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Estimating Body Composition of Lizards from Total Body Electrical Conductivity and Total Body Water

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Nondestructive methods of estimating body composition are crucial for measuring energy budgets of free-ranging animals. However, most methods have proved to be either difficult or inaccurate for estimating lipid mass, particularly in small animals. I validated the use of total body electrical conductivity (TOBEC) and total body water (TBW) to estimate lean mass and nonpolar lipid mass in the lizard *Sceloporus undulatus*. Regression models based on either TOBEC or TBW were able to predict dry lean mass and wet lean mass within 5% of actual values. Estimates of nonpolar lipid mass, derived by subtracting predicted wet lean mass from total body mass, were highly correlated with actual nonpolar lipid mass. When total nonpolar lipid mass was greater than 0.1 g, the average percent error in predicted nonpolar lipid was 30% and 15% for the TOBEC and TBW methods, respectively. A sensitivity analysis suggests that, in most cases, TBW can be used to estimate nonpolar lipid mass within 15% of actual lipid mass. Both TOBEC and TBW measurements are relatively easy methods of tracking qualitative changes in body composition within individuals, but TBW should be used when quantitative estimates of nonpolar lipid mass are desired.

THE principal goal of life historians is to understand the processes by which individuals acquire and allocate energy to explain the phenotypes that result from these processes (Dunham et al., 1989; Stearns, 1992). In nature, rates of acquisition and allocation processes differ frequently, such that animals are in positive or negative energy balance for periods of time. Nonpolar lipids represent a dynamic pool in which energy is stored, during periods of energy surplus, for use during periods of energy deficit (Derickson, 1976a; Fitzpatrick, 1976; Mrosovsky, 1976). The amount of nonpolar lipid reserves has a tremendous impact on an organism's ability to survive (Gosler, 1996; Alonso-Mejia et al., 1997) and reproduce (Humphries and Boutin, 1996; Henen, 1997; Jonsson et al., 1997). Moreover, the potential to store energy can influence the lifecycle, including age at maturity and frequency of reproduction (Reznick and Braun, 1987). Thus, data on lipid cycles of organisms in nature are critical for understanding the evolution of life histories.

Obtaining accurate data on lipid cycles is notoriously problematical. Accurate measures of nonpolar lipid content are possible for even minute samples using destructive methods, such as lipid extraction (Christie, 1982; Gardner et al., 1985). However, destructive methods constrain investigators to drawing inferences about lipid cycles in individuals by comparing nonpolar lipid levels among groups of individuals sampled at different times (e.g., Ballinger et al., 1992; Benabib, 1994; Scott et al., 1995), or among individuals of different ages or stages (e.g., Taghon et al., 1994; Heulett et al., 1995). If individuals of a population do not cycle uniformly, actual patterns of lipid cycling may be obscured, and spurious patterns may emerge. For example, consider a species that reproduces iteroparously. Individuals accumulate large fat reserves in nonreproductive years, which are then depleted in reproductive years. If individuals in a population do not reproduce in the same years, destructive sampling would yield a highly variable distribution of nonpolar lipid levels, with little information about the manner in which lipids cycle within individuals.

Several nondestructive methods of estimating nonpolar lipids have been tried with varying levels of success. Estimators such as changes in body mass (Humphries and Boutin, 1996) and condition indices (Gosler, 1996; Jakob et al., 1996) have been used to estimate changes in nonpolar lipid. Like lipid extraction, these estimators attempt to measure changes in nonpolar lipids directly. Unfortunately, the accuracy of methods based on body mass and condition indices is largely unknown and depends on both allometry and the relative allocation of energy to lean versus lipid mass. More recently, a method based on absorption of cyclopropane gas by animals has been shown to be highly accurate (Henen, 1991; Gessaman et al., 1998) but is difficult and labor intensive. Thus, the cyclopropane method is not likely to be a practical means of gathering an adequate amount of data in a reasonable duration.

The most practical nondestructive methods of estimating nonpolar lipid content are indirect methods. Indirect methods estimate lipid-free mass, or wet lean mass (M_w), from which nonpolar lipid mass (M_n) is derived. Usually, a regression model, describing the relationship between some independent variable and M_w, is used to predict the M_w of an individual. The predicted value of M_w is subtracted from the animal's body mass (M_b) to yield an estimate of M_n for the individual. Success of an indirect method depends on the strength of the relationship between the independent variable and M_w, as well as the accuracy with which the independent variable is measured.

Total body electrical conductivity (TOBEC) is an accurate predictor of the M_w of animals (Walsberg, 1988; Castro et al., 1990; Fischer et al. 1996). Electrical conductivity of wet lean tissue is much greater than that of nonpolar lipid; therefore, TOBEC of an organism is largely a function of the amount of M_w it contains. Walsberg (1988) demonstrated that there is a very strong relationship between M_w and TOBEC, which is linear in some organisms and exponential in others. He concluded that TOBEC could be used to predict M_n in small birds and mammals. Subsequently, investigators discovered that very small inaccuracies in estimates of M_w can lead to large inaccuracies in corresponding estimates of M_n due to the small proportion of M_b that is comprised of nonpolar lipid relative to lean tissue (Henen, 1991; Morton et al., 1991). Inaccuracy in the estimate of M_w is more problematical for smaller organisms, because the absolute amount of nonpolar lipid generally decreases with body size. Overall, validations of the use of TOBEC to estimate M_n have yielded mixed results. Some investigators have been able to accurately estimate M_n of birds and rats from TOBEC (Roby, 1991; Skagen et al., 1993; Guggenbuhl, 1995), but other investigators have not been able to do so (Bell et al., 1994; Asch and Roby, 1995; Lyons and Haig 1995). To date, the smallest organism for which the TOBEC method has been validated is the bluegill, *Lepomis macrochirus* [≈25–106 g (Fischer et al., 1996)].

Total body water (TBW) has also been used to estimate M_n (Boyd et al., 1993). Since nonpolar lipids are hydrophobic, TBW tends to be negatively correlated with M_n (Farley and Robbins, 1994), and positively correlated with M_w (Torbit et al., 1985). Thus, measures of TBW, such as those obtained from isotope dilution (Halliday and Miller, 1977; Schoeller et al., 1986), may be a practical nondestructive method of estimating nonpolar lipid content of a large number of organisms in a relatively short time period. To date, the TBW method has been applied mainly in studies of human body composition. As a result, this method has been validated by comparing the predictions of M_n from the TBW method to predictions derived from other nondestructive methods, such as anthropometry (Kabir and Forsum, 1993; Sheng et al., 1994; Fuller et al., 1996), total body potassium (Sheng et al., 1994; Hopkinson et al., 1997), TOBEC (de Bruin et al., 1994a; Sheng et al., 1994), magnetic resonance imaging (Sohlstrom et al., 1993), and bioelectrical impedance analysis (Gales et al., 1994; Fuller et al., 1996). The accuracy of the TBW method in small organisms is unknown.

In this paper, I evaluate the performances of the TOBEC method and the TBW method in estimating body composition in the lizard *Sceloporus undulatus.* Adult *S. undulatus* range from 5–15 g, which is considerably smaller than organisms on which these methods have been applied previously. I demonstrate that either TOBEC or TBW can be used to estimate qualitative changes in M_n of lizards. Furthermore, TBW predicts M_w and M_n with sufficient accuracy to construct energy budgets for free-ranging lizards.

MATERIALS AND METHODS

Study animals.—A total of 37 lizards were collected from the Savannah River Site, Aiken County, South Carolina. Ten lizards were collected in fall of 1995, and 27 were collected in summer of 1996. All lizards were kept in the laboratory for several months prior to measurements. Lizards were housed in 38-liter aquaria contained in an environmental chamber with an ambient temperature of 20 C. Full spectrum lighting was used to create a light cycle of 14L:10D, and each cage was heated by a 60W incandescent bulb during photophase. Vitamin-dusted crickets and water were available ad libitum. The 37 animals represented a wide range of body lengths (61–77 mm; $\mu = 68 \pm 1$ mm) and body masses (4.65–14.36; $\mu = 9.39 \pm 0.90$ g).

Measurement of TOBEC.—I measured TOBEC with an Animal Body Composition Analysis System (base unit Model SA-3000 and detection chamber Model 3044, EM-Scan, Springfield, IL). Prior to measurement, lizards were weighed to the nearest 0.01 g. Because body position affects TOBEC measurements greatly (Fiorotto et al., 1987), individuals were cooled until mobility was reduced (mean body temperature = 6.7 ± 2.2 C). For each reading, a lizard was placed on its dorsal surface on a plastic carriage, and inserted into the detection chamber for a minimum of 5 sec. Every effort was made to position lizards identically within the chamber. The heads of lizards were placed flush with a mark on the carriage. The carriage was inserted in the chamber such that a second mark was aligned with the edge of the chamber. Initial tests revealed that this position in the chamber resulted in maximum TOBEC values for an individual. Five successive readings were averaged to determine a raw TOBEC score for each animal. Generally, lizards remained motionless during the readings, but those that changed position were returned to the original position before the next reading. A minimum of three calibration readings were made before, during, and after animal measures, using a calibration rod supplied with the detection chamber. The electrical conductivity (E) of the empty carriage was measured 3–5 times at the beginning and the end of the study. The final TOBEC score was determined by subtracting the average score for the empty carriage from the average score for the carriage plus the animal. Prior to analysis, final TOBEC scores were corrected for snout–vent length (L) by the following transformation (EM-SCAN, Inc., unpubl.): $\sqrt{E \cdot L}$.

A blind test of the consistency of the TOBEC measurement procedure was performed. One animal was randomly selected and the procedure described above was repeated three times at ≥ 10 min intervals. To prevent any bias, I was unaware of the previous scores for the animal.

Body composition analysis.—Immediately after TOBEC readings, animals were killed by freezing. Frozen carcasses were freeze-dried for 72 to 144 h and weighed to the nearest 0.1 mg. The TBW of each lizard was calculated as the difference between wet body mass and dry body mass. Bodies were ground in a Wiley Mill and then homogenized with a mortar and pestle. Two aliquots, averaging 0.7 g, from each carcass were used to determine nonpolar lipid concentration by petroleum ether extraction. The samples were placed in preweighed cellulose thimbles and dried to a constant mass at 55 C. Nonpolar lipids were extracted with Soxtec HT2 and HT6 extraction units (Perstorp Analytical, Silver Spring, MD). Samples were oven-dried again and weighed. The difference in sample mass before and after extraction was used to calculate nonpolar lipid content (percent lipid) of each sample. Aliquot measures of percent lipid were averaged and multiplied by animal dry mass to determine total M_n of each animal. Measures of M_n from chemical extraction were used to evaluate the accuracy of estimates based on TOBEC and TBW.

Construction of regression models to predict body composition.—Regression models were constructed to predict M_w and dry lean mass (M_d) in *S. undulatus.* Two models were used to predict each variable, one based on TOBEC and the other based on TBW. Stepwise multiple linear regression analysis was performed using wet mass and either TBW or TOBEC as independent variables. Regression equations were used to predict M_w and M_d for the 37 lizards. For each lizard, M_w was subtracted from M_b to yield a predicted M_n. Thus, two predicted values of M_n were generated for each lizard, one based on a regression model including TOBEC and the other based on a regression model including TBW. For both methods, Pearson product-moment correlation and paired *t*-tests were used to compare predicted M_n to actual M_n determined by extraction. Statistical analyses were performed with Statistica for Windows. All descriptive statistics are reported as mean ± 95% confidence interval.

Sensitivity analyses.—Sensitivity analyses were performed to determine the effect of measurement error in regression model parameters on the accuracy of M_n predictions. A computer program, written and compiled in the C programming language, was used to systematically vary the parameters (TOBEC, TBW, and M_b) of both regression models and compare predicted M_n to a reference value for a hypothetical lizard. Results of the analyses were sensitive to M_b and nonpolar lipid content of the animal, so calculations were made for an animal of average mass (9 g) over the entire range of percent lipid observed in the 37 lizards.

RESULTS

Body composition.—The lizards represented a broad range of body compositions. Average water content was 72.3 ± 1.2%, and individuals ranged from well-hydrated (81.3% water) to severely dehydrated (63.6% water). Likewise, nonpolar lipid content was quite variable; average nonpolar lipid content was 4.3 ± 1.1%, and ranged from 0.5–11.8% of total body mass. Dry lean content averaged 23.4 ± 0.6%, ranging from 17.7–27.3% of total body mass.

TOBEC.—Total body electrical conductivity was repeatable within individuals. The average coefficient of variation for five measures of each lizard was 1.6 ± 0.2% (n = 37). Results of the

TABLE 1. MULTIPLE REGRESSION MODELS OF DRY LEAN MASS (M_d) AND WET LEAN MASS (M_w) CONSTRUCTED FROM STEPWISE LINEAR REGRESSION OF DATA ON TOTAL BODY ELECTRICAL CONDUCTIVITY (TOBEC) AND TOTAL-BODY WATER (TBW).

Method	Model	r^2	p
TOBEC	$M_d = (0.004 \cdot \text{TOBEC}) + (0.18 M_b) - 0.14$	0.944	<0.00001
	$M_w = (0.01 \cdot \text{TOBEC}) + (0.79 \cdot M_b) - 0.02$	0.991	<0.00001
TBW	$M_d = (0.38 \cdot \text{TBW}) - (0.27 \cdot M_b) + 0.38$	0.944	<0.00001
	$M_w = (0.78 \cdot \text{TBW}) + (0.34 \cdot M_b) + 0.38$	0.997	<0.00001

entire TOBEC procedure were also repeatable. The coefficient of variation for final TOBEC values from the blind trial was 0.7% (n = 3). As expected, there was a strong correlation between TOBEC and TBW (n = 37, $t = 7.65$, $r^2 = 0.62$, $P \ll 0.0001$).

Regression models of dry lean mass.—In both analyses (TOBEC and TBW), stepwise linear regression retained all independent variables and produced statistically significant models (Table 1). The 95% prediction intervals for TOBEC and TBW models were the same (± 0.28 g of M_d). On average, predicted values of M_d based on TOBEC and TBW deviated from actual values by 5.8 ± 1.8% and 5.3% ± 1.4%, respectively. Average percent error in predicted M_d was 0.8 ± 2.6% and 1.1 ± 2.2% for TOBEC and TBW models, respectively. In both cases, average percent error in predicted M_d was not significantly different from zero (two sided *t*-test, $P > 0.05$), indicating that the residuals were dispersed evenly above and below the regression line.

Regression models of wet lean mass.—Both TBW and TOBEC were highly correlated with M_w (Fig. 1). Examination of residuals indicated two points were extreme outliers. Because outliers can have a large effect on regression coefficients in analyses of small sample sizes, outlying points were removed for the purpose of generating regression models. Subsequently, all data points were used to determine the accuracy of predictions derived from the regression models.

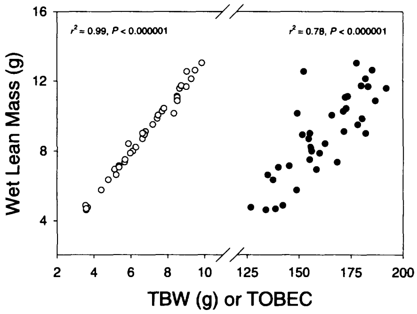

Fig. 1. The relationship between wet lean mass and two estimators of body composition, total body water (TBW) and total body electrical conductivity (TOBEC). Both TBW and TOBEC were significantly correlated with wet lean mass.

In both analyses (TOBEC and TBW), stepwise linear regression retained all variables, and produced statistically significant models (Table 1). The 95% prediction intervals were ± 0.44 g and ± 0.27 g of M_w for TOBEC and TBW models, respectively. The TBW model was slightly better at predicting M_w than was the TOBEC model. Residual M_w from the TOBEC model was significantly correlated with percent body water ($t = 3.13$, $r^2 = 0.23$, $P = 0.004$) but was not correlated with body temperature ($t = 0.18$, $r^2 = 0.001$, $P = 0.86$). No correlation was found between residual M_w from the TBW model and percent body water ($t = -0.97$, $r^2 = 0.28$, $P = 0.34$).

Accuracy of lipid mass estimates.—Predicted M_n derived from both the TOBEC model and the TBW model were highly correlated with actual M_n (Fig. 2). Paired *t*-tests established that predicted M_n and actual M_n were not significantly different in either case (TOBEC: $t = -0.83$, df = 36, $P > 0.05$; TBW: $t = -0.12$, df = 36, $P > 0.05$). Also, predicted values from the TOBEC model and those from the TBW model were not significantly different ($t = -0.76$, df = 36, $P > 0.05$). Mean error, expressed in percent lipid, was 0.2% (± 0.4%) and 0.6% (± 0.7%) for the TBW and TOBEC predictions, respectively. However, deviation of predicted M_n from actual M_n was considerable in some cases (Table 2), largely due to individuals consisting of < 0.5% lipid. When individuals with less than 0.1 g of nonpolar lipid were excluded (n = 8), average percent error in the estimation of M_n by TBW and TOBEC models was 14.7% (± 9.8%) and 30.3% (± 18.5%), respectively.

The sensitivity analysis indicates that TOBEC and TBW must be measured with great accuracy to obtain accurate estimates of M_n in cases where nonpolar lipid comprises a small portion

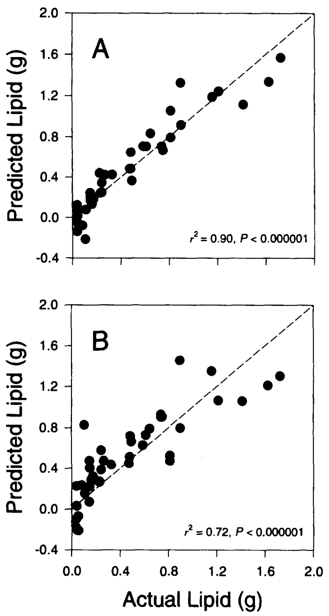

Fig. 2. The relationship between nonpolar lipid mass predicted from indirect estimation and nonpolar lipid mass measured by lipid extraction. Predicted nonpolar lipid mass was determined using two different regression models for estimating wet lean mass: (A) a model incorporating total body water and wet mass (M_w = 0.78 · TBW + 0.34 · M_b + 0.38); and (B) a model incorporating total body electrical conductivity and wet mass (M_w = 0.01 · TOBEC + 0.79 · M_b − 0.02).

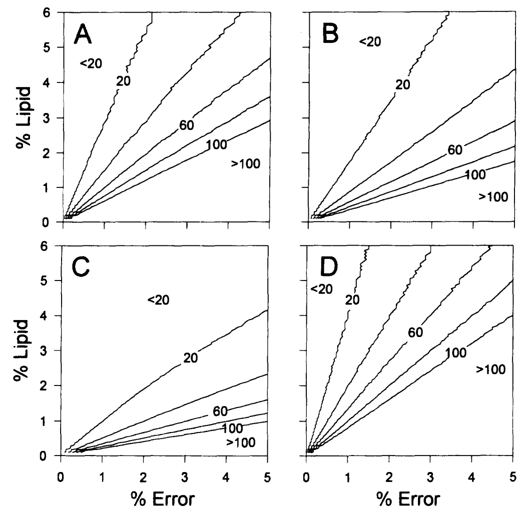

Fig. 3. Contour plots detailing the results of sensitivity analyses for regression models to predict wet lean mass. Contour lines denote the percent error in the estimation of nonpolar lipid mass resulting from the percent error in the independent variable (abscissa), given an individual's percent lipid (ordinate). Independent variables in the TBW model included TBW and wet mass (plots A and B, respectively), and those for the TOBEC model included TOBEC and wet mass (plots C and D, respectively). All analyses were performed for a 9 g lizard (see text for details).

of M_b (Fig. 3). For example, when body composition is only 1% lipid, 1% error in TBW and TOBEC would result in 60% and 20% error in M_n, respectively. However, for an individual of average percent lipid (4.3%), a 1% error in TBW and TOBEC would result in 13% and 4% error in M_n, respectively. Because percent lipid was positively correlated with body mass (t = 8.65, r^2 = 0.68, $P \ll 0.0001$), both methods are likely to be more accurate on larger individuals. Estimates of M_n for lizards weighing ≥ 9 g should be relatively accurate, because these individuals had greater than average lipid content (≥ 4%), whereas lizards weighing ≤ 6 g had less than 1% lipid.

DISCUSSION

Prediction of lean mass.—As expected, the TOBEC model predicted M_w accurately in *S. undulatus.* Previous studies have demonstrated that, except in rare cases (e.g., Lyons and Haig 1995), a strong relationship exists between TOBEC and M_w (Walsberg, 1988; Castro et al., 1990; Fischer et al., 1996). However, water content of M_w varies, and energy content of lean tissue is determined from M_d to eliminate po-

TABLE 2. ESTIMATES OF BODY COMPOSITION FOR THE 37 LIZARDS USED IN THE VALIDATION STUDY. Values in parentheses are 95% confidence intervals.

Body compartment	Actual (g)	Predicted TBW (g)	Percent error	Predicted TOBEC (g)	Percent error
Dry lean	2.18	2.19	1.1	2.18	−0.8
	(±0.20)	(±0.19)	(±2.2)	(±0.18)	(±2.6)
Wet lean	8.91	8.91	0.1	8.88	−0.2
	(±0.77)	(±0.77)	(±0.6)	(±0.76)	(±0.9)
Lipid	0.48	0.48	−29.0	0.51	−2.5
	(±0.15)	(±0.16)	(±41.2)	(±0.14)	(±75.8)

tential errors caused by variation in hydration (Congdon et al., 1982). Therefore, measurements of M_d, as opposed to M_w, are necessary to calculate the energy allocated to growth in field studies of energetics. The ability to predict M_w does not guarantee that M_d can be predicted. TOBEC, and obviously TBW, are sensitive to variation in hydration state (Cochran et al., 1989; Roby, 1991). Indeed, residual variation in TOBEC of lizards was significantly correlated with percent body water. Still, TOBEC and TBW models produced accurate predictions of M_d. Why not use M_b to predict M_d in cases where the two are highly correlated? A regression model based on wet mass may be an adequate estimator of M_d, but short-term (i.e., daily to weekly) fluctuations in M_b are likely to result from changes in body water, and in some cases M_n, rather than changes in M_d. Thus, M_b is not a suitable estimator of M_d in field studies.

Prediction of nonpolar lipid mass from TOBEC.—Estimates of M_n are always less accurate than the measures of M_w from which they are derived (Skagen et al., 1993; Asch and Roby, 1995; Fischer et al., 1996). This is partly because of the indirect nature of the calculation of M_n and the relative amount of M_n and M_w in animals. For most organisms, the proportion of body mass comprised of M_w is several times greater than that comprised of M_n. Therefore, any amount of error in M_w is magnified in the calculation of M_n, when M_w is subtracted from M_b (Morton et al., 1991). The very small average percent error ($< 1\%$) in the estimation of M_w resulted in a surprisingly large percent error in predicted M_n (Table 2). Other studies have shown that measures of M_w must be extremely accurate to predict M_n accurately (Henen, 1991; Skagen et al., 1993; Fischer et al., 1996).

The accuracy and precision of the TOBEC method depends on a great number of factors. Body geometry and positioning have severe effects on TOBEC (Fiorotto et al., 1987); therefore, individuals must be positioned identically and remain motionless throughout the procedure. Although anesthesia has been used (Guggenbuhl, 1995; Fischer et al., 1996), reducing the body temperature of ectotherms may be an easier, faster, and safer way to immobilize the subject. I found no relationship between body temperature and residual M_w, suggesting that TOBEC was not altered by cooling lizards. Because acute exposure to low body temperature may induce other physiological effects in ectotherms, animals should not be chilled below body temperatures that are experienced in nature (e.g., average nighttime body temperature). Also, variation in hydration state can potentially reduce the accuracy of M_w estimates (Cochran et al., 1989), by altering the concentration of ions in the body fluid. The TOBEC of an animal is known to be sensitive to changes in ion concentration (de Bruin et al., 1994b) and is highly correlated with TBW (Fiorotto et al., 1987; Cochran et al., 1988; Keim et al., 1988). However, hydration state may not always have consistent effects on TOBEC. I found a positive correlation between residuals of M_w and percent body water, but other studies have found either a negative relationship (Roby, 1991) or no relationship at all (Skagen et al., 1993; Asch and Roby, 1995). Still, another study reported that changes in extracellular fluid volume, by injection of saline, had no effect on TOBEC (Battistini et al. 1993). Daily fluctuation in hydration will make it difficult to track short-term changes in body composition but are less problematic for monitoring seasonal changes in M_w, which are likely to be driven by changes in M_d and M_n as well as water content. A reevaluation of regression coefficients on a regular basis will help increase the accuracy of M_w predictions in long-term studies (Bell et al., 1994).

If taken carefully, measures of TOBEC are very precise. The average coefficient of variation (V) for individuals in this study was 1.6%, and blind measurements indicated repeatability between trials. Assuming that 1.6% is a reasonable estimate of the magnitude of error in TOBEC measures, the TOBEC model would be capable of estimating M_n with less than 10% error. For example, 1.6% error in the TOBEC of a lizard of average percent lipid would result in 6% error in M_n. However, the high precision of TOBEC measures does not guarantee that they are accurate, and the coefficient of variation may underestimate measurement error in TOBEC. Based on results of the sensitivity analysis (Fig. 3), I calculated an expected value of percent error in M_n for data collected in this study. For each individual, I used actual percent lipid and assumed that measurement error in TOBEC was equal to the V in TOBEC for that individual. The analysis predicted an average absolute deviation in M_n of 17.0 ± 7.5%, which is significantly different from the observed deviation in M_n ($t = 3.96$, df = 36, $P < 0.001$). The discrepancy may mean that measures of TOBEC are less accurate than precise (percent error in TOBEC $> V$), that one or more key variables were excluded from the TOBEC model, or that measurement error in M_b also contributed to the inaccuracy of predicted M_n. The third possibility is a likely one, considering that the accuracy of predicted M_n is very sensitive to measurement error in M_b (Fig. 3). Therefore, studies should be designed to eliminate sources of error in wet mass and TOBEC by measuring an animal with a clear digestive tract (Bachman, 1994).

If the TOBEC method is to be of use in field studies of lipid cycling, it must be accurate enough to detect seasonal and annual changes in lipid masses of individuals. The TOBEC model provided estimates of M_n in *S. undulatus* that were highly correlated with actual M_n (Fig. 2). On average, TOBEC was able to predict percent lipid with an error of 0.6% ± 0.7%. This level of accuracy is sufficient to perceive lipid cycles in free-ranging lizards. For example, whole body lipids of *S. undulatus* in a Kansas population varied from 100 mg M_d^{-1} ($\approx$2.4%) to 300 mg M_d^{-1} ($\approx$7.2%) during the active season (Derickson, 1976b). Also, fat bodies in other sceloporine lizards undergo great fluctuations in mass [e.g., from 4 to 153 mg (Derickson, 1974)] within a single season. Given the magnitude of lipid cycles in lizards, TOBEC should be a useful method for assessing seasonal variation in nonpolar lipids in free-ranging individuals. Previously, the smallest organism for which TOBEC models provided useful estimates of M_n was 25 g (Fischer et al., 1996). Clearly, the TOBEC method must be carefully evaluated in each species, to assess the utility of the method.

Prediction of nonpolar lipid mass from TBW.—Ecologists have made a greater effort to validate the TOBEC method than the TBW method, but TBW is a more accurate estimator of M_w than is TOBEC. In the few existing studies, a significant negative correlation between TBW and M_n was discovered (Table 1; Torbit et al., 1985; Farley and Robbins, 1994). Moreover, M_n estimates from TBW are consistent with those based on anthropometry (Kabir and Forsum, 1993; Sheng et al., 1994), total body potassium (Sheng et al., 1994), underwater weighing (Sohlstrom et al., 1993), and TOBEC (de Bruin et al., 1994a; Sheng et al., 1994). Likewise, the TBW method provided accurate predictions of M_n in *S. undulatus* (Table 2; Fig. 2). Average error in percent lipid (0.2 ± 0.4%) was one-third of that for the TOBEC method. For animals with greater than 0.1 g of nonpolar lipid, TBW predicted M_n with an average percent error of 15%. Furthermore, the mean and variance of predicted M_n and actual M_n were almost identical (Table 2), suggesting that the TBW method is a valuable tool for determining nonpolar lipid levels in a population of animals.

Use of isotope dilution space to measure TBW is a potentially powerful tool for obtaining nondestructive estimates of M_n in *S. undulatus*. However, care must be taken to minimize the error associated with isotopic estimates of TBW. The sensitivity analysis indicates that a very small error in TBW ($\approx$1%) can lead to a large error in M_n (Fig. 3). TBW can be measured as deuterium dilution space to better than ± 0.5% (Halliday and Miller, 1977), but an overestimation by 1–4% occurs frequently when hydrogen isotopes are used to determine TBW (Halliday and Miller, 1977; Nagy and Costa, 1980). More accurate measures of TBW can be obtained by ^{18}O enrichment instead of ^{2}H or ^{3}H enrichment (Halliday and Miller, 1977; Nagy and Costa, 1980). Additionally, one can minimize error associated with dilution space measurements of TBW by ensuring the digestive tract is clear (K. A. Nagy, unpubl.) and using isotope ratio mass spectrometry for sample analysis (Nagy, 1989).

Advantages of indirect methods.—Indirect methods of estimating M_n, such as TBW and TOBEC, are inherently less accurate than direct methods (Henen, 1991; Morton et al., 1991). Error in estimates of M_n from TOBEC and TBW are typically greater than 10%. In comparison, estimation of M_n by cyclopropane absorption yields percent errors of $\leq$ 5% (Gessaman et al., 1998; Henen, 1991). Therefore, comparing the performances of TOBEC and TBW methods to other methods might lead one to conclude that TOBEC and TBW methods are not suitable for use in studies of energy allocation. However, the accuracy of a technique cannot be the only consideration in choosing a suitable method for ecological studies.

The TOBEC and TBW methods have several advantages over other techniques for estimating M_n. Both methods are easy to learn and relatively rapid; several dozen individuals can be measured in one or two days. The cyclopropane method is more accurate but requires considerable time and technical skill (Henen, 1991; Gessaman et al., 1998). Lipid extraction techniques are destructive, and preclude measurement of lipid cycles within individuals. The ease and speed of TBW and TOBEC measurements permit application of both methods in conjunction to increase one's confidence in estimates of body composition. At present, the TBW and TOBEC methods are the most practical means of obtaining data on lipid cycles of free-ranging individuals. Incorporating these methods into field studies of energetics will permit ecologists to better resolve the mechanisms that underlie life-history phenomena.

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