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Weber, T.P.; Piersma, T.; Weber, Thomas P.

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Basal Metabolic Rate and the Mass of Tissues Differing in Metabolic Scope: Migration-Related Covariation between Individual Knots *Calidris canutus*

Author(s): Thomas P. Weber and Theunis Piersma

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Basal metabolic rate and the mass of tissues differing in metabolic scope: migration-related covariation between individual Knots *Calidris canutus*

Thomas P. Weber and Theunis Piersma

Weber, T. P. and Piersma, T. 1996. Basal metabolic rate and the mass of tissues differing in metabolic scope: migration-related covariation between individual Knots *Calidris canutus*. – J. Avian Biol. 27: 215–224.

To examine whether variability in the basal metabolic rate (BMR) of migrant shorebirds is a function of a variably sized metabolic machinery or of temporal changes in metabolic intensities at the tissue level, BMR, body composition and activity of cytochrome-c oxidase (CCO, a marker for maximum tissue respiration) were measured in 14 captive Knots *Calidris canutus islandica* in late spring, during the period of mass loss after the migratory body mass peak. Although the body mass cycle of captive birds closely followed the changes of free-living conspecifics, their fat-free mass of muscles and organs was somewhat lower and their fat content higher. BMR significantly declined during mass loss, as did the fat-free dry mass. BMR was an allometric function of both body mass (exponent = 0.687) and lean dry mass (exponent = 1.132). Fat-free dry mass of heart and flight muscle decreased with the loss of fat. CCO-activity was determined in heart, flight muscle, leg muscle, liver and kidney. It was highest in heart and flight muscle and low in the other tissues. CCO-activity was not correlated with total fat mass. Intraspecific migration-related variation in BMR seems better explained by variation in the mass of organs with a high metabolic scope (as indicated by high CCO-activity), than by variation in the intensity of tissue metabolism.

T. P. Weber, Zoological Laboratory, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands, and BSRC/NERC Ecology and Behaviour Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K. T. Piersma (corresponding author), Netherlands Institute for Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands, and Zoological Laboratory, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands. E-mail: theunis@nioz.nl

The basal metabolic rate (BMR), i.e. the rate of energy utilization of postabsorptive homeothermic animals under thermoneutral conditions (Aschoff and Pohl 1970), is a key concept in the study of avian energetics. BMR is commonly used as a reference point to evaluate energy intake or expenditure. For example, values for the ratio between daily energy expenditure and BMR during breeding (Drent and Daan 1980, Bryant and Tatner 1991), and between sustained metabolic rate and BMR (Peterson et al. 1990), usually fall between 1.5 and 5. Short term peak rates can yield much higher ratios (Bartholomew 1977, Peterson et al. 1990).

However, even when BMR is measured in a standardized way (see e.g. Aschoff and Pohl 1970, Hayes et al. 1992), it varies in the course of the annual cycle of a bird. In fact, seasonally variable BMR-levels (e.g. Wijnandts 1984, Dietz et al. 1992) may explain part of the inter- and intraspecific variation in the reported ratios between energy expenditure and BMR. Recently, Piersma et al. (1995) demonstrated that BMR of captive individuals of a long-distance migrant shorebird species, the Knot *Calidris canutus*, peaked when body mass was high during the period of northward migration. BMR of Knots in early June increased by 40% above their average level during the rest of the year.

Long-distance flight is energetically demanding. A multitude of physiological mechanisms allows birds to meet these demands, many related to the storage and subsequent mobilization of large amounts of fat (Blem 1990, Ramenofsky 1990, Jenni-Eiermann and Jenni 1992). There is also considerable flexibility in organ (including muscle) sizes (Davidson and Evans 1986, Evans et al. 1992, Piersma et al. 1996). For example, large fuel stores may require a large musculature for transport (Pennycuik 1978, Piersma 1990, Piersma and Jukema 1990, Lindström and Piersma 1993). Studies on mammals have indicated that a large part of BMR is generated by the brain and by the organs in the abdominal cavity (Aschoff et al. 1971, Schmidt-Nielsen 1990). However, since muscle tissue accounts for some 20% of the lean mass of Knots (pers. obs.), even small variations in muscle mass and muscle respiration could affect BMR. The high level of muscular activity during long-distance migration also requires a high level of support in the form of fuel supply and waste degradation by the organs in the abdominal cavity (Kersten and Piersma 1987, Jenni-Eiermann and Jenni 1991). It is therefore possible that migration-related changes in the size of these organs additionally explain variable BMR-levels (Piersma et al. 1996).

Enzymatic or biochemical characteristics have been employed frequently to investigate tissue-specific responses to exercise, environment and events in the annual cycle (Holloszy 1975, Marsh 1981, Dawson et al. 1983, Lundgren and Kiessling 1985, 1986, Butler and Turner 1988, Carey et al. 1989, Saunders and Klemm 1994, O'Connor 1995). An indirect method to quantify tissue respiration is measurement of the activity of cytochrome-c oxidase (CCO), the terminal step in the electron transport system. This approach is implicitly based on the concept of symmorphosis, which states that morphological structures, biochemical pathways and enzyme concentrations will develop and be maintained to the degree required for meeting maximum demands (Taylor and Weibel 1981; a critique of this concept is given by Dudley and Gans 1991). The enzymatic approach offers a straightforward alternative to conventional *in vitro* respirometry which is affected by the method of tissue preparation (Oikawa and Itazawa 1983) and medium composition (Krebs 1950). *In vitro* respirometry gives oxygen consumption rates at basal level, whereas, according to the principle of symmorphosis, enzyme activities should reflect maximum levels. Yet, in mammals CCO-activity seems well correlated with *in vitro* oxygen consumption rates for different tissues (Simon and Robin 1971), a relationship which, to our knowledge, has not been established for other enzymes frequently used as markers for metabolic activity. The way in which physiological adjustments affect BMR depends on the extent to which BMR is a function of (1) the mass of the metabolically active parts of the body, (2) the intensity of tissue metabolism,

or (3) a combination of (1) and (2). If BMR is only a function of the mass of metabolically active (fat-free) tissue (e.g. Scott and Evans 1992) measuring the trajectories of fuel gain in terms of fat and fat-free tissue (Lindström and Piersma 1993, Van der Meer and Piersma 1994) would be the only requirement for estimates of migration-related changes in basal expenditure and metabolic scope, parameters which are relevant for understanding constraints on fuel deposition rates (Lindström 1991). To test hypotheses (1)–(3), we have measured BMR, CCO-activity in selected tissues and the dynamics of body composition in captive Knots during and after the period of peak mass in connection with their (non-executed) northward migration. The necessity to relate seasonal changes in metabolic performance and body composition to known circannual history forced us to use partially restrained, rather than free-living, individuals.

Material and methods

Experimental animals

Measurements were carried out during June 1992 on 14 adult Knots of the subspecies *C. c. islandica* (see Piersma et al. 1991, Davidson and Wilson 1992, Piersma and Davidson 1992 for reviews of this and other subspecies, and their migrations and annual cycles). The birds had been captured in the Wadden Sea during October–March, 1–3.5 years earlier, and were kept in outdoor aviaries (2 m × 4 m × 1.8 m) at the Zoological Laboratory in Haren, The Netherlands (53°11'N, 06°36'E), in groups of 4–8 individuals. Floors of the aviaries were covered with regularly refreshed sand. A 30 cm by 60 cm basin with continuously flowing water gave the birds the opportunity to drink and bathe. The birds were fed protein-rich (45% of fresh mass) trout food pellets *ad libitum*. At weekly intervals all the birds were weighed to the nearest 0.1 g on a Sartorius electronic balance, checked for injuries and scored for body-moult. All the Knots maintained a clear circannual body mass cycle and a normal pattern of moult and plumage changes (Piersma et al. 1995). They showed enhanced flight activity in the cages during the normal migration periods (pers. obs.).

BMR measurements

Twenty-four hours prior to the experiment, the birds were transferred to a separate outdoor cage, where they were kept without food but with fresh water. Since the throughput time of food in the gut of Knots is 2 h at most, this procedure ensured that the birds were post-absorptive at the start of the oxygen measurements. Before entering the respiratory chambers of 15 or 20 l,

the birds were weighed to the nearest 0.1 g, and then placed individually in temperature-controlled cabinets at 25°C, i.e. within the thermoneutral zone of Knots (Wiersma et al. 1993). Dry air was pumped into the sealed chamber. Flow rates were set at approximately 90 l/h (for the 15 l chamber) or 120 l/h (20 l chamber) and measured on the dry inlet air with a Mass Flow Controller (Brooks Instruments, Veenendaal, The Netherlands, Model 5850E) with an accuracy of 0.4%. Gas analyses were performed on samples of inlet and outlet gases, both dried with a molecular sieve (3 Å, granules approximately 2 mm; Merck). The oxygen concentration of the dried ingoing and outflowing air was measured by an oxygen analyser (AMETEK Applied Electrochemistry Pittsburgh, PA, USA, Models S-3A and S-3A/II) with an accuracy of 0.002%. Calibration was performed using dry, oil-free air mixtures. The use of a mass flow meter made it unnecessary to transform the data to standard temperature and pressure. Oxygen consumption in ml/h was calculated after Hill (1972). As post-absorptive Knots had an RQ of approximately 0.73 (T. Piersma, L. Bruinzeel and P. Wiersma, unpubl. data), the energy equivalent per volume oxygen was taken as 20 kJ/l O₂ (Gessaman and Nagy 1988, Schmidt-Nielsen 1990).

The measurements started between 18 and 20 hours, and ended around 09 hours the following morning, when the birds were reweighed. The data were checked immediately and BMR was estimated from a 60 min running mean of oxygen consumption rate (see Piersma et al. 1995 for details). The birds were then killed by cervical dislocation. Parts of flight muscle, leg muscle, kidney, liver and the whole heart were dissected in this fixed sequence. They were frozen in liquid nitrogen as quickly as possible, the whole procedure taking a maximum of 10 min. The tissue samples were stored at -80°C until further analysis. The rest of the carcass was either directly dissected into body components, or first stored in air-tight plastic bags at -18°C for a few weeks before dissection.

Body composition analysis

All flight and contour feathers were plucked and the naked carcass dissected. The sex of the bird was determined by gonadal inspection. The different organs were taken out (see Summers et al. 1992, Piersma et al. 1996 for details) and dried separately to constant mass at 55–60°C. Flight muscle refers here to the combination of the *m. pectoralis* and the *m. supracoracoideus*. Each component was reweighed, packed in filter paper and the fat extracted in a Soxhlet apparatus using petroleum-ether (boiling traject 40–60°C) as a solvent for 8 to 24 h depending on the size of the component, dried at 55–60°C and weighed again. The loss of dry

mass during fat extraction was taken as the extracted fat mass. The values for leg muscle, flight muscle, kidney and liver were corrected for the parts removed for the enzyme assays. The fat-free dry mass of the heart was estimated from fresh mass on the basis of data from carcass analyses of other samples of Knots (T. Piersma, unpubl. data).

To evaluate the absolute masses of the various body parts, the values for the captive birds were compared with the values of a similarly analysed wild Knot, collected a few days after its arrival in the breeding areas in the Canadian High Arctic on 10 June 1990 (Alert, Ellesmere Island, 82°30'N, 62°W).

Measurement of CCO-activity

Tissue samples were thawed, weighed, washed in isotonic medium and then transferred into 30 ml of cold Na-K-Phosphate buffer (0.03 M) at pH 7.4. Aprotinin (final concentration 2 µg/ml) and phenylmethylsulfonylfluoride (PMSF; final concentration 100 µg/ml) were added as proteinase inhibitors. The tissues were then homogenized mechanically for 2 min with an Ultrathurax® homogeniser. Two samples of 500 µl of each tissue were taken and n-dodecyl b-D-maltosid added as detergent (final concentration 0.75%). The samples were incubated for 15 min at room temperature and subsequently centrifuged at 0–4°C at 1200 g for 20 min. The supernatant, avoiding the surface lipid layer, was used for analysis of the CCO-activity. The assay method used is based on the oxidation of reduced cytochrome-c (Cooperstein and Lazarov 1950, Simon and Robin 1971). The reaction was monitored by following the decline in absorbency at 550 nm of a solution of reduced cytochrome-c using a Beckmann DU-7 spectrophotometer. Assays were conducted at room temperature (20–22°C). Samples were diluted until a linear decrease in absorbency over 2–3 min could be observed. CCO-activity was expressed as nmol of cytochrome-c oxidised per min per mg of extracted tissue protein. A zeroth order reaction was assumed. We used a millimolar extinction coefficient of 29.5 for reduced cytochrome-c (Goolish and Adelman 1987). The protein concentration of the supernatant was determined with the Pierce BCA-Proteinassay® (Pierce, Rockford, Illinois, USA), using bicinchoninic acid (Smith et al. 1985).

Statistics

Statistical analyses were carried out in StatView®. We used one-tailed significance tests where we had a hypothesis about the direction of a correlation.

Table 1. Summary data for sex (F = female, M = male), mass and BMR of the captive Knots used in this study. We failed to identify the sex of #620.

Bird ID	Sex	Peak mass (g)	Mass at measurement (g)	Days after peak mass	BMR (W)
#412	F	195.0	192.3	2	0.91
#001	F	182.2	179.0	4	0.97
#237	F	163.4	155.7	4	0.95
#371	M	176.4	163.3	4	1.33
#408	F	192.1	146.4	8	1.42
#370	M	164.5	154.9	9	0.93
#406	F	186.5	177.5	15	0.97
#372	F	192.2	165.6	20	0.84
#620	?	164.6	110.9	21	0.65
#405	F	184.6	113.9	21	0.80
#621	M	214.2	98.3	24	0.65
#154	M	165.8	124.8	28	1.00
#373	F	178.4	132.1	28	0.81
#616	M	152.7	93.6	31	0.68

Results

The Knots reached maximum body mass between 22 and 29 May (Table 1), exactly as their free-living conspecifics (Wilson and Morrison 1992). Peak body mass varied between 152.7 and 214.2 g (mean = 179.5 g, SD = 16.3). There was no difference in peak mass between the sexes; the mean was 174.7 g for males (SD = 23.61, N = 5), and 184.3 g for females (SD = 10.13, N = 8; Student's t-test, $t = 1.026$, $P = 0.33$). Body mass immediately before the BMR measurements varied between 93.6 and 192.3 g. The mass loss after migration-related peak levels is a consequence of voluntary reductions in food intake (cf. Kersten and Piersma 1987). Average mass loss, based on the individual rates, was 2.17 g/day (SD = 1.45). The highest rate of loss was incurred by bird #408 with 5.71 g/day.

Among the individuals, body mass decreased significantly with time (Fig. 1A) (body mass [g] = 181.3 - 2.44 time [days], N = 14; $r^2 = 0.619$; slope: SE = 0.552, $t = -4.419$, $P = 0.0008$). Most of this mass loss was attributable to fat since the slope of the relationship between total fat and time was -1.81 g/day (N = 14; $r^2 = 0.602$; SE = 0.425, $t = -4.260$, $P = 0.0011$, Fig. 1C). These two slopes are not significantly different ($t = 0.42$, $df = 24$, $P > 0.25$). Total fat-free dry mass (FFDM) also decreased significantly with time (Fig. 1B) (FFDM [g] = 41.21 - 0.27 time [days], N = 14; $r^2 = 0.47$; slope: SE = 0.081, $t = -3.266$, $P = 0.007$). BMR measurements were conducted from 2 to 31 days after the date of peak mass (Table 1).

BMR varied between 0.65 W and 1.42 W, and showed a significant decrease with time after peak mass (Fig. 2) (BMR [W] = 1.131 - 0.014 time [days], N = 14; $r^2 = 0.37$; $t = -2.652$, $P = 0.0211$). BMR showed a significant linear relationship with total body mass in a double-logarithmic scale (Fig. 3A) ($\log \text{BMR [W]} = -1.523 + 0.687 \log \text{body mass [g]}$, N = 14; $r^2 = 0.442$; intercept: SE = 0.478, $t = -3.184$, $P = 0.0079$; slope:

SE = 0.223, $t = 3.082$, $P = 0.0095$). This slope is statistically indistinguishable from the slope (0.729) of an interspecific regression for six shorebird species, not including the Knot (Kersten and Piersma 1987).

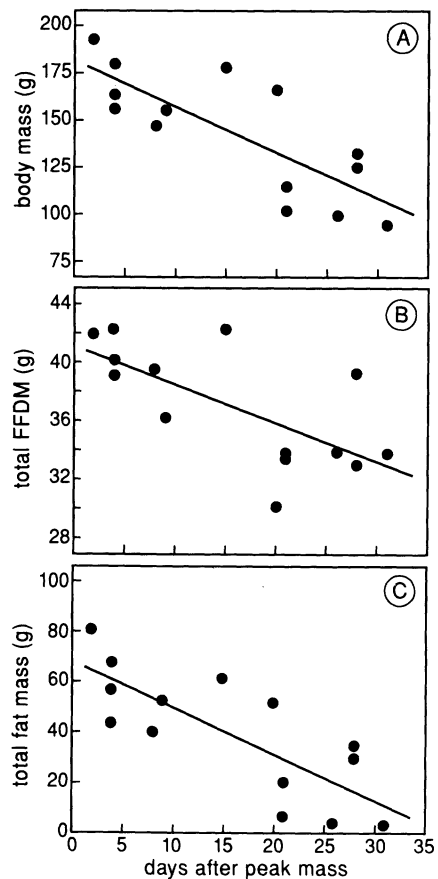


Fig. 1. Body mass (A), fat-free dry mass (B), and fat mass (C) of captive Knots as a function of the number of days since body mass showed its peak value (for the putative northward migration).

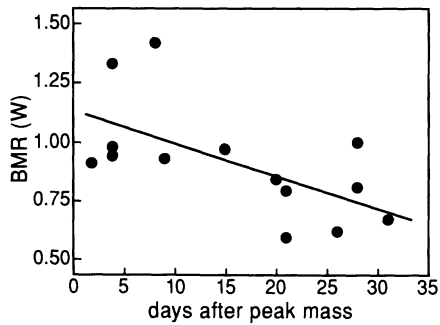


Fig. 2. BMR of Knots as a function of the number of days since body mass showed its peak value (for the putative northward migration).

Total body mass includes many components with low or no metabolic activity, especially fat (Scott and Evans 1992) and water. Fat-free dry mass rather than total body mass should reflect the metabolically active tissue (Piersma et al. 1996). The slope of the double-log regression between BMR and fat-free dry mass was statistically significant in a one-tailed test (Fig. 3B) ($\log \text{BMR [W]} = -1.823 + 1.132 \log \text{FFDM [g]}$, $N = 14$; $r^2 = 0.255$; intercept: $\text{SE} = 0.875$, $t = -2.082$, one-tailed $P < 0.05$; slope: $\text{SE} = 0.559$, $t = 2.026$, one-tailed $P < 0.05$). Remarkably, however, BMR and total fat mass showed an even stronger relationship ($\log \text{BMR [W]} = -0.296 + 0.17 \log \text{fat [g]}$, $N = 14$; $r^2 = 0.538$; slope:

$\text{SE} = 0.046$, $t = 3.736$, $P = 0.0028$). Regressing the residuals of the double-log regression of BMR and fat-free dry mass against the logarithm of fat mass gives a significant relationship in a one-tailed test (residuals = $-0.151 + 0.105 \log \text{fat [g]}$, $N = 14$, $r^2 = 0.272$; $t = 2.119$, one-tailed $P < 0.05$).

To answer the question which physiological mechanism may cause the observed change in BMR with body mass and time, we looked for variations in fat-free dry mass and CCO-activity of the tissues. To take interindividual differences in the timing of programmed mass changes into account, we used total fat as the independent (temporal) axis (Van der Meer and Piersma 1994). Fat mass and FFDM showed a significant positive correlation ($r = 0.669$, $P = 0.0072$). Of the tissues used in the enzyme assay, only the fat-free dry mass of heart ($r = 0.664$, $P = 0.0079$) and flight muscle ($r = 0.742$, $P = 0.0015$) showed significant correlations with fat mass (Fig. 4); the value for leg muscle mass was $r = 0.166$, $P = 0.58$, for liver $r = 0.27$, $P = 0.36$, and for kidney $r = 0.248$, $P = 0.4$.

To get a general idea about the relative metabolic intensity characterizing the investigated tissues, all the values of the CCO-activity for each tissue type were pooled and the mean calculated (Fig. 5). There are significant differences in activity; because the variances are not homogeneous the original data were log-transformed to carry out an ANOVA ($F = 24.921$, $P < 0.0001$). Two groups of tissues can be distinguished clearly: heart and flight muscle have a much higher activity than the three other tissues. There is no significant difference in the activity of heart and flight muscle (Scheffé's test, $P = 0.79$) and no significant difference between the other three tissues (Scheffé's test for all pairs, $P = 0.99$). Heart and flight muscle are significantly different from the three other tissues (Scheffé's test, $P < 0.0001$ for all pairs).

Generally, the values for the CCO-activities were variable. Using untransformed data, the coefficients of variation (SD/mean) are 66.5% for heart, 80.8% for flight muscle, 54.3% for leg muscle, 46.8% for liver and 44.4% for kidney. To see if some of this variation is a consequence of changes in total body mass and fat load, Fig. 6 gives CCO-activity in relation to fat mass. None of the tissues exhibited a significant correlation between CCO-activity and fat load ($N = 14$; heart: $r = -0.008$, $P = 0.98$; flight muscle: $r = -0.269$, $P = 0.36$; leg muscle: $r = 0.343$, $P = 0.31$; liver: $r = 0.334$, $P = 0.25$; kidney: $r = 0.148$, $P = 0.62$).

In heart and flight muscle, protein content per gram fresh mass varied neither with body mass (heart: $r = -0.449$, $P = 0.11$; flight muscle: $r = 0.018$, $P = 0.95$) nor with fat mass (heart: $r = -0.373$, $P = 0.19$; flight muscle: $r = 0.054$, $P = 0.86$). The mean level for heart muscle ($N = 14$) was 0.103 ± 0.011 g protein/g and for flight muscle ($N = 13$) 0.139 ± 0.043 g protein/g (mean \pm SD).

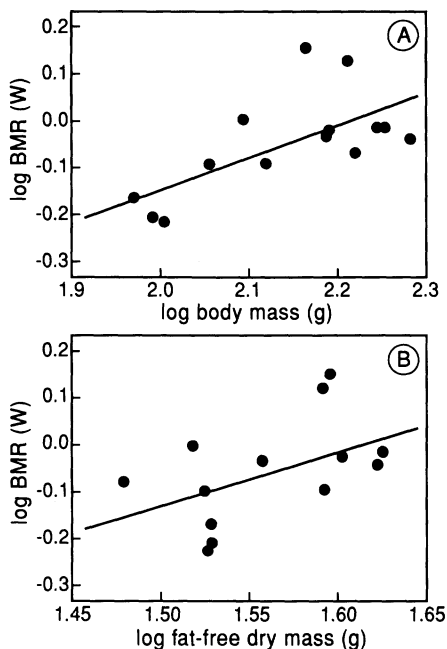


Fig. 3. Allometric relationship between log BMR and log body mass (A) and the log total fat-free dry mass (B) of captive Knots from the northward migration and breeding season period.

Discussion

This study confirms that BMR of Knots varies a great deal during the migration season (Piersma et al. 1995). The measured values of BMR are mostly lower than

1 W, the predicted value of BMR for a shorebird such as a Knot (Kersten and Piersma 1987). One critical assumption in many studies on avian energetics is that the metabolic rates of captive birds are similar to those

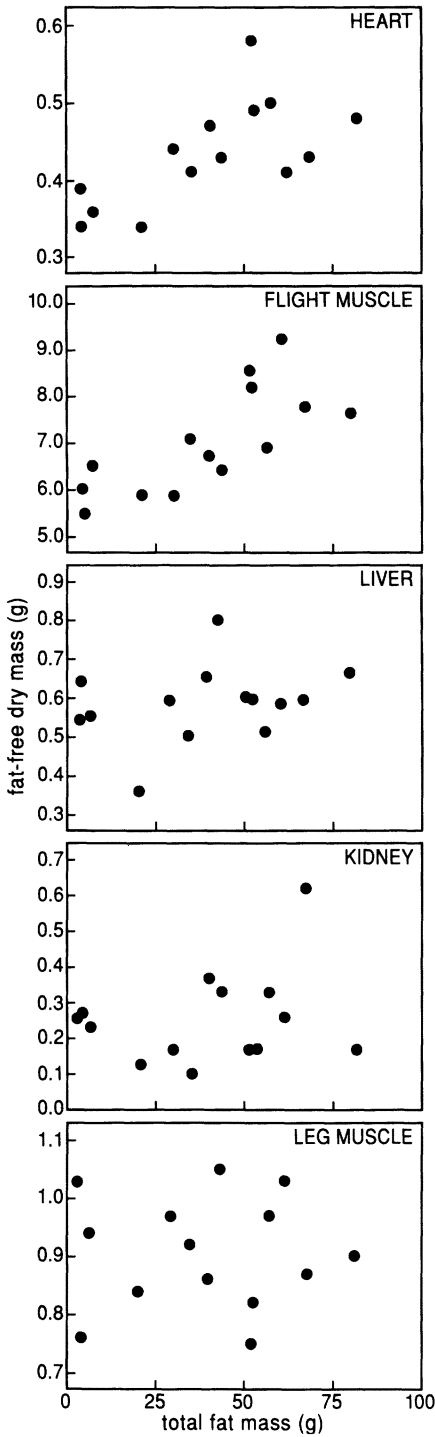


Fig. 4. The fat-free dry masses of the tissues used in the enzyme assays, as a function of total fat mass of Knots.

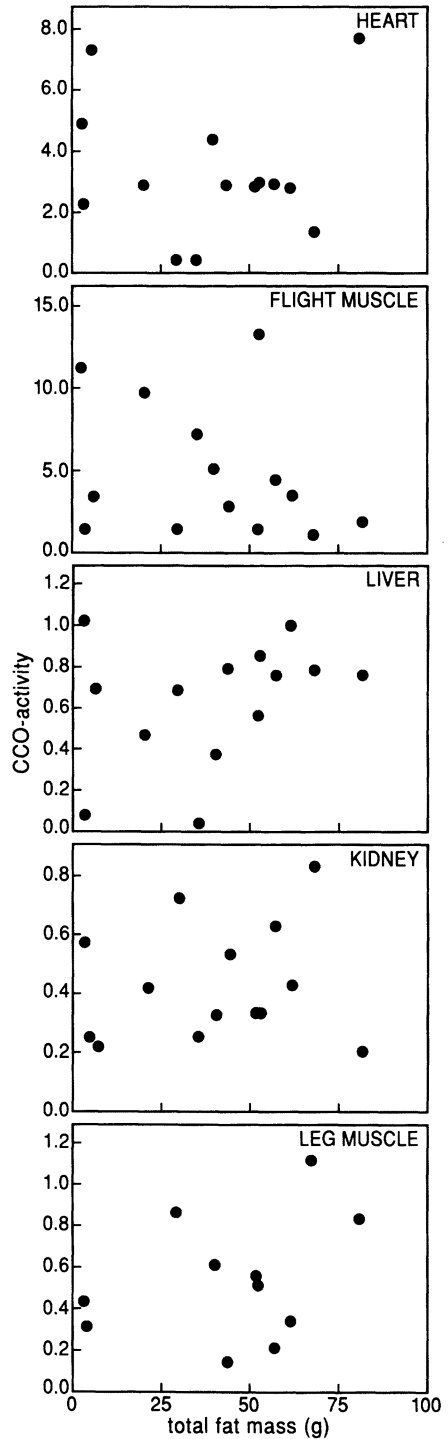


Fig. 5. The CCO-activity (in nmol substrate oxidized per min per mg of extracted tissue protein) of the tissues (of Knots) used in the enzyme assay as a function of total fat mass.

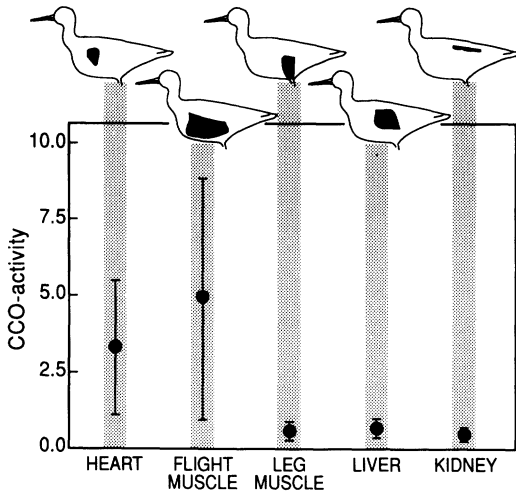


Fig. 6. Mean CCO-activity (in nmol substrate oxidized per min per mg of extracted tissue protein, \pm SD) for five tissue types. Values for all individual Knots were pooled.

of free-living birds. This is less critical here, because we are interested in changes in BMR rather than in absolute levels. Weathers et al. (1983) compared Apapane *Himantone sanguinea* living for one year in captivity with freshly captured birds. They found no significant impact of captivity on BMR, body mass or body temperature. Warkentin and West (1990) reported a significant increase in BMR of Merlins *Falco columbarius* during long-term captivity. The different activity level and food supply of captive birds compared to wild birds could lead to changes in body composition and, consequently, to changes in metabolic rates (Piersma et al. 1996). Only detailed body composition analyses of wild and captive birds in comparable situations can provide satisfying answers. The basic body composition data of the Knots used in our measurements are presented in Table 2, and compared to the data of the single comparably analysed free-living Knot from the same time of the year. The best comparison is for birds of similar body mass, and this has been done by contrasting the average values for #373 and #154 with bird #1052 from Ellesmere Island. Although bill length and body mass are similar, the fat content of the captive Knots is rather larger than that of the wild bird, and the fat-free mass of the different tissues smaller. The differences between captive and wild birds are most pronounced in fat-free dry masses of liver and kidney.

This does not mean that our results for captive Knots bear no relevance to the biology of free-living birds: the seasonal changes in body mass closely parallel those of their free-living conspecifics (Piersma et al. 1995). It only means that the absolute levels of BMR and tissue metabolism may rather be biased downwards (Piersma et al. 1996). Any intraspecific differences found, indicate intraspecific differences of wild birds as well. At least qualitatively, the seasonal body mass and BMR

cycles of free-living Knots are maintained by birds in captivity (Piersma et al. 1995), and we have evidence that such circannual cycles are endogenously controlled (Cadée et al. 1996).

The slope of the allometric relationship between BMR and body mass is close to the value of 0.67 deduced by Heusner (1982) on the basis of theoretical considerations. However, the basic assumption of his argument (no change in body composition), is clearly not met. The slope of BMR on fat-free dry mass is considerably steeper than 0.67. Similarly high values were found by Daan et al. (1989) for an intraindividual comparison in Kestrels *Falco tinnunculus*. This means that heavy birds have an energy turnover considerably above that expected from homomorphic change (Heusner 1987). It is likely that this is a consequence of a disproportionate increase in the mass of metabolically active tissue.

Interestingly, the ranking of the tissues according to enzyme activity differs from the rank order of oxygen consumption reported in the literature. Field et al. (1939) and Simon and Robin (1971) found the highest oxygen consumption rates in heart and kidney. In contrast, Else and Hurlbert (1981) found only very small differences in CCO-activity between liver, kidney and heart of the mouse *Mus musculus*, but they calculated activity with respect to wet mass. Kidney and liver had very low activities in our experiments. Differences between birds and mammals, and differences in the content of extractable protein of organs could possibly explain some of the discrepancies.

Although we found no significant fat-mass related changes in mass-specific aerobic capacity, mass-specific changes in metabolic rate cannot be completely ruled out. CCO-activity values were variable and sample sizes were quite low. Dimauro et al. (1987) report that coefficients of variation of CCO-activities of human tissues are in the range of 32–42%. Given that the determination of protein is a further experimental step that introduces some error, our values and their variability (CV = 44–81%) are not unusual. Note further that there is a non-linear, accelerating relationship between CCO-activity and tissue respiration (Simon and Robin 1971). Small changes in enzyme concentrations can, therefore, go together with large changes in oxygen consumption. The enzyme assay may not have been sensitive enough.

Lundgren and Kiessling (1986) showed a higher activity of CCO in the pectoralis muscle of migratory Reed Warblers *Acrocephalus scirpaesus* than in pre-migratory individuals. In contrast, there were no migration-related changes in mass-specific oxidative capacity of pectoralis muscles in Semipalmated Sandpipers *Calidris pusilla* (Driedzic et al. 1993) and Gray Catbirds *Dumetella carolinensis* (Marsh 1981). Furthermore, no changes in oxidative capacity of the pectoralis muscle associated with winter acclimatization have been de-

Table 2. Body composition of the captive Knots used in this study, and a comparison between a wild-captured bird after arrival from migration on the breeding grounds in arctic Canada (10 June 1990, Alert, Ellesmere Island, NWT) and the average of two similarly weighing experimental birds (#373 and #154). Note that total body mass of the wild bird has been adjusted to the average water% of fat-free mass of the, slightly dehydrated, captive birds (63.2%). Bill length in mm, all mass values in g. FFDM = fat-free dry mass.

Variable	All captive birds (n = 14)		#373 & #154 (captivity)	#1052 (Alert)	Ratio Alert/captivity
	mean	SD			
Bill length	31.85	2.35	31.0	32.8	
Body mass	142.74	31.24	128.45	128.19	
Total fat mass	39.44	23.52	36.55	12.47	0.34
Total FFDM	37.98	3.58	36.55	42.55	1.16
FFDM-5 organs	9.22	1.21	8.56	11.77	1.37
Breast FFDM	6.97	1.06	6.46	8.47	1.31
Breast Fat	1.63	0.83	1.46	0.98	
Leg FFDM	0.97	0.19	0.95	1.36	1.43
Leg Fat	0.79	0.39	0.85	0.45	
Heart FFDM	0.43	0.06	0.43	0.61	1.42
Heart Fat	–	–	–	0.02	
Liver FFDM	0.58	0.09	0.54	0.89	1.65
Liver Fat	0.24	0.12	0.33	0.14	
Kidney FFDM	0.26	0.16	0.19	0.44	2.32
Kidney Fat	0.16	0.10	0.25	0.07	
Rest FFDM	30.36	2.86	28.00	30.78	1.10
Rest Fat	36.62	22.33	29.22	10.83	

tected for two finch species (Marsh and Dawson 1982, Carey et al. 1989). The observation that the residuals of the regression of BMR on FFDM were correlated with fat mass may suggest that (1) birds carrying high fat loads show increased metabolic intensity of the active tissue or that (2) fat is not entirely metabolically inert, or (3) a combination of (1) and (2).

If we consider CCO-activity as an indicator of metabolic scope, our results showed that the “temporal” (as indicated by fat mass) decline in BMR corresponds with a decrease in the mass of those organs with the highest metabolic scope, i.e. heart and flight muscle. These two tissues comprise the most important parts of the machinery for active flight (Burton 1990). In double-log regressions, fat-free dry masses of heart and flight muscle were both positively correlated with BMR, but only for heart the relationship with BMR was statistically significant ($\log \text{BMR} [\text{W}] = 0.298 + 0.948 \log \text{FFDM}_{\text{heart}} [\text{g}]$; $N = 14$; $r^2 = 0.344$, $t = 2.511$, $P = 0.027$). We propose that migration-related variations in BMR are mainly due to variations in the size of organs capable of generating a high working level. This mechanism would provide the causal basis for the empirical correlations between BMR and maximum sustained working level reported in the literature (Daan et al. 1990).

Migration-related changes in BMR thus represent secondary adjustments to the demands of migration, because the tissues that vary in mass are precisely those that enable a high flight performance. The present findings extend the conclusion of Piersma et al. (1996) that intraspecific variations in BMR of overwintering Knots (i.e. individuals that are not actively migrating) are correlated with, and effects of variations in, the size of the nutritional organs (the gut, liver and kidneys).

Depending on seasonal context, adaptive size variation in the nutritional as well as the “exercise” organs (muscles and heart) can thus modify the level of BMR.

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References

- Aschoff, J. and Pohl, H. 1970. Der Ruheumsatz von Vögeln als Funktion der Tageszeit und der Körpergrösse. – *J. Orn.* 111: 38–47.
- , Günther, B. and Kramer, K. 1971. Energieaushalt und Temperaturregulation. – Urban & Schwarzenberg, Berlin.
- Bartholomew, G. A. 1977. Energy metabolism. – In: Gordon, M. S. (Ed.). *Animal physiology: principles and adaptations*. Third ed. Macmillan, New York, pp. 57–110.
- Blem, C. R. 1990. Avian energy storage. – *Current Ornithol.* 7: 59–113.

- Bryant, D. M. and Tatner, P. 1991. Intraspecific variation in avian energy expenditure: correlates and constraints. – *Ibis* 133: 236–245.
- Burton, R. 1990. Bird flight. An illustrated study of birds' aerial mastery. – Facts on File, New York.
- Butler, P. J. and Turner, D. L. 1988. Effect of training on maximal oxygen uptake and aerobic capacity of locomotory muscles in tufted ducks (*Aythya fuligula*). – *J. Physiol. (Lond.)* 401: 347–359.
- Cadée, N., Piersma, T. and Daan, S. 1996. Endogenous circannual rhythmicity in a non-passerine migrant, the Knot *Calidris canutus*. – *Ardea* 84: in press.
- Carey, C., Marsh, R. L., Bekoff, A., Johnston, R. M. and Olin, A. M. 1989. Enzyme activities in muscles of seasonally acclimatized house finches. – In: Bech, C. and Reinertsen, R. E. (Eds.). *Physiology of cold adaptation in birds*. Plenum Press, New York, pp. 95–104.
- Cooperstein, S. J. and Lazarov, A. 1950. A microspectrophotometric method for the determination of cytochrome oxidase. – *J. Biol. Chem.* 189: 665–670.
- Daan, S., Masman, D. and Groenewold, A. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. – *Am. J. Physiol.* 28: R333–R340.
- , Masman, D., Strijkstra, A. and Verhulst, S. 1989. Intraspecific allometry of basal metabolic rate: relations with body size, temperature, composition, and circadian phase in the kestrel, *Falco tinnunculus*. – *J. Biol. Rhythms* 4: 267–283.
- Davidson, N. C. and Evans, P. R. 1986. Prebreeding accumulation of fat and muscle protein by arctic-breeding shorebirds. – *Acta XIX Congr. Int. Ornithol. (Ottawa)*: 342–352.
- and Wilson, J. R. 1992. The migration system of European-wintering Knots *Calidris canutus islandica*. – *Wader Study Group Bull.* 64, Suppl.: 39–51.
- Dawson, W. R., Marsh, R. L. and Yacoe, M. E. 1983. Metabolic adjustments of small passerines for migration and cold. – *Am. J. Physiol.* 245: R755–R767.
- Dietz, M. W., Daan, S. and Masman, D. 1992. Energy requirements for molt in the Kestrel *Falco tinnunculus*. – *Physiol. Zool.* 65: 1217–1235.
- Dimauro, S., Servidei, S., Zeviani, M., Dirocco, M., Devivo, D. C., Didonato, S., Uziel, G., Berry, K., Hoganson, G., Johnsen, S. D. and Johnsen, P. C. 1987. Cytochrome-c oxidase deficiency in Leigh's syndrome. – *Ann. Neurol.* 22: 498–506.
- Drent, R. H. and Daan, S. 1980. The prudent parent: energetic adjustments in avian breeding. – *Ardea* 68: 225–252.
- Driedzic, W. R., Crowe, H. L., Hicklin, P. W. and Sephton, D. H. 1993. Adaptations in pectoralis muscle, heart mass, and energy metabolism during premigratory fattening in semipalmated sandpipers (*Calidris pusilla*). – *Can. J. Zool.* 71: 1602–1608.
- Dudley, R. and Gans, C. 1991. A critique of symmorphosis and optimality models in physiology. – *Physiol. Zool.* 64: 627–637.
- Else, P. L. and Hurlbert, A. J. 1981. Comparison of the "mammal machine" and the "reptile machine": energy production. – *Am. J. Physiol.* 240: R3–R9.
- Evans, P. R., Davidson, N. C., Uttley, J. D. and Evans, R. D. 1992. Premigratory hypertrophy of flight muscles: an ultrastructural study. – *Ornis Scand.* 23: 238–243.
- Field, J., Belding, H. S. and Martin, A. W. 1939. An analysis of the relation between basal metabolism and summated tissue respiration in the rat. – *J. Cell. Comp. Physiol.* 14: 143–157.
- Gessaman, J. A. and Nagy, K. A. 1988. Energy metabolism: errors in gas-exchange conversion factors. – *Physiol. Zool.* 61: 507–513.
- Goolish, E. M. and Adelman, I. R. 1987. Tissue-specific cytochrome oxidase activity in largemouth bass: the metabolic costs of feeding and growth. – *Physiol. Zool.* 60: 454–464.
- Hayes, J. P., Speakman, J. R. and Racey, P. A. 1992. Sampling bias in respirometry. – *Physiol. Zool.* 65: 604–619.
- Heusner, A. A. 1982. Energy metabolism and body size. I. Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? – *Respir. Physiol.* 48: 1–12.
- 1987. What does the power function reveal about structure and function in animals of different size? – *Annu. Rev. Physiol.* 49: 121–133.
- Hill, R. N. 1972. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. – *J. Appl. Physiol.* 33: 261–263.
- Holloszy, J. O. 1975. Adaptation of skeletal muscle to endurance exercise. – *Med. Sci. Sports* 7: 155–164.
- and Jenni, L. 1991. Metabolic responses to flight and fasting in night migrating passerines. – *J. Comp. Physiol.* 161B: 465–474.
- Jenni-Eiermann, S. and Jenni, L. 1992. High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates? – *Physiol. Zool.* 65: 112–123.
- Kersten, M. and Piersma, T. 1987. High levels of energy expenditure in shorebirds; metabolic adaptations to an energetically expensive way of life. – *Ardea* 75: 175–187.
- Krebs, H. A. 1950. Body size and tissue respiration. – *Biochim. Biophys. Acta* 4: 249–269.
- Lindström, Å. 1991. Maximum fat deposition rates in migrating birds. – *Ornis Scand.* 22: 12–19.
- and Piersma, T. 1993. Mass changes in migrating birds: the evidence for fat and protein storage re-examined. – *Ibis* 135: 70–78.
- Lundgren, B. O. and Kiessling, K. H. 1985. Seasonal variation in catabolic enzyme activities in breast muscle of some migratory birds. – *Oecologia* 66: 468–471.
- and Kiessling, K. H. 1986. Catabolic enzyme activities in the pectoralis muscle of premigratory and migratory juvenile Reed Warblers *Acrocephalus scirpaes* (Herm.). – *Oecologia* 68: 529–532.
- Marsh, R. L. 1981. Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the Gray Catbird (*Dumetella carolinensis*). – *J. Comp. Physiol.* 141: 417–423.
- and Dawson, W. R. 1982. Substrate metabolism in seasonally acclimatized American Goldfinches. – *Am. J. Physiol.* 242: R563.
- O'Connor, T. P. 1995. Seasonal acclimatization of lipid mobilization and catabolism in House Finches (*Carpodacus mexicanus*). – *Physiol. Zool.* 68: 985–1005.
- Oikawa, S. and Itazawa, Y. 1983. Examination of techniques for manometric determination of the rate of tissue respiration. – *Bull. Jpn. Soc. Sci. Fisheries* 49: 23–26.
- Pennycuik, C. J. 1978. Fifteen testable predictions about bird flight. – *Oikos* 30: 165–176.
- Peterson, C. C., Nagy, K. A. and Diamond, J. A. 1990. Sustained metabolic scope. – *Proc. Natl. Acad. Sci. USA* 87: 2324–2328.
- Piersma, T. 1990. Pre-migratory "fattening" usually involves more than the deposition of fat alone. – *Ring. & Migr.* 11: 113–115.
- and Davidson, N. C. 1992. The migrations and annual cycles of five subspecies of Knots in perspective. – *Wader Study Group Bull.* 64, Suppl.: 187–197.
- and Jukema, J. 1990. Budgeting the flight of a long-distance migrant: changes in nutrient reserve levels of Bar-tailed Godwits at successive spring staging sites. – *Ardea* 78: 315–337.
- Drent, R. and Wiersma, P. 1991. Temperate-versus tropical wintering in the world's northernmost breeder, the knot: metabolic scope and resource levels restrict subspecific options. – *Acta XX Congr. Internat. Ornithol. (Christchurch)*: 761–772.
- , Cadée, N. and Daan, S. 1995. Seasonality in basal metabolic rate and thermal conductance in a long-distance

- migrant, the Knot (*Calidris canutus*). – J. Comp. Physiol. 165B: 37–45.
- , Bruinzeel, L., Drent, R., Kersten, M., Van der Meer, J. and Wiersma, P. 1996. Variability in basal metabolic rate of a long-distance migrant shorebird (Knot *Calidris canutus*) reflects shifts in organ size. – Physiol. Zool. 69: 191–217.
- Ramenofsky, M. 1990. Fat storage and fat metabolism in relation to migration. – In: Gwinner, E. (Ed.). Bird migration: physiology and ecophysiology. Springer-Verlag, Berlin, pp. 214–231.
- Saunders, D. K. and Klemm, R. D. 1994. Seasonal changes in the metabolic properties of muscle in blue-winged teal, *Anas discors*. – Comp. Biochem. Physiol. 107A: 63–68.
- Scott, I. and Evans, P. R. 1992. The metabolic output of avian (*Sturnus vulgaris*, *Calidris alpina*) adipose tissue, liver and skeletal muscle: implications for BMR/body mass relationships. – Comp. Biochem. Physiol. 106A: 329–332.
- Schmidt-Nielsen, K. 1990. Animal physiology. – Cambridge University Press, Cambridge.
- Simon, L. E. and Robin, E. D. 1971. Relationship of cytochrome oxidase activity to vertebrate total and organ oxygen consumption. – Int. J. Biochem. 2: 569–573.
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olsen, B. J. and Klenk, D. C. 1985. Measurement of protein using bicinchoninic acid. – Anal. Biochem. 150: 76–85.
- Summers, R. W., Underhill, L. G., Nicoll, M., Rae, R. and Piersma, T. 1992. Seasonal, size and age-related patterns in body-mass and composition of Purple Sandpipers *Calidris maritima* in Britain. – Ibis 134: 346–354.
- Taylor, C. R. and Weibel, E. R. 1981. Design of the mammalian respiratory system. I. Problem and strategy. – Respir. Physiol. 44: 1–10.
- Van der Meer, J. and Piersma, T. 1994. Physiologically inspired regression models for estimating and predicting nutrient stores and their composition in birds. – Physiol. Zool. 67: 305–329.
- Warkentin, I. G. and West, N. H. 1990. Impact of long-term captivity on basal metabolism in birds. – Comp. Biochem. Physiol. 96A: 379–381.
- Weathers, W. W., Weathers, D. L. and Van Riper, C., III. 1983. Basal metabolism of the Apapane: comparison of freshly caught birds with long-term captives. – Auk 100: 977–978.
- Wiersma, P., Bruinzeel, L. and Piersma, T. 1993. Energy savings in waders: studies on the insulation of Knots. – Limosa 66: 41–52.
- Wilson, J. R. and Morrison, R. I. G. 1992. Staging studies of Knots *Calidris canutus islandica* in Iceland in the early 1970s: body mass patterns. – Wader Study Group Bull. 64, Suppl.: 129–136.
- Wijnandts, H. 1984. Ecological energetics of the Long-eared Owl (*Asio otus*). – Ardea 72: 1–92.

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