as a first approximation for the cost of fat deposition in growing bats. The net efficiency of fat deposition (70 to 79%) in growing rats and pigs (Pullar and Webster, 1977; van Es, 1977) could be used as a testable prediction.

5.4.2. Protein Deposition

No cost estimates of protein deposition have been reported for bats. Estimates of protein deposition can be derived from nitrogen balance studies or body composition analysis (Thorbeck, 1977). Reported costs of protein deposition in laboratory and domestic animals, which average 2.3 kJ of metabolizable energy per kJ protein deposited (Pullar and Webster, 1977), could be used as a first order prediction for growing bats. The net efficiency of protein deposition in growing rats and pigs (36 to 77%) (Pullar and Webster, 1977; Thorbeck, 1977) varies considerably with age, but is the highest during the early period of growth.

6. COMPARISON OF METHODS

A time-budget model that incorporated occupied roost temperatures, energy conserved by clustering, and times spent foraging and roosting, indicated that daily energy expenditure in Myotis lucifugus was only 5.5% less than an estimate of metabolizable energy derived from nightly food intake (Kunz, 1980). Although differing considerably in methods and assumptions, this close agreement lends confidence to both methods for estimating daily energy expenditure in free-ranging bats.

There have been no independent assessments of daily energy expenditure in free-ranging bats, where DLW was one of the methods. Apparent discrepancies in estimates of daily energy expenditure for bats derived from DLW (Helversen and Reyer, 1984) and predictions based on Kunz’s (1980) allometric equation could reflect different methods, different life histories, and biases characteristic of interspecific allometric equations (Harvey and Mace, 1982; Huesner, 1982; Calder, 1984). Weathers et al. (1984) and Williams and Nagy (1984) reported that estimates of daily energy expenditure for birds derived from time budgets were 20 to 40% lower than those derived from DLW measurements. Hulversen and Reyer (1984) suggested that differences between estimates of daily energy expenditure derived from DLW turnover for the nectar feeding bat Anoura caudifer and
the allometric equation for bats (Kunz, 1980) could reflect a higher pace of life for flower-visiting bats. It is certainly reasonable that the costs associated with high speed flight and aerial maneuvers, during flower visitation, could explain the higher estimated daily energy expenditure (Helversen and Reyer, 1984), since Kunz's equation was based on limited data and included only one flower-visiting bat (Leptonycteris sanborni—Howell, 1979).

Relationships between food availability and food demand are important for understanding the ecological energetics and evolution of a species. Efforts should be made to include measurements of food availability in studies of energy expenditure (Chapters 12 and 14). This can be especially important in assessing individual variability in energy turnover. Moreover, failure to acknowledge the effect of food availability may account for discrepancies in estimates of daily energy expenditure derived from time-budget and DLW studies (Bryant and Westerterp, 1983).

7. SUMMARY

In this chapter we review methods of analyzing energy budgets of free-ranging bats. These include the use of doubly labeled water (DLW), food consumption, and time-budget analysis. For the DLW method, practical considerations include choice of appropriate subjects, decisions on cost of isotopes and their analyses, and verification of underlying assumptions. Potential errors associated with this method can be as high as ± 70%, although careful choice of the study animal and the sampling period can reduce the overall error to less than 10%. Estimates of food consumption include gravimetric and isotope methods. Limitations of the gravimetric method include low recapture rates after feeding, rapid food passage, and difficulties in establishing the number of feeding periods. Isotope methods for determining food intake are limited to animals that obtain their water from food and metabolic water. Time-budget analysis may involve direct observations, radiotelemetry, microclimate analysis, and laboratory-derived estimates of the energy expenditure for different activities. This approach is limited by the sensitivity of animals to direct observation, the imprecision in establishing time budgets for individuals, and the difficulty in quantifying energy costs for various activities.

Isotope methods offer considerable potential for estimating energy budgets of free-ranging bats. Use of DLW can yield direct estimates of daily energy expenditure, foraging costs, and changes in total body water (including estimates of milk intake of sucklings and milk output of lactating mothers). When estimates of daily milk output/input are combined with estimates of energy content of milk, it becomes possible to quantify the energetics of lactation and postnatal growth. In the final analysis, energy budget studies of bats can best be accomplished by using a combination of methods, including time-budgets, food consumption, and doubly labeled water.

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