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ESTIMATION OF LIPIDS AND LEAN MASS OF MIGRATING SANDPIPERS[1]

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Abstract. Estimation of lean mass and lipid levels in birds involves the derivation of predictive equations that relate morphological measurements and, more recently, total body electrical conductivity (TOBEC) indices to known lean and lipid masses. Using cross-validation techniques, we evaluated the ability of several published and new predictive equations to estimate lean and lipid mass of Semipalmated Sandpipers (*Calidris pusilla*) and White-rumped Sandpipers (*C. fuscicollis*). We also tested ideas of Morton et al. (1991), who stated that current statistical approaches to TOBEC methodology misrepresent precision in estimating body fat. Three published interspecific equations using TOBEC indices predicted lean and lipid masses of our sample of birds with average errors of 8–28% and 53–155%, respectively. A new two-species equation relating lean mass and TOBEC indices revealed average errors of 4.6% and 23.2% in predicting lean and lipid mass, respectively. New intraspecific equations that estimate lipid mass directly from body mass, morphological measurements, and TOBEC indices yielded about a 13% error in lipid estimates. Body mass and morphological measurements explained a substantial portion of the variance (about 90%) in fat mass of both species. Addition of TOBEC indices improved the predictive model more for the smaller than for the larger sandpiper. TOBEC indices explained an additional 7.8% and 2.6% of the variance in fat mass and reduced the minimum breadth of prediction intervals by 0.95 g (32%) and 0.39 g (13%) for Semipalmated and White-rumped Sandpipers, respectively. The breadth of prediction intervals for models used to predict fat levels of individual birds must be considered when interpreting the resultant lipid estimates.

Key words: Lipids; lipid estimation; energetics; total body electrical conductivity; TOBEC; sandpipers.

INTRODUCTION

Knowledge of lipid dynamics is central to understanding many ecological relationships between animals and their habitats, and in turn contributes to a pool of knowledge essential to wise management of wildlife resources. Body condition indices based on estimated lipids have been developed for waterfowl (Ringelman and Szymczak 1985) and shorebirds (Page and Middleton 1972, Davidson 1983, Piersma and Van Brederode 1990) using measures of body mass and morphological features. Walsberg (1988) introduced the TOBEC (total body electrical conductivity) methodology as a promising and nonlethal way to determine fat stores in wild animals. In this method, an EM-SCAN Small Animal Composition Analyzer differentiates lean mass from lipids based on electrical conductivity patterns and provides signal output based on lean body mass. (Use of trade names of commercial products does not constitute endorsement of the U.S. Government.) Walsberg (1988) presented a curvilinear relationship between signal output and lean body mass for a sample of 14 species of birds. Castro et al. (1990), expanding on Walsberg's (1988) study, presented another interspecific curve relating lean mass and signal output, and suggested that the technology is useful in intraspecific studies.

Walsberg (1988) and Castro et al. (1990) suggested that TOBEC estimates of lean mass are useful in estimating lipids, yet neither proceeded to the next step, presumably to estimate lipid reserves by subtracting estimated lean body mass from total body mass. Morton et al. (1991) introduced concern that the coefficients of determination for lean mass regressions misrepresent the precision with which lipid mass can be estimated. The absolute error is the same for both lean and lipid mass, but the error represents a larger fraction of lipid than lean mass. Thus, the simple algebraic manipulation disregards the variation associated with lipid mass. We agree that this basic point is central to understanding

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the contribution of current TOBEC methodology in lipid estimation.

There are convincing arguments about the positive attributes of TOBEC technology. It is rapid, noninvasive and nonlethal, and therefore is a marked improvement over costly and lethal laboratory lipid extractions (Walsberg 1988, Castro et al. 1990). Field applications of TOBEC technology, however, are logistically more difficult and expensive than taking standard field measurements of body mass and morphological traits. We wanted to quantify the contribution of this technology to our ability to estimate lipids and to use this perspective in our decisions on whether to invest further resources in this technology. Such a decision could be based on a benefit-to-cost analysis incorporating a quantified estimate of the improvement to predictive models, the purchase price and cost of operation of the technology, and the level of precision necessary to address the ecological questions of interest.

This paper (1) describes how well published interspecific equations predict lean mass, and by calculation, lipid mass of a new sample of birds, thereby testing the ideas of Morton et al. (1991), (2) evaluates the contribution of current TOBEC methodology to lipid estimation beyond using body mass and standard morphological measurements, and (3) generates preliminary equations to estimate lipid stores in two species of small shorebirds, the Semipalmated Sandpiper (*Calidris pusilla*) and White-rumped Sandpiper (*C. fuscicollis*).

METHODS

DEVELOPMENT OF PREDICTIVE EQUATIONS

Semipalmated Sandpipers (*Calidris pusilla*) and White-rumped Sandpipers (*C. fuscicollis*) were captured with mist nets at Quivira National Wildlife Refuge, Stafford County, Kansas. Measurements taken immediately for each bird included body mass (0.1 g, Ohaus Electronic Balance C305), tarsus length (0.1 mm), wing length (flattened, 1 mm), total head length (0.1 mm), and culmen (0.1 mm). We calculated measurement error (expressed as a percentage of the measurement; $n = 48$) for two independent observers as 1.9% for tarsus, 1.1% for wing length, 0.8% for total head length, and 1.6% for culmen.

TOBEC was measured with an EM-SCAN SA-1 Small Animal Body Composition Analyzer following procedures used by Walsberg (1988) and Castro et al. (1990). The power supply was an DC/AC inverter producing current from a 12-volt automobile battery. Birds were positioned on their backs with mid-sternum at the half-way mark on a Plexiglass plate (corresponding to the half-way point when inserted into the chamber). The shorebirds were relatively docile when handled and were immobilized using firm elastic bands around the legs and across the sternum. An index of lean body mass (I_{LM}) was calculated as a trimmed mean (Mosteller and Rourke 1973), the average of four of five readings of

$$I_{LM} = (S - E)/R$$

where S = measurement with sample, E = measurement of the empty chamber, and R = reference or calibration number (see Walsberg 1988, Castro et al. 1990). By eliminating the one reading that was most different from the other four readings, we were able to improve mean precision (where precision is represented by $P = SE/\bar{x}$; Andrew and Mapstone 1987) of this estimate from 0.025 to 0.017.

Birds over a broad range of body size were collected, sacrificed by thoracic compression (AOU 1988), double-bagged and stored frozen until laboratory analyses were conducted. Whole, feathered birds were weighed and contents of the gastrointestinal tracts were weighed and discarded. Carcasses were sectioned and oven-dried to constant mass at ca. 90°C. After drying, samples were homogenized with an electric coffee grinder and placed in cellulose thimbles (dry homogenate was divided into two or more thimbles when homogenate > 10 g). Lipids were extracted using petroleum ether in a modified Soxhlet apparatus (Dobush et al. 1985). Laboratory-derived values for lean mass (observed lean mass) included water, feathers, and ingesta (contents of the digestive tract), and were derived by subtracting extracted lipids from total body mass measured at time of capture. We included ingesta in our observed lean mass values because it is detected by TOBEC, because preliminary models were substantially better when including rather than omitting it, and because there would be no information on ingesta without laboratory analyses for future application of the models.

We used several published and newly generated equations to predict lean and lipid masses and compared these predicted values with actual values. We tested published equations with data

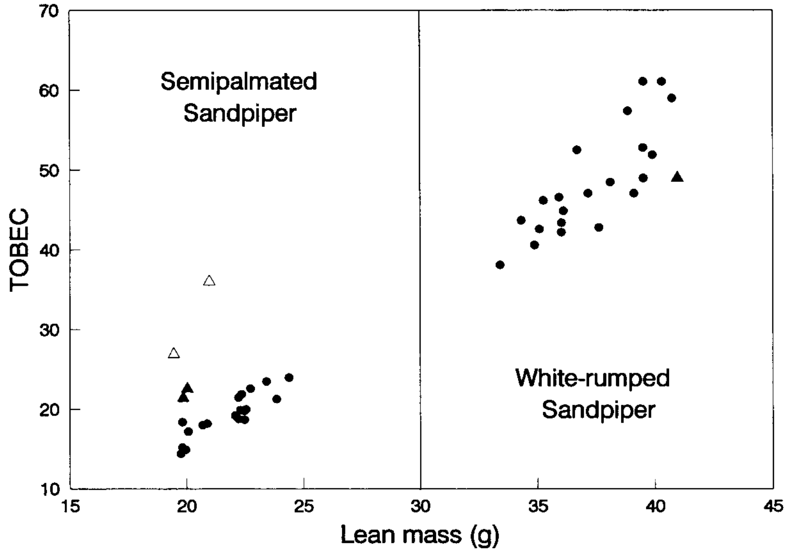

FIGURE 1. The relation between total body electrical conductivity (TOBEC) indices and observed lean mass of 24 Semipalmated Sandpipers and 22 White-rumped Sandpipers. Open triangles represent outlying values that were omitted to yield the subsample (n = 44) used to test published equations and to generate two-species models. Closed triangles represent additional outliers that were omitted from single-species models.

that fell within the range of values used in generating the equations. For some analyses, we used inverse regression procedures to predict lean mass from equations relating lean mass and TOBEC values. We calculated 95% prediction intervals for inverse regression for mean lean mass according to Zar (1984). We then calculated lipid mass using the simple formula of $FM = BM - LM$, where FM is lipid or fat mass, BM represents total body mass at time of capture, and LM is TOBEC-estimated lean mass. For other analyses, we predicted lipid mass directly from body mass and morphological measurements.

We evaluated new predictive equations for lean and lipid mass using the prediction sums of squares (PRESS) procedure for cross-validation (Neter et al. 1989). With this procedure, each data point is predicted from the least squares fitted regression function developed from the remaining $n - 1$ data points.

In preliminary analyses, we identified outlying values of TOBEC with large residuals and eliminated those individual birds from subsequent analyses. First, we eliminated two Semipalmated Sandpipers for which there were unexplainably high TOBEC readings (Fig. 1). For single-species models, we further eliminated data for two Semipalmated Sandpipers and one White-rumped Sandpiper that had high leverage in regression models relating TOBEC and lean mass. Although these data points were not outliers in later models, we consistently omitted them so that we could compare models. We did not use weighted least squares regression analysis because we found the accuracy of weighted TOBEC-lean mass models to be no greater than that of ordinary least squares regressions, with or without outliers (Willett and Singer 1988).

We calculated 95% confidence and prediction intervals for multiple regression models (Neter et al. 1989) at mean and at maximum values for independent variables. All statistical analyses were performed using SYSTAT 5.0. Means ± SE are reported unless otherwise specified.

EFFECTS OF METAL BANDS

We determined an index of lean body mass (as above) both before and after metal USFWS bands were applied to individual birds that were released after processing. We used the EM-SCAN Model SA-1 for sandpipers captured at Quivira National Wildlife Refuge, Kansas, and a Model SA-2 for sandpipers captured in wetlands in Clark, Kingsbury, and Lake counties, South Dakota. We expressed the effect of metal bands as the percent difference in readings, or

$$(\mathrm{abs}((I_{LM\text{-}B} - I_{LM\text{-}UB})/I_{LM\text{-}UB})\cdot 100),$$

where $I_{LM\text{-}B}$ is the TOBEC index for the banded bird and $I_{LM\text{-}UB}$ is the TOBEC index for the unbanded reading.

RESULTS

Our collected sample included 24 Semipalmated Sandpipers (14 males and 10 females) and 22 White-rumped Sandpipers (WRSA; 11 males and 11 females). Mean body mass was 26.0 ± 0.61 g (range 21.0–30.4 g) and 46.4 ± 1.20 g (range 37.3–54.9 g) for Semipalmated Sandpipers and White-rumped Sandpipers, respectively. Mean lipid mass was 4.5 ± 0.54 g (range 1.2–9.4 g) and 8.9 ± 0.88 g (range 2.6–14.5 g), representing 16.6 ± 1.71% (range 5.3–32.2%) and 18.5 ± 1.91% (range 6.9–27.6%) of whole body mass, respectively. Lean body masses averaged 21.5 ± 0.30 g (range 19.4–24.4 g) and 37.5 ± 0.48 g (range 33.4–40.9 g) for Semipalmated Sandpipers and White-rumped Sandpipers, respectively. Ingesta averaged 1.1 ± 0.4 g and 1.9 ± 0.13 g, respectively. Total body water (expressed as percent of lean mass) averaged 63.3 ± 0.17% (range 61.2–64.5%) for Semipalmated Sandpipers and 64.3 ± 0.16% (range 62.9–66.2% for White-rumped Sandpipers.

TESTS OF EXISTING EQUATIONS

We tested the predictive power of three interspecific equations and one single-species equation that relate lean body mass and I_{LM}. Three equations were generated across a wide range of body sizes and several species (Walsberg 1988, Castro et al. 1990, Scott et al. 1991) and one equation was generated with Dunlin (*Calidris alpina*) only (Scott et al. 1991; Table 1). The interspecific equations have high coefficients of determination (the I_{LM} reading explained 95–99% of the variance in lean body mass), indicating strong relationships between lean body mass and I_{LM}. A lower r^2 (0.71) was reported for the Dunlin single-species equation.

When the values of lean body mass predicted by the equations of Walsberg (1988) and Castro et al. (1990) were compared with actual lean body mass of our birds, we found high correlations (Table 1). The Castro equation yielded the smallest average error (8% for both species) between predicted and observed values of lean body mass of the four equations tested. The Castro equation was slightly better for the smaller Semipalmated Sandpiper than the larger White-rumped Sandpiper, whereas the Walsberg equation provided a better prediction of the larger White-rumped Sandpiper than the smaller Semipalmated Sandpiper (Table 1). Even though White-rumped Sandpipers fall in the same range of body sizes as Dunlin, the species-specific equation for Dunlin yielded an average of 26% error in predicting lean mass.

Using the equation that gave the best prediction of lean mass, the Castro equation with Semipalmated Sandpipers (on average 7% error), the resultant lipid values deviated on average 50% (Table 1). Lipid calculations using the remaining equations resulted in average errors in lipid mass ranging from 111% to 196% (Table 1).

Using inverse regression, we generated three new equations, one interspecific and two species-specific, to relate lean mass and TOBEC readings and evaluated their performances in predicting lean and lipid masses using cross-validation. There were no relationships between residuals and total body water (% lean mass) nor ingesta mass. Species-specific models yielded estimates of mean lean mass and 95% prediction intervals of 21.7 ± 1.70 and 37.3 ± 3.46 for Semipalmated and White-rumped Sandpipers. Not surprisingly, the new equations, tested with the same set of species for which they were generated, yielded a smaller percent error than earlier published equations in predicting both lean and lipid masses (Table 2). However, even though lean masses were predicted with an average of 3.3–4.6% error, calculated lipids were in error by 17.4–23.6%. The expression of fat as a percentage of body mass had such a high error (nearly 200%) that it was unreliable.

DEVELOPMENT OF PREDICTIVE EQUATIONS

In Semipalmated Sandpipers, several linear dimensions (wing length, total head length, culmen, and tarsus) correlated with lean body mass (Table 3), and in White-rumped Sandpipers, wing length was significantly correlated with lean mass. In both of these species, body mass was highly correlated with fat mass. We built single-species multiple regression models to predict fat mass with forward-selection procedures, including independent variables that explained the most additional variation and for which measurement errors were lowest. In these models, TOBEC values were not used to estimate lean mass as in earlier procedures (hereafter Type A models), but

TABLE 1. Tests of three interspecific and one intraspecific equation that predict lean body mass from TOBEC readings, based on laboratory analyses of 22 Semipalmated Sandpipers (SESA) and 22 White-rumped Sandpipers (WRSA). Calculated lipid masses were determined by subtracting predicted lean body mass from total body mass.

Study	Walsberg (1988)	Castro et al. (1990)	Scott et al. (1991)	Scott et al. (1991)[a]
Equation[b]	$LM = 15.70 + 0.58X - 0.0006X^2$	$LM = (X + 42.66)/2.71$	$LM = 17.56 + 0.67X - 0.0008X^2$	$LM = 21.4 + 0.53X$
Lean body mass (g)	18–160	18–90	28–410	28–50
Number of species	14	8	4	1
Sample size	25	38	33	11
r^2	0.99	0.95	0.99	0.71
Tests of equations				
Correlation (r)[c]	0.98	0.98	0.78	0.78
Percent error, lean body mass ($\bar{x} \pm SE$)				
SESA, $n = 22$	27 ± 1.0	7 ± 1.1		
WRSA, $n = 22$	21 ± 1.5	10 ± 0.8	28 ± 1.3	26 ± 1.2
Both, $n = 44$	24 ± 1.1	8 ± 0.7		
Percent error, calculated lipid mass ($\bar{x} \pm SE$)				
SESA, $n = 22$	196 ± 35.2	50 ± 15.0		
WRSA, $n = 22$	111 ± 14.3	56 ± 7.3	155 ± 20.0	141 ± 18.7
Both, $n = 44$	153 ± 19.9	53 ± 8.3		

[a] Intraspecific curve for Dunlin.
[b] LM = lean body mass; $X = I_{LM}$ = TOBEC reading.
[c] Correlation coefficient for predicted and observed values for lean body mass for 44 birds (Walsberg and Castro) or 22 WRSAs (Scott equations).

TABLE 2. Cross-validation tests of equations that predict lean body mass from TOBEC readings, based on laboratory analyses of 22 Semipalmated Sandpipers (SESA) and 22 White-rumped Sandpipers (WRSA). Calculated lipid masses were determined by subtracting predicted lean body mass from total body mass.

	SESA/WRSA combined	SESA	WRSA
Equation[a]	LM = $(X + 19.985)/$ 1.829	LM = $(X + 17.226)/$ 1.680	LM = $(X + 46.823)/$ 2.554
Lean body mass (g)	19.7–41.0	19.7–24.4	33.4–40.7
Sample size	44	20	21
r^2	0.954	0.786	0.682
r^2_c[b]	0.949	0.730	0.618
Tests of equations			
Percent error, lean body mass ($\bar{x}$ ± SE)			
SESA	4.1 ± 0.90	3.3 ± 0.42	
WRSA	5.1 ± 0.76		3.5 ± 0.52
Both species	4.6 ± 0.59		
Percent error, calculated lipid mass ($\bar{x}$ ± SE)			
SESA	22.7 ± 4.69	23.6 ± 4.35	
WRSA	23.6 ± 2.93		17.4 ± 2.64
Both species	23.2 ± 2.73		

[a] LM = lean body mass; $X = I_{LM}$ = TOBEC reading.
[b] Cross-validation r^2.

rather to correct for individual differences in body size or lean mass (Morton et al. 1991), hereafter Type B models.

Body mass alone explained a substantial portion (76 and 89%) of the variance in fat mass of the two species (Table 4). For Semipalmated Sandpipers, addition of wing length and total head length explained an additional 11.6% of the variance in fat mass, and TOBEC readings explained an additional 7.8% after wing length and total head length. Wing length explained an additional 2.4% of the variance for White-rumped Sandpipers and TOBEC readings an additional 2.6% after wing length (Table 4). If morphological measurements were not included in the models, TOBEC explained an additional 17.2% and 4.8% of the variance after body mass for Semipalmated and White-rumped Sandpipers, respectively.

Multiple regression models using fat mass as the dependent variable (Type B models) yielded lower errors in predicting fat than Type A models (Fig. 2). In cross-validation tests of several models, the lowest absolute error was associated with multiple regression models that included body mass, morphological measures and TOBEC values (Table 5). Inclusion of TOBEC indices enabled us to reduce the average error in estimating fat by 0.48 g (15.9% of fat mass) for Semipalmated Sandpipers and 0.16 g (2.4% of fat mass) for White-rumped Sandpipers. The relationship between predicted and observed fat levels is presented in Fig. 3.

In general, the 95% confidence and prediction intervals at mean and maximum values of independent variables (minimum and maximum intervals) narrowed as additional predictors were incorporated into the model (Table 4). Models based on body mass alone had broad 95% prediction intervals, spanning ca. 3 g around the fat estimate, even though associated r^2 values were high. Addition of morphological measurements reduced widths of minimum prediction intervals by 25% for Semipalmated Sandpipers and 19% for White-rumped Sandpipers. Addition of TOBEC indices further reduced the width of minimum prediction intervals by 32% for Semipalmated Sandpipers and 13% for White-rumped Sandpipers.

PRECISION AND ACCURACY IN TOBEC METHODOLOGY

Measurements of I_{LM} for White-rumped Sandpipers were significantly more precise ($t = 2.845$, df = 40, $P < 0.01$) than measurements of I_{LM} for Semipalmated Sandpipers, although precision was good for both species ($P = 0.02$ and 0.01 for Semipalmated Sandpipers and White-rumped Sandpipers, respectively; see Methods). The model SA-1 was more precise than the SA-2 for measuring TOBEC indices of unbanded Semipalmated Sandpipers ($t = -2.523$, df = 43, $P =$

TABLE 3. Correlation matrix between body mass, lean mass, fat mass, TOBEC readings, and four linear dimensions of Semipalmated Sandpipers (SESA) and White-rumped Sandpipers (WRSA) in central Kansas during migration.

Species (n)		Lean mass	Wing	Total head	Culmen	Tarsus	TOBEC	Fat
SESA (22)	Body mass	0.44*	0.13	0.38*	0.36*	0.40*	0.31	0.88**
	Lean mass		0.41*	0.70**	0.72**	0.70**	0.68**	0.04
	Wing			0.17	0.25	0.16	0.03	0.07
	Total head				0.93**	0.79**	0.46*	0.05
	Culmen					0.77**	0.40*	0.02
	Tarsus						0.52**	0.08
	TOBEC							0.01
WRSA (22)	Body mass	0.79**	0.13	0.01	0.01	0.04	0.64**	0.94**
	Lean mass		0.41*	0.13	0.02	0.14	0.78**	0.53**
	Wing			0.42*	0.48**	0.47*	0.45*	0.05
	Total head				0.80**	0.48**	0.10	0.08
	Culmen					0.49**	0.14	0.13
	Tarsus						0.35*	0.02
	TOBEC							0.45*
BOTH (44)	Body mass	0.96**	0.91**	0.89**	0.86**	0.83**	0.94**	0.80**
	Lean mass		0.98**	0.95**	0.92**	0.89**	0.98**	0.59**
	Wing			0.96**	0.93**	0.88**	0.95**	0.53**
	Total head				0.98**	0.93**	0.92**	0.51**
	Culmen					0.93**	0.90**	0.48**
	Tarsus						0.88**	0.48**
	TOBEC							0.60**

* $P < 0.05$.
** $P < 0.01$.

0.02), whereas the two models did not differ in precision of White-rumped Sandpiper measurements ($t = -0.524$, df $= 22$, $P = 0.606$).

Accuracy of TOBEC methodology (expressed as absolute error in estimating lean mass) was greater for Semipalmated Sandpipers than for White-rumped Sandpipers ($t = -2.726$, df $= 40$, $P = 0.01$). Because we collected birds for laboratory analyses only in conjunction with the SA-1, we were not able to compare accuracy of the SA-1 and SA-2.

EFFECTS OF METAL BANDS

We determined I_{LM} for 102 birds before and after applying aluminum USFWS bands. The Model SA-1 was used for 17 Semipalmated Sandpipers and eight White-rumped Sandpipers in Kansas, and a Model SA-2 was used for 18 Least Sandpipers (*Calidris minutilla*), 26 Semipalmated Sandpipers, 15 White-rumped Sandpipers, one Dunlin, three Pectoral Sandpipers (*C. melanotos*), 13 Semipalmated Plovers (*Charadrius semipalmatus*), and one Killdeer (*C. vociferous*) in South Dakota. There was no significant difference in precision of readings due to presence of bands for any species/model combination.

Of 102 pairs of I_{LM} estimates for banded and unbanded birds, 4.9% differed by >50%. For the remaining pairs, $I_{LM\text{-}B}$ differed from $I_{LM\text{-}UB}$ on average by 9.8% ± 1.05 (Fig. 4). When species were ranked according to body size, absolute differences between $I_{LM\text{-}B}$ and $I_{LM\text{-}UB}$ did not vary with body size ($F = 2.70$, df $= 2, 95$, $P = 0.104$). However, when differences were expressed as a percent of $I_{LM\text{-}UB}$, they decreased with increasing body size ($F = 14.17$, df $= 2, 95$, $P < 0.001$). Model of EM-SCAN machine had no effect on absolute ($F = 0.39$, df $= 2, 95$, $P = 0.54$) or percent differences ($F = 1.57$, df $= 2, 95$, $P = 0.21$).

DISCUSSION

Existing published equations relating lean mass and TOBEC indices (Type A models) in birds were not useful in estimating lipid mass in our sample of shorebirds. Morton et al. (1991) explain that when lipid is calculated from total body mass and estimates of lean mass, the prediction error is different and often far greater for lipids than lean mass. Our calculations support the concern of Morton et al. (1991) both for published equations and for our own equations. Type

A models were less accurate for individuals with low fat levels (also see Roby 1991).

Our data suggest that there is considerable error in estimating fat for individual birds even when using our most inclusive Type B models. Whereas current TOBEC methodology may provide reasonable estimates of lean mass, as was demonstrated in early work (Bracco et al. 1983, Presta et al. 1983, Fiorotto et al. 1987, Van Loan and Mayclin 1987, Walsberg 1988, Cochran et al. 1989, this study), its usefulness in predicting lipid mass for individual birds is uncertain.

High coefficients of determination for equations that relate lean mass to TOBEC indices did not indicate the relative extent of error in predicting lipid mass. In fact, absolute errors in predicted lean and lipid masses for interspecific equations with $r^2 \geq 0.95$ were greater than for single-species equations with $r^2 \leq 0.80$. Correlation coefficients measure "the residual variance relative to the total variation in the data" (Ehrenberg 1975), but do not indicate whether the residual scatter is the same for two correlations. It follows that regression equations cannot be evaluated for their usefulness simply by comparing their coefficients of determination.

In addition, strong correlations between observed and predicted lean masses do not necessarily suggest that prediction errors are low. Our tests of published equations yielded strong correlations ($r = 0.98$) between observed and predicted lean mass for two equations, yet average errors in predicting lean mass ranged to nearly 30%. Similarly, in a cross-validation study of TOBEC measurements of humans (Van Loan et al. 1987), correlation coefficients were high (0.99), but paired *t*-tests indicated significant differences in predicted and observed lean body masses.

WHAT IS THE APPROPRIATE FORM OF A PREDICTIVE MODEL?

From these comparisons, we conclude that body fat is not estimated accurately from regression models developed from lean mass and TOBEC readings and subsequent algebraic manipulations. According to Morton et al. (1991), this problem of erroneous partitioning of variation can be circumvented by using a multiple regression model to predict lipid mass with total body mass and TOBEC values as independent variables. In such a model, TOBEC values would be used to correct for individual differences in body size rather than to measure lean mass.

TABLE 4. Equations to predict fat mass of Semipalmated Sandpipers and White-rumped Sandpipers from total body mass, morphological measurements, and TOBEC readings. Estimates, 95% confidence intervals and 95% prediction intervals (PI) are reported for mean values of independent variables (mean $\bar{X}$) and maximum values of independent variables (maximum $\bar{X}$).

Equation[a]	r^2_{adj}	SEE	Fat estimate (g) ± 95% CI (95% PI)	
			Mean $\bar{X}$	Maximum $\bar{X}$
Semipalmated Sandpipers ($n = 20$)				
$FM = -17.071 + 0.823BM$	0.760	1.357	4.7 ± 0.64 (2.92)	8.4 ± 1.18 (3.09)
$FM = 2.473 + 0.832BM - 0.211W$	0.775	1.314	4.7 ± 0.62 (2.84)	7.7 ± 1.47 (3.14)
$FM = 18.248 + 0.957BM - 0.184W - 0.554TH$	0.868	1.007	4.7 ± 0.47 (2.19)	6.7 ± 1.29 (2.50)
$FM = 6.979 + 1.036BM - 0.142W - 0.231TH - 0.382I_{LM}$	0.958	0.568	4.7 ± 0.27 (1.24)	6.4 ± 0.73 (1.42)
White-rumped Sandpipers ($n = 21$)				
$FM = -23.509 + 0.700BM$	0.886	1.409	8.8 ± 0.64 (3.02)	14.9 ± 1.21 (3.19)
$FM = 14.013 + 0.710BM - 0.309W$	0.907	1.276	8.8 ± 0.58 (2.75)	13.1 ± 2.04 (3.37)
$FM = -9.069 + 0.826BM - 0.103W - 0.157I_{LM}$	0.932	1.089	8.8 ± 0.50 (2.35)	13.4 ± 1.76 (2.90)

[a] FM = fat mass, BM = total body mass, W = flattened wing length, TH = total head length, I_{LM} = TOBEC reading.

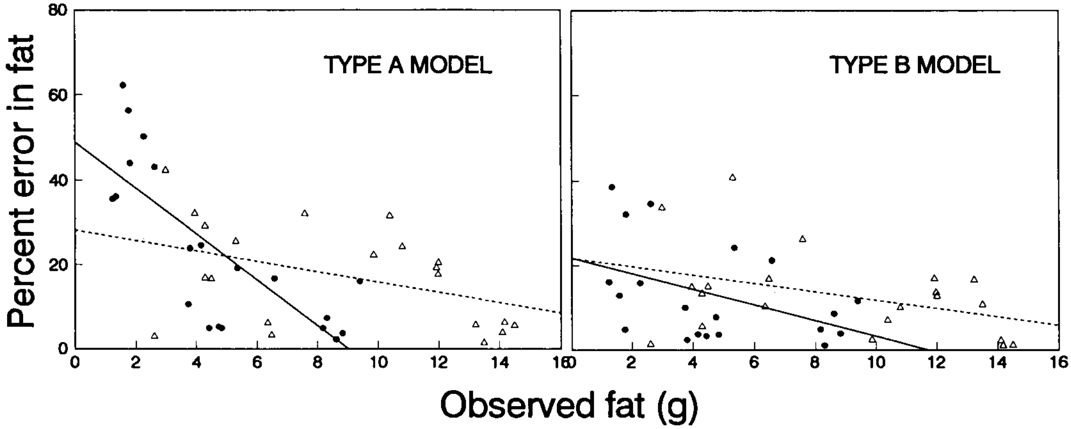

FIGURE 2. The relation between observed body fat and percent error in predicting body fat using two types of models. Model A predicts lean mass from the TOBEC reading using inverse regression, then calculates body fat mass as FM = BM − LM, where FM is fat mass, BM is total body mass, and LM is lean body mass. With the type B model, FM is predicted directly from BM, morphological measurements, the TOBEC readings, using the most inclusive equation in Table 4. Solid circles and solid line represent data points and regression line for Semipalmated Sandpipers. Open triangles and dashed line are data points and regression line for White-rumped Sandpipers. Regression lines are plotted to show trends in data.

There has been considerable discussion regarding assignment of dependent and independent variables in generating calibration equations. In previous papers (Walsberg 1988, Castro et al. 1990), lean mass rather than TOBEC was assigned as the independent variable based on assumptions of standard Model I regression (i.e., that the independent variable X is measured without error, does not vary at random, and is under the control of the investigator [Sokal and Rohlf 1981]). Because precision and distribution of TOBEC was virtually unknown, it was assigned as the dependent, random, and normally distributed variable. To examine implications of

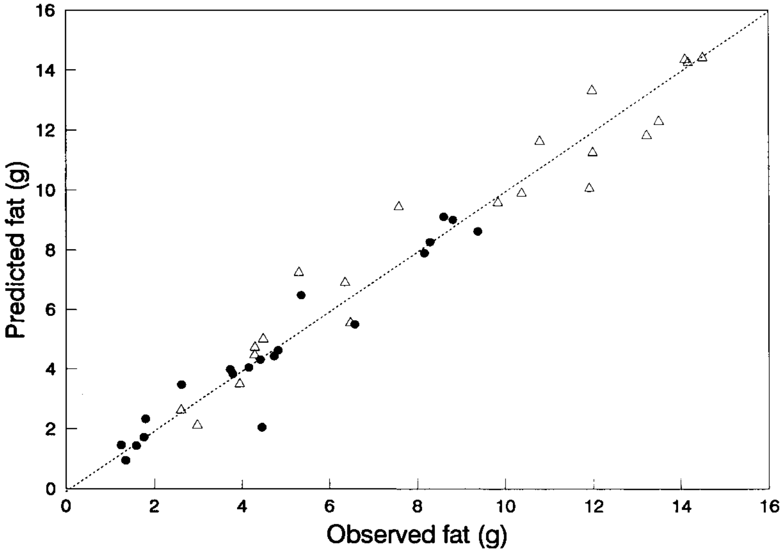

FIGURE 3. The relation between observed body fat measured by extraction and body fat estimated using multiple regression models for 20 Semipalmated Sandpipers (solid circles; $r^2 = 0.966$) and 21 White-rumped Sandpipers (open triangles; $r^2 = 0.943$) collected in Kansas during spring 1992. The most inclusive equations in Table 4 are presented. The dashed line is $y = x$.

TABLE 5. Tests of Castro et al. (1990) equation and cross-validation tests of several newly generated equations to predict fat of Semipalmated Sandpipers (SESA) and White-rumped Sandpipers (WRSA) using predictive models of two basic types. With Model Type A, lean mass (LM) was estimated from TOBEC readings by inverse regression, and fat mass (FM) was calculated as FM = BM − LM, where BM = total body mass. Type B models estimate fat directly from body mass (BM), morphological measurements, and TOBEC readings.

Source model generation					Model testing		
Model type[a]	Number of species (n)	Predictors[b]	r^2	r^2_c	(n)	Absolute error (g) ($\bar{x}$ ± 95% CI)	Percent error in fat ($\bar{x}$ ± 95% CI)
			Semipalmated Sandpipers				
A_L	8 (38)	I_{LM}	0.95	—	22	1.40 ± 2.145	50.4 ± 145.99
A	2 (44)	I_{LM}	0.95	0.95	22	0.79 ± 1.468	22.7 ± 45.41
A	1 (20)	I_{LM}	0.79	0.73	20	0.71 ± 0.826	23.6 ± 40.61
B	1 (20)	BM, W, TH	0.89	0.86	20	0.96 ± 1.339	29.0 ± 54.10
B	1 (20)	BM, W, TH, I_{LM}	0.97	0.95	20	0.48 ± 0.849	13.1 ± 23.97
			White-rumped Sandpipers				
A_L	8 (38)	I_{LM}	0.95	—	22	3.85 ± 3.229	55.5 ± 71.21
A	2 (44)	I_{LM}	0.95	0.95	22	1.95 ± 2.945	23.6 ± 28.54
A	1 (21)	I_{LM}	0.68	0.62	21	1.32 ± 1.939	17.4 ± 25.16
B	1 (21)	BM, W	0.92	0.89	21	1.14 ± 1.600	15.4 ± 23.80
B	1 (21)	BM, W, I_{LM}	0.94	0.92	21	0.98 ± 1.498	13.0 ± 21.81

[a] L = from published literature (Castro et al. 1990).

[b] BM = total body mass, W = flattened wing length, TH = total head length, I_{LM} = TOBEC reading. See Tables 1, 2, and 4 for equations.

the model form, we compared 95% prediction intervals for mean lean mass of models with TOBEC as the dependent (inverse prediction) and as the independent variable. The 95% prediction intervals of the inverse prediction models were larger than the model built with TOBEC as the independent variable (mean lean mass ± 95% PI = 21.7 g ± 1.45 for Semipalmated Sandpipers and 37.3 g ± 2.70 for White-rumped Sandpipers), probably because the inverse prediction model reflects the measurement error in TOBEC.

When formulating an equation incorporating fat, total body mass, other morphological measurements, and possibly TOBEC, one could ar-

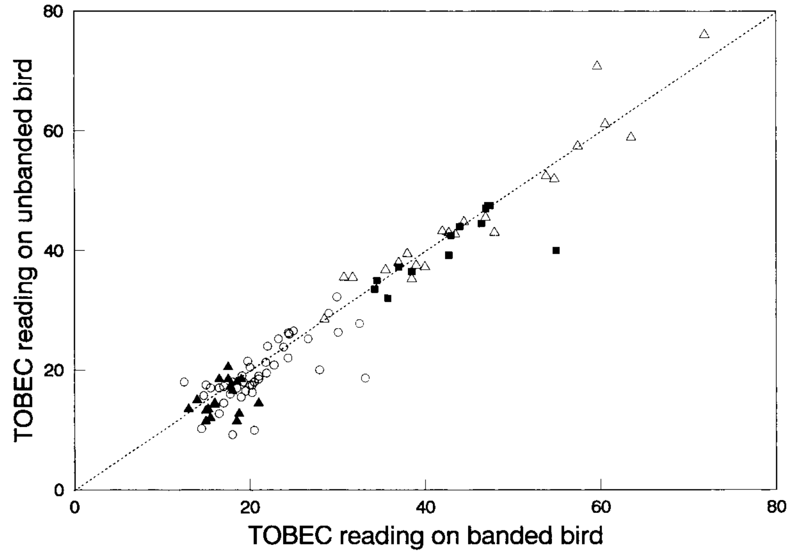

FIGURE 4. The relation between total body electrical conductivity (TOBEC) indices of 18 Least Sandpipers (solid triangles), 43 Semipalmated Sandpipers (open circles), 13 Semipalmated Plovers (solid squares), and 22 White-rumped Sandpipers (open triangles) taken when unbanded and when banded. Data point for one White-rumped Sandpiper (43.8, 236.1) is not depicted. Dashed line is $y = x$.

gue (endlessly) which of these variables should be assigned as the independent variable(s), that is, (1) has the least measurement error, (2) is under most control of the investigator, and (3) does not vary at random. First, although laboratory extractions are regarded as precise and accurate measurements of fat, we know from observer reliability tests that other measurements are also highly precise. Second, an observation is controlled only when collections and subsequent measurements are made at predetermined regular intervals for variable(s) of interest rather than when collections measurements are made at random (Ricker 1973). In this study, we attempted to collect birds over a range of body sizes, but in doing so we considered only total body mass, not fat, lean mass, or other morphological features. Finally, there is natural variation within each of these variables. Because we want to use the equation to predict fat content, and because there is no clear reason for doing otherwise, we chose to assign fat as the dependent variable.

Sokal and Rohlf (1981) argue that a Model II regression is more appropriate than standard Model I regression when using two continuous variables that are distributed according to the bivariate normal distribution (i.e., vary mutually and naturally). This is the case with all variables we could consider in a model, suggesting that a Model II regression is most appropriate. It is unclear, however, how to perform a multiple regression using Model II regression techniques, although Ricker (1973) discusses use of geometric mean regression for Model II regression for bivariate data. Furthermore, Sokal and Rohlf (1981) concluded that when the correlation is high ($r^2 > 0.77$) between two variables, the slopes computed using Model I and Model II regressions will be similar. We therefore chose Model I regression.

HOW ACCURATE ARE LIPID ESTIMATES?

Type A models, formulated to relate lean mass and TOBEC indices and then to estimate lipid mass, do not allow an assessment of the accuracy of lipid estimations. Coefficients of determination for such equations give limited indication of error, and it is not possible to compute prediction intervals for lipid estimates. Cross-validation tests suggested that Type B models which incorporate fat mass directly into the model are more accurate than Type A models, in part because other morphological measurements can be included (Morton et al. 1991). Type B models also allow the determination of prediction intervals for the lipid estimates.

Consideration of prediction as well as confidence intervals is essential for interpretation of lipid estimates. Here, we report prediction intervals for new estimates of individual birds because individuals are the sample of interest for many ecological and energetics studies. With any predictive equation, the accuracy of a prediction of the mean for an entire population is greater than that for an individual (Zar 1984). The ecological question dictates which interval is pertinent. If one is interested in estimating the mean of a new population, confidence intervals are relevant; if the question necessitates estimating lipid for new individual birds, prediction intervals are appropriate. For example, the minimum and maximum confidence limits for our most inclusive models indicate that the mean fat mass of a new sample of Semipalmated Sandpipers and White-rumped Sandpipers would lie within ca. 0.3–0.7 g and 0.5–1.8 g of true fat, or within 6–18% and 8–29% of the actual range of fat observed in the two species, with $P = 0.95$. On the other hand, the prediction intervals indicate that a single new observation of Semipalmated Sandpiper can be expected to lie within ca. 1.2–1.4 g and of White-rumped Sandpiper to lie within ca. 2.4–2.9 g of observed fat with $P = 0.95$. The prediction intervals span 30–35% and 39–48% (minimum and maximum) of the measured range of fat in Semipalmated Sandpipers and White-rumped Sandpipers. Additionally, it could be argued that many applications will require simultaneous predictions for a number of individuals with simultaneous prediction intervals or tolerance intervals, which are wider than those for a single individual (Vardeman 1992).

Because prediction intervals associated with lipid estimates are broad, interpretation should be made with caution. For example, conclusions about whether equations generated from birds collected in one geographic area are useful in another region (Dunn et al. 1988, Castro and Myers 1990) should be made only if prediction intervals are reasonably narrow. If prediction intervals are wide, lack of concordance may suggest that the equation is not useful even in source geographic regions. Using our sample of Semipalmated Sandpipers collected in Kansas, we tested equations to predict fat that were generated in the same and in different geographic regions. The Page and Middleton (1972) equation, which relates wing length and lean mass of Semipalmated Sandpipers collected in Ontario during migration, and the Castro et al. (1990) equation, which relates I_{LM} and lean mass of Kansas migrants, performed similarly. The Ontario equation predicted lipids with an average error of 46 ± 11.0%, while the Kansas equation was 50 ± 15.0% in error.

EFFECT OF BODY SIZE ON PRECISION AND ACCURACY OF I_{LM}.

Lean mass estimates of both Semipalmated Sandpipers (ca. 22 g lean mass) and White-rumped Sandpipers (ca. 38 g lean mass) were reasonably accurate, with average errors of only 3.3% and 3.5%, respectively. In this study, small body size of the subject did not reduce accuracy of lean mass or lipid estimates, contrary to suggestions by Walsberg (1988) and Roby (1991) that TOBEC is less accurate below 40–50 g body mass. In fact, TOBEC methodology improved our lipid estimates of the smaller Semipalmated Sandpiper more than the larger White-rumped Sandpiper. Regression results in Castro et al. (1990) show a tighter fit of the data to the regression line at smaller than larger body sizes, suggesting that percent error may not change along the line.

For the smallest birds (<20 g), subject body size appears to compromise precision of the I_{LM} estimate when using Model SA-2, but not when using the SA-1. This may be because the software associated with the SA-2 (and not the SA-1) rounds the I_{LM} index to the nearest integer, thereby yielding larger rounding errors for smaller birds.

A necessary attribute for accurate predictions of I_{LM} of a subject is a cross-sectional area that is uniform along its length (EM-SCAN representative, pers. comm.). In general, birds do not have this attribute, suggesting that some error is likely regardless of body size. Error due to this source may be smaller in mammals, such as rodents, that more closely fit this requirement.

ERROR ASSOCIATED WITH METAL BANDS

An additional source of error not incorporated in prediction equations can be expected if birds are banded with aluminum USFWS bands for identification before determining I_{LM}. We can expect most I_{LM} to have an additional 10% error, which would yield a larger resultant error in predictions using Model Type A than in Type B. Scott et al. (1991) also report that metal identification bands affect TOBEC readings, but Castro et al. (1990) and Roby (1991) found no significant effects.

ECONOMIC EVALUATION

For our species, EM-SCAN improved body fat estimates by 7.8% and 2.4% and reduced the breadth of 95% prediction intervals by 32% and 13%, respectively. At a January 1993 cost of $6,800, (EM-SCAN, Inc.) for a model SA-2, plus an additional $2,600 for a computer and software to use the SA-2, that represents an initial cost of $1,200–4,000 per 1% improvement of the fat estimate and $3,000–7,200 for a 10% reduction in prediction intervals. Additional costs include laboratory analyses for developing calibration curves and field costs incurred in using this technology over and above taking standard morphological measurements.

It is clear that calibration curves should be generated within each study and that existing curves cannot be applied to different species. It is feasible that many factors are responsible for the unexplained variance in the model (for example, changeable environmental conditions, detailed aspects of subject positioning, hydration state of the subject [Roby 1991], or composition of stomach contents) and that these factors might be eliminated or reduced. We also would expect that increasing sample sizes across the entire range of independent variable values in the model building data set would yield greater accuracy and narrower prediction intervals to some degree, thereby influencing the above cost-benefit balance. These predictive models might be improved further by incorporating principal components analysis (Freeman and Jackson 1990; Lougheed et al. 1991; J. Morton, pers. comm.) on morphological measurements (and sex/age if discernable in the field) to generate indices of overall body size.

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