SYMPOSIUM

Availability of Water Affects Renewal of Tissues in Migratory Blackcaps During Stopover

Ortal Mizrahy,[1],*,[†] Ulf Bauchinger,*,[‡] Sarah E. Aamidor,* Scott R. McWilliams[‡] and Berry Pinshow[2],*

*Mitrani Department of Desert Ecology, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, 84990 Midreshet Ben-Gurion; [†]Institute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; [‡]Department Natural Resources Science, 105 Coastal Institute in Kingston, University of Rhode Island, Kingston, RI 02881, USA

From the symposium "Environment, Energetics, and Fitness: A Symposium Honoring Donald W. Thomas" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2011, at Salt Lake City, Utah.

[1]E-mail: ortal.mizrahy@mail.huji.ac.il
[2]E-mail: pinshow@bgu.ac.il

Synopsis Migrating blackcaps (*Sylvia atricapilla*) were used to test the predictions that (1) the rebuilding of the digestive tract, as reflected by mass-specific consumption of food on the first 2–3 days of a stopover, is faster in birds with access to drinking water than in birds without, and (2) that adipose tissue and pectoral muscles grow faster and to a greater extent in birds with unlimited access to water. We simulated migratory stopover in two experiments. In Experiment I, each of 31 birds was randomly assigned to one of three experimental groups for 6 days. Along with mealworms (∼64% water) *ad libitum*, Group 1 received drinking water *ad libitum*; Group 2 had 0.5 h/day access to water; and Group 3 had no access to water. In Experiment II, 30 birds were offered a mixed diet for insectivorous birds (∼33% water) *ad libitum* for 6 days, while randomly assigned to two groups: (1) Water *ad libitum*—control; and (2) 30 min access to water twice a day. We measured lean mass and fat mass using dual energy X-ray absorptiometry, as well as body mass (m_b), pectoral muscle index (PMI), and daily intake of food and water. Mean daily water intake was significantly different among the groups in both experiments. However, the availability of drinking water positively affected the rates of gain of lean and fat mass only in birds fed with the mixed, relatively dry diet. Furthermore, mass-specific daily food intake was affected by the availability of drinking water only in the mixed diet experiment, in which birds with unlimited access to drinking water reached an asymptote, 1 day earlier than birds in the water-restricted group. We suggest that in birds consuming diets with low water content, the lack of sufficient drinking water may result in slower rebuilding of the digestive tract, or may influence biochemical processes in the gut that result in slower growth of tissue. Although blackcaps obtained sufficient water from preformed and metabolic water to renew lost tissues when eating mealworms, given access to water, the birds drank prodigiously. Our results also suggest that if drinking water is unavailable to migrating blackcaps, their choices are restricted to water-rich foods, which may constrain their rate of feeding and thus the rate at which they deposit fat. Consequently, drinking water may have an important influence on birds' migratory strategies with respect to habitat selection, use of energy, and the saving of time.

Introduction

Blackcaps (*Sylvia atricapilla*) migrate in autumn from Eurasia to Africa, across the Mediterranean Sea and Sahara Desert, to their wintering grounds south of the Sahel region in Africa, and return in the spring to their breeding grounds (Cramp and Brooks 1992). To complete their long journey, comprised of relatively short periods of flight followed by longer periods at stopovers where energy reserves are renewed, the birds must accumulate fuel before and during migration. At stopovers, which take up about 87% of the total time of migration (Hedenström and Alerstam 1997), feeding rates are influenced by the physiological state of the bird and

the environmental conditions, such as availability of food (reviewed by Lindström 2003); these, in turn, affect the speed of migration (Alerstam and Lindström 1990). The duration of migration may be critical, especially in the spring, since early arrival on the breeding grounds is advantageous for birds competing for resources and seeking good territories (Alerstam and Lindström 1990; Hedenström and Alerstam 1998).

During migratory flight, catabolism of fat and protein from organs and tissues fulfill the need for fuel as well as other physiological requirements (Fry et al. 1972; Marsh 1984; Lindström and Piersma 1993; Karasov and Pinshow 1998; Bauchinger et al. 2009). The sources of most of the protein catabolized in the flight are the splanchnic organs and the pectoral muscles. During flight, the mass of these organs is significantly reduced (Hume and Biebach 1996; Biebach 1998; Bauchinger and Biebach 1998; Battley et al. 2000, 2001; Schwilch et al. 2002; Biebach and Bauchinger 2003; Bauchinger et al. 2005). Upon arrival at a stopover, a bird must first rebuild its atrophied digestive system, a process reported to take 2–3 days (Hume and Biebach 1996; Biebach 1998; Karasov and Pinshow 1998, 2000; Karasov et al. 2004), in order to reach maximum ability to take in food (Lindström 1991; Murphy 1996; Bauchinger et al. 2009). The size of the digestive system influences digestive efficiency, rate of intake of metabolizable energy and, subsequently, gain in body mass (m_b) (Karasov 1996; Karasov and McWilliams 2005; McWilliams and Karasov 2005). For example, Karasov and Pinshow (2000) found that blackcaps deprived of food under laboratory conditions (to simulate fasting during a migratory flight), gained m_b rapidly only after 3 days of re-feeding.

Only after a bird begins to rebuild its digestive system does the restoration of other organs that were reduced in size and mass during migratory flight occur (Bauchinger and Biebach 2001; Bauchinger et al. 2005). Recently Bauchinger and McWilliams (2009), using carbon turnover to estimate protein turnover in garden warblers, concluded that differences between tissues in the rate of rebuilding are primarily related to differences in rate of protein turnover. These differences in turnover rate mean that the digestive system, which is mostly protein that has a turnover rate very high compared to other organs and tissues, such as pectoral muscle, is likely a primary source of water during migration (see below). We used the rate of protein turnover reported by Bauchinger and McWilliams (2009) to determine minimum energy requirements for the re-building of tissue during stopover.

During migratory flight, fat provides ~95% of the energy required (Klaassen and Biebach 1994; Jenni and Jenni-Eiermann 1998), but accounts for ~73% of the loss in mass (Klaassen and Biebach 1994), while protein provides the remaining energy, and accounts for ~27% of the loss of mass. This is because fat is stored without attending water in dedicated adipose tissues, and it produces 8–10 times more energy per gram wet mass than does protein or carbohydrate (Jenni and Jenni-Eiermann 1998). Furthermore, through respiration, the aerobic catabolism of fat brings about higher water loss per unit dry mass than does catabolism of protein (Jenni and Jenni-Eiermann 1998). In contrast, protein has no special storage tissues, and contains ~70% structural water (Jenni and Jenni-Eiermann 1998). Accordingly, catabolism of protein from functional organs, such as pectoral muscles and the digestive tract, actually makes available more water per unit energy than does catabolism of fat (Klaassen 1996; Bauchinger and Biebach 1998; Jenni and Jenni-Eiermann 1998).

Theoretical studies suggest that flight distance is affected by a bird's water balance (Carmi et al. 1992, 1995; Klaassen 1995, 1996), while empirical studies show that access to drinking water influences accumulation of energy during stopover (Sykes 1983; Yalda and Fordes 1995, 1996; Sapir et al. 2004; Tsurim et al. 2008). For example, Sykes (1983) found that restricting intake of water leads to decreased food intake in domestic fowl (*Gallus gallus*), and Sapir et al. (2004) found that blackcaps given free access to drinking water increased in m_b, and fattened faster than did birds with no drinking water. Also, Tsurim et al. (2008) reported that availability of water strongly affects choice of diet and the intake of and food and energy in blackcaps, and hence their rate of accumulation of fat during migratory stopover. Blackcaps with free access to water preferred the fat-rich fruits of *Pistacia atlantica* that are low in water content, to a water-rich insect-based diet. However, when deprived of water, they preferred to eat insects. This suggested that availability of water at a stopover site affects the rate of fat deposition and the bird's fat level at the end of the stopover period.

When birds have no drinking water available and are possibly at risk of dehydration, as in migratory flight (Fogden 1972), catabolism of tissue that produces large amounts of water per unit energy may reduce water stress; this was posited as the "water hypothesis" discussed by Klaassen (1996), Bauchinger and Biebach (1998), and Jenni and

Jenni-Eiermann (1998). The idea was given credence when migrating birds on the ground in the Sahara desert were found to have high-fat reserves, but low-protein reserves (Bauchinger et al. 2005), possibly indicating that the birds mitigated dehydration by catabolizing protein to maintain water balance (Klaassen 2004).

We examined the hypothesis that availability of water during stopover affects the rebuilding of the digestive tract, assuming that this rebuilding takes place first, and only once it has been achieved does the consumed food serve to restore other tissues, or be deposited as fat. We further hypothesized that the rate and extent of rebuilding lost tissues, mainly fat and pectoral muscle, are also affected by the availability of drinking water. Using freshly caught migrating blackcaps, we tested the prediction that rebuilding of the digestive tract is faster in birds with access to drinking water than in birds without, and that they reach maximum digestive capacity earlier. We also predicted that fat tissues and pectoral muscle will be renewed faster and to a greater extent in birds with unlimited water intake. We did two experiments to test these predictions in birds re-feeding after a period of starvation that simulated the obligatory fast experienced during migratory flight.

Methods

Birds and maintenance

The blackcap is the most common species of passerine migrant that passes through Israel (Shirihai 1996). During their northward spring migration, in March and April 2008 and 2009, we mist-netted 61 blackcaps (33 females and 28 males) at Midreshet Ben-Gurion (30°52′N, 34°47′E). Birds were banded and individually housed in $35.5 \times 24.7 \times 37$ cm cages that were placed in an out-door aviary. After no less than 3 days, we transferred the birds, with their cages, to a temperature-controlled room where they were maintained at a daytime air temperature (T_a) of $35 \pm 2°C$ and a nighttime T_a of $15.0 \pm 2°C$, under a photoperiod regime that followed the natural, out-door light cycle. After capture, the birds were fed, *ad libitum*, on a mixed diet designed for insectivorous birds (Gwinner et al. 1988), which contained 25% commercial dry insect-based food ("Fettmischung fein", Aleckwa Tiernahrung, Altrip, and Germany), 30% minced hard boiled eggs, 21% cheese curd, 15% bread crumbs, 6% ground egg shells, 3% minced beef heart, and 0.53% vitamins (Quikon multivitamin, Bocholt, Germany) and water was available *ad*

libitum as well. After habituation for 2 days in the temperature-controlled animal room, the birds were fasted for 1–2 days and then provided with one of two diets that differed in the amount of water available to them for 6 days; a simulated stopover period.

Experimental procedures

In 2008 (Experiment I: mealworm diet), 31 birds were randomly assigned to one of three groups that differed in the amount of drinking water offered to them: Group 1 (control)—water *ad libitum*, $n = 10$; Group 2—30 min access to water per day, in the morning, $n = 10$; and Group 3—no access to drinking water, $n = 11$. All three groups were offered live mealworms (~64% water) *ad libitum* during the 6-day experiment.

In 2009 (Experiment II: mixed diet), 30 birds were randomly assigned to two groups that differed in the amount of water made available to them: Group 1 (control)—water *ad libitum*; and Group 2—30 min access to water twice a day; the birds were offered water at 07:00 and again at 13:00. The minimum time the birds needed to drink enough to maintain m_b above 13 g was 30 min twice a day (our unpublished data). All the birds in this experiment were offered the mixed diet (~33% water). Food was prepared once for the entire experiment and stored in a freezer at $-20°C$. Each day, one to two portions were defrosted.

In both experiments, all birds were deprived of food and water for 1 or 2 days to simulate in-flight starvation (Gwinner et al. 1985; Hume and Biebach 1996; Karasov and Pinshow 2000). This period was followed by a simulated stopover period of 6 days, during which all birds were offered food (mealworms in Experiment I and the mixed diet in Experiment II) *ad libitum*. Birds with m_b <16 g after 1 day of food deprivation were not deprived of food for a second day. We detected no significant effect of the duration of fasting (1 or 2 days as a covariate) on any of the results; therefore, this covariate was not included in the reported statistical models. At the end of each experiment, the birds were allowed 1–4 days to recover with the mixed food and water *ad libitum* before release at their place of capture.

We tested our prediction concerning the effect of drinking water on the anabolism of fat and lean mass by using dual-energy X-ray absorptiometry (DEXA) to follow the changes in the composition of the birds' bodies. We used a Lunar PIXImus[TM] 2 (General Electric Medical Systems, Fitchburg, WI, USA) following Korine et al. (2004) who calibrated the same DEXA device with small birds,

including blackcaps. In Experiment I, each bird was scanned four times; twice on the morning of Day 0 and twice on the morning of Day 6, at the end of the experiment. The mean of each pair of scans was used for calculations. In Experiment II, the birds were scanned on Day 2 as well, 48 h after they were fed, making a total of six scans. During scanning, the birds were lightly anaesthetized with Isoflurane$^®$.

An indicator of pectoral muscle size, the pectoral muscle index (PMI), was also obtained using a "muscle meter" [Wiersma et al. (2005) (Max-Planck Research Centre for Ornithology, Seewiesen)], a simple device that measures the distance from the surface of the breast to the crest of the sternum to ±0.1 mm. Each morning, three measurements were made on each bird, and the average was used. PMI, measured in millimeters by the muscle meter, is usually positive, but with a negative relationship to the actual size of the pectoral muscle; namely, the smaller the PMI, the bigger the pectoral muscles. Therefore, the results in Figs. 1C and 3C are multiplied by −1 to make the changes in PMI intuitively clearer.

Daily intake of food and water were measured by the difference in mass of food and water offered in the morning, and that of the orts and leftover water removed from the cages 10–15 min before lights-out in the evening. To correct for evaporative water loss, five dishes of water and two dishes of food were placed randomly in the climate room and weighed at the same time as those in the cages. Evaporative water loss from live mealworms was negligible. To quantify the amount of water in mealworms, two packets of 5 g of fresh mealworms were desiccated at 60°C to constant mass and, assuming that the change in mass was due solely to loss of water, the mean of the value of 64% was used in the calculations that follow. To quantify the amount of water in the mixed diet, aliquots were dried in an oven at 60°C to constant mass, weighing them before and after desiccation. We found that water accounted for 33% of the mass of food provided to birds in this experiment.

Total water intake was calculated as the intake of drinking water, corrected for evaporation and pre-formed water from food, using the measured values.

Statistical analysis

We used repeated-measures (RM) ANOVA to analyze differences in $\overline{m}_b$, PMI, intake of water and food, and the DEXA data for lean mass and fat mass, with sampling day as the within-subject variable, and groups with different access to water as a between-subject factor. All tests were two-tailed, and were done after the data were tested for normality of distribution (Kolmogorov–Smirnov test), and homoscedasticity (Levene's test), or sphericity (Mauchly's test). The Huynh-Feldt Epsilon correction was used when the assumption of sphericity was not met. Results for the interactions are given only when statistically significant. When statistically significant results were found, Tukey's post hoc test was used to compare between the treatment groups within each day and between experimental days within each treatment group. In addition, when the interaction between treatments and days was significant, we used least-squares linear regression for further analysis. In Experiment II, m_b and PMI were regressed against days of stopover for each group separately. Owing to the small sample size, no regression analysis was done for lean mass and fat mass in Experiment II, despite the significant interaction between treatment and day (see "Results" section). Values are presented as means ± SD. All statistical analyses were done using STATISTICA 7$^®$ or SigmaPlot$^®$ 11.0.

Results

Experiment I

Consumption of food and water

Mass-specific food intake (food intake/m_b) by blackcaps (Fig. 1A) was not affected by the availability of drinking water (RM ANOVA, treatment: $F_{2,28} = 0.76$, NS). However, food intake/m_b changed significantly during the simulated stopover (RM ANOVA, time: $F_{5,140} = 50.17$, $P < 0.001$). Namely, in all three groups, mean daily food intake/m_b almost doubled from Day 0 (0.53 ± 0.11 g/g) to Day 1 (0.82 ± 0.12 g/g) (Tukey's post hoc test, $P < 0.0001$), remained high on Day 2 (0.83 ± 0.11 g/g) and Day 3 (0.76 ± 0.12 g/g) (Tukey's post hoc test, $P = 0.11$), and declined on Days 4 (0.66 ± 0.11 g/g) and 5 (0.58 ± 0.02 g/g) (Tukey's post hoc test, $P < 0.001$).

The interaction term, treatment × day, for total daily water intake was significant (Fig. 1B; RM ANOVA, $F_{10,140} = 2.69$, $P < 0.01$); mean intake of water was higher in the control group (13.04 ± 2.88 g) than in the water-restricted (8.39 ± 1.06 g) or water-deprived groups (7.50 ± 0.85 g) (RM ANOVA, treatment: $F_{2,28} = 27.57$, $P < 0.001$; Tukey's post hoc test, $P < 0.001$), but did not differ between the latter two groups (Tukey's post hoc test, $P = 0.551$). Given free access to water, birds drank a quantity equivalent to 67–85% of their m_b every day, while birds with restricted access to water had a total daily water intake of 32–48% of their m_b. In addition, the bird's water intake changed
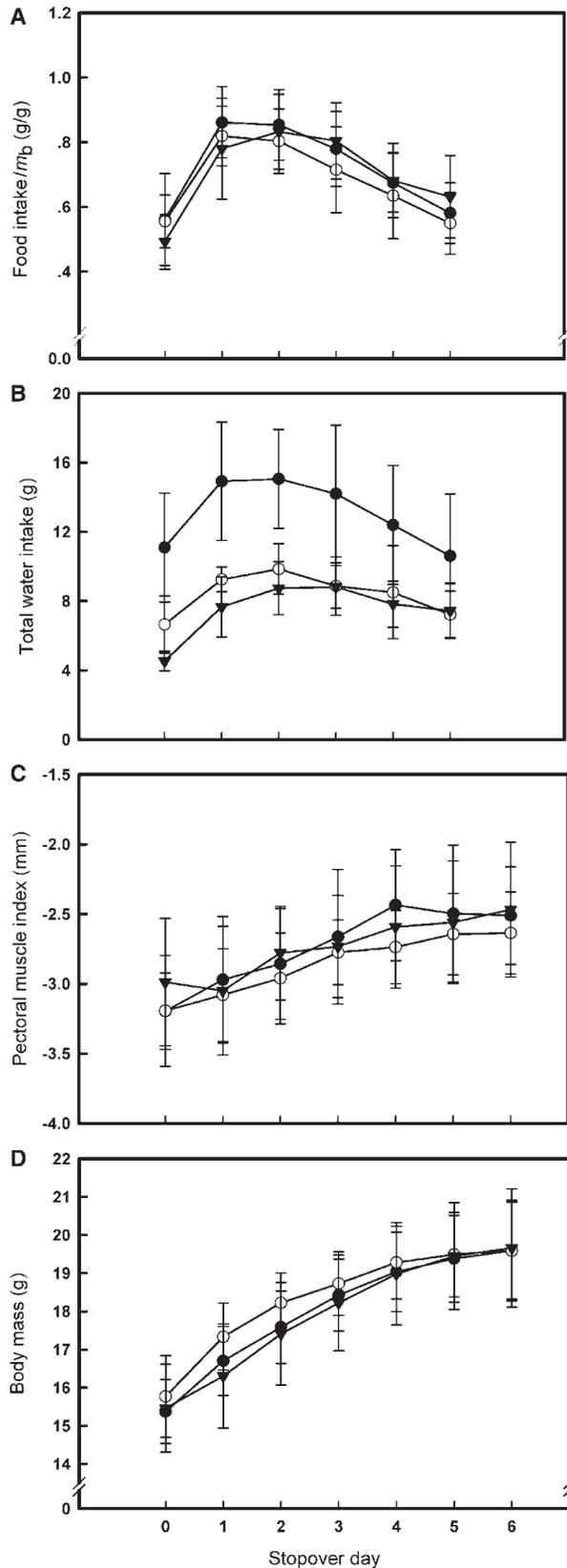
Fig. 1 (**A**) Body mass (m_b) specific food intake, (**B**) total water intake, (**C**) PMI (mm × −1), and (**D**) Body mass (m_b) in blackcaps (*S. atricapilla*) in relation to water availability and "stopover" day during 6 days of feeding, following a 2-day fast, for three experimental groups of birds: water *ad libitum* [control group

significantly during the simulated stopover (RM ANOVA, time: $F_{5,140} = 26.97$, $P < 0.001$). A post hoc test showed that water intake of the birds from the control group increased from Day 0 to Day 1 (Tukey's post hoc test, Day 0: 11.09 ± 0.64 g/g, Day 1: 14.91 ± 0.70 g/g, $P < 0.001$), remained high on Day 2 (15.05 ± 0.64 g/g) and Day 3 (14.19 ± 0.80 g/g) (Tukey's post hoc test, $P = 0.99$), and declined on Days 4 (12.39 ± 0.82 g/g) and 5 (10.60 ± 0.74 g/g) (Tukey's post hoc test, $P < 0.05$). The water intake of the birds from the water-restricted group also increased from Day 0 to Day 1 and Day 2 (Tukey's post hoc test, Day 0: 6.65 ± 0.64 g/g, Day 1: 9.24 ± 0.70 g/g, Day 2: 9.84 ± 0.64 g/g, $P < 0.05$), and decreased on Days 3, 4, and 5 (Day 3: 8.86 ± 0.80 g/g, Day 4: 8.50 ± 0.82 g/g, Day 5: 7.21 ± 0.74 g/g) to a similar amount of water intake like on Day 0 (Tukey's post hoc test, NS). The water intake of the water-deprived group increased from Day 0 to Day 1 (Tukey's post hoc test, Day 0: 4.52 ± 0.61 g/g, Day 1: 7.66 ± 0.67 g/g, $P < 0.001$) and remained relatively high until the last day of the experiment (Tukey's post hoc test, NS).

PMI

PMI in the groups of birds was not affected by the availability of drinking water (Fig. 1C; RM ANOVA, treatment: $F_{2,28} = 0.49$, NS). There was, however, a significant increase in PMI in all birds during the experiment (RM ANOVA, time: $F_{6,168} = 34.17$, $P < 0.001$). PMI of the control group increased from -3.196 ± 0.12 mm on Day 0 to -2.497 ± 0.12 mm on Day 6 (PMI $= -3.222 + 0.122$ day, $r^2 = 0.269$, $P < 0.001$). PMI of the water-restricted group increased from -3.193 ± 0.12 mm on Day 0 to -2.636 ± 0.12 mm on Day 6 (PMI $= -3.256 + 0.099$ day, $r^2 = 0.307$, $P < 0.001$), and PMI of the water-deprived group increased from -2.998 ± 0.12 mm on Day 0 to -2.468 ± 0.12 mm on Day 6 (PMI $= -3.156 + 0.101$ day, $r^2 = 0.181$, $P < 0.001$).

(filled circle), $n = 10$], 0.5 h water/day [water-restricted group (open circle), $n = 10$], and no water [water-deprived group (filled inverted triangle), $n = 11$]. Data are means ± SD. (**A**) No difference was found among the three experimental groups on any day; (**B**) Intake of water by the control group was higher than in the water-restricted and water-deprived groups; (**C**) PMI increased significantly in all groups, but there was no difference in the rate of change among groups; (**D**) Body mass increased significantly in all groups, but there was no difference in the rate of change among groups. See text for statistical details.
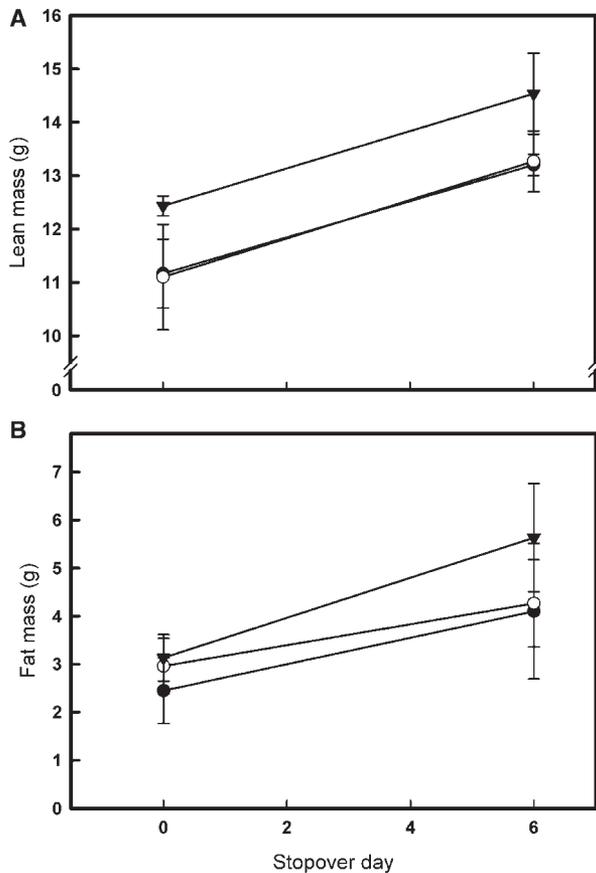
Fig. 2 (**A**) Lean mass and (**B**) fat mass in blackcaps (*S. atricapilla*) in relation to water availability and "stopover" day during 6 days of feeding, following a 2-day fast, for three experimental groups of birds: water *ad libitum* [control group (filled circle), $n = 10$], 0.5 h water/day [water-restricted group (open circle), $n = 10$], and no water [water-deprived group (filled inverted triangle), $n = 11$]. Data are means ± SD. (A) Lean mass increased significantly in all groups, but there was no difference in the rate of change among groups. (B) Fat mass increased significantly in all groups, but there was no difference in the rate of change among groups. See text for further details.

Body mass

Gain in m_b by blackcaps was not affected by the availability of drinking water (RM ANOVA, treatment: $F_{2,28} = 0.33$, NS). Mean $\overline{m}_b$ in all groups increased continuously from Day 0 to Day 5 (15.5 ± 0.18 g to 19.5 ± 0.23 g. Tukey's post hoc test, $P < 0.001$), but did not change on Day 6 (19.5 ± 0.23 g to 19.7 ± 0.26 g. Tukey's post hoc test, $P = 0.098$).

Lean mass and fat mass

Change in lean mass (Fig. 2A) did not differ among the three experimental groups (RM ANOVA, treatment: $F_{2,28} = 1.92$, NS); however, lean mass increased significantly from the first to the last day in all groups (RM ANOVA, time: $F_{1,28} = 307.22$, $P < 0.001$). Fat mass increased significantly from Day 0 to Day 6 in all groups (Fig. 2B) (RM ANOVA, time: $F_{1,28} = 180.52$, $P < 0.001$), but it was not affected by the availability of drinking water (RM ANOVA, treatment: $F_{2,28} = 1.94$, NS).

Experiment II

Consumption of food and water

The interaction of mass-specific food intake × day (Fig. 3A) was significant (RM ANOVA, interaction: $F_{5,105} = 3.72$, $P < 0.01$), and a post hoc test between group and day showed that mass-specific food intake in the control group reached an asymptote on Day 1 (Tukey's post hoc test, Day 0: 0.37 ± 0.15 g/g, Day 1–5: 0.63 ± 0.11 g/g, $P < 0.001$), while in the water-restricted group, it reached an asymptote only on Day 2 (Tukey's post hoc test, Day 0–1: 0.35 ± 0.14 g/g, Day 2–5: 0.61 ± 0.21 g/g, $P < 0.05$). Intake of water differed between the two experimental groups (Fig. 3B; RM ANOVA, treatment: $F_{1,21} = 24.19$, $P < 0.001$). Given free access to water, birds drank a quantity equivalent to 90–105% of their m_b, while birds with restricted access drank 40–53% of their m_b. In addition, the bird's water intake changed significantly during days of the simulated stopover (RM ANOVA, time: $F_{5,105} = 115.23$, $P < 0.001$). A post hoc test showed that water intake of the birds from the control group increased from Day 0 to Day 1 (Tukey's post hoc test, Day 0: 10.135 ± 0.99 g/g, Day 2: 15.15 ± 0.90 g/g, $P < 0.001$), and reached an asymptote on that day, while in the water-restricted group, water intake did not change significantly from Day 0 untill the last day of the experiment (Tukey's post hoc test, Day 0: 4.62 ± 1.36 g/g, Day 6: 8.68 ± 2.48 g/g, NS).

PMI

Daily increase in PMI differed significantly between the two groups (Fig. 3C; RM ANOVA, interaction: $F_{6,126} = 9.22$, $P < 0.001$). PMI of control birds increased significantly during the simulated stopover days; from -3.719 ± 0.14 mm on Day 0 to -3.076 ± 0.49 mm on Day 6 (PMI $= -3.757 + 0.0949$ day, $r^2 = 0.143$, $P < 0.001$), while the PMI of birds from the water-restricted group remained unchanged (PMI $= -3.710 - 0.0377$ day, $r^2 = 0.025$, NS). In addition, mean PMI was not different between groups on Day 0 (RM ANOVA, $F_{1,21} = 0.32$, NS), Day 1 ($F_{1,21} = 1.33$, NS), or Day 2 ($F_{1,21} = 3.28$, NS). However, from Day 3, mean
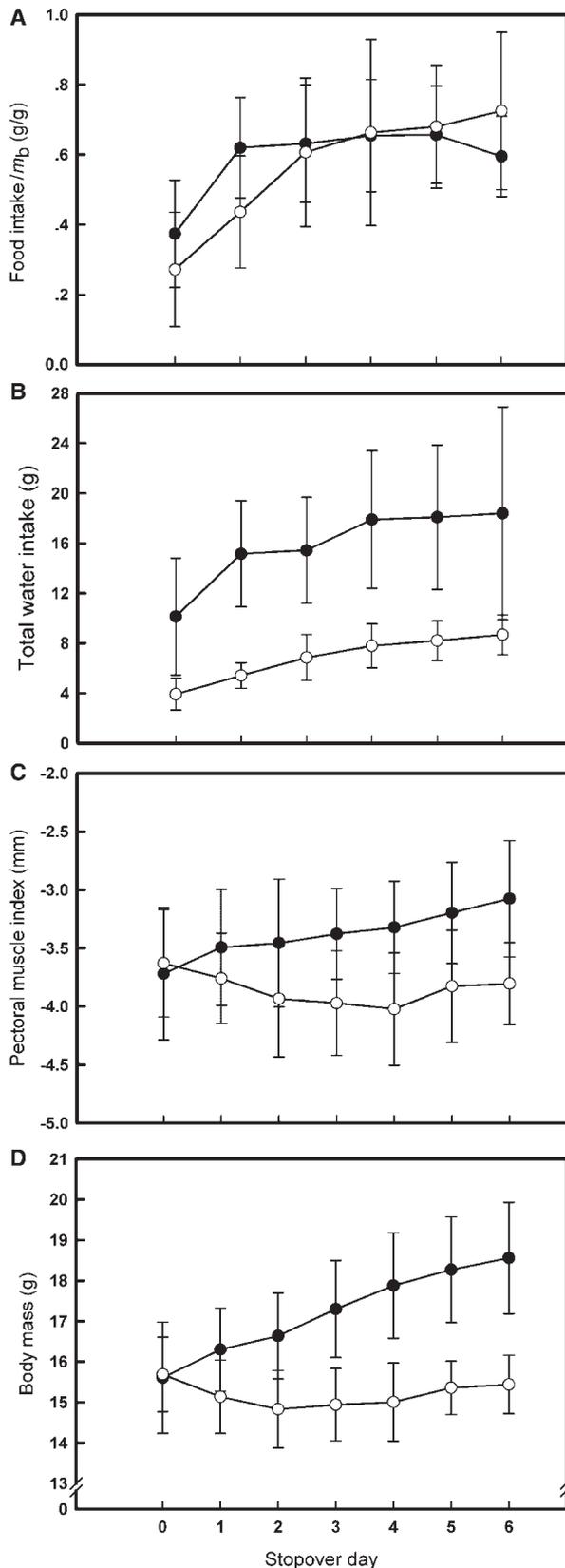
Fig. 3 (**A**) Body mass (m_b)-specific food consumption, (**B**) total water intake, (**C**) PMI (mm × −1), and (**D**) m_b in blackcaps (*S. atricapilla*) in relation to water availability and "stopover" day during 6 days of feeding, following a 2-day fast, for two experimental groups of birds: water *ad libitum* [control group

PMI differed between the two groups ($F_{1,21} = 7.61$, $P < 0.05$).

Body mass

The rate of m_b gain was different between the two groups (RM ANOVA, interaction: $F_{6,126} = 13.71$, $P < 0.001$). $\overline{m}_b$ was not different between groups at the beginning of the experiment (Day 0) (RM ANOVA, $F_{1,21} = 0.001$, NS), but from Day 1, $\overline{m}_b$ of birds offered water *ad libitum* increased (from 15.60 ± 0.35 g on Day 0 to 18.56 ± 1.37 g on Day 6; $m_b = 15.193 \pm 0.511$ day, $r^2 = 0.413$, $P < 0.001$), while $\overline{m}_b$ of birds from the water-restricted group remained unchanged (15.63 ± 1.14 g on Day 0; 15.44 ± 0.72 g on Day 6; $m_b = 15.286 - 0.0296$ day, $r^2 = 0.004$, NS).

Lean mass and fat mass

The rate of gain in lean mass in the birds was positively affected by the availability of drinking water (Fig. 4A; RM ANOVA, interaction: $F_{2,30} = 8.31$, $P < 0.01$). Mean lean mass on Day 0 was not different between groups ($F_{1,15} = 0.02$, NS), but differed on Day 2 ($F_{1,15} = 7.68$, $P < 0.05$) and on Day 6 ($F_{1,15} = 21.88$, $P < 0.001$). Lean mass of the control group increased significantly ($F_{1,15} = 15.25$, $P < 0.01$) from 12.02 ± 0.67 g on Day 0 to 14.27 ± 0.74 g on Day 6 (Tukey's post hoc test, $P < 0.001$), while in the water-restricted group lean mass remained constant, from 12.08 ± 0.77 g on the Day 0 to 12.70 ± 0.44 g on Day 6 (Tukey's post hoc test, $P = 0.44$).

The rate at which blackcaps gained fat was also positively affected by the availability of drinking water (Fig. 4B; RM ANOVA, interaction: $F_{2,30} = 9.65$, $P < 0.001$). Mean mass of fat on Day 0 was not different between groups ($F_{1,15} = 0.50$, NS), but it differed on Day 2 ($F_{1,15} = 5.16$, $P < 0.05$) and on Day 6 ($F_{1,15} = 19.66$, $P < 0.001$). Furthermore, mass of fat in the control group did not increase from Day 0 to Day 2 (3.52 ± 1.06 g to 3.10 ± 0.67 g, Tukey's post hoc test, $P = 0.61$), but increased significantly from Day 2 to Day 6 (3.10 ± 0.67 g to 4.56 ± 1.16 g, Tukey's post hoc test, $P < 0.001$).

(filled circle), $n = 15$], and 0.5 h water/twice a day [water-restricted group (open circle), $n = 15$]. Data are means ± SD. (A) Birds from the control group reached an asymptote on Day 1 of the experiment, while birds from the water restricted group reached an asymptote on Day 2. (B) Intake of water by the control group was higher than in the water-restricted group. (C) The rate of change in PMI differed between groups. (D) The rate of m_b gain differed between groups. See text for further details.
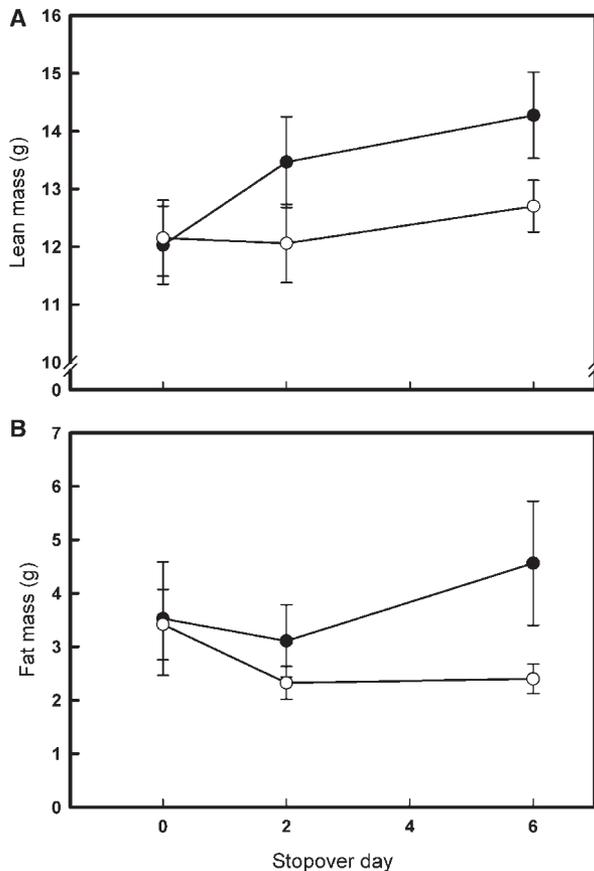
Fig. 4 (A) Lean mass, and (B) fat mass in blackcaps (*Sylvia atricapilla*) in relation to water availability and "stopover" day during 6 days of feeding, following a 2-day fast, for two experimental groups of birds: water *ad libitum* (control group (filled circle), $n = 15$), and 0.5 h water/twice a day (water-restricted group (open circle), $n = 15$). Data are means $\pm$ SD. (A) The rate in lean mass gain was different between groups. (B) The rate in fat mass gain differed between groups. See text for further details.

Fat mass of the water-restricted group did not increase during the experiment (3.18 ± 0.69 g on Day 0 to 2.45 ± 0.25 g on Day 2 and to 2.40 ± 0.27 g on Day 6, Tukey's post hoc test, $P = 0.12$).

Discussion

Availability of water during simulated stopover significantly affected the renewal of tissues in blackcaps. Moreover, water content of food in addition to availability of drinking water affected their body condition. When the water content of food was high (Experiment I: mealworms) availability of drinking water had no effect on body condition. However, when water content of the food was low (Experiment II: mixed diet), access to drinking water had a significant affect on condition. Birds that did not have free access to drinking water when their

food water content was low, had lower m_b, less increase in PMI, and no increase in lean mass and fat mass over the stimulated stopover period.

Intake of water differed among groups in both experiments; birds with free access to water drank significantly more than did birds with limited access. However, no differences were found in intake of food among groups in either experiment, except in Experiment II, in which food intake in the group with unlimited access to drinking water reached an asymptote, 1 day earlier than the group with limited access. This led us to conclude that birds eating the mixed diet and with restricted access to water, re-established digestive capacity slower than did those birds with free access to water, as indicated by the slower increase in food consumption in the former group on Day 1. We suggest that the renewal of the previously reduced digestive tract may be slower when water is limited. Interestingly, Tsurim et al. (2008) found that food consumption in blackcaps was positively correlated with water intake. They showed that when only a few mealworms (0.14 g) were offered to blackcaps, along with *Pistacia atlantica* drupes *ad libitum*, water-deprived birds ate 4-fold less fruits per day than did birds with *ad libitum* access to drinking water. Six birds with unlimited access to water ate 5.74 ± 1.98 (SD) g/day, while seven water-deprived birds ate 1.30 ± 0.23 (SD) g/day. However, Tsurim's and colleagues' experiment differed from the present study in that their birds were deprived of water for only 1 day and they were not examined during tissue renewal. Also, the birds fed only fruit of *P. atlantica* that, because of its high content of fat and fiber, was probably more difficult for to consume and digest when no water was available than was the mixed diet in the present study. Furthermore, the birds in the present study had limited access to drinking water (30 min twice a day) and were not completely deprived of water, as were those of Tsurim et al. (2008). In addition to the above, Sykes (1983) reported that when laying, hens who were provided with only 30% of their normal intake of water, they consumed ~30% less food. The dissimilarity between our results and those of Sykes (1983) likely arises from the fact that Sykes' study was on domestic chickens that are clearly non-migratory and in a steadier nutritional state than our stopover migrants, and also their diet probably differed from our bird's diet in its electrolyte content. Several lines of evidence suggest that digestive constraints, and not availability of drinking water, limited daily food intake of blackcaps immediately after fasting. On Day 1 of Experiment II, the mean

food consumption of both groups of birds was 5.5 g, while on Days 2, 3, 4, 5, and 6, the mean food intake was 11.6, 10.4, 11.1, and 13.9 g, respectively. In addition, availability of water had no effect on m_b gain when mealworms were available *ad libitum*; in fact all the birds increased in m_b at the same rate. However, when the birds ate relatively dry food (the mixed diet), those with unlimited access to drinking water increased in m_b, while birds with restricted access either lost m_b, or it remained constant. These results are similar to those of Tsurim et al. (2008), who found that blackcaps provided with *P. atlantica* drupes *ad libitum,* along with a restricted quantity of mealworms, gained less mass when deprived of drinking water than did birds that had mealworms and drinking water *ad libitum.* Sapir et al. (2004) also found that free-living blackcaps given free access to drinking water, increased m_b faster than did birds with no available drinking water, which lost m_b.

All these results support the notion that drinking water has positive effects on the birds' digestive processes (see also Yasar and Forbes 1999), allowing m_b to increase faster. The conclusion that we draw from the above is that, if drinking water is available at migratory stopover, birds can refuel faster than they can without access to drinking water. This seems to apply especially to the first days of stopover, when the digestive tract is under reconstruction. Thus, drinking water available to migratory birds may shorten their sojourn, and enable them to continue migration sooner than if drinking water is limited.

During preparation for long-distance migration, passerine birds increase pectoral muscle mass (Marsh 1981; Bauchinger and Biebach 2006), which serves as one of the organs in which protein is catabolized during flight (Bauchinger and Biebach 2001). In the present study, PMI of blackcaps provided with the mixed diet was positively correlated with the availability of drinking water, suggesting that its availability positively influences the increase in the mass of flight muscles. Also, Wiersma et al. (2005), using the same ''Muscle Meter'' to study the relation between daily energy expenditure, foraging costs, and physiological variables (mass changes, time and energy budgets, pectoral muscle size) of starlings to their harsh environment, found that pectoral muscle size was directly related to availability of food. The rate of renewal of pectoral muscle is likely a determinant of the time a bird needs to spend at stopover in order to start the next leg of the flight with adequate muscle. Slow renewal of flight muscle may cause a delay in migration that

would be disadvantageous, assuming that birds follow a time-minimizing strategy (Alerstam and Lindström 1990).

We also found that gain in lean mass was strongly affected by availability of water to birds fed with the mixed diet. Birds provided with water *ad libitum* renewed tissue more rapidly than did birds with restricted access to drinking water. After 3 days of stimulated stopover, lean mass, which is largely determined by the mass of internal organs and pectoral muscle, was higher in blackcaps that were not limited in access to drinking water. Apparently, only after birds complete renewing their digestive system does the deposition of fat begin, since the size of the digestive system influences fuel gain (Karasov 1996). Indeed, we found that fat mass in birds in both experiments did not increase until Day 2. However, from Day 2 until the last day of the experiment, fat mass in blackcaps provided with the mixed diet increased faster, and to a greater extent, when provided with water *ad libitum* than when they were water-restricted. Similarly, Tsurim et al. (2008) found that blackcaps, provided with *P. atlantica* fruit and a restricted quantity of mealworms, gained less fat than did birds offered mealworms and drinking water *ad libitum*. In addition, Sapir et al. (2004) found that blackcaps deposited fat faster when they were given free access to drinking water than when they lacked drinking water.

To summarize, contrary to our predictions, when mealworms were available *ad libitum* to blackcaps, availability of water did not affect m_b, pectoral muscle size, or rate of accumulation of lean mass or fat during stopover. The fact that availability of drinking water did not affect these physiological responses suggests that preformed and metabolic water gained through having a diet high in water-content was adequate for blackcaps to maintain a high digestive activity and consequently to renew tissue and energy reserves during stopover at the same rate as if they had unlimited access to drinking water. Furthermore, availability of water affected food intake only on Day 1 and only in birds fed with the mixed diet. Nevertheless, the rate of deposition of lean tissue and fat was affected when mealworms were not made available to blackcaps and when they had limited drinking water. Possibly, the lack of drinking water resulted in lower digestive efficiency through slower development of the digestive organs and/or by increasing the mean ''viscosity'' of the gut's contents thereby, bringing about a slower rate of passage of food through the tract (Yasar and Forbes 1999), or through lower efficiency in digesting food (Yalda and Forbes 1995). Another

possibility is that lack of drinking water may influence biochemical processes in the gut, such as the rate of entry of digestive juices into food matter, which might result in slower digestion (Forbes 2003). Perhaps for that reason, despite similar food consumption in birds with unrestricted and birds with restricted drinking water there were differences in m_b, and increases in fat and lean tissue, between the groups. An alternative explanation might be behavioral adjustment of the birds to limitation of water by reducing activity and thereby using water more frugally. Birds with limited access to drinking water may have fed more during the time that water was served to them, that is, 30 min twice a day.

Our results also imply that if drinking water is unavailable to migrating blackcaps their choices of food are restricted, which may constrain the rate of fat deposition, which is in agreement with earlier studies (Tsurim et al. 2008). Consequently, drinking water could have an important influence on the birds' migratory strategy with respect to habitat selection, use of energy, and time saved (Lundberg et al. 1981; Moller 1994; Norris et al. 2004; Smith and Moore 2005).

Acknowledgments

Funding

References

Alerstam T, Lindström Å. 1990. Optimal bird migration: the relative importance of time, energy, and safety. In: Gwinner E, editor. Bird migration: the physiology and ecophysiology. Berlin: Springer. p. 331–51.

Battley PF, Dietz MW, Piersma T, Dekinga A, Tang SX, Hulsman K. 2001. Is long distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. Physiol Biochem Zool 74:435–49.

Battley PF, Piersma T, Dietz MW, Tang SX, Dekinga A, Hulsman K. 2000. Empirical evidence for differential organ reductions during trans-oceanic bird flight. Proc R Soc B 267:191–5.

Bauchinger U, Biebach H. 1998. The role of protein during migration in passerine birds. Biol Cons Fauna 102:299–305.

Bauchinger U, Biebach H. 2001. Differential catabolism of muscle protein in garden warblers (Sylvia borin): flight and leg muscle act as a protein source during long-distance migration. J Comp Physiol B 171:293–301.

Bauchinger U, Biebach H. 2006. Transition between moult and migration in a long-distance migratory passerine: organ flexibility in the African wintering area. J Ornithol 147:266–73.

Bauchinger U, McWilliams S. 2009. Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. Physiol Biochem Zool 82:787–97.

Bauchinger U, Wohlmann A, Biebach H. 2005. Flexible remodeling of organ size during spring migration of the garden warbler (Sylvia borin). Zoology 108:97–106.

Bauchinger U, Van't Hof T, Biebach H. 2009. Food availability during migratory stopover affects testis growth and reproductive behaviour in a migratory passerine. Horm Behav 55:425–33.

Biebach H. 1998. Phenotypic organ flexibility in garden warblers Sylvia borin during long-distance migration. J Avian Biol 29:529–35.

Biebach H, Bauchinger U. 2003. Energetic savings by organ adjustment during long migratory flights in garden warblers (Sylvia borin). In: Berthold P, Gwinner E, Sonnenschein E, editors. Avian Migration. Berlin: Springer. p. 269–80.

Carmi N, Pinshow B, Porter WP, Jaeger J. 1992. Water and energy limitations on flight duration in small migrating birds. Auk 109:268–76.

Cramp S, Brooks DJ. 1992. Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic, Vol. VI. Warblers. Oxford: Oxford University Press.

Fogden MPL. 1972. Premigratory dehydration in the reed warbler Acrocephalus scirpaceus and water as a factor limiting migratory range. Ibis 114:548–52.

Forbes JM. 2003. Wet foods for poultry. Avian Poultry Biol Rev 14:175–93.

Fry CH, Ferguson-Lee IJ, Dowsett RJ. 1972. Flight muscle hypertrophy and ecophysiological variation of yellow wagtail Motacilla flava races at Lake Chad. J Zool 167:293–306.

Gwinner E, Biebach H, von Kries I. 1985. Food availability affects migratory restlessness in caged garden warblers (Sylvia borin). Nature 72:51–2.

Gwinner E, Schwabl H, Schwablbenzinger I. 1988. Effects of food-deprivation on migratory restlessness and diurnal activity in the garden warbler Sylvia borin. Oecologia 77:321–6.

Hedenström A, Alerstam T. 1997. Optimum fuel loads in migratory birds. distinguishing between time and energy minimization. J Theor Biol 189:227–34.

Hedenström A, Alerstam T. 1998. How fast can birds migrate? J Avian Biol 29:424–32.

Hume ID, Biebach H. 1996. Digestive tract functions in the long-distance migratory garden warbler, *Sylvia borin*. J Comp Physiol B 166:388–95.

Jenni L, Jenni-Eiermann S. 1998. Fuel supply and metabolic constraints in migrating birds. J Avian Biol 29:521–8.

Karasov WH. 1996. Digestive plasticity in avian energetics and feeding ecology. In: Carey C, editor. Avian energetics and nutritional ecology. New York: Chapman and Hall. p. 61–84.

Karasov WH, McWilliams SR. 2005. Digestive constraint in mammalian and avian ecology. In: Starck JM, Wang T, editors. Physiological and ecological consequences of feeding in vertebrates. Enfield, New Hampshire: Science Publishers Inc.

Karasov WH, Pinshow B. 1998. Changes in lean mass and in organs of nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. Physiol Zool 71:435–48.

Karasov WH, Pinshow B. 2000. Test for physiological limitation to nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. Physiol Biochem Zool 73:335–43.

Karasov WH, Pinshow B, Starck JM, Afik D. 2004. Anatomical and histological changes in the alimentary tract of migrating blackcaps (*Sylvia atricapilla*): a comparison among fed, fasted, food-restricted, and refed birds. Physiol Biochem Zool 77:149–60.

Klaassen M. 1995. Water and energy limitations on flightrRange. Auk 112:260–2.

Klaassen M. 1996. Metabolic constraints on long-distance migration in birds. J Exp Biol 199:57–64.

Klaassen M. 2004. May dehydration risk govern long-distance migratory behaviour? J Avian Biol 35:4–6.

Klaassen M, Biebach H. 1994. Energetics of fattening and starvation in the long distance migratory Garden warbler, *Sylvia borin*, during the migratory phase. J Comp Physiol B 164:362–71.

Korine C, Daniel S, van Tets IG, Yosef R, Pinshow B. 2004. Measuring fat mass in small birds by dual-energy X-ray absorptiometry. Physiol Biochem Zool 73:522–9.

Lindström Å. 1991. Maximum fat deposition rates in migrating birds. Ornis Scand 22:12–19.

Lindström Å. 2003. Fuel deposition rates in migrating birds: causes, constraints and consequences. In: Berthold P, Gwinner E, Sonnenschein E, editors. Avian migration. Berlin: Springer. p. 307–20.

Lindström Å, Piersma T. 1993. Mass changes in migrating birds: the evidence for fat and protein storage re-examined. Ibis 135:70–8.

Lundberg A, Alatalo RV, Carlson A, Ulfstrand S. 1981. Biometry, habitat distribution and breeding success in the pied flycatcher *Ficedula hypoleuca*. Ornis Scand 12:68–79.

Marsh RL. 1981. Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the Gray Catbird (*Dumetella carolinenesis*). J Comp Physiol 141:417–32.

Marsh RL. 1984. Adaptations of the gray catbird *Dumetella carolinensis* to long distance migration: flight muscle hypertrophy associated with elevated body mass. Physiol Zool 57:105–17.

McWilliams SR, Karasov WH. 2005. Migration takes guts: digestive physiology of migratory birds and its ecological significance. In: Marra P, Greenberg R, editors. Birds of two worlds. Washington, DC: Smithsonian Institution Press. p. 67–78.

Moller AP. 1994. Phenotype dependent arrival time and its consequences in a migratory bird. Behav Ecol Sociobiol 35:115–22.

Murphy ME. 1996. Nutrition and metabolism. In: Carey C, editor. Avian energetics and nutritional ecology. New York: Chapman and Hall. p. 31–60.

Norris DR, Marra PP, Kyser TK, Sherry TW, Ratcliffe LM. 2004. Tropical winter habitat limits reproductive success on temperate breeding grounds in a migratory bird. Proc R Soc B 271:59–64.

Sapir N, Tsurim I, Gal B, Abramsky Z. 2004. The effect of water availability on fuel deposition of two staging Sylvia warblers. J Avian Biol 35:25–32.

Schwilch R, Grattarola A, Spina F, Jenni L. 2002. Protein loss during long-distance migratory flight in passerine birds: adaptation or constraint. J Exp Biol 205:687–95.

Shirihai H. 1996. The birds of Israel. London: Academic Press.

Smith RJ, Moore FR. 2005. Arrival timing and seasonal reproductive performance in a long-distance migratory landbird. Behav Ecol Sociobiol 57:231–9.

Sykes AH. 1983. Food intake and its control. In: Physiology and biochemistry of domestic fowl, Vol. 4. New York: Academic Press. p. 1–29.

Tsurim I, Sapir N, Belmaker J, Shanni I, Izhaki I, Wojciechowski MS, Karasov WH, Pinshow B. 2008. Drinking water boosts food intake rate, body mass increase and fat accumulation in migratory blackcaps (*Sylvia atricapilla*). Oecologia 156:21–30.

Wiersma P, Salomons M, Verhulst S. 2005. Metabolic adjustments to increasing foraging costs of starlings in a closed economy. J Exp Biol 208:4099–108.

Yalda AY, Forbes JM. 1995. Food-intake and growth in chickens given food in the wet form with and without access to drinking-water. Brit Poultry Sci 36:357–69.

Yalda AY, Forbes JM. 1996. Effects of food intake, soaking time, enzyme and cornflour addition on the digestibility of the diet and performance of broilers given wet food. Brit Poultry Sci 37:797–807.

Yasar S, Forbes J M. 1999. Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. Br Poultry Sci 40:65–76.