Determining Seabird Body Condition Using Nonlethal Measures

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ABSTRACT

Energy stores are critical for successful breeding, and longitudinal studies require nonlethal methods to measure energy stores ("body condition"). Nonlethal techniques for measuring energy reserves are seldom verified independently. We compare body mass, size-corrected mass (SCM), plasma lipids, and isotopic dilution with extracted total body lipid content in three seabird species (thick-billed murres *Uria lomvia*, all four measures; northern fulmars *Fulmarus glacialis*, three measures; and black-legged kittiwakes *Rissa tridactyla*, two measures). SCM and body mass were better predictors of total body lipids for the species with high percent lipids (fulmars; $R^2 = 0.5$–0.6) than for the species with low percent lipids (murres and kittiwakes; $R^2 = 0.2$–0.4). The relationship between SCM and percent body lipids, which we argue is often a better measure of condition, was also poor ($R^2 < 0.2$) for species with low lipids. In a literature comparison of 17 bird species, percent lipids was the only predictor of the strength of the relationship between mass and total body lipids; we suggest that SCM be used as an index of energy stores only when lipids exceed 15% of body mass. Across all three species we measured, SCM based on the ordinary least squares regression of mass on the first principal component outperformed other measures. Isotopic dilution was a better predictor of both total body lipids and percent body lipids than were mass, SCM, or plasma lipids in murres. Total body lipids decreased through the breeding season at both sites, while total and neutral plasma lipid concentrations increased at one site but not another, suggesting mobilization of lipid stores for breeding. A literature review showed substantial variation in the reliability of plasma markers, and we recommend isotopic dilution (oxygen-18, plateau) for determination of energy reserves in birds where lipid content is below 15%.

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Introduction

Use of body condition index as a proxy for energy reserves is common in zoology, especially when it is not possible (e.g., longitudinal studies) or desired (e.g., endangered wildlife) to kill animals and determine body composition directly (Speakman 2001). While some authors measure oxygen carriage capacity (hematocrit) or some aspect of immunocompetence and call these measures "body condition," by far the most common usage of "body condition" is as a synonym for the size of energy stores (Dabbert et al. 1997; Gosler and Harper 2000), and it is that definition that we use here. Total body lipids are usually considered the gold standard for body condition (most studies listed in table A1, available in the online edition of *Physiological and Biochemical Zoology*). In some cases, such as when a female is producing an egg and the largest egg possible will provide the highest offspring fitness, total body lipids provide a good representation of body condition. However, often a more accurate measure would be percent body lipids; a skinny elephant has higher total lipid stores than a plump mouse, but the latter is likely in better body condition (Schamber et al. 2009). Given that both percent and total body lipids can be appropriate depending on the context, we consider both metrics.

Most approaches for calculating body condition use mass corrected for body size to separate aspects of body mass that are due to structural size from aspects associated with energy stores, thereby implicitly accepting that it is the relative size of energy stores (percent lipids) that is more relevant (Green 2001; Peig and Green 2009, 2010). The term "body condition index" suggests that the term means something more than mass, when in fact body condition indexes are usually highly correlated with mass (Schamber et al. 2009; Ndlovu et al. 2010). To emphasize that "body condition index" is closely linked to mass and because of the variation in usage of "body condition," we use the term "size-corrected mass" (SCM) to mean what is traditionally referred to as "body condition index." There are many different equations for calculating SCM (Green 2001; Peig and Green 2009, 2010), and a purpose of this article is to determine which equation best predicts energy stores.

Alternatives to SCM for determining total lipid reserves are

plasma parameters, such as metabolites and hormones (e.g., glucose, triacylglycerides, uric acid, nonesterified fatty acids; corticosterone; leptin), which are often touted to provide nonlethal indexes of total body lipids (Kitaysky et al. 1999*a*, 1999*b*; Angelier et al. 2009; Buck et al. 2007; table A1). Nonetheless, circulating metabolites potentially represent mobilization of energy stores rather than the stores themselves, which is likely why plasma techniques seem to work in some cases but not in others (table A1). Other nondestructive alternatives to plasma or SCM methods are total body electrical conductivity, magnetic resonance spectroscopy, dual x-ray absorptiometry, and isotope dilution, but cost and portability of some of these techniques are still prohibitive for many studies (Wirestam et al. 2008; Seewagen and Guglielmo 2010). Isotope dilution is based on the idea that because lipids are hydrophobic, percent body water is negatively associated with total body lipids (table A1). Indeed, percent body water predicts total body lipids more accurately, on average, than mass-length residuals, total body electric conductivity, or plasma measures (table A1).

To compare the relative predictability of different nondestructive measures of total body lipids, we studied thick-billed murres (*Uria lomvia*; body mass, SCM, plasma, isotope dilution), northern fulmars (*Fulmarus glacialis*; all but isotope dilution), and black-legged kittiwakes (*Rissa tridactyla*; body mass, SCM). Particular emphasis is placed on comparing the various SCMs described in "Comparison of Equations for Determining SCM." For each animal, we extracted and measured total body lipids. Although metabolites, such as fatty acids and triglycerides, represent mobilization of stores, there could be a correlation between lipid mobilization and total energy stores at a particular time of year, and we tested for the presence of that correlation as a possible nonlethal technique for measuring total body lipids. The primary objective was to validate nonlethal measures of total body lipids, but we also wished to use these measures to study variation in lipid stores over the breeding season at two study sites. During endurance exercise such as flight, lipids are mobilized and transported through the circulation in the form of low-density lipoproteins or plasma neutral lipids (Jenni-Eiermann and Jenni 1992; McWilliams et al. 2004), but few data exist outside of migration (e.g., Golet and Irons 1999; Vézina and Williams 2003). Because energy demands peak during chick rearing (Elliott and Gaston 2005; Paredes et al. 2005; Elliott et al. 2008*a*, 2009*b*) and neutral lipids are likely the main transport molecule (McWilliams et al. 2004), we predicted that neutral plasma lipid concentrations should be higher during chick rearing than during incubation (Croll et al. 1991; Gaston and Perin 1993; Weimerskirch and Lys 2000; Gaston and Hipfner 2006*a*, 2006*b*). We also used the techniques developed above to estimate total body lipid stores and relate changes in those values to changes in plasma lipid stores. Understanding the factors (breeding status, sex) influencing plasma lipid stores could provide insight into the conditions under which plasma lipids would be useful indexes of energy stores.

Material and Methods

To examine the validity of various metrics for determining energy stores in seabirds, we collected seabirds in 2002 and 2006 and measured total body lipids of the entire carcass in both years. During June–August 2002, we collected 42 thick-billed murres (14 during each sampling period: July 5–9, July 27, and August 9–10), 30 black-legged kittiwakes (12 on July 13–14 and 18 on August 2–4), and 26 northern fulmars (20 on June 14–21 and 11 on July 31) from a breeding colony at Prince Leopold Island, Nunavut, Canada (74′02°N, 90′00°W; see Jacobs et al. 2010 for details). In all cases, we established breeding status by monitoring sites daily for presence of an egg or chick, and birds were taken only when they were actively and continuously incubating the egg or brooding the chick. We took blood samples from birds killed using a guillotine, and serum was frozen. We measured the wing chord, tarsus, tube length (for fulmars only), bill depth at the nares, and culmen of each specimen. For fulmars, we also measured the length of the tube. During July–August 2006, we injected eight murres with deuterium and oxygen-18 to examine the feasibility of using isotopic dilution to measure body lipids. We collected an additional eight murres to examine the relationship between total body water and body lipids in more detail. We extracted lipids and measured total body lipids in all 16 birds. We plucked and dissected specimens from both 2002 and 2006 at the laboratory and then extracted and measured total body lipids for each specimen.

To examine lipid dynamics at another site in another year and thereby determine whether trends found in one year would continue under different circumstances, we collected plasma samples in June–August 2003 from 117 thick-billed murres breeding at Coats Island, Nunavut, Canada (62′15°N, 83′00°W). We froze specimens and plasma for all studies at −20°C. We obtained all permits required to conduct research (Canadian Wildlife Service Permit NUN-SCI-01-03, Wildlife Research Permit WL000174, Government of Nunavut Permit 02M00G02, Animal Care Permit BL-172).

Lipid Measurements

We measured total body lipids for each carcass by summing the contributions of lipid from five tissues (Jacobs et al. 2010). We conducted lipid extractions on five tissue groups: breast muscle, liver, skin, combined visceral organs (except liver), and remaining carcass (including bones). Each tissue was homogenized in Folch reagent (Jacobs et al. 2010). After filtration (Whatman 1) to remove solid material, 0.25% KCl was added to help remove any remaining aqueous components. The samples were placed in a water bath at 70°C for 5 min to separate aqueous and organic phases. After removal of the aqueous phase, the organic phase was evaporated and total body lipids were weighed (± 0.001 g). Based on the fat mass in each subsample, total fat mass was determined for each whole tissue.

Plasma lipids were determined using Folch reagent following standard methods (Jacobs et al. 2009). We divided total plasma lipids into three classes: neutral lipids, nonesterified fatty acids, and phospholipids. The neutral lipid fraction was eluted by flowing chloroform : isopropanol (2 : 1 v/v) through the columns. The nonesterized fatty acid fraction was eluted by flowing isopropyl ether : acetic acid (98 : 2 v/v) through the columns. We eluted the phospholipid fraction by flowing methanol through the columns. An internal standard (17 : 0, 30 mg/100 mL hexane) was added to each lipid fraction. These classes were then separated to analyze their fatty acid composition. Fatty acids were analyzed using GC (Hewlett-Packard 5890 series II with Hewlett-Packard 7673 autosampler and flame-ionization detector; Jacobs et al. 2009).

We also used total lipid and neutral lipid of plasma samples of thick-billed murres from Prince Leopold Island in 2002 ($N = 40$) and from Coats Island in 2003 ($N = 117$) to examine differences in breeding stage and colony (Jacobs et al. 2009). The sex of adult thick-billed murres was identified using DNA markers (Elliott et al. 2010). Based on the reproductive activity in which birds were engaged at the time of sampling, data for each sex were organized into four sampling periods: early incubation (<16 d after mean laying date), late incubation (≥16 d after mean laying date), early chick rearing (<11 d after mean hatching date), and late chick rearing (≥11 d after mean hatching date).

Isotope Dilution Method

The relative concentration of isotopes in the blood before and after injection with the isotope provides evidence of the dilution space of the isotope, which is, for oxygen and hydrogen, primarily total body water. Oxygen-18 is usually a better measure of total body water than deuterium (Speakman 2001).

We examined whether total body water (assessed by isotope dilution) accurately predicted total body lipids and what type of isotope (deuterium vs. oxygen-18) or equation (plateau method or intercept method) better predicted total body lipids. Specifically, at Coats Island during July 18–August 10, 2006, we injected 38 murres with 0.5 or 1.0 mL of doubly labeled water (50% H_2O^{18} and 25% D_2O) approximately 1 cm deep into the brood patch, using a 27-G needle attached to a 1-cc syringe. The injectate eventually equilibrates with body water, and the isotopic concentration of the equilibrium blood sample allows for estimation of the amount of body water (i.e., total body water) that the injectate has been diluted into. Equilibrium blood samples were taken 90–180 min after injection from the tarsal vein. Eight of the birds were sampled every 30–90 min from the brachial vein, weighed, and killed after 240 min by cervical dislocation. Another eight birds were killed using the same method but without being injected, due to logistical constraints (they were used in this study only to compare body water and lipid content). The entire body was then freeze-dried to determine the body water content and subsampled to determine lipid content. The birds were considered completely freeze-dried after their weights remained constant over two weighing sessions 24 h apart. Feathers were plucked and weighed prior to freeze-drying. The percent of body water was defined as the difference between the weight in the field (after subtracting the weight of the feathers) and the weight in the laboratory after freeze-drying (when the feathers were already removed). The remaining 30 birds were released following injection, and they returned immediately to their breeding site. Deuterium and oxygen-18 values were elevated 30–60 min after injection (fig. 2), during which period the injectate had not yet exchanged from the blood to the other body fluids, and fell off after 200 min ($t_{18} = 3.92$, $P = 0.001$). Thus, the plateau occurred 90–180 min after injection, justifying our use of that time period for equilibrium blood sampling.

Background blood samples were obtained from the tarsal vein in a separate group of 16 birds (seven on July 14, two on July 26, and seven on August 5; the oxygen-18 measurement was not made by the laboratory for one of the July 14 birds, so the sample size for oxygen-18 is 15). The average background concentrations were 1,993.64 ± 0.51 ppm of oxygen-18 and 153.26 ± 0.46 ppm of deuterium, with no difference among dates ($P > 0.6$). All blood samples were collected into one to three 60-μL capillary tubes that were flame-sealed for laboratory processing after the field season. To account for volume, the difference between equilibrium and background levels was doubled for all murres injected with only 0.5 mL. The equations for the intercept and plateau body water followed Speakman (1997).

Statistical Analysis

We considered the first principal component (PC1) of a principal component analysis (SYSTAT, ver. 8.0) including culmen, tarsus, bill depth, and wing length to determine size. In general, all size measures were highly correlated, and PC1 explained >60% of the variation in those size characters. Backward stepwise multiple regression analysis (*F-stat* entry criterion, 4.00) was used to determine which size and mass variables were the best predictors of total body lipid for each species. We used a general linear model with reproductive period, sex, and colony as independent variables to examine variation in total lipid and neutral lipid concentrations. To determine whether there were differences in the plasma fatty acid signatures of thick-billed murres sampled during the same period on Prince Leopold Island and Coats Island, we used principal component analysis and discriminant function analysis (SYSTAT, ver. 8.0). The results of discriminant function analyses (F statistics), using the concentrations of the different fatty acids or the principal components of those, were similar. All statistical results with P values less than 0.05 were considered significant. Data presented are means ± SE. Raw data are presented in appendix B, available in the online edition of *Physiological and Biochemical Zoology* as a PDF.

Comparison of Equations for Determining SCM

Initial approaches for determining SCM used ratio methods (mass/body size), but these approaches are biased when body size and mass are not directly proportional (which they seldom are). When body size and mass are not directly proportional, ratio-based SCM indexes will depend on size, so large animals will appear to have higher or lower energy stores just because they are structurally larger. Despite this drawback, ratio methods continue to be used (e.g., Hannah et al. 2008; Ndlovu et al. 2010).

More often, current approaches consider SCM the residual of mass on body size regression (Green 2001). Body size is usually cubed, or the cube root of mass is taken, to try to convert linear measurements of size into variables compatible with mass. Nonetheless, exponents in allometric relationships between linear proxies for size and mass often differ from three (Jakob et al. 1996; Elliott and Gaston 2008). Many authors calculate the residual of the logarithm of body mass onto the logarithm of linear size, thereby allowing the length-mass exponent to vary away from three. Because animals are not simple geometric objects, single linear measures are unlikely to explain all of the variation in volume. Combining several measures into a single variable using principal component analysis can improve size estimates. Reduced major axis regression is sometimes considered more appropriate than least squares regression because there is uncertainty in both size and mass. For these reasons, Green (2001) argued that the best estimate of total body lipids (SCM) is the residual from the reduced major axis regression of log mass on the first principal component.

To determine which of several different equations for SCM most accurately reflected total body lipids and percent lipids, we calculated 84 (murres, kittiwakes) or 98 (fulmars) indexes. We considered combinations of ratio and residual (reduced major axis and least squares) methods with linear, power ($\text{mass}^{1/3}$ and size^{3}), and logarithmic relationships. We also examined the relationships reported by Schamber et al. (2009), so that nine avian groups were covered. We report R^2 values rather than attempting to account for parsimony (e.g., information criterion methods) because all SCM models have the same number of parameters (two), and therefore ranking from R^2 values will be identical to rankings from information criterion methods.

Finally, Peig and Green (2009, 2010) suggested a scaled mass index that overcomes some of the drawbacks of both residual and ratio methods. The index is based on scaling properties and is calculated as scaled mass index, $M_i(L_o/L_i)^{b\text{RMA}}$, where M_i is the mass of the individual, L_i is the length of the animal, L_o is the average size of all individuals in the sample, and bRMA is the reduced major axis slope of the $\ln L_i$-$\ln M_i$ plot. The length is chosen as the size index that correlates best with mass. Although Peig and Green (2009, 2010) suggest that percent and total body lipids are less useful indexes of condition than scaled body components, we correlate the scaled mass index with total and percent body lipids for consistency and because most authors continue to consider those indexes to be the gold standard.

Interspecies Comparison

We completed a literature review of avian studies that report correlations between body mass and total body lipids. We searched the Web of Science using the expression "body condition." While our search was not exhaustive (23 species), it likely provides a representative sample of studies. We considered the strength of the relationship among different metrics for determining total body lipids. We calculated whether the strength of the relationship between mass and total body lipids depended on mass, variation in body mass, percent lipids, or variation in percent lipids. Variation in the calculation of SCM prevented a direct interspecies comparison of SCM (as opposed to body mass alone) validity for all 23 species. We considered null ($k = 0$), linear ($k = 2$), and threshold ($k = 3$) functions using the corrected Akaike's Information Criterion (cAIC),

$$2k - 2\ln(L) + \frac{2k(k+1)}{n-k-1},$$

where k is the number of parameters in the model, n is the sample size, and L is the log likelihood.

Results

External Measures

Skin lipid mass was the most accurate predictor of total body lipid (table 1). Liver lipid mass (fulmars, kittiwakes) and breast muscle lipid mass (murres, kittiwakes) were not correlated with total body lipids. The species with the highest percent of body lipids (fulmars) had higher adjusted R^2 values for models of total body lipids using external measures (maximum $R^2 = 0.72$) than the other two species (murres, 0.36; kittiwakes, 0.30).

Comparisons of Different Equations for Calculating SCM

In kittiwakes, none of the 56 equations for SCM (i.e., excluding size alone) predicted total or percent lipids better than the logarithm of mass alone (table A2, available in the online edition of *Physiological and Biochemical Zoology*). In murres and fulmars, the best equation for SCM increased the variation in total or percent body lipids explained by only about 10% compared to mass alone (total body lipids, murres: $R^2 = 0.37$ vs. 0.28; fulmars: $R^2 = 0.71$ vs. 0.58; percent body lipids, murres: $R^2 = 0.26$ vs. 0.14; fulmars: $R^2 = 0.60$ vs. 0.41). For all three species, ordinary least squares (OLS) almost always outperformed both reduced major axis (higher R^2 value for 115 out of 128 comparisons shown in table A2, pairwise comparison of each value in bold with the equivalent value not in bold; $z = -12.8$, $P < 0.00001$) and ratio methods (95 out of 128 comparisons shown in table A2, pairwise comparison of each value shown as M/A versus each value shown as $mA + b$, z value = 8.41; $P < 0.00001$). Although SCM using wing length performed similarly to PC1 for murres (wing length, total lip-

Table 1: Proportion of variance in total body lipids explained (R^2) by nonlethal external and lethal predictors of total body lipid in three seabird species

Predictor variable(s)	Thick-billed murres ($N = 42$)	Black-legged kittiwakes ($N = 31$)	Northern fulmars ($N = 26$)
Mass	.27* (.14)	.24* (.05)	.52** (.41**)
Mass, culmen	.36*,[a]	.30*,[c]	.56*
Mass, culmen, wing	.35*	.29*	.57*
Mass, culmen, wing, tarsus	.35*	.24*	.67*
Mass, culmen, tarsus, tube depth	NA	NA	.72*,[b]
Breast muscle lipid mass	.00 (.03)	.13 (.48**)	.64** (.41**)
Visceral organ lipid mass	.35** (.30**)	.13 (.52**)	.68** (.78**)
Liver lipid mass	.49** (.28**)	.04 (.31**)	.04 (.08)
Skin lipid mass	.94** (.84**)	.91** (.70**)	.90** (.87**)
Carcass lipid mass	.67** (.68**)	.78** (.80**)	.57** (.70**)
Total body water (direct)	.78 (.71)	...	...
Total body water (oxygen-18)	.58 (.52)	...	...
Total body water (deuterium)	.52 (.48)	...	...
Average body mass (g)	875	359	722
Average percent body lipids (%)	6.6	3.1	13.0

Note. Also shown are the percent body lipids and total mass for each species. Relationships of arcsin-transformed percent lipid in each component with arcsin-transformed percent total body lipids are shown in parentheses. NA = not applicable.

*$P < 0.05$.

**$P < 0.001$.

[a]Total lipid = −275.79 + 0.191(mass) − 4.95(culmen).

[b]Total lipid = 138.14 + 0.35(mass) − 8.50(culmen) − 4.81(tarsus) + 14.03(tube depth).

[c]Total lipid = −65.39 + 0.057(mass) + 2.03(culmen).

ids: $R^2 = 0.30$; PC1, total lipids: $R^2 = 0.30$; wing length, percent lipids: $R^2 = 0.17$; PC1, percent lipids: $R^2 = 0.16$; all for OLS residuals on log-transformed data, denoted as $\log(M) = m\log(\text{PC1 or wing}) + b$; table A2) and tarsus outperformed PC1 for fulmars (tarsus, total lipids: $R^2 = 0.69$; PC1, total lipids: $R^2 = 0.51$; tarsus, percent lipids: $R^2 = 0.60$; PC1, percent lipids: $R^2 = 0.40$; all for OLS residuals on log-transformed data, denoted as $\log(M) = m\log(\text{PC1 or tarsus}) + b$; table A2), PC1 did better on average than single measures (16 out of 20 comparisons for murres and fulmars; table A2). There was little difference in either single size measures or PC1 whether values were left untransformed, cubed, cube rooted, or log transformed (see, e.g., OLS residuals for table A2).

Out of nine avian groups (our own and those reported by Schamber et al. [2009]), the residual of mass on PC1 predicted total and percent body lipids better than the residual of body mass on single measures of size in six groups (no difference in a seventh group) and better than ratio measures in five groups for total body lipids and six groups for percent body lipids (no difference in an additional group for each; table 2). Similarly, PC1 had equal or better performance than all single morphometrics in four out of six waterfowl groups for percent lipids (three out of six waterfowl groups for total lipids) and outperformed the average of single morphometrics (15 out of 18 comparisons; binomial test $P = 0.004$; tables 2 and 3 in Schamber et al. 2009). Residual methods outperformed ratio methods for the same morphometric (wing, tarsus, etc.) in the six waterfowl groups, using paired comparisons (14 out of 18 paired comparisons for each morphometric; binomial test $P = 0.02$; table 3 in Schamber et al. 2009). In all nine avian groups, the amount of variance explained by ratio methods was also more variable (more likely to be 0) than residual methods (table A1; Schamber et al. 2009). The scaled mass index generally did no better than standard measures at predicting total or percent lipids (table 2).

Interspecies Comparison

Across 17 studies, the relationship between body mass and lipid stores increased with percent body lipids ($R^2 = 0.42$, $t_{15} = 2.70$, $P = 0.02$) but was unrelated ($P > 0.05$) to body mass ($R^2 = 0.03$) or variability (coefficient of variation) in mass ($R^2 = 0.04$) or lipid stores ($R^2 = 0.24$; table A1; fig. 1). There appeared to be a threshold at 15%–20% of body mass, as a stepwise function was more parsimonious than a linear function (fig. 1). Specifically, a stepwise function was more parsimonious than a linear function (ΔcAIC = 3.09; ΔcAIC = 7.84 over null model). Thus, body mass predicted total lipids with increasing confidence as lipids became a larger proportion of body mass, to about 15% of body mass, at which point the R^2 was constant at about 0.7. Plasma lipids (cholesterol, triglycerides, or fatty acids) predicted total or percent body lipids well in a few cases, but results tended to be variable (table A1).

Table 2: Proportion of variance (R^2) in total and percent body lipids explained by body mass alone, ratio-based size-corrected mass (SCM) indexes, residual-based SCM, and residual-based SCM using principal component 1 (PC1)

	Total body lipids					Percent body lipids				
Species	Mass	Ratio	Residual (single)	Residual (PC1)	SMI	Mass	Ratio	Residual (single)	Residual (PC1)	SMI
Black-legged kittiwake	.24	.15	.17	.12	.03	.05	.02	.03	.01	.06
Thick-billed murre	.28	.18	.28	.32	.11	.14	.09	.14	.18	.04
Northern fulmar	.58	.52	.52	.53	.03	.41	.40	.39	.40	.01
Harlequin duck	.83	.61	.68	.79	...	.78	.58	.64	.75	...
Northern pintail	.52	.58	.54	.58	...	.46	.44	.49	.53	...
American wigeon	.27	.31	.31	.38	...	.18	.25	.23	.29	...
Lesser scaup	.27	.24	.26	.23	...	.19	.17	.19	.19	...
Barrow's goldeneye (male)	.25	.22	.20	.20	...	.19	.20	.15	.14	...
Barrow's goldeneye (female)	.22	.03	.28	.31	...	.16	.03	.22	.25	...

Note. "Ratio" and "residual (single)" are averages across all size measures reported. Values for three seabirds are derived from table A2, available in the online edition of *Physiological and Biochemical Zoology*. Values for five waterfowl are from Schamber et al. (2009). SMI, scaled mass index.

Other Nonlethal Measures: Plasma Lipids and Isotopic Dilution

For murres, the best prediction using a single fatty acid was obtained from neutral lipid 20 : 5 ($R^2 = 0.32$). For fulmars, the best prediction using a single fatty acid was obtained from plasma lipid 20 : 1 ($R^2 = 0.43$). In the only species examined, murres, total plasma lipids were not correlated with body lipids ($R^2 = 0.02$ for total body lipids and $R^2 = 0.02$ for percent body lipids; $P > 0.5$), while plasma triglycerides were ($R^2 = 0.28$ for total body lipids and $R^2 = 0.18$ for percent body lipids; $P > 0.5$). Leptin did not correlate with total body lipids, using a heterologous bioassay (app. C, available in the online edition of *Physiological and Biochemical Zoology*).

Isotopic dilution was strongly correlated with total body water content, with oxygen-18 and the plateau method having both the highest correlation (R^2) and the closest absolute agreement (to the 1 : 1 line; fig. 2). Deuterium estimates averaged 107.0% ± 0.9% (plateau) or 104.0% ± 0.9% (intercept) of measured (freeze-dried) total body water, while oxygen-18 estimates averaged 100.2% ± 0.5% (plateau) or 95.2% ± 0.8% (intercept) of measured (freeze-dried) total body water. As total body water content was strongly correlated with total body lipids (table 1), isotopic dilution was also strongly correlated with total body lipids (deuterium, $R^2 = 0.52$; oxygen-18, $R^2 = 0.58$) and percent body lipids (deuterium, $R^2 = 0.48$; oxygen-18, $R^2 = 0.52$).

Plasma Lipids: Variation in Time and Space and between the Sexes

There was a significant effect of sampling period (incubation vs. chick rearing) on total plasma lipids (period, $F_{3,136} = 6.53$, $P < 0.001$; colony, $F_{1,136} = 0.37$, $P = 0.54$; sex, $F_{1,137} = 0.42$, $P = 0.52$; colony × sex, $F_{1,136} = 0.21$, $P = 0.78$) and neutral plasma lipids (period, $F_{3,157} = 5.02$, $P = 0.002$; colony, $F_{1,157} = 4.14$, $P = 0.04$; sex, $F_{1,157} = 0.83$, $P = 0.37$; colony × sex, $F_{1,157} = 0.16$, $P = 0.82$; fig. 3). There was a significant difference in the distributions of the plasma lipid signatures between murres breeding in the high and low Arctic sites ($F_{17,139} = 22.7$, $P < 0.001$; fig. 4). At the low Arctic site, the greatest change in fatty acid signatures occurred between incubation and chick rearing, as there was no difference in the signatures during chick rearing (early incubation vs. late incubation, $F_{5,47} = 17.32$, $P < 0.001$; late incubation vs. late chick rearing, $F_{5,19} = 54.64$, $P < 0.001$; all other $P > 0.05$). Fatty acids signatures differed between the sexes at the low Arctic site ($F_{5,96} = 5.60$, $P < 0.0001$) but not at the high Arctic site ($F_{5,34} = 1.69$, $P = 0.16$). Plasma lipid signatures differed be-

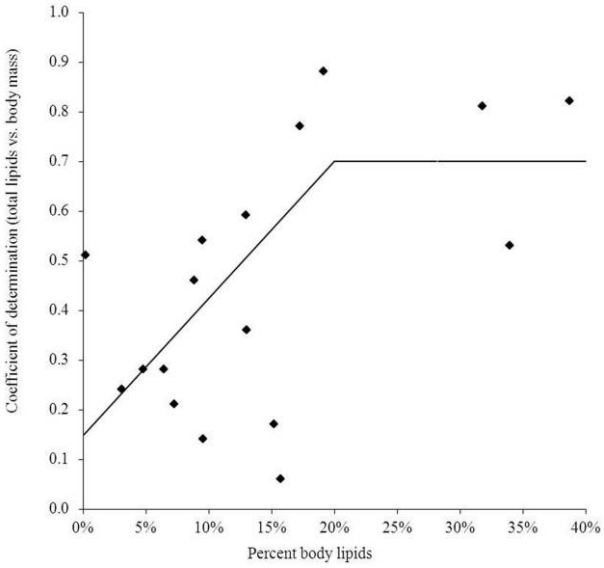

Figure 1. A comparison across avian studies of the strength of the relationship (R^2 value) between total body lipids and body mass. The strength of the relationship increases with percent of total body mass that is lipids for the species being tested. A stepwise function was more parsimonious than a linear model or the null model (see text). Data are from table A1 in the online edition of *Physiological and Biochemical Zoology*.

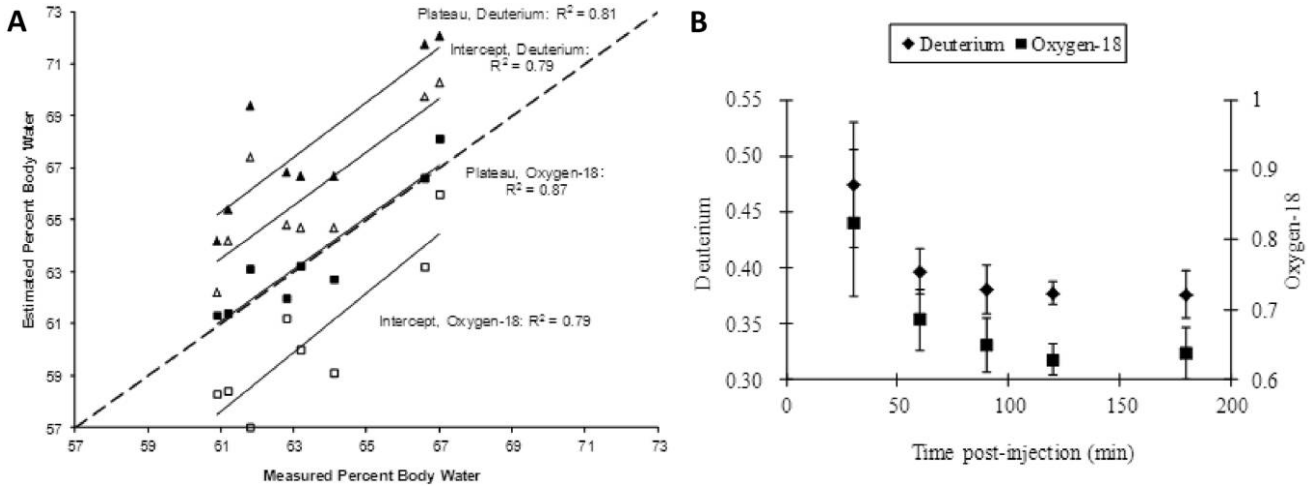

Figure 2. *A*, Estimated percent body water (from four dilution techniques: deuterium = triangles, oxygen-18 = squares, plateau = filled, intercept = open) against measured percent body water (freeze-dried featherless carcass relative to fresh featherless carcass). Dashed line represents the 1 : 1 line. *B*, Isotopic concentrations ± SE (enrichment in ppm per g of adult bird) after injection against resting time. Values for birds injected with 0.5 mL of isotopes (instead of 1.0 mL) were doubled.

tween sites, stages, and sexes (figs. 3, 4), and it was not readily apparent how those signatures could be converted into a reliable index of total or percent lipid stores.

Discussion

What Is the Best Method for Measuring Body Energy Stores?

Skin lipids were consistently the best index of total body lipids. Among nonlethal measures, body mass was a better predictor of total body lipids for species with high percent lipids. The strength of the relationship between mass and total body lipids (R^2 value) increased with percent of body lipids across a wide range of bird species (table A1; fig. 1). Similarly, in our own study, the species with high percent lipids (fulmars) had a much stronger relationship than the species with low percent lipids (murres and kittiwakes; table 1). For species with low percent lipids, variation in nonlipid components (gut contents, lean mass) obscures the relationship between body mass and lipid mass.

As was the case for five waterfowl species (Schamber et al. 2009), the relationships between mass and percent body lipids, likely a better measure of energy reserves, were generally poorer than relationships between mass and total body lipids (table A2). Multivariate models had much stronger relationships than simple regressions (tables 1, A1). We suspect Type I errors, as there were few consistent patterns and, in any case, the more parameters that are added, the better the fit will inevitably be (tables 1, A1). We do not recommend multivariate models for this reason and because they would need validation each time they are used.

SCM was a slightly better predictor of total and percent lipids than mass alone, explaining at most 5%–10% more variation in lipid-rich species (R^2 increased by about 0.05–0.10; table 2). Nonetheless, in kittiwakes, where body mass variation is due primarily to lean body components (Jacobs et al. 2010), none of the 84 proxies for SCM predicted total or percent lipids significantly (tables 2, A2). Given that SCM is heavily dependent on mass, we suggest that SCM is a reliable index (e.g., $R^2 > 0.3$) only where body mass is also a reliable index, which was primarily in birds with percent lipids greater than 15% (the point where $R^2 = 0.50$ in fig. 1). In all three seabird species and six waterfowl species, OLS almost always outperformed both reduced major axis and ratio methods. Also, ratio methods are biased, and reduced major axis is believed to provide a less biased estimate than OLS because it allows for variation in both size and mass (Green 2001; Speakman 2001). We suggest that OLS may be a better method because it specifically minimizes variation in the *Y*-axis (mass) and it is those residuals that are used. Scaled mass index generally did not correlate well with total or percent body lipids (table 2).

Although wing length outperformed PC1 in murres (table A2) and tarsus outperformed PC1 in fulmars (table A2), PC1 did better on average than single measures (16 out of 20 comparisons for murres and fulmars), and we suggest that PC1 is a better measure of length because it is hard to know a priori what single morphometric to use (table 2). Wing length, the most common metric used, was better than PC1 in only two out of six cases (table A2; Schamber et al. 2009). There was no difference in either single size measures or PC1 whether values were left untransformed, cubed, cube rooted, or log transformed (table A1). Peig and Green (2009, 2010) recommend using the size measure that correlates best with mass rather than PC1, at least partially because their method requires log transforming the size measure, which is not straightforward when the size measure includes negative values (i.e., PC1). Using that criteria, we would select bill depth for kittiwakes, culmen for fulmars, and wing chord for murres; only one of these

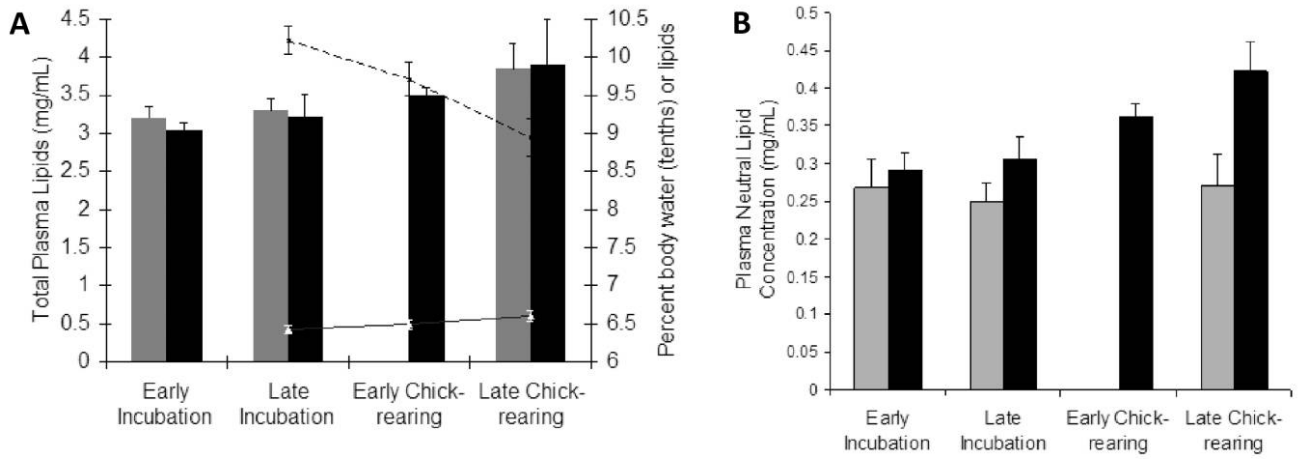

Figure 3. Plasma total lipids (*A*) and neutral lipids (*B*; ±SE) during different reproductive stages for thick-billed murres on Prince Leopold Island (gray bars) and Coats Island (black bars) in 2002 and 2003, respectively. Also shown in *A* are the percent body water (solid line, in tenths to maintain the same axis labels) and lipids (dashed line) for murres at Coats Island in 2006.

measures (wing chord for murres) results in a SCM that is better than PC1, illustrating the difficulty of a priori choosing a single size measure to generate SCM. We recommend use of SCM using transformed or untransformed mass residuals on PC1 of body size and only for birds with percent lipids above 15% (table A1).

SCM predicted total body lipids and, to a lesser extent, percent body lipids marginally better than mass alone (maximum improvement of R^2 of about 0.1) in three seabird species (this study) and five waterfowl species (Schamber et al. 2009). Nonetheless, SCM was highly correlated with body mass, and the idea that SCM means something different from mass (e.g., Ndlovu et al. 2010) was not supported. SCM is best thought of as a size-independent measure of mass, with all the caveats involved in using body mass as a metric of quality (Green 2001; Peig and Green 2009, 2010). Many studies use SCM (residual of mass on PC1) as an indicator of energy stores in kittiwakes, often citing Golet and Irons (1999), where the R^2 is 0.06 (e.g., Moe et al. 2002; Helfenstein et al. 2004; Chastel et al. 2005). Although SCM was a poor predictor of total body lipids in kittiwakes (Table 1), SCM may still be a good indicator of condition because in kittiwakes muscle mass is reduced to a greater degree than lipid mass during stress (Jacobs et al. 2010), and lean mass, rather than lipid mass, may be a better indicator of body condition in this species. Furthermore, SCM may be improved for kittiwakes by including head-bill as a structural measure, as head-bill correlates better with mass than other structural features and also best distinguishes the sexes (Golet and Irons 1999).

Plasma fatty acids correlated with total body lipids (table A1). Nonetheless, the variation in 20 : 5 fatty acid, which correlated with body mass in the high Arctic, was unable to explain likely lipid trajectories for murres at the low Arctic colony. Because plasma and dietary fatty acids are related, the usefulness of fatty acid indexes will be reduced for interannual or interpopulation comparisons when shifts in diet occur. In environments where diet is constant, however, fatty acid analysis allows researchers to collect serial nonlethal samples showing changes in the energy stores in response to specific activities or season. Similarly, while plasma cholesterol or triglycerides sometimes correlated with total body lipids, often they did not (table A1). Presumably, variation in those metrics represents mobilization of energy stores or recent food intake, and they do not appear to be useful metrics in cases where the energy or nutritional status of the bird is not already known and accounted for. Other nonlethal measures, such as total body electrical conductivity,

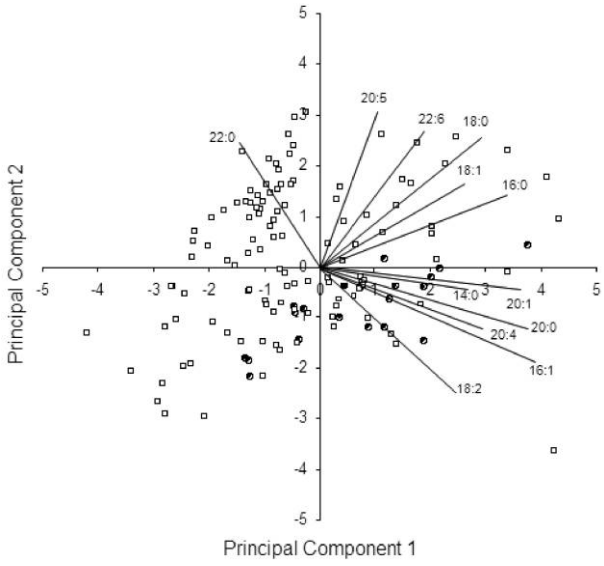

Figure 4. Component 1 versus component 2 from a principal component analysis of plasma lipid signatures from breeding thick-billed murres on Prince Leopold Island (circles) and Coats Island (squares).

are seldom good predictors of lipids on their own but can be useful when employed in conjunction with external methods (table A1). The recent use of medical resonance is very promising where access to the necessary hardware is practical (Budge et al. 2001; Wirestam et al. 2008).

Both deuterium dilution and oxygen-18 dilution were strongly correlated with total body lipids, as were a combination of plasma fatty acids and external measurements. Within isotopic techniques, oxygen-18 using the plateau method showed the strongest agreement, which coincides with past studies. We show significant elevation of isotopes after 60 min, the usual plateau sampling time for seabirds (Harding et al. 2009; Welcker et al. 2009, 2010). We suggest that future studies consider using a plateau of 90 min, although effects on subject behavior need to be considered (Schultner et al. 2010; Hinsley et al. 2011). Isotope dilution is likely a useful method for determining body composition (percent lipids) in species where lipids are below 15% and SCM is no longer reliable.

Plasma Lipid Trends

Plasma neutral and total lipids were highest during chick rearing at the low Arctic site, although it is not possible to strictly separate year from site effects. At that site, the largest change occurred between late incubation and early chick rearing, a period when breeding murres typically (including our study year) lose mass at that study site (Croll et al. 1991; Gaston and Perin 1993; Gaston and Hipfner 2006*a*, 2006*b*; Elliott et al. 2008*a*). Presumably, lipids from the energy stores are mobilized to fuel the increased energetic needs of provisioning offspring. At the high Arctic site in 2002, total plasma lipid concentrations did not increase significantly during the breeding season (fig. 3). The environmental conditions in the high Arctic in 2002 were particularly challenging throughout the breeding season because of heavy ice (Gaston et al. 2005*a*, 2005*b*). Thus, birds may have mobilized lipids earlier in 2002 to overcome poor feeding opportunities.

Fatty acid signatures of adults breeding at two different sites in two different years were different, although because the sites were sampled in different years, it is not possible to strictly separate year effects from site effects (fig. 4). Nonetheless, patterns correspond to known differences in prey composition between the two sites (Gaston and Bradstreet 1993; Gaston et al. 2005*a*, 2005*b*; Elliott et al. 2008*b*). For example, fatty acid signatures in the low Arctic changed during the season, while those in the high Arctic did not, which corresponds to data from prey deliveries and stable isotopes (Gaston and Nettleship 1981; Woo et al. 2008; Elliott et al. 2009*a*, 2009*b*, 2010). The sex difference in fatty acid signatures in the low Arctic also corresponds to the observed differences in chick-provisioning and foraging behavior between the sexes in the low Arctic (Elliott et al. 2010); its absence in the high Arctic supports the idea that trends are colony specific and that at colonies where adults self-feed on different prey items than they feed their chicks, males tend to forage during the day because of diel variation in prey availability (Elliott et al. 2010). Energy demands of egg laying may also play a role (Hipfner 2001; Hipfner and Gaston 2002; Hipfner et al. 2003; Jacobs et al. 2009).

Measuring energy reserves, lipid dynamics, and diet could be an excellent strategy for monitoring the effects of changing food availability, including changes due to overfishing or climate. Energy reserves connect foraging success to reproductive success and individual survival (Green et al. 2005; Kato et al. 2008). However, birds may be able to buffer the effects of variable food supplies by altering their foraging effort (Burger and Piatt 1990); such extra energy expenditure should be reflected physiologically. Hence, using an index of energy reserves coupled with an understanding of how lipids are mobilized can provide a sensitive early-detection tool for monitoring the health of marine ecosystems (Gaston et al. 2003; Montevecchi 2007; Beaulieu et al. 2009; Burke and Montevecchi 2009; Ropert-Coudert et al. 2009).

As plasma lipids varied with sex, colony, and time of year, it was unclear how they could be used to replace SCM or isotopic dilution as indexes of total or percent body lipids. Similarly, as reported above, relationships between plasma and body lipids often contradict one another. Use of such indexes requires a detailed understanding of the ecological and physiological context for a particular species at a particular place and at a particular time. For instance, plasma lipids were highest during chick rearing, when total body lipids were lowest, likely because of extensive energy mobilization at that time, especially for females (fig. 3; see Elliott et al. 2008*a*, 2010). A study using plasma lipids as an index of total body condition that did not consider breeding stage would assume that chick-rearing birds had high total/percent body lipids when they actually had low total/percent body lipids. Thus, SCM and isotopic dilution remain the best indexes of total and percent body lipids.

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