

# EVALUATION OF LIPID INDICES OF THE WOOD THRUSH<sup>1</sup>

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**Abstract.** Many techniques for assessing lipid reserves have been used, but techniques are seldom evaluated. We evaluated five common methods (fat scoring, regression residuals of body mass vs. morphological measurements, quotients of body mass divided by morphological measurements, total body electrical conductivity, and water content) for assessing lipid reserves in the Wood Thrush (*Hylocichla mustelina*) in Rhode Island. Lipid content ranged from 3 to 11%, water content ranged from 64 to 72%, and fat score ranged from 0 to 4. Mean total body electrical conductivity (TOBEC) was moderately correlated with lean body mass. Percentage water and fat score were highly correlated with extracted lipid mass and percentage lipid content. Body mass, tail  $\times$  mass regression residuals, and quotient of body mass/tail length were highly correlated with lean body mass. Morphological measurements were correlated with lean mass only when in association with body mass, but not considered alone. Combining TOBEC readings with body mass and morphological measurements produced regression models with similar predictive abilities as previous studies using TOBEC, but TOBEC provided little or no additional predictive ability. TOBEC did not significantly improve body fat predictive models and does not appear accurate in predicting lipid mass of individual small birds. Fat score and percentage water are useful indices of body fat in Wood Thrush.

**Key words:** *Body fat; fat scoring; Hylocichla mustelina; lean body mass; lipid; solvent extraction; total body electrical conductivity; Wood Thrush.*

## INTRODUCTION

Lipid is the major form of energy storage in birds (Griminger 1986, Walsberg 1988), is the first to be mobilized during times of need (Blem 1990), and is commonly a limiting nutrient during the annual cycle (Johnson et al. 1985). Consequently, lipid storage is important for avian reproduction (Drobney 1980, Krapu 1981, Walsberg 1983), migration (Blem 1980, Biebach et al. 1986), and survival (Lima 1986, Blem 1990; but see Kremetz et al. 1989). Accurate measurement of lipid content in birds is a valuable tool for examining processes in avian ecology, energetics, and behavior. Many techniques have been used to assess lipid content of birds, yet the techniques are infrequently evaluated.

Techniques can be classified as invasive or non-invasive. Invasive techniques include solvent lipid extraction of homogenized birds and determination of fractional water content. The four

most common non-invasive methods for evaluating lipid reserves in birds are: (1) fat scoring, (2) determining the quotient of body mass divided by morphological measurements, (3) calculating regression equations of body mass vs. morphological measurements, and (4) measuring total body electrical conductivity (TOBEC).

Solvent lipid extraction of homogenized birds is the most accurate technique for quantifying lipid reserves, but is expensive, time consuming, and requires sacrificing individuals. Water content is often inversely correlated with lipid content because lipids are water-insoluble and hence, little water accompanies the storage of fat compared to protein and carbohydrate (Bailey 1979, Wishart 1979, Johnson et al. 1985). Measurement of water content also requires sacrificing individuals, but is less expensive and time consuming than lipid extraction.

Non-invasive techniques for estimating lipid content are attractive because they are more humane and allow subsequent measurement of the same individuals. Fat scoring (Helms and Drury 1960, Kremetz and Pendleton 1990) is a visual index of subcutaneous lipid depots used fre-

<sup>1</sup> Received 24 January 1994. Accepted 12 April 1994.  
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quently by field ornithologists. It is unobtrusive, inexpensive, and quick, but may be inaccurate in some species (Krementz and Pendleton 1990). Body mass divided by a morphological measure, or the residuals of body mass regressed against more than one morphological measure, can provide a quantitative index of lipid content (Odum et al. 1964, Owen and Cook 1977, Iverson and Vohs 1982, Ringelman and Szymczak 1985, Ellegren 1989). TOBEC is a non-invasive technique that determines electrical conductivity from the change in the phase relation of voltage and current in a 5 MHz-frequency oscillating magnetic signal passed through a coil (Walsberg 1988, EM-SCAN 1991). TOBEC has provided accurate indices of lean body mass for several species of birds (Walsberg 1988, Castro et al. 1990, Morton et al. 1991, Roby 1991, Scott et al. 1991). Lean body mass was then subtracted from total body mass to estimate lipid mass. However, the precision of this technique to estimate lipid content has not been adequately addressed (but see Skagen et al. 1993). Many techniques have been used to estimate lipid content in birds and the relative accuracy of each technique may vary with species, but seldom have all techniques been evaluated simultaneously in a single species.

We evaluated the accuracy of techniques for estimating lipid content in the Wood Thrush (*Hylocichla mustelina*) by comparing lipid indices to results of solvent lipid extraction. The Wood Thrush is particularly appropriate because it is a long-distance Neotropical migratory bird which has experienced recent population declines throughout its range (Robbins 1979, Robbins et al. 1989, Askins et al. 1990). Migration is energetically demanding and requires adequate lipid depots, especially for passerines which have a smaller margin for survival of environmental extremes compared to larger migrants (Stuebe and Ketterson 1982, Blem 1990). Consequently, optimal lipid storage is particularly important for Wood Thrushes and other Neotropical migrants.

## MATERIALS AND METHODS

We collected 21 Wood Thrush in Washington County, Rhode Island from 16 June to 14 September 1992. Birds were captured in mist-nets. Lipid depots of captured birds were examined and assigned a 0–5 fat score (Helms and Drury 1960). Fat scores were assigned based on visual examination of furcular and abdominal lipid de-

pots. For example, a fat score of 0 indicates no visible lipid depots and 5 indicates bulging furcular and abdominal lipid depots. We measured wing chord and tail length on all birds to the nearest 0.1 mm and weighed birds to the nearest 0.1 g on a Pesola spring scale.

Birds were restrained with rubber bands and placed in an SA-2 Small Animal Body Composition Analyzer (EM-SCAN, 3420 Constitution Drive, Springfield, Illinois 62707). We recorded mean TOBEC readings from 16 replicate measures on live birds, then sacrificed the birds using thoracic compression (AOU 1988), and immediately took 16 additional measures on dead birds. We examined skull pneumatization and plumage to determine age class (Pyle et al. 1987). Carcasses were then immediately frozen.

Partially thawed carcasses were plucked and the contents of the crop, esophagus, gizzard, and intestines were removed. Gonads were examined to determine sex of birds and carcasses were weighed to the nearest 0.1 g and freeze dried for 24 hr to constant mass. Freeze drying is preferable to other techniques because it eliminates evaporation of short-chain lipids (Blem 1990). Dried samples were homogenized using a Waring blender. Lipids were extracted from 2.0 g aliquot samples in duplicate using a Soxhlet extraction apparatus with chloroform as the solvent. Reflux time was 12–16 hr. The extract was weighed and lean body mass calculated as total wet mass of the bird minus total mass of extracted lipids. We also performed two additional replicate extractions from each of five carcasses using petroleum ether as the solvent to examine the effects of solvent on amount of lipid extracted.

We calculated percentage lipid content in each bird as:

$$(\text{lipid mass/plucked body mass}) \times 100,$$

and calculated percentage water content as:

$$((\text{wet mass-dry mass})/\text{wet mass}) \times 100.$$

We tested all variables for normality by examining Shapiro-Wilk's *W*-statistics and normal probability plots (SAS Institute 1985). Distributions of lipid mass, percentage lipid content, and percentage water were non-normal ( $P \leq 0.01$ , Shapiro-Wilks' test), but unimodal. Distributions of all other variables were normal ( $P > 0.01$ , Shapiro-Wilks' test).

We regressed body mass against each morphological measure using simple linear regres-

sion (SAS Institute 1985) to obtain regression residuals. We calculated Pearson product-moment correlation coefficients ( $r$ ) and associated significance probabilities (SAS Institute 1985) for all combinations of variables. We used forward-selection multiple regression to determine the amount of variation in extracted lipid mass, lean body mass, and percentage lipid content explained by combinations of variables. We used regression analyses because results are easily interpretable, current TOBEC technology uses regression to predict lean body mass (EM-SCAN 1991), and most studies estimating lipid reserves in birds have used regression analyses (Blem 1990). We incorporated morphological measurements into regression equations predicting lipid and lean body mass so that our results would be consistent with previous studies and TOBEC manufacturer suggestions (EM-SCAN 1991).

We evaluated the performance of our regression equations in predicting lean body mass and lipid mass using cross-validation procedures (INFLUENCE option, PROC REG, SAS Institute 1985). For each regression equation, we calculated the coefficient of determination from cross-validation tests by subtracting predicted residual sum of squares from total sum of squares and dividing by total sum of squares. We calculated absolute error (predicted residuals) and percent error (absolute error/observed mass) for all equations using results of cross-validation tests. We calculated 95% confidence and 95% prediction intervals for regression models at both mean and maximum values for the independent variables so that we could evaluate effects of variable addition on both minimum and maximum widths of intervals.

We used two-factor main-effects analysis of variance (SAS Institute 1985) to compare lipid mass and percentage lipid content between sexes and age classes. We then calculated the power of these tests for a significance level of  $\alpha = 0.05$  (Cohen 1969). We examined the precision of TOBEC measurements by plotting the number of replicate TOBEC readings vs. coefficient of variation on a subset of six arbitrarily-selected birds. We used paired  $t$ -tests (SAS Institute 1985) to compare mean TOBEC readings from live birds to mean readings from recently sacrificed birds and to compare extracted lipid mass between solvents. Although several of our variables were non-normal, we chose to use parametric statistics because most variables were normally distrib-

uted and these statistics are robust under violations of normality (Boneau 1960, Donaldson 1968, Harris 1985).

## RESULTS

### EVALUATION OF TECHNIQUES

Mean body mass of the 21 Wood Thrush was 52.3 g (SD = 3.2, range 45.7–57.6 g) and mean fat score was 1.6 (SD = 1.2, range 0–4). Mean water content was 69.7% (SD = 2.2%, range 64.0–71.8%) and mean percentage lipid content was 4.8% (SD = 2.4%, range 2.9–11.2%). The mean coefficient of variation from replicate lipid extraction samples was 1.56% (SD = 1.36%, range 0.06–5.69%).

Percentage water content and fat score were highly correlated with both extracted lipid mass and percentage lipid content (Table 1). TOBEC was only moderately correlated with lean body mass (Table 1). Surprisingly, TOBEC was also moderately correlated with lipid mass, and lean body mass was not correlated with lipid mass (Table 1). Body mass, tail  $\times$  mass regression residuals, and body mass/tail length were highly correlated with lean body mass (Table 1). As expected, fat score and percentage water content were not correlated with lean body mass (Table 1). Using tail length to correct for body mass variation was slightly better than using other morphological measurements when predicting lipid content or lean mass.

Our regression equations predicting lean body mass and lipid mass had high coefficients of determination (Table 2). Body mass explained the largest portion of the variation in lean body mass and fat score explained the largest portion of the variation in lipid mass (Table 2). Addition of total body electrical conductivity increased the overall coefficient of determination little, or not at all, in equations predicting lean body mass and lipid mass (Table 2). Our model incorporating total body electrical conductivity to predict lipid mass had a coefficient of determination from cross-validation tests that was lower than simpler models predicting lipid mass (Table 2).

The percent error in models predicting lean body mass was 1.9% (Table 3). The model with the lowest absolute error in predicting lean body mass was the model including only body mass (Table 3). Inclusion of morphological measurements and total body electrical conductivity did not reduce absolute error, but did reduce widths

TABLE 1. Correlations ( $r$ ) between lipid mass (g), lipid content (%), lean body mass (g), and predictor variables for 21 Wood Thrush from Washington County, Rhode Island, June–September 1992.

	Lipid mass	Lipid %	Lean mass	Fat score	Body mass	Water %	TOBEC live <sup>a</sup>	TOBEC dead <sup>b</sup>	Tail	Wing chord	Mass/tail
Lipid %	1.00**										
Lean mass	0.14	0.07									
Fat score	0.72**	0.74**	0.03								
Body mass	0.53*	0.48*	0.91**	0.32							
Water %	-0.93**	-0.95**	0.07	-0.73**	-0.32						
TOBEC live <sup>a</sup>	0.51*	0.48*	0.52*	0.48*	0.65**	-0.29					
TOBEC dead <sup>b</sup>	0.13	0.09	0.51*	0.04	0.49*	-0.06	0.40				
Tail	-0.10	-0.13	0.18	-0.28	0.11	0.07	-0.01	0.34			
Wing chord	0.19	0.20	-0.09	-0.29	-0.01	-0.19	-0.25	-0.05	0.44*		
Mass/tail	0.54*	0.54*	0.78**	0.47*	0.89**	-0.33	0.60**	0.29	-0.36	-0.19	
Residuals <sup>c</sup>	0.54*	0.50*	0.90**	0.36	0.99**	-0.33	0.66**	0.45*	0.00	-0.05	0.93**

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

<sup>a</sup> Mean total body electrical conductivity from live birds.

<sup>b</sup> Mean total body electrical conductivity from recently sacrificed birds.

<sup>c</sup> Residuals from simple linear regression analysis of tail length vs. body mass.

of minimum prediction intervals (Table 3). However, addition of morphological measurements reduced the width of the minimum prediction intervals for models predicting lean body mass by only 2%. Addition of TOBEC indices further reduced the width of the minimum prediction interval for models predicting lean body mass by only 5%.

Percent error for models predicting lipid mass approached 30% (Table 3). Inclusion of body mass and morphological measurements reduced the average error in estimating lipid mass by 0.13 g (4.2% of fat mass). Addition of body mass reduced the width of the minimum prediction interval for predicting lipid mass by 8%. Addition of morphological measurements further reduced the width of the minimum prediction interval for models predicting lipid mass by 24%. However, addition of TOBEC indices resulted in an expansion of the minimum prediction interval for predicting lipid mass (Table 3).

We failed to find a difference ( $t = -1.21$ ,  $P = 0.238$ ,  $n = 21$ ) between mean TOBEC readings from live and dead birds, but TOBEC readings from dead birds were not correlated with live TOBEC readings (Table 1). The coefficient of variation of TOBEC readings averaged  $3.2 \pm 1.4\%$  ( $n = 288$ , 18 individuals). The mean coefficient of variation in TOBEC readings of six arbitrarily-selected individuals stopped increasing at 2.4% after nine replicates. Absolute error in lipid mass predictions from cross-validation tests of a regression equation including only TOBEC was strongly correlated with actual lipid mass ( $r = 0.81$ ,  $P = 0.0001$ ,  $n = 21$ ) and body mass ( $r = 0.51$ ,  $P = 0.02$ ,  $n = 21$ ).

#### FACTORS AFFECTING MEASUREMENT OF LIPID CONTENT

Morphological measurements were correlated with lean mass only when in association with body mass (Table 1), but were not correlated ( $0.14 < r < 0.26$ ,  $P > 0.10$ ) with lean mass when considered alone. Body mass was correlated with lipid mass (Table 1).

We found no differences in lipid mass between male ( $\bar{x} = 2.64 \pm 1.51$  g,  $n = 12$ ) and female ( $\bar{x} = 1.93 \pm 0.47$  g,  $n = 7$ ) birds ( $F = 1.45$ ,  $P = 0.245$ ,  $df = 18$ ,  $power = 0.22$ ) or percentage lipid content (male  $\bar{x} = 5.05 \pm 2.79$ , female  $\bar{x} = 3.85 \pm 0.85$ ,  $F = 1.21$ ,  $P = 0.288$ ,  $df = 17$ ,  $power = 0.18$ ). In addition, we found no differences between hatch year ( $\bar{x} = 2.09 \pm 0.44$  g,  $n = 6$ ) and

TABLE 2. Relationship between lean body mass (g), lipid mass (g), and predictor variables for 21 Wood Thrush from Washington County, Rhode Island, June–September 1992.

Regression equation <sup>a</sup>	R <sup>2</sup>	R <sup>2b</sup>
LBM = 8.9 + 0.78BM	0.83	0.79
LBM = 12.88 + 0.77BM - 0.16W + 0.19T	0.86	0.73
LBM = 17.8 + 0.77BM - 0.32W + 0.4T - 0.03EC	0.90	0.74
LM = 1.19 + 0.81FS	0.52	0.38
LM = -5.75 + 0.69FS + 0.14BM	0.62	0.45
LM = -22.93 + 0.80FS + 0.13BM + 0.22W - 0.09T	0.78	0.53
LM = 152.2 + 0.75FS + 3.68BM + 0.33W - 2.77T - 246.3MT	0.82	0.60
LM = 140.7 + 0.74FS + 3.44BM + 0.32W - 2.60T - 230.4MT + 0.007EC	0.83	0.46

<sup>a</sup> LBM = lean body mass, BM = body mass, W = wing chord, T = tail length, EC = total body electrical conductivity, LM = lipid mass, FS = fat score, MT = mass/tail.

<sup>b</sup> Coefficient of determination from cross-validation tests.

after hatch year ( $\bar{x} = 2.61 \pm 1.52$  g,  $n = 15$ ) birds in lipid mass ( $F = 0.66$ ,  $P = 0.427$ ,  $df = 20$ ,  $power = 0.12$ ) or percentage lipid content (hatch year  $\bar{x} = 4.09 \pm 0.75$ , after hatch year  $\bar{x} = 5.03 \pm 2.79$ ,  $F = 0.64$ ,  $P = 0.433$ ,  $df = 19$ ,  $power = 0.12$ ). Lipid mass and percentage lipid content were not correlated with date of capture ( $r < 0.40$ ,  $P > 0.10$ ). Lipid mass extracted was greater ( $t = 4.69$ ,  $P = 0.018$ ,  $n = 5$ ) using chloroform solvent compared to petroleum ether, but material extracted was strongly correlated ( $r = 0.9868$ ,  $P = 0.0018$ ,  $n = 5$ ) between solvents.

## DISCUSSION

### EVALUATION OF TECHNIQUES

Traditional measurement of lipid content in birds requires sacrificing birds. Attempts to predict lipid content using non-invasive techniques have had varying success, depending on species and methods used. Many studies have used linear regression to predict lipid content from mass and morphological measurements. These method-

ologies are most apparent in the waterfowl and game bird literature (Ringelman and Szymczak 1985, Blem 1990). Recently, measures of electrical conductivity have been used to estimate lean body mass and lipid mass (Walsberg 1988, Castro et al. 1990, Morton et al. 1991, Roby 1991, Scott et al. 1991; but see Skagen et al. 1993). Fat scoring provides only a qualitative index of body fat, but is sufficiently accurate for predicting lipid mass in some species.

Fat scores out-performed TOBEC and morphological regression techniques for predicting lipid mass and percentage lipid content in Wood Thrush. Our correlation between fat score and lipid mass was similar to that for five other passerines (Krementz and Pendleton 1990). Fat scores are easy to measure, unobtrusive, and provide a relatively accurate fat index suitable for most studies examining body condition in Wood Thrush. Fat scoring does have limitations (Krementz and Pendleton 1990) and researchers using fat scores should minimize observer variability and obtain large sample sizes to increase

TABLE 3. Absolute error (g) and percent error of cross-validation test results of equations predicting lean body mass (g) and lipid mass (g) in 21 Wood Thrush from Washington County, Rhode Island, June–September 1992. Estimates (g), 95% confidence intervals, and 95% prediction intervals (PI) are reported for mean and maximum values of the independent variables.

Model <sup>a</sup>	Absolute error ( $\bar{x} \pm 95\%$ CI)	Percent error ( $\bar{x} \pm 95\%$ CI)	Estimate + 95% CI (95% PI)	
			Mean $\bar{x}$	Maximum $\bar{x}$
LBM = BM	0.93 ± 0.365	1.9 ± 0.72	49.8 ± 0.52 (2.45)	54.0 ± 1.04 (2.61)
LBM = BM, W, T	0.94 ± 0.472	1.9 ± 0.91	49.8 ± 0.51 (2.40)	53.7 ± 1.44 (2.75)
LBM = BM, W, T, EC	0.97 ± 0.446	1.9 ± 0.86	49.8 ± 0.49 (2.28)	52.6 ± 1.91 (2.94)
LM = FS	0.77 ± 0.305	31.0 ± 9.37	2.5 ± 0.43 (2.01)	4.4 ± 1.01 (2.20)
LM = FS, BM	0.69 ± 0.305	27.2 ± 9.78	2.5 ± 0.39 (1.85)	4.9 ± 1.02 (2.07)
LM = FS, BM, W, T	0.68 ± 0.261	29.6 ± 11.38	2.5 ± 0.32 (1.50)	6.0 ± 1.22 (1.90)
LM = FS, BM, W, T, MT	0.64 ± 0.234	26.8 ± 9.93	2.6 ± 0.33 (1.40)	7.0 ± 1.58 (2.08)
LM = FS, BM, W, T, MT, EC	0.72 ± 0.281	30.2 ± 11.93	2.6 ± 0.35 (1.45)	7.0 ± 1.65 (2.17)

<sup>a</sup> Equations are in Table 2.

accuracy. Error in predicting lipid mass can be further reduced by including morphological measures with fat score in a regression model.

Lean body mass was predicted with an average of 1.9% error using body mass alone. TOBEC indices did not reduce error in predicting lean body mass. Lipid mass was predicted with an average error of 27–30%. Precision of TOBEC indices has been questioned for estimating lipid content of individual birds (Walsberg 1988, Morton et al. 1991, Skagen et al. 1993) because error represents a much larger fraction of lipid mass than lean body mass (Skagen et al. 1993), especially in small passerine birds which generally have lower percentage lipid content compared to waterfowl and shorebirds. We agree with Walsberg (1988) that TOBEC is not useful for predicting lipid content of individual small birds.

The coefficients of determination from our multiple regression models incorporating TOBEC, body mass, and morphological measurements were comparable to previous studies ( $R^2 > 0.83$ ). Although coefficients of determinations of our models were relatively high, cross-validation tests revealed low accuracy in predicting lipid mass. Coefficients of determination are not proper indicators of error (Skagen et al. 1993) when evaluating the usefulness of regression equations. Our data suggest that lipid mass is not estimated accurately from regression models incorporating TOBEC indices and morphological measurements (also see Skagen et al. 1993), and that TOBEC indices add little to the predictive ability. Similarly, TOBEC readings explained an additional 2.4–7.8% of the variance in lipid mass for sandpipers (Skagen et al. 1993).

Previous studies have used TOBEC on larger species (Scott et al. 1991) or for interspecific studies (Walsberg 1988, Castro et al. 1990, Roby 1991). Future studies need to examine the influence of body size on accuracy of TOBEC. In addition, high coefficients of determination for models relating TOBEC to lean body mass do not ensure low predictive errors (Skagen et al. 1993).

We did not detect lower conductivities in dead Wood Thrush compared to live birds as reported by Castro et al. (1990). However, TOBEC readings from recently sacrificed birds are not useful for predicting lean body mass, probably because electrolytic conductivity is sensitive to changes in subject temperature (Walsberg 1988, Castro et al. 1990, EM-SCAN 1991).

Our coefficient of variation on replicate TOBEC readings ( $\bar{x} = 3.2\%$ ) was slightly greater than the  $\leq 1\%$  specified by current TOBEC equipment (EM-SCAN 1991). Our data suggest that studies using TOBEC can increase precision by using the mean of  $\geq 9$  replicate readings.

#### FACTORS AFFECTING MEASUREMENT OF LIPID CONTENT

Our correlation between lipid mass and body mass ( $r = 0.53$ ) was less than that reported for other species (Blem 1990). Body mass divided by a morphological measurement can offer an improvement over the use of body mass alone in predicting lipid mass (Ringelman and Szymczak 1985). Body mass/tail length outperformed ( $r = 0.54$ ) body mass/wing length, but was less accurate than percentage water and fat score as a predictor of extracted lipid mass in Wood Thrush. Tail length showed the most promise for correcting for body mass variation in multiple regressions predicting lipid content in Wood Thrush. Our regression models, incorporating body mass and morphological measurements (Table 2), accounted for a similar proportion of the variation in lipid mass compared to previous studies (Blem 1990).

In our study, percentage water was inversely correlated ( $r = -0.93$ ) with lipid mass. In a variety of species, percentage water is a good indicator of lipid mass (Blem 1990). Non-invasive techniques for estimating percentage water content should be evaluated as a measure of lipid content in small passerines. We failed to find differences between sexes or age classes in any measure of lipid content, but power was poor for these comparisons. Hence, we cannot draw strong inferences about possible sex and age differences in lipid content.

Lipids chemically extracted from carcasses can vary depending on the solvent used (Christie 1982, Dobush et al. 1985). For example, petroleum ether extracts fewer polar lipids compared to chloroform mixtures (Hagan et al. 1967, Dobush et al. 1985). Hence, chloroform may extract more total lipids compared to petroleum ether and the difference should be most profound in carcasses with small lipid depots. Indeed, chloroform extracted more total lipids from Wood Thrush compared to petroleum ether and the difference was largest in individuals which had low lipid content. However, we believe that our

comparisons are valid because amounts of lipid extracted using the two solvents were strongly correlated ( $r = 0.9868$ ,  $P = 0.0018$ ,  $n = 5$ ) and all extractions presented in our results were performed using the same solvent.

We recommend that fat scoring be used for studies of body condition of small passerines in which workers can amass large sample sizes, have low observer variability, and do not require exact prediction of fat content of individual birds. Our results suggest that TOBEC, while correlated with lean body mass, does not further improve the accuracy of predicting lipid mass for individuals of small species. Percentage water content was highly correlated to lipid mass and future studies should evaluate the accuracy in predicting lipid mass of non-invasive techniques for estimating water content.

#### ACKNOWLEDGMENTS

We thank R. F. Cheney for assistance with field work and C. A. Millard and M. C. Wallace for helping pluck birds. M. K. Ito and S.-I. Huang assisted with the fat extractions. T. E. Martin, F. R. Moore, L. J. Petit, S. K. Skagen, B. S. Cade, and D. G. Krementz provided helpful comments that improved the manuscript. Funding was provided by the Faculty Assistance Grant Fund of the University of Rhode Island (URI) Foundation, the URI College of Resource Development, and URI Department of Natural Resources Science. We especially thank E. F. Patric and W. R. Wright for assistance with funding. Permission to collect Wood Thrush was obtained in U.S. Fish and Wildlife Service Permit #PRT-770007 and URI Institutional Animal Care and Use Committee Protocol #A91-92-026. Contribution #2908 of the College of Resource Development, University of Rhode Island with support from the Rhode Island Agricultural Experiment Station.

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