Ornis Fennica 95: 41–47. 2018

Brief report

Estimation of fat reserves of Robins (*Erithacus rubecula*) migrating through the southern coast of the Baltic Sea in spring

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Received 18 May 2017, accepted 7 December 2017

Fat is the main source of energy during bird migration, and the visual fat scoring is widely used in the studies on, e.g., stopover ecology of migratory species. Yet, visual fat scoring can lead to wrong conclusion due to potentially different pattern of fat distribution in species that differ in size or even individuals being during active migration and those at stopovers. To overcome limitations of this method, we developed the predictive equation for estimating fat reserves in spring-migrating Robins – one of the most numerous migratory species in Europe. The equation allows estimating the amount of fat reserves based on body mass of birds, which was the best predictor of fat mass accumulated by Robins. The predictive value of the equation was reasonable with the coefficient of determination equal to 0.56 and the standard error of estimation *SEE* = 0.36. This equation was tested on the sample of adult and immature Robins captured during spring migration. The studied species is a typical short/medium distance migrant employing short step migration, which does not require large fat reserves. The mean estimated fat mass in spring-migrating Robins varied from 0.7g in individuals with the lowest fat reserves (fat score 0) to almost two-fold higher amount, i.e., 1.38 g, in birds with the high fat score (fat score 4). In the fattest Robins, fat composed about 8% of their total body mass.

1. Introduction

Fat is the main source of energy used by birds during migration (Lindström & Alerstam 1992, Piersma 1998). The amount of accumulated fat in migratory birds is affected by opposite selection pressures, i.e., predator avoidance and endurance migratory flight. Low fat reserves, and consequently lower body mass, increase take off speed and thus help to avoid attacks by predators (Kullberg *et al.* 1996, 1998, Lind *et al.* 1999), while higher fat reserves enable long-distance migratory flight and survival during the periods, when there is no possibility of their replenishment (Lindström & Piersma 1993). The amount of fat reserves varies greatly depending on migration strategy employed by the species (Salewski *et al.* 2009).

Hence, the analysis of fat reserves is widely used in the research on stopover ecology (Bibby & Green 1981, Wojciechowski *et al.* 2014), estimating the potential distance of the nonstop flight (Ellegren & Fransson 1992, Kaiser 1992, Ożarowska 2015), migration phenology (Pilastro & Spina 1997, Prop *et al.* 2003) or survival rate (Evans 1969, Houston & McNamara 1993). The assessment of the amount of fat accumulated by birds plays a crucial role in such studies. In the field studies, visual assessment according to different scoring systems is usually applied to estimate the amount of fat reserves in captured individuals (e.g., Busse & Kania 1970, Kaiser 1993, Meissner 2009).

Otherwise, it can be measured by sacrificing individuals or by the use of technically complex methods, like dual-energy X-ray absorptiometry (DEXA) (Mazess *et al.* 1990). Hence, due to its simplicity in use, the visual fat scoring is nowadays widely applied (Rogers 2003, Salewski *et al.* 2009). Yet, this method sometimes may lead to wrong conclusions because of potentially different patterns of fat distribution during the phase of burning and rebuilding of fat reserves (Åkesson *et al*. 1992). Moreover, visual fat scoring methods may be used in relatively small species with thin skin, through which fat amount may be assessed (Krementz & Pendleton 1990). To overcome this limitation, alternative approaches based on the predictive equations have been proposed. Since such approaches require a sufficient sample of fresh carcass, such equations are available just for a few species of passerines (Carpenter *et al.* 1993, Wojciechowski *et al.* 2014), waders (Castro & Myers 1990, Piersma & Brederode 1990) and ducks (Meissner *et al.* 2012).

Based on fat mass extracted from dead individuals and their linear measurements we aimed to develop, and then test on a sample of alive birds, an equation to estimate the amount of fat reserves in one of the most numerous migratory species in Europe – the Robin (*Erithacus rubecula*).

2. Material and methods

We studied the amount of fat in spring migrating Robins based on two samples, i.e., dead and alive individuals. The first sample was collected during the species spring migration in 2014–2015 and included 74 fresh, complete carcasses (collected up to 4 hours after death of an individual) of 15 adult and 59 immature birds found along the main road on the Hel Peninsula (54°75’ N, 18°54’ E, southern Baltic coast), all probably killed by passing cars. These individuals were collected during the morning hours and were used for deriving the predictive equation for assessing the fat mass.

The second sample was composed of 6 862 individuals captured at Wicie ringing station (54°29’ N, 16°27’ E; central part of the Polish Baltic coast) during spring migration in 2011–2013. This sample was used for testing the predictive equation developed on the basis of laboratory analyses of dead birds. The period when these two samples were collected (April) covered the main part of Robin spring migration in this part of Europe (Zaniewicz & Rosińska 2015).

All Robins were aged according to plumage characteristics given by Svensson (1992). Their wing length (maximum chord) was measured using a ruler with accuracy to the nearest millimetre and they were weighed with accuracy to the nearest 0.1 g with an electronic balance. In individuals captured alive during spring migration, the fat score was checked visually according to the 9 point scale (Busse & Kania 1970 – a modified version of Helms’ & Drury’s (1960) scale). The fat score of Robins caught on migration in the studied region usually did not exceed the score 4 (authors’ unpubl. data). In the sample of 6 862 Robins captured alive in Wicie ringing station, only 0.1% individuals had larger fat reserves than that score. In dead birds fat score was not assessed as it becomes difficult to check this score properly after the death of an animal.

In carcasses total head and tarsus length were measured with a calliper to the nearest 0.1 mm (for details see: Busse & Meissner 2015). No later than 4 hours after finding, the corpses were frozen at –20 °C before further laboratory processing. Frozen carcasses were thawed overnight at room temperature before the next procedures. The carcass without feathers, head and tarsi was cut into small pieces using scissors, and afterwards the sample was weighed again (sample wet mass). The sample was dried in a lab dryer at 60 °C to a constant mass (sample dry mass). In order to deter-

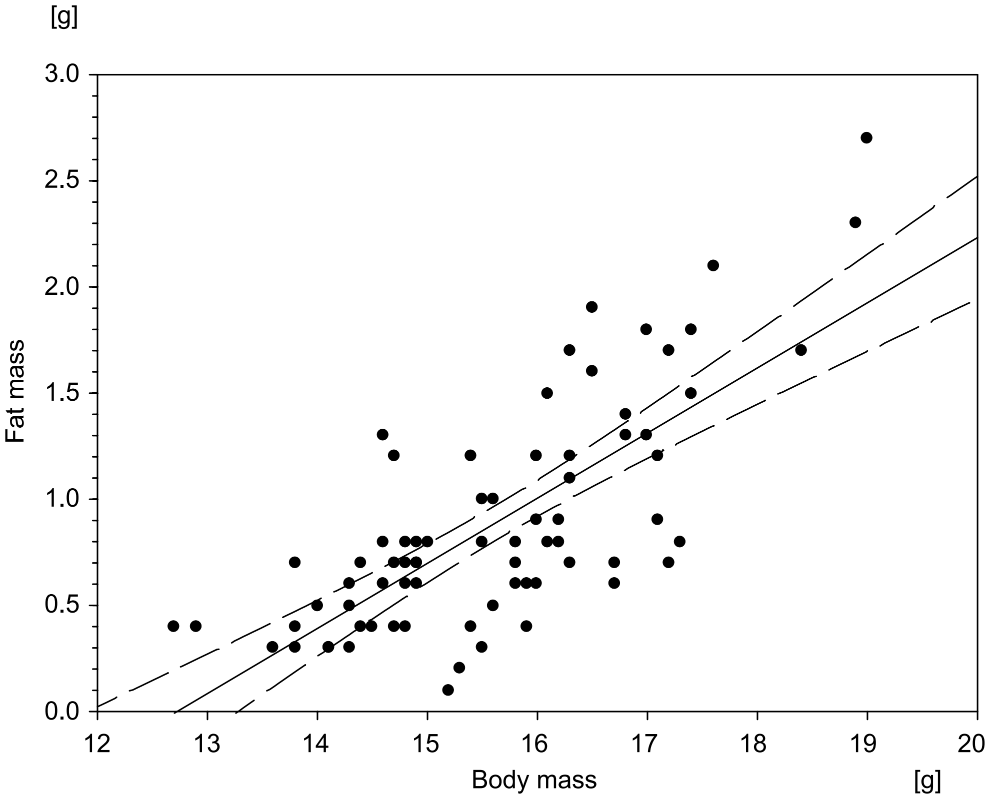

Fig. 1. Relationship between body mass and fat mass in spring migrating Robins (based on the sample of dead birds). The regression line (solid line) is shown. Dashed lines indicate 95% confidence interval.

mine the fat content, extraction of each sample in a Soxhlet apparatus was performed for at least 6 hours using petroleum ether as a solvent (Dobush *et al.* 1985). The fat mass was determined by subtracting the mass of extracted sample from sample dry mass.

2.1. Statistical analyses

The pattern of body mass change was checked to detect potential non-linear effect. The visual inspection of the scatter plot revealed a linear relationship between body mass and extracted fat mass. Moreover the head, wing and tarsus lengths were not correlated ($r < 0.16$ and $p > 0.17$ in all cases). Hence, to estimate fat mass, multiple regression equations were derived based on body mass, total head length, wing and tarsus length of dead individuals. The inclusion of a given measurement into the model was based on forward selection in stepwise procedure with default minimum partial F to enter the model equal to 3.84 and maximum partial F to remove: 2.71.

The obtained equation was applied to the sample of alive Robins captured during spring migration. The sample of alive birds (100 adults and 100 immatures) comprised individuals randomly picked from the group representing each fat score class from 0 to 3. Due to small sample size of birds representing fat class 4, 100 randomly selected immatures and all available, i.e., 33 adults were included into this group. The relationship between body mass and fat score was checked and GLM statistics was applied to determine the relationship between the mean estimated fat mass and fat score classes. The analyses were performed using STATISTICA 12.5 software (StatSoft 2014).

Table 1. Estimated fat mass of Robins representing different fat scores. Birds were captured at Wicie ringing station in 2011–2013. Similar letters indicate the difference in fat mass which was not statistically significant (Tukey's post hoc test, $p > 0.05$).

Fat score	N	Mean body mass (g)	Mean estimated mass (g)	Range	SD	% of fat	Tukey's post hoc test
0	200	15.00	0.70	0.06–1.56	0.29	4.68	a
1	200	15.71	0.92	0.27–1.59	0.26	5.85	b
2	200	16.59	1.19	0.42–2.17	0.31	7.17	c
3	200	17.07	1.33	0.27–2.45	0.39	7.82	d
4	133	17.21	1.38	0.61–2.42	0.33	8.02	d

3. Results

There were no statistically significant differences between adult and immature Robins in total head, tarsus and wing lengths in the sample of dead birds from the Hel Peninsula (t-test, $p > 0.211$ in all cases) and in wing length of individuals captured alive at Wicie ringing station (t-test, $t = 1.39$, $p = 0.165$). Moreover, the mean mass of fat extracted from adult and immature Robins did not differ significantly (t-test, $t = 0.79$, $p = 0.43$). There was also no interaction between age and body mass (Wald $\chi^2 = 0.33$, $p = 0.56$). Therefore, the two age groups were pooled in all further analyses.

Body mass was the only predictor of fat mass accumulated by spring migrating Robins (Wald $\chi^2 = 133.5$; $p < 0.001$). Either linear measurements or age had no significant influence on estimated fat mass ($p > 0.102$ in all cases) and there was no significant correlation of fat mass with other linear measurements (Pearson r; $p > 0.17$ in all cases). Hence, the regression function for estimating fat mass in Robins was:

Fat mass = 0.307 Body Mass – 3.904

The predictive value of the model was reasonable with the coefficient of determination (R^2) equal to 0.56 and the standard error of estimation $SEE = 0.36$. There was also strong relationship between body and fat mass (Fig. 1) and 85% of all individuals were within a band of ± 0.5 g and 100% within ± 1g running along the regression line. According to the regression coefficient, with the increase in body mass of 1 g, the fat mass increased by 0.307 g ($SE = 0.032$, $t_{72} = 9.605$, $p < 0.001$).

The mean estimated fat mass in Robins caught at the Wicie ringing station varied from 0.7 g in individuals with fat score 0 to almost two-fold higher amount, i.e., 1.38 g, in birds with fat score 4 (Table 1). There was moderate correlation between body mass and fat score (correlation coefficient: $r = 0.61$). The relationship between the estimated fat mass and fat score was significant (GLM, $F_{1,931} = 553.8$, $p < 0.001$). Mean fat mass of Robins representing following fat scores: 0, 1, 2, and 3 differed significantly between each other, while there was no such difference between individuals of fat scores 3 and 4. In the fattest Robins, fat composed 8% of their total body mass (Table 1).

4. Discussion

The relationship between the changes in body mass and its composition is complex and depends on homeostatic balance among three main components of the bird body – fat, protein and water. Since fat provides eight times more energy than protein per unit of the fresh mass (Jenni & Jenni-Eiermann 1998), it is the main source of energy that plays a crucial role during migration. In small passerines, flight is powered mostly by fat (85–95%) and in lesser extent by protein (5–15%) (Jenni & Jenni-Eiermann 1998, Klaassen *et al.* 2000). According to the predictive equation for estimating fat reserves in spring-migrating Robins developed in our study, over a half of the variation in fat mass was determined by body mass.

Although the precision of this estimation is lower when compared to such equations developed for some passerines, like the Blackcap (*Sylvia atricapilla*) ($R^2 = 0.84$) (Korine *et al.* 2004), yet quite similar to that for the Garden Warbler (*Sylvia borin*) ($R^2 = 0.60$) (Barboutis *et al.* 2011). This result is also consistent with the data given in the review article by Labocha & Hayes (2012), who reported that in most studies predictive equations explained slightly more than 50% of the variation in fat content in birds. It might be that sex of birds could explain some part of the variation but the Robin is a monomorphic species. On the other hand, in the Blackcap, in which sexual dimorphism is obvious and some populations are long distance migrants (Wojciechowski *et al.* 2014), the variation of body mass and fat mass did not differ between males and females ($F_{1,20747} = 0.173$, $p = 0.68$).

Validation of fat scoring in Robins based on the predictive equation showed that estimated fat amounts were significantly different among scores from 0 to 3, while there was no such difference between higher scores: 3 and 4. These results may indicate that the ranges of estimated fat amounts in scores 3 and 4 are too wide to rely exclusively on visual scoring system in these two classes. They also show that using visual scoring method for this species may only roughly estimate the amount of fat reserves.

The Robin is a typical medium or even short distance, night migrant, resting and/or foraging to replenish energy resources during the day time (Szulc-Olech 1965, Bolshakov & Rezvyi 1998). Moreover, this species employs short step migration (Pettersson & Hasselquist 1985). It was shown that such strategy does not require large amounts of fat (Pettersson & Hasselquist 1985), which was confirmed by the results of our study. This may be also the reason why the relationship between estimated fat mass and body mass was linear and did not show two phases of fattening, i.e., initially slow rate of mass gain or even decrease in body mass observed in migrating birds at stopover sites, followed by rapid body mass increase due to high rate of fattening, which were reported in long-distance migrants (Barboutis *et al.* 2011, Wojciechowski *et al.* 2014).

Usually predictive equations for fat mass assessment in birds should be used only in certain periods or regions because the proportion of fat and non-fat components may differ within a given species seasonally and geographically (Biebach 1990, Åkesson *et al.* 1992, Olsen & Cox 2003). Migrating Robins as short/medium distance migrants carry low fat reserves either in the spring or autumn migration period (Åkesson *et al.* 1992), yet for the reasons given above our predictive equation should be used with caution for individuals captured during autumn migration.

Punarinnan kevätmuutonaikaisen rasvavaraston estimointi – uusi ennustemalli

Rasva on muuttavien lintujen tärkein energian lähde. Rasvan määrän silmämääräistä arviointia käytetään paljon esimerkiksi muuton välilaskujen tutkimuksessa. Silmämääräinen arviointi ei kuitenkaan ota huomioon rasvan erilaista jakautumista kehon eri osiin. Esimerkiksi linnun koko tai muuton vaihe voi vaikuttaa rasvan jakautumiseen. Kehitimme laskennallisen ennusteen rasvan määrälle kevätmuuttavilla punarinnoilla. Tässä yhtälössä rasvan määrä ennustetaan painon avulla. Paino selitti 56 % (virhe 36 %) rasvan määrän vaihtelusta.

Testasimme mallia sekä aikusilla että nuorilla linnuilla. Punarinta on keskimatkan muuttaja, joka etenee lyhyissä muuttojaksoisa, eikä tarvitse isoja rasvavarastoja. Havaitsimmekin että rasvan määrä vaihteli 0.7 g (luokka 0) ja 1.38g: n (luokka 4) välillä, suurimmillaan vain 8 % ruumiinpainosta.

Acknowledgements. We would like to thank all ringers and volunteers involved in the field work at the Wicie ringing station. We are also grateful to the Board of the Bird Migration Research Foundation for financial support. As last but not least we thank Olga Ponieważ and Katarzyna Roziewska who were involved in data collection.

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