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AN EVALUATION OF THE METHOD OF ESTIMATING BODY FAT IN BIRDS BY QUANTIFYING VISIBLE SUBCUTANEOUS FAT

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Abstract.—Field ornithologists frequently quantify the fat reserves of small- to medium-sized birds by assigning a numerical rank to visible subcutaneous fat. Despite the significance and widespread application of this method, relatively few studies have tested its underlying assumptions. Using wintering Dark-eyed Juncos, *Junco hyemalis,* the visible fat-class method was evaluated in four ways. (1) The relationship between visible fat class and lipid index (angular transformation of g lipid/g lean dry mass) contained a significant linear term; nonlinear terms were nonsignificant. At least in statistical terms, these fat-class data closely approach interval data. (2) The Y-intercept of the lipid index-fat class relationship was significantly different from zero, indicating the presence of unseen lipid reserves at 0 fat class; the method therefore has limited utility in studies of birds involving severe food limitation. (3) Wintering juncos in southwest Indiana lost 43% of their visible fat overnight, suggesting that fat class methodology can measure short-term changes in body fat of small birds. (4) Important sources of error include inconsistent positioning of the bird during classification of visible fat, mistaking nonfat tissue (e.g., gut) for fat, difficulties with classification of fat in dead specimens, and observer bias.

UNA EVALUACIÓN DEL MÉTODO PARA ESTIMAR LA CANTIDAD DE GRASA EN AVES, MEDIANTE LA CUANTIFICACIÓN DE LA GRASA SUBCUTANEA VISIBLE

Sinopsis.—Los ornitólogos frecuentemente cuantifican la reserva de grasa de aves, de pequeño a mediano tamaño, asignando un rango numérico a la cantidad de grasa subcutánea visible. A pesar de lo significativo y la amplia aplicación de este método, muy pocos estudios han puesto a prueba sus postulados. Utilizando individuos invernales de *Junco hyemalis,* evaluamos el método de la cantidad de grasa subcutánea en cuatro formas. (1) La relación entre el rango asignado a la cantidad de grasa observada y un índice de grasa (transformación angular de g lípidos/g de masa seca limpia) conteniendo términos lineares significativos; no resultaron significativos los términos no lineares. Al menos en términos estadísticos, los datos en rangos particulares de grasa, se acercan muy marcadamente a los datos de intérvalos. (2) El intercepto en Y de la relación del índice-grasa y rango de lípidos resultó significativamente diferente de cero, indicando la presencia de una reserva de grasa en el rango 0; este método tienen una utilidad limitada en estudio de aves que envuelven una marcada carestía de alimentos. (3) Las aves estudiadas en el sur de Indiana, perdieron el 43% de la grasa visible durante la noche, lo que sugiere que el método de asignar rangos tan sólo puede medir cambios a corto alcance, en aves pequeñas. (4) Otras fuentes importantes de error incluye el colocar en el rango equivocado a un ave, confundir tejido no-adiposo con grasa, dificultad en la clasificación de grasa en animales muertos y sesgo por parte del observador.

Fat reserves play important roles in the wintering, migratory and breeding phases of the annual physiological cycles of birds (Blem 1990). Ornithologists working in the field and laboratory commonly estimate

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the size of the reserve by assigning a numerical rank (fat class) to visible subcutaneous fat (e.g., Blanchard 1941, McCabe 1943, Rogers et al. 1989, Weise 1956, Wolfson 1954). Despite the frequent use of the fat class method, few studies have reliably tested its underlying assumptions (but see Busse 1970, 1974; cf. Lehikoinen 1987; also Brooks 1968; Rogers 1987). For example, it has been suggested that the method yields data on an ordinal but not an interval scale, i.e., the classes can be ordered along a continuum unambiguously, but the intervals in actual body fat between adjacent classes are unknown (Hailman 1965, 1969). Therefore, the entire relationship between fat class and true lipid content may not be perfectly linear, as has been previously assumed. Second, no study has tested the assumption that birds with no visible fat have exhausted metabolically available body lipids. Third, no study of which I am aware has estimated the sensitivity of the method by attempting to measure slight changes in the fat reserve in the same set of individuals. Finally, there has been no consistent study of important sources of error in experienced workers.

I evaluated the fat class method in four ways with data on the Dark-eyed Junco (*Junco hyemalis*). I measured the differences in chemically-determined lipid index among six quantitative fat classes. I tested the assumption that 0 fat class represents a complete absence of usable body lipid by comparing lipid index of birds in 0 fat class with literature estimates of lipid index of birds with no metabolically available lipid. I estimated the sensitivity of the method by employing it to measure the overnight reduction in the evening fat reserve of wintering juncos. Finally, I identified sources of error encountered during 15 yr of experience classifying fat in North American birds. I did not evaluate the effect of naive observers on fat class data (Krementz and Pendleton 1990); such analyses are of limited value in that inexperience rather than true observer effects are assessed. This paper instead focuses on how the method performs after significant experience with it.

METHODS

Study site and populations.—Ninety-two juncos in their first winter were studied. All captures were made in the winters of 1982–1983, 1983–1984, and 1984–1985. Juncos were collected, in a study of the regulation of winter fattening, from natural winter populations in southern Michigan (MI), southwestern Indiana (IN) and central Tennessee (TN). Study sites are described in detail by Rogers (1988). Preliminary analysis showed that the sexes were similar in both lipid index and visible fat class over a broad range of these variables (Rogers 1988); hence data from males and females were pooled.

Determination of visible subcutaneous fat class.—Visible subcutaneous fat is here defined as the fat visible underneath the transparent skin in the furcular depression and the abdominal region, the latter extending from the posterior end of the sternum to the cloaca. These two regions correspond to the claviculo-coracoid and ischio-pubic/transverse abdominal subcutaneous fat organs in the White-crowned Sparrow, *Zonotrichia leucophrys* (King and Farner 1965).

Birds captured in mist nets or ground traps were brought to the laboratory (MI, IN) or an automobile (TN) and examined within 1.5 h of capture. Each bird was held in the left hand, ventral side up, with the first two fingers of the left hand on the ventral (first finger) or dorsal (second finger) side of the neck. The first finger pressed against the base of the bill so that the bill pointed forward at approximately 45° above the extended longitudinal axis of the bird. The first finger of the right hand was held lightly against the left side of the pectoral musculature while the right thumb lightly held the tail in its natural position. The last three fingers of the right hand (excluding the last finger in small-bodied species, e.g., hummingbirds, kinglets, chickadees) supported the bird's body dorsally. Birds were held gently to avoid injury, but firmly to avoid escape.

With the bird held in the above position, the ventral contour feathers were blown aside and the subcutaneous fat observed in the two defined areas was classified as follows (after Nolan and Ketterson 1983). 0 = no visible fat on abdomen (A) or in furcular depression (F). 1 = F < 33% full, A < 50% covered. 2 = F 33–66% full, A 50–100% covered but fat layer not even with pectoral region. 3 = F filled and fat flush with pectoral musculature, A completely covered, fat layer flush with pectoral musculature, thus neither F nor A bulging outward from pectoral musculature. 4 = as in 3 with F or A bulging. 5 = both F and A bulging. Intermediate classes were also recognized in birds that did not meet criteria of below and above classes, e.g., 1.5 exceeded 1.0 criteria but did not meet 2.0 criteria. Intermediate classes were also recognized in birds that, for example, showed furcular fat of class 2.0 and abdominal fat of class 1.0 (fat class of 1.5). A fluorescent or incandescent light (MI, IN) or incident daylight (TN) provided illumination. Subcutaneous fat was recognized by its yellow or orange-yellow color, which contrasts with the dark red color of muscle. Examined birds were killed humanely within 2–4 h of capture and stored on dry ice pending laboratory storage at −20 C.

Determination of lipid index.—Lyophilized carcasses were cut into small pieces with a scissors and converted into a fine homogenate in a high-speed blender fitted with sharp rotating blades in a small (approximately 500 ml) blending chamber. The "blending regime" consisted of six 10-s periods with 10 s of hand mixing with a stainless-steel spatula between periods, terminated with 30 s of continuous blending. Neutral lipids were extracted from two 1-g samples/bird using reagent-grade petroleum ether in Soxhlet extractors. Lipid index (LI) is average g lipid/g lean dry mass for the two samples.

Estimation of sensitivity.—I estimated sensitivity of the fat class method in a general way by determining whether or not it could measure the normal overnight reduction in the winter fat reserve of small birds (Farner et al. 1961, Lehikoinen 1987). On each of 17 days (December–early March 1987–1988), juncos were captured in mist nets as they entered a large evergreen roost in southwest Indiana (described by Rogers and Rogers 1990). Juncos were weighed on an analytical balance (nearest 0.01 g) and their fat classes were determined. Next, birds were held overnight in small cages in an unheated and uninsulated building (Webb and Rogers 1988). At dawn, birds were weighed and their fat class determined without reference to evening records.

RESULTS

Statistical relationship between visible fat class and lipid index.—LI and not visible fat class is the appropriate dependent variable because more than one value of LI (Y) was available for each fat class (X; after Sokal and Rohlf 1981). All P-values are 2-tailed.

The relationship between fat class and LI was linear (Fig. 1). Polynomial regression (weighted by sample size) yielded a significant linear term ($P < 0.05$); neither the quadratic nor the cubic term was significant ($P < 0.20$). When a simple linear regression was performed, the significant ($P < 0.0005$) linear term explained 97.4% of the variation in LI (Fig. 1). Regression of LI treated with the angular transformation yielded closely similar results (Fig. 1).

Regression was repeated using the individual bird as the unit of analysis. The linear regression equation was virtually the same as that derived from analysis of means although the R^2 was lower (51.1%) in the analysis of individuals. Again, the linear term was highly significant ($P < 0.0001$); both the quadratic and cubic terms were nonsignificant ($P < 0.80$). Analysis of angular-transformed LI yielded closely similar results.

Lipid index at 0 fat class.—Of 92 birds examined, only 1 (1.1%) showed absolutely no visible fat. The Y-intercept of the fat class-LI regression provides an estimate of LI at 0 fat class, however. This Y-intercept was significantly different from 0 ($P < 0.0005$, Fig. 1). More importantly, this estimate is well above LI values for birds known to have exhausted their lipid reserves (e.g., approximately 0.04 for the junco, computed from data in Helms et al. 1967; 0.06 for 7–10 d-old Red-winged Blackbird *Aegelaius phoenecius,* nestlings known to have starved to death, J. D. Hengeveld and C. M. Rogers, unpubl. data). Assuming that these LI values are reliable estimates of polar phospholipids unavailable to intermediary metabolism, unseen lipid reserves represented by an LI of approximately 0.19 remained metabolically available at 0 fat class.

Quantitative relationship between visible fat class and lipid index.—Although statistical analysis indicated the relationship between these variables to be linear, the absolute difference in LI between successive fat classes was not constant (Fig. 1). This result is of course expected from the departure of the above R^2 value from exactly 100%.

Overnight change in fat class.—As expected, wintering juncos lost body mass overnight; a substantial reduction in evening fat class was concomitant with mass loss. Juncos lost an average of 9.5 ± 0.02% (SD) of evening body mass and 43.1 ± 0.20% of evening visible fat reserve. Of 265 juncos examined, none was classified as having greater dawn than

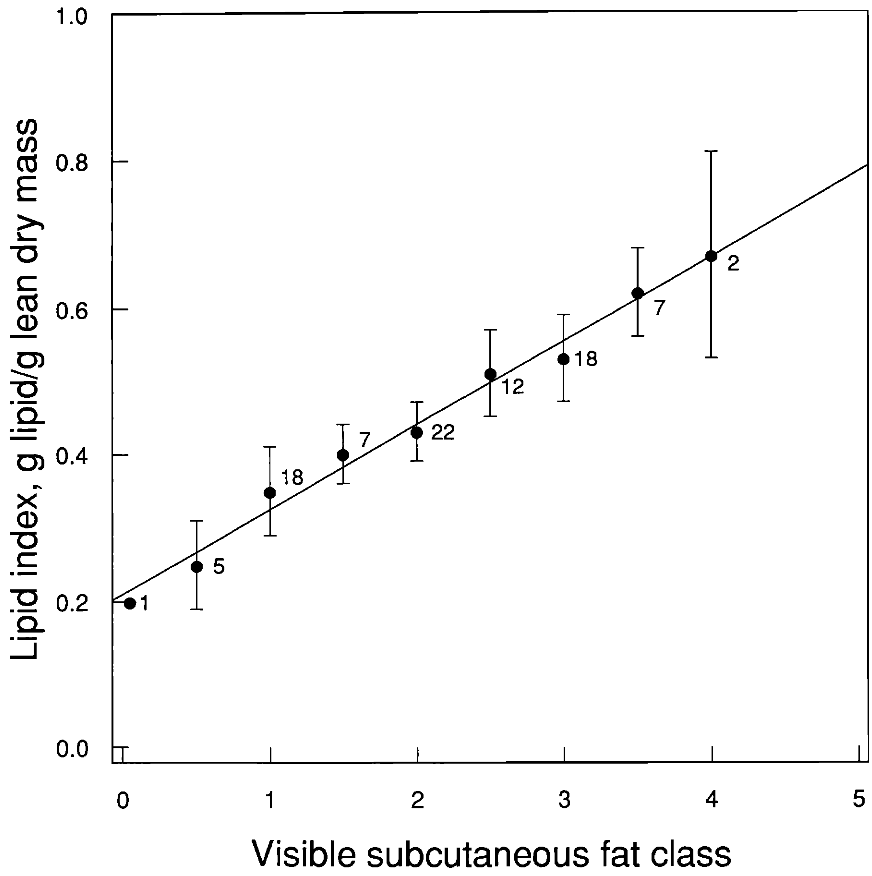

FIGURE 1. Graphical relationship between visible subcutaneous fat class (fat class) and lipid index (LI). Points indicate $\bar{x} \pm 2$ SE; sample sizes are adjacent to means. Simple linear regression equation: Y = 0.2286 + 0.1057(fat class), untransformed LI; Y = 0.5056 + 0.1095(fat class), LI transformed with angular transformation. See text for R^2 values. Confidence intervals for untransformed LI means: 0.08, 0.06, 0.05, 0.04, 0.07, 0.06, 0.07, 0.89.

evening visible fat, and 15 (6.7%) were placed in the same evening and dawn fat class.

DISCUSSION

Applying the Fat Class Method

I now discuss these results in terms of what they suggest for reliable application of the fat class method. As stated above, this discussion deals with what can be accomplished with a significant amount of practice. In the conclusion I recommend simple steps for rapidly gaining proficiency.

Ordinal or interval data.—Hailman (1965, 1969) argued that fat class data are ordinal, not interval, data. In the present study, the difference in LI between successive fat classes varied widely, with a minimum of 0.018 and a maximum of 0.093. Strictly speaking, this result supports Hailman's contention. A true interval scale is not suggested by these results, nor is such a scale likely to be obtained by any investigator. In contrast, the lack of a significant nonlinear term indicates that these data closely approach a true interval scale.

What type of statistical tests are appropriate for fat class data? Hailman (1965, 1969) suggested that due to the nature of ordinal data, only nonparametric tests are appropriate. In contrast, there is at least one important situation in which nonparametric methods cannot be used: multivariate analysis of potentially interacting independent variables. Given a highly predictive, linear relationship of the type in Figure 1, I suggest parametric (including multivariate) statistical procedures can be applied legitimately to fat class data. In addition, parametric tests are appropriate if body mass differences between successive fat classes are uniform, i.e., linear (e.g., Weise 1956). If, however, nonlinear fat class-LI or fat class-mass terms are found or suspected, nonparametric tests would seem most appropriate.

Lipid index at 0 fat class.—The lipid index at 0 fat class was well above that of birds (including juncos) known to be without metabolically available lipid. These additional lipids probably represent unseen, hence unclassified, subcutaneous fat (e.g., on sites in the dorsal and/or humeral areas) and/or unseen fat located in the abdominal cavity. Therefore the method is of limited application in studies of birds that recently experienced severe energy limitation.

Estimation of sensitivity.—Wintering juncos lost 43% of the evening fat class by dawn. These data are not biased by prior knowledge of evening fat class because I did not refer to evening fat classes during dawn examinations; furthermore, the moderate-to-large numbers of birds examined/trap effort (>16) prevented any bias caused by remembering the fat class of particular birds. I have no "true" value however, for the overnight change in the junco fat reserve (e.g., a value derived from chemical lipid extraction). Without such data, these results only encourage the idea that overnight fat loss can be measured by changes in visible fat.

Sources of error.—In the course of determining visible fat class in over 6000 wintering, migrating or breeding birds of over 120 North American bird species, I have identified several sources of error involved with the fat class method. Several factors can lead to overestimation, at least compared to the "standard" situation used here. Neck constriction may reduce the furcular depression in size if the bird is held in an improper position; furcular fat then appears to fill a greater proportion of the furcular depression than under standard circumstances. Correct neck placement is particularly difficult in very small species such as hummingbirds and kinglets, and in species that struggle frequently and intensely such as Northern Cardinals, *Cardinalis cardinalis*. The intestinal folds seen through the abdominal skin may be mistaken for abdominal fat (more likely in medium- and large-sized species with an opaque skin such as doves, ducks, hawks, owls and large woodpeckers, although this mistake can be a problem with small-bodied species molting the ventral contour feathers). I caution strongly against classifying fat in birds that have been dead for more than several minutes. In such specimens, subcutaneous fat becomes difficult to distinguish from surrounding nonfat tissue and the morphology of visible fat changes, leading to possible overestimation. One factor leading to underestimation is, of course, neck extension, where the problem is opposite in nature to that of neck constriction.

Another potential problem with visible fat classes is the subjectivity involved and the associated lack of repeatability, or precision, among different observers. I suggest that a major result of this study is not necessarily the exact statistical relationship between between fat class and lipid content. A more important finding is that the relationship between these variables may be rigorously quantified; the parameters of the relationship might vary among researchers. Accordingly, I suggest that caution be exercised in studies that pool fat-class data across observers. In particular, if collection of representative specimens is possible, research groups might consider searching for such effects with statistical analysis, and correcting for observer effects either directly or with statistical correction factors.

Conclusion.—Ornithologists who control the above sources of error can use the present method to obtain a general estimate of body fat in small birds that exhibit at least a minimum amount of visible subcutaneous fat. The above descriptions of the visual appearance of subcutaneous fat should make the method fairly easy to master. Competence can be quickly gained by development and adjustment of standard curves for species under study. If its limitations are recognized (e.g., possible inter-observer bias), quantification of visible fat is a ready and useful alternative in situations when study populations cannot be reduced significantly by collection. Such situations are common, and include studies of breeding biology, ethology, migration, mark-recapture and endangered species. The method when properly applied supplies a reasonable alternative to techniques such as the electromagnetic method (Walsberg 1988), which require equipment not yet readily available.

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