Travels in a changing world flexibility and constraints in migration and breeding of the barnacle goose

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Chapter 2

Evaluation of the deuterium dilution method to estimate body composition in the barnacle goose: accuracy and minimum equilibration time

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Abstract

We examined body composition in barnacle geese (*Branta leucopsis*) by proximate carcass analysis and by deuterium isotope dilution. We studied the effect of isotope equilibration time on the accuracy of total body water (TBW) estimates and evaluated models to predict fat-free mass (FFM) and fat mass (FM) from different measurements varying in their level of invasiveness.

Deuterium enrichment determined at 45, 90 and 180 min after isotope injection did not differ significantly. At all sampling intervals isotope dilution spaces (TBWd) consistently overestimated body water determined by carcass analysis (TBWc). However, variance in the deviation from actual TBW was higher at the 45 min sampling interval, whereas variability was the same at 90 min and 180 min, indicating that 90 min is sufficient time to allow for adequate equilibration. At 90 min equilibration time, deuterium isotope dilution overestimated TBWc by 7.1% ± 2.6% ($P < 0.001$, paired t-test, n = 20). This overestimate was consistent over the range of TBW studied and TBWc could thus be predicted from TBWd ($r^2 = 0.976$, $P < 0.001$).

Variation in TBWc and TBWd explained, respectively, 99% and 98% of the variation in FFM. FM could be predicted with a relative error of ca. 10% from TBW estimates in combination with body mass (BM). In contrast, BM and external body measurements allowed only poor prediction. Abdominal fat fresh mass was highly correlated to total FM and, if the carcass is available, allows simple means of fat prediction without the necessity to dissect the entire specimen.

Introduction

Somatic stores are a key factor in defining an animal's body condition, and much of the variation in survival and reproduction has been attributed to the optimisation of body reserves (Blem 1990; Carey 1996a; Houston et al. 2007). Because of the central importance of energy and nutrient storage, their assessment has become an increasingly important aspect in current research (Brown 1996; Stevenson and Woods 2006). Birds in particular face the energetic dilemma of high energy expenditure for activity and maintenance and limitations of storage abilities due to their aerial lifestyle (McNab 2002).

A variety of methods have been applied to assess fat mass (FM) and fat-free mass (FFM) in vivo and vitro (overviews in Blem 1990; Brown 1996; Gessaman 1999; Speakman 2001; Stevenson and Woods 2006). These methods differ in accuracy to measure the variable of interest and in the degree of invasiveness for the study animal. Although proximate body composition analysis is regarded as the most accurate method, and the standard against which all other methods are evaluated, it obviously represents the most invasive, i.e. lethal, avenue. Additionally, it is a labour- and time-intensive method. From such carcass analyses researchers have recognised that an animal's body water content represents a fairly stable proportion of the FFM because lipids are stored nearly free of water (Pace and Rathbun 1945; Odum et al. 1964; Ellis and Jehl 1991). While some animals can experience large changes in body mass and composition, particularly during extensive periods of fasting, the relative water content in the fat-free mass (H_2O_{FFM}) seems not significantly affected (Groscolas et al. 1991; Cherel et al. 1992). Consequently, estimating total body water (TBW) content enables prediction of FFM and subsequently, in combination with total body mass (BM), FM. Indeed, many studies used body water content estimates to successfully predict body stores (Campbell and Leatherland 1980; Miller 1989; overview table VI in Blem 1990; Boos et al. 2000; but see Jamieson et al. 2006). Using calibrated regressions with dissectible fat depots, like abdominal and leg fat pads, represents another method to estimate total FM in carcasses without the necessity to analyse the entire specimen (Thomas et al. 1983; Piersma 1984; Boos et al. 2000; Jamieson et al. 2006).

The refinement of isotope analysis techniques has yielded non-destructive means to estimate TBW by the principle of isotope dilution, allowing longitudinal studies and work where killing the animal is not an option. This method relies on releasing isotopically labelled water molecules into the body water pool and after mixing determining isotope concentrations in body fluids (usually blood) of a single timed sample ('plateau approach') or a series of samples ('intercept approach') to derive an estimate of TBW (Speakman et al. 2001). Because it involves only one sampling event and a shorter experimental period the 'plateau approach' is less invasive for the animal. Additionally, a single sample reduces costs for material and analysis. However, using the 'plateau approach' appropriate timing of the single sample is important, i.e. after mixing of the marker with the body water is completed and before washout of the marker becomes effective. To reduce the latter,

captive animals are deprived of food and water during the equilibrium period. The time a wild animal is held in captivity can crucially affect its performance, in particular during the breeding period when parental care for a clutch or brood has to be provided. Our general research goal is to employ isotope dilution to assess body composition of incubating barnacle geese (*Branta leucopsis*) in the field. To this end, we want to reduce the equilibrium time without affecting data quality. Furthermore, it is known that the isotope-dilution method generally overestimates the actual TBW volume, because part of the labelled atoms exchange with non-aqueous body constituents (Culebras and Moore 1977; Schoeller et al. 1980). A review of studies employing hydrogen isotopes in four bird species revealed a considerable variation by which the dilution space differed from actual TBW ranging from underestimates of 2.3% to overestimates of 18% (Table 3 in Speakman et al. 2001). As has been noted frequently (Gessaman 1999; Shaffer et al. 2006; Mata et al. 2006) there is clearly a need for more bird studies evaluating estimates of TBW and other body components measured by isotope dilution against standard proximate body composition analysis.

In the present study on captive barnacle geese, we compared estimates of TBW, FFM and FM measured by proximate carcass analysis and by deuterium isotope dilution. Our specific objectives were to: (1) assess how equilibration time may compromise accuracy of the dilution method; (2) determine the level of overestimation of TBW by deuterium dilution; (3) evaluate the accuracy of predictions of FFM and FM from TBW and/or other predictor variables (BM, external morphological measurements, abdominal fat) depending on lethal and non-lethal approaches.

Methods

Animals and experimental setup

Animals were taken from a stock of barnacle geese kept at the Biological Centre of the University of Groningen in Haren, The Netherlands. Geese were kept on grassland while receiving *ad libitum* supplementary food (a mixture of grain and pellets). A total of 21 adult birds (≥ 2 years old), consisting of 10 females and 11 males, were selected to achieve maximal range in body condition; the body condition criterion was residual body mass from a regression of body mass on the first principal component from a factor analysis including tarsus and total head length. To further increase the variation in body condition a sub-sample of 2 females and 2 males were kept separately from the stock on grassland of lower food quality and with only limited supplementary food for 2 weeks prior to the experiment. Their average mass loss during this period was 210 ± 72.5 SD g. All animals were used in the carcass analysis, but only 10 of the 11 males for the isotope dilution space measurements, because of leakage of the isotope mixture through the puncture hole of the thin skin in 1 male. Birds were sexed by cloacal inspection and confirmed by examination of gonads during dissection. Most birds (16) were collected February to mid March 2006, the others (4 males, 1 female) on 21 April 2005.

To standardise treatment, animals were put in bird cages with no access to food but access to drinking water on the evening before the isotope-dilution experiment. The next morning (ca. 15 hours later), 2 hours before administration of the isotope solution, drinking water was removed until the end of the experiment, 4–6 hours later. The birds were intraperitoneal injected with a 99.9% deuterium isotope solution (Sigma Chemicals) using 1.0 ml insulin syringes. The actual mass of each volume injected was determined by weighing the syringe before and after injection on an analytical balance (Mettler AG204) to the nearest 0.1 mg. Average dose mass was 1.1153 ± 0.0021 SD g (n = 20). Blood samples were collected from 9 females and 7 males at each of the following sampling times: 45, 90 and 180 min after injection. Additionally, 1 female bird and 3 males were sampled at 90 min. To estimate deuterium background levels, blood samples prior to isotope administration were taken from 3 female and 3 male birds. Blood was collected from the brachial and intertarsal veins and stored in flame sealed micro-capillaries. After the last blood sample was taken, birds were fully anesthetized with 3 ml intraperitoneal injected Nembutal (natriumpentobarbital 60 mg/ml), followed by cervical dislocation 10–15 min later. Body mass was then measured to the nearest 1 g and carcasses were placed in plastic bags and refrigerated until being further processed next day or double-packed and frozen at –20°C until dissection and body composition analysis.

Daily care and management of the animals, as well as the experimental protocol was approved by the animal experimentation committee of the University of Groningen, license DEC 4081B.

Isotope analyses

The blood in the capillary tube was distilled in a vacuum line where water vapor was cryogenically trapped in a quartz tube using liquid nitrogen. After complete transfer, the vacuum system was brought to room pressure by admitting dry nitrogen. The insert was then quickly brought into a standard vial for automatic injection and sealed with a septum. During the sample preparation, internal water standards (gravimetrically prepared from pure deuterated water and also stored in flame-sealed capillaries) covering the entire enrichment range of the blood samples were distilled alongside the samples. This way, possible systematic effects on isotope enrichment due to the distillation process were accounted for. Such effects were also monitored in every batch by comparing the distilled standard waters with the same waters that were introduced into the vials directly. The actual δ^2H measurements were performed in automatic batches using a Hekatech High Temperature Pyrolysis unit (Gehre et al. 2004) in which the injected water reacted with the glassy carbon available in the reactor according to: $H_2O + C \rightarrow H_2 + CO$. The H_2 and CO gas, emerging into a continuous He flow through the system, were then led through a GC column to separate the two gases in time, and fed into a GVI Isoprime Isotope Ratio Mass Spectrometer for the actual isotope analysis. For the analysis of δ^2H (from the H_2 gas emerging first from the GC column) every sample was injected typically 6 times from the same vial into the furnace in 0.2 μl quantities. Memory effects of the HTP oven were corrected for using a memory correction

algorithm similar to the one described by Olsen et al. (2006). In the complete analysis scheme, several quality checks were incorporated. The isotope scales were calibrated using multiple distilled samples of two of the standard waters (being at the lower and higher end of the sample range, respectively), whereas the measured δ^2H values for a third standard, representing the expected midrange of blood samples, were used as quality "target" and had to be measured within 1% of its assigned values in order to meet the quality criterion for the batch. All sample analyses were run at least in duplicate, more times if values differed by more than 2.5%, and we used the average of values differing from each other by less than 2.5%.

Measurement of the hydrogen dilution space (TBWd)

Using the plateau approach (Speakman 1997) and employing equation (1) the hydrogen dilution space (TBWd) was calculated by taking into account the quantity of the dose (Q_d, mol), the ^2H concentration of the dose (C_d, atom %), the ^2H background concentration (C_b, atom %), and the ^2H concentration of individual blood samples (C_i, atom %) taken at the various sampling intervals.

$$TBWd = 18.02 \cdot Q_d \cdot (C_d – C_i) / (C_i – C_b) \quad \text{(Eqn. 1)}$$

Background levels of δ^2H measured in six birds prior to dose administration averaged 4.11‰ with a range of 33.01‰, which represented only 0.60% of the average measured deuterium enrichment after dose injection (5532‰, n=52). Therefore, we applied this average background value for all birds.

Dissection and body composition analysis

Fresh or thawed carcasses were weighed, all feathers plucked and re-weighed, the difference being plumage fresh mass. All skin was removed together with associated subcutaneous fat. The following organs were dissected out, weighed and analysed for water and fat content: left flight muscle complex (*pectoralis* and *supracoracoideus*), left leg musculature (attached to the *tibiotarsus* and *femur*), abdominal fat (a discrete deposit in the abdominal cavity, excluding mesenteric fat adhering to the intestines), gizzard, intestines (incl. mesenteric fat and *caeca*), heart, liver, spleen and kidneys. Prior to analysis the oesophagus, gizzard and intestines were emptied and reweighed. The total wet content excised from these organs was 20 ± 9 SD g. The right flight and right leg musculature were excised and retained for other work and their contributions to dry and fat free masses were estimated via the equivalent masses of their left counterparts. Organs were cut into small pieces of ca. 1 cm^3 and bones of the skeleton were broken to expose marrow and brain before oven-dried at 60°C until constant mass (7–15 days). Total body water from the carcass analyses (TBWc) was calculated as the carcass fresh mass after plucking minus the sum of all dried tissues, thereby accounting for general water loss during dissection. Thus water absorbed by feathers is intentionally not included in TBWc because it is not part of the body water pool into the isotope marker can be diluted. Lipids were

extracted from the tissues with a soxhlet apparatus using petroleum ether as solvent. We refer to whole body fat-free mass (FFM) as total wet lean mass, including feathers and skeleton, calculated from fresh body mass (BM) minus extractable fat mass (FM).

Calculations and statistics

Statistical analyses were performed with SPSS 14. All results are reported as mean ± 1 SD, unless stated otherwise, and were considered to be significant at $P < 0.05$. For all parametric tests assumption of normality and homogeneity of variances were evaluated using the Kolmogorov-Smirnov test and Levene's test, respectively (Zar 1999). As a measure of structural size we derived scores of the first principal component (PC1) from a factor analyses based on four external measurements: the length of tarsus, total head (i.e., including bill), maximum wing chord and keel (measured from the anterior tip of the *carina* to the end of the *sternum*, at the transition with the abdominal cavity). Tarsus was measured with callipers to the nearest 0.1 mm; all other variables were measured with a ruler at 1 mm accuracy. Variables had a similar factor loading (0.72 - 0.94) on PC1, which explained 73% of the total variance. We used ANOVA to test for mean differences among sex, and GLM, with PC1 included as covariate.

Differences in estimated dilution space with time after isotope injection were compared by a repeated measure ANOVA. Two-tailed paired t-tests were used to compare means of isotope dilution space (TBWd) and body water based on carcass analysis (TBWc). We used estimated TBW and other predictor variables (see below) to predict FM and FFM by two approaches: (A) multiple regression analyses and (B) assuming a constant FFM hydration.

A) We applied a stepwise backward elimination procedure in the multiple regression analyses. Starting with the non-destructively obtained variables tarsus, head, wing, keel, BM and sex as basic model, we extended the set of predictor variables and included either TBWd and dry BM or TBWc and dry BM to investigate if FM predictions could be improved. Finally, we took the set of predictor variables from the basic model and included additionally abdominal fat fresh mass (i.e., of the dissected fat pad, not chemically extracted fat). Before we included abdominal fat in multiple regressions, we tested if a curvilinear relationship between FM and abdominal fat would be more appropriate than a linear fit (Piersma 1984). Although the coefficient of determination increased slightly from $r^2 = 0.90$ to $r^2 = 0.92$, adding a quadratic term did not significantly improve a linear fit of FM to abdominal fat ($P = 0.06$).

We used double cross-validation to evaluate the robustness and replicability of regression equations following the procedure described by Guan et al. (2004). Briefly, subjects from the original data set were randomly assigned to two groups, equal or similar in number and sex-ratio of subjects. The statistically significant predictor variables derived from the original full data set were applied in both subgroups to develop predictive equations and derive coefficients of determination (r_{11}^2 and r_{22}^2; first subscript number refers to subsample's data and second

subscript number to subsample's regression coefficients). Standardised regression coefficients and Z scores of predictor variables and of the response variable were used in all cross-validation procedures. The predictors' regression coefficients were crossed over the two subsamples to produce equations and coefficients of determination (r_{12}^2 and r_{21}^2) from actual group data using the regression coefficients from the other group for the predictions. Using this double cross procedure two shrinkage values were calculated: $r_{11}^2 - r_{12}^2$ and $r_{22}^2 - r_{21}^2$. The more closely the shrinkage estimate approaches zero, the greater the degree of stability across subsamples. Furthermore, two invariance coefficients were derived by correlating the predicted values of subsample 1 with the predicted values of subsample 1 using the regression coefficients of subsample 2 ($r_{11–12}$) and *vice versa* ($r_{22–21}$). As these invariance coefficients approach one, more confidence can be obtained in the replicability of the results.

B) As alternative to calibrated regression equations and assuming a constant water content in the FFM (H_2O_{FFM} = TBW:FFM = constant) the individual FFM and FM can be inferred from following equations:

$$FFM = \frac{TBW}{H_2O_{FFM}} \quad \text{(Eqn. 2)}$$

$$FM = BM - FFM \quad \text{(Eqn. 3)}$$

We will refer to this approach as the Pace and Rathbun (1945) approach (*sensu* Mata et al. 2006).

Results

Body composition by carcass analysis

Carcass analyses are listed in Table 2.1. Animals in this study covered a broad scale of body mass and composition, ranging twofold in body mass and from 2% to 25% in lipid content. Males were larger than females according to PC1, the first principal component from a factor analysis including tarsus, wing, skull and keel length ($F_{1,19} = 31.18$, $P < 0.001$). Significant differences between sexes were also found for body mass, TBW and FFM. However, these were caused by the sex-related differences in structural size. Thus, when sex was tested together with PC1 in one analysis to explain differences in BM, TBW and FFM, only PC1 explained a significant part ($P < 0.05$ in all models) whereas variation due to sex became non-significant. Females in our sample tended to have higher fat loads (FM:BM, $F_{1,19} = 3.39$, $P = 0.08$).

TBW measured by deuterium dilution and the effect of equilibration time

Within-individual variation in deuterium isotope enrichment occurred mainly between the first two sampling events and was independent of the size of the indi-

Table 2.1. Whole body composition by carcass analysis. Given are body mass (BM), total body water (TBWc), fat-free mass (FFM), fat mass (FM) and fractions (%) of water and fat content for sexes combined and males and females separately.

	Total	Males		Females	
	n=21	n=11	Range	n=10	Range
BM (g)	1,995 ± 241*	2,104 ± 183	1,893-2,515	1,876 ± 248	1,479-2,185
TBWc (g)	1,121 ± 152*	1,213 ± 112	1,079-1,457	1,020 ± 125	799-1,209
FFM (g)	1,773 ± 224*	1,907 ± 159	1,729-2,273	1,625 ± 193	1,263-1,901
FM (g)	222.5 ± 110.7	196.4 ± 99.0	37.1-330.6	251.2 ± 120.9	95.6-539.6
TBWc:BM (%)	56.2 ± 3.9 [a]	57.7 ± 3.7	52.9-62.9	54.6 ± 3.5	46.9-59.3
TBWc:FFM (%)	63.2 ± 1.1 [a]	63.5 ± 1.3	61.8-65.4	62.8 ± .8	61.1-63.8
FM:BM (%)	11.1 ± 5.1	9.2 ± 4.5	2.0-15.3	13.1 ± 5.2	6.5-24.7

* Significant differences between sexes ($P < 0.05$). [a] Including fresh plumage mass in BM; if plumage is excluded from BM, the fractions are TBWc:BM = 60.6 ± 4.1% and TBWc:FFM = 68.8 ± .8%.

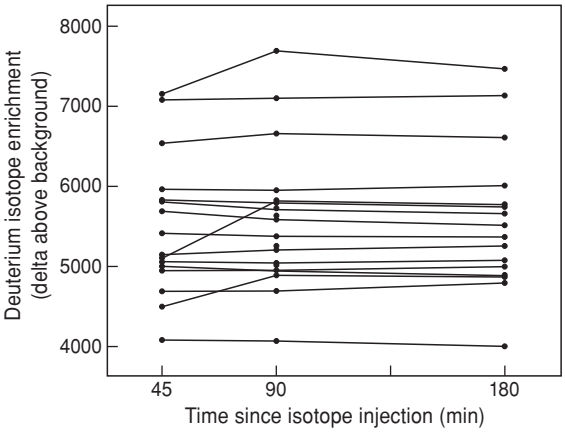

Figure 2.1. Deuterium isotope enrichment determined for 16 animals sampled at 45 min, 90 min and 180 min after isotope injection plus another 4 animals sampled at 90 min after dose administration.

vidual body water pool (Figure 2.1). However, a pronounced increase over this period was restricted to a few individuals only, and overall changes between sampling times were too low to be significant (repeated measures ANOVA, $F_{2,30} = 1.894$, $P = 0.168$).

At all sampling intervals carcass body water was significantly overestimated by the deuterium dilution space ($P < 0.001$, paired *t*-test), ranging from average values of 9.2% at 45 min to 7.1% at 90 min (Table 2.2). This overestimate was consistent over the range of TBWc studied ($P > 0.05$ for all regression models at the various

Table 2.2. Deuterium dilution space (TBWd) measured at different sampling intervals and in relation to total body water from carcass analysis (TBWc). Data present means ± SD. Further are given coefficients of determination (r^2) together with the standard errors of the estimates (SEE, i.e. the root mean square errors) from linear regressions of TBWc vs. TBWd ($P < 0.001$ for all regressions).

	45 min	90 min		180 min
	n=16	n=16	n=20*	n=16
TBWd (g)	1,198 ± 185	1,179 ± 187	1,190 ± 169	1,182 ± 185
TBWd:TBWc	1.092 ± .054	1.073 ± .027	1.071 ± .026	1.076 ± .024
$r^2_{TBWc–TBWd}$ (SEE in g)	.90 (51)	.98 (22)	.98 (23)	.98 (20)

* Includes four birds which were not measured at 45 and 180 min.

sampling times). The accuracy to predict TBW from deuterium dilution, was on average slightly better at 90 and 180 min than at 45 min but this difference was not significant (repeated measures ANOVA, $F_{2,30} = 2.130$, $P = 0.136$). Variance of the ratio of TBWd:TBWc was significantly different between sampling events (Levene test: $F_{2,49} = 4.473$, $P = 0.016$). The error in the deviation of TBWd from TBWc was twice as high at 45 min compared to 90 and 180 min after dose administration (Table 2.2) leading to a reduced precision of TBW predictions from isotope measurements at this early sampling stage. The following regression equations were derived to predict TBWc from TBWd:

at 90 min (n = 20): $TBWc = 96.034 + 0.852 \cdot TBWd$ (Eqn. 4)
at 180 min (n=16): $TBWc = 94.544 + 0.848 \cdot TBWd.$ (Eqn. 5)

Further on, when using deuterium dilution space as predictor variable to estimate body composition, we employed TBWd values measured at 90 min.

Estimates of body composition by lethal and non-lethal methods

TBW determined either from carcass analysis or isotope dilution was a very strong single predictor for FFM explaining 98-99% of the variation in FFM (Figure 2.2). Table 2.3 compares the outcome of multiple regression analyses using TBW measured by deuterium isotope dilution and carcass analysis and further predictors related to different levels of invasiveness. The errors in the prediction of FM and FFM (calculated as deviations of predicted from observed values) following the Pace and Rathbun (1945) approach are also listed in Table 2.3. For the latter approach we applied individual estimates of TBW from the established relationship between TBWc and TBWd (measured at 90 min equilibrium time) and a H_2O_{FFM} of 63.2% (i.e., the average from our sample). We presented only predictive equations for FM because the absolute error of prediction was the same for FM and FFM, regardless if the one or other component was taken as response variable. This was due to same

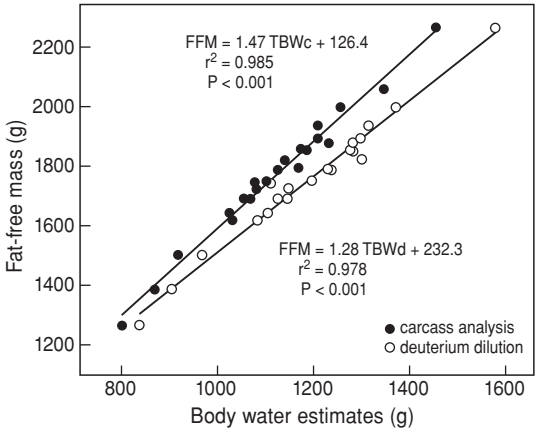

Figure 2.2. Relationships between fat-free mass and body water determined by carcass analysis (closed circles, n = 21) and deuterium dilution (open circles, n = 20).

significant predictors (model 1 to 3) for both FM and FFM and the fact that they add up exactly to BM. Thus FFM was calculated as FFM = BM – FM. Also, we were mainly interested in the variation of FM (the relatively smaller of both components) and to what extent it can be accounted for by the various regression models.

Much of the variation in FM was unaccounted for by model (1) based on body mass and external body measurements. Furthermore, relatively strong r^2 shrinkage and low invariance coefficients indicate lower replicability of the prediction when applied to different sub-samples compared to the other models. FM was not significantly related to any of the potential predictors offered to model (1) separately. Only the combination of BM and a structural measurement (keel) revealed a significant relationship with FM. The combined variables dry BM (i.e. BM – TBW) and TBW determined from isotope dilution (model 2) or carcass analysis (model 3) both explained a large part of the variation in FM (92% and 97%, respectively). Moreover, the results from a cross-validation showed good replicability of the respective equations. When dry BM was replaced by BM in models (2) and (3) errors of prediction and r^2 were the same. However, such alternative models suffered notably from multicollinearity of the predictors (see VIF in Table 2.3) making them less robust. Abdominal fat fresh mass was highly correlated to total FM and, if the single predictor in the model, accounted for 90% of the variation in FM. The variation increased slightly to 93% when head length was added to this model.

Table 2.3. Predictive equations for total fat mass (FM in g) based on non-lethal and lethal measurements in 21 barnacle geese (n=20 if TBWd is used in the equation). Modelling started with non-invasively measurable predictors in model no. 1: tarsus, head, wing, keel, BM and sex. This set of potential predictors was expanded by TBWd, dryBM (i.e. BM - TBWd) and TBWc, dryBM (i.e. BM - TBWc) in model no. 2 and 3, respectively. For model no. 4 the same predictors as for model 1 were applied with addition of abdominal fat fresh mass F_{abd}. Shown are the final models containing only significant predictor variables derived by stepwise backward multiple regressions. $P < 0.001$ for all models except no. 1, $P = 0.008$. Summary statistics include coefficients of determination (r^2), standard error of the estimate (SEE), absolute and relative error of predicted FM and FFM calculated as deviations of predicted from observed values (mean ± SD), variance inflation factors of predictors (VIF) and statistics from a cross-validation procedure (see methods for details). FFM was calculated as BM – FM (see also text). Outcomes of the Pace and Rathbun (1945) approach are given in the last row (n=20).

			Absolute error FM and FFM	Error FFM	Error FM	VIF	Shrinkage		Invariance coefficients	
	r^2	SEE	(g)	(%)	(%)		$r_{11}^2–r_{12}^2$	$r_{22}^2–r_{21}^2$	$r_{11–12}$	$r_{22–21}$
Multiple regression models										
Non-lethal										
1) FM = 368.7+ 0.329·BM - 6.997·Keel	0.418	89.1	69.9 ± 44.8	4.0 ± 2.8	37.3 ± 24.9	1.42	0.19	0.11	0.84	0.74
2) FM = -200.6 - 0.266·TBWd + 0.936·dryBM	0.918	33.7	21.3 ± 23.3	1.2 ± 1.4	10.1 ± 10.1	1.15[a]	0.02	0.01	0.99	0.99
Lethal										
3) FM = -57.48 - 0.418·TBWc + 0.856·dryBM	0.966	21.6	16.0 ± 12.3	0.9 ± 0.8	10.6 ± 11.4	1.18[b]	0.14	0.12	0.93	0.94
4) FM = -382.7 + 4.492·F_{abd} + 5.223·Head	0.931	30.1	24.9 ± 13.8	1.4 ± 0.8	16.0 ± 18.4	1.01	0.01	0.01	1.00	1.00
Pace and Rathbun (1945) approach			22.1 ± 26.0	1.3 ± 1.5	12.7 ± 17.1					

[a] When dryBM is replaced by BM in the model VIF = 4.51.
[b] When dryBM is replaced by BM in the model VIF = 3.68.

Discussion

We found a strong relationship between TBW measured by carcass desiccation and by deuterium isotope dilution in barnacle geese, which was consistent over a large range in body composition. With knowledge of this relationship and of the minimum equilibrium time needed for the isotope dose to mix completely with the body water pool, TBW can be very accurately predicted by deuterium isotope dilution as non-lethal method. Furthermore, an accurate estimate of TBW was a strong predictor of FFM and, in combination with BM, FM. However, the accuracy of the estimates of FFM and FM for additional samples on the basis of TBW measured isotopically or by desiccation largely depends on the variation of the relative water content in the FFM.

Body composition by carcass analysis
The dissected barnacle geese showed pronounced sexual differences in structural size and, related to this, differences in BM, FFM and TBW. Higher fat loads in females may be expected in geese, in particular during the pre-breeding phase, in anticipation of egg production and incubation (Raveling 1979).

Fat-free mass hydration in birds - how variable is it?
Whenever total body water, or an estimate thereof, is used to predict FFM, the variation of H_2O_{FFM} is of crucial relevance, because it comprises the other major error source, beside the error of the TBW estimate. Wang et al. (1999) reviewed this issue for adult mammals, and concluded that species share a relatively constant H_2O_{FFM}, in spite of differences in body mass ranging by a factor of 10^4. The relatively higher water content in the lean body component of the young growing organism until it reaches chemical maturity is well known (e.g., Arnould et al. 1996 for mammals; Bech and Østnes 1999 for birds), and thus we will restrict ourselves to mature birds in the following discussion.

When comparing results of H_2O_{FFM} among studies first of all attention has to be paid to possible differences in methodologies and definitions applied in these studies. Researchers often include water adsorbed to the feathers in the amount of TBW, which may amount to ca. 1-2.5% of TBW (Crum et al. 1985; Mata et al. 2006). We intentionally did not so because this 'external water' is not in exchange with the body water pool estimated by isotope dilution (Crum et al. 1985). Further, FFM may be differently defined among studies, excluding, for instance, plumage (e.g., Boos et al. 2000; Mata et al. 2006) or bones and plumage (e.g., Groscolas et al. 1991) from the FFM component. Consequently, estimates of H_2O_{FFM} from those studies (71% – 73%) are necessarily considerable higher than the value reported here.

In Table 2.4 we compiled data on H_2O_{FFM} reported or calculated from studies of waterfowl carcass analyses using comparable methods and definitions as applied in the present study. H_2O_{FFM} can be reasonably stable over different seasonal and/or physiological stages within a given study. As in our study no sex-related differences are indicated. Variation between studies can be, however, considerable.

Table 2.4. Data on H_2O_{FFM} in adult waterfowl. In most cases H_2O_{FFM} was calculated from mean values of BM, FM and TBW (except: from individual values in Korte (1988); Campbell and Leatherland (1980) gave mean values directly). We used only samples (means) where the same birds were analysed for all of those body components; n=sample size, se=standard error.

species / source	period of collection	H2OFFM (%)			
Lesser Snow Goose (*Anser c. caerulescens*) (Campbell and Leatherland 1980)	through the year	69.3 (se=0.06)[a] (sexes pooled)			
		Females	n	Males	n
Barnacle Goose (this study)	winter - spring	62.8 (se=0.2)	10	63.5 (se=0.4)	11
Brent Goose (*Branta bernicla*) (Korte 1988)	winter - spring	69.0 (se=0.6)	14	68.4 (se=0.5)	21
Canada Goose	autumn migration	65.2	6	63.5	9
(*Branta canadensis*)	midwinter	62.6	5	62.9	10
(Raveling 1979)	spring migration	65.7	11	65.8	5
	pre-lay	63.4	4	65.4	5
	mid-incubation	65.4	2	66.5	1
	hatch day	66.1	9	65.2	6
	early moult	68.7	8	68.1	9
	mid-moult	67.5	2		
	mean	65.6		65.3	
Common Eider	2-3 weeks pre-lay	63.6	4		
(*Somateria mollissima*)	pre-lay breeders	64.4	7		
(Parker and Holm 1990)	pre-lay non-breeders	66.5	2		
	post-lay	63.9	7		
	hatch day	64.6	8		
	mean	64.6			
Black Duck	pre-lay	64.5	4		
(*Anas rubripes*)	laying	64.3	12		
(Reinecke et al. 1982)	post-lay	65.0	16		
	moult	63.8	3		
	autumn	63.2	7		
	winter	60.3	11		
	mean	63.5			
Lesser Scaup	pre-moult	73.3	21		
(*Aythya affinis*)	moult	70.8	24		
(Austin and)	post-moult	71.5	8		
Fredrickson 1987	migratory	70.8	32		
	mean	71.6			
All studies	mean	66.6 (se=1.2)[b]	7		

[a] TBW component included water adsorbed to feathers. Source was unclear about the actual sample size related to the reported mean and standard error of 0.693 ± 0.06. Translated into % this standard error would equal a questionable se = 0.0006%. We suspect 0.06 being the correct value.

[b] Mean of studies' means; for studies where data for both sexes were available the average was used.

Compared to other studies our values (mean = 63.2%) are at the lower range of H_2O_{FFM} values reported so far. Although the geese had access to water during the captive period until 4-6 hours before termination of the experiment, due to general capture stress they may have not made sufficient use of it and experienced a certain degree of dehydration. Birds can tolerate notable water losses under restrained conditions. For instance, Davidson (1984) noted a decrease of H_2O_{FFM} of 0.8-1% per hour over the first four hours after capture in knots (*Calidris canutus*) and dunlins (*C. alpina*), i.e. from 66.7% to 63.5% and from 65.8% to 61.8%, respectively. Interestingly, after the first 4 hours Dunlins appeared to fully compensate further water loss by metabolically produced water, whereas in knots dehydration continued (at lower rate) until 60.9% at 24 h after capture.

We conclude that both methodological factors and tolerance for a (temporarily) negative water balance in birds may contribute to the considerable variation in H_2O_{FFM} reported among studies. Nevertheless, homeostasis is crucial for organismal functioning. Therefore, under unrestrained circumstances birds will probably strive to avoid dehydration and keep their water balance within small margins. Currently, it is not possible to rule out if and to what extent variation in H_2O_{FFM} reflects truly species-specific diversity or varying sampling procedures and other methodological differences. Future applications of the isotope dilution method to estimate body composition would benefit from a better understanding of general patterns of variation in H_2O_{FFM} and how it maybe related to species and/or physiological state.

TBW measured by deuterium dilution and the effect of equilibration time

In the present study TBWd overestimated TBWc by 7.1% (at 90 min equilibrium time) consistently over a large range of TBWc; and can thus be corrected according to the values given here. Speakman et al. (2001, Table 3) calculated an average of 4.7% by which actual TBW was overestimated by hydrogen isotope dilution in birds, based on nine studies on four species. Additional studies using hydrogen isotopes together with the 'plateau approach' reported 8.1% for the chicken *Gallus gallus* (Mata et al. 2006), 8.4% for glaucous-winged gull *Larus glaucescens* nestlings (Hughes et al. 1987) and 3.3% for the glaucous gull *Larus hyperboreus* (Shaffer et al. 2006). However, at least part of the variation among studies is due to methodological factors. For instance, the study of Degen et al. (1981), which was included in the average calculated by Speakman et al. (2001), as well as Hughes et al. (1987) and Shaffer et al. (2006) included water adsorbed to feathers in TBWc. This plumage water cannot be accounted for by isotope dilution. As a consequence, the difference of TBWd to TBWc becomes smaller. Thus, an average value by which carcass TBW is overestimated by the hydrogen dilution space in birds certainly exceeds the 4.7% given by Speakman et al. (2001).

Our results indicate that mixing of marker solution and body water was not completed after 45 min. Average levels and variability were the same at 180 min and 90 min, indicating that 90 min is sufficient time to allow for adequate equilibration. Apparently, compared to an earlier estimate of about 4 hours for this

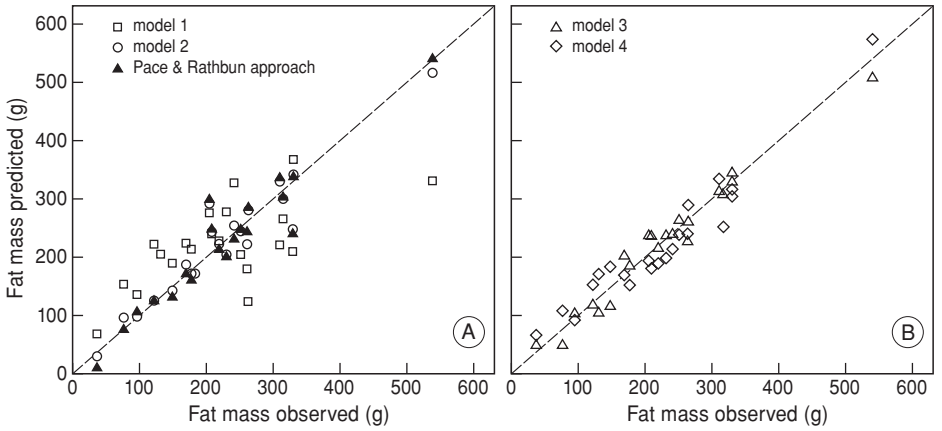

Figure 2.3. Relationships between fat mass determined by carcass analysis and predictions of models presented in Table 2.3. Panel (A) refers to non-lethal methods, panel (B) to lethal methods. The dashed line represents a relationship of Y=X.

species (Nolet et al. 1992) equilibration time can be considerably reduced without compromising accuracy of the TBW estimate, thereby reducing disturbance to the animal.

Estimates of body composition by lethal and non-lethal methods

Regression models to predict body composition based on BM in combination with other external morphological parameters performed poorly in the present study on the barnacle goose (Table 2.3 and Figure 2.3). BM alone was unable to explain a significant part in FM variation. In contrast Skagen et al. (1993) found in two sandpiper species 76% and 89% of the variance in FM explained by BM. There are two main reasons why BM alone may be a weak predictor for FM. First, changes in BM can involve, beside fat, appreciable amounts of protein, and the relative contribution of both components may vary over periods of mass change. Studies on geese provided good examples (Newton 1977; Raveling 1979; Prop and Spaans 2004) where such changes were related to seasonal and physiological stages like migration, reproduction and moult. Second, study subjects usually differ in structural size and such differences are generally unrelated to lipid contents, although some relationship may occur when fat is deposited in the bone marrow, which can reach significant levels in waterfowl (Hutchinson and Owen 1984). Additional incorporation of structural measurements can improve the predictive power of the model but does account for only part of the overall structural size differences. Because FFM includes virtually all structural mass and, at the same time, nearly all water, a measure of TBW accounts much better for differences due to structural mass than most morphological measurements. Indeed, variation in TBW determined from carcass analysis or isotope dilution both explained a large part of the variation in FFM and, consequently, in FM.

Abdominal fat fresh mass was highly correlated to total FM and, if the carcass is available, allows simple means of fat prediction without the necessity to dissect the entire specimen. Model (1) represented the least invasive of the methods tested in Table 2.3. However, the error of prediction was highest, equating to 37% and 4.0% of actual FM and FFM, respectively. Using estimates of TBW from deuterium isotope dilution and either a regression or Pace and Rathbun (1945) approach as alternative non-destructive methods reduced the relative error of the FM and FFM estimate to 10 – 13% and 1.2 – 1.3%, respectively.

Differences in accuracy of the regression and the Pace and Rathbun (1945) approach depend on how much individual animals in the study sample deviate from the assumption of a constant FFM hydration. Variation in H_2O_{FFM} was low in our sample (Table 2.1) resulting in a similar accuracy of both approaches (Table 2.3 and Figure 2.3). Irrespective of which approach is applied, prediction accuracy for a new sample will be reduced if H_2O_{FFM} differs between calibration sample and new sample. A direct measurement of H_2O_{FFM} in a sub-sample of the study population is always advisable to ensure confidence about resulting predictions. If this is not possible, we suggest to apply an average value of 66.6% for studies on adult waterfowl, as calculated from the species-specific studies listed in table 2.4, when using the Pace and Rathbun (1945) approach.

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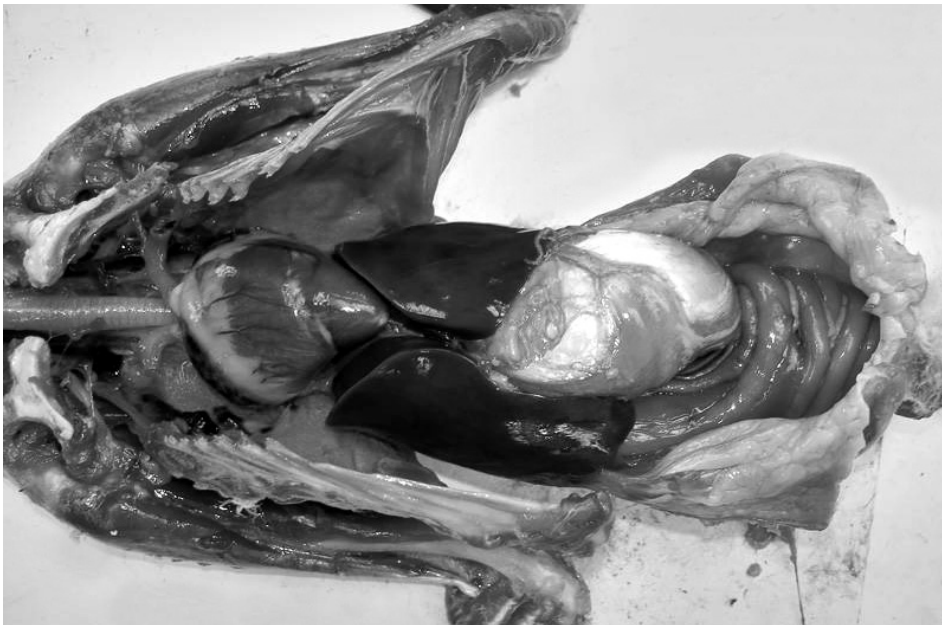

Photo by René Adelerhof.