ORNIS SVECICA 5:75–79, 1995 https://doi.org/10.34080/os.v5.23007

Correlation between some body components and visible fat index in the Willow Warbler *Phylloscopus trochilus* (L.)

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Abstract

The correlations between some body components and visual as well as chemically extracted fat deposits of migratory Willow Warblers were studied. This study shows that the use of body mass and visual fat index are good predictors of fat deposition in the Willow Warbler. The results also indicate that body water and fat content explain most of the variation of total body mass, followed by carcass-dry mass and pectoralis muscle dry-mass (spring birds). A significant, positive correlation between pectoralis muscle dry-mass and total extracted fat content in spring birds suggests a possible muscle hypertrophy associated with fat deposition. This may be an adaptation to carry heavy fat loads during migratory flights.

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Received 21 September 1993, Accepted 3 May 1995, Edited by J.-Å. Nilsson

Introduction

Bird migration has many intriguing aspects such as orientation problems and energetics of long-distance flights. During recent years several migration studies of passerine birds have focused on the connection between migratory strategies and energy storage and utilisation (e.g. Marsh 1983, Pettersson & Hasselquist 1985, Biebach *et al.* 1985, Hedenström & Pettersson 1986, Moore & Kerlinger 1987, Karlsson *et al.* 1988, Alerstam & Lindström 1990, Loria & Moore 1990, Åkesson *et al.* 1992). The aim of these studies has been to examine how the migratory strategy of a species depends on food availability and fuel deposition. In field studies of migration strategies and stopover ecology an important method is to estimate the amount of subcutaneously deposited fat. Usually the amount of fat is estimated visually according to some classification scale (e.g. Pettersson & Hasselquist 1985, Kaiser 1993). Visual amount of fat generally shows a positive correlation with body mass, but for more detailed studies of stopover and migration strategies it is valuable to know in more detail the relationship between visual and actual amount of fat deposited subcutaneously as well as intraviscerally. However, calibration studies dealing with the relationship between visual methods of fat classification and true amount of body fat are scarce (but see e.g. Rogers 1991, Kaiser 1993). The aim of the present study was to examine the relationship between visual fat content and other body components, such as total fat mass, water content and flight muscle mass in the Willow Warbler *Phylloscopus trochilus* during spring and autumn migration in Sweden.

Material and methods

The Willow Warbler is a long distance migrant, which winters in tropical Africa (e.g. Moreau 1972, Hedenström & Pettersson 1987). In Scandinavia the birds arrive on spring migration in mid-April to late May, and autumn migration begins in late July and culminates in the second half of August (Hedenström & Pettersson 1984).

All investigated birds were collected at Ottenby Bird Observatory (56.12 N, 16.24 E) during spring and autumn migration (May and August). All birds collected in August were first year birds. Soon after capture the body mass of the birds was measured with a Pesola spring balance to the nearest 0.1 g. The birds were caught early in the morning and therefore the variation of body mass should represent fuel reserves rather than gut content. Birds were selected on the basis of fat score in order to get data across a wide range of fat scores rather than a random sample of the population. The amount of visible subcutaneous fat was classified according to a seven grade scale (0-6; Pettersson & Hasselquist 1985). A "zero" means no visible fat at all while a "six" means that the whole belly is covered with a thick swelling of fat and fat also covers the tracheal pit and surrounding areas. The maximum length of the left wing was measured according to Svensson (1984).

The two pectoral muscles were removed and their mass was measured. The carcasses and pectoral muscles were freeze-dried for 48 hours for determination of water content. Finally, the fat was Soxhlet-extracted from the water-free residues in diethylether for 24 hours.

In the statistical analyses the material was divided into spring birds (n= 8) and autumn birds (n=15). When there was no significant difference between spring and autumn birds the two data sets were combined.

Results

In both spring and autumn birds, there was a significant correlation between extracted fat mass and visual fat class (spring birds: r=0.92, df=6, P<0.01; autumn birds: r=0.85, df=13, P<0.001). However, the slopes of the linear regressions differed significantly between spring and autumn birds ($F_{1,19}$= 7.44, P<0.05; Fig. 1). These results indicate that visual fat classification is a good predictor of the actual fat content, but that the functional relationships may differ between spring and autumn. There was also a significant correlation between extracted fat mass and total body mass (r=0.87, df=21, P<0.001; Fig. 2). The slopes of the linear regressions between spring and autumn birds did not differ significantly ($F_{1,19}$=2.28, P>0.05). However, the total body mass may be influenced by other factors, such as size and muscle mass of each individual bird. Therefore, a multiple regression analysis was made with total body mass of the bird as the dependent variable and as independent variables extracted fat mass, fat-free dry mass of the carcass, fat-free dry mass of the pectoralis muscle, total water mass and wing length. For the spring birds there was a combined r-value of 0.999 (P<0.001), with total water mass as the predominant factor (partial F=1859.9, P<0.001), followed by total extracted fat mass (partial F= 1316.7, P<0.001), fat-free dry mass of the carcass (partial F=79.0, P<0.05) and fat-free dry mass of the muscle (partial F=33.0, P<0.05). The wing length did not enter the model (partial F=3.0, P>0.05). The pattern was nearly the same for the autumn birds with a combined r-value of 0.999 (P<0.001), but with total extracted fat mass as the predominant factor (partial

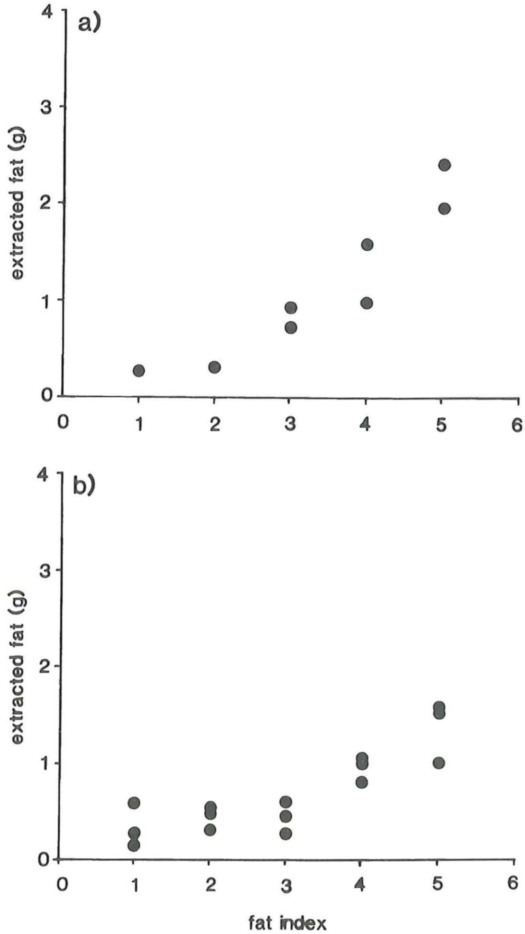

Fig. 1. The relationship between amount of extracted fat (y) and visual fat class (x) in the Willow Warbler. (a) spring birds: regression equation y=–0.56+0.50x, r=0.92, P<0.01, n=8; (b) first year birds in the autumn: regression equation y=–0.08+0.26x, r=0.85, P<0.001, n=15.

Relationen mellan extraherat fett (y) och visuell fettklass (x) hos lövsångaren. (a) vårflyttare, (b) årsungar på hösten. Regressionerna enligt ovan.

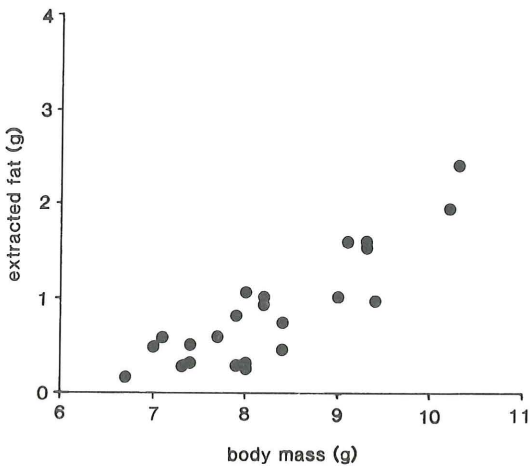

Fig. 2. The relationship between amount of extracted fat (y) and total body mass (x) in the Willow Warbler. Data from spring and autumn are combined as there was no significant difference between the seasons. Regression equation y=–3.56+0.53x, r=0.87, P<0.001, n=23.

Relationen mellan mängden extraherat fett (y) och totala kroppsvikten (x) hos lövsångaren. Data från vår och höst har slagits ihop eftersom det inte fanns någon signifikant skillnad mellan säsongerna. Regressionen enligt ovan.

F=1282.1, P<0.001), followed by total water mass (partial F= 202.1, P<0.001), fat-free dry mass of the carcass (partial F=35.8, P<0.001). Fat-free dry mass of the muscle did not enter the model (partial F=3.2, P>0.05) and as for the spring birds the wing length did not enter the model (partial F= 0.7, P>0.05). These results indicate that variation in total fat and water content are the main contributors to the variation of the total body mass. However, there was a positive correlation between fat-free dry mass of the carcass and wing length (spring birds: r= 0.72, df=6, P<0.05; autumn birds: r=0.91, df=12, P<0.001), which indicates that the wing length reflects the birds' specific size.

We found a positive correlation between total extracted fat mass and fat-free dry mass of the pectoral muscle in spring birds (r=0.83, df=6, P<0.05), while in autumn birds this correlation was not significant (r=0.50, df=13, P>0.05).

Discussion

We found a positive relationship between visual fat class and total extracted fat in both spring and autumn for the Willow Warbler. However, the slope of regressions differed between seasons, and in spring the slope was about twice that in the autumn (Fig. 1). This implies that in spring about 0.5 g fat is deposited per unit of fat class, while in autumn only 0.26 g fat is deposited per unit of fat class. Why this difference between spring and autumn birds emerges is unclear, but it may be associated with the pattern by which fat is deposited around the body or that the birds are in different stages of migration. For instance, in the spring the birds presumably arrive directly from a long flight over the Baltic Sea and if they do not burn fat uniformly from different fat deposits on the body, the fat scoring may underestimate the true amount of fat as compared to the autumn birds, when the birds still may accumulate fat in a uniform pattern. The pattern of fuel deposition and retrieval during migration is not clearly understood and warrants further investigation. Maybe the very detailed fat classing developed by Kaiser (1993) may be a path to increased understanding in this respect.

There is a tendency of curvilinearity of the relationship between fat class and amount of extracted fat (cf. Fig .1), with very small differences in extracted amount of fat between the low fat classes (1–2). This pattern is found also in other species (Kaiser 1993). Hence, it may be difficult to accurately estimate the amount of fat visually in the lowest classes. From fat class 3 there seems to be a more linear relationship between fat class and true amount of fat (cf. Fig. 1).

Some authors have shown a possible flight muscle (pectoralis) hypertrophy associated with premigratory fattening (e.g. Fry *et al.*1972, Marsh 1981, 1984, Lindström & Piersma 1993), presumably in order to compensate for the increased power requirements of flight due to the fuel load. However, Baggott (1975) found this muscle hypertrophy in the Willow Warbler to be associated with a recovery after the post-juvenile moult. Pennycuick (1975) also suggested that the flight muscle itself could serve as an energy reserve during migratory flights. In our study, we found a positive correlation between fat mass and flight muscle mass in autumn birds. Carpenter *et al.* (1993) reported that migrant Rufous Hummingbirds *Selasphorous rufus* probably catabolize flight muscle protein during migratory flights. However, to recover the muscle protein seems to be a very slow process as compared with gaining mass as fat (Carpenter *et al.* 1993). Therefore, if migrating birds are time-selected (*sensu* Alerstam & Lindström 1990), muscle protein should be avoided as fuel for flight as the time lost at the next stopover to recover the flight muscle is significant. Nevertheless, that some protein are catabolized besides fat during migratory flights seems to be a general phenomenon (e.g. Klaassen & Biebach 1994), but why this is so is unclear. Maybe, the size of the flight muscle is adjusted so it matches the fuel load in an optimal way (cf. Pennycuick 1975).

For ringers using the visual fat index, body mass and wing length as tools in studies of stopover and migration strategies, it is of interest to know if the visual fat index is a useful predictor of body mass, and if the body size (wing length) contributes to the variation in body mass. Although our sample sizes are small, the data indicate that in first year birds caught in autumn, both the visual fat index (partial F=24.4, $P<0.001$) and wing length (partial F=12.51, $P<0.01$) significantly contributed to the variation in total body mass. For the birds caught in spring only the visual fat index contributed to the variation in total body mass (partial F=13.07, $P<0.05$), but notice that in spring the sample size was only 8 birds.

In conclusion, this study supports the use of visual fat index and body mass as reliable measures of the fat deposition in the Willow Warbler. The results may be applied to several other species of passerines with a similar fat deposition pattern as the Willow Warbler. For species such as pipits *Anthus* spp. and wagtails *Motacilla* spp., where the fat deposition is more difficult to score visually, new morphological studies should be carried out in order to decide the validity of the fat index.

The results from this study are also in agreement with suggestions of possible muscle hypertrophy in migratory birds associated with fuel deposition. However, more detailed studies are needed in order to clarify the effect on flight muscle size from factors such as moult stage, age and site of capture.

Acknowledgement

We are grateful to Susanne Åkesson and two anonymous referees for comments on an early version of the manuscript. This is contribution no. 151 from Ottenby Bird Observatory.

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Sammanfattning

Korrelation mellan visuellt fettindex och verklig fetthalt hos lövsångare Phylloscopus trochilus

Flyttfåglar delar normalt upp sin flyttningsresa i flygetapper i riktning mot destinationen och däremellan längre eller kortare rastningsperioder för bränsleupplagring. Flygbränslet består till största delen av fett, som lagras på kroppen. Detta fett kan ses under huden på fågelns buk om man försiktigt blåser undan fjädrarna. Vid studier av flyttfåglars rastningsekologi utgör klassificering av fettmängd tillsammans med vägning och mätning av fågelns storlek (ving- eller tarslängd) den information som rutinmässigt registreras av ringmärkare. Men hur väl representerar den visuella fettskalan den verkliga mängen fett som fågeln har? Detta kan bara tillförlitligt undersökas på döda fåglar genom att på kemisk väg extrahera det inlagrade fettet. I denna studie jämför vi den s.k. visuella fettskalan med extraherad fettmängd hos lövsångare *Phylloscopus trochilus* insamlade under vår- och höstflyttning vid Ottenby, Öland. Fåglarna samlades in för analyser av muskelmorfologi hos flyttfåglar, vilket gav oss tillfälle att använda fågelkropparna för fettextraktion. Fåglarna valdes så att individer med fettklass 1–5 blev representerade i materialet. Hos lövsångare under både vår- och höstflyttning förelåg en signifikant positiv korrelation mellan extraherad mängd (massa) fett och visuellt klassificerad fettmängd (Fig. 1). Materialet uppvisade likaså en signifikant positiv korrelation mellan extraherad fettmängd och total kroppsmassa (Fig. 2). En fågels totala kroppsmassa kan bero av en rad faktorer, t. ex. storlek (här representerad av vinglängd), vatteninnehåll, fett- och muskelmassa. För att undersöka vilka faktorer som bäst förklarar variationen hos den totala kroppsmassan gjorde vi en multipel regressionsanalys med total kroppsmassa som beroende variabel. Hos fåglar fångade på våren visade det sig att vattenmängd bäst förklarade variationen i total kroppsmassa, följd av total massa extraherat fett, fettfri torrmassa (exklusive flygmuskler) samt fettfri torrmassa av flygmusklerna. Mönstret var likartat hos fåglar fångade på hösten, men totala massan extraherat fett bytte plats med vattenmängd som den dominerande faktorn. Vinglängd kom inte med i modellen som att signifikant förklara variation i total kroppsvikt. Det var dock en signifikant positiv korrelation mellan fettfri torrmassa och vinglängd både hos vår- och höstfåglar.

Sammanfattningsvis fann vi ett tydligt samband mellan visuellt klassificerad fettmängd och verklig fettmängd hos lövsångare. Den visuella metoden är alltså ett värdefullt hjälpmedel när det gäller att bedöma hur fet en fågel är eller om en individ lagrar upp fett mellan upprepade kontroller på en rastplats.