

## Heterothermy in small, migrating passerine birds during stopover: use of hypothermia at rest accelerates fuel accumulation

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### SUMMARY

For small endothermic animals, heterothermy serves as an energy-saving mechanism for survival in challenging environments, but it may also accelerate fat accumulation in individuals preparing for fuel-demanding activities. This is the first study to demonstrate adaptive hypothermic responses in migrating passerines. While monitoring body temperature ( $T_b$ ) of eight blackcaps (*Sylvia atricapilla*) by radiotelemetry, we found that during daytime  $T_b=42.5\pm 0.4^\circ\text{C}$  (mean  $\pm$  s.d.); at night  $T_b$  decreased to a minimum between 33 and 40°C. We determined the lower limit for normothermy at 37.4°C and found that on 12 out of 34 bird-nights of observations under semi-natural conditions blackcaps reduced their  $T_b$  below normothermic resting levels with minimum values of 33 and 34.5°C compared with rest-phase normothermic  $T_b$  of 38.8 $\pm$ 0.8°C. In birds of body mass ( $m_b$ ) <16.3 g, minimum  $T_b$  at night correlated with the individual's  $m_b$  ( $r=0.67$ ,  $P<0.01$ ,  $N=17$ ), but this was not the case in birds with  $m_b>16.3$  g. Minimum nocturnal  $T_b$  did not correlate with night-time air temperature ( $T_a$ ). Measurements of metabolic rate in birds subjected to a  $T_a$  of 15°C showed that hypothermia of this magnitude can lead to a reduction of some 30% in energy expenditure compared with birds remaining normothermic. Our data suggest that by reducing the  $T_b-T_a$  gradient, blackcaps accelerate their rate of fuel accumulation at a stopover. When body energy reserves are low blackcaps may achieve this reduction by entering hypothermia. Since hypothermia, as seen in blackcaps, may lead to significant energy savings and facilitate body mass gain, we predict that it is common among small migrating passerines.

Key words: heterothermy, hypothermia, passerine bird, migration, stopover, *Sylvia atricapilla*.

### INTRODUCTION

Each year, billions of birds fly in autumn from their breeding grounds in Eurasia to wintering grounds in Africa and return in spring, often covering many thousands of kilometers (Moreau, 1972; Biebach et al., 2000). In small passerine birds, fuel for migratory flight consists mainly of fat (between 85 and 95%) and to a lesser extent of protein (5–15%) (Klaassen and Biebach, 1994; Klaassen et al., 2000). Fuel used during flight is amassed before migration and restored while birds sojourn at stopover sites. During migration, most of these normally diurnal birds change their activity rhythm and fly at night (Berthold, 1996). If a bird's fuel reserves are adequate, it takes off at dusk and flies non-stop, often for several hundred kilometers at a stretch (Biebach et al., 1986; Biebach et al., 2000). However, this daily rhythm changes when fuel stores run low, and the bird lands to refuel. At stopovers, birds return to diurnality (Berthold, 1996), and may maintain this activity rhythm for several days, until restored energy reserves are sufficient to resume flight (Biebach et al., 1986). Such changes in activity rhythms may occur several times during the migratory journey and are quantitatively related to a bird's fuel reserves (Biebach, 1996).

During migration, songbirds apparently expend more than twice as much energy at stopovers as in flight because the total time spent at stopovers exceeds the time spent in flight by as much as sevenfold (Hedenström and Ålerstam, 1997; Wikelski et al., 2003; Bowlin et al., 2005). It is unlikely that birds can directly reduce the energy spent in flapping flight, although they can save energy by choosing

when and where in the air-column to fly (Carmi et al., 1992). Theoretically, however, there are ways in which a bird might directly reduce energy expenditure during stopover, either while resting or while feeding to refuel. Studies on the rates of fat accumulation in birds, both before and during migration, have addressed potentially important means of saving energy, including changes in feeding behavior (e.g. Gwinner et al., 1985), food choice and digestive physiology (for reviews, see Bairlein, 2002; McWilliams et al., 2004), endocrine regulation of fattening (Wingfield et al., 1990), and energy balance during the fattening process (Klaassen and Biebach, 1994). However, whether migrating birds are able to reduce energy costs while refueling at stopovers, by becoming hypothermic or even entering torpor at rest, has not been explored other than in hummingbirds (Carpenter and Hixon, 1988; Hiebert, 1993). Facultative reductions in body temperature ( $T_b$ ) do, however, seem to be common in birds (McKechnie and Lovegrove, 2002).

Passerine birds typically maintain  $T_b$  between 39°C and 44°C, which, in terms of metabolic energy, is expensive (Prinzinger et al., 1991). However,  $T_b$  normally fluctuates through the day and usually decreases by 1–3°C during normothermic rest, with a concomitant saving of energy through decreased metabolic rate (MR) (Prinzinger et al., 1991; Dawson and Whittow, 2000). However, when faced with unfavorable environmental conditions, many birds may decrease their rest-phase  $T_b$  significantly below normothermic values. This state, depending on its depth, is called rest-phase hypothermia ( $T_b$  lowered by 3–10°C) or torpor ( $T_b$  lowered by

>10°C) (Reinertsen, 1996; McKechnie and Lovegrove, 2002) [but see also Schleucher (Schleucher, 2004) and Schleucher and Prinzinger (Schleucher and Prinzinger, 2006)]. In non-migrating passerine species,  $T_b$  was found to decrease in birds exposed to very low air temperatures ( $T_a$ ), or after several weeks of acclimatization to winter conditions (for reviews, see Reinertsen, 1996; Welton et al., 2002). In passerines, torpor has been reported only in nectarivores and insectivores and was described as an adaptation related to variable food availability (McKechnie and Lovegrove, 2003). Here, we adhere to the nomenclature in the 'Glossary of Terms for Thermal Physiology' (IUPS Thermal Commission, 2001), wherein heterothermy is defined as 'a pattern of  $T_b$  regulation that exceeds in range that characteristic for homeothermy', while hypothermia is 'the condition of a temperature regulator when core temperature is below its range specified for the normal active state of the species', being either regulated or forced (pathological).

Bearing in mind that several species of small, non-migrating passerine birds are capable of significantly lowering  $T_b$  at rest, and that hummingbirds use torpor to facilitate fuel accumulation during migration (Carpenter and Hixon, 1988; Hiebert, 1993), we investigated whether passerine migrants use hypothermia while preparing for their next flight-leg at stopovers. We did this by measuring daily changes in  $T_b$  in blackcaps (*Sylvia atricapilla*), a very common Palearctic passerine migrant, under semi-natural conditions using radiotelemetry at a stopover site at Midreshet Ben-Gurion, Israel, while they fed in a large outdoor aviary. We hypothesized that, while at rest during a stopover, blackcaps lower their  $T_b$  below their normothermic rest-phase  $T_b$  and become hypothermic, thereby facilitating fuel deposition by reducing nocturnal energy expenditure. We tested the prediction that birds that use hypothermia at rest would increase body mass ( $m_b$ ) faster during stopover than those that do not.

## MATERIALS AND METHODS

### Animals

Blackcaps (*Sylvia atricapilla* L. 1758) breed throughout Europe, wintering mainly in northern Africa, south to the Sahel (Cramp, 1992), and in spring are one of the most numerous passerine species that stopover in Israel. Body mass of blackcaps during the breeding season is 16–20 g (Cramp, 1992), but during migration  $m_b$  may range from <13 g observed in very lean individuals to >27 g in the heaviest birds captured after a 10-day stay at a springtime stopover in Eilat, Southern Israel (personal observations). In April 2007, during the spring migration season, we mist-netted blackcaps in a plantation of *Pistacia atlantica* on the Sede Boqer Campus of Ben-Gurion University at Midreshet Ben-Gurion (30°52'N, 34°46'E) in the Negev Desert highlands. Upon capture, the birds weighed between 14.9 and 17.4 g and their wrist-to-wing tip length ranged between 71 and 80 mm; we found no correlation between these two measures (Pearson product moment correlation:  $P=0.9$ ). After capture, birds were ringed with standard aluminum rings and transferred to outdoor flight cages (five to a cage 6×2×2.5 m, and three to a cage 3×2×2.5 m). Birds were kept in the flight cages for at least 2 days to allow them to habituate to the semi-natural conditions, which we verified by observing that they ate freely and maintained body mass. The flight cages had a groundcover of grasses and annuals to attract insects. Since blackcaps eat fruit and insects during migration (personal observations) (Karasov and Pinshow, 2000), we supplied them daily with fruit *ad libitum* and 5–10 g of mealworms, offered in small feeders. The flight cages were covered with 70% shade netting and contained branches to perch on; the ground cover provided shelter and additional shade for birds.

### Measurements under semi-natural conditions

Body temperature was measured with implantable radio transmitters (model BD-2N, Holohil Systems, Carp, Ontario, Canada). Transmitters were previously calibrated between 10°C and 50°C in a controlled-temperature water bath, against a mercury-in-glass thermometer with an accuracy of ±0.1°C traceable to the US NIST. Thereafter, we entered the calibration data (temperature and corresponding pulse interval) into the memory of the receiver that calculated  $T_b$  with built-in software. Before implantation, transmitters were coated with sterilized, pure paraffin wax, and their final mass ranged between 0.8 g and 0.9 g. We implanted transmitters intraperitoneally in all eight birds (five males and three females) under inhalation anesthesia (Isoflurane<sup>®</sup>; Minrad, Inc., Bethlehem, PA, USA), and allowed the birds to fully recover from anesthesia before returning them to the aviaries and beginning temperature recording. Radio signals were recorded with a logging receiver (Lotek model SRX-400A W21AST with Event\_Log<sup>®</sup> software, Newmarket, Ontario, Canada).

We observed daily changes in  $T_b$  while birds were rebuilding their body stores as they fed in the aviary, simulating a migratory stopover. We recorded  $T_b$  in blackcaps over periods of 4–12 days per bird, resulting in 59 bird-days and 34 bird-nights of observations. During observations in the aviaries, birds were exposed to the natural photoperiod, namely sunrise at 06:00 h (±15 min), sunset at 19:00 h (±10 min), and  $T_a$ .  $T_a$  was recorded continuously with two calibrated iButton<sup>®</sup> data loggers (model DS1921, Maxim Integrated Products, Sunnyvale, CA, USA), each suspended from the ceiling of a cage in a well-ventilated, white, open-ended cardboard tube (20 cm long, and 8.5 cm diameter) to minimize effects of incident radiation.

Every day, at approximately 09:00 h, each bird was caught with a hand net and weighed to ±0.1 g with an electronic balance (Scout; Ohaus Corporation, Florham Park, NJ, USA), and the previous day's  $T_b$  data were downloaded.

### Measurements of metabolic rate

Metabolic rates were measured by indirect calorimetry with a multiple-channel, open-flow respirometry system (Qubit Systems, Kingston, ON, Canada), comprising a differential oxygen analyzer (DOX, S104 Differential Oxygen Analyzer, Qubit Systems, Kingston, ON, Canada) and a CO<sub>2</sub> analyzer (LI-6252, LI-COR Inc., Lincoln, NE, USA). In brief, dry, CO<sub>2</sub>-free air was supplied to up to four 2.1 l air-tight metabolic chambers at a time, with flow rates controlled upstream and maintained at 1.2–1.5 l min<sup>-1</sup> through each chamber. Three or four chambers, each containing a single bird, were placed in a controlled temperature cabinet (Precision Incubator 850, Thermo Scientific, Waltham, MA, USA), for overnight measurement. Gases leaving the metabolic chambers were selected sequentially for analysis for 20 min, with reference air being sampled for 8.3 min between birds. Thus, the air from the chamber of each bird was sampled every 64–104 min per night. Full details of the system are provided by Marom et al. (Marom et al., 2006). During gas exchange measurements, birds were held at  $T_a=15^\circ\text{C}$ , approximating the average natural night-time  $T_a$  in the outdoor flight cages.

We measured MR and  $T_b$  during the rest phase in six out of the eight blackcaps. Food deprivation, which leads to depletion of body energy reserves, serves as a cue to reduce  $T_b$  in heterothermic animals [for avian examples see Graf et al. (Graf et al., 1989); Hohtola et al. (Hohtola et al., 1991); McKechnie and Lovegrove (McKechnie and Lovegrove, 2003)] and mimics the physiological changes that occur during the migratory flight (Hume and Biebach, 1996; Karasov and Pinshow, 1998; Karasov et al., 2004). Thus, before

measurement, depending on their initial  $m_b$ , blackcaps were deprived of food for 6 to 24 h to stimulate them to lower  $T_b$  in response to reduced energy reserves, but ensuring that no blackcap weighed less than ~15 g when it was placed in a metabolic chamber. We began measurements of MR approximately 0.5 h before sunset;  $T_b$  was measured by telemetry, as described above. Birds were weighed before and after gas exchange measurements, and to calculate mass-specific MR we assumed that  $m_b$  decreased linearly between weighings.

### Data analysis

We distinguished between nocturnal normothermy and hypothermia following McKechnie et al. (McKechnie et al., 2007), by assuming that normothermic nocturnal  $T_b$  is normally distributed and centered on the modal nocturnal  $T_b$  value. Using individual  $T_b$  data averaged every minute for each of seven out of eight birds, we fitted the normal distribution curve shaped by the data equal to or higher than the mode. To account for variability in the data sets that could be caused by  $T_b$  values of active birds that were in migratory restlessness (*Zugunruhe*), we calculated the average modal value for all birds and the average standard deviation (s.d.) for all curves. Using these averages we set the lower limit for the normothermic  $T_b$  values as the average modal value minus 2 averaged standard deviations.

We assessed the relationship between patterns of thermoregulation and refueling rates by calculating the  $m_b$  change between consecutive weighings of the birds caged outdoors, and related this difference to the difference between minimum  $T_b$  at night and the concurrent  $T_a$ . Since birds were weighed only once a day at approximately 09:00 h, we conservatively used morning  $m_b$  data to examine the relationship between the  $m_b$  of individuals and their nocturnal minimum  $T_b$  recorded on the night preceding the weighing.

To evaluate whether the observed relationship between  $T_b$  and  $m_b$  did not result simply from the size differences among individual birds, we additionally analyzed the relationship between minimum  $T_b$  at night and a calculated body condition index. The body condition index used was the ratio of  $m_b$  to a relatively unchanging linear measure of the animal's size, i.e. the length of a folded wing, from a carpal joint to the tip of the longest primary feather, which is a good body size predictor in blackcaps (Gosler et al., 1998). Since changes in  $m_b$  during a stopover result from simultaneous and predictable changes in lean and fat masses (Gannes, 2002; Wojciechowski et al., 2005), we chose to analyze and report changes of total  $m_b$ .

Our objective was to determine whether blackcaps use hypothermia at stopovers and, if so, what are the potential benefits of this behavior. Thus, we treated each bird-night as an independent data point and indicated the number of observations ( $N$ ) in our analyses of the differences between normothermic and hypothermic  $T_b$ , the effects of  $T_b$  and  $T_b - T_a$  differences on  $m_b$  change, and the correlation between MR and minimum  $T_b$ . For the analysis of minimum metabolic rates, for each bird we used the lowest MR recorded for a 20 min interval at night.

Since it appeared that the influence of  $m_b$  on minimum nocturnal  $T_b$  may be different in lighter and in heavier blackcaps, we applied the method of Pinshow et al. (Pinshow et al., 1976) to objectively divide data into two subsets with which we could calculate separate correlations between  $m_b$  and  $T_b$ . In brief, a range of body masses was chosen that was broad enough to clearly include the point at which the relationship between  $m_b$  and  $T_b$  changed. The data points were then successively divided into two groups, a higher  $m_b$  group and a lower  $m_b$  group, and the corresponding pair of least-squares linear regression equations, and their pooled mean squares (PMS)

were calculated. This process was repeated, and the pair of regression lines with the lowest PMS was considered to best determine the two subsets of data, one above and the other equal to or below the highest  $m_b$  of lighter birds. Then, we tested the correlation between  $m_b$  and nocturnal minimum  $T_b$  within the given subsets using methods described below. If the data were normally distributed and homoscedastic, we used Student's  $t$ -test to compare means, or the Pearson product moment correlation to describe the correlation between two variables. For data that were not normally distributed or homoscedastic, we used the Mann-Whitney  $U$ - or Spearman rank order correlation tests. A probability of  $P < 0.05$  was chosen as the lowest acceptable level of significance, and  $0.05 < P \leq 0.1$  was taken to indicate a trend. Data are presented as means  $\pm$  s.d.

## RESULTS

### Air temperature

Air temperature during data collection ranged between 6.0°C and 35.0°C. The highest daytime (35.0°C) and nighttime (21.0°C)  $T_a$  values were associated with the occurrence of Sha'arav conditions, i.e. hot, dry desert winds. The lowest night-time  $T_a$  values (~6.0°C) occurred on the same days as the lowest daytime maxima (18.5°C and 20.5°C). During this period, there were two afternoon hail storms which resulted in a substantial drop in  $T_a$  by ~8°C in 30 min. As a result, during the period of experiments, the circadian amplitude of  $T_a$  varied between 7.5°C and 21°C.

### Hypothermia versus normothermy

In distinguishing between nocturnal normothermy and hypothermia, the average modal  $T_b$  value was 39.7 $\pm$ 0.7°C for all birds, and the average standard deviation for all curves was 1.2 $\pm$ 0.4°C. We calculated the lower limit for normothermic  $T_b$  to be 37.4°C (Fig. 1). This is >5°C lower than the average normothermic  $T_b$  observed during the day in all birds (see next section). On several occasions, while making pilot measurements of  $T_b$  in a climate chamber at  $T_a = 15.0 \pm 2.0$ °C, we observed the behavior of birds as they became hypothermic, eventually lowering their  $T_b$  to approximately 34°C. During that time, the responses of hypothermic blackcaps to auditory or tactile stimuli were sluggish compared with those of normothermic individuals.

### Regulation of body temperature under semi-natural conditions

By day, the birds'  $T_b$  values were relatively constant and averaged 42.5 $\pm$ 0.4°C ( $N=59$ ). In all blackcaps,  $T_b$  began to decrease almost immediately after dusk and was labile at night. Often, irrespective of the lowest  $T_b$  attained, the decrease in  $T_b$  was continuous until birds commenced rewarming, a process that lasted between 1 h and 8.5 h. Examples of  $T_b$  and  $T_a$  recordings for two blackcaps, one recorded for 5 days and one for 3 days, are shown in Fig. 2. We recorded patterns of  $T_b$  on eight nights in five individuals, which indicated that these birds aroused for part of the night and remained active with  $T_b$  relatively high, yet significantly lower than their own day-time active values [41.4 $\pm$ 0.7°C ( $N=8$ ) and 42.3 $\pm$ 0.4°C ( $N=38$ ), respectively, Student's  $t$ -test:  $t = -4.81$ , d.f. = 44,  $P < 0.001$ ]. Although few, our observations of the birds' behavior suggest that these high nocturnal  $T_b$  values reflect activity associated with *Zugunruhe* (Fig. 3) because we observed them to fly skyward in their flight cages. These individuals began their rest-phase drop in  $T_b$  before dusk, but aroused after an hour or two. Nevertheless, nocturnal restlessness did not preclude them from lowering their  $T_b$  in the later part of the night to levels characteristic of nocturnal hypothermia (Fig. 3).

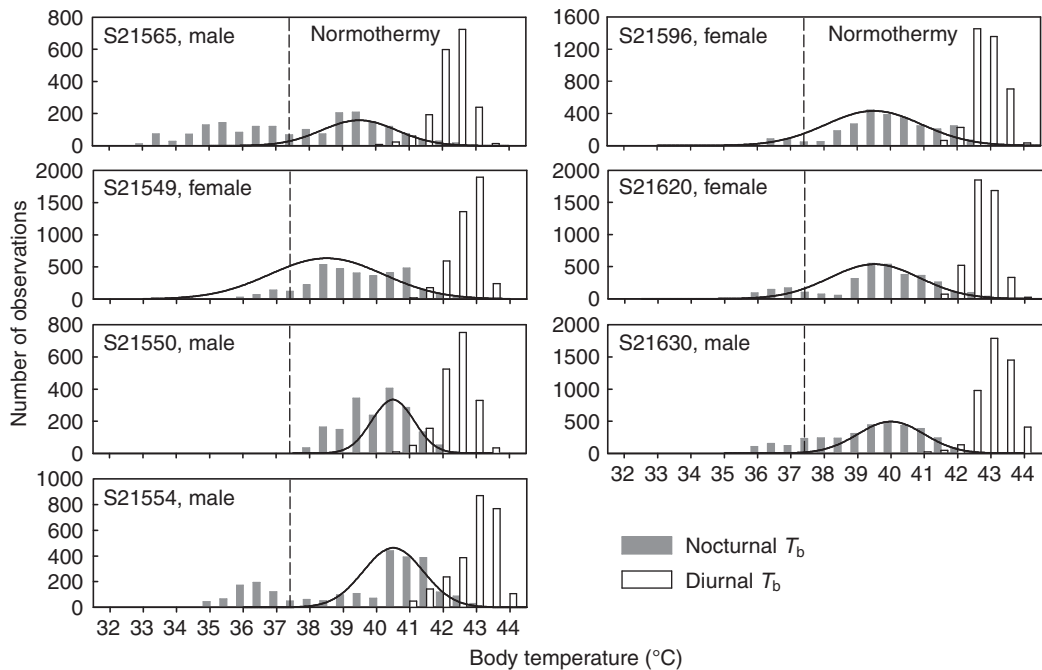


Fig. 1. Histograms of the nocturnal (gray) and diurnal (white) body temperatures ( $T_b$ ) of seven blackcaps (*Sylvia atricapilla*) observed in an aviary. The normal distribution curves (black lines) were fitted to the data that were equal or higher than the modal nocturnal  $T_b$ , which we assumed to be the normothermic  $T_b$ . The vertical, dashed line shows the lower limit of the normothermy, estimated to be 37.4°C (see text for details and criteria for making this estimation).

Minimum rest-phase normothermic  $T_b$  values (those above 37.4°C) in the flight cages averaged  $38.8 \pm 0.8^\circ\text{C}$  ( $N=22$ ) whereas those of hypothermic birds were reduced by some 3°C, and averaged  $35.5 \pm 1.2^\circ\text{C}$  ( $N=12$ ; Student's  $t$ -test:  $t=9.82$ , d.f.=32,  $P<0.001$ ). The minimum  $T_b$  observed during the study was 33.0°C in one individual at a mean night-time  $T_a$  of 11.4°C. Birds generally began to increase their  $T_b$  approximately 1.5 h before civil twilight, and were normothermic at daybreak. Blackcaps rewarmed to normothermy at an average rate of  $0.10 \pm 0.08^\circ\text{C min}^{-1}$  ( $N=32$ ). We found that minimum  $T_b$  at night did not correlate with  $T_a$ .

#### Relationship between body temperature and body mass

In blackcaps of  $m_b < 16.3$  g there was a significant correlation between minimum  $T_b$  at night and  $m_b$  measured the following morning (Pearson product moment correlation:  $r=0.67$ ,  $P<0.01$ ,  $N=17$ ; Fig. 4A), whereas in heavier birds, we found no such correlation. The lowest  $T_b$  values (33.0°C and 34.5°C) were recorded in the lightest blackcaps (13.5 g and 13.7 g, respectively). Since we did not weigh birds in the evening, we do not know the relationship between evening  $m_b$  and minimum  $T_b$  at night. The positive correlation between minimum  $T_b$  at night and a ratio of changing  $m_b$  to the constant measure of body size (length of a folded wing) in individual blackcaps supports the above observation. Birds with a better body condition index maintained higher minimum  $T_b$  at night (Pearson product moment correlation:  $r=0.44$ ,  $P<0.05$ ,  $N=32$ ; Fig. 4B).

To examine whether the use of hypothermia could potentially affect the rate of  $m_b$  increase in refueling blackcaps, we plotted the change in  $m_b$  over 24 h against the difference between the minimum nocturnal  $T_b$  and the corresponding  $T_a$  on the night between weighings. When all available data for 24 h changes of  $m_b$  and  $T_b - T_a$  differences were analyzed (Fig. 5A), we found no correlation between the variables. However, since  $m_b$  may either increase or

decrease during a stopover, in analyzing only data where  $m_b$  increased between weighings on consecutive days, we found a negative trend, indicating that the reduction of night-time  $T_b - T_a$  may affect  $m_b$  gain during refueling (Pearson product moment correlation:  $r=-0.40$ ,  $P=0.1$ ,  $N=18$ ; Fig. 5B). Next, we selected only data for birds that lowered their  $T_b$  below normothermic rest-phase values and found that during those bird-nights the change in  $m_b$  was negatively correlated with  $T_b - T_a$  (Pearson product moment correlation:  $r=-0.77$ ;  $P<0.05$ ,  $N=7$ ; Fig. 5C).

#### Metabolic cost of the night rest

During 15 measurements of MR at  $T_a=15^\circ\text{C}$ , the blackcaps' minimum  $T_b$  ranged between 33.4°C and 39.1°C, not differing from the minimum  $T_b$  values recorded overnight in the caged birds (Mann-Whitney  $U$ -test:  $P>0.05$ ). By our criteria, five individuals became hypothermic and their minimum MR was correlated with their minimum nocturnal  $T_b$  (Spearman rank order correlation:  $r=0.54$ ,  $P<0.05$ ,  $N=15$ ; Fig. 6). By becoming hypothermic, the birds significantly reduced their energy expenditure from  $30.22 \pm 1.31 \text{ mW g}^{-1}$ , observed in normothermic birds, to  $25.94 \pm 3.00 \text{ mW g}^{-1}$  (Mann-Whitney  $U$ -test:  $Z=-2.45$ ,  $P>0.05$ ,  $N=15$ ). However, the difference between the highest ( $32.74 \text{ mW g}^{-1}$ ) and the lowest ( $21.82 \text{ mW g}^{-1}$ ) minimum MR values at night was greater, and translates to a >30% reduction in minimum MR in birds that became hypothermic compared with ones that did not. There was also a positive correlation between minimum  $T_b$  at night and  $m_b$  before MR measurements (Spearman rank order correlation:  $r=0.70$ ,  $P<0.01$ ,  $N=15$ ).

#### DISCUSSION

The present data support our hypothesis that small passerine birds are heterothermic and use hypothermia while resting at a migratory stopover, decreasing their  $T_b$  by up to 10°C below normothermic

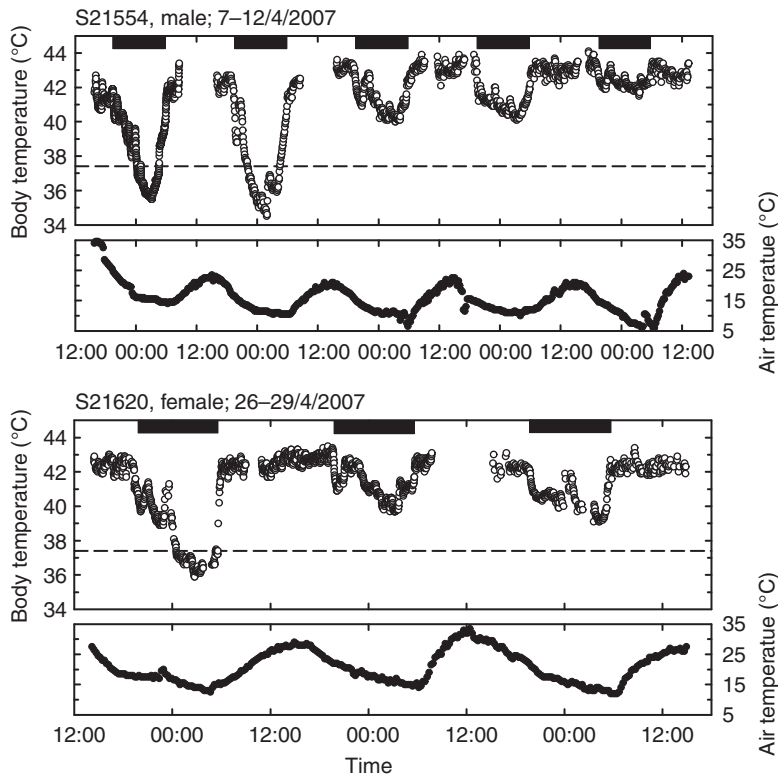


Fig. 2. Changes in body temperature ( $T_b$ , open symbols), between 7 and 12 April 2007 and between 26 and 29 April 2007 in two blackcaps (*Sylvia atricapilla*) under semi-natural conditions (data obtained by radiotelemetry). During measurements birds were held in a large aviary with food available *ad libitum*. Filled symbols represent air temperature during the measurement period. Black horizontal bars indicate hours of darkness. Missing values are from times when the battery was changed in the receiver and data were being downloaded. The dashed horizontal line indicates the lower limit of normothermy of 37.4°C (see text for details).

rest-phase  $T_b$ . While active, blackcaps defended a stable, high  $T_b$  in the range typical of passerine birds (Prinzinger et al., 1991). A decrease in rest-phase  $T_b$  of approximately 5°C at  $T_a=15^\circ\text{C}$  was associated with an over 30% reduction in energy expenditure, quantitatively similar to the energy savings observed by McKechnie and Lovegrove (McKechnie and Lovegrove, 2001) in food-restricted, heterothermic speckled mousebirds under similar conditions.

The fact that in the present study the lowest  $T_b$  values were observed in the birds of least  $m_b$  could be construed to indicate that

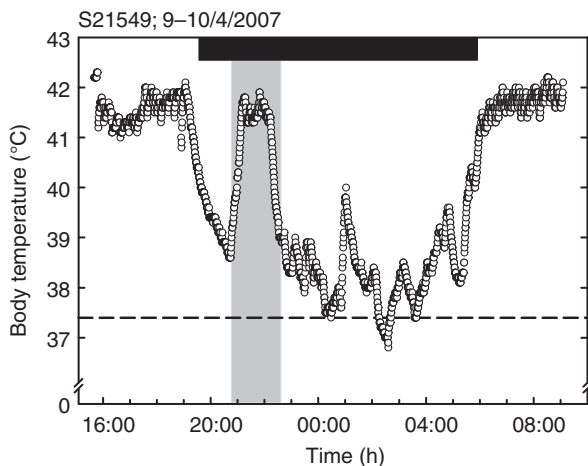


Fig. 3. Body temperature ( $T_b$ ) of a blackcap (*Sylvia atricapilla*) on the night of 9–10 April 2007. The black horizontal bar at the top of the figure indicates hours of darkness. The vertical gray bar indicates the relatively high nocturnal  $T_b$  values that may be associated with nocturnal migratory restlessness (*Zugunruhe*). The dashed horizontal line indicates the lower limit of normothermy of 37.4°C (see text for details).

our observations were of a pathological hypothermic response in emaciated or protein starved birds that do not have large enough energy resources to maintain nocturnal normothermy. However, if that were true, we would expect them not to be able to return to normothermy of their own accord, but they did.

Birds, especially those that have crossed wide ecological barriers, land at stopovers with diminished body energy reserves. Blackcaps with  $m_b < 13\text{g}$  are not unusual in Eilat during spring migration (Reuven Yosef, International Birding and Research Center, Eilat, and our own unpublished data). Additionally, because they exploit splanchnic protein during flight, birds commence their stopover with atrophied gastrointestinal organs (Karasov et al., 2004). Consequently, their digestive efficiency is significantly lower than in the later part of the stopover when net fuel accumulation occurs (Hume and Biebach, 1996; Karasov and Pinshow, 2000; Gannes, 2002). In this initial stage, hypothermia may play a crucial role; birds that forage by day would benefit from lowered energy expenditure at night while waiting for sunup to recommence rebuilding their tissues and energy stores.

Our experiments on caged birds did not reveal whether the low  $m_b$ , usually associated with low body energy stores (Krementz and Pendleton, 1990; Wojciechowski et al., 2005), or the need for reduced energy expenditure during refueling determine the decrease in  $T_b$  at night. However, we did find that lighter birds with lower body condition indices regulate their  $T_b$  at a lower level (Fig. 4A,B), and that the rate of increase in  $m_b$  may be related to the reduction in the  $T_b-T_a$  gradient, the latter leading to decreased MR. Moreover, focusing only on blackcaps that lowered  $T_b$  below normothermic levels, we found that reducing the difference in  $T_b-T_a$  at night led to a greater increase of  $m_b$  over those specific 24 h (Fig. 5C). Taking into account that small migrating passerine birds experience large variations in  $m_b$  during their flight–refueling cycle, we presume that under natural conditions these birds benefit from hypothermia during stopover while rebuilding their energy stores. By their nature, long

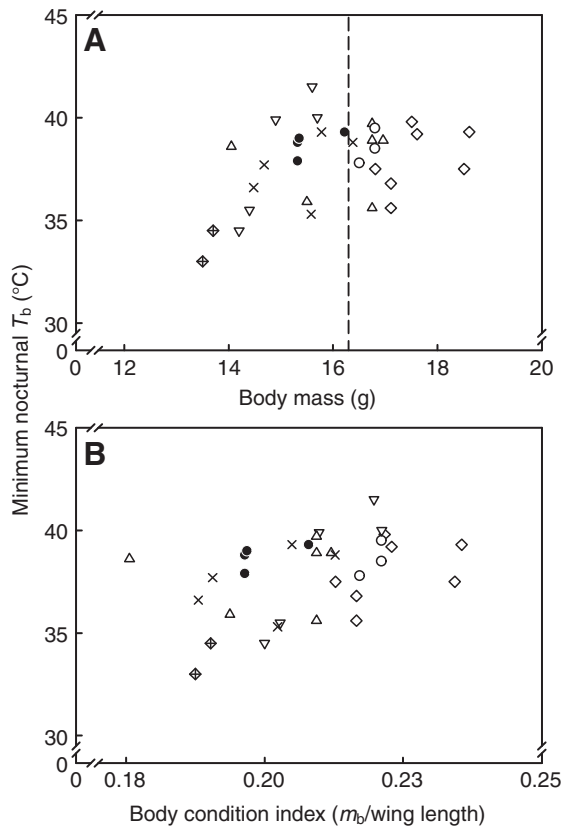


Fig. 4. (A) The relationship between body mass ( $m_b$ ) measured in the morning, and minimum body temperature ( $T_b$ ) recorded during the preceding night in blackcaps (*Sylvia atricapilla*) in an aviary with food available *ad libitum*. In birds of  $m_b < 16.3$  g, minimum  $T_b$  at night correlated with the individual's  $m_b$  (Pearson product moment correlation:  $r = -0.67$ ,  $P < 0.01$ ,  $N = 17$ ); this was not the case in birds with  $m_b > 16.3$  g. The dashed vertical line indicates the division between lighter and heavier birds at  $m_b = 16.3$  g. (B) The relationship between body condition index (ratio of  $m_b$  to length of a folded wing) and minimum nocturnal  $T_b$  during the preceding night. Minimum nocturnal  $T_b$  is positively correlated with morning body condition index of blackcaps feeding in the aviary (Pearson product moment correlation:  $r = 0.44$ ,  $P < 0.05$ ,  $N = 32$ ). Each symbol indicates an individual bird.

flights over broad ecological barriers lead to depletion of a bird's fat reserves within a short period (Schaub and Jenni, 2000; Battley et al., 2001; Pennycuik and Battley, 2003). In addition to fat, protein in organs that are inactive during flight also serve as a source of fuel for flight (Hume and Biebach, 1996; Karasov et al., 2004; Bauchinger et al., 2005). Organs of the gastrointestinal tract undergo acute atrophy (Karasov et al., 2004; Bauchinger et al., 2005) that may lead to their dysfunction during the beginning of a stopover (Hume and Biebach, 1996; Gannes, 2002; Karasov and Pinshow, 2000; Tracy et al., 2005). Thus, low body mass upon arrival at a stopover, associated with impaired capacity for assimilation of nutrients, mean that birds may compensate for the high costs of  $T_b$  regulation and for low body energy stores by entering controlled hypothermia while resting at the stopover.

Although small migrating birds could benefit in terms of energy by lowering their  $T_b$  below normothermic levels while resting at stopovers, this connection has been made only for migrating hummingbirds (Carpenter and Hixon, 1988; Hiebert, 1993). During their autumn migration, the incidence of torpor in rufous

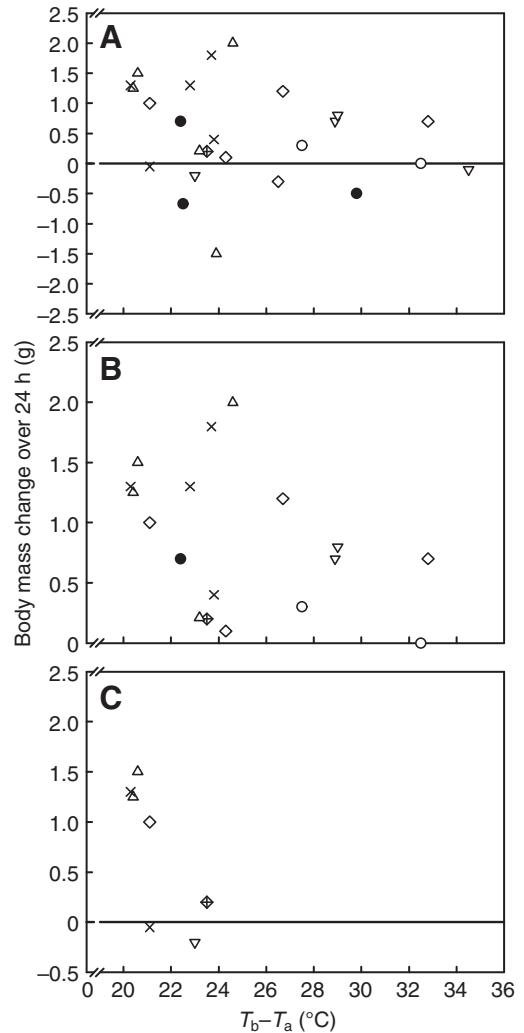


Fig. 5. Relationship between body mass change over 24 h and the minimum body temperature-ambient temperature difference ( $T_b - T_a$ ) during the night between weighings of blackcaps (*Sylvia atricapilla*) in an aviary with food available *ad libitum*. Each symbol indicates a different individual. (A) Data for all 25 nights of measurement; (B) data only from birds that increased  $m_b$  overnight ( $N = 18$ ); and (C) data for birds that lowered their  $T_b$  below normothermy ( $N = 7$ ). There was no correlation between values for all birds, however, there was a negative trend (Pearson product moment correlation:  $r = -0.40$ ,  $P = 0.1$ ) when only birds that increased their  $m_b$  over 24 h were taken into account. For birds that were hypothermic at night there was a negative correlation (Pearson product moment correlation:  $r = -0.77$ ;  $P < 0.05$ ) between  $m_b$  change overnight and the  $T_b - T_a$  difference.

hummingbirds (*Selasphorus rufus*) was directly related to mean body mass, suggesting that they conserve energy by using torpor during premigratory fattening (Hiebert, 1993). There is also evidence that use of daily torpor in heterothermic mammals, such as microchiropteran bats, facilitates pre-hibernatory fattening (Speakman and Rowland, 1999). More recently, Butler and Woakes (Butler and Woakes, 2001) suggested that lowered  $T_b$  might be an important mechanism of energy conservation in migrating barnacle geese (*Branta leucopsis*).

Heterothermy is not the only mechanism whereby small passerines can reduce their energy requirements at stopover sites. Birds may also save energy by behavioral adjustments, among which huddling is important (Le Maho, 1977; Boix-Hinzen and Lovegrove,

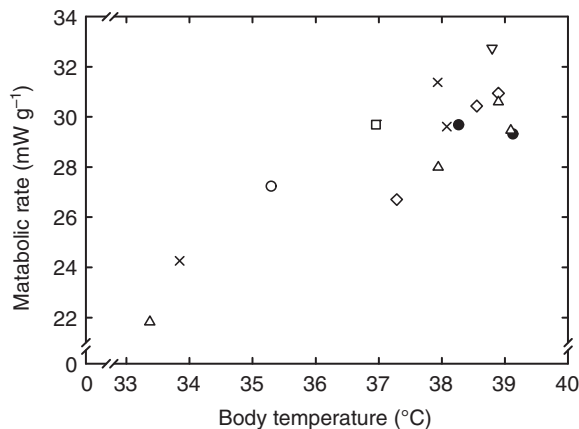


Fig. 6. Relationship between mass specific metabolic rate and body temperature in seven blackcaps (*Sylvia atricapilla*) over 15 nights of measurement at an air temperature of 15°C. Each symbol indicates an individual bird. There was a positive correlation between these values (Spearman rank order correlation:  $r=0.54$ ,  $P<0.05$ ,  $N=15$ ).

1998; Heinrich, 2003; McKechnie et al., 2006). Huddling was previously observed in many passerines, but was mainly perceived as a strategy to survive under harsh, wintertime conditions (Steen; 1958; Smith, 1972; Heinrich, 2003). Recently, we described this behavior in blackcaps huddling at rest at migratory stopovers in Israel and Poland (Wojciechowski et al., 2008). Based on our limited observations of huddling, in the present study we cannot quantitatively determine whether huddling significantly contributed to the observed changes in  $T_b$  and estimated energy savings, but since birds could huddle while hypothermic, doing both together would probably lead to additive energy savings.

Based on our initial respirometry measurements (M.S.W. and B.P., unpublished), we calculated that huddling blackcaps save up to 30% energy overnight compared with the energy expenditure of normothermic solitary blackcaps. This is consistent with data for other species, where, depending on group size and  $T_a$ , birds may reduce their metabolic demands by 50% while resting in a huddle compared with birds resting alone (Chaplin, 1982; Du Plessis and Williams, 1994; Boix-Hinzen and Lovegrove, 1998; McKechnie and Lovegrove, 2001).

McKechnie and Lovegrove (McKechnie and Lovegrove, 2001) pointed out that patterns of thermoregulation, where  $T_b$  is labile and lacking a clearly defended set point as in speckled mousebirds, may be associated with huddling behavior. Comparing our data with that of McKechnie and Lovegrove (McKechnie and Lovegrove, 2001) and of McKechnie et al. (McKechnie et al., 2004) leaves an impression of close similarity between the patterns of thermoregulation in blackcaps at migratory stopover and that of speckled and white-backed mousebirds in terms of interactions between physiological and behavioral mechanisms of energy conservation. Both blackcaps and mousebirds lack an obvious set-point for nocturnal  $T_b$  regulation, both let their  $T_b$  drop well below the normothermic levels observed in other avian taxa, but not as far as in hummingbirds that undergo deep, mammal-like torpor with  $T_b$  decreasing by as much as 30°C (for a review, see McKechnie and Lovegrove, 2002). Both, blackcaps and mousebirds, also huddle while resting, which may be additionally beneficial for maintaining  $T_b$ .

The rate of rewarming from torpor to normothermic  $T_b$  is, on average, lower in birds than in mammals and this difference

increases with increasing  $m_b$  (McKechnie and Wolf, 2004). It was found in mammals that active thermogenesis at low  $T_b$  values is a prerequisite for the arousal from torpor (Geiser et al., 2002; Jefimow et al., 2004). Although there is some evidence for non-shivering thermogenesis (NST) in birds, so far no specialized organ has been identified and it appears that, in birds, muscles are its main source (Bicudo et al., 2001). The average rate of rewarming observed in blackcaps was much slower than allometrically predicted for a 15 g bird by McKechnie and Wolf (McKechnie and Wolf, 2004), namely,  $0.10\pm 0.08^\circ\text{C min}^{-1}$  versus  $0.49^\circ\text{C min}^{-1}$ . Keeping in mind that migratory flight brings about significant atrophy of flight muscles and other organs that could potentially be involved in both shivering and non-shivering thermogenesis (Karasov et al., 2004; Bauchinger et al., 2005), we suggest that the observed deviations from normothermy, as well as huddling behavior, might result from decreased capacity for thermoregulatory heat production in heterothermic birds, blackcaps among them.

The frequent use of heterothermy by blackcaps implies that its benefits exceed its potential costs, as suggested for small mammals by Humphries et al. (Humphries et al., 2003). We propose that both heterothermy, known to accelerate fattening prior to hibernation in mammals and in refueling migratory hummingbirds, as well as huddling, serve to save energy in refueling passerine migrants that, in turn, might shorten their stopover sojourn.

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#### LIST OF ABBREVIATIONS

$m_b$	body mass
MR	metabolic rate
PMS	pooled mean squares
$T_b$	body temperature
$T_a$	air temperature

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