A New Multi-Category Classification of Subcutaneous Fat Deposits of Songbirds (Una Nueva Clasificación, con Multi-categorías, para los Depósitos de Grasa en Aves Canoras)

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A NEW MULTI-CATEGORY CLASSIFICATION OF SUBCUTANEOUS FAT DEPOSITS OF SONGBIRDS

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Abstract.—A new fat scoring technique for small birds is introduced using 31 classes (nine main classes with up to four subclasses each; Fig. 1). In contrast to many other methods, fat score and respective fat load (as determined by the Soxhlet method) correlate very well. Furthermore, variability in the calculated regression lines for nine different species is low. This effective new method can easily be learned by handling approximately 100 individual birds and substantially improves precision of studies on fat-deposition in migratory birds.

UNA NUEVA CLASIFICACIÓN, CON MULTI-CATEGORÍAS, PARA LOS DEPÓSITOS DE GRASA EN AVES CANORAS

Sinopsis.—Se presenta una nueva técnica para clasificar los depósitos de grasas. La misma utiliza 31 clases, de las cuales 9 son principales, y éstas a su vez tienen un máximo de cuatro subclases cada una (Fig. 1). A diferencia de otros métodos, la clasificación de la grasa, y su respectiva carga de grasa (como es determinada por el método Soxhlet), se correlacionan muy bien. Más aún, la variabilidad en el cálculo de líneas de regresión para nueve especies de aves canoras, resultó bajo. Este nuevo método es fácil de aprender con una muestra de aproximadamente 100 aves y mejora sustancialmente la precisión de estudios sobre depósitos de grasa en pájaros migratorios.

Subcutaneous fat deposits of birds can be estimated visually. Different authors (Davidson 1984, Pettersson and Hasselquist 1985) have developed a series of classification methods to estimate visible fat deposits (here "fat score"). These methods, however, are rather rough and so far the accuracy of the various techniques has not been investigated (Blem 1990), although fat scores determined by different authors are, to some extent, convertible. Most of the fat score techniques use only 4–7 classes (Bernis 1966, Busse 1970, Busse and Kania 1970, Cherry 1982, Frelin 1978, Fry et al. 1970, Helms and Drury 1960, Wolfson 1945). I developed a much finer scale of 31 classes (Fig. 1) during studies in the long-term monitoring program of migratory bird populations (MRI-Programme) of the Vogelwarte Radolfzell (Berthold and Schlenker 1975, Kaiser 1992). This method was developed to increase the precision and accuracy of estimating fat scores. A more precise estimation of visible fat deposits may enable one to calculate actual fat load more accurately in future studies as well as to determine precisely physiological capabilities of birds, such as flight range and overnight or overwinter survival.

The new scoring method was tested on the following points: 1) the correlation between visible fat deposits and the actual amount of stored fat; 2) interspecific variability; 3) accuracy (i.e., repeatability); and 4) "user friendliness," particularly, how fast the method can be learned and how easily it can be applied.

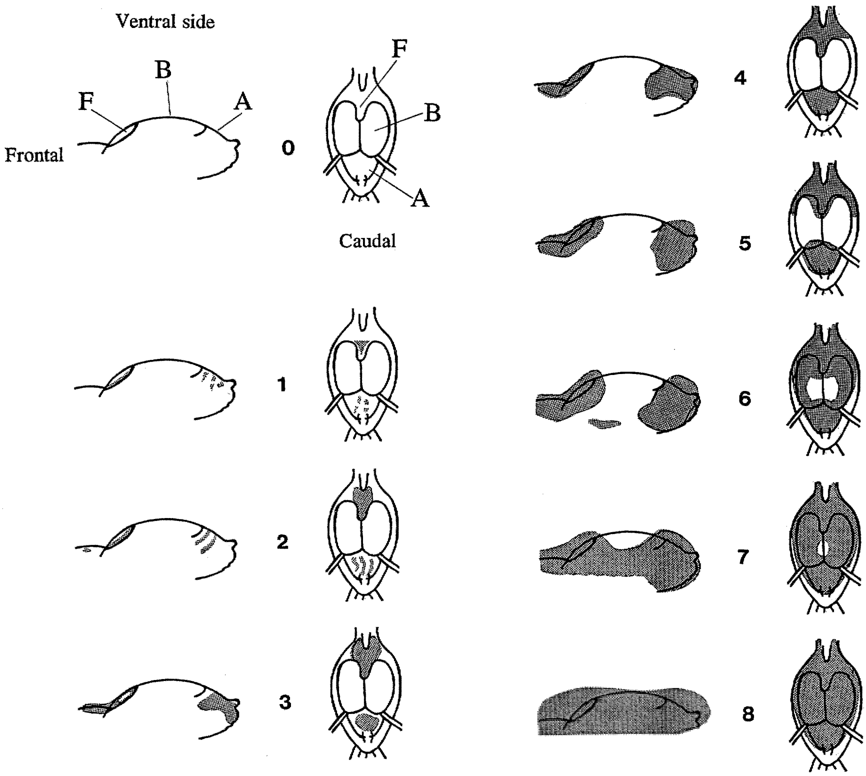

FIGURE 1. Main fat score classes (0–8). F = furcular depression (interclavicular depression), B = breast muscles, A = abdomen, stippled = fat.

METHODS

The fat scoring method for small birds developed here follows the basic approach of a method introduced by Helms and Drury (1960) (Fig. 1). During the determination process, two of the most important visible fat deposits are checked, the furcular (interclavicular depression, "tracheal pit") and the abdominal (see Table 1, Fig. 2). A specific positioning of the body is necessary to make the determinations. The bird is laid on its back in one hand, and the legs are held by the other hand. The neck must be stretched slightly so that the furcular deposit is well visible, and the feathers must be blown aside. Additional requirements are (1) the use of bright light, which intensifies the contrast between yellowish fat layers and red muscle tissue, and (2) the determination of the amount of visible fat before the bird is weighed to avoid biasing the measuring process. Within the framework of a long-term study (Kaiser 1992), the correlation of visible fat deposits and actual fat load was determined on 302 small passerines of 28 species (Table 2). Sample sizes varied among species because most of the birds were sampled as fresh netting mortalities.

TABLE 1. Description of the 31 fat classes (furcular depression and abdomen).

Main class	Subclass	Furcular depression	Abdomen	Color of the considered areas
	0.00	no fat	no fat	dark red
0	0.25	barest trace, very narrow stripe	fat deposits not yet delimited	red
	0.50	small stripe	fat deposits not yet delimited	red
	0.75	wedge-shaped	small trace, patchy	light red
1	1.00	wide wedge	trace, very small, stripes around intestinal loops (<1 mm)	light red
	1.25	half of furcular depression is covered	trace, stripes 1 mm wide	yellow-red
	1.50	almost completely covered with fat	trace, stripes smaller than intestinal loops	yellow-red
	1.75	small amount, almost completely covered with fat	wide stripes (2 mm)	yellowish
2	2.00	completely covered, shape deeply concave	slips of visceral fat, area between intestinal loops completely filled	light yellow
	2.25	completely covered, shape deeply concave	some subcutaneous lipid, not yet forming a pad	light yellow
	2.50	completely covered, shape deeply concave	very small pad	light yellow
	2.75	completely covered, shape deeply concave	small pad, at least 2 or 3 intestinal loops still visible	light yellow
3	3.00	moderate fat reserves cover ends of interclavicles	flat pad, one loop still visible	light yellow
	3.25	concave	slightly rounded pad, one loop sometimes visible	yolk-yellow
	3.50	still concave	slightly bulging, loops completely covered	yolk-yellow

TABLE 1. Continued.

Main class	Subclass	Furcular depression	Abdomen	Color of the considered areas
	3.75	almost filled	bulging	yolk-yellow
4	4.00	filled up to distal portion of interclavicles	conspicuously bulging (2–4 mm)	·
	4.25	filled up to distal portion of interclavicles	further increase in bulge (4–5 mm)	·
	4.50	filled up to distal portion of interclavicles	abdominal structures completely covered and bulging	·
	4.75	slightly bulging with central depression (concave)	abdominal structures completely covered and bulging	·
5	5.00	convex bulge	extreme convex bulge, increasing thickness	·
	5.25	just covering flight muscles from either furc. or abdomen	extreme convex bulge, increasing thickness	·
	5.50	covering border of flight muscles a few mm	covering border of flight muscles a few mm	·
6	6.00	covering flight muscles by several mm	covering abdominal part of flight muscles by several mm	·
	6.50	fat reaches flight muscles from sides of wings		·
	6.75	fat covering flight muscles conspicuously		·
7	7.00	three quarters of flight muscles covered		·
	7.25	large rounded fat-free area in middle of breast		·
	7.50	small rounded fat-free area (red)		·
	7.75	very small fat-free area still visible		·
8	8.00	flight muscles not visible, fat layer covers underside/ventral side of the bird completely		·

TABLE 2. Number of birds taken to measure fat content and visible fat deposition.

Species	*n*	Mean body mass (g)	Range in visible fat deposition (classes)
Bearded Tit (*Panurus biarmicus*)	3	14.2	2.25–3.00
Blackbird (*Turdus merula*)	23	88.6	0.25–3.50
Blackcap (*Sylvia atricapilla*)	30	18.3	0.75–5.25
Blue-Tit (*Parus caeruleus*)	33	11.0	0.00–3.50
Chaffinch (*Fringilla coelebs*)	1	16.1	1.00
Chiffchaff (*Phylloscopus collybita*)	39	7.4	0.50–4.00
Coal Tit (*Parus ater*)	3	9.0	0.25–2.00
Dunnock (*Prunella modularis*)	9	18.9	1.25–3.75
Garden Warbler (*Sylvia borin*)	22	19.8	1.00–7.25
Grasshopper Warbler (*Locustella naevia*)	2	10.9	0.25–0.75
Great Reed-Warbler (*Acrocephalus arundinaceus*)	1	26.6	1.00
Great-Tit (*Parus major*)	3	16.8	2.25–3.75
Icterine Warbler (*Hippolais icterina*)	1	13.5	2.00
Marsh Tit (*Parus palustris*)	1	11.7	3.25
Marsh Warbler (*Acrocephalus palustris*)	1	11.9	1.25
Moustached Warbler (*Acrocephalus melanopogon*)	1	10.9	2.25
Penduline-Tit (*Remiz pendulinus*)	2	9.3	2.25–3.50
Reed Bunting (*Emberiza schoeniclus*)	16	18.4	0.00–4.75
Reed-Warbler (*Acrocephalus scirpaceus*)	53	11.6	0.25–6.25
Robin (*Erithacus rubecula*)	21	16.4	1.25–4.25
Sand Martin (*Riparia riparia*)	1	14.0	2.50
Sedge Warbler (*Acrocephalus schoenobaenus*)	4	11.1	0.75–3.25
Short-toed Treecreeper (*Certhia brachydactyla*)	2	9.2	1.50–4.00
Song Thrush (*Turdus philomelos*)	10	70.9	0.50–4.75
Starling (*Sturnus vulgaris*)	2	76.3	1.25–2.25
Willow Warbler (*Phylloscopus trochilus*)	11	8.7	0.50–3.50
Wren (*Troglodytes troglodytes*)	1	8.6	5.00
Yellow Wagtail (*Motacilla flava*)	6	16.0	2.50–4.50

Birds were examined only by the author. The amount of stored fat was determined by a Soxhlet extraction after 3 wk of drying at approximately 70 C (Kaiser 1992, for description of methods see Dobush et al. 1985). In the results section I use indices of the fat content based on lean dry mass (LDM), as by definition, the fat-LDM-index is free of fluctuations of body mass components such as water and fat. The fat content (fat-LDM-index) is calculated as a percentage of LDM (g fat/g LDM × 100).

The fat scoring technique gives highly repeatable results under field conditions. Repeated measurements by the same observer were analyzed using 77 birds caught twice within 5 h. In 98% of the cases, differences in estimations of visible fat deposition were less than one class, and in 61% were less than two subclasses, with an average in visible fat class of 2.5 (SE = 0.17) during the first capture and of 2.6 (SE = 0.16) during the second. Variations found after greater recapture intervals may partly be explained by diurnal variation or by changes in behavior after first

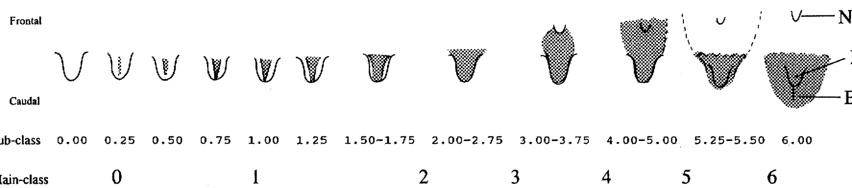

FIGURE 2. Visible fat deposition at the furcular region (ventral view). For description of subclasses see Table 1. F = furcular depression, N = neck, B = breast muscles, stippled = fat.

capture. To test variation among observers, the fat scoring technique was tested by 15 banders. On average, inter-observer variability amounts to only 1–2 intermediate classes when scoring the same bird. Experience with approximately 100 birds is usually required to learn the method, which is equivalent to only 1–2 d banding at our field stations.

RESULTS AND DISCUSSION

There was a strong relationship between fat score and fat content. As fat score is an ordinally scaled variable (Hailman 1969), ranks were used for nonparametric correlation. As the scores are ranks, the relationship is linear. In addition, the exponential function does not explain significantly more variance than ranked values (with $r = 0.81$, $R^2 = 0.66$, $P = 0.00001$, SE = 0.39; ln(fat load) = 1.64 + 0.48 × fat score cp. with data in Fig. 3). Thus, regression analysis was performed on transformed data of all 302 birds combined (Fig. 3).

Variance of individual measurements was remarkably low within each species (Table 3). Correlation and the goodness-of-fit measures range from $R^2 = 0.50$ for species with a smaller range of fat scores to $R^2 = 0.82$ for species with large amounts of migratory fat deposition. The independent variable "species" does not affect the prediction of fat scores (analysis of covariance, $P = 0.36$, nine species, see Table 3). For two species, Garden Warbler (*Sylvia borin*) and Blackcap (*S. atricapilla*), interspecific differences in the correlation of fat content and estimated fat values is shown in Figure 4.

My results are considerably different from those of other authors using different kinds of analysis (usually the data are not transformed into ranks and the standard error of estimation is not given). The regression lines and the goodness-of-fit measures published so far ($R^2 = 0.19$, absolute fat content vs. fat score, 100 birds, five species [Krementz and Pendleton 1990]; $R^2 = 0.58$, fat score vs. relative fat content, 63 birds, 11 species [Rogers 1987]; $R^2 = 0.51$, lipid index vs. fat score, 92 birds, one species, [Rogers 1991]; and $R^2 = 0.64$, fat score vs. actual fat load, seven species, [Rogers and Smith, in press]), demonstrate the importance of a standardized method for calculating relative fat content (based on LDM), especially if different species are combined. The results of Rogers's (1987, 1991) studies and especially of the investigations presented here dem-

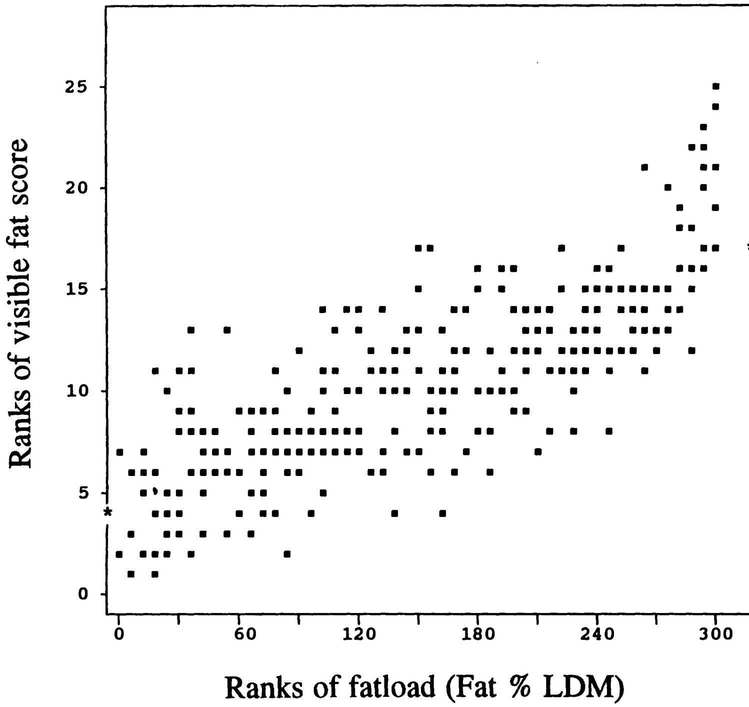

FIGURE 3. Correlation between visible fat deposits (fat score) and fat load (% LDM), 28 species combined. Data were transformed into ranks. Spearman rank correlation values are for fat load = 4.34 + 0.04 × ranks of fat score ($P < 0.00001$, $r = 0.79$, $R^2 = 0.62$, $n = 302$).

onstrate a clear correlation between fat score and fat content. If the data are not transformed into ranks, the relationship between fat score and relative fat load is linear in lean to intermediate birds, but exponential in fatter birds (Fig. 4). This is due to the wider range of scores and to the fact that the scale has a maximum value (Greenwood 1992). Rogers's (1991) calculations were based on four main fat classes only (his highest class 4 indicating a bulging furcular depression or abdominal region, which is equivalent to class 4.75 in the score presented here). Higher fat classes up to a score of 8 are not unusual in migratory birds, however.

In contrast to their value in statistical analysis, rank regressions cannot be used to estimate fat loads directly from a graph, as can be seen from Figure 3. On the other hand, untransformed data are very well suited for such an estimation, see Figure 4, provided species-specific mean LDM is known (i.e., 5.0 g for the Blackcap and 5.2 g for the Garden Warbler).

The fat scoring according to the method presented here is a useful approach to a quantitative estimate of fat deposits in songbirds in the field. The method has several advantages: 1) absolute fat content can be

TABLE 3. Variance of individual measurements. Correlation of rank values between visible fat score and fat load for individual species. Results are presented for species with more than 11 individuals.

Species	*n*	*r*	R^2	SE of est.	Slope	Inter-cept	*P*
Blackbird	23	0.79	0.63	1.61	0.30	1.51	0.00001
Blackcap	30	0.82	0.67	2.07	0.33	1.08	0.00001
Blue Tit	33	0.73	0.53	2.50	0.27	2.95	0.00001
Chiffchaff	39	0.82	0.67	2.08	0.26	2.56	0.00001
Garden Warbler	22	0.91	0.82	1.62	0.53	1.05	0.00001
Reed Bunting	16	0.71	0.50	2.09	0.42	2.40	0.0002
Reed Warbler	53	0.84	0.70	2.58	0.25	2.64	0.00001
Robin	21	0.80	0.64	1.65	0.35	2.36	0.00001
Willow Warbler	11	0.91	0.82	0.96	0.59	1.00	0.0001

calculated with good precision from relative fat score data using a standard regression line/curve and corresponding LDM; 2) in comparison with other methods that estimate migratory fat deposits, the fat scoring technique is both accurate and easy to use, especially in field studies; and 3) the detection of small differences in visible fat deposition may be used to

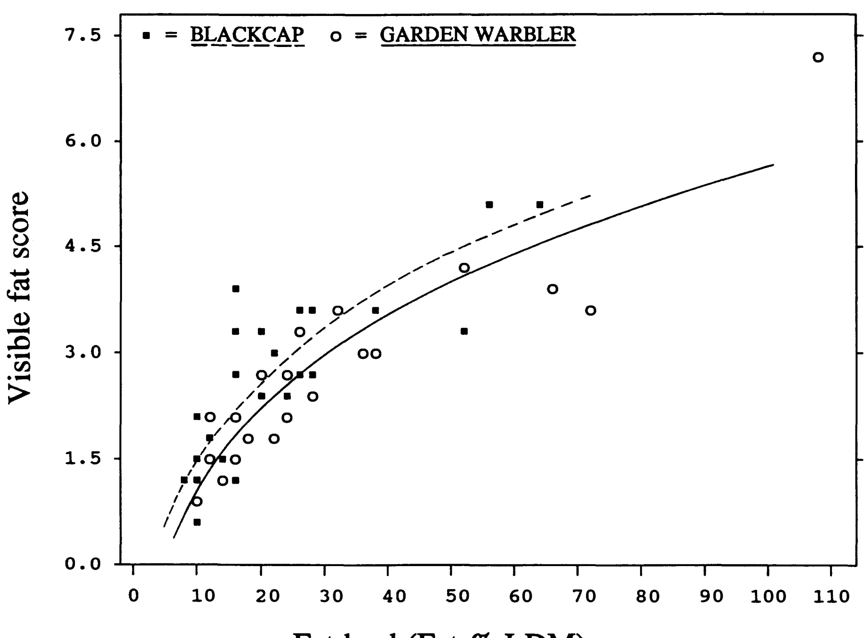

FIGURE 4. Correlation between visible fat deposits (fat score) and fat load (% LDM) for Garden Warbler and Blackcap. Data were not transformed into ranks. For statistics of rank values see Table 3.

establish seasonal changes in the physiological condition of individuals independent of absolute body size or mass fluctuations during the course of day, for example. Other methods that determine fat load fairly accurately (by direct or indirect measurements) such as: 1) calculating increases in total body mass with regard to lean body mass; 2) extracting stored fat biochemically in the lab (Dobush et al. 1985); and 3) measuring electrical conductivity in an electromagnetic field (TOBEC) of living birds (Castro et al. 1990, Walsberg 1988) show at least one major disadvantage each. The main disadvantages include: determination of fat deposits from body mass is not exact and must be corrected by the covariate "time of day" because of diurnal variations in body mass (method 1); the method is destructive and elaborate (method 2); it requires a minimum body mass of approximately 20 g (method 3); or it must be averaged from a series of highly standardized measurements and/or is expensive (method 3). The "fat scanner" (TOBEC) is used to determine lean body mass. From this measurement a highly imprecise value for the percentage of body fat is derived. In an experiment to compare the two methods using 18 Starlings (*Sturnus vulgaris*), the visible fat score technique described here (Fig. 1, Table 1) gave correlations of $R^2 = 0.85$ and 0.92, respectively (fat load vs. fat score, linear regression and logarithmic transformation), whereas the "animal body composition analyzer" (EM-Scan Model SA-2) yielded correlation values of $R^2 = 0.56$, and 0.56 (T. Meijer and F. Möhring, unpubl. data).

I therefore suggest that the multi-category classification of visible subcutaneous fat deposits be tested by other researchers in order to establish if there are any important shortcomings of the method or if it can be widely used for the determination of fat deposits in songbirds. Fat scoring works very well with all sizes (Table 2) of passerines. The method can also be used in the field without the need of elaborate equipment. The only requirements are detailed instructions.

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33rd publication from the MRI-Programme.

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