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An evaluation of methods used to estimate carcass composition of common eiders *Somateria mollissima*

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To examine how endogenous reserves may influence avian life history, it is often necessary to quantify carcass composition. However, proximate analyses are expensive, time-consuming and difficult to perform under field conditions. Consequently, carcass composition is often estimated from easily measured data. We evaluate methods of estimating carcass composition of the common eider duck *Somateria mollissima*. We measured, dissected and completed proximate analyses of 92 eiders. Predictive models were derived using multiple regressions of 70 birds, while the remaining 22 were used as an independent test of the models. Each model's accuracy was evaluated by comparing estimates against known values of protein and lipids, using root mean square error (RMSE). Abdominal and leg fat pad mass were highly correlated with total lipid ($r = 0.92$), and body mass was highly correlated with total protein ($r = 0.80$). Models that used body mass, fat depots and/or muscle group data were the most accurate (lipids adjusted $R^2 = 0.93$, RMSE = 14.60; protein adjusted $R^2 = 0.74$, RMSE = 11.14). By using these equations it is possible, using dissection data, to accurately estimate carcass composition of eiders. If dissection data are not available, one can still estimate carcass composition using equations that require only morphometrics although in our lipid analysis such equations had relatively low accuracy (lipids adjusted $R^2 = 0.54$, RMSE = 32.74).

Key words: body condition, carcass composition, common eiders, estimation models, Somateria mollissima

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Knowledge of body condition can provide insight into avian life history. Birds commonly store fat to ensure that their future energetic needs are met (Griminger 1986), and protein can also be catabolised in times of energy or nutrient shortage (King & Murphy 1985). The amount of reserves that a bird stores is a trade-off between starvation risk and predation risk (Lima 1986). Reserve levels are also influenced by food availability (Joyner et al. 1984, Oosterhuis & van Dijk 2002), environmental conditions (Whyte & Bolen 1984a, Lovvorn 1994), and life history stage (Korschgen 1977, McLandress & Raveling 1981, Parker & Holm 1990).

Body condition is a general term that refers to a bird's ability to meet current and future energy needs (Owen & Cook 1977). It is often inferred from body mass, condition indices or mass of muscles and/or lipid depots (e.g. breast muscle, abdominal fat). To measure carcass composition, it is standard practice to extract lipids from dry tissue using a Soxhlet apparatus, with a solvent such as petroleum ether, and then to combust lean dry tissue to determine the protein content of a carcass (Reynolds & Kunz 2001). Such proximate analyses are expensive, time-consuming and difficult to perform under most field conditions. They also require that birds be sacrificed, and this prevents repeated measurements of the same bird over time and often limits sample size. Consequently, many researchers have attempted to estimate carcass composition using more easily obtained data.

A common technique for indexing size of nutrient reserves is to derive predictive models from regressions of carcass lipids and protein against measurements of body mass, abdominal fat pad mass, or breast muscle mass (Thomas et al. 1983, Piersma 1984, Miller 1989, Boos et al. 2000). Sometimes ratios of body mass over a measure of body size are used as predictor variables in regressions or as indices of body condition on their own (e.g. Wishart 1979, Whyte & Bolen 1984b). However, using ratios as a means of expressing body condition should be done only when the two variables are related isometrically, and they rarely are (Packard & Boardman 1999).

Although several studies have investigated ways to estimate carcass composition of waterfowl, there are no published studies examining any of the sea ducks (Mer-

gini tribe). Sea ducks may have unique physiologies because they spend most of their lives in the marine environment, with several species inhabiting polar environments. The body mass of common eiders *Somateria mollissima* is known to fluctuate greatly throughout the year (Gorman & Milne 1971, Milne 1976), and it is suspected that eiders in poor condition during winter and spring refrain from breeding in summer (Coulson 1984, Oosterhuis & van Dijk 2002). However, little is known about their body condition in winter (Gorman & Milne 1971, Korschgen 1977). In this study, we evaluate several methods for estimating lipid and protein content of northern common eiders *S. m. borealis* collected during the non-breeding season in Greenland. Specifically, we evaluate models taken from the literature and models we derived from various external morphological measurements and dissection data. Finally, we determine the minimum analysis necessary to rigorously quantify endogenous reserve levels of sea ducks.

Material and methods

Collections

Inuit hunters and fishermen collected 748 common eiders as part of a subsistence harvest from the waters of the southwest coast of Greenland. The birds were either shot or retrieved drowned from fishnets where they were caught unintentionally. From this sample, 92 birds were selected for complete carcass analysis of lipid and protein. They were selected to ensure that a wide range of endogenous reserve levels, and sex and age classes were analysed (18 first-year females; 28 after-first-year females; 15 first-year males; 31 after-first-year males). We also ensured that these 92 birds had not sustained any damage during collection that would prevent us from gathering all the data.

Carcass analysis

Ducks were aged by plumage characteristics (Baker 1993) and length of the bursa of Fabricius (Mather & Esler 1999), and sexed by syrinx morphology (Beer 1963). The total length of the head and bill and the length of the tarso-metatarsus bone were measured to the near-

est 0.1 mm using calipers. Flattened wing length was measured with a wing board to the nearest mm. Birds were weighed to the nearest 1 g with an electronic scale.

Eiders were plucked, except for the head region, and reweighed. Breast muscles (both *pectoralis major* and *minor*), leg muscles (all muscles that originate or insert in the femur or tibiotarsus bones), and leg fat pad were dissected from the right side of each bird and weighed. The gizzard, heart and abdominal fat pad were extracted and weighed. The digestive system was removed, emptied of its contents and reweighed. For exact methods of dissection see Jamieson et al. (submitted). After dissection, all excised tissues were returned to the carcass and refrozen.

Dissected birds were shipped frozen to the Avian Energetics Laboratory at the University of Western Ontario, London, ON. There, each bird was thawed and cut into ~2-cm³ pieces and subsequently dried to a constant weight in an oven at 80°C. Constant weight was achieved if the bird lost < 1 g per day in the oven (~ 1 week). After dry carcass mass was measured, each bird was homogenised using a hand meat grinder. From each homogenate, a 10-g sample was placed in a single thickness cellulose thimble and placed in a Soxhlet extractor for 16 hours. Petroleum ether was used as the solvent because it extracts mostly storage lipids rather than structural lipids (e.g. phospholipids) or non-lipid compounds (Dobush et al. 1985). After 16 hours of extraction, each sample was dried at 80°C in an oven, weighed and returned to the extractor for another four hours. This process was repeated until a weight change of < 0.01 g was obtained. The lean dry homogenate was placed in a crucible and heated in a muffle furnace for 16 hours at 620°C, to burn off the non-mineral content of the sample. Dry ash weight was recorded. Total carcass water, lipid and protein were calculated as:

$$\text{Total carcass water} = \text{body mass} - \text{dry carcass mass} \quad (1)$$

$$\text{Total carcass lipid} = \text{dry carcass mass} - \text{lean carcass dry mass} \quad (2)$$

$$\text{Total carcass protein} = \text{lean carcass dry mass} - \text{ash mass} \quad (3)$$

Model development

The assumption of normality was checked using the Kolmogorov-Smirnov test (Zar 1999). Homoscedasticity was examined using Levene's test (SYSTAT Software Inc. 2002). Linearity was inspected using residual plots of total carcass lipids and protein, and of body mass (Zar 1999). Total body mass was used rather than body mass

minus digestive system contents because it was most relevant to field studies where the entire bird is weighed.

Approximately 80% of the birds were randomly selected from each age/sex class and this source group was used to derive models that estimated total lipids and protein (Tabachnick & Fidell 2001). The remaining 20% (test group) provided an independent means of evaluating the performance of predictive models through cross-validation. We ran both unadjusted and Bonferroni adjusted t-tests to ensure that there were no significant differences between carcasses of test and source groups. We compared their carcass composition, morphometrics and masses of muscles groups, organs and lipid depots.

We also considered using a principle component analysis to index body size and ran some preliminary analyses with PC1 as an independent variable. However, we were concerned that this approach might obscure some morphological variation that might otherwise be significant in the regression analyses. Furthermore, initial analyses using PC1 to index body size did not increase the accuracy of the resulting predictive equations. Therefore, we felt it was appropriate to use individual morphometrics.

To estimate total carcass lipid and protein, we applied stepwise backwards multiple regression using combinations of 12 independent variables (Table 1). At each step the variable with the highest P-value was removed from the analysis and the regression was rerun. This process was repeated until only those variables that contributed significantly to explaining variation in the dependent variable remained ($P < 0.05$). Regressions were run using: a) all independent variables, b) only independent variables that could be measured on live birds, and c) independent variables that were strongly correlated with the dependent variable (Pearson correlation coefficients

Table 1. Independent variables included in various combinations in stepwise backwards multiple regressions used to estimate total carcass lipid and total carcass protein of non-breeding common eiders.

Independent variables
Age
Sex
Body mass (in g)
Right breast muscle mass (in g)
Right leg muscle mass (in g)
Gizzard mass (in g)
Heart mass (in g)
Right leg fat pad mass (in g)
Abdominal fat pad mass (in g)
Tarso-metatarsus bone length (in mm)
Flattened wing length (in mm)
Head-bill length (in mm)

Table 2. Carcass components of northern common eiders wintering in Greenland. Ducks were randomly divided into a source (N = 70) or test (N = 22) group and compared using both unadjusted and Bonferroni adjusted t-tests. No significant differences were found between the two groups.

	Source Group		Test Group	
	Mean ± SE	Range	Mean ± SE	Range
Total carcass lipid (in g)	182.0 ± 10.0	33.3 - 415.2	178.8 ± 18.1	54.9 - 386.6
Total carcass protein (in g)	384.2 ± 4.3	313.6 - 482.8	391.6 ± 8.5	338.9 - 493.8
Total carcass water (in g)	1086 ± 13	908 - 1388	1134 ± 23	1001 - 1426
Body mass (in g)	1907 ± 26	1542 - 2484	1966 ± 47	1674 - 2547
Breast muscles (in g)	157.5 ± 2.1	123.7 - 202.9	159.6 ± 3.2	132.8 - 183.2
Leg muscles (in g)	75.2 ± 0.9	59.3 - 91.4	76.1 ± 1.7	60.4 - 100.0
Gizzard (in g)	67.2 ± 1.7	40.3 - 102.7	64.4 ± 2.4	49.0 - 89.6
Heart (in g)	22.0 ± 0.6	12.4 - 35.6	15.7 ± 1.1	15.7 - 33.5
Leg fat (in g)	5.2 ± 0.3	0.0 - 14.6	5.0 ± 0.7	0.1 - 14.0
Abdominal fat (in g)	6.4 ± 0.7	0.0 - 24.6	5.2 ± 1.1	0.0 - 24.1
Tarso-metatarsus bone (in mm)	50.0 ± 0.2	44.2 - 58.9	50.3 ± 0.4	47.3 - 52.7
Flattened wing (in mm)	279 ± 1	256 - 295	280 ± 2	265 - 293
Head-bill (in mm)	118.7 ± 0.4	108.9 - 128.6	119.0 ± 1.0	111.6 - 129.2

>0.60). These variables were run both individually and together as a group.

There are several predictive models involving water content that have been applied to birds (Child & Marshall 1970, Campbell & Leatherland 1980, Briggs & Thornton 1988). Percent water content is often correlated negatively with lipid content of a carcass (e.g. Briggs & Thornton 1988, Miller 1989). Therefore, we also ran a regression that included percent water to estimate total lipid content. We applied two previous published methods, the Child-Marshall model (1970) to estimate lipid content and the Campbell-Leatherland model (1980) to estimate lipid and protein content. The Child-Marshall model uses the ratio of carcass water to fat-free weight, whereas the Campbell-Leatherland model uses: 1) the ratio of carcass water to carcass protein, and 2) the ratio of total carcass lipid plus total carcass protein to dry carcass weight (Child & Marshall 1970, Campbell & Leatherland 1980).

All regressions originally included dummy variables for age (1 = first-year; 0 = after-first-year) and sex (0 = female; 1 = male) because during preliminary analyses we found that there were significant differences between the slopes and intercepts of the age and sex classes when body mass or masses of individual fat pads were regressed against carcass lipid.

Traditional condition indices (e.g. body mass divided by a measure of body size) were not used because the data were not related isometrically; that is, they did not produce straight lines through the origin when plotted against each other (Packard & Boardman 1999). We also refrained from using residuals as our data did not meet all the necessary assumptions (Green 2001).

Model evaluation

Derived models were applied to the morphological and dissection data of the test group (N = 22), whose lipid and protein content were known, and total carcass lipid and protein were estimated. The accuracy of each model was evaluated by comparing estimates against known values of protein and lipids from each carcass. This was done by calculating the root mean square error of prediction (RMSE; Olden & Jackson 2000) with a small value indicating a model of high accuracy. Each model was ranked according to its RMSE, which was calculated as follows:

$$RMSE = \sqrt{\sum (y_{\text{estimated}} - y_{\text{actual}})^2 / N} \quad (4)$$

where $y_{\text{estimated}}$ = estimated lipid mass (or protein mass), y_{actual} = measured lipid mass (or protein mass), and N = sample size of source data set.

Table 3. Pearson correlation coefficients of the relationships between various carcass components and total carcass lipid and protein of non-breeding common eiders.

Carcass component	Total carcass lipid	Total carcass protein
Body mass	0.62	0.80
Breast muscles	0.44	0.74
Leg muscles	0.51	0.69
Gizzard	0.20	0.54
Heart	0.29	0.17
Leg fat	0.92	0.25
Abdominal fat	0.92	0.22
Tarso-metatarsus bone	0.04	0.44
Flattened wing	0.23	0.41
Head-bill	0.01	0.59

Table 4. Models derived using backward stepwise multiple regressions to estimate total carcass lipid (TCL) of common eiders during the non-breeding season. Models were ranked according to their root mean square error of prediction (RMSE; see 'Model evaluation'). All models originally included the variables sex and age.

Original variables used to predict TCL	Model	Model	Adjusted R ²	RMSE	Rank
All variables [†]	1	= -246.84 +0.04 BM +13.50 LGF +6.14 ABF +0.90 WG	0.93	14.60	1
Body mass, body size [‡]	2	= -100.13 -39.38 SEX +0.32 BM +2.72 WG -8.92 HD	0.61	38.93	8
Body size, fat pads [§]	3	= -127.78 -18.00 AGE +13.42 LGF +6.73 ABF +1.72 HD	0.92	17.04	2
Body mass	4	= -277.19 -57.26 SEX -36.31 AGE +0.26 BM	0.54	32.74	6
Leg fat pad	5	= 54.82 -18.45 AGE +25.98 LGF	0.86	19.17	3
Abdominal fat pad	6	= 118.78 -30.77 AGE +11.74 ABF	0.88	21.71	4
% water	7	= 1104.73 -1348.13 % water	0.48	33.10	7
Campbell-Leatherland				31.67	5
Child-Marshall				44.57	9

[†] All variables = body mass (BM), right breast (BST), right leg muscles (LGM), gizzard (GIZ), heart (HRT), right leg fat pad (LGF), abdominal fat pad (ABF), tarso-metatarsus bone (TAR), flattened wing (WG) and head-bill (HD).

[‡] Body size = TAR, WG and HD.

[§] Fat pads = LGF and ABF.

All statistical analyses were carried out using SYSTAT 10.2 (SYSTAT Inc. 2002).

Results

Data for body mass, total carcass lipid and total carcass protein met assumptions of normality (Kolmogorov-Smirnov test: $P > 0.05$), homoscedasticity (Levene's test: $P > 0.05$), and linearity. Strong multicollinearity was not found among the independent variables (variance inflation factors ranged within 1.1-5.0).

There were no statistically significant differences between carcass components of the source and test groups (Table 2).

The variables of abdominal fat pad mass, leg fat pad mass, and body mass were strongly correlated to total carcass lipids (Pearson correlation coefficients > 0.60 ; Table 3). The locomotory muscles (breast and leg) and body mass were strongly correlated with total carcass

protein (Pearson correlation coefficients > 0.60 ; see Table 3). Due to the high correlations with the dependent variables, these variables were run in their own regression analyses. Lipid and protein contents were not strongly correlated to any measure of body size.

Seven different models were produced using backwards stepwise multiple regressions to estimate total carcass lipids (Table 4). Model 1, which included body mass, leg fat pad mass, abdominal fat pad mass, and flattened wing length, most accurately estimated total carcass lipids (see Table 4). Previously published methods (Marshall-Child Method, Campbell-Leatherland Method, and percent water content) all scored lower in comparison to models containing fat pad weights, as did models derived using only external measures from live birds (see Table 4).

Six different models were derived to estimate total carcass protein (Table 5). Model 8 had the highest accuracy for predicting total carcass protein, which included body mass, breast muscle mass, gizzard mass and

Table 5. Models derived using backward stepwise multiple regressions to estimate total carcass protein (TCP) of common eiders during the non-breeding season. Models were ranked according to their root mean square error of prediction (RMSE; see 'Model evaluation'). All models originally included the variables sex and age.

Original variables used to predict TCP	Model	Model	Adjusted R ²	RMSE	Rank
All variables [†]	8	= 73.92 +0.08 BM +0.82 BST +0.52 GIZ -1.06 ABF	0.74	11.14	1
Body mass, body size [‡]	9	= -197.78 +0.10 BM +3.25 HD	0.71	13.27	3
Body size, locomotory muscles [§]	10	= 130.15 +20.22 SEX +0.79 BST +1.59 LGM	0.63	14.15	4
Breast muscle	11	= 166.28 +19.74 SEX +1.32 BST	0.60	14.87	5
Leg muscle	12	= 146.71 +24.72 SEX +2.99 LGM	0.59	15.03	6
Body mass	13	= 158.30 +16.72 SEX +0.11 BM	0.67	13.20	2
Campbell-Leatherland				15.21	7

[†] All variables = body mass (BM), right breast (BST), right leg muscles (LGM), gizzard (GIZ), heart (HRT), right leg fat pad (LGF), abdominal fat pad (ABF), tarso-metatarsus bone (TAR), flattened wing (WG) and head-bill (HD).

[‡] Body size = TAR, WG and HD.

[§] Locomotory muscles = BST and LGM.

abdominal fat pad mass (see Table 5). Models using only variables easily measured on living birds, i.e. models 9 and 13, scored higher than models that included only muscle groups (see Table 5). The Campbell-Leatherland Method ranked low when compared to derived models (see Table 5).

Discussion

The primary goal of our study was to derive models using relatively easily measured data to estimate carcass composition of common eiders. However, we found poor fit between simple external measurements of eiders and actual carcass composition, in particular lipid content. We did, however, find that it is possible to accurately estimate the carcass composition of northern common eiders through dissection using models derived from proximate carcass analyses. Among this sample of common eider ducks, Model 1 most accurately estimated total carcass lipid (Adjusted $R^2 = 0.93$, RMSE = 14.60). Variables in Model 1 that significantly contributed to the equation included body mass, leg fat pad mass, abdominal fat pad mass, and flattened wing length; all parameters easily quantified through dissection. Total carcass protein was most accurately estimated by Model 8 which included the independent variables of body mass, breast muscles mass, gizzard mass and abdominal fat pad mass (Adjusted $R^2 = 0.74$, RMSE = 11.14).

Both models 1 and 8 were derived by entering all 12 measured variables into the regression analysis and removing those that did not contribute significantly to explaining the variance observed in carcass composition. Recall that the goal of our study was to develop models to estimate carcass composition and not to make any inferences about biological relationships between variables. Therefore, it was appropriate to include variables that did not appear obviously related to the dependent variable because they may add significantly to the model for unknown reasons (e.g. abdominal fat pad mass explaining some of the observed variation in total carcass protein).

Despite their strong predictive power, we caution that the specific models developed here should be used only to estimate the composition of common eiders that fall within the range of the data used to derive the equation (Zar 1999). Models derived for one population may not be appropriate for others of the same or similar species (Castro & Myers 1990, Sparling et al. 1992). Therefore, the models developed in our study should be applied to northern common eiders that weigh 1,542-2,602 g (the range of body masses included in this study) and only

to non-breeding birds. Eiders undergo large physiological changes during the breeding season, which could influence the accuracy of the models. However, the results of our study have several general implications concerning the methods used to study endogenous reserves of other bird species, for example the use of fat pad mass as an index of body condition.

The abdominal fat pad and leg fat pads from dissected eiders were good indices of body condition for several reasons. First, fat pads consist primarily of lipids that are available as an energy source (i.e. triglycerides) rather than structural lipids (e.g. phospholipids). Second, they can be easily removed without causing much damage to the carcass, and this might increase the participation of hunters offering their take for research purposes. Finally, both abdominal and leg fat pad were highly correlated with total carcass lipid (see Table 3). Abdominal fat pad has been found to be highly related to total carcass lipids in other species of waterfowl (red-billed teal *Anas erythrorhyncha*: Woodall 1978; Canada geese *Branta canadensis* and lesser snow geese *Chen caerulescens caerulescens*: Thomas et al. 1983; ring-necked ducks *Aythya collaris*: Hohman & Taylor 1986; northern pintails *Anas acuta*: Miller 1989). However, leg fat pad is often overlooked as a possible index of body condition, even though it was also strongly related to total carcass lipids in our study (see Table 3). The leg fat pad is more easily removed than abdominal fat because it is well-defined and not entwined in the internal mesentery or organs.

Models that incorporated external structural measures and body mass predicted carcass protein more accurately than they did carcass lipid content (RMSE for protein ranged within 13.20-13.27 and adjusted R^2 ranged within 0.67-0.71, while RMSE for lipids ranged within 32.74-38.93 and adjusted R^2 ranged within 0.54-0.61). This has also been found in other waterfowl studies (maned ducks *Chenonetta jubata*: Briggs 1989; northern pintails: Miller 1989; mallards *Anas platyrhynchos*: Boos et al. 2000).

We found that it was not possible to accurately predict total carcass lipids from percent carcass water (Adjusted $R^2 = 0.48$, RMSE = 33.10). Unlike Miller (1989) and Johnson et al. (1985), we found that the Child-Marshall and Campbell-Leatherland models did not accurately predict carcass composition, nor did percent water content. Many of the eiders examined in our study had drowned in fishing nets (N = 52). Even though we could account for water held in feathers, we were not able to account for any water contained in their lungs and air sacs. We speculated that this additional water contributed to the inaccuracy of the models predicting carcass

lipids from carcass water among our sample of drowned eiders. However, this does not appear to be the case. Independently, we ran the Child-Marshall and Campbell-Leatherland models on a sample of birds that had been shot, and the new RMSE values were even greater than those calculated from drowned and shot birds combined (58.18 compared to 44.57 for the Child-Marshall Model; 45.67 compared to 31.67 for the Campbell-Leatherland Model).

In conclusion, methods using only measures of structural size and body mass were insufficient to predict lipid content in northern eiders. In contrast, the models derived from measures of fat depots and muscle groups attained through simple dissection gave accurate estimates of both lipid and protein content which would be sufficient for detailed studies of avian energetics. We suggest that these methods should be considered to avoid the time and significant costs associated with proximate carcass analyses (i.e. protein, lipid and ash) of all birds within a sample.

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