



Fat and pectoral muscle in migrating Sedge Warblers *Acrocephalus schoenobaenus*

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Increases in fat and pectoral muscle mass are important physiological changes associated with migration, but the extent to which these are linked is uncertain. The relationship between fat and pectoral muscle in first-year Sedge Warblers *Acrocephalus schoenobaenus* was investigated using the carcasses of 20 birds that died by flying into the lighthouse on Bardsey Island, North Wales, UK, in autumn 1996, and data for fat and pectoral muscle scores from 3,281 Sedge Warblers ringed while on migration through the Wetland Trust Reserve at Elms Farm, Sussex, UK, between the end of July and early October in the years 2000, 2001 and 2002. For the Bardsey sample, the mass of tracheal pit fat (claviculo-coracoid fat body) correlated with the masses of fat at other body sites, and was a good measure of overall fat levels. Lean dry pectoral muscle mass did not correlate with the mass of tracheal pit fat, suggesting that pectoral muscle mass and fat mass are independent measures of body size. The link between fat and pectoral muscle mass was investigated further using the Elms Farm data. Wing length, time of capture, fat score and pectoral muscle score all made significant contributions to overall body mass. Although fat and pectoral muscle scores were correlated overall, analysis by year and fat score range supported the idea that these can vary independently. Analysis of fat and pectoral muscle scores by different ringers suggested that fat scores were consistently applied in different years; however, pectoral muscle scores may be harder to standardise between ringers. In summary, we suggest that fat and pectoral muscle mass increase independently in preparation for migration, but the factors which determine variation in pectoral muscle scores in relation to fat scores in different years are unknown.

Birds can undergo substantial physiological changes in preparation for migration. Fat is the most efficient fuel for flight and large, subcutaneous fat deposits can be accumulated, particularly in passerines, in preparation for long-distance flights. Substantial changes in other body organs can also occur, with the mass of gut and associated organs being substantially reduced in some species in preparation for migration (Jehl 1997, Piersma & Lindstrom 1997, Piersma 1998, Piersma & Gill 1998, Piersma *et al* 1999). The increase in body mass due to fat accumulation, which for some species can be well over 50% of normal body mass, necessitates greater power output by the pectoral muscles (Pennycuik 1998). To achieve this, pectoral muscle mass also increases in preparation for migration (Fry *et al* 1972, Kendall *et al* 1973, Marsh 1984, Driedzic *et al* 1993, Lindstrom & Piersma 1993, Cantos *et al* 1994). In addition to providing increased power for flight, the extra muscle mass can also be utilised for fuel (Pennycuik

1998) and during migratory flight pectoral muscle mass decreases as the fat fuel load is used up (Klaassen *et al* 1997, Pennycuik 1998). The metabolism of protein, whether from the pectoral muscle or other tissues such as leg muscle and gut, is also required to facilitate the metabolic conversion of fat to energy. Clearly, both fat and pectoral muscle should increase in mass in preparation for migration, particularly in species which reach their destination via a few long-haul flights. However, it is not clear whether pectoral muscle mass increases as a consequence of (or in proportion to) fat accumulation or increases as part of preparation for migration, regardless of the amount of fat deposited. Addressing this issue would increase our understanding of the mechanisms and timing of fat and pectoral muscle deposition in relation to bird migration.

In a recent study of Redwings which died as a result of flying into Bardsey lighthouse while on autumn migration, tracheal pit fat mass, a measure of overall fat levels, did not correlate with lean dry pectoral muscle mass (Redfern *et al* 2000). Other species also suffer mortality by this route while on migration and 20 Sedge

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Warblers from the autumn of 1996 were available for analysis. Evidence from ringing studies suggest that the migration strategy of Sedge Warblers is to reach their sub-Saharan destination via a small number of long, non-stop flights (Peach 2002). Therefore, this species is an ideal one to test the relationship between fat accumulation and pectoral muscle mass. Each year, large numbers of Sedge Warblers are caught for ringing at a ringing site at Elms Farm, near Icklesham, Sussex, on the south coast of England; since 2000, visual scores for both fat and pectoral muscle mass have been recorded in Sedge Warblers passing through the site. The availability of the Bardsey samples and the Elms Farm data presents an opportunity to examine the relationship between fat levels and pectoral muscle mass, and their contribution to overall body mass, in migrating Sedge Warblers. In particular, it is not clear whether fat and pectoral muscle scores should be used as independent measures of condition and migratory preparation. Therefore, the aim of this study was to elucidate the relationships between pectoral muscle mass, fat levels and body mass in the Bardsey casualties, and between fat score, pectoral muscle score and body mass in the Sedge Warblers caught for ringing while passing through the Elms Farm site. In addition, since the Elms Farm data were generated by many ringers, this provided an opportunity to investigate consistency in fat and muscle scoring between ringers.

METHODS

This study is based on an analysis of 20 Sedge Warblers which died as a result of flying into the lighthouse on Bardsey Island, Gwynedd, UK (52° 45'N 4° 45'W), during August-September 1996, and 3,281 first-year Sedge Warblers ringed on passage through the Wetland Trust reserve at Elms Farm, Icklesham, Sussex, UK (50° 54'N, 0° 40'E) within the period 25 July to 6 October in the years 2000 (1,099 birds), 2001 (1,260 birds) and 2002 (922 birds).

Bardsey data

Bardsey is an island of 180 ha situated three km from the Llyn Peninsula, north Wales. Birds which died as a result of flying into the main light were collected and stored frozen at -20°C in sealed plastic bags. Mass (to the nearest 0.1 g) and wing length (to the nearest mm) were recorded within a few hours of death, and the birds were aged as first-year (Euring age code 3) using criteria described by Svensson (Svensson 1992). Samples were thawed five years later, reweighed and analysed by dissection. Fat levels were initially assessed, after

thawing, on the basis of visible fat in the "tracheal pit" (claviculo-coracoid fat body) using the scale defined by Gosler (Gosler 1996) but with the upper range extended by three points. This scale was adopted by the Biometrics Working Group (BWG) of the British & Irish Ringing Scheme and is referred to here as the BWG scale (Redfern & Clark 2001). As in Gosler's study (Gosler 1996), interpolated intermediate scores (0.5, 1.5, 2.5 etc) were also used. Birds were sexed by examination of the gonads. The claviculo-coracoid fat body (tracheal pit fat) was removed and weighed. Additional fat depots comprising the subcutaneous fat running along the posterior edge of the sternum (transverse abdominal fat, referred to here as "substernal fat"), and visceral (intra-abdominal) fat associated with the intestine and omentum were also dissected and weighed. Right and left pectoral muscles were then dissected from the carcasses, minced and dried for three days in a vacuum oven at 80°C. The dried tissue was crushed to a powder using a tight-fitting steel pestle and mortar (BDH, Poole, UK), redried for 24 h under vacuum at 80°C and weighed to determine dry mass. Lipid was extracted from the dried samples by repeated inversion for 24 h with 5.5 ml petroleum ether (boiling point 40-60°C) in sealed glass tubes at room temperature; samples were then centrifuged at 500g, and the petroleum-ether supernatant discarded. This lipid extraction procedure (Redfern *et al* 2000) was repeated twice more. After allowing residual solvent to evaporate, samples were dried under vacuum for 2 h at 80°C and re-weighed to determine lean dry pectoral muscle mass.

Elms Farm ringing data

First-year Sedge Warblers caught for ringing in mist nets at Elms Farm, Icklesham, Sussex, were aged as above. Wing length (maximum chord, Redfern & Clark 2001) to the nearest mm (or in some cases to the nearest 0.5 mm), mass to the nearest 0.1 g and hour of weighing (British Summer Time) were recorded. Mass was measured using Pesola spring balances in 2000 and 2001 (with mass recorded by interpolation to 0.1 g), and electronic top pan balances (weighing to 0.1g) in 2002. All balances were regularly calibrated. Tracheal pit fat and pectoral muscle condition were scored using the European Science Foundation (ESF) system devised by Kaiser (Kaiser 1993, Bairlein 1995, Redfern & Clark 2001).

Data analysis

Statistical procedures were done using Systat version 10 (SPSS Inc, Chicago), except where specified otherwise. Where appropriate, normality of data was tested using the Kolmogorov-Smirnov test (Lillefors modification).

For analysis of the Bardsey data, *t*-tests were used to test for sex differences in wing length, weight, fat and pectoral muscle mass. Pearson's correlation coefficient was used to analyse relationships between variables, and multiple regression was used to analyse the contribution of different variables to overall body mass. For multiple regression of the Bardsey sample, body mass data were used in the original scale because the residuals from the analysis did not depart significantly from a normal distribution.

For the Elms Farm data, the Kolmogorov-Smirnov two-sample test was used to compare the distributions of fat and pectoral muscle scores between the two halves of the July-October capture period. For analysis of the multi-way frequency tables summarizing fat and pectoral muscle scores by year, log-linear modeling (SPSS Version 11.0) was used to ask if the frequencies in each cell could be explained by the three parameters (fat score, pectoral muscle score and year) in combination with interactions between them (fat by pectoral muscle, fat by year, pectoral muscle by year, and pectoral muscle by fat by year) [a 'fully saturated' model]. Since few birds were given pectoral muscle scores of 0 or fat scores of 6 or more, the number of zero cells in the table would prevent meaningful analysis. Therefore, for log-linear modelling, pectoral muscle scores of 0 were pooled with pectoral muscle score 1, and fat scores of 8, 7 and 6 were pooled with fat score 5. The non-parametric Kruskal-Wallis test was used to test for differences in mean fat and pectoral muscle scores between years. Correlations between fat and pectoral muscle scores were analysed non-parametrically using Kendall's Tau-B to take account of ties, using SPSS version 11.0.

General linear models and Analysis of Variance (ANOVA) were used to investigate the contributions of wing length, fat score, pectoral muscle score, time of capture, year and date of capture to body mass for the Elms Farm birds. Fat score, pectoral muscle score and year of capture were included as categorical variables. Data for body mass of the Elms Farm birds were not normally distributed and skewed to the right; therefore, for analysis of these data, the Box-Cox method was used to estimate the best power transformation to produce data with a closer approximation to the normal distribution. The transformation was: $-1/(\text{mass})^{2.584}$. This general linear modeling approach allows the relative magnitudes of the effects on body mass of each different variable (wing length, fat score, pectoral muscle score etc) to be estimated, independently of the remaining variables. Least-squares parameter estimates and confidence limits were back-transformed to the original scale. Linear relationships between body mass and wing length, and body mass and time of processing were

confirmed by fitting polynomial contrasts. One-way ANOVA on a subset of the data was used to analyse the consistency of fat and pectoral muscle scoring between ringers (see Results). Bonferroni corrections were applied to maintain an 'experiment-wise' significance level of 0.05 when multiple pairwise comparisons were performed.

RESULTS

Bardsey Sedge Warblers

The 20 birds in the sample comprised 11 females and 9 males. Mean wing length for males (65.8 mm) and females (63.8 mm) differed significantly ($t_{18} = -3.5$, $P = 0.002$), but there were no sex differences in weight or fat levels ($P > 0.2$). Although wing length correlated with lean dry pectoral muscle mass ($r_{19} = 0.494$, $P < 0.03$), with the available sample size the difference in mean lean dry pectoral muscle mass between males (0.36 g) and females (0.34 g) just failed to reach the conventional boundary ($P = 0.05$) for statistical significance ($t_{18} = -2.016$, $P = 0.059$).

Body mass of these birds ranged from 11.0 to 19.1 g and fat scores (BWG system) ranged from 3 to 8. To obtain objective estimates of fat mass in the tracheal pit, the claviculo-coracoid fat body in each bird was dissected and weighed. The mass of this fat showed an approximately linear relationship to fat score (Fig 1a). Fat deposits were visible at other subcutaneous sites and within the abdominal cavity. Subcutaneous fat present as a band below the sternum (substernal fat) and fat surrounding the intestines (visceral fat) was also dissected and weighed. The masses of these fat deposits correlated with the mass of tracheal pit fat ($r_{19} = 0.886$ and $r_{19} = 0.956$, for substernal and visceral fat, respectively, $P < 0.001$, Fig 1b). Extrapolation of the regression lines relating substernal or visceral fat to tracheal pit fat suggest that both visceral and substernal fat deposits would be exhausted when the tracheal pit is empty. This suggests that tracheal pit fat mass or score can be used as a reliable indicator of overall fat levels.

One bird in the sample was exceptionally fat and weighed 19.1 g. This bird had subcutaneous fat completely surrounding the body due to the coalescence of discrete (membrane-delimited) fat deposits. The separate subcutaneous fat deposits representing tracheal pit fat and substernal fat were dissected and weighed as for the other birds. However, the remaining fat deposits around the body were also dissected and weighed. The total mass of dissectable fat bodies (including visceral, substernal and tracheal pit) was 8.1 g, thus accounting for most of the excess mass compared to birds with fat

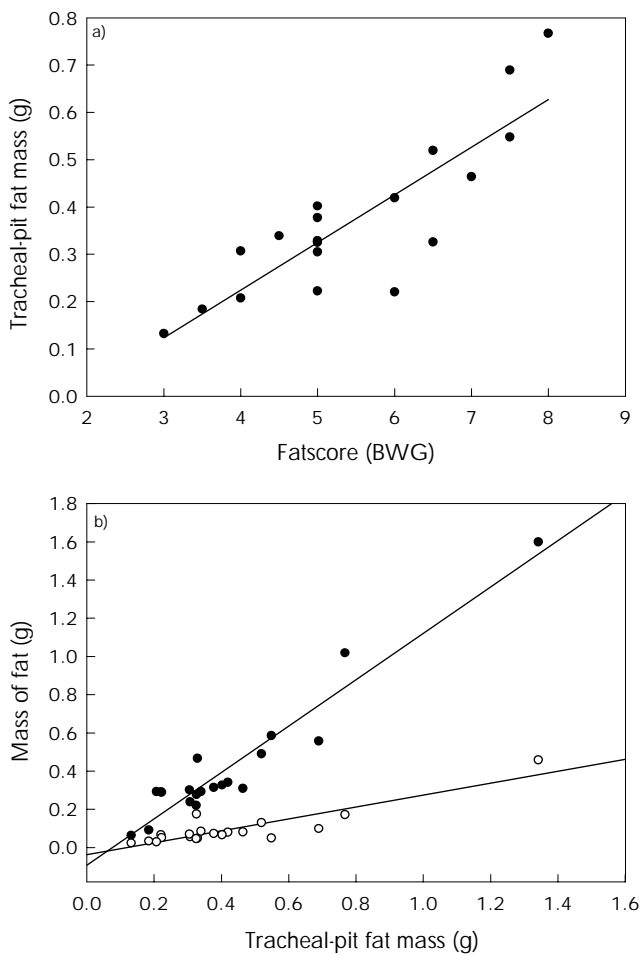


Figure 1. The mass of fat deposits in the Bardsey Sedge Warblers. a) the mass of tracheal pit fat in relation to fat score. The line is a fitted linear regression. b) relationships between the mass of fat in the tracheal pit and mass of substernal fat (○) and visceral fat (●). The lines were fitted by linear regression.

score 0 (mean mass 10.04 g, estimated from the Elms Farm data).

Petroleum-ether extraction of the dried pectoral muscles gives an estimate of the amount of fuel fat present in the muscle tissue. Extractable fat comprised 9.14% of pectoral muscle dry mass (range: 4.9 - 15.6). However, pectoral muscle fat (whether expressed in absolute values or as a percentage of pectoral muscle dry mass) did not correlate with tracheal pit fat mass ($r_{19} = 0.258$, $P > 0.2$).

Lean dry pectoral muscle mass did not correlate with tracheal pit fat mass ($r_{19} = -0.178$, $P > 0.5$, Fig 2), suggesting that these are independent measures of body size. The contribution of fat mass (represented by the tracheal pit fat mass), lean dry pectoral muscle mass, pectoral muscle fat and wing length to overall body mass was investigated using multiple regression. Only tracheal pit fat and lean dry pectoral muscle mass contributed

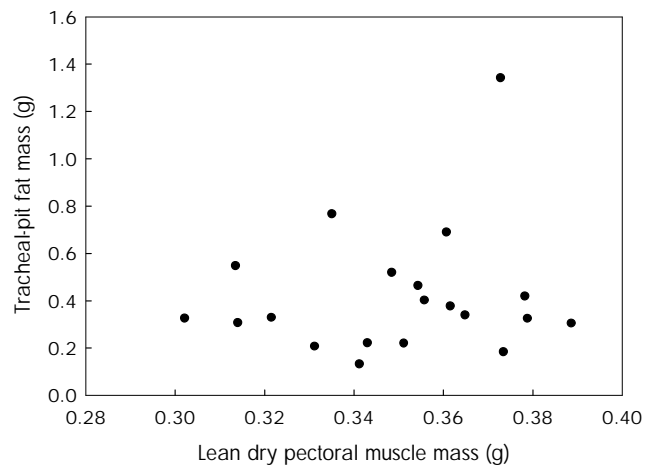


Figure 2. The relationship between the masses of tracheal pit fat and lean dry pectoral muscle in the Bardsey Sedge Warblers. There was no significant correlation between tracheal pit fat mass and lean dry pectoral muscle mass ($r_{19} = -0.178$, $P > 0.5$). Lean dry pectoral muscle mass correlated with wing length, but there was also no significant correlation between tracheal pit fat mass and the residuals of lean dry pectoral muscle mass regressed against wing length ($r_{19} = 0.22$, $P = 0.35$).

appreciably to body mass, although the effect of lean dry pectoral muscle mass was marginal, contributing only 2% to the variance in body mass compared to 88% for tracheal pit fat (Table 1). Wing length did not correlate with body mass in this sample.

Elms Farm Sedge Warblers

The results from the Bardsey Sedge Warblers indicated that pectoral muscle mass and fat mass were independent measures of body condition. Therefore, it is possible that the processes of fat accumulation and pectoral muscle hypertrophy are separate elements of migratory preparation and otherwise not directly linked. To test this idea further, data were analysed for fat scores and pectoral muscle scores from 3,281 first-year Sedge Warblers caught for ringing at Elms Farm. Sample sizes, dates, and mean wing lengths and weights are given in Table 2. In this analysis, we first consider the distribution of fat and pectoral muscle scores within and between years, then the relationships between fat and pectoral muscle scores, the relationships between fat and pectoral muscle scores and body mass, and, finally, we ask whether variation between ringers makes a significant contribution to variation of fat and pectoral muscle scores in relation to body mass.

Fat and pectoral muscle scores

Fat scores (ESF system) in the Elms Farm birds ranged from 0-8; although only 14 birds (0.4%) had fat scores greater than 6, 12 of these were from year 2000. To find

Table 1. Summary of stepwise multiple regression analysis of body mass of Bardsey Sedge Warblers against measures of fat load, muscle and wing length.

Effect	Coefficient	SE	df	F	P
Terms included					
Tracheal pit fat	6.483	0.546	1	140.771	<0.001
Lean dry pectoral muscle mass	11.776	6.116	1	3.708	0.071
Terms dropping out					
	Partial	Correlation			
Wing length	-0.206		1	0.706	0.413
Pectoral muscle fat	0.161		1	0.425	0.524

Table 2. Summary of data on ringing dates, biometrics and sample sizes for Sedge Warblers ringed at Elms Farm.

Year	2000	2001	2002
Dates	3 Aug - 6 Oct	25 Jul - 30 Sep	29 Jul - 30 Sep
Mean wing length (range) mm	64.7 (60 - 70)	64.8 (60 - 70)	64.8 (60 - 69)
Mean weight (range) g	10.88 (8.4 - 18.6)	10.76 (8.6 - 16.1)	10.81 (8.8 - 15.1)
Sample size	1,099	1,260	922

out whether or not there were changes in fat and pectoral muscle scores during seasonal progression, data were divided into two categories of approximately equal periods; birds caught between 25 July and 29 August (period 1), and between 30 August and 6 October (period 2). For the three years combined, there was a greater number of birds with fat scores greater than the median score (2) in period 1 than in period 2, resulting in a slightly higher mean fat score for period 1 (2.213) than period 2 (2.039); this was also the case when the data for each year were analysed separately. The difference in fat score distributions between period 1 and period 2 was statistically significant (two-sample Kolmogorov-Smirnov test, $P < 0.001$ for data overall and each year analysed separately) (Fig 3a). Thus, fatter birds were encountered earlier in the season.

With respect to pectoral muscle scores, the majority of Sedge Warblers (74%) had scores of 2 with the rest assigned pectoral muscle scores of 0 (0.3%), 1 (18%) and 3 (8%). Unlike fat scores, there was no difference in distribution of pectoral muscle scores between period 1 and period 2, either for all data together (two-sample Kolmogorov-Smirnov test, $P > 0.8$) or within each year ($P > 0.27$) (Fig 3b).

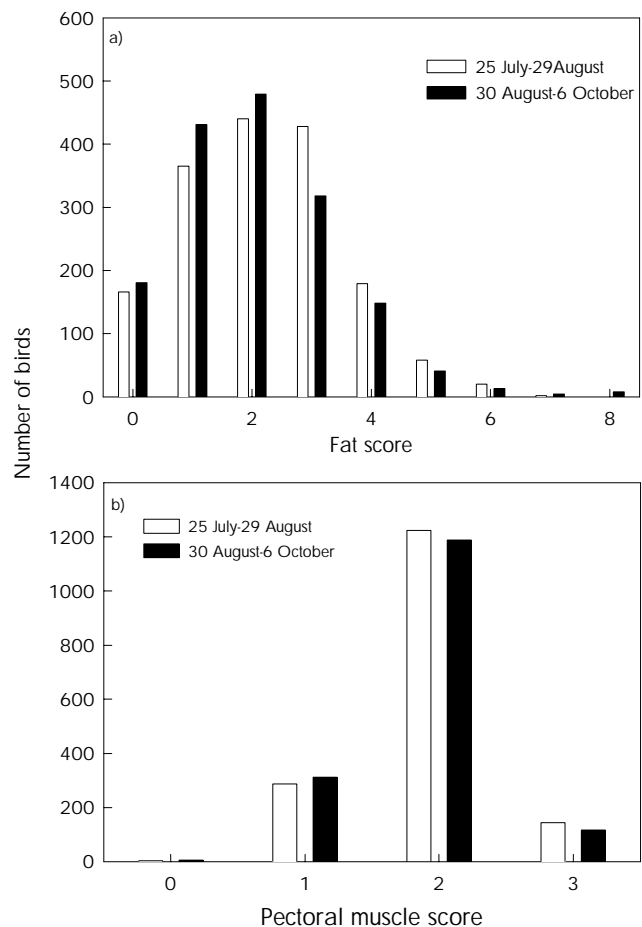


Figure 3. The distributions of a) fat scores and b) pectoral muscle scores for first-year Sedge Warblers ringed at Elms Farm, Sussex, 2000 - 2002, in periods 1 (25 July - 29 August, white bars) and 2 (30 August - 6 October, black bars).

Relationships between fat and pectoral muscle scores

The relative frequencies of fat and pectoral muscle scores in each of the three years are plotted in Fig 4. These data suggest that the distribution of fat scores differs for each pectoral muscle score class, and between years. A log-linear analysis showed that all terms in the fully saturated model (see *Methods*) made a significant ($P < 0.0001$) contribution to cell frequencies. This indicates that there were significant differences in the relationships between fat scores and pectoral muscle scores between years. There were significant differences in fat score between years (Kruskal-Wallis test, $P < 0.0015$), with the highest mean score (2.25) in 2000. There was also significant differences in pectoral muscle scores between years (Kruskal-Wallis, $P < 0.00001$), again with the highest mean score in 2000.

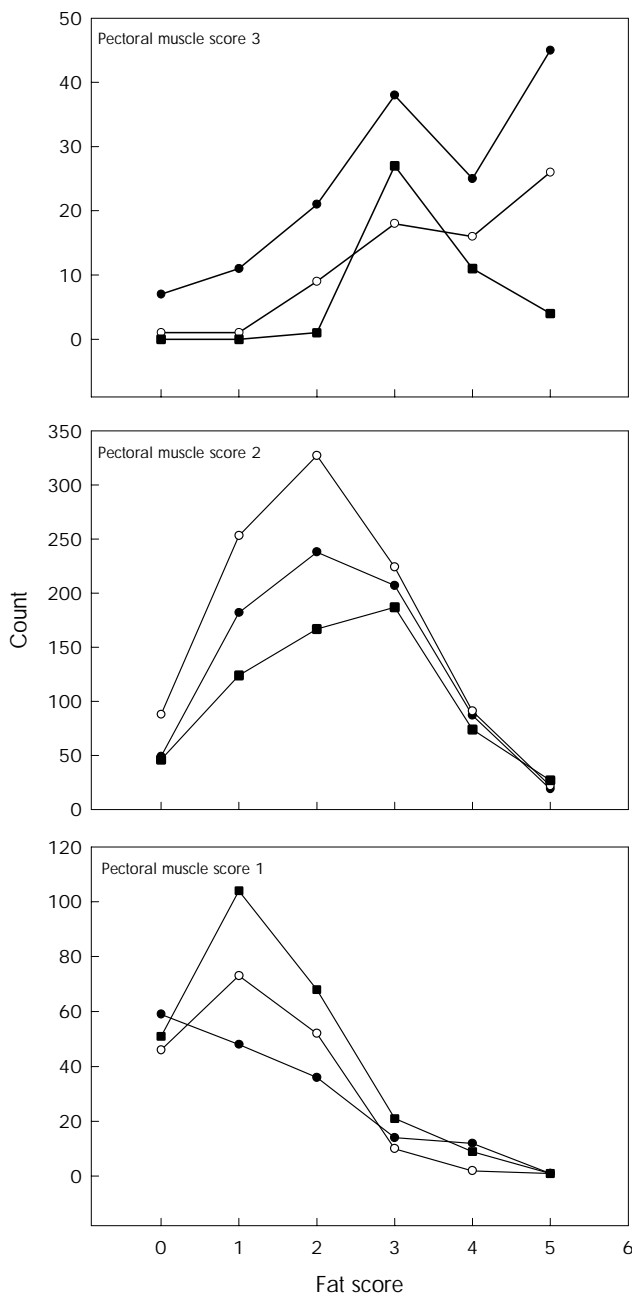


Figure 4. Distributions of fat scores at each pectoral muscle score class in year 2000 (●), 2001 (○) and 2002 (■). For this analysis, pectoral muscle scores of 0 were pooled with score class 1, and fat score class 5 were pooled with all scores greater than 5.

Although the BWG fat-score system differs slightly from the ESF system, in the score range 3-8 the two systems record similar amounts of fat. This is illustrated in Fig 5 where body mass is plotted against BWG fat score for the Bardsey sample in comparison with mean body mass plotted against ESF fat score for the Elms Farm birds. Therefore, in view of the lack of correlation

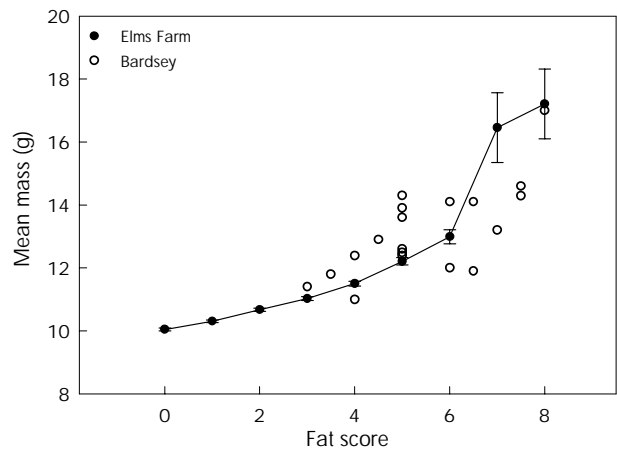


Figure 5. Mean body mass for Elms Farm Sedge Warblers at each ESF fat score (●), after allowing for variation in wing length, time of capture, pectoral muscle score, and year effects. Points are least-squares values from the general linear model \pm standard error. The open symbols (○) are body masses for the Bardsey Sedge Warblers plotted against BWG fat score for comparison.

between tracheal pit fat and lean dry pectoral muscle mass in the Bardsey sample (which had fat scores of 3-8), the Elms Farm data for each year were analysed by non-parametric correlation (Kendall's Tau-B) in two groups: birds with fat scores 0-2, and birds with fat scores 3-8. This analysis showed a statistically significant positive correlation between fat and pectoral muscle scores for both fat ranges in 2000 and 2001, but only for the low fat (fat scores 0-2) range in 2002 (Table 3).

Body mass in relation to fat and pectoral muscle scores

Both fat and pectoral muscle mass are likely to make major contributions to variation in body mass between individual birds. Time of capture and body size (estimated by wing length) are also likely to be contributing factors. For the data overall, a general linear model was used to investigate the separate contributions of wing length, fat score, pectoral muscle score, time of capture, year and date of capture on transformed body mass. Fat and pectoral muscle scores, wing length, time of capture, date of capture and year all made statistically significant contributions to variation in body mass; however, date and year of capture made relatively small contributions compared to the other variables (Table 4). Body mass was linearly related to wing length and increased by 0.225 g for every millimetre increase in wing length (Fig 6a). Similarly, body mass was also linearly related to time of weighing (polynomial contrast of order 1, $F_{1,3244} = 14.28$, $P = 0.00016$), and increased by 0.083 g per h (Fig 6b). As the autumn progressed, average body

Table 3. Rank correlation between fat and pectoral muscle scores for Sedge Warblers ringed at Elms Farm.

Year		Correlation*	Probability**	Sample Size
2000	Fat scores 0-2	0.248	< 0.001	651
	Fat scores 3-8	0.274	< 0.001	448
2001	Fat scores 0-2	0.179	< 0.001	850
	Fat scores 3-8	0.302	< 0.001	410
2002	Fat scores 0-2	0.183	< 0.001	561
	Fat scores 3-8	0.023	0.65	361

*Kendall's Rank Correlation Coefficient Tau-B

** For 6 comparisons, applying Bonferroni's correction requires $P < 0.008$ for significance at the 0.05 (5%) level overall.**Table 4.** Summary of a general linear model of transformed body mass against potential explanatory variables for Sedge Warblers ringed at Elms Farm.

Variable	df	F-ratio	P
Fat score	8	289.83	< 0.000001
Pectoral muscle score	3	13.03	< 0.000001
Wing	1	566.211	< 0.000001
Time	1	82.545	< 0.000001
Year	2	3.961	0.019
Date (day of capture)	1	5.005	0.025
Error	3,257		

mass (after correction for other variables) decreased by 0.0017 g per day. Variation of body mass with fat score and pectoral muscle score are shown in Figs 5 and 7a. Between fat scores 0-6 there was an approximately linear increase in body mass with increasing fat score (0.48 g increase in body mass per fat score), but body mass for scores of 7 and 8 was substantially higher. For pectoral muscle scores, with the exception of score 0 where the sample size was only nine birds, there was an increase in body mass of 0.17 g per pectoral muscle score for scores 1-3. With respect to year, mean body mass (corrected for variation in wing length, time of capture, fat and muscle scores) was slightly lower in 2000 than in 2001 or 2002, a difference that was statistically significant (Fig 7b, Bonferroni-corrected, $P < 0.01$).

Variation between ringers

The Elms Farm Sedge Warblers were processed by 107 different ringers, with 17 ringers each processing 50 or more birds (max 197 birds) and 10 ringers processing 100 or more each. To investigate variability between ringers with respect to scoring fat and pectoral muscle, a subset of the data was analysed; this consisted of birds processed by the 10 ringers who accounted for 100 or

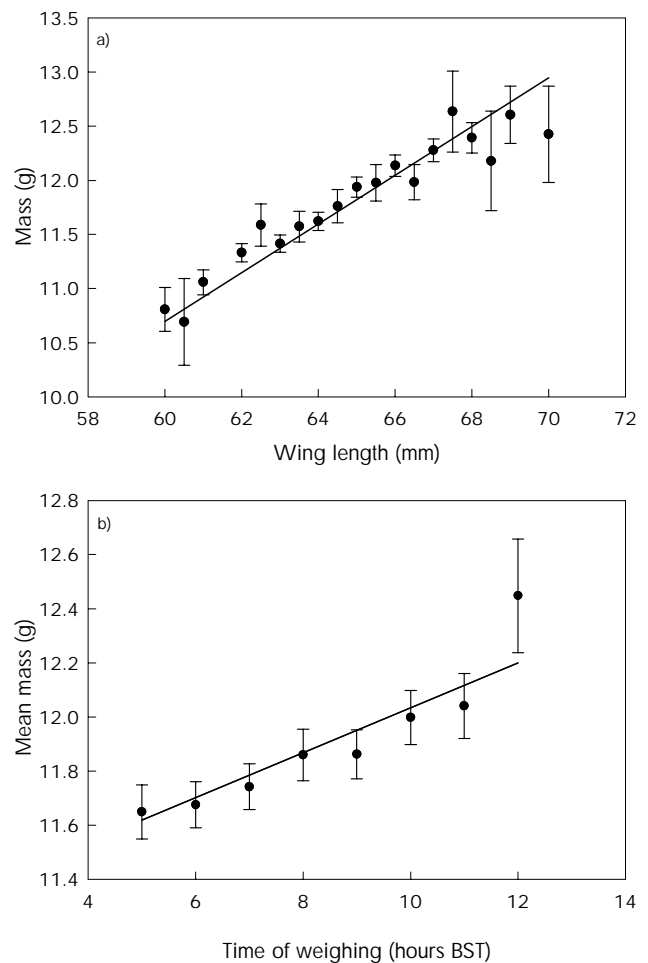


Figure 6. Body mass of Elms Farm Sedge Warblers in relation to a) wing length and b) time of weighing. Each point is the mean least-squares value from the general linear model \pm standard error, and is therefore corrected for variation in fat score, pectoral muscle score, year and capture date effects, and for variation in time of weighing in a) and for variation in wing length in b). The means were obtained by running the general linear model with wing length or time of weighing as categorical variables, respectively, and the lines were fitted from the appropriate coefficients of the general linear model after reanalysis with wing length and time of weighing as continuous variables. In b), only data for times between 0500 and 1200 hrs are shown as the sample sizes for times outside this range are small (six birds or fewer). For the points shown, the sample size ranges from 26 (1200 hrs) to 850 (0700 hrs).

more birds each, giving a sample size of 1,565 birds spread over the three years. For the dataset as a whole, variation in body weight correlated to a large extent with fat score and pectoral muscle mass, and neither score correlated with wing length ($r_{1564} = 0.028$, $P > 0.1$, non-significant). Therefore, for the 10-ringer subset, variation between ringers in assigning fat scores and pectoral muscle scores was analysed by separate one-way ANOVAs on the residuals from a linear regression of mass (Box-Cox

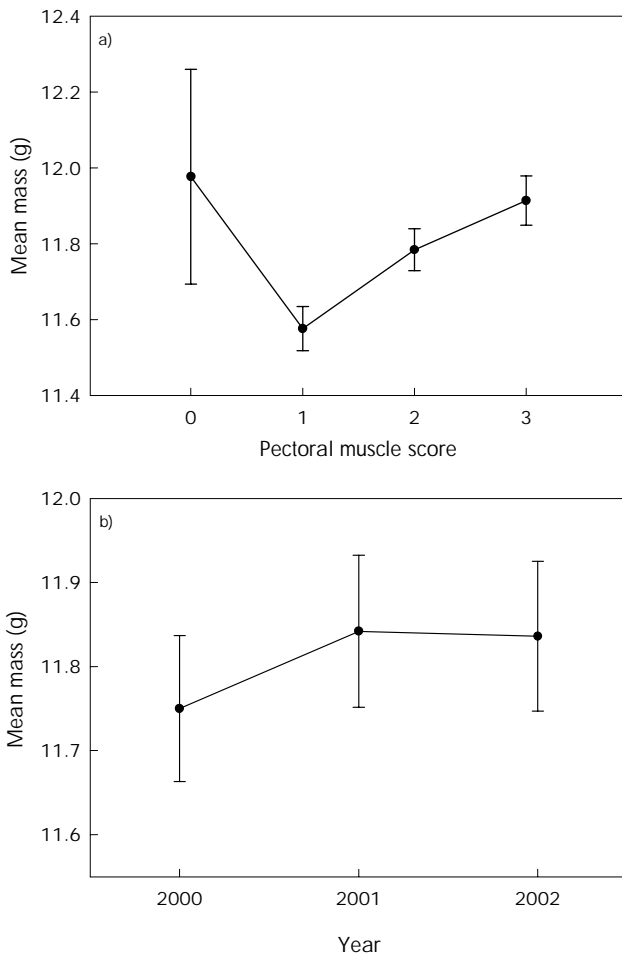


Figure 7. Body mass of Elms Farm Sedge Warblers in relation to a) pectoral muscle score and b) year. Each point is the mean least-squares value from the general linear model \pm standard error, and is therefore corrected for variation in fat score, wing length, time of weighing, capture date, and for year effects in a) and for variation in pectoral muscle score in b).

transformed) on fat score and pectoral muscle score, respectively. For each ringer, the mean mass residuals calculated for each fat score and pectoral muscle score should be 0. For fat-score-mass residuals, there was no significant difference between ringers ($F_{9,1554} = 0.675$, $P > 0.7$). However, for the pectoral-muscle-score-mass residuals, there was a significant difference between ringers ($F_{9,1554} = 3.021$, $P = 0.0014$); the residuals for one of the 10 ringers was significantly different from 0 ($F_{1,1554} = 8.95$, $P = 0.0028$; since 10 such tests were made, applying the Bonferroni correction, P must be < 0.005 to achieve an 'experiment-wise' significance level of 0.05). Since this ringer's residuals were positive, masses were higher than expected from the pectoral muscle scores, implying that this ringer had a more conservative approach when applying pectoral muscle scores.

As described in the previous section, for the whole dataset there was a small but significant difference in body mass between years after correction for fat and pectoral muscle scores, wing length, time and date of capture. Since all balances used were carefully calibrated, the variation in mass between years cannot be explained by calibration differences between years. The subset of data for 10 ringers was therefore analysed to see if variation between ringers in interpolation of weights on Pesola balances (used in 2000 and 2001) could explain this difference in body mass between years. With this subset, there was no significant variation in body mass between years. However, the term accounting for variation due to ringers was significant (Table 5). Nevertheless, when tested individually, none of the least-squared mean masses from each ringer differed significantly from the mean of all 10 ringers (Bonferroni-corrected $P > 0.05$). Furthermore, there was no consistent pattern of variation between years for each ringer that might indicate greater error arising from the use of Pesolas compared to electronic balances (results not shown).

DISCUSSION

The data analysed in this study represent cross-sectional views of fat deposition and pectoral muscle mass in two groups or populations of migrating Sedge Warblers. Both sets of data relate to Sedge Warblers during their autumn migration, and these are likely to be birds originating from within the UK (Peach 2002). However, there are important differences between the two groups: the Bardsey casualties were birds migrating at night, whereas the Elms Farm captures had either reached a temporary stopover point or were in the process of increasing body condition at south-coast fattening sites in preparation

Table 5. Summary of a general linear model of the effect of individual ringers on transformed body-mass data for Sedge Warblers ringed at Elms Farm. Data are a subset for 10 ringers who had each processed 100 or more Sedge Warblers over the three-year period.

Source	df	F-ratio	P
Fat score	7	148.669	< 0.000001
Pectoral muscle score	2	11.703	0.000010
Wing	1	260.198	< 0.000001
Time	1	13.355	0.000266
Ringer	9	3.83	0.000083
Year	2	1.154	0.316
Date (day of capture)	1	4.958	0.026
Error	1,539		

for longer-haul flights across to continental Europe and sub-Saharan Africa (Peach 2002). The lowest fat score (BWG system) in the Bardsey sample was 3, unlike the wider range of fat scores in the (larger) Elms Farm sample in which 10% of birds had a zero fat score, and this emphasises the difference in activity between the two groups. Compared to the Bardsey birds, the Sedge Warblers caught at Elms Farm will have included birds at early stages of fattening as well as those ready to depart. Ringing studies have shown that the south coast of England is an important area for premigratory fattening with birds moving east-west (and *vice versa*) in search of food (Insley & Boswell 1978, Peach 2002).

Two features of the Bardsey sample are of particular interest. First, the relationship between fat in the tracheal pit and fat at other major sites, particularly subcutaneous fat below the sternum and visceral fat associated with the intestines, indicates that when the tracheal pit is empty of fat, fat at other sites would also be depleted. This suggests that the amount of tracheal pit fat is an excellent indicator of overall fat levels. Second, there was no apparent correlation between tracheal pit fat mass (used as an indicator of overall fat) and lean dry mass of the pectoral muscle. In this respect, the results are similar to those for the Bardsey Redwings (Redfern *et al* 2000) which also showed no correlation between tracheal pit fat mass and lean dry pectoral muscle mass. This lack of correlation between pectoral muscle mass and fat levels is surprising, given the need for greater power output from the pectoral muscles to account for the increased mass of body fat.

The fat and pectoral muscle score data from Sedge Warblers passing through the south coast at Elms Farm provided an opportunity to examine further the relationship between fat and pectoral muscle mass. Although the resolution of visual scores is considerably less than that obtainable from direct measurements of fat mass and lean dry pectoral muscle mass, a much larger sample was available for analysis than could be obtained from natural casualties. For the Elms Farm sample overall, there was a clear correlation between fat and pectoral muscle scores. However, when the sample was analysed in relation to two fat ranges, a low-fat range and a high-fat range comparable to the Bardsey sample, fat score and pectoral muscle scores for the high-fat range correlated in two of the three years analysed. This supports an interpretation of the Bardsey data that while increases in fat and pectoral muscle mass are part of the preparation for migration, they are not necessarily linked and can vary independently. Other studies, although not directly related to migration, have shown that fat and lean body mass in birds can vary independently; for example, Great Tits *Parus major* breeding in marginal

habitats maintain fat reserves at the expense of pectoral muscle (Riddington & Gosler 1995), and in Greenish Warblers *Phylloscopus trochiloides*, fat can be accumulated at the expense of lean body mass to ensure short-term survival under conditions of food shortage (Katti & Price 1999).

With respect to mass increases in preparation for migration, studies on captive Red Knots *Calidris canutus* show that pectoral muscle hypertrophy is not driven by 'power training' (ie increased work load, when flying, as a result of a greater body mass due to fat accumulation) but is driven by endogenous factors (Dietz *et al* 1999). In other species, pectoral muscle hypertrophy can coincide with a loss of fat stores (Jehl 1997, Brown & Saunders 1998). Studies in which individual birds have been studied under laboratory conditions, simulating migratory flight, demonstrate that pectoral muscle mass is used up during flight (Lindstrom *et al* 2000) and there is clearly an important metabolic link between changes in fuel stores (fat) and pectoral muscle mass during flight and subsequent replenishment. However, carrying large fuel loads during migratory flights may be energetically less costly than had been thought (Kvist *et al* 2001) and it is possible that there is a threshold of pectoral muscle mass increase which is sufficient to power flight over the range of body fat accumulation which individuals achieve. The issue here is the extent to which overall fat levels and pectoral muscle mass vary independently between individuals, and the factors responsible for this variation. Therefore, although the hormonal or endogenous mechanisms initiating migratory drive will also stimulate increases in body fat and pectoral muscle mass in preparation for long flights, the relative extent of fat deposition and pectoral muscle hypertrophy may depend on dietary and other factors. The Mealy Plum Aphid *Hyalopterus pruni* is an important food source for Sedge Warblers fattening in preparation for migration; the abundance of these insects on reeds *Phragmites australis* (a secondary host - the primary host plants are fruit trees such as plum and blackthorn) can vary substantially from year to year, and determines the stopover duration of Sedge Warblers passing through (Bibby *et al* 1976). Aphids are rich in sugars extracted from the host plant (Bibby *et al* 1976) and it is possible that in aphid-poor years fat accumulation takes priority over pectoral muscle hypertrophy once pectoral muscle mass is sufficient. To address this issue, it will be necessary to monitor annual variation in aphid abundance in relation to fat and pectoral muscle scores in migrating Sedge Warblers.

This study also provided an opportunity to examine the contribution of fat score, pectoral muscle score, wing length and time of capture to overall body mass. Of

these variables, fat score contributed greatest to change in body mass, contributing 0.5 g per unit increase in fat score between scores of 0-6. The ESF fat score system is clearly not linear with respect to body mass, with scores of 7 and 8 making a greater contribution. Pectoral muscle score (for scores 1-3) contributed about the same to overall body mass as each millimetre increase in wing length. Time of capture also made a considerable contribution, and if the 0.083 g per hour reflects the diurnal weight increase as birds move through the area feeding (rather than a result of heavier birds arriving later), this would be equivalent to 1-1.5 units of fat score during the period 0500 to 1300 hrs. There was also a small but statistically significant contribution of capture year and date of capture to variation in body mass. Surprisingly, birds in 2000 had an overall lower body mass once fat score, pectoral muscle score, wing length and time and date of capture had been taken into consideration, despite the fact that birds in that year had higher fat scores and pectoral muscle scores than in 2001 or 2002. The reason for this is not clear; it is possible that when food is abundant, both fat and pectoral muscle increase while other body components are reduced or kept to a minimum to maintain a favourable wing loading (fuel stores and power output being the priority). Alternatively, it may be a statistical artifact of the general linear model: an over-compensation to body mass resulting from the higher fat loads in that year coupled with the relatively coarse resolution of fat scores. This issue, and the reason for the small reduction in corrected body mass with seasonal progression, will require further work to resolve.

Estimating the relative amounts of fat and pectoral muscle mass with visual scores is subjective. However, the ringers who contributed over half of the data were consistent in their application of fat scores. Fat scores are relatively easy to assign against objective criteria and there is clearly a high level of agreement between ringers. Conversely, unambiguously assigning a pectoral muscle score is rather more difficult, and one of the 10 ringers applied pectoral muscle scores more conservatively than the others. Given the important contribution of pectoral muscle mass to overall body mass, it would be useful to have a more objective method of measurement. Although ultrasound techniques have been used to assess pectoral muscle thickness in wild birds (Newton 1993, Dietz *et al* 1999, Lindstrom *et al* 2000), the equipment is expensive and not specifically designed for measuring wild birds. Leaving aside the problems of cost, it may be feasible to design and build appropriate equipment and analytical software if problems of obtaining probe contact with the skin with minimum stress to the bird can be overcome.

This analysis has shown that there can be important changes of body condition between years in migrating Sedge Warblers. We need to know a great deal more about how the diet of these birds varies from year to year, and how this is affected by environmental factors. Similarly, our understanding of how diet may affect the physiological balances between fat and protein deposition is at best rudimentary.

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