ORIGINAL ARTICLE

Body composition of American Woodcock during fall staging: a validation of the non-invasive deuterium dilution method

Clayton L. Graham[1] · Scott R. McWilliams[1]

Abstract

We conducted the first validation of the deuterium dilution method as a nonlethal technique for estimating the body composition of a shorebird during fall staging as birds fatten in preparation for their migration. For this validation, we captured male ($n=12$) and female ($n=8$) American Woodcock (*Scolopax minor*) in the fall of 2018 and 2019 in southern New England, USA. We developed predictive models for estimating body composition of woodcock given each bird's body mass and deuterium space, a proxy for total body water space, estimated using the deuterium dilution method. Accuracy and precision of these predictive models were assessed by comparing the model predictions with directly measured body composition. The top models predicted whole-body lean and fat mass with a relative error of 1.37% and 11.26%, respectively. In comparison, conventionally used morphology-based condition indices were poor predictors of fat mass in fall-staging woodcock. We applied this method to accurately estimate body composition dynamics of woodcock during fall staging, a period of fattening in the annual cycle that is pertinent to subsequent migratory movements, phenology, survival, and habitat management for woodcock. This nonlethal technique also has broad applicability to other migratory birds that show substantial changes in body composition as they prepare for key life-history events such as migration.

Keywords American Woodcock · Body composition · Deuterium dilution · Premigratory · Scolopax minor · Upland gamebirds

Zusammenfassung

Körperzusammensetzung von Kanadaschnepfen in herbstlichen Rastgebieten: eine Validierung der nichtinvasiven Deuterium-Verdünnungsmethode

Wir führten die erste Validierung der Deuterium-Verdünnungsmethode als nichtletale Technik zur Einschätzung der Körperzusammensetzung bei einer Limikolenart am herbstlichen Rastplatz durch, wenn die Vögel sich in Vorbereitung auf den Zug Fettreserven anlegen. Zur Durchführung dieser Validierung fingen wir Männchen ($n=12$) und Weibchen ($n=8$) der Kanadaschnepfe *Scolopax minor* im Herbst 2018 und 2019 im südlichen Neuengland, USA. Wir entwickelten Vorhersagemodelle zur Einschätzung der Körperzusammensetzung der Kanadaschnepfen anhand von Körpermasse und Deuteriumanteil eines jeden Vogels; letzteres diente als stellvertretendes Maß für den Gesamtwassergehalt des Körpers und wurde mittels der Deuterium-Verdünnungsmethode geschätzt. Genauigkeit und Präzision dieser Vorhersagemodelle wurden durch Vergleich der Modellvorhersagen mit der direkt gemessenen Körperzusammensetzung bewertet. Die besten Modelle sagten die Fett- und Magermasse des Körpers mit einem relativen Fehler von 1,37% beziehungsweise 11,26% voraus. Verglichen hiermit lieferten konventionelle morphologische Konditionsindizes nur schlechte Vorhersagen der Fettmasse der Kanadaschnepfen an herbstlichen Rast- und Sammelplätzen. Die Anwendung dieser Methode ermöglichte die korrekte Einschätzung der Körperkonditionsdynamik von Kanadaschnepfen während der herbstlichen Rast- und

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Sammelphase, einer Zeit im Jahreszyklus, während der Fettreserven gebildet werden und die daher relevant für spätere Zugbewegungen, Phänologie und Überlebensraten sowie das Habitatmanagement für die Kanadaschnepfe ist. Diese nicht-letale Technik hat außerdem ein breites Anwendungspotenzial bei anderen Zugvögeln, bei denen starken Veränderungen in der Körperzusammensetzung in Vorbereitung auf wichtige biologische Ereignisse, wie zum Beispiel den Zug, auftreten.

Introduction

Many birds alter their body composition in preparation for seasonal changes, and the extent of these seasonal changes in body composition often has important implications for their ecology and management (Karasov and Pinshow 1998; Williams et al. 1999; Battley et al. 2001; Servello et al. 2005). For instance, the body condition of birds just prior to migration affects the timing of their departure, rate of migration, and distance traveled prior to resting at a stopover site (Deppe et al. 2015; Buler et al. 2017). For northern-breeding waterfowl and shorebirds, body condition of females at staging areas during spring migration affects the timing of arrival at the breeding grounds and subsequent reproductive success (Alisauskas and Ankney 1992; Tulp et al. 2009; Eichhorn et al. 2010). Body condition also may indicate the quality of a given environment, because, for example, anthropogenic disturbance and development, and exposure to disease and parasites, can restrict access to higher quality resources and thus reduce the rate of fat deposition at stopover sites used during migration (Klaassen et al. 2012). Therefore, understanding the dynamics of body composition of birds across seasons provides important insights into how birds prepare for key life-history events such as migration and how this is influenced by the environment.

A variety of methods have been developed to assess body composition of animals (Blem 1990; Speakman et al. 2001). Directly measuring body composition (e.g., fat-free or lean mass, fat mass) is most accurate but requires killing the animal (Ellis and Jehl 1991). Indirect methods use nonlethal techniques to estimate body composition, although all such methods must be validated (Speakman et al. 2001; McWilliams and Whitman 2013). The deuterium dilution method is an indirect method that uses the dilution of injected heavy water within an animal to estimate total body water (Speakman et al. 2001). Given that an individual's total body water is stored almost entirely within their lean (fat-free) mass, and fat is relatively water free (<3% water), both lean and fat mass of an animal can be predicted given an estimate of total body water and measurement of body mass (Speakman et al. 2001; Eichhorn and Visser 2008). To date, the deuterium dilution method has been validated and successfully used to estimate body composition in six species of passerines (Karasov and Pinshow 1998; McWilliams and Whitman 2013; Mitchell et al. 2011; Rae et al. 2009), Barnacle Geese (Eichhorn and Visser 2008), and Common Eider (Beuth et al. 2016). Our goal was to validate the deuterium dilution method for estimating body composition (i.e., both wet lean mass and fat mass) of fall pre-migratory American Woodcock (*Scolopax minor*) that inhabit southern New England as they fatten in preparation for their fall migration. To our knowledge, this is the first validation of the deuterium dilution method for estimating body composition of a shorebird, and the first estimates of fattening rates of woodcock during fall staging.

Methods

Field methods

During September 1–November 20, 2018 and 2019, 110 American Woodcock were captured using hand nets or mist nets at roosting sites within five fields in Rhode Island Department of Environmental Management Areas and a Nature Conservancy preserve (see Online Resource 1 for location information). Each bird was sexed in the field using their wing chord, culmen length, and width of outer three primaries, and aged using molt limits and secondary feather patterns as hatch-year (HY) or after-hatch-year (AHY) (Mendall and Aldous 1943; Sheldon 1967). We also visually estimated the fat score (0–5; Helms and Drury 1960) and measured the weight of each bird to include these covariates within our predictive models. We selected the 20 woodcock used for the validation study from the larger group of captured birds ($n = 110$), so that individuals represented the weight range of males ($130 - 180$ g) and females ($170 - 230$ g) encountered during fall in southern New England. We similarly selected an additional 28 woodcock to which we applied the deuterium dilution method (details below) and then immediately released these individuals. We used the predictive models developed from the 20 validation birds to estimate the body composition of these additional 28 released bird and then estimated the timing and rate of fattening of woodcock ($n = 48$) during fall staging.

We directly measured total body water and body composition (lean and fat mass) of each of the 20 validation birds, and estimated deuterium space (a proxy for total body water space) of each of the 20 validation birds plus the additional 28 released birds following the methods described in Beuth et al. (2016). In brief, while still in the field and within 30 min of capture, we used a prefilled 1 mL insulin syringe (22004270, Fisher Scientific, Pittsburgh, PA, USA) to inject each woodcock in the pectoral muscle with 297 ± 0.99 mg

(mean $\pm$ SE) of 99.9% deuterium oxide (Sigma-Aldrich, St. Louis, MO, USA). Each syringe was weighed before and after injection to determine the exact amount of deuterium injected. After injection, birds were placed in cloth bags for 60 ± 3 min to allow the deuterium to equilibrate in their body pool of water. We then collected ca. 100 µL of blood into heparinized capillary tubes (22-362566, Fisher Scientific, Pittsburgh, PA, USA) after brachial venipuncture. The bird was placed back in the bird bag, while we flame-sealed the glass heparinized capillary tubes. All blood samples in the sealed glass tubes were stored at 4.4 ℃ until laboratory analyses. We then released the 28 woodcock used only to estimate the timing and rate of fall fattening, and euthanized via cervical dislocation each of the 20 woodcock selected for the validation study. Cervical dislocation was used to euthanize woodcock in order to not adulterate carcasses with chemical euthanasia, or lose any water from decapitation. We placed carcasses within two Ziploc freezer bags and stored frozen specimens (-17 ℃) until we completed total carcass analysis in the laboratory. In sum, we were able to estimate body composition of 48 woodcock throughout the fall-staging period. All methods were approved by the University of Rhode Island Institutional Animal Care and Use Committee (Protocol No. AN 10-02-017).

Laboratory methods

A deuterium validation study involves developing a predictive relationship between body composition measured directly from carcass analyses and deuterium space (a proxy for total body water space) estimated by deuterium dilution, as described in Eichhorn and Visser (2008) and McWilliams and Whitman (2013). Below, we first describe the direct measurement of each body component from the carcass analysis, then the methods used to estimate deuterium space from the deuterium dilution, and finally the statistical analyses used to develop the predictive relationship.

Thawed validation birds were weighed, shaved, and plucked free of their feathers, and then, the shaved carcass was reweighed to determine by difference each bird's feather mass. We homogenized each shaved carcass four times (Floor Mixer, Hobart, Troy, OH, USA) and then dried 410 g samples of homogenized carcass in aluminum trays at 90 ℃ until the homogenate reached a constant mass. Water content (%) of each bird was determined as the difference in weight between the fresh and dried homogenate divided by the fresh homogenate multiplied by 100. Total body water (TBW) was the percent water content multiplied by the shaved carcass mass.

We dried an additional 410 g samples of each homogenized carcass in aluminum trays at 60 ℃ until constant mass (Dobush et al. 1985). Each dried sample was then further homogenized using a mortar and pestle. Ten grams of dried

homogenate was placed in two previously dried (60 ℃) ceramic thimbles and refluxed in a Soxhlet extractor with petroleum ether for a minimum of 8 h (Dobush et al. 1985). The thimbles were removed, dried for 4 h at 60 ℃, and then reweighed. Fat content (%) of each thimble was determined by taking the difference between the weight of dry sample prior to extraction and the fat-free dry sample divided by the dry sample mass multiplied by 100. We used the mean % fat content of the replicates multiplied by the shaved carcass mass to determine the total whole-body fat mass. Two 5.0 g samples of lean-dry sample were placed within a dry ash oven at 550 ℃ to determine mineral (ash) content. Total whole-body lean-dry mass was estimated as the difference between shaved carcass dry mass and the combined total fat mass and mineral content.

Each pair of replicate fat-extracted samples from each carcass was combined and we used a mortar and pestle to further homogenize each lean-dry sample. We measured 31 mg replicates of lean, dry homogenate into 4×6 mm aluminum capsules (Costech, Valencia, CA, USA) and then directly measured nitrogen content using a continuous-flow isotope ratio mass spectrometer (Elementar Americas, Mount Laurel, NJ, USA). Protein content was determined by multiplying the nitrogen content by 6.25 (Parker and Holm 1990). As expected, total protein estimated from nitrogen content was closely related to total lean-dry mass across individuals ($r^2 = 0.91$, $P < 0.001$), so we report below only the results for total lean-dry mass.

Each blood sample was micro distilled following methods outlined in Nagy (1983) to separate the blood water from the whole blood sample. Using an FT-IR spectrophotometer (L160000A Perkin-Elmer, Norwalk, CT, USA), we measured deuterium concentration in five 1 uL subsamples of blood water, with the last three samples retained and the first two samples discarded to reduce carryover from previous samples. Distilled water was used to correct for background deuterium in our samples. Deuterium concentration was determined by using Beer's Law to convert peak heights into parts per millions of D_2O, given a known set of standards (Karasov et al. 1988). We converted deuterium enrichment in parts per million (ppm) to atom percent concentration using the following equation:

$$\text{Atom \%} = (100 + 0.0001557 \times (X/1000 + 1) \\ /(1 + 0.0001557 \times (X/1000 + 1), \quad (1)$$

where 0.0001557 was the mole fraction of deuterium in Vienna Standard Mean Ocean Water (Coplen et al. 2002) and X was the measured deuterium enrichment (ppm) of the sample.

We used the following equation from Karasov and Pinshow (1998), McWilliams and Whitman (2013), and Beuth et al. (2016) to estimate deuterium space (S, a proxy for

total body water space) of the 20 American Woodcock used for the validation study plus the 28 birds that were injected with deuterium, bled approximately 60 min after injection, and then released

$$E = 100 \times \{0.999 \times (B/20)/[0.999 \times (B/20) + 0.001 \times (B/18) + (S/18)]\}, \quad (2)$$

where E was the measured enrichment (atom %) of deuterium in the blood water, 0.999 was the proportion of injected solution that was deuterated water, 0.001 was the proportion of injected solution that was unlabeled water, B was the measured injection mass in grams, 20 was the molar mass of deuterated water, 18 was the molar mass of unlabeled water, and S was deuterium space in grams. We multiplied the measured deuterium enrichment from the FT-IR spectrophotometer by 1.16 to correct for a consistent underprediction of the deuterium space ($50 - 80\%$ is biologically reasonable; McWilliams and Whitman 2013). This equation can be rearranged given we know the actual amount of deuterium injected (B) and measured enrichment (E) to estimate the deuterium space (S),

$$S(g) = 18 \times \{(\{100 \times [0.999 \times (B/20)]\}/E) - [0.999 \times (B/20)] - [0.001 \times (B/18)]\}. \quad (3)$$

Statistical analysis

For the validation study, we used multiple regression analysis to create predictive models to estimate wet lean (fat-free) and fat mass of woodcock given their deuterium space (S), and body mass, structural size, age, and sex. Structural size (wing chord, culmen length, and width of outer three primaries) was condensed into orthogonal estimates using a principal component analysis (PCA), which were then used in the multiple regression analysis. The categorical variables 'age' and 'sex' were coded in the regression analysis as "1" and "2" (for AHY and HY, and female and male, respectively). A Levene's test was used to check for normality and homoscedasticity for both the regression and PCA. We used the R 3.3.2 statistical open-source software (www.r-project.org), and the segmented and caret packages to perform all statistical analyses with an alpha level of 0.05 for all statistical tests (Kuhn 2008; Muggeo 2008; R Development Core Team 2017). We used a Welch's t test corrected for unequal variance to test for mean differences in body mass, body composition, and structural size measures between male and female woodcock.

Models were selected using Akaike's Information Criterion corrected for small sample sizes (AIC_c), with a Leave-One-Out Cross Validation for model selection. Accuracy and precision of all predictive models were assessed in part by r^2 values and the root-mean-square error (RMSE). We calculated RMSE using the following equation: $\sqrt{(\Sigma(\sqrt{p} - \sqrt{m})2/n)}$, where ($\sqrt{p}$) is the predicted value, ($\sqrt{m}$) was the measured value, and n was the number of birds over which the squared difference between predicted and measured was summed (Olden and Jackson 2000). Since coefficients of determination such as RMSE are not always indicative of error when evaluating the usefulness of regression equations (e.g., Skagen et al. 1993), we also present absolute and relative errors of the predictive models (McWilliams and Whitman 2013). Each individual bird's absolute error (g) was calculated as |predicted − measured| and the relative error (%) as (absolute error/measured)×100.

We used the top predictive models generated from the validation birds to estimate the body composition of the 28 released birds. We then pooled estimates of body composition for released and validation birds ($n = 48$) and fit a nonlinear curve to describe the overall change in body composition during the fall (Sept − Nov). We used piecewise linear regression to estimate the date of fat deposition initiation and then used linear regression to estimate the rate of fattening of woodcock during the fall-staging period (~ Mid Sept − Nov).

Results

Capture of woodcock

Validation females ($n = 8$) included two HY and six AHY birds and body mass averaged 200.4 ± 6.71 g (range $170 - 230$ g; Table 1). Validation males ($n = 12$) included eight HY and four AHY birds and body mass averaged 149.5 ± 4.22 g (range $134 - 180$ g; Table 1).

Body composition and size of woodcock

Female woodcock were on average 51 g heavier, had 32 g more wet lean mass, had 24 g more total body water, and were structurally larger than males (Table 1). Male and female woodcock did not differ in their fat mass (Table 1). Total body water for woodcock was $62.36 \pm 1.22\%$ and $60.24 \pm 1.36\%$ for males and females, respectively. After hatch year and hatch-year birds did not differ in their body components or structural measures ($p > 0.05$). The first principal component (PC1) accounted for 81% of the variance with PC1 = culmen (0.552) + primary width (0.581) + wing chord (0.597). Principal component two (PC2) accounted for 12% of the variance with PC2 = culmen (0.813) + primary width (− 0.533) + wing chord (-0.233). Principal component three (PC3) accounted for 7% of the variance with PC3 = culmen (− 0.183) + primary width (− 0.615) + wing chord (0.766). Given that PC1 explained > 80% of the

Table 1 Body mass, body composition, and structural size measures (mean ± SE) of male and female woodcock caught from September to November 2018 and 2019, in Rhode Island, USA, and used for the validation of the deuterium dilution method

	Male $n = 12$ Mean ± SE	Female $n = 8$ Mean ± SE	t	p value
Body mass (g)	152.0 ± 4.22	200.4 ± 6.71	6.1	< 0.001
Wet lean mass (g)	118.7 ± 2.14	150.6 ± 3.59	7.62	< 0.001
Dry lean mass (g)	26.14 ± 1.58	34.33 ± 1.64	3.59	0.002
Fat mass (g)	20.19 ± 2.81	31.31 ± 4.63	2.05	0.06
Water mass (g)	92.57 ± 2.24	116.22 ± 3.04	6.27	< 0.001
Feather mass (g)	9.99 ± 0.53	11.75 ± 0.33	2.81	0.01
Ash mass (g)	4.21 ± 0.24	6.12 ± 0.43	3.89	0.002
Culmen (mm)	6.55 ± 0.05	7.17 ± 0.07	6.84	< 0.001
Wing chord (mm)	122.4 ± 0.57	130.8 ± 0.45	11.44	< 0.001
Primary width (mm)	10.08 ± 0.29	12.62 ± 0.32	5.87	< 0.001

Statistical results (t and p values) compare males and females for each trait

variance in structural size, we used only PC1 in our validation models.

Predictive models for estimating body composition of woodcock

Deuterium space (S) estimated using the deuterium dilution method was strongly correlated with TBW where $S = (26.973/(E \times 1.16)) - 0.269429$, $r^2 = 0.98$. As expected, the deuterium space overpredicted TBW by 9.82 ± 0.9851%, which is within the overprediction range of previous studies (8.63 – 20.86%; Karasov and Pinshow 1998; Speakman et al. 2001; Eichhorn and Visser 2008; McWilliams and Whitman 2013; Beuth et al. 2016).

The best-supported model for predicting wet lean mass (Table 2) included age, body mass, fat score, and deuterium space (Model 1). The most parsimonious model (fewest covariates) to predict wet lean mass included body mass,

fat score, and deuterium space (Model 2), although this model was not as well supported given the lower delta AIC (> 2.0). Deuterium space alone was not as predictive as our best-supported model with nearly double the relative error (Model 5, Fig. 1).

The best-supported and most parsimonious model for predicting fat mass (Table 3) included body mass, fat score, and deuterium space (Model 1). Final predictive models using the deuterium dilution method predicted wet lean mass with an absolute error of 2.07 g and relative error of 1.37%, and fat mass with an absolute error of 3.44 g and 11.26% relative error (Table 4). Models with fat score alone were not as accurate at predicting fat mass (absolute error of 6.74 g, relative error of 27.38%) (Table 3, Model 6). Predictive models using fat score to estimate fat mass explained only 51% of the variation in fat mass (Table 3, Model 6), whereas final predictive models using the deuterium dilution method to estimate fat mass (Table 4) explained 92% of the variation in fat mass (Fig. 2).

Rates and timing of fattening during fall staging for woodcock

The 28 released birds were captured between September 3 and November 5, 2018 ($n = 13$) and 2019 ($n = 15$). In early September, woodcock are completing their pre-basic molt (Owen and Krohn 1973) and the subset of individuals captured during that time were apparently losing weight. Woodcock started to build fat stores after September 22 at a rate of on average 0.42 ± 0.09 g fat per day^{-1} ($r^2 = 0.38$, $p = < 0.001$; Fig. 3). Estimated total lean-dry mass was not correlated with date ($r^2 = 0.02$, $p = 0.60$), indicating that woodcock did not build lean mass prior to fall migration. In most years, woodcock depart Rhode Island in fall during mid to late November (i.e., after Julian date 320; Graham 2020).

Table 2 Top six a priori candidate models used to estimate wet lean mass of American Woodcock captured in Rhode Island USA, during the fall of 2018 and 2019, given measured age, body mass (mb), fat score (fat), estimated deuterium space (S), a proxy for total body water space, structural size (first principal component, PC1), and sex

Model no	Wet lean mass models	AIC$_c$[a]	ΔAIC[a]	RMSE (g)	Abs ± SE (g)	Rel ± SE (%)
1	age + mb + fat + S	104.3	0	3.21	2.58 ± 0.57	1.96 ± 0.44
2	mb + fat + S	108.1	3.87	3.15	2.81 ± 0.72	2.14 ± 0.48
3	age + mb + fat + S + PC1	108.8	4.48	3.28	2.68 ± 0.75	2.04 ± 0.47
4	age + mb + fat + S + sex	108.9	4.64	3.39	2.74 ± 0.77	2.09 ± 0.46
5	S	142.4	38.13	7.85	6.41 ± 1.80	4.87 ± 1.09
6	age + S	145.5	41.18	8.54	6.76 ± 1.95	5.14 ± 1.15

Root-mean-square error (RMSE g), and absolute (Abs g) and relative (Rel %) errors ± standard errors (SE) are provided as indicators of model accuracy and precision

[a] AIC$_c$ Akaike information criterion corrected for small sample size; ΔAIC$_c$ = differences in AIC$_c$ from top model
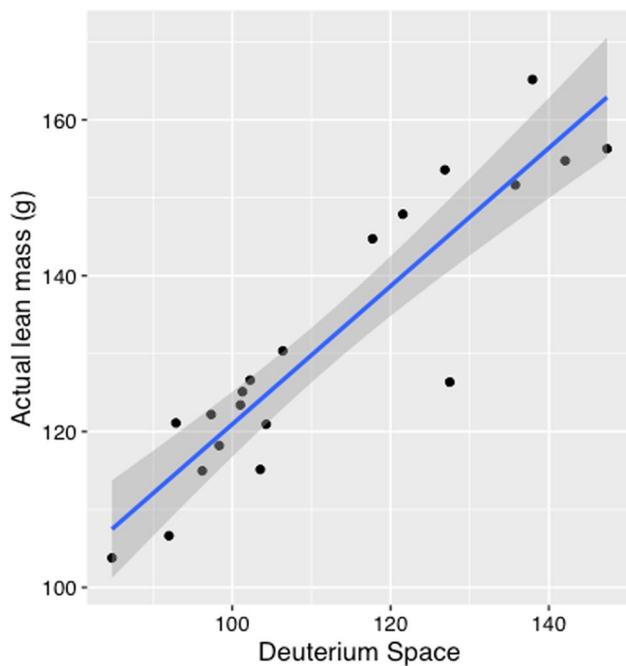
Fig. 1 Relationship between predicted deuterium space and actual lean mass measured by chemical extraction for American Woodcock caught in southern New England. Regression line and confidence intervals are for predicted deuterium space (see Table 3) in relation to actual wet lean mass

Discussion

We present the first validation of the deuterium dilution method to estimate the body composition of a shorebird, the American Woodcock, during their fall pre-migration fattening period. The resulting predictive models for nonlethally estimating whole-body fat and wet lean mass of woodcock require the visual estimation of both fat score and age, and the measurement of body mass and deuterium space, a proxy for total body water space. The predictive power of our model (relative error of $1.37 \pm 0.31\%$ for lean mass and $11.26 \pm 2.52\%$ for fat mass) is similar to previous studies that have used the deuterium dilution method to estimate body composition of birds other than shorebirds. Beuth et al. (2016) predicted the wet lean and fat mass of Common Eider with a relative error of $2.0 \pm 0.4\%$ and $20.2 \pm 3.9\%$, respectively. Eichhorn and Visser (2008) predicted the wet lean and fat mass of barnacle geese with a relative error of $1.2 \pm 1.4\%$ and $10.1 \pm 10.1\%$, respectively. McWilliams and Whitman (2013) predicted the wet lean and fat mass for three passerines with a relative error of $0.96 \pm 0.70 - 5.31 \pm 1.55\%$ and $26.36 \pm 18.6 - 34.13 \pm 10.25\%$, respectively. We advise caution in the use of our predictive models for other shorebird species, as no previous study directly compares the accuracy and precision of inter- and intra- specific models for

Table 3 Top a priori candidate models used to estimate fat mass of American Woodcock captured in Rhode Island USA, during the fall of 2018 and 2019, given measured age, body mass (mb), fat score (fat), estimated deuterium space (S), a proxy for total body water space, structural size (first principal component, PC1), and sex

Model No	Total fat models	AIC$_c$[a]	ΔAIC[a]	RMSE (g)	Abs ± SE (g)	Rel ± SE (%)
1	mb + fat + S	119.5	0	4.33	3.52 ± 0.78	14.3 ± 3.2
2	age + mb + fat + S	122.2	2.67	4.57	3.72 ± 0.83	15.12 ± 3.38
3	mb + fat + S + PC1	123.5	4.03	4.53	3.69 ± 0.82	14.96 ± 3.34
4	mb + fat + S + sex	123.6	4.06	4.84	3.86 ± 0.86	15.67 ± 3.5
5	mb + S	125.4	5.94	5.16	4.25 ± 0.95	17.12 ± 3.82
6	mb + S + age	128.5	9.30	5.47	4.41 ± 0.99	17.89 ± 4.00
7	mb + fat + PC1	148.3	18.76	8.41	6.25 ± 1.4	25.36 ± 5.67
8	fat	149	29.47	9.08	6.74 ± 1.51	27.38 ± 6.12

Root-mean-square error (RMSE g), absolute (Abs g), and relative (Rel %) errors ± standard errors (SE) are provided as indicators of model accuracy

[a]AIC$_c$ = Akaike information criterion corrected for small sample size, ΔAIC$_c$ = Differences in AIC$_c$ from top model

Table 4 Final predictive models for estimating wet lean and fat mass of American Woodcock given age, body mass (mb), fat score, and estimated deuterium space (S), a proxy for total body water space, based on measurements from all validation woodcock ($n = 20$) collected during fall 2018 and 2019 in Rhode Island, USA

Final predictive models	RMSE (g)	Abs ± SE (g)	Rel ± SE (%)
Fat mass = − 5.3599 + 0.5358 (mb) + 7.6506 (fat) + − 0.5765 (S)	3.44	2.77 ± 0.62	11.26 ± 2.52
Wet lean mass = 3.676 + 3.2529 (age) + 0.478 (mb) + -8.5239 (fat) + 0.4221 (S)	2.07	1.80 ± 0.47	1.37 ± 0.31

Root-mean-square error (RMSE g), and absolute (Abs g), and relative (Rel %) errors ± standard errors (SE) are provided as indicators of model accuracy and precision
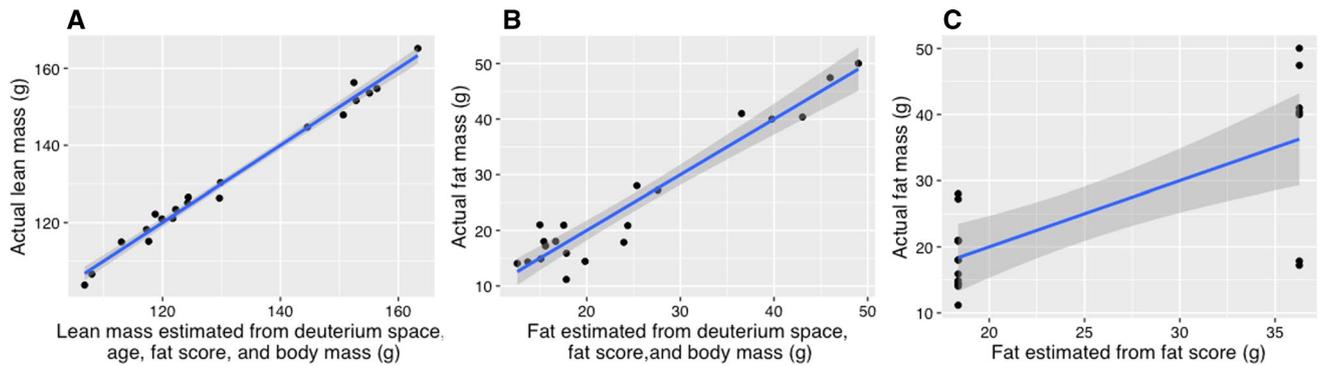
Fig. 2 Relationship between predicted lean mass and actual lean mass (**A**) and predicted fat mass and actual fat mass (**B** and **C**) measured by chemical extraction for American Woodcock caught in southern New England. Regression line and confidence intervals are for (**A**) predicted wet lean mass (g) given age, body mass, fat score, and deuterium space (see Table 4) in relation to actual wet lean mass; (**B**) predicted fat mass (g) given body mass, fat score, and deuterium space (see Table 4) in relation to actual fat mass; (**C**) predicted fat mass (g) given only fat score (Table 3, Model 4) in relation to actual fat mass
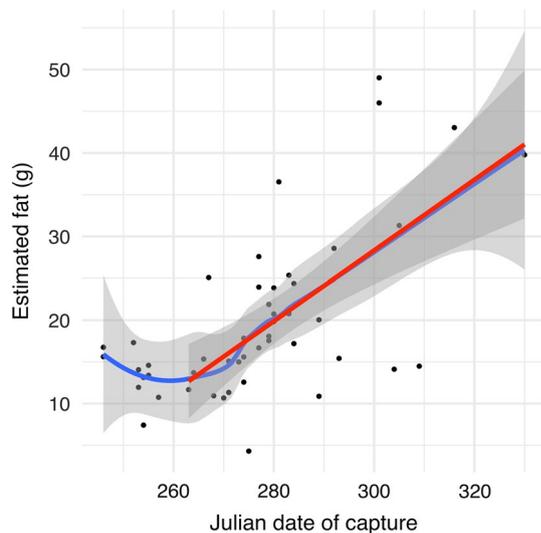
Fig. 3 Estimated whole-body fat of 48 American Woodcock during fall in southern New England based on the deuterium dilution method. Changes in whole-body fat starting in early September (blue line: nonlinear best-fit regression) and after day 265 (September 22) when birds initiated fattening (red line: linear regression). The slope of the red line estimates the rate of fat accumulation during fall staging (0.42 ± 0.09 g fat day^{-1}). Confidence intervals are shown by the gray bands (color figure online)

shorebird species like that accomplished by McWilliams and Whitman (2013) for small-bodied passerine species. The deuterium dilution method provides reasonably accurate estimates of body composition for a variety of birds—within $1-3\%$ for fat-free lean mass and $10-34\%$ for fat mass—and this accuracy is adequate enough to detect seasonal changes in body composition, given that the whole-body wet lean and fat mass of migratory birds can change seasonally by as much as 25–50% (Blem 1990; Piersma and Van Brederode 1990; Seewagen and Guglielmo 2011).

The estimates of fat mass for woodcock during their fall pre-migration fattening period that we obtained using the deuterium dilution method were more accurate than those obtained using other nonlethal techniques. For example, fat score predicted whole-body fat mass with a relative error of $27.38 \pm 6.12\%$ (Table 3, Model 6) and was much less accurate than our best-fit model using body mass, fat score, and deuterium space (Table 3, Model 1 and Fig. 1). In contrast, McWilliams and Whitman (2013) found that fat mass of songbirds with fat scores greater than 1 could be more accurately predicted given only fat score compared to using total body electrical conductivity (TOBEC) or the deuterium dilution method. However, most of these songbirds had fat scores of < 1.0 and the fat mass of these leaner birds was more accurately predicted using the deuterium dilution method. In comparison, body mass, fat score, and the first principal component predicted whole-body fat mass for fall woodcock with a relative error of $25.36 \pm 5.67\%$ (Table 3, Model 5), which is more than double the error of our best-fit model that included deuterium space (Table 3, Model 1). Fat score may be a poor predictor of actual fat mass within woodcock, because like other birds, they may deposit fat subcutaneously or in areas not visible when assessing fat score (Seewagen and Slayton 2008; Labocha and Hayes 2012). TOBEC has been used to estimate body composition of several species of shorebirds, although relative error for predicted fat mass was, for example, $30-40\%$ for intraspecific models and 46–73% for interspecific models (Lyons and Haig 1995). In general, TOBEC is not accurate for estimating fat mass for birds lighter than 215 g, making TOBEC unsuitable for the analysis of body composition of woodcock (Morton et al. 1991; McWilliams and Whitman 2013). We conclude that the deuterium dilution method provides the most accurate tundra-tested (McWilliams and Whitman

2013) field method to predict fat mass of woodcock and likely most other shorebirds.

Understanding the dynamics of body composition of woodcock as well as other migratory birds is important to managers and biologists, because fat and protein stores affect migratory movement, phenology, and success (Stutchbury et al. 2011; Bruggink et al. 2013; Seewagen et al. 2011). For instance, woodcock initiates migration only after acquiring enough fat stores and completing their pre-basic molt (Owen Jr and Krohn 1973). Fall-staging woodcock that inhabited poorer-quality landscapes (e.g., lower densities of earthworms) moved greater distances in search of food and this may delay the accumulation of fat and affect the tempo and pace of their subsequent migration (Doherty et al. 2010). Many migratory birds shift their diets and thus habitats in late-summer to track shifts in resource availability, and this may affect their ability to acquire sufficient fuel stores to use during fall migration (Connors 1984; Leinaas and Ambrose 1999; Churchwell et al. 2018). For example, fall-staging semipalmated sandpipers in the Bay of Fundy selected foraging sites based on the availability of lipid-rich *Corophium volutator* and biofilms to be able to deposit enough fat to fuel their non-stop flight to South America (Maillet and Weber 2006; Quinn and Hamilton 2012). Many species of post-breeding songbirds also substantially switch their habitat use during fall, in this case from mature forest to early successional habitat where high fruit densities advance the molt and body condition of birds (Vitz and Rodewald 2006; Stoleson 2013). Thus, providing adequate resources and appropriately managing habitat for birds as they prepare for migration require knowing where and when birds initiate their preparations for migration (i.e., the dynamics of body composition) and how best to manage habitat to provide the resources they need.

Although survival rates are relatively high for woodcock in the fall in comparison to the spring, a reduction in fat stores and body mass is associated with increased mortality and may in turn reduce recruitment rates (McAuley et al. 2005). For example, a reo-virus outbreak in Virginia and New Jersey substantially reduced the body condition of woodcock, resulting in a die- off of these local populations (Docherty et al. 1994). Survival rates may also be reduced during summer droughts, and drought conditions which continue into fall staging may slow or delay molting strategies and subsequently inhibit the accumulation of fat stores for fall migration (Sepik et al. 1993). Thus, information on the dynamics of woodcock body composition can provide managers information on population susceptibility to periodic disturbances (e.g., drought and disease), and how these events affect population dynamics.

We estimated that woodcock in southern New England fattened at a rate of 0.42 ± 0.09 g fat per day^{-1}, a 0.24% increase in body mass per day, during the fall pre-migration period. Woodcock during fall migration in the central flyway flew 784–1,693 km over 15–48 days to their wintering grounds, and this relatively deliberate pace of migration requires lower fattening rates than other shorebirds that migrate further and more rapidly (Myatt and Krementz 2007). Sharp-tailed Sandpipers (*Calidris acuminata*) with average pre-migratory body masses of 70.8 g for males and 57.6 for females had similar fattening rates during the early pre-migratory fattening period (males: 0.4 ± 0.2 g day^{-1}, females: 0.3 ± 0.2 g day^{-1}) but then accelerated their fattening rate starting in mid-September as they prepared to migrate on average 7100 km (Lindström et al. 2011); males: 4.2 ± 0.3 g day^{-1} or 6.3% increase relative to lean body mass (LBM) per day; females: 3.2 ± 0.3 g day^{-1} or 5.7% of LBM per day). Red Knots (*Calidris canutus*; mean body mass: 118 g) staging in fall at Ottenby, Sweden deposited fat on average by 2.8% of LBM per day^{-1} (Helseth et al. 2005). Fall-staging Red-Necked Phalaropes (*Phalaropus lobatus*: body mass range: 30.4–63.6 g) deposited fat at a rate of 1.0 g per day in preparation for their long-distance flight from New Brunswick to South America (Mercier 1985). In general, our estimates of fattening rates of fall-staging woodcock in southern New England conform to the trend observed by others that shorebirds departing higher latitudes and covering larger migratory distances deposit fat at a greater rate than shorebirds migrating from lower latitudes (Aharon-Rotman et al. 2016).

The deuterium dilution method and the predictive models presented here provide biologists an accurate and repeatable method to nonlethally estimate lean and fat mass of woodcock at fall staging and stopover locations. This method also provides managers and ecologists an effective tool to address how habitat management practices affect the body composition dynamics of woodcock and their preparation for fall migration (Moore and Yong 1991; Petit 2000). Future validation studies are needed to estimate the body composition of male and female woodcock during the spring, as their physiology and body composition dynamics during spring may differ from that in fall, because it includes preparation for migration as well as breeding.

Declarations

References

Aharon-Rotman Y, Gosbell K, Minton C, Klaassen M (2016) Why fly the extra mile? Latitudinal trend in migratory fuel deposition rate as driver of trans-equatorial long-distance migration. Ecol Evol 6:6616–6624. https://doi.org/10.1002/ece3.2388

Alisauskas RT, Ankney CD (1992) The cost of egg laying and its relationship to nutrient reserves in waterfowl. In: Batt BDJ, Afton AD, Anderson MG, Ankney CD, Johnson DH, Kadlec JA, Krapu GL (eds) Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis, pp 30–61

Battley PF, Dietz MW, Piersma T et al (2001) Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. Physiol Biochem Zool 74:435–449. https://doi.org/10.1086/320432

Beuth JM, Paton PWC, Osenkowski JE, McWilliams SR (2016) Validating the deuterium dilution method to measure body composition of common eider. Wildl Soc Bull 40:456–463. https://doi.org/10.1002/wsb.667

Blem CR (1990) Avian energy storage. In: Power M (ed) Current ornithology. Plenum Press, New York, pp 59–113

Bruggink JG, Oppelt EJ, Doherty KE et al (2013) Fall survival of American woodcock in the western Great Lakes Region. J Wildl Manage 77:1021–1030. https://doi.org/10.1002/jwmg.547

Buler JJ, Lyon RJ, Smolinsky JA et al (2017) Body mass and wing shape explain variability in broad-scale bird species distributions of migratory passerines along an ecological barrier during stopover. Oecologia 185:205–212. https://doi.org/10.1007/s00442-017-3936-y

Connors PG (1984) Ecology of shorebirds in the Alaskan Beaufort littoral zone. In: Barnes PW, Shell DM, Reimnitz E (eds) The Alaskan Beaufort Sea: ecosystems and environments. Academic Press, New York, pp 403–416

Coplen TB, Hopple JA, Böhlke JK, Peiser HS, Rieder SE, Krouse HR, Rosman KJR, Ding T, Vocke Jr. RD, Révész KM, Lamberty A, Taylor P, De Bièvre P (2002) Compilation of minimum and maximum isotope ratios of selected elements in naturally occurring terrestrial materials and reagents. U.S. Geological Survey Water-Resources Investigations Report 01-4222

Churchwell RT, Kendall S, Brown SC et al (2018) The first hop: use of Beaufort Sea deltas by hatch-year semipalmated sandpipers. Estuaries Coasts 41:280–292. https://doi.org/10.1007/s12237-017-0272-8

Deppe JL, Ward MP, Bolus RT et al (2015) Fat, weather, and date affect migratory songbirds' departure decisions, routes, and time it takes to cross the Gulf of Mexico. Proc Natl Acad Sci 112:6331–6338. https://doi.org/10.1073/pnas.1503381112

Dobush GR, Davison C, Krementz DG (1985) The effect of apparatus, extraction time, and solvent type on lipid extraction of snow geese. Can J Zool 63:1917–1920. https://doi.org/10.1139/z85-285

Docherty DE, Converse KA, Hansen WR, Norman GW (1994) American woodcock (*Scolopax minor*) mortality associated with a reovirus. Avian Dis 38:899–904. https://doi.org/10.2307/1592132

Doherty KE, Andersen DE, Meunier J et al (2010) Foraging location quality as a predictor of fidelity to a diurnal site for adult female American woodcock *Scolopax minor*. Wildlife Biol 16:379–388. https://doi.org/10.2981/09-100

Eichhorn G, Visser GH (2008) Evaluation of the deuterium dilution method to estimate body composition in the Barnacle Goose: accuracy and minimum equilibration time. Physiol Biochem Zool 81:508–518. https://doi.org/10.1086/588170

Eichhorn G, van der Jeugd HP, Meijer HAJ, Drent RH (2010) Fueling incubation: differential use of body stores in arctic- and temperate-breeding Barnacle Geese (*Branta leucopsis*). Auk 127:162–172. https://doi.org/10.1525/auk.2009.09057

Ellis HI, Jehl JR (1991) Total body water and body composition in phalaropes and other birds. Physiol Zool 64:973–984. https://doi.org/10.1086/physzool.64.4.30157952

Graham, CL (2020) The influence of pre-migratory body composition and habitat quality on fall migration in the American Woodcock (Scolopax minor). M.Sc Thesis. University of Rhode Island, Kingston

Helms CW, Drury WH (1960) Winter and migratory weight and fat field studies on some North American buntings. Bird-Banding 31:1. https://doi.org/10.2307/4510793

Helseth A, Lindström Å, Stervander M (2005) Southward migration and fuel deposition of Red Knots Calidris canutus. Ardea 93:213–224

Karasov WH, Han LR, Munger JC (1988) Measurement of ^2H_2O by IR absorbance in doubly labeled H_2O studies of energy expenditure. Am J Physiol 255:R174–R177

Karasov WH, Pinshow B (1998) Changes in lean mass and in organs of nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. Physiol Zool 71:435–448. https://doi.org/10.1086/515428

Klaassen M, Hoye BJ, Nolet BA, Buttemer WA (2012) Ecophysiology of avian migration in the face of current global hazards. Philos Trans R Soc B Biol Sci 367:1719–1732

Kuhn M (2008) Building predictive models in R using the caret package. J Stat Softw 28:1–26. https://doi.org/10.18637/jss.v028.i05

Labocha MK, Hayes JP (2012) Morphometric indices of body condition in birds: a review. J Ornithol 153:1–22. https://doi.org/10.1007/s10336-011-0706-1

Leinaas HP, Ambrose WG (1999) Decision between small and large prey: reduced energy acquisition by pre-migratory purple sandpipers, Calidris maritima, on Svalbard. Polar Biol 22:264–270. https://doi.org/10.1007/s003000050419

Lindström Å, Gill RE, Jamieson SE et al (2011) A puzzling migratory detour: are fueling conditions in Alaska driving the movement of

juvenile sharp-tailed sandpipers? Condor 113:129–139. https://doi.org/10.1525/cond.2011.090171

Lyons JE, Haig SM (1995) Fat content and stopover ecology of spring migrant Semipalmated Sandpipers in South Carolina. Condor 97:427–437

Maillet D, Weber JM (2006) Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. J Exp Biol 209:2686–2695. https://doi.org/10.1242/jeb.02299

McAuley DG, Longcore JR, Clugston DA et al (2005) Effects of hunting on survival of American Woodcock in The Northeast. J Wildl Manage 69:1565–1577. https://doi.org/10.2193/0022-541X(2005)69[1565:EOHOSO]2.0.CO;2

McWilliams SR, Whitman M (2013) Non-destructive techniques to assess body composition of birds: a review and validation study. J Ornithol 154:597–618. https://doi.org/10.1007/s10336-013-0946-3

Mendall HL, Aldous CM (1943) The ecology and management of the American woodcock. Maine Cooperative Wildlife Research Unit. University of Maine, Orono

Mercier FM (1985) Fat reserves and migration of Red-necked Phalaropes (*Phalaropus lobatus*) in the Quoddy region, New Brunswick, Canada. Can J Zool 63:2810–2816. https://doi.org/10.1139/z85-420

Mitchell GW, Guglielmo CG, Wheelwright NT, Freeman-Gallant CR, Norris DR (2011) Early life events carry over to influence pre-migratory condition in a free-living songbird. PLoS ONE 6:e28838

Moore FR, Yong W (1991) Evidence of food-based competition among passerine migrants during stopover. Behav Ecol Sociobiol 28:85–90. https://doi.org/10.1007/BF00180984

Morton JM, Kirkpatrick RL, Smith EP (1991) Comments on estimating total body lipids from measures of lean mass. Condor 93:463–465. https://doi.org/10.2307/1368970

Muggeo VM (2008) Segmented: an R package to fit regression models with broken-line relationships. R News 8:20–25

Myatt NA, Krementz DG (2007) Fall migration and habitat use of American Woodcock in the Central United States. J Wildl Manage 71:1197–1205. https://doi.org/10.2193/2006-154

Nagy KA (1983) The doubly labeled water (3HH18O) method: a guide to its use. UCLA Publication no 12-1417. UCLA, Los Angeles, CA

Owen RB Jr, Krohn WB (1973) Molt patterns and weight changes of the American Woodcock. Wilson Bull 85:31–41

Olden JD, Jackson DA (2000) Torturing data for the sake of generality: how valid are our regression models? Ecoscience 7:501–510

Parker H, Holm H (1990) Patterns of nutrient and energy expenditure in female common eiders nesting in the high arctic. Auk 107:660–668

Petit DR (2000) Habitat use by landbirds along nearctic-neotropical migration routes: implications for conservation of stopover habitats. Stud Avian Biol 20:15–33

Piersma T, Van Brederode NE (1990) The estimation of fat reserves in coastal waders before their departure from northwest Africa in spring. Ardea 78:221–236

Quinn JT, Hamilton DJ (2012) Variation in diet of Semipalmated Sandpipers (*Calidris pusilla*) during stopover in the upper Bay of Fundy, Canada. Can J Zool 90:1181–1190. https://doi.org/10.1139/Z2012-086

R Development Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rae LF, Mitchell GW, Mauck RA, Guglielmo CG, Norris DR (2009) Radio transmitters do not affect the body condition of Savannah Sparrows during the fall premigratory period. J Field Ornithol 80:419–426

Seewagen CL, Guglielmo CG (2011) Quantitative magnetic resonance analysis and a morphometric predictive model reveal lean body mass changes in migrating Nearctic-Neotropical passerines. J Comp Physiol B 181:413–421. https://doi.org/10.1007/s00360-010-0534-2

Seewagen CL, Slayton EJ (2008) Mass changes of migratory landbirds during stopovers in a New York City park. Wilson J Ornithol 120:296–303. https://doi.org/10.1676/07-087.1

Seewagen CL, Sheppard CD, Slayton EJ, Guglielmo CG (2011) Plasma metabolites and mass changes of migratory landbirds indicate adequate stopover refueling in a heavily urbanized landscape. Condor 113:284–297. https://doi.org/10.1525/cond.2011.100136

Sepik GF, McAuley DG, Longcore JR (1993) Critical review of the current knowledge of the biology of the American Woodcock and its management on the breeding grounds. In: Sepik GF, Longcore JR (eds) Proceedings eigth woodcock symposium. pp 98–104

Servello F, Hellgren EC, McWilliams SR (2005) Techniques for wildlife nutritional research. In: Braun E (ed) Research and management techniques for wildlife and habitat. The Wildlife Society, Washington, pp 554–590

Sheldon WG (1967) The book of American Woodcock. University of Massachusetts Press, Amherst

Skagen SK, Knopf FL, Cade BS (1993) Estimation of lipids and lean mass of migrating sandpipers. Condor 95:944–956

Speakman JR, Visser GH, Ward S, Krol E (2001) The isotope dilution method for the evaluation of body composition. In: Speakman JR (ed) Body composition analysis of animals: a handbook of non-destructive methods. Cambridge University Press, Cambridge, pp 56–98

Stoleson SH (2013) Condition varies with habitat choice in postbreeding forest birds. Auk 130:417–428. https://doi.org/10.1525/auk.2013.12214

Stutchbury BJM, Gow EA, Done T et al (2011) Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. Proc R Soc B Biol Sci 278:131–137. https://doi.org/10.1098/rspb.2010.1220

Tulp I, Schekkerman H, Klaassen RHG et al (2009) Body condition of shorebirds upon arrival at their Siberian breeding grounds. Polar Biol 32:481–491. https://doi.org/10.1007/s00300-008-0543-8

Vitz AC, Rodewald AD (2006) Can regenerating clearcuts benefit mature-forest songbirds? An examination of post-breeding ecology. Biol Conserv 127:477–486. https://doi.org/10.1016/j.biocon.2005.09.011

Williams TD, Guglielmo CG, Martyniuk CJ (1999) Plasma lipid metabolites provide information on mass change over several days in captive Western Sandpipers. Auk 116:994–1000. https://doi.org/10.2307/4089679