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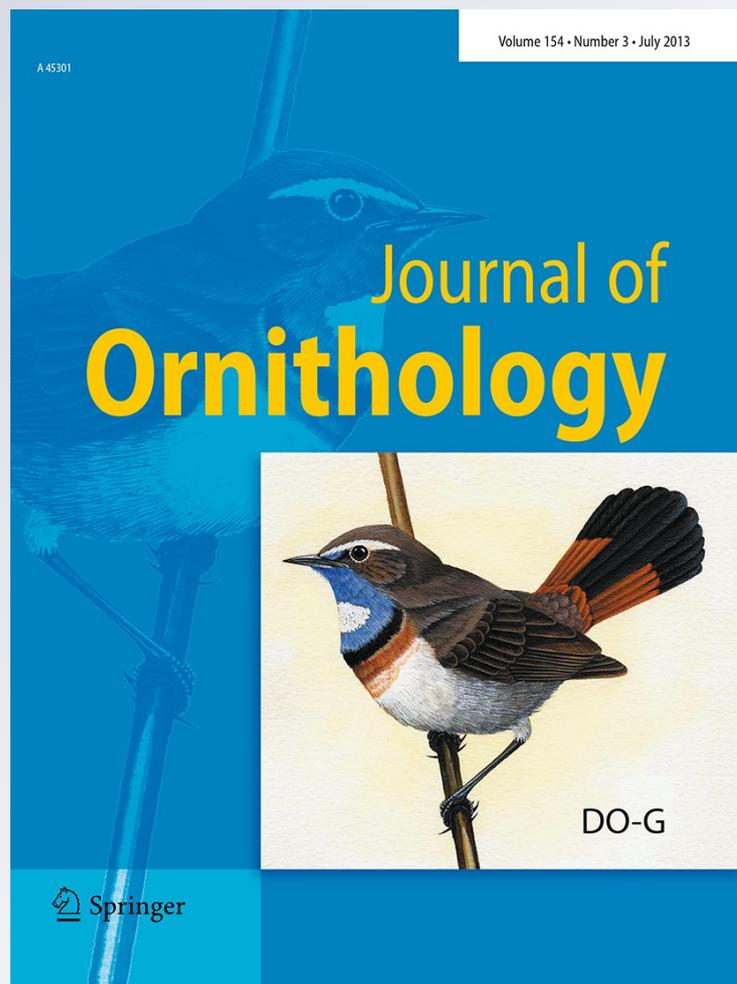
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# Non-destructive techniques to assess body composition of birds: a review and validation study

Scott R. McWilliams · Megan Whitman

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**Abstract** Body composition of birds is often used to assess in part the “condition” of individuals in the context of their life history and ecology. We describe contemporary non-destructive (non-lethal) techniques that are available for estimating the body composition of free-living birds. We critically evaluate the strengths and weaknesses of these techniques in the context of bird studies. Although most contemporary techniques are based on theory and first principles, assessing their accuracy and precision requires empirical calibrations. We summarize the results of recent validation studies on songbirds and discuss their implications. Deuterium dilution was the best single technique among those compared for measuring lean and fat dynamics in small songbird species that averaged 9–29 g, although we advocate technique(s) that independently estimate each body component. Interspecific models that estimated lean mass using total body electrical conductivity and structural measure(s), and estimated fat mass using deuterium dilution were as accurate (within 0.3–1.1 g of actual lean mass and 0.2–0.9 g of actual fat mass, respectively, depending on bird species) as intraspecific models for songbirds that averaged 13–29 g in body mass. Thus,

separate models for each bird species may not be necessary, and the development and testing of interspecific models for estimating body composition is warranted. Several factors, including body size and physiological state, required accuracy and precision, and the scope of predictions must be carefully considered when any of these non-destructive techniques are used to measure the body composition of birds.

**Keywords** Songbird body composition · Non-destructive techniques · Total body electrical conductivity · Deuterium · Fat score · DEXA · QMR

## Zusammenfassung

### Nicht-destructive Techniken zur Ermittlung der Körperzusammensetzung von Vögeln: eine kritische Übersicht

Oft wird die Körperzusammensetzung von Vögeln zur ansatzweisen Bewertung der “Kondition” von Individuen im Kontext von Lebenszyklus und Ökologie herangezogen. Hier stellen wir nicht-destructive (nicht tödliche) Techniken vor, die derzeit zur Ermittlung der Körperzusammensetzung freilebender Vögel zur Verfügung stehen. Die Stärken und Schwächen dieser Methoden werden vor dem Hintergrund ornithologischer Studien kritisch erläutert. Obwohl die meisten heute verwendeten Techniken auf wissenschaftlichen Theorien und Annahmen begründet sind, ist zur Beurteilung ihrer Richtigkeit und Präzision ein empirischer Abgleich notwendig. Hier fassen wir die Ergebnisse neuerer Studien an Singvögeln zusammen und diskutieren deren Bedeutung. Im Vergleich der Techniken zur Messung der “Fett-Mager-Dynamik” bei kleinen Singvogelarten (im Schnitt 9–29 g) schnitt die Bestimmung des Deuteriumgehalts im Körperwasser am besten ab, allerdings würden wir Methoden

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empfehlen, welche die einzelnen Körperbestandteile einzeln ermitteln. Interspezifische Modelle, welche die Magermasse anhand von TOBEC (Total Body Electrical Conductivity, Gesamtkörperleitfähigkeit) und strukturellen Messgrößen und die Fettmasse anhand des Deuteriumanteils abschätzten, waren gleichermaßen genau (je nach Vogelart zwischen 0.3–1.1 g um den tatsächlichen Wert der Magermasse bzw. 0.2–0.9 g um den tatsächlichen Wert der Fettmasse) wie intraspezifische Modelle für Singvögel mit einer durchschnittlichen Körpermasse von 13–29 g. Daher sind separate Modelle für jede Vogelart möglicherweise nicht notwendig und stattdessen wäre die weitere Entwicklung und Erprobung interspezifischer Modelle zur Schätzung der Körperzusammensetzung wünschenswert. Mehrere Faktoren-unter anderem Körpergröße, physiologischer Zustand, die angestrebte Messgenauigkeit aber auch die Reichweite der daraus gezogenen Schlussfolgerungen-müssen sorgfältig in Betracht gezogen werden, wenn eine solche nicht-destruktive Technik zur Ermittlung der Körperzusammensetzung von Vögeln eingesetzt wird.

## Introduction

Ornithologists measure the body composition of birds for a variety of good reasons (Brown 1996). For example, birds during migration must rest and refuel at stopover sites (e.g., Bairlein 1983; Moore et al. 1990; Yong and Moore 1997) for varying lengths of time (e.g., 2–7 days, Parrish 1997; 2–4 days, Gannes 2002), and measuring the dynamics of body composition in songbirds at these stopover sites provides a useful way to assess habitat quality and the effectiveness of habitat management programs that are designed to protect and conserve habitat for migrating birds (Bensch and Nielsen 1999; Dunn 2000; Petit 2000; McWilliams and Karasov 2005; Moore and Kerlinger 1987; Moore and Yong 1991; Parrish 2000). Multitudes of waterfowl studies have reported the body composition of females in spring because the extent of their nutrient stores often determines investment in reproduction (e.g., Campbell and Leatherland 1980; Drent and Daan 1980; Alisauskas and Ankney 1992; Eichhorn et al. 2010). Thus, accurate and precise measures of body composition are of interest for many different reasons, although there are many options and considerations involved in deciding how to measure body composition of wild birds.

Early scholars of avian body composition assumed that body stores consisted entirely of fat and that lean mass was the structural part of the body and was, as such, invariant (Odum et al. 1964; Hicks 1967). More recent studies, however, suggest that body stores include both

lean and fat components (Ellis and Jehl 1991; van der Meer and Piersma 1994; Klaassen et al. 1997; Piersma and Lindström 1997; Gannes 1999). The number of whole-body components considered depends on level of biological organization (Reynolds and Kunz 2001; Stevenson and Woods 2006). Common practice in ornithological studies wherein body composition is destructively measured is to divide the bird into four components [water, fat, protein, other (e.g., ash, feathers)] or three components (water, fat, fat-free), although many non-destructive methods require a simpler two-component model of body composition (fat, fat-free or “lean”) (Speakman 2001; Servello et al. 2005). The lack of accurate non-destructive methods to measure lean and fat mass in wild birds was proposed as one of the most limiting aspects of avian ecological energetics (Gessaman et al. 1998). Here, we describe non-destructive (non-lethal) techniques that are available for estimating body composition of free-living birds with an emphasis on songbirds, and we critically evaluate the strengths and weaknesses of these techniques in the context of bird studies.

## Condition indices

Non-destructive techniques, by definition, provide an indirect estimate of body composition in that total fat, protein or some other body component is not directly measured as can be done with destructive techniques. “Condition indices” are an important subset of both non-destructive and destructive techniques (Johnson et al. 1985; Servello et al. 2005). Such indices provide a measure that is correlated with some body component (e.g., a relative fat score may relate to whole-body fat mass) as opposed to providing a direct measure or estimate of the quantity of the body component. Although condition indices that use a ratio of body mass to a linear measure of body size are easy to compute and have long been used in avian studies (Johnson et al. 1985), there are serious limitations with such indices (see Packard and Boardman 1999; Hayes and Shonkwiler 2001; Servello et al. 2005; Stevenson and Woods 2006; Peig and Green 2010; Labocha and Hayes 2012). Most importantly, condition indices based on a ratio of body mass to body size are often misleading because changes in the index value are often confounded by the effects of body size, and such ratio variables are plagued by statistical and inferential problems (Jakob et al. 1996; Hayes and Shonkwiler 2001; Labocha and Hayes 2012). Labocha and Hayes (2012) provide a thorough treatment of the pros and cons of using a ratio of body mass to body size and other morphometric indices to estimate body condition of birds.

## Description of hydrostatics, aerostatics, and bioelectrical impedance analysis

Many of the methods used to non-destructively estimate body composition were originally developed for humans, so it is worth briefly mentioning a few of the contemporary standard approaches used for humans that have not yet been used for bird studies. The gold standard for non-destructively measuring human body composition is water or air volume displacement. The subject is placed in a water bath or sealed air-tight chamber (e.g., the Bod Pod), and the measured volume of water or air displaced along with his/her body mass are used to estimate percentage body fat given standardized equations and corrections for lung and gut air volume [see Heyward and Wagner (2004) for detailed methods]. However, most wild birds are unlikely to tolerate submergence long enough for the necessary measurements to be made, which limits the hydrostatic method's utility in bird studies. In principle, air displacement could work for birds, although we are not aware of any manufacturers considering the production of "Bird Pods."

Bioelectrical impedance analysis (BIA) is another common measure used to estimate body composition of humans as well as a few wild mammals, although we are not aware of any BIA studies conducted with birds. The BIA apparatus involves measuring electrical conductivity between two discrete points on the surface of the animal. Measured conductivity can then be used to estimate the lean mass of the animal because conductivity is directly related to the mass of dissolved electrolytes (primarily potassium, sodium, and chloride ions) in tissues, with lean tissue containing most body water and, thus, most dissolved electrolytes. Hildebrand et al. (1998) compared BIA and the deuterium (deuterium oxide; hereafter  $D_2O$ ) dilution method (see following section for more details) for estimating the body composition of bears and concluded that  $D_2O$  dilution was the more accurate and therefore more preferred method. The biggest problem with BIA for applications on wild animals, including birds, is that it is sensitive to electrode placement, body temperature, skin insulation, body shape and size, hydration state, gut fill, and activity of the animal (Marken-Lichtenbelt 2001). Given that all these variables are difficult to control for wild birds, the potential use of BIA by ornithologists interested in estimating the body composition of birds is greatly limited.

The remainder of this article focuses on those methods that have been used to estimate the body composition of free-living birds. We also report the results of a cross-validation study that uses a combination of methods to separately estimate different body components. Such a combined-methods approach has been endorsed by the

American Society for Exercise Physiologists (Heyward 2001) and avoids problems associated with compounding of error estimates when some components are estimated by subtraction.

## Total body electrical conductivity, $D_2O$ dilution, and fat score: the methods and a validation study

The methods

Three other non-invasive techniques [total body electrical conductivity (hereafter TOBEC), dilution of the stable isotope  $D_2O$ , and fat scoring] have been more commonly used by ornithologists for estimating lean and fat stores in small migratory birds, although the TOBEC and  $D_2O$  dilution techniques should work in principle with animals regardless of size and species.

A TOBEC device measures the electrical conductivity of the whole animal within a detection chamber by causing a change in the electromagnetic inductance of a solenoid (Walsberg 1988; Burger 1997). The primary determinant of signal output from a TOBEC device is lean body mass because the electrical conductivity of lipids is only about 4–5 % of lean tissues (Pethig 1979). The primary advantages of TOBEC for estimating lean mass of birds are that it is entirely noninvasive, measurements can be quickly made (<3 min), the instrument is portable and easy to use in the field, and it is one of the few techniques that can be used to measure short-term changes in body composition of the same individuals (e.g., Karasov and Pinshow 1998). The primary disadvantages of TOBEC are that it can only be used on certain wildlife that can fit into the detection chamber and tolerate being restrained in a fixed position during the measurement, the manufacturer has not produced new devices since 2006, and many factors must be controlled to achieve reliably accurate estimates. Although avian researchers have had variable success with TOBEC (Castro et al. 1990; Roby 1991; Purvis et al. 1999), recent advances in the method used to restrain small birds within the TOBEC device have yielded estimates of lean mass within on average 7 % of actual lean mass measured by chemical extraction (Karasov and Pinshow 1998). TOBEC has proven less successful in estimating fat mass (Morton et al. 1991; Skagen et al. 1993; Conway et al. 1994; Asch and Roby 1995; Lyons and Haig 1995), primarily because the absolute error of predicted fat mass is of the same magnitude as that of predicted lean mass when only TOBEC is used to estimate body composition (Burger 1997). In addition, the accuracy of TOBEC is influenced by the position of the bird during measurement, type of restraint used during measurement, hydration state of the bird, whether the bird was alive or dead during measurement,

and the TOBEC model used (Walsberg 1988; Castro et al. 1990; Roby 1991). Here we propose a method for using TOBEC that controls for these factors and provides reasonably accurate and precise estimates of lean mass.

Hydrogen isotope-labeled water can be used to measure total body water which is closely related to lean mass ( $r^2 > 0.95$ ; Child and Marshall 1970; Campbell and Leatherland 1980; Eichhorn and Visser 2008) as well as fat mass ( $r^2 > 0.95$ ; Bailey 1979; Johnson et al. 1985; Piersma and Klaassen 1999). The uneven distribution of water among body tissues (body fat contains almost no water, body protein contains on average about 70 % water) means the fatter an animal becomes the lower the water content as a percentage of body mass. Thus, in principle, body fat can be estimated by measuring the body mass and water content of any animal. We used D<sub>2</sub>O, a stable isotope of water, to estimate total body water by measuring the isotopic enrichment of a blood sample taken from an individual at some specified time point after the injection of a known amount of D<sub>2</sub>O (Speakman et al. 2001). D<sub>2</sub>O dilution has been used to estimate fat mass in a songbird and geese within on average 16 and 10 %, respectively, of the actual fat mass measured by chemical extraction (Karasov and Pinshow 1998; Eichhorn and Visser 2008).

Alternatively, ornithologists often estimate fat mass using a categorical fat score that corresponds to relative amounts of visual fat deposits in the furcular and abdominal regions of a bird (Helms and Drury 1960; Kaiser 1993). Though favored among many field biologists, it is a highly subjective index and yields variable results among species and among observers (Krementz and Pendelton 1990; Rogers 1991, 2003). In our validation study, we compared the accuracy and precision of the D<sub>2</sub>O dilution and fat score techniques for estimating fat mass in several species of small songbird.

Whereas interspecific models using TOBEC or isotopic water dilution to estimate body composition in mammals are common (Fiorotto et al. 1987; Walsberg 1988; Farley and Robbins 1994; Koteja 1996), interspecific models are rarely used in avian studies. Early attempts at using interspecific models to predict the lean or fat mass of birds given TOBEC had limited success (Walsberg 1988; Asch and Roby 1995; Lyons and Haig 1995; Spengler et al. 1995). Because TOBEC is influenced by the conductivity, mass, and geometry of the subject (Fiorotto et al. 1987), and thus is highly affected by the bird's position within the measurement chamber (Roby 1991; Asch and Roby 1995), most ornithologists have used separate intraspecific models to measure body composition of each bird species (Scott et al. 2001). In this validation study, we compared the accuracy and precision of intraspecific and interspecific models for estimating body composition of several species of small passerine birds.

## The validation study

The accurate use of any non-destructive technique for estimating the body composition of free-living birds requires a validation study (Scott et al. 2001). One validation approach involves building predictive models for estimating lean and fat mass using a subset of birds (the calibration birds) and then using the models to predict lean and fat mass in another subset of birds that were not used to develop the predictive models (the validation birds). Only one previous validation study has simultaneously used both TOBEC and D<sub>2</sub>O dilution to estimate the lean and fat mass of a bird (Karasov and Pinshow 1998), and no previous study has simultaneously used these two techniques plus fat score to measure body composition in songbird species that differ in body size. We used the TOBEC, D<sub>2</sub>O dilution, and fat score techniques to estimate lean and/or fat mass of Black-throated Blue Warblers (*Setophaga caerulescens*), Yellow-rumped Warblers (*Setophaga coronata*), Red-eyed Vireos (*Vireo olivaceus*), and White-throated Sparrows (*Zonotrichia albicollis*). The primary goal of this validation study was to evaluate the accuracy and precision of both intra- and interspecific models for estimating the lean and fat mass of small passerine birds using the TOBEC, D<sub>2</sub>O, and fat score techniques.

## Study area and study species

All field work was conducted on Block Island, Rhode Island (41°12'N, 71°35'W), a 2,900-ha island located approximately 19 km off the southern coast of Rhode Island, USA. The study site was located within Clayhead Preserve, a 70-ha conservation area at the northeast end of Block Island [for more details about the study area see Parrish 1997; Enser 2002; Whitman 2002; Smith et al. 2007]. We selected four passerine species that represented a broad range of body sizes, migration strategies, and foraging types. All are relatively common fall migrants on Block Island (Parrish 1997; Reinert et al. 2002), and all breed in temperate forests in the USA and Canada. *Setophaga caerulescens* are the smallest of the four study species, averaging about 10 g (range 9.3–11.0 g; Holmes et al. 2005). They are long-distance migrants that winter as far south as Venezuela, and they forage primarily on insects during the breeding season but eat some fruit during migration (Holmes et al. 2005). *Setophaga coronata* are slightly larger than *S. caerulescens*, averaging about 12 g (range 9.5–19.7 g; Hunt and Flaspohler 1998). They are short-distance migrants that winter in the southern USA, and they forage primarily on insects during the breeding season but become omnivorous during migration (Afik and Karasov 1995; Podlesak et al. 2005). *Vireo olivaceus* average about 18 g (range 14.3–29.5 g; Cimprich et al.

2000). They are long-distance migrants that winter as far south as the Amazon basin and forage primarily on insects during the breeding season but become omnivorous during migration (Parrish 1997; Cimprich et al. 2000). *Zonotrichia albicollis* average about 25 g (range 22.5–36.1 g; Falls and Kopachena 2010). They are the shortest-distance migrant of the four study species, and they are generally considered to be granivorous, although they consume some fruit during migration (Falls and Kopachena 2010).

#### Field work: D<sub>2</sub>O and TOBEC protocols

We operated mist nets every fair-weather day starting at 0600 hours from 4 September to 9 November 1999. During this study period, we caught individuals of each of the four study species during a portion of this period as follows: *S. caerulescens*, 09 September–13 October; *S. coronata*, 12 September–06 November; *V. olivaceus*, 16 September–21 October; *Z. albicollis* 26 September–10 November. Individuals used in this experiment were caught during a subset of days as follows: *S. caerulescens*, 24 September–13 October; *S. coronata*, 08 October–25 October; *V. olivaceus*, 24 September–03 October; *Z. albicollis* 29 September–19 October. Upon capture, birds were banded, fat score visually estimated (0–5 scale, whole- and half-unit intervals; Helms and Drury 1960), gender and age determined (after Pyle 1997), and wing chord and bill length, width, and depth (Pyle 1997), and body mass ( $m_b \pm 0.1$  g) measured. Soon after the measurements were completed, we injected  $51.9 \pm 0.4$  (standard error,  $n = 29$ ) mg of 99.9 % D<sub>2</sub>O water (Aldrich Chemical Co., Milwaukee, WI) into the pectoral muscle of each bird. After injection, we placed the individual in a cloth bag where it rested undisturbed for 55 min until TOBEC measurements were taken.

We used an EM-SCAN SA-3000 small animal body composition analyzer with a model 3044 detection chamber (Em-Scan, Springfield, IL) to measure the TOBEC of each bird. We did not use the smallest available detection chamber (model 3030) because it would have precluded interspecific comparison (only *S. caerulescens* would fit inside it). All TOBEC measurements were conducted in a small cabin at the field site. Mean ambient temperature in the cabin at 0700 hours was  $12.1 \pm 0.8$  °C ( $n = 28$ ; range 3.0–20.1 °C). We turned on the TOBEC device at least 1 h before use to ensure that the electromagnetic field within the detection chamber had stabilized. All birds were measured 55 min after capture to minimize the effect of digesta in the gut on TOBEC values (mean retention time of digesta in similar-sized songbirds was  $54.8 \pm 6.0$  min; McWilliams and Karasov 1998); gavaging 1.5 g distilled water into the foregut of *Z. albicollis* increased TOBEC  $E$  values by >30 % (Fraterrigo, McWilliams, and Karasov, unpublished). For the TOBEC measurement, we placed

each unanesthetized bird within a custom-built Plexiglas cylinder (warbler cylinder: inner diameter 31 mm, length 19 cm; vireo and sparrow cylinder: inner diameter 34.5 mm; length 18 cm; both cylinders were made out of 3-mm-thick Plexiglas) to adequately restrain individuals during the measurement. Each bird was positioned carefully in the cylinder on its back with its keel and bill parallel and pointing upward, and legs tucked flat against the stomach. We inserted a removable plunger in one end of the tube and secured it to ensure that the bird was sufficiently immobilized. We measured TOBEC of the cylinder with the bird three times followed by three measurements of the cylinder without the bird. The corrected TOBEC value was then calculated for the bird by subtracting the mean value of the empty cylinder from the mean value of the cylinder with the bird. For a given bird, we completed this entire procedure for measuring TOBEC within 3 min.

Sixty minutes after the initial D<sub>2</sub>O injection and immediately following the TOBEC measurements, we took a blood sample (50–100 µL) from the brachial vein of each bird after puncture with a 27-gauge needle. Capillary tubes with blood samples were flame-sealed and then refrigerated until laboratory analysis. Birds were immediately killed after blood sampling and stored frozen (–20 °C) until carcass analysis. All fieldwork described here was done by a single investigator (MLW) who had extensive banding experience and was authorized to conduct the work by the U.S. Fish and Wildlife Service (no. MB003201), Rhode Island Department of Environmental Management (no. 99-27), the University of Rhode Island IACUC (no. AN02-03-022), and the Block Island office of The Nature Conservancy.

#### Laboratory analysis of field samples

Whole-animal body composition analysis was conducted in the laboratory to compare estimated and measured composition as part of the validation study. Standard methods, as outlined in Alisauskas and Ankney (1992); Reynolds and Kunz (2001), and Servello et al. (2005), were used. In the laboratory, we weighed thawed carcasses, plucked them clean, and then reweighed all carcasses. Carcasses were freeze-dried until a constant mass was achieved, then ground in a small blender, dried at 50 °C for at least 3 h, and reweighed. We refluxed each dried, ground sample in a ceramic thimble (30 × 80 mm, medium porosity) with petroleum ether for 6 h (Dobush et al. 1985) using a Soxhlet apparatus to remove fat. The insoluble residue of the sample and thimble were dried at 50 °C for 3 h, reweighed, and then burned at 550 °C to determine ash content. Water content of the bird was determined as the difference between  $m_b$  at time of death and carcass dry mass plus feathers. Body fat is the mass of extracted fat measured as the difference between a dried, ground sample

before and after refluxing with petroleum ether. Wet lean mass was calculated as the  $m_b$  of the bird at the time of death minus the fat content.

We used infrared spectrophotometry to measure D<sub>2</sub>O dilution enrichment of water in microdistilled blood samples (following Karasov et al. 1988). Measurements were made using a Perkin–Elmer Spectrum One FTIR spectrophotometer and a Miracle SR ATR accessory with a ZnSe crystal (Perkin–Elmer Corp, Norwalk, CT). D<sub>2</sub>O dilution enrichment ( $E$ , atom %) of the microdistilled samples is a function of molar masses of D<sub>2</sub>O (20 g/mol), unlabeled H<sub>2</sub>O (18 g/mol), and the injection of 0.052 g of 99.9 % enriched water into a D<sub>2</sub>O space in the bird ( $S$ , g) as in (Karasov and Pinshow 1998):

$$E = 100 \times \{0.999(0.052 \text{ g} / 20) / [0.999(0.052 \text{ g} / 20) + 0.001(0.052 \text{ g} / 18) + S/18]\}$$

We verified the mass of D<sub>2</sub>O injected into each bird (0.052 g) by weighing 29 samples from capillary tubes that were injected with the same volume as were the birds and that were randomly selected from daily samples taken in the field over the course of the 2-month study. Solving the formula for the D<sub>2</sub>O space in the bird yields:  $S = 4.6753/E - 0.0467$ . We excluded seven of our 67 original birds from subsequent analysis because our estimates of D<sub>2</sub>O space were biologically unreasonable, either greater than 80 % of  $m_b$  ( $n = 5$ ) or less than 50 % of  $m_b$  ( $n = 2$ ) (Kontogiannis 1968; Karasov and Pinshow 1998). For all four species combined, D<sub>2</sub>O space overestimated actual water content (by  $12.6 \pm 0.8$  %,  $n = 60$ ), as has been found in many other animals (Karasov and Pinshow 1998; Speakman et al. 2001; Mata et al. 2006; Eichhorn and Visser 2008).

#### Procedures used for developing predictive models

We used eight to ten birds of each species to develop intraspecific regression models for estimating lean mass from TOBEC, fat mass from D<sub>2</sub>O space, and fat mass given the fat score. To ensure that the birds used to build each model spanned the full range of  $m_b$  for that species, we assigned the heaviest and the lightest individuals of each of the four species to the calibration group used to build the model, and then six to eight additional birds of each species were randomly selected and assigned to the calibration group. Individuals of each species not used to develop the calibration curve ( $n = 4$ –7) were used as an independent validation group.

#### Predicting body fat given D<sub>2</sub>O

More complete descriptions of the D<sub>2</sub>O dilution approach are available in Campbell and Leatherland (1980) and

Karasov and Pinshow (1998), so we only provide an overview here. We used nonlinear regression to develop predictive models for estimating body fat given D<sub>2</sub>O space and body mass using the following equation:  $F = (m_b - S - [S/C_2]) / (1 - [C_1/C_2])$  where  $F$  is dry fat (g),  $m_b$  is body mass (g),  $S$  is the deuterium space (a proxy for body water space) (g),  $C_1$  is the ratio of deuterated water mass to dry fat mass, and  $C_2$  is the ratio of deuterated water mass to fat-free dry mass (Karasov and Pinshow 1998). We used nonlinear regression to first estimate  $C_1$  and  $C_2$  for each of the four species. For all four species, when the data were first fit to the calibration equation, the value for  $C_1$  was not significantly different from zero and its confidence interval included zero. Empirical measurements confirm that fat of songbirds has very little water content (e.g.,  $0.17 \pm 0.07$  g H<sub>2</sub>O/g dry fat in Blackcaps, *Sylvia atricapilla*; Karasov and Pinshow 1998). Setting the value of  $C_1$  to zero in the calibration equation yields the simple model we then used to estimate fat mass given the measured D<sub>2</sub>O space and  $m_b$ :  $F = (m_b - S - [S/C_2])$ .

#### Predicting lean mass given TOBEC

We used linear regression to develop predictive models for estimating lean mass using TOBEC. We used a correlation matrix to determine which variables should be considered for inclusion in our predictive models for estimating lean mass. Several variables were significantly correlated with lean mass, including, in rank order:  $m_b$ , bill depth, and TOBEC  $E$  value. The Spearman rank correlation of fat score with these variables revealed only one significant correlation, namely, fat score with dry fat (g) ( $r^2 = 0.899$ ,  $P < 0.0001$ ,  $n = 27$ ). To determine the “best” regression model for predicting lean mass given the measured variables, we used backward elimination regression (Zar 1999). This approach confirmed that only the TOBEC  $E$  value, tarsus,  $m_b$ , and bill depth were required for estimating lean mass.

For each of the four species, we present here four intraspecific models for predicting lean mass that included different combinations of independent variables: (1) TOBEC only; (2) TOBEC and tarsus; (3) TOBEC, tarsus, and  $m_b$ ; (4) tarsus and  $m_b$ . We excluded bill depth from the intraspecific models but included it in the interspecific models because bill depth was significantly correlated with lean mass only when all four species were considered. We present four interspecific models for predicting lean mass that include different combinations of independent variables: (1) TOBEC only; (2) TOBEC, tarsus, and bill depth; (3) TOBEC, bill depth, and  $m_b$ ; (4) bill depth and  $m_b$ . For both intra- and interspecific models, we verified that simple linear regression models provided a better fit than models that included second or higher polynomials.

### Predicting body fat given fat score

We used the Model I linear regression to develop predictive models for estimating fat mass given the fat score. We present three models for each of the four species that included different combinations of independent variables: (1) fat score only; (2) fat score and  $m_b$ ; (3) fat score,  $m_b$ , and tarsus. As with the TOBEC models, we excluded tarsus length from the interspecific model and included bill depth. We present three interspecific models for predicting fat mass that included different combinations of independent variables: (1) fat score only; (2) fat score and  $m_b$ ; (3) fat score,  $m_b$ , and bill depth.

### Assessing the predictive models

To evaluate the accuracy and precision of all the predictive models, we present  $r^2$  values and the standard errors of the estimate (square root of the mean square error) from non-linear curve fitting for fat mass measured by chemical extraction on D<sub>2</sub>O space, from least squares linear regression of lean mass measured by chemical extraction on the TOBEC  $E$  value, and from least squares linear regression of fat mass measured by chemical extraction on the fat score. Since coefficients of determination 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. Absolute and relative errors were estimated by including individuals from a separate validation group as unknowns in the predictive equations that were developed using the calibration group. Absolute error was then calculated as |predicted – actual|, and relative error as  $[100 \times (|predicted - actual|)/actual]$ .

All statistical analyses were done with SYSTAT (Wilkinson 1992). We decided that data would be considered outliers and removed if the values for the studentized residuals were statistically significant (see SYSTAT, Wilkinson 1992). We thus removed one such datum from the calibration and validation data sets of TOBEC analysis for *Z. albicollis*. For all analyses, two-tailed tests were used and a  $P$  value of  $< 0.05$  was considered statistically significant. Results are presented as mean values  $\pm 1$  SE, and  $n$  as the number of birds used.

## Results

### Body composition of songbirds

We used data from the carcass analysis of 60 individuals (Table 1) to calibrate and validate predictive models for

estimating body composition of the four species of migratory songbird. Body mass ranged from 8.7 to 28.5 g across species, spanning a range of  $m_b$  typical of many migratory songbirds (Parrish 1997). Fat mass ranged from 0.42 to 5.82 g (6–32 % of total  $m_b$  depending on bird species). The fat score ranged from 0 to 5 across species. *V. olivaceus*, the longest-distance migrant of the four study species, averaged a greater fat mass than any of the other species. Ash mass increased with  $m_b$ . Water content comprised on average  $57.3 \pm 0.58$  % (SE;  $n = 60$ , range 47.2–65.1 %) of  $m_b$  for the four bird species.

Using TOBEC to directly estimate lean mass: intraspecific models

Although the model EM-SCAN SA-3000 analyzer with the model 3044 detection chamber is reportedly able to estimate lean mass in individuals as small as 3 (Anonymous 1993) to 5 g (Biebach 1996; Scott et al. 2001), we could not establish a significant predictive relationship between TOBEC measurements and the lean mass of *S. caerulescens* ( $m_b$  range 8–13 g; Tables 1, 2). The one intraspecific model for *S. caerulescens* that was significant included only tarsus and  $m_b$  as variables ( $r^2 = 0.76$ ,  $P = 0.027$ ). Because of the inability to calibrate any model that included TOBEC, *S. caerulescens* were excluded from all subsequent interspecific analyses that used TOBEC.

Intraspecific models that included only TOBEC to predict lean mass for *S. coronata*, *V. olivaceus*, and *Z. albicollis* explained between 50 and 70 % of the variation in lean mass (Table 2). The SE of the estimate for these three intraspecific models ranged from 0.84 g in *S. coronata* to 1.25 g in *Z. albicollis*, or 3–5 % relative error. Intraspecific models that incorporated tarsus and TOBEC to predict lean mass explained more variation in lean mass than those using only TOBEC, as indicated by the increased  $r^2$  values and decreased SE of the estimate for each of the three species (Table 2). However, for *S. coronata* and *Z. albicollis*, the absolute and relative errors were higher for models that incorporated tarsus and TOBEC compared to models with only TOBEC.

The most conspicuous increase in coefficients of determination for the intraspecific models occurred when  $m_b$  was included: for all three species,  $r^2$  values increased from 5 to 17 % and the SE of the estimate decreased. Both the absolute and relative error for these models decreased for *S. coronata* and *V. olivaceus* (but not for *Z. albicollis*) compared to models with only TOBEC or TOBEC and tarsus. Deleting TOBEC from the regression models (compare models 3 and 4 for each species) had relatively small effects on the  $r^2$  values, the SE of the estimate, and the absolute and relative errors.

**Table 1** Body composition and morphometrics of four species of migratory songbirds<sup>a</sup> captured during the fall migration on Block Island, RI, USA

Body composition and morphometric measures	<i>S. caeruleus</i> ( <i>n</i> = 12)	<i>S. coronata</i> ( <i>n</i> = 15)	<i>V. olivaceus</i> ( <i>n</i> = 17)	<i>Z. albicollis</i> ( <i>n</i> = 16)
Body mass (g)	10.50 ± 0.35 (8.7–12.5)	13.29 ± 0.52 (9.6–17.2)	18.45 ± 0.67 (13.2–23.0)	23.85 ± 0.64 (19.2–28.5)
Lean mass (g)	8.89 ± 0.18 (8.14–10.41)	11.47 ± 0.25 (9.51–13.22)	15.70 ± 0.34 (13.05–18.14)	22.25 ± 0.51 (18.89–25.43)
Fat mass (g)	1.61 ± 0.24 (0.42–3.41)	1.81 ± 0.28 (0.09–3.98)	2.75 ± 0.43 (0.15–5.82)	1.74 ± 0.29 (0.30–4.50)
Fat score (0–5)	2.13 ± 0.50 (0–5.0)	1.87 ± 0.34 (0–3.5)	2.09 ± 0.40 (0–4.0)	1.13 ± 0.30 (0–3.5)
Ash (g)	0.31 ± 0.01 (0.27–0.35)	0.39 ± 0.01 (0.34–0.44)	0.63 ± .06 (0.49–1.64)	0.91 ± 0.02 (0.73–1.06)
Water (g)	5.8 ± 0.1 (4.8–6.9)	7.4 ± 0.2 (6.1–8.7)	10.4 ± 0.2 (8.6–12.2)	14.4 ± 0.3 (12.2–16.6)
Tarsus length (mm)	18.64 ± 0.11 (17.89–19.11)	18.44 ± 0.15 (17.11–19.36)	17.98 ± 0.17 (16.71–19.21)	22.87 ± 0.24 (20.50–24.08)
Bill length (mm)	6.81 ± 0.06 (6.53–7.25)	7.05 ± 0.07 (6.61–7.61)	8.88 ± 0.11 (8.02–9.61)	8.12 ± 0.09 (7.55–8.88)
Bill depth (mm)	2.70 ± 0.07 (2.35–3.04)	3.00 ± 0.04 (2.72–3.00)	4.13 ± 0.04 (3.88–4.51)	6.23 ± 0.07 (5.80–6.73)
Bill width (mm)	2.99 ± 0.05 (2.70–3.25)	2.85 ± 0.05 (2.55–3.27)	3.87 ± 0.08 (3.17–4.35)	5.12 ± 0.09 (4.63–5.97)

Values are presented as the mean ± standard error (SE), with the range given in parentheses

<sup>a</sup> The four study species include: Black-throated Blue Warbler (*Setophaga caeruleus*), a small, insectivorous long-distance migrant; Yellow-rumped Warbler (*S. coronata*), a larger, omnivorous short-distance migrant; Red-eyed Vireo (*Vireo olivaceus*), a larger, omnivorous long-distance migrant; the White-throated Sparrow (*Zonotrichia albicollis*), a large, granivorous short-distance migrant

Using TOBEC to directly estimate lean mass: interspecific models

Data for *S. coronata*, *V. olivaceus*, and *Z. albicollis*, the three species used to build the intraspecific models (*n* = 29), were combined to build each of the four interspecific models (Table 3). The interspecific model that included only TOBEC to predict lean mass explained 83 % of the variation in lean mass (Table 3). The SE of the estimate (2.0 g) was twofold higher than that of any of the other three interspecific models. The interspecific model that incorporated TOBEC, tarsus, and bill depth (Model 2, Table 3) had a 13 % higher predictive value, a lower SE of the estimate, and lower absolute and relative errors for each of the three species than the model using only TOBEC (Model 1). The SE of the estimate was lower and the *r*<sup>2</sup> higher when the interspecific model included TOBEC, bill depth, and *m*<sub>b</sub> (Model 3, Table 3). Compared to Model 2, the absolute and relative errors of Model 3 decreased slightly for *V. olivaceus*, increased slightly for *S. coronata*, and increased further for *Z. albicollis*. Removing TOBEC from Model 3 had a relatively small effect on the *r*<sup>2</sup> and SE of the estimate (Model 4, Table 3), decreased the absolute

and relative errors for *V. olivaceus*, and increased both error terms for *S. coronata* and *Z. albicollis*.

Using D<sub>2</sub>O to directly estimate fat mass

We used eight *S. caeruleus*, ten *S. coronata*, ten *V. olivaceus*, and ten *Z. albicollis* to build non-linear models for estimating body fat given the D<sub>2</sub>O space and *m*<sub>b</sub>. Non-linear curve fitting of the four intraspecific models [all of the form  $F = (m_b - S - [S/C_2])$ ; see section “Predicting body fat given D<sub>2</sub>O” for terms of equation] yielded values for *C*<sub>2</sub> of 2.41–3.28 depending on the species (Table 4). Using these estimated values for the ratio of D<sub>2</sub>O mass to fat-free dry mass, we built predictive models for estimating fat mass from measured *m*<sub>b</sub> and D<sub>2</sub>O space for each of the four species and for all species combined. The predictive models for each species explained a significant amount of the variation in fat mass (*r*<sup>2</sup> values of 0.77–0.98; Table 4). When these intraspecific predictive models were used to estimate fat mass for the validation birds, the absolute error was 0.25–0.86 g or between 26 and 50 % of measured fat mass depending on bird species.

The interspecific predictive model estimated the fat mass of validation birds from each of the four species to be

**Table 2** Coefficient of determination ( $r^2$ ), SE of the estimate (g), and absolute (Abs, g) and relative errors (Rel %) for intraspecific regression models for estimating lean mass (LM) given: (1) only total

body electrical conductivity (TOBEC); (2) TOBEC and tarsus length; (3) TOBEC, tarsus length, and body mass ( $m_b$ ); and (4) tarsus length and  $m_b$

Model	<i>S. caerulescens</i>				<i>S. coronata</i>				<i>V. olivaceus</i>				<i>Z. albicollis</i>			
	$r^2$	SE (g)	Error		$r^2$	SE (g)	Error		$r^2$	SE (g)	Error		$r^2$	SE (g)	Error	
			Abs (g)	Rel (%)			Abs (g)	Rel (%)			Abs (g)	Rel (%)			Abs (g)	Rel (%)
1	0.143	0.654	–	–	0.508	0.837	0.467 (0.184)	3.95 (1.56)	0.607	1.053	0.794 (0.218)	4.81 (1.29)	0.711	1.246	0.696 (0.233)	3.30 (1.16)
2	0.358	0.620	–	–	0.822	0.538	0.815 (0.313)	6.83 (2.50)	0.673	1.027	0.797 (0.224)	4.79 (1.32)	0.825	1.050	1.058 (0.121)	4.77 (0.59)
3	0.781	0.405	–	–	0.980	0.195	0.329 (0.106)	2.79 (0.92)	0.722	1.022	0.451 (0.135)	2.75 (0.80)	0.954	0.587	1.111 (0.367)	5.31 (1.82)
4	0.763	0.377	0.739 (0.161)	8.53 (1.65)	0.980	0.200	0.303 (0.103)	2.62 (0.92)	0.668	1.035	0.469 (0.095)	2.90 (0.60)	0.949	0.565	1.050 (0.293)	4.96 (1.49)

We used the following number of birds for the calibration and validation of the four models: Black-throated Blue Warbler (*S. caerulescens*), 8 and 4 respectively; Yellow-rumped Warbler (*S. coronata*), 10 and 5, respectively; Red-eyed Vireo (*V. olivaceus*), 10 and 7, respectively, and White-throated Sparrow (*Z. albicollis*), 9 and 6 respectively; Absolute (Abs, g) and relative errors (Rel %) for *S. caerulescens* are not reported for three of the four intraspecific regression models because they were not statistically significant ( $P > 0.05$ )

within 0.44–0.63 g, or between 16 and 49 % of the measured fat mass (Table 4). Predicted fat mass using the interspecific model (“All four” model in Table 4) was closely related to actual fat mass measured by chemical extraction (Fig. 1a). Data from 21 validation birds were used to determine the absolute (0.51 g) and relative error (30 %) for this interspecific predictive model (Table 4).

Using fat score to directly estimate fat mass

We used eight *S. caerulescens*, 10 *S. coronata*, 10 *V. olivaceus*, and 10 *Z. albicollis* to build predictive models for estimating body fat given (1) visual fat score, (2) fat score and  $m_b$ , and (3) fat score,  $m_b$ , and a structural measurement (Table 5). We built the three separate models for each of the four species and for an interspecific model that included all four species. Intraspecific models for estimating fat mass yielded  $r^2$  values of 0.83–0.98, depending on the specific model and bird species (Table 5). When these models were used to estimate fat mass for the validation birds, the absolute error was 0.15–0.46 g or between 13 and 36 % of measured fat mass. Relative error from the intraspecific models was highest for *S. caerulescens*, lowest for *V. olivaceus*, and intermediate for *S. coronata* and *Z. albicollis*. Compared to the model that included only fat score (Model 1, Table 5), including fat score and  $m_b$  (Model 2, Table 5), or fat score,  $m_b$ , and tarsus length (Model 3, Table 5) had relatively little effect on the  $r^2$  values and standard errors of the estimate for *S. caerulescens* and *Z. albicollis*, whereas it had a larger effect for *S. coronata* and *V. olivaceus*.

The interspecific model that included only fat score (Fig. 1b; Table 5) predicted the fat mass of each species less accurately and precisely than did the interspecific models that also included  $m_b$  or  $m_b$  and tarsus. Absolute and relative errors for fat mass were generally lower for the intraspecific compared to interspecific models, although this was not true for Models 2 and 3 for *Z. albicollis* (Table 5). Data from 21 validation birds were then used to determine the absolute and relative errors of the interspecific models (“All four” model in Table 5). The most accurate of these models were those that included  $m_b$  or  $m_b$  and bill depth. The interspecific predictive models as a group estimated fat mass of validation birds within 0.16–0.66 g or between 9–42 % of the measured fat mass (Table 5). For comparison we also estimated fat mass by subtraction of predicted lean mass given TOBEC from measured  $m_b$  (Fig. 1c).

Using a dual approach for independent estimates of fat and lean mass

The above approaches involve using one technique (TOBEC or D<sub>2</sub>O dilution) to estimate one body component (e.g., fat) and then indirectly estimating the other body component (e.g., lean mass) by subtraction from total  $m_b$ . We indirectly estimated the fat mass of each bird by subtracting the lean mass, estimated by the best predictive intraspecific model using TOBEC (Model 3, Table 2), from total  $m_b$  for each individual (Table 6). We also indirectly estimated the lean mass of each bird by subtracting the fat mass, estimated by the predictive model using D<sub>2</sub>O dilution

**Table 3** Coefficient of determination, standard error of the estimate, and absolute and relative errors of the four interspecific regression models for estimating lean mass<sup>a</sup>

Model <sup>a</sup>	$r^2$	SE of the estimate (g)	<i>S. coronata</i>		<i>V. olivaceus</i>		<i>Z. albicollis</i>	
			Abs (g)	Rel (%)	Abs (g)	Rel (%)	Abs (g)	Rel (%)
1	0.830	2.009	0.785 (0.323)	6.62 (2.69)	2.176 (0.720)	13.44 (4.53)	0.607 (0.148)	2.97 (0.74)
2	0.963	0.977	0.255 (0.092)	2.14 (0.79)	0.856 (0.195)	5.28 (1.21)	0.312 (0.083)	1.39 (0.38)
3	0.975	0.797	0.355 (0.110)	3.05 (0.99)	0.803 (0.185)	4.89 (1.14)	0.547 (0.240)	2.57 (1.11)
4	0.974	0.792	0.427 (0.192)	3.73 (1.72)	0.472 (0.128)	2.95 (0.81)	0.723 (0.186)	3.12 (0.73)

The validation group consisted of 5 *S. coronata*, 7 *V. olivaceus*, and 6 *Z. albicollis*. *S. caerulescens* were excluded from these interspecific models because the three regression models that included TOBEC were not statistically significant ( $P > 0.05$ )

<sup>a</sup> The four regression models for estimating lean mass include the following different combinations of independent variables: (1) only total body electrical conductivity (TOBEC); (2) TOBEC, tarsus length, and bill depth; (3) TOBEC, bill depth, and body mass ( $m_b$ ); and (4) bill depth and  $m_b$ . These models were built using 10 *S. coronata*, 10 *V. olivaceus*, and 9 *Z. albicollis*

**Table 4** Coefficient of determination, standard error of the estimate, and absolute and relative errors for intra- and interspecific models for estimating fat mass given the deuterium dilution space and body mass

Species	$r^2$	SE of the estimate (g)	Intraspecific model <sup>a</sup>		Interspecific model <sup>a</sup>	
			Abs $\pm$ SE (g)	Rel $\pm$ SE (%)	Abs $\pm$ SE (g)	Rel $\pm$ SE (%)
<i>S. caerulescens</i>	0.97	0.33	0.568 $\pm$ 0.107	50.06 $\pm$ 19.96	0.625 $\pm$ 0.082	49.38 $\pm$ 17.89
<i>S. coronata</i>	0.98	0.28	0.249 $\pm$ 0.094	26.36 $\pm$ 18.60	0.528 $\pm$ 0.081	33.49 $\pm$ 9.09
<i>V. olivaceus</i>	0.89	1.05	0.857 $\pm$ 0.250	34.13 $\pm$ 10.25	0.496 $\pm$ 0.211	15.89 $\pm$ 6.27
<i>Z. albicollis</i>	0.77	1.08	0.436 $\pm$ 0.147	28.42 $\pm$ 12.46	0.435 $\pm$ 0.147	28.40 $\pm$ 12.44
All four	0.86	0.87	–	–	0.511 $\pm$ 0.074	30.03 $\pm$ 5.79

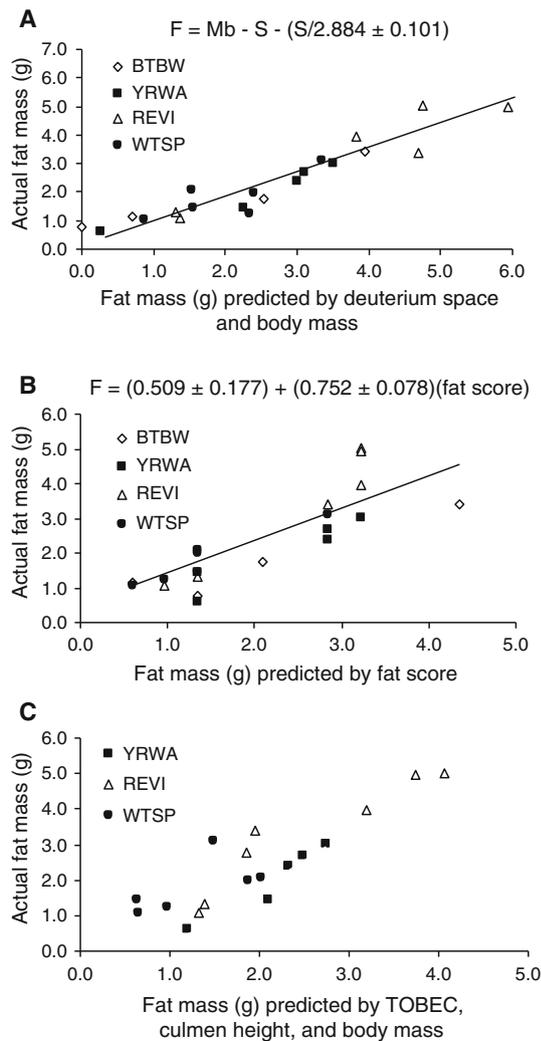
<sup>a</sup> These models were built using 8 *S. caerulescens*, 10 *S. coronata*, 10 *V. olivaceus*, and 10 *Z. albicollis*. Intraspecific models were tested with 4–6 individuals of the same species used to build the model. The interspecific model was built using the 38 calibration birds from all four species and was applied to each of the four species separately. Thus, the absolute and relative errors for the interspecific model were determined using 4–6 individuals when calculated separately for each species and using 21 individuals when calculated for all four species combined

(Table 4), from the  $m_b$  of an individual (Table 6). An alternative approach is to use both TOBEC and D<sub>2</sub>O dilution (“dual approach”) to independently estimate each body component within the same individual. To avoid problems of statistical dependence of estimates of both lean mass and fat mass on  $m_b$  and because all models using D<sub>2</sub>O dilution to estimate fat mass included  $m_b$ , we used a TOBEC model for estimating lean mass that did not include  $m_b$  in the model. We directly estimated lean mass using TOBEC (Model 2, Table 2) and fat mass using D<sub>2</sub>O dilution (from Table 4), and then compared the accuracy and precision of estimated fat and lean mass using this dual method with that of both single methods (Table 6). In general, for *S. coronata* and *Z. albicollis*, but not *V. olivaceus*, the dual approach yielded more accurate and precise estimates of fat mass than did the TOBEC-only approach (Table 5). The relative errors of lean mass estimates were higher for *S. coronata* and *V. olivaceus* but

lower for *Z. albicollis* using this dual approach compared to using only TOBEC (Table 5).

Estimates of lean and fat mass given the complete dataset

Having determined the absolute and relative errors for the intraspecific and interspecific models, we used both calibration and validation birds to develop the final predictive models that can be used in subsequent studies to estimate fat and lean mass of songbirds (Table 7; “Appendix”). Intraspecific models that included only TOBEC explained between 50–76 % of the variation in lean mass of the complete dataset for each species (Model 1, Table 7). Models that included both TOBEC and tarsus to predict lean mass (Model 2, Table 7) explained slightly more of the variation in lean mass (55–85 %) than those that included only TOBEC (Model 1, Table 7). For a given bird species, the coefficients



**Fig. 1** Relationship between predicted fat mass and actual fat mass measured by chemical extraction for birds caught on Block Island, RI. Bird species include Black-throated Blue Warbler (BTBW, *Setophaga caerulea*), Yellow-rumped Warbler (YRWA, *S. coronata*), Red-eyed Vireo (REVI, *Vireo olivaceus*), and White-throated Sparrow (WTSP, *Zonotrichia albicollis*). Equation and regression lines are for: **a** predicting actual fat mass ( $F$ , g) given body mass ( $m_b$ ) and deuterium space ( $S$ ) (interspecific model for all four species in Table 4), **b** predicting actual fat mass given fat score (interspecific Model 1 for all four species in Table 5), **c** indirectly estimating fat mass by subtracting predicted lean mass (interspecific Model 3 for three species in Table 3) from measured  $m_b$ . No predictive equation or regression line is shown because fat mass was estimated by subtraction and not by linear regression

of determination increased by 6–45 % in regression models that included  $m_b$  (Models 3 and 4, Table 7) compared to models that included only TOBEC (Model 1) or TOBEC and tarsus (Model 2). Deleting TOBEC from the model that included  $m_b$  and tarsus length had a negligible effect on the  $r^2$  value and standard error of the estimate (Model 3 vs. 4).

The interspecific models that were built using the complete dataset (“Appendix”) had higher coefficients of

determination than their respective intraspecific models (Table 7), although for *V. olivaceus* and *Z. albicollis* the standard errors of the estimate for intraspecific Models 2, 3, and 4 were similar to their respective interspecific models. The intraspecific models for *S. coronata* consistently predicted lean mass more accurately than the interspecific models. The intraspecific models as well as the interspecific model for estimating fat mass using deuterium dilution and built with the entire dataset explained 82–98 % of the variation in fat mass (Table 7). The intraspecific models for *S. caerulea* and *S. coronata* consistently predicted fat mass more accurately than the interspecific model (i.e., standard error of the estimate was 0.34 and 0.53 g compared to 0.78 g). For *V. olivaceus* and *Z. albicollis*, the intraspecific models consistently predicted fat mass less accurately than the interspecific model.

## Discussion

We validated the use of three non-destructive methods for estimating body composition in four species of migratory songbirds. In the following sections we discuss the accuracy and precision of the intra- and interspecific models that we developed using TOBEC,  $D_2O$  dilution, and fat score. We compare the results from this study to those of previous avian studies that incorporate one or more of the methods, we describe the potential of two relatively new imaging techniques for estimating the body composition of songbirds, and we provide recommendations about which of the models and techniques would be most appropriate for use in other studies given certain objectives.

### Intraspecific TOBEC models for directly estimating lean mass

The intraspecific models that included TOBEC did not accurately estimate the lean mass of *S. caerulea*, the smallest of our four species. Body size also affected the accuracy of estimates of body composition of birds in previous studies using TOBEC (Castro et al. 1990; Roby 1991; Asch and Roby 1995). Our results suggest that the model 3040 TOBEC detection chamber with the model SA 3000 base unit (Em-Scan) cannot accurately estimate the lean mass of birds smaller than about 10 g, although it is effective for slightly larger songbirds, such as *S. coronata*.

For the other three songbird species included in our study ( $m_b$  range 9.6–28.5 g), the intraspecific TOBEC models estimated lean mass within 0.3–1.1 g of the actual lean mass, or with a relative error of 2.6–6.8 %, depending on the bird species. The lean mass of similar-sized songbirds can change as much as by 18–42 % of the total body mass during migration [Willow Warbler, *Phylloscopus*

**Table 5** Coefficient of determination, standard error of the estimate, and absolute and relative errors for intra- and interspecific regression models for estimating fat mass<sup>a</sup> given: (1) only fat score, or (2) fat score and body mass ( $m_b$ )

Species	Model	$r^2$	SE of estimate (g)	Intraspecific model		Interspecific model	
				Absolute error $\pm$ SE (g)	Relative error $\pm$ SE (%)	Absolute error $\pm$ SE (g)	Relative error $\pm$ SE (%)
<i>S. caeruleus</i>	1	0.894	0.250	0.459 $\pm$ 0.104	30.27 $\pm$ 6.31	0.599 $\pm$ 0.126	42.85 $\pm$ 12.98
	2	0.896	0.271	0.445 $\pm$ 0.106	28.65 $\pm$ 5.56	0.436 $\pm$ 0.217	30.11 $\pm$ 19.78
	3	0.903	0.292	0.446 $\pm$ 0.074	36.02 $\pm$ 15.13	0.459 $\pm$ 0.147	37.69 $\pm$ 16.15
<i>S. coronata</i>	1	0.830	0.504	0.279 $\pm$ 0.096	27.64 $\pm$ 18.13	0.334 $\pm$ 0.117	32.49 $\pm$ 22.68
	2	0.982	0.176	0.152 $\pm$ 0.084	17.95 $\pm$ 15.09	0.207 $\pm$ 0.082	20.66 $\pm$ 12.55
	3	0.982	0.189	0.220 $\pm$ 0.079	20.59 $\pm$ 14.69	0.337 $\pm$ 0.091	31.53 $\pm$ 20.69
<i>V. olivaceus</i>	1	0.852	0.781	0.433 $\pm$ 0.192	13.23 $\pm$ 4.19	0.823 $\pm$ 0.317	19.51 $\pm$ 5.54
	2	0.918	0.620	0.361 $\pm$ 0.082	13.72 $\pm$ 4.26	0.660 $\pm$ 0.261	16.22 $\pm$ 4.22
	3	0.921	0.657	0.311 $\pm$ 0.089	13.24 $\pm$ 5.25	0.463 $\pm$ 0.152	15.96 $\pm$ 3.82
<i>Z. albicollis</i>	1	0.921	0.411	0.357 $\pm$ 0.069	21.45 $\pm$ 5.56	0.410 $\pm$ 0.101	24.72 $\pm$ 6.25
	2	0.922	0.438	0.325 $\pm$ 0.064	19.86 $\pm$ 5.65	0.164 $\pm$ 0.047	9.33 $\pm$ 2.75
	3	0.929	0.450	0.339 $\pm$ 0.106	21.19 $\pm$ 7.49	0.190 $\pm$ 0.071	9.49 $\pm$ 3.37
All four	1	0.722	0.736	–	–	0.546 $\pm$ 0.105	28.53 $\pm$ 6.15
	2	0.811	0.616	–	–	0.368 $\pm$ 0.094	17.96 $\pm$ 4.81
	3	0.875	0.508	–	–	0.354 $\pm$ 0.061	21.96 $\pm$ 5.99

The three models for estimating fat mass given the fat score include different combinations of independent variables: (1) fat score only; (2) fat score and  $m_b$ ; (3) fat score,  $m_b$ , and tarsus. For the intraspecific models, Model (3) estimates fat mass given fat score,  $m_b$  and tarsus length. For the interspecific models, Model (3) estimates fat mass given fat score,  $m_b$ , and bill depth. The following number of birds were used for the calibration and validation of the three models: *S. caeruleus*, 8 and 4, respectively; *S. coronata*, 10 and 5, respectively; *V. olivaceus*, 10 and 7, respectively; *Z. albicollis*, 10 and 6, respectively

**Table 6** Absolute and relative error of direct and indirect estimates of lean and fat mass for three species of migratory songbirds

Species	Body component	TOBEC only		D <sub>2</sub> O dilution only		Dual approach	
		Absolute error $\pm$ SE (g)	Relative error $\pm$ SE (%)	Absolute error $\pm$ SE (g)	Relative error $\pm$ SE (%)	Absolute error $\pm$ SE (g)	Relative error $\pm$ SE (%)
<i>S. coronata</i>	Fat	0.329 $\pm$ 0.106	20.57 $\pm$ 8.58	0.249 $\pm$ 0.094	26.36 $\pm$ 18.60	0.249 $\pm$ 0.094	26.36 $\pm$ 18.60
	Lean	0.329 $\pm$ 0.106	2.79 $\pm$ 0.92	0.249 $\pm$ 0.094	2.16 $\pm$ 0.86	0.815 $\pm$ 0.313	6.83 $\pm$ 2.50
<i>V. olivaceus</i>	Fat	0.451 $\pm$ 0.135	17.86 $\pm$ 4.75	0.857 $\pm$ 0.250	34.13 $\pm$ 10.25	0.857 $\pm$ 0.250	34.13 $\pm$ 10.25
	Lean	0.451 $\pm$ 0.135	2.48 $\pm$ 0.80	0.857 $\pm$ 0.250	5.31 $\pm$ 1.55	0.797 $\pm$ 0.224	4.79 $\pm$ 1.32
<i>Z. albicollis</i>	Fat	1.111 $\pm$ 0.367	75.40 $\pm$ 2.84	0.436 $\pm$ 0.147	28.42 $\pm$ 12.46	0.436 $\pm$ 0.147	28.42 $\pm$ 12.46
	Lean	1.111 $\pm$ 0.367	5.31 $\pm$ 1.82	0.436 $\pm$ 0.147	1.96 $\pm$ 0.70	1.058 $\pm$ 0.121	4.77 $\pm$ 0.59

D<sub>2</sub>O, Deuterium oxide

Sample sizes for the calibration and validation were the same as in Tables 2 and 4. Using TOBEC, direct estimates of lean mass were calculated using intraspecific Model 3 [TOBEC, tarsus length, and body mass ( $m_b$ ); see Table 2] and then fat mass was calculated by subtracting lean mass from  $m_b$ . Using the D<sub>2</sub>O dilution method, direct estimates of fat mass were calculated using a predictive model for each species (see Table 4), and then lean mass was calculated by subtracting estimated fat mass from  $m_b$ . Using TOBEC and D<sub>2</sub>O together (“dual approach”), direct estimates of lean and fat mass were calculated separately using intraspecific Model 2 (TOBEC and tarsus length; see Table 2) to estimate lean mass, and the predictive model for estimating fat mass given D<sub>2</sub>O space and  $m_b$  (in Table 4)

*trochilus* (Biebach 1990); Garden Warbler, *Sylvia borin*, (Bairlein 1991; Bauchinger and Biebach 2001); Thrush Nightingale, *Luscinia luscinia*, (Klaassen et al. 1997); Blackcap (Karasov and Pinshow 1998)]. Thus, given the results of our study, TOBEC can be effectively used to study the dynamics of lean mass in such songbirds. This conclusion is supported by other recent research which has shown that intraspecific TOBEC models can accurately

estimate the lean mass of other bird species (body mass range 13–95 g) (Castro et al. 1990; Scott et al. 1991; Skagen et al. 1993; Lyons and Haig 1995; Burger 1997; Karasov and Pinshow 1998).

Choosing one intraspecific TOBEC model for estimating the lean mass of a given species is problematic not only because the model with the lowest absolute and relative errors does not always have the lowest  $r^2$  value, but also

**Table 7** Coefficient of determination and standard error of the estimate for intra- and inter-specific regression models for estimating lean and fat mass of songbirds<sup>a</sup>

Species	Model 1		Model 2		Model 3		Model 4		D <sub>2</sub> O model	
	<i>r</i> <sup>2</sup>	SE of estimate (g)	<i>r</i> <sup>2</sup>	SE of estimate (g)						
<i>S. caeruleus</i>									0.92	0.53
<i>S. coronata</i>	0.50	0.72	0.69	0.60	0.95	0.25	0.94	0.25	0.98	0.34
<i>V. olivaceus</i>	0.52	0.99	0.55	0.99	0.74	0.78	0.70	0.81	0.92	0.99
<i>Z. albicollis</i>	0.76	1.06	0.85	0.88	0.91	0.71	0.91	0.70	0.82	0.90
Interspecific	0.84	1.87	0.97	0.86	0.98	0.71	0.98	0.73	0.90	0.78

Regressions for lean mass were calibrated using 47 individuals from three species: *S. coronata*, 15; *V. olivaceus*, 17; *Z. albicollis*, 15. Regressions for fat mass were calibrated using 59 individuals from four species: *S. caeruleus*, 12; *S. coronata*, 15; *V. olivaceus*, 16; *Z. albicollis*, *n* = 16. Both intraspecific and interspecific models for estimating fat mass were of the form:  $F = (m_b - S - [S/C_2])$  (see text for details)

<sup>a</sup> Intraspecific models for estimating lean mass are: (1) only TOBEC; (2) TOBEC and tarsus length; (3) TOBEC, tarsus length, and body mass ( $m_b$ ); (4) tarsus length and  $m_b$ . Interspecific models include (*n* = 47): (1) only TOBEC; (2) TOBEC, tarsus length, and bill depth; (3) TOBEC, bill depth, and  $m_b$ ; (4) bill depth and  $m_b$ . All models were statistically significant ( $P < 0.003$ ) and each model is shown in the “Appendix”

because the best model for a particular species is not necessarily the best model for another species. A comparison of only the  $r^2$  values would indicate that the intraspecific models for predicting lean mass in all three species were most improved by including  $m_b$  in the predictive model (Models 1 and 2 compared to Models 3 and 4, Table 2). Although the  $r^2$  values of the linear regression models for estimating lean mass indicate the “goodness of fit” of a particular model, estimates of the absolute and relative errors of these models are necessary for evaluating the predictive power of the model with new data (Skagen et al. 1993; Lyons and Haig 1995; Spengler et al. 1995; Burger 1997). Given that more often the primary objective is to develop predictive models for estimating body composition, we recommend more emphasis on absolute and relative error when different models are to be compared.

In general, the intraspecific model that included TOBEC, tarsus length, and  $m_b$  (Model 3) estimated lean mass the most accurately and with the highest precision. Structural measures have improved TOBEC estimates of body composition in other studies (Conway et al. 1994; Burger 1997). For all three species, intraspecific models that included TOBEC, tarsus length, and  $m_b$  (Model 3) had similar  $r^2$  values, standard error of the estimate, and absolute and relative error compared to models with only  $m_b$  and tarsus length (Model 4, Table 3). Authors of previous studies reported similar results and so concluded that TOBEC was not necessary for estimating the body composition of birds (Skagen et al. 1993; Conway et al. 1994; Lyons and Haig 1995; Burger 1997). For example, Burger (1997) found that TOBEC did not significantly increase the accuracy of lean mass estimates and recommended against buying the relatively expensive TOBEC device. Other researchers have drawn similar conclusions in using TOBEC to estimate lean mass (Morton et al. 1991) and to

estimate fat mass (Skagen et al. 1993; Conway et al. 1994). In the following sections we discuss how TOBEC can be effectively used to independently estimate lean mass if a second method is used to estimate fat mass.

#### Intraspecific TOBEC models for indirectly estimating fat mass

The fat mass of the three larger species (*S. coronata*, *V. olivaceus*, and *Z. albicollis*) was indirectly estimated by subtracting TOBEC estimates of lean mass from the measured total  $m_b$ . Fat mass in *S. coronata* and *V. olivaceus* was indirectly estimated with a 0.3–0.5 g absolute error and relative error of 17.9–20.6 %. This level of accuracy in estimating fat mass to within less than 0.5 g is likely to be sufficient for many studies of wild migratory birds because the fat mass of *S. coronata* and *V. olivaceus*, the two mid-sized species in our study, averaged 1.81–2.75 g. In other studies, fat mass has been found to comprise up to 80 % of the total  $m_b$  among long-distance migrants (Blem 1990; Sandberg and Moore 1996; Klaassen et al. 1997). In general, however, TOBEC indirectly estimated fat mass less accurately than the direct estimates of fat mass using D<sub>2</sub>O dilution or the fat score (Table 6). For *Z. albicollis*, indirectly estimating fat mass using TOBEC yielded absolute and relative errors that were two- to threefold higher than the absolute and relative errors for *S. coronata* and *V. olivaceus*, and threefold higher than the errors from models estimating fat mass in *Z. albicollis* using D<sub>2</sub>O dilution or fat score.

These interspecific differences in the accuracy and precision of fat mass estimated using TOBEC are related to two issues. First, when TOBEC is used to directly estimate lean mass and indirectly estimate fat mass, the measurement error associated with estimates of lean mass is the

same when applied to fat mass (Burger 1997). Thus, the relative errors of fat mass are usually much greater than those for lean mass because fat mass is by and large proportionally smaller than lean mass in animals (Morton et al. 1991; Skagen et al. 1993). Second, our four species differed in the proportion of  $m_b$  composed of fat. Although the  $m_b$  of *S. coronata* and *V. olivaceus* was on average 10 and 5 g less than that of *Z. albicollis* (Table 1), respectively, both had as much or more fat than *Z. albicollis*. As a result, the relative error of the indirect estimate of fat mass for *Z. albicollis* was exceptionally high compared to that for *S. coronata* and *V. olivaceus*.

There has been considerable debate as to the effectiveness of using TOBEC to estimate fat mass (Scott et al. 2001). Although TOBEC has been successfully used to estimate fat mass of much larger birds than the four species in this study [e.g., Black-legged Kittiwake, *Rissa tridactyla*: body mass 326–392 g and fat mass 16–36 g (Golet and Irons 1999); Northern Bobwhite, *Colinus virginianus*: body mass 172–277 g and fat mass 10–50 g (Roby 1991)], most studies have concluded that TOBEC is inadequate for estimating fat mass in smaller-sized (<215 g) bird species (range approx. 10–215 g; Morton et al. 1991; Conway et al. 1994; Asch and Roby 1995; Lyons and Haig 1995; Spengler et al. 1995; Burger 1997). We found that TOBEC accurately estimated fat mass in only two of our four study species. Indirect estimates of fat mass using TOBEC yielded a relative error of 75 % for *Z. albicollis*, and TOBEC proved ineffective for estimating both the fat and lean mass of *S. caerulescens*. Based on these results, we conclude that TOBEC is not a reliable technique for estimating fat mass of small songbirds.

#### Intraspecific D<sub>2</sub>O models for directly estimating fat mass

We found that the D<sub>2</sub>O model could be used to estimate the fat mass of the four bird species with an absolute and relative error of 0.2–0.9 g and 26–50 %, respectively, depending on the bird species. Importantly, because TOBEC could not accurately predict the body composition of *S. caerulescens*, the D<sub>2</sub>O dilution technique provided an accurate method for estimating body composition of this smallest of our study species. Absolute errors of fat mass estimates using D<sub>2</sub>O were lower for *S. coronata* and *Z. albicollis* than the absolute errors from indirect estimates of fat mass using TOBEC (Table 7). In the only other published study that has used D<sub>2</sub>O to estimate fat mass and TOBEC to estimate lean mass in songbirds, Karasov and Pinshow (1998) estimated fat mass in Blackcaps with an absolute and relative error of 0.4 g and 16 % ( $m_b$  17.3 ± 0.5 g). Deuterium has also been used to accurately estimate the fat mass of Barnacle Geese (Eichhorn and Visser 2008)

as well as other wildlife species, such as White-tailed Deer (*Odocoileus virginianus*, Rumpler et al. 1987) and three species of bear (*Ursa americanus*, *U. arctos*, *U. maritimus*, Farley and Robbins 1994).

#### Intraspecific D<sub>2</sub>O models for indirectly estimating lean mass

Lean mass was indirectly estimated by subtracting D<sub>2</sub>O estimates of fat mass from the total  $m_b$ . The D<sub>2</sub>O dilution method indirectly estimated the lean mass of *S. caerulescens* with an absolute and relative error of 0.57 g (±0.11 SE) and 6.72 % (±0.01), respectively. This method indirectly estimated the lean mass of *S. coronata* and *Z. albicollis* more accurately than the direct estimates of lean mass using TOBEC (Table 7). In contrast, D<sub>2</sub>O dilution estimated the lean mass of *V. olivaceus* less accurately than did TOBEC. In summary, if only one technique is used to estimate fat and lean mass, then our results suggest that D<sub>2</sub>O dilution is the best single technique for measuring body composition dynamics in most small songbird species.

#### Estimating fat mass using the fat score

Fat scoring is a popular method for visually estimating subcutaneous fat of songbirds in the field (Krementz and Pendelton 1990) although it does provide only a qualitative index of body fat (Conway et al. 1994). Unfortunately, fat scoring can have high variability among observers (Krementz and Pendelton 1990; Conway et al. 1994). Even within a group of four bird banders given the same set of instructions, Krementz and Pendelton (1990) found that banders assigned different fat scores to the same bird and that the assigned fat scores did not correspond to the actual fat mass in the birds. Within-observer variation can be reduced by exposing the observer to the full range of fat scores for the species in question before the study begins (Krementz and Pendelton 1990). Although such extensive training to minimize variation within and among observers would be ideal, familiarizing bird banders at a banding station such as ours on Block Island with the full range of fat scores for each of the 60–70 species caught during fall migration (see Parrish 1997) would be a monumental task.

We eliminated inter-observer variation in our study by using only one bander to assign fat scores to all birds. In general, the fat score estimated fat mass with more consistent accuracy (0.15–0.46 g; 13–36 % relative error) than did the D<sub>2</sub>O dilution (0.25–0.86 g; 26–50 %) or TOBEC (0.33–1.11 g; 18–75 %) techniques. Including  $m_b$  and structural measures along with fat score in the predictive models usually yielded lower absolute and relative errors than models using only fat score, as was also found by

Conway et al. (1994) and Seewagen (2007). Clearly, fat scoring alone can not be used to estimate both lean and fat mass of the same bird, although it could be used in conjunction with another technique that estimated lean mass (e.g., D<sub>2</sub>O dilution or TOBEC). Thus, fat scoring can accurately estimate fat mass of small songbirds as long as subcutaneous fat provides a good index of body fat (Rogers 1991, 2003) and if necessary precautions and protocols are used.

#### Comparison of intraspecific and interspecific models

Selecting which technique and model to use for estimating body composition is difficult, particularly when many species are being studied (Lyons and Haig 1995). Few studies have developed and tested interspecific models for estimating the body composition of birds, although researchers studying marine mammals have regularly applied models developed for one species to other species (e.g., Coltman et al. 1998). Many prominent researchers have concluded that species-specific models should not be used for estimating the body composition of birds spanning a wide range of body masses (Roby 1991; Scott et al. 1991; Skagen et al. 1993; Asch and Roby 1995; Lyons and Haig 1995). Em-Scan, the manufacturer of the TOBEC device, recommends that different calibrations be developed for each species being studied—especially when species have distinctly different body shapes (e.g., among mammals, birds, and fish; Anonymous 1995). The four species of songbirds in this study differed by only 20 g in  $m_b$ , and their body shape is similar and typical of the majority of small, migratory songbird species.

A primary goal of our study was to compare the accuracy and precision of intraspecific and interspecific models for estimating lean and fat mass of songbirds. If interspecific models are reasonably accurate for estimating body composition of songbirds, then this would broaden the applicability of these techniques considerably. In general, we found that the interspecific models that estimated lean mass using TOBEC and fat mass using D<sub>2</sub>O dilution or the fat score compared quite favorably with estimates from our intraspecific models for all species except *S. caeruleus*.

The range of absolute errors and relative errors from the intra- and interspecific models for estimating lean mass given TOBEC indicate that the interspecific models estimated lean mass as well as or better than the intraspecific models for *S. coronata* and *Z. albicollis*, but not for *V. olivaceus* (Tables 2, 3). Intra- and interspecific models that included  $m_b$  and structural measures (Models 3 and 4) had lower absolute and relative errors than those using only TOBEC (Model 1) to estimate lean mass, although there are some exceptions for *Z. albicollis*. These results demonstrate that interspecific models for estimating lean mass

given TOBEC and structural measure(s) can be used to accurately predict the lean mass of songbirds that average 13–23 g in body mass.

Similarly, the interspecific models estimating fat mass given D<sub>2</sub>O dilution compared favorably to the estimates by intraspecific models (Table 4), although this also depended on bird species. For *S. caeruleus* and *Z. albicollis*, absolute and relative errors of the interspecific models for estimating fat mass were very similar to those of the intraspecific models. For *V. olivaceus*, the interspecific model produced estimates of fat mass with almost half the absolute and relative errors of the intraspecific model, and clearly provided the better estimate of fat mass. In contrast, for *S. coronata*, absolute and relative errors of the interspecific models increased slightly compared to the intraspecific model. Even the interspecific model for estimating fat mass using D<sub>2</sub>O dilution that was built and tested with birds from all four species provided reasonably good estimates of fat mass (0.5 g absolute error; 30 % relative error) in songbirds ranging in  $m_b$  from 9 to 29 g. Thus, interspecific models for estimating fat mass given D<sub>2</sub>O dilution can be as accurate as intraspecific models for songbirds within this size range.

In contrast, the interspecific models that used only the fat score to estimate fat mass were less accurate than the intraspecific models for each of the four species (Table 5). Differences in absolute errors between intraspecific and interspecific models that included only fat score ranged from 0.06 g (in *Z. albicollis*) to 0.39 g (in *V. olivaceus*). Differences among relative errors between intraspecific and interspecific models ranged from 3 % (in *Z. albicollis*) to 12 % (in *S. caeruleus*). The poorer performance of these interspecific models that used fat score to estimate body fat is not surprising given that the same fat score represents different amounts of actual fat for birds of different body size. Thus, the interspecific models that used the fat score to estimate body fat estimated actual fat mass less accurately compared to the intraspecific models.

#### Single versus dual techniques for estimating body composition of songbirds

Deciding which technique and model to use for estimating body composition of wild birds will largely be dictated by the accuracy and precision required to meet the objectives of the particular study, and will depend on the context and goals of the investigator (Castro et al. 1990; Spengler et al. 1995). In short, estimates of body composition are only useful if the errors associated with a particular predictive model are smaller than the variation in the body component that is being estimated (Scott et al. 2001). In theory, one technique (i.e., TOBEC or D<sub>2</sub>O dilution) may suffice for

estimating body composition of an individual at a single point in time (e.g., at capture during migration, at a certain stage of juvenile development). Each of the three techniques that we used in this study has certain advantages and disadvantages compared to the other techniques (Table 8).

One advantage of using TOBEC to estimate lean mass is that the method can be performed repeatedly on the same individual within a short period of time (e.g., hourly, daily). In contrast, D<sub>2</sub>O dilution is difficult to use repeatedly with the same individual because corrections must be made for residual isotope in the body water which requires additional blood sampling (Tatner 1990; Speakman et al. 2001). Although the predictive models for estimating lean mass that included and excluded TOBEC were similarly accurate, measuring changes in lean mass using a model that includes only  $m_b$  and a structural measurement assumes that any change in  $m_b$  is due entirely to changes in lean mass. Because we know this is not necessarily true, using a method such as TOBEC that directly estimates the lean mass of individuals is necessary when both lean and fat mass are changing over time.

Unlike TOBEC, D<sub>2</sub>O dilution can be accurately used over a wide range of animal body sizes, and it is not affected by factors such as changes in ambient temperatures (Table 8). In addition, unlike visual fat scoring, D<sub>2</sub>O dilution can directly and accurately estimate fat mass using either intraspecific or interspecific models. D<sub>2</sub>O dilution has the additional advantage of being a much more objective technique compared to visual fat scoring and TOBEC. The primary disadvantage of the D<sub>2</sub>O technique is the laboratory effort required to process samples, although several laboratories will measure D<sub>2</sub>O concentration in blood samples for a cost. Our results suggest that D<sub>2</sub>O dilution is the best single technique for estimating fat mass

and lean mass of small songbirds (also see Karasov and Pinshow 1998).

Visual fat scoring provides a quick and relatively accurate method for estimating subcutaneous fat in an individual. Concern about variation among observers and among species can be at least in part mediated with proper training. However, comparing estimates of the fat score between studies is difficult, especially when researchers use different fat score scales (e.g., a 0–5 scale vs. a 0–4 or 0–7 scale). Recent efforts to standardize fat score scales with detailed descriptions of each score may minimize this variation among observers, but will not eliminate it altogether. If researchers require accurate estimates of whole body fat rather than just of subcutaneous fat, or if researchers want to compare actual fat mass among studies, then either D<sub>2</sub>O dilution or TOBEC must be used.

The biggest weakness with using only one technique to directly estimate one body component and then indirectly estimate the other body component is that it is difficult to accurately study the dynamics of lean and fat mass over time if both components are simultaneously changing within a bird. Abundant evidence suggests that mass change in songbirds is due to changes in both lean and fat mass (e.g., Lindström and Piersma 1993; Klaassen and Biebach 1994; Karasov and Pinshow 1998; Klaassen et al. 2000; Bauchinger et al. 2011). Thus, if the research objective is to study the dynamics of both lean and fat mass, then ideally two techniques would be used to ensure independent measures of lean and fat mass in individuals.

One option for estimating changes in lean and fat mass would be to use TOBEC or D<sub>2</sub>O dilution to estimate lean mass and the fat score to estimate fat mass. We found that the TOBEC and D<sub>2</sub>O dilution techniques estimated lean mass with a similar accuracy (Tables 2, 3, 6). Thus,

**Table 8** Summary of the pros and cons of the five primary methods currently being used for measuring body composition of whole live birds

Method	Body mass or size	Tundra certified <sup>a</sup>	Accuracy <sup>b</sup>	Pros	Cons
TOBEC	>10 g	Yes, but temperature sensitive	0.8–1.1 g	Fast, repeatable with practice	Positioning, temperature
D <sub>2</sub> O dilution	Unlimited	Yes	0.2–0.9 g	Objective	Extensive lab analysis
Fat score	>1 rank fat score	Yes	0.3–0.5 g	Fast, repeatable with practice	Species-specific, observer bias
DEXA	8 × 6.5 cm area	No	<i>L</i> : 0.2–0.4 g <i>F</i> : 0.5–0.9 g	Measures lean and fat mass, and bone density	Expensive, heavy, slow
QMR (EchoMRI <sup>TM</sup> )	10–200 g	No	<i>L</i> : 0.2–0.4 g <i>F</i> : 0.1–0.9 g	Fast, objective; lean and fat mass, and total body water	Expensive, heavy, temperature

DEXA, Dual-energy X-ray absorptiometry (DEXA); QMR, quantitative magnetic resonance imaging (EchoMRI, Singapore/Houston, TX)

<sup>a</sup> The first three methods are “tundra certified” meaning they are applicable to field conditions such as the arctic tundra where at best only a generator is available for electricity. See text for full description and evaluation of these methods

<sup>b</sup> Accuracy of whole-body lean mass (*L*) or fat mass (*F*) estimated from validation studies (see text). The first three methods directly estimate the *L* or *F* component, with the other component indirectly estimated by subtraction from whole body mass

deciding between these two techniques for estimating lean mass would depend mostly on convenience and the availability of the method and required material, and how frequently lean mass must be measured. Fat scoring can provide an independent and accurate estimate of fat mass (Table 5) when used in conjunction with either TOBEC or D<sub>2</sub>O as long as changes in body fat are accurately indexed by visible changes in subcutaneous fat. Unfortunately, this latter assumption may often not be true.

Birds captured soon after arrival at a stopover site often have depleted their fat stores and so are mostly lean (Biebach et al. 1986; Moore and Kerlinger 1987; Moore and Simons 1992). In such situations, fat scoring may often be ineffectual because restoration of fat stores while at the stopover site will likely include fat in areas not detected by fat scoring (Wiersma and Piersma 1995). An alternative approach for measuring changes in body composition in songbirds over time would be to use TOBEC to estimate lean mass and D<sub>2</sub>O dilution to estimate fat mass. We found that TOBEC estimated lean mass within an absolute and relative error of 0.8–1.1 g and 5–7 %, while D<sub>2</sub>O dilution can estimate fat mass within an absolute and relative error of 0.2–0.9 g and 26–34 % relative error (Table 8). It is this combination of TOBEC and D<sub>2</sub>O dilution techniques that we advocate using for studies of body composition dynamics in migrating songbirds.

In summary, whereas each of these three techniques is useful in certain contexts, using a single technique to study the dynamics of two body components that may be changing simultaneously is difficult to do accurately. Using one technique to estimate lean mass and another technique to estimate fat mass ensures independent measures. Furthermore, because the interspecific models were in many cases as accurate as the intraspecific models, we conclude that species-specific predictive models are not necessarily a prerequisite for accurately and nondestructively estimating body composition dynamics in songbirds.

Dual-energy X-ray absorptiometry and quantitative magnet resonance: can one method really do it all?

There are two more recent developments in technology that warrant mention, although to date they are very expensive (\$35–>\$100,000 US) and heavy instruments (>25 kg) that limit their utility to most ornithologists for use in the field. We mention them here because they offer a single method that can independently estimate several body components (e.g., mass of lean, fat, bone, water), and technological innovations could make them more accessible and useful for field ornithologists in the near future.

Dual energy X-ray absorptiometry (DEXA) uses X-ray to measure bone, fat, and lean composition of animals the size of mice (0.01 kg) to humans weighing 150 kg,

although different units must be used across this size range of animals. DEXA instruments send two X-ray beams (40, 70 keV) through motionless subjects and then measure the extent of attenuation in separate small pixels across the whole animal. DEXA is successful in measuring body composition because the relative extent of attenuation of the two beams depends on the composition of tissue. Sophisticated (proprietary) algorithms are used to estimate for each pixel the relative proportion of fat, lean, and bone tissue; these individual estimates are then summed to estimate body composition of the whole animal. The output includes an estimate of bone, fat, and lean composition of the animal as well as a color-coded two-dimensional image of the spatial pattern of the body components. Results of recent validation studies with the small-animal (10–50 g) model of DEXA (Lunar PIXImus Densitometer; GE Medical Systems, GE Healthcare, Little Chalfont, UK) suggest that the body composition of small birds can be estimated with reasonable accuracy and precision (relative error for fat mass  $0.25 \pm 6.27$  %) as long as a correction for feathers is made (Korine et al. 2004). This feather correction is apparently necessary because DEXA perceives feathers as “fat.” Nagy (2001) provides a review of validation studies conducted on mammals and their estimates of accuracy and precision for estimating body composition. The primary disadvantages of the current small-animal units most useful to ornithologists are that the animal must remain motionless (usually anesthetized or dead) during the entire scan (5 min), the imaging area is relatively small (80 × 65 mm), and the units are heavy (>27 kg) and expensive (>\$35,000 US).

Medical-grade magnetic resonance imaging (MRI) and quantitative magnetic resonance (QMR) use nuclear magnetic resonance to measure total body fat, lean muscle tissue, free water, and total body water in live animals the size of fruit flies to rats to humans (Tinsley et al. 2004). These instruments magnetically align the spin of hydrogen nuclei in tissues and then produce an alternating orthogonal magnetic field that perturbs the alignment of each atom's nucleus in the animal. As these perturbed hydrogen atoms return to their original alignment, the instrument measures the amplitude, duration, and spatial distribution of radio frequency signals produced by the perturbed (resonating) hydrogen atoms in tissues. Standards (such as canola oil, muscle, saline) are used to establish reference spectra for each tissue type so that the instrument can then convert the amplitude, duration, and spatial distribution of these radio frequency signals to direct estimates of mass of total body water, lean and fat tissue in the whole animal.

The output from medical MRI instruments includes serial diagnostic images of various types of soft tissues that can be integrated to produce a three-dimensional image of, for example, torn knee ligaments and cartilage, or in the

case of birds, the whole-body distribution of body fat and lean muscle tissue. Tinsley et al. (2004) and Taicher et al. (2003) concluded that QMR was more accurate and precise than DEXA for estimating the whole body composition of mice. Wirestam et al. (2009) and Hedenström et al. (2009) used a standard medical MRI instrument to measure the spatial distribution of body fat and the correspondence between MRI-estimated body fat and fat score in a small passerine (*Phylloscopus trochilus*).

The output from QMR instruments (e.g., EchoMRI™) includes an estimate of total body fat, lean muscle tissue, free water, and total body water of the animal, but no information about spatial distribution. Almost all previously published validation studies that have assessed accuracy and precision of the body composition estimates from QMR instruments were conducted on rats and mice (Taicher et al. 2003; Tinsley et al. 2004). A recent validation study with the EchoMRI™ for European Starlings (*Sturnus vulgaris*), Zebra Finch (*Taeniopygia guttata*), and House Sparrows (*Passer domesticus*) revealed extremely accurate and precise estimates of body composition: for example, absolute (and relative error) for House Sparrows was  $0.13 \pm 0.02$  g ( $11.4 \pm 3.6$  %) for body fat,  $0.24 \pm 0.05$  g ( $1.1 \pm 0.3$  %) for lean tissue, and  $0.31 \pm 0.08$  g ( $2.0 \pm 0.5$  %) for total body water (Guglielmo et al. 2011). The primary disadvantages of the current small-animal units are that the animal must tolerate confinement in holding devices during the entire scan (1–3 min; although unlike DEXA and TOBEC the animal can move during scanning so the confinement is relatively modest) and the imaging area is a cylinder which restricts the size range that one instrument can effectively measure (about 10–200 g bird for EchoMRI-B™; Guglielmo et al. 2011). A new Flexi™ option is currently available from Echo Medical which introduces a smaller insert antenna that enables measurements of birds weighing 1–10 g, including hummingbirds. Perhaps most important, the EchoMRI-B™ and other similar units are heavy (>200 kg), very expensive (>\$100,000), and the magnet must be maintained within a modest temperature range (18–25 C) which may limit field studies.

“Best” method(s) for non-destructively estimating the body composition of birds

Table 8 summarizes the five primary methods that are currently being used for measuring body composition of whole live birds. The first three are “tundra certified,” meaning they are applicable to field conditions such as the arctic tundra where at best only a generator is available for electricity. Recognize, however, that TOBEC is temperature sensitive, which complicates its use in colder, tundra-like conditions. The first three methods provide only a single value (*E* value, D<sub>2</sub>O space, fat score) so that

estimates of more than one body component must be done by subtraction from  $m_b$ , whereas the latter two methods provide simultaneous, independent estimates of several body components, including lean and fat mass.

DEXA and QMR are large, expensive instruments that can independently and accurately estimate lean and fat mass of live animals. The imaging area of the small-unit version of DEXA is too small for many bird studies, requires immobilizing the animal, and is not as accurate and precise as estimates from QMR. The latest QMR units are the best new technology for estimating body composition of small songbirds. However, the QMR units are very heavy, expensive, and must be maintained at modest temperature which restricts its use to sites close to the QMR unit that are temperature controlled (e.g., in a laboratory or portable heavy-duty trailer with adequate temperature controls).

Our validation study suggests that TOBEC estimates the lean mass of songbirds weighing 10–30 g with accuracy of about 1 g, but that it is not effective for estimating the lean mass of smaller songbirds such as *S. caeruleus*. D<sub>2</sub>O dilution provided more accurate and precise estimates of lean mass than TOBEC, and the fat score provided as accurate and precise estimates of fat mass as the D<sub>2</sub>O method when only a single species was considered. D<sub>2</sub>O is a more objective method than fat score although it requires significant laboratory analysis of samples. The primary advantages of D<sub>2</sub>O over fat score is that the former is applicable over a wide range of body sizes, and the interspecific models were as accurate as intraspecific models for estimating fat mass (fat score is essentially species specific and still requires a validation study to estimate actual fat mass). The use of TOBEC to estimate lean mass and D<sub>2</sub>O dilution (or fat score) to estimate fat mass offers a reasonable “dual” approach for measuring the body composition dynamics of songbirds with accuracy of about 1 g for lean mass and 0.5 g for fat mass.

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Appendix

See Table 9.

**Table 9** Intra- and interspecific models for estimating fat mass ( $F$ ) given deuterium dilution space ( $S$ ) and body mass ( $m_b$ ) using all 59 individuals: Black-throated Blue Warbler (*Setophaga caerulescens*,  $n = 12$ ); Yellow-rumped Warbler (*S. coronata*,  $n = 15$ ); Red-eyed Vireo (*Vireo olivaceus*,  $n = 16$ ); White-throated Sparrow (*Zonotrichia albicollis*,  $n = 16$ ). Intra- and interspecific models for estimating lean mass (LM) given total body electrical conductivity (TOBEC), tarsus length (T) or bill depth (BD), and/or  $m_b$  using 47 individuals: *S. coronata* ( $n = 15$ ); *V. olivaceus* ( $n = 17$ ); *Z. albicollis* ( $n = 15$ )

Species	Estimating fat mass	Model	Estimating lean mass
<i>S. caerulescens</i>	$F = m_b - S - \{S/[2.696 (\pm 0.170)]\}$		(TOBEC models not significant for <i>S. caerulescens</i> )
<i>S. coronata</i>	$F = m_b - S - \{S/[2.458 (\pm 0.065)]\}$	1	LM = $(9.84 \pm 0.49) + (0.047 \pm 0.013)(\text{TOBEC})$
		2	LM = $(-4.30 \pm 5.27) + (0.036 \pm 0.012)(\text{TOBEC}) + (0.789 \pm 0.293)(\text{T})$
		3	LM = $(2.05 \pm 2.35) + (0.007 \pm 0.006)(\text{TOBEC}) + (0.207 \pm 0.144)(\text{T}) + (0.403 \pm 0.053)(m_b)$
		4	LM = $(2.03 \pm 2.40) + (0.193 \pm 0.146)(\text{T}) + (0.442 \pm 0.043)(m_b)$
<i>V. olivaceus</i>	$F = m_b - S - \{S/[3.017 (\pm 0.191)]\}$	1	LM = $(12.85 \pm 0.75) + (0.025 \pm 0.006)(\text{TOBEC})$
		2	LM = $(19.74 \pm 6.74) + (0.027 \pm 0.007)(\text{TOBEC}) + (-0.398 \pm 0.387)(\text{T})$
		3	LM = $(11.52 \pm 5.99) + (0.011 \pm 0.008)(\text{TOBEC}) + (-0.156 \pm 0.318)(\text{T}) + (0.313 \pm 0.103)(m_b)$
		4	LM = $(7.46 \pm 5.45) + (0.030 \pm 0.299)(\text{T}) + (0.418 \pm 0.074)(m_b)$
<i>Z. albicollis</i>	$F = m_b - S - \{S/[2.854 (\pm 0.111)]\}$	1	LM = $(14.56 \pm 1.21) + (0.045 \pm 0.007)(\text{TOBEC})$
		2	LM = $(-0.48 \pm 5.73) + (0.039 \pm 0.006)(\text{TOBEC}) + (0.704 \pm 0.264)(\text{T})$
		3	LM = $(-3.89 \pm 4.78) + (0.011 \pm 0.011)(\text{TOBEC}) + (0.563 \pm 0.219)(\text{T}) + (0.475 \pm 0.173)(m_b)$
		4	LM = $(-5.36 \pm 4.51) + (0.551 \pm 0.218)(\text{T}) + (0.625 \pm 0.075)(m_b)$
All four	$F = m_b - S - \{S/[2.829 (\pm 0.070)]\}$	1	LM = $(9.39 \pm 0.53) + (0.066 \pm 0.004)(\text{TOBEC})$
		2	LM = $(0.30 \pm 1.47) + (0.029 \pm 0.004)(\text{TOBEC}) + (0.261 \pm 0.110)(\text{T}) + (1.786 \pm 0.264)(\text{BD})$
		3	LM = $(0.88 \pm 0.66) + (0.009 \pm 0.005)(\text{TOBEC}) + (1.779 \pm 0.171)(\text{BD}) + (0.362 \pm 0.068)(m_b)$
		4	LM = $(-0.14 \pm 0.41) + (1.803 \pm 0.175)(\text{BD}) + (0.462 \pm 0.047)(m_b)$

See text for description of models

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