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Reduced body mass gain in small passerines during migratory stopover under simulated heat wave conditions

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ABSTRACT

For birds that migrate long distances, maximizing the rate of refueling at stopovers is advantageous, but ambient conditions may adversely influence this vital process. We simulated a 3-day migratory stopover for garden warblers (*Sylvia borin*) and compared body temperatures (T_b) and rates of refueling under conditions of a heat wave ($T_a = 40$ °C by day, and 15 °C at night) with those under more moderate conditions ($T_a = 27$ °C by day, and 15 °C at night). We measured T_b with implanted thermo-sensitive radio transmitters. Birds had significantly lower rates of body mass gain on the first day of stopover (repeated measures mixed model ANOVA, $p = 0.002$) affecting body mass during the entire stopover ($p = 0.034$) and higher maximum T_b during the day when exposed to high T_a than when exposed to moderate T_a ($p = 0.002$). In addition, the birds exposed to high T_a by day had significantly lower minimum T_b at night than those exposed to moderate daytime T_a ($p = 0.048$), even though T_a at night was the same for both groups. We interpret this lower nighttime T_b to be a means of saving energy to compensate for elevated daytime thermoregulatory requirements, while higher T_b by day may reduce protein turnover. All effects on T_b were significantly more pronounced during the first day of stopover than on days two and three, which may be linked to the rate of renewal of digestive function during stopover. Our results suggest that environmental factors, such as high T_a, constrain migratory body mass gain. Extreme high T_a and heat waves are predicted to increase due to global climate change, and thus are likely to pose increasing constraints on regaining body mass during stopover and therefore migratory performance in migratory birds.

1. Introduction

In order to exploit favorable habitats during different seasons of the year, hundreds of bird species migrate thousands of kilometers, often flying over broad ecological barriers. Depending on the distance, birds are on the move from weeks to months, typically alternating between flight and sojourn at stopover sites where they spend time refueling. Indeed, migrants spend about 85% of their migration time at stopovers compared to 15% in active flight, and twice as much energy at stopovers than in flight (Hedenstöm and Alerstam, 1997, Wikelski et al., 2003). During spring migration, when early arrival at the breeding area is beneficial in terms of annual reproductive success (Newton, 2006; Newton, 2008), efficient rebuilding of body tissues is likely to be under selective pressure (Alerstam and Lindström, 1990; Lindström and Alerstam, 1992; Hedenström and Alerstam, 1997). Therefore maximizing the rate of refueling at a stopover is adaptive because it facilitates early resumption of migration.

Adaptive behavioral and physiological responses such as hyperphagia, diet selection (Bairlein 2002; McWilliams et al., 2004) and/or heterothermy (increased body temperature (T_b) at high ambient temperature (T_a) (Tieleman and Williams, 1999) and decreased T_b at low T_a (Prinzinger et al., 1991; McKechnie and Lovegrove, 2002)) make it possible for birds to maximize their rate of refueling. Whereas heterothermic responses depend on the environment, food selection and rate of intake may depend on the state of the digestive tract (Karasov and Pinshow, 2000; McWilliams et al., 2004; Karasov and McWilliams 2005; McWilliams and Karasov, 2005). Passerine birds within the Afro-Palearctic migration system face the challenge of flying across a major ecological barrier, the Sahara desert. This effort has been reported to result in the catabolism of half of the digestive tract and liver mass (Hume and Biebach, 1996; Biebach, 1998; Karasov

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Abbreviations: m_b, body mass; $m_{b\ change}$, body mass change over 24 h; T_a, ambient temperature; T_b, body temperature; $T_{b\ day}$, daytime body temperature; $T_{b\ night}$, nighttime body temperature; $T_{b\ range\ day/night}$, range of body temperature between daytime and nighttime; $T_{b\ min}$, ten minute mean around the T_b minima of the night; $T_{b\ max}$, ten minute mean around the T_b maxima of the day; $T_{b\ range\ peaks}$, range of T_b between $T_{b\ min}$ and $T_{b\ max}$; $T_{a\ 40/15\ °C}$, ambient temperature regime with peak value of 40 °C during day and constant value of 15 °C during night; $T_{a\ 27/15\ °C}$, ambient temperature regime with peak value of 27 °C during day and constant value of 15 °C during night.

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and Pinshow, 1998; Schwilch et al., 2002; Karasov et al., 2004; Bauchinger et al., 2005). Consequently, these birds need to rebuild their digestive tract before they can rebuild depleted fat stores. Rebuilding the digestive tract in the garden warbler (*Sylvia borin*), and the similar sized blackcap (*Sylvia atricapilla),* requires about two days, after which assimilation rate, food intake rate and body mass (m_b) gain resume normal values (Hume and Biebach, 1996; Karasov et al., 2004).

Passerine birds are capable of hypothermic responses (Prinzinger et al., 1991; Reinersten, 1996; McKechnie and Lovegrove, 2002), and recent investigations suggest that such responses are a strategy used during migratory stopover (Wojciechowski and Pinshow, 2009). Blackcaps maintained under semi-natural conditions at a stopover site in the Negev desert had T_b reduced below normothermic levels, which the authors associated with low m_b rather than with nighttime T_a. The authors estimated that the energy saved due to hypothermia accounts for up to 30% of the energy required to maintain normothermic T_b (Wojciechowski and Pinshow, 2009). In contrast, birds that are exposed to high T_a face increased costs for thermoregulation and maintenance of water balance (Calder and King, 1974; Tieleman and Williams, 1999). Protracted periods of high T_a, as commonly occurring in regions north of the Sahara desert belt during spring and autumn (Frich et al. 2002), can cause mass mortality among birds (McKechnie and Wolf, 2010). In the present study we examined how a migratory passerine species responds to high daily $T_{a,}$ during stopover after crossing the Sahara, how high $T_{a,}$ affects T_b, and how the birds' capacity to refuel is influenced by high T_a.

In spring 2007, we caught garden warblers in the Negev desert, kept them until they maintained constant body mass (m_b), and then deprived them of food and water for two days. Two days of food deprivation is a common manipulation used to simulate an in-flight starvation period (Gwinner et al., 1985, Gwinner et al., 1988; Bauchinger et al., 2008), and is known to cause reduced digestive tract and liver masses (Hume and Biebach 1996, Biebach 1998, Karasov and Pinshow 1998, Karasov et al. 2004). Birds were subsequently offered food and water ad libitum for three days, to simulate a stopover, while they were exposed to either high or moderate daytime temperatures *(*$T_{a\ day}$ 40 °C and $T_{a\ day}$ 27 °C, respectively), whereas nighttime temperature ($T_{a\ night}$) was the same (15 °C) for both treatments. We predicted that birds held at high daytime T_a increase energy expenditure to maintain homeothermy, and thus have slower body mass gain ($\mathrm{m_{b\ gain}}$) and increased $T_{b\ day}$, to minimize energy use for thermoregulation, or a combination of both.

2. Materials and methods

2.1. Animals and transmitters

Migrating garden warblers were captured at the beginning of May in a small *Pistacia atlantica* plantation on the Sede Boqer campus of Ben-Gurion University (30°52′N, 34°47′E) in the Negev desert. Mass passage of this species in the region takes place at the beginning of May (Shirihai, 1996), when hot daytime T_a already occurs regularly in this desert region, with sporadic heat waves (locally known as sha'arav or hamsin conditions). Birds were maintained in individual outdoor aviaries ($2 \times 3 \times 3$ m) and supplied with mealworms and water ad libitum until morning m_b was consistent (~5 days after capture). A thermo-sensitive radio transmitter (BD-2N, Holohil Systems, Ontario, Canada) was implanted in the peritoneal cavity of each bird under Isoflurane anesthesia and the small incision was sutured. Birds were transferred into individual cages ($36 \times 25 \times 37$ cm) in a temperature-controlled room and were allowed to recover for two days before the start of the experimental simulation of in-flight starvation. Reported m_bs were corrected for transmitter mass (~0.9 g). Prior to implantation, transmitters were calibrated in a water bath between 15 °C and 50 °C against a mercury-in-glass thermometer (accuracy ± 0.1 °C traceable to the US NIST). Radio signals were recorded with a LOTEK receiver (SRX-400A W21AST with Event Log software, Newmarket, Ontario, Canada). Successive T_b readings for each bird and transmitter were recorded continuously, with approximately one reading per minute (range 0.33 to 2 readings per minute for each transmitter).

2.2. Experimental design and T_a manipulation

Ambient temperature manipulation lasted 15 days and consisted of three consecutive five-day periods that each included a transition day, and then four days with a consistent day/night temperature regime. The first period had a daytime T_a of 27.5 °C and 15 °C at night ($T_{a\ 27/15\ °C}$). The second was 40 °C by day and 15 °C at night ($T_{a\ 40/15\ °C}$). The third was a repetition of the $T_{a\ 27/15\ °C}$ regime. We gradually increased T_a in the morning and decreased it in the evening to simulate natural patterns, with more pronounced changes for $T_{a\ 40/15\ °C}$ than for $T_{a\ 27/15\ °C}$ (Fig. 1). T_a was measured at five-minute intervals with four calibrated iButton data loggers (DS1921, Maxim Integrated Products, Sunnyvale, USA), and the temperature profile was plotted over 24 h periods (Fig. 1). Birds were exposed to T_a 33.75 °C by day and 15 °C at night during the transition day.

We implanted transmitters in 12 birds, but only nine birds were used in experiments; one bird lost all its tail feathers and began to molt, and transmitters failed in two others. The remaining nine birds were exposed to two successive T_a regimes, randomly assigned to start with $T_{a\ 27/15\ °C}$ or $T_{a\ 40/15\ °C}$, and were either deprived of food and water for two days to simulate in-flight starvation during each of the two periods ('stopover group', $n = 7$), or were offered ad libitum food and water every day ('constant food group', $n = 2$). Two of the seven birds of the stopover group were first exposed to $T_{a\ 27/15\ °C}$ and then to $T_{a\ 40/15\ °C}$; the remaining five birds of the stopover group were first exposed to $T_{a\ 40/15\ °C,}$ and then $T_{a\ 27/15\ °C}$. The two birds of the constant food group saw $T_{a\ 40/15\ °C}$, before $T_{a\ 27/15\ °C}$. The birds in the stopover group were deprived of food for the transition day and the first day of each T_a period. After these two days of fasting the birds were fed ad libitum for at least three days to simulate a 3-day stopover (days 1, 2 and 3). We allowed the birds that were exposed to $T_{a\ 40/15\ °C}$ a fourth day of stopover with ad libitum food and water, so they had sufficient time to regain m_b to pre-fast conditions. Such an additional day was not necessary for the $T_{a\ 27/15\ °C}$ period.

2.3. Maintenance and measurements

Light was matched to the natural light conditions for May 17 (mean for the experiment) and held constant throughout (L:D; 14 h 45 min:9 h 15 min). Light intensity during night was 0.1–0.2 lx and ~250 lx during day as measured in the bird cages (Digital Illuminance Meter B 360, LMT Lichtmesstechnik, Berlin, Germany). Throughout the experiment, birds were fed a standard diet described by Gwinner et al. (1988) which consisted of 30% hard boiled eggs,

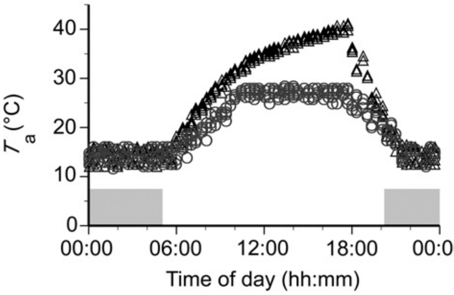

Fig. 1. Experimental manipulation of ambient temperature (T_a) plotted against time of day in heat wave experiment with garden warblers (*Sylvia borin*). Average data for three consecutive days are presented for $T_{a\ 40/15\ °C}$ (black triangles) and for $T_{a\ 27/15\ °C}$ (grey circles). Grey areas indicate night (lights off). Temperature was increased beginning at 06:00 h and was decreased from 18:00 h. Nighttime T_a is similar in both groups.

21% curd, 15% bread crumbs, 6% ground egg shells and 3% beef heart, but without the 25% commercial insect food. A single batch of this standard diet was prepared for the entire experiment and then stored frozen in daily portions. Birds were always fed twice a day to avoid the food becoming desiccated. Food and fresh water were provided two hours after lights on at 07:15 h and at 13:00 h. When food was provided in the morning, birds were weighed to ± 0.1 g and cages were cleaned. Body mass change for each day was calculated as the 24 h difference between m_b measurements.

We recorded the time that we spent in the temperature chamber, and all T_b recordings during this interval, plus an additional 15 min thereafter, were removed from the data set to avoid biased T_b readings due to handling disturbance. We calculated the mean T_b for the entire day ($\overline{T}_{b,\ day}$), mean T_b for the entire night ($\overline{T}_{b,\ night}$), $T_{b\ max}$ (ten minute mean around the maximum T_b of the day) and $T_{b\ min}$ (ten minute mean around the minimum T_b of the night). For each of these measurements we also calculated the range, either as $T_{b\ range\ day/night}$, or $T_{b\ range\ peaks}$.

We used an infrared camera (XNiteIRBoardCam; www.maxmax.com) to record nocturnal migratory restlessness (*Zugunruhe*) for all birds on each of the 3 nights of stopover under both T_a regimes. Two-minute intervals of video recording were analyzed for every 20-minute period throughout the night. Each two-minute interval was scored as 'not active' if the bird moved less than 5 times and did not change the position of its legs. An interval was scored as 'active' if the bird moved more than five times without changing the position of its legs, or if the bird changed the position of its legs, hopped or fluttered in the cage. Each 20-min interval was counted as "with *Zugunruhe*" when the two-minute interval between 18–20 min was scored as active, otherwise the time step was counted as without *Zugunruhe*. All consecutive twenty-minute intervals were treated alike and the final quantity of *Zugunruhe* is presented as percentage of active 20-min intervals per total number of 20-min intervals per night.

2.4. Statistics

All variables were tested for normality of distribution (Kolmogorov–Smirnov Test) and for homogeneity of variance (Bartlett's test). Body mass for the two stopover groups that differed in the sequence of T_a regimes was compared by paired sample t-tests. We used a repeated measures mixed model ANOVA to test for differences in measurements of all T_b, m_b and $m_{b\ change}$. In the model, we accounted for the sequence of T_a regimes (either $T_{a\ 27/15\ °C}$ followed by $T_{a\ 40/15\ °C}$, or $T_{a\ 40/15\ °C}$ followed by $T_{a\ 27/15\ °C}$) and the effect of first vs. second experimental period. We tested for differences (main effects) between treatment (constant food vs. stopover), T_a ($T_{a\ 40/15\ °C}$ vs. $T_{a\ 27/15\ °C}$), days (days 1 to 4) and the interactions $T_a \times$ treatment, $T_a \times$ day and day × treatment. When interactions were not significant, the interaction with the highest *p*-value was removed and the analysis was repeated. Given that we consistently detected significant differences between treatment groups (constant food and stopover; the 'full model'), we also tested for differences between T_a regimes, day, and their interaction ($T_a \times$ day) for only the stopover group. Main effects and interactions were tested in the analysis of the full model without further *post-hoc* analysis. *Post hoc* analysis with Bonferroni corrections for multiple testing was done for the stopover group. The original data are presented as mean ± SD, and mean ± SE for estimated marginal means in the figures.

3. Results

3.1. Ambient temperature manipulation

Mean maximum daytime T_a for 10 min was 40.1 °C (± 0.4; $n = 5$) at $T_{a\ 40/15\ °C}$ and 28.3 °C (± 0.2; $n = 5$) at $T_{a\ 27/15\ °C}$. The mean hourly daytime maximum was 39.7 °C (± 0.5; $n = 5$) for $T_{a\ 40/15\ °C}$ and 27.5 °C (± 0.3; $n = 5$) for $T_{a\ 27/15\ °C}$. The mean daytime T_a was 31.3 °C (± 0.4;

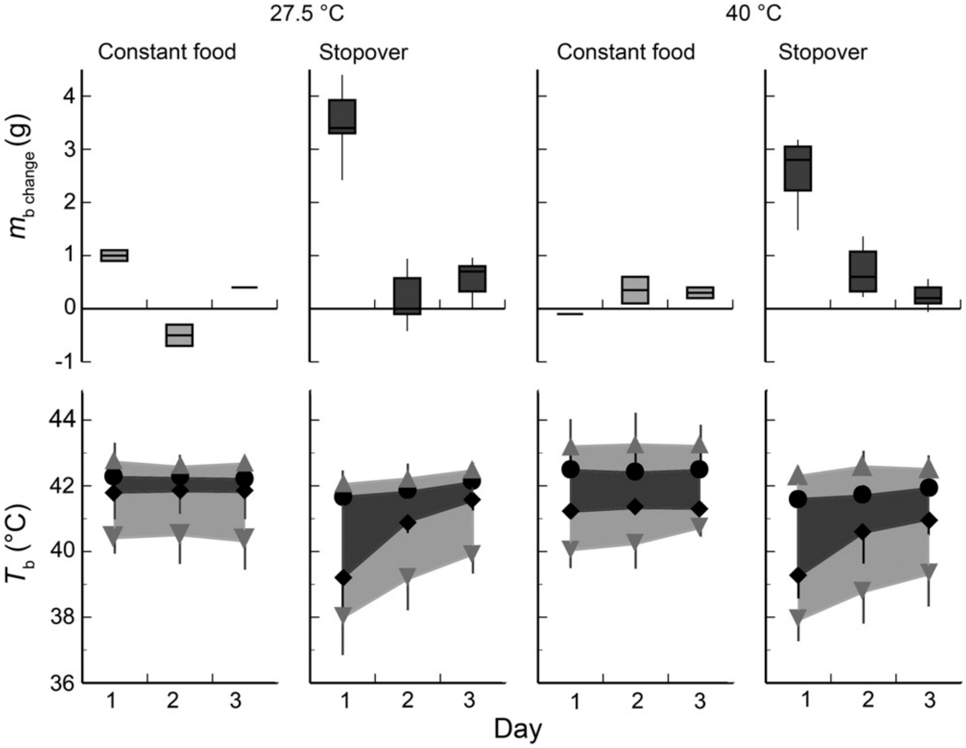

Fig. 2. Median change in body mass ($m_{b\ change}$) and mean body temperature (T_b) for garden warblers plotted over the three experimental days for a constant food group and an experimental stopover group at $T_{a\ 27/15\ °C}$ on the left, and for constant food group and stopover group at $T_{a\ 40/15\ °C}$ on the right. See text for details. Boxes represent quartiles and error bars percentiles. T_bs are means ± SD for $T_{b\ day}$ (black circles), $T_{b\ night}$ (black diamonds), $T_{b\ max}$ (gray triangles), $T_{b\ min}$ (gray triangles upside down). Dark gray areas indicate the $T_{b\ range\ day/night}$, light gray areas indicate T_b range peaks for the constant food group at $T_{a\ 27/15\ °C}$ (1st column) and at $T_{a\ 40/15\ °C}$ (3rd column) were not been deprived of food before day 1. The stopover group at, $T_{a\ 27/15\ °C}$ (1st column) and at $T_{a\ 40/15\ °C}$ (3rd column), were deprived of food for 2 d prior to experimental day 1.

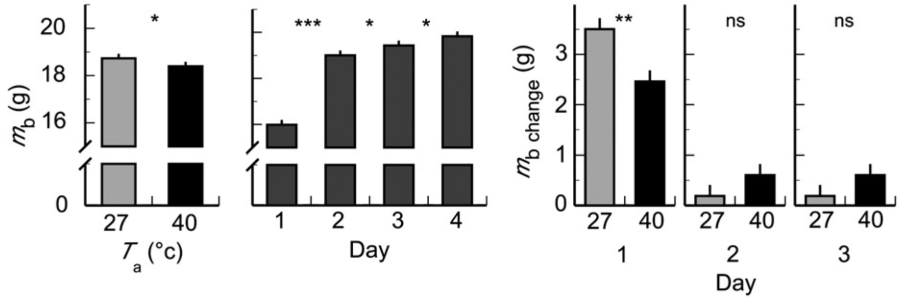

Fig. 3. Least squares means for body mass (m_b) and $m_{b\ change}$ for garden warblers of the stopover group only (see text for details). Body mass is presented for the main effects in our statistical model and plotted for each T_a regime (on the left) and for each stopover day (middle panel). Body mass change is plotted in a combined graph for stopover day and T_a (right panel; representing the significant interaction term $T_a \times$ day). Only differences between T_a for each day are presented. Error bars represent SE. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, ns = not significant. Indicated significances are Bonferroni corrected. Light gray bars give means at $T_{a\ 27/15\ °C}$, black bars are means for $T_{a\ 40/15\ °C}$, dark gray are means for both T_a regimes combined.

$n=5$) for $T_{a\ 40/15\ °C}$ vs. 23.9 °C (±0.5; $n=5$) for $T_{a\ 27/15\ °C}$. Nighttime T_b was constant at about 15 °C (see Fig. 1).

3.2. Data and distribution

All variables were normally distributed and variances met the criteria for homogeneity (for all $p>0.05$). Means for the original data are summarized in Fig. 2.

3.3. Body mass

Mean m_b on the morning of the first day of stopover was 15.7 ± 0.6 g for $T_{a\ 27/15\ °C}$ and 16.1 ± 0.6 g for $T_{a\ 40/15\ °C}$, which were not significantly different (paired sample T-test, $t_{1,6}=0.8$, $p=0.44$). While m_b remained constant over time in the constant food groups for both T_a regimes, it increased in both stopover groups with the highest increase between day 1 and 2, and a higher increase in $T_{a\ 27/15\ °C}$ birds than $T_{a\ 40/15\ °C}$ birds (Figs. 2 and 3). Treatment groups (constant food and stopover) differed in m_b and $m_{b\ change}$ across days as indicated by significant $T_a \times$ day and treatment × day interactions (Table 1). The higher mean m_b for the constant food group at the beginning of the three day period was expected, given that the constant food group was not deprived of food while the stopover group was deprived of food for two days (Fig. 2). Analyses of the stopover group alone revealed that m_b significantly changed between days and across T_a (Table 1, Fig. 3) whereas $m_{b\ change}$ differed over the stopover days and T_a in a more complicated way; there was a significant interaction between T_a and days (Table 1, Fig. 2). Post-hoc analysis revealed significant differences in $m_{b\ change}$ between the two T_a regimes on the first day of stopover only, whereas $m_{b\ change}$ was not significantly different between the two T_a regimes on stopover days two and three (Fig. 3). Mean m_b change over the entire stopover period (day 1 to day 4) was 4.2 ± 0.2 g for $T_{a\ 27/15\ °C}$ and 3.5 ± 0.3 g for $T_{a\ 40/15\ °C}$, which was not significantly different (paired sample T-test, $t_{1,6}=1.7$, $p=0.136$). Under heat wave conditions ($T_{a\ 40/15\ °C}$) birds showed a 26.8% gain in m_b, whereas bird maintained under $T_{a\ 27/15\ °C}$ revealed a 21.9% increase in m_b.

3.4. Body temperature measurements

The $T_{b\ range\ day/night}$ of the treatment groups was significantly different between days and treatment but not between the two T_a regimes (Table 1). For the stopover group, $T_{b\ range\ day/night}$ was significantly different among the three experimental days, but not between the two T_a regimes (Figs. 2 and 4). $T_{b\ range\ peak}$ was different between treatment groups, T_a and days, and both these dependent variables remained significantly different between T_a and day when only the stopover group was considered (Table 1, Fig. 2). In the constant food groups the range was smaller compared to the stopover groups, and it was always smaller in both treatments at $T_{a\ 27/15\ °C}$ compared to the corresponding treatment groups at $T_{a\ 40/15\ °C}$ (Table 1).

Mean $T_{b\ day}$ was consistently different between the days when both the constant food and stopover group, or only the stopover group, were considered (Table 1). In contrast, $T_{b\ night}$ differed over the stopover days and between T_a in a more complicated way when both

Table 1

Summary of statistical results for repeated measures analysis and the main effects testing for differences between T_a, day and treatment (treatment refers to test between constant food group and stopover group, referred to in the text as 'full model'; six columns on the left). Included are all possible and significant interactions; and similar analysis for stopover group only (three columns at the right) testing for differences between T_a, day and the interaction term T_a* day. T_a refers to test between $T_{a\ 27/15\ °C}$ and $T_{a\ 40/15\ °C}$. Day refers to test between stopover day 1, 2 and 3. For each column results are presented as F-value with degrees of freedom (DF) and p-value.

Rm mixed model	Constant food group and stopover group ('full model')						Stopover group only		
	Treatment	T_a	Days	T_a* day	Treatment* day	Treatment* T_a	T_a	Day	T_a* day
	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value	F_{DF} p-value
m_b [a](mean, g)	$21.9_{1,6}$ 0.003	$9.5_{1,52}$ 0.003	$57.5_{3,52}$ <0.001	$3.5_{3,52}$ **0.022**	$30.6_{3,52}$ <**0.001**		$4.8_{1,44}$ **0.034**	$158.7_{3,44}$ <**0.001**	
$m_{b\ change}$ (mean, g)	$49.5_{1,43}$ 0.003	$0.2_{1,43}$ 0.704	$38.1_{2,43}$ <0.001	$14.0_{2,43}$ <**0.001**	$16.7_{3,43}$ <**0.001**		$0.1_{1,34}$ 0.718	$98.5_{2,34}$ <0.001	$7.8_{2,34}$ **0.002**
$T_{b\ range\ day/night}$ (mean, °C)	$11.4_{1,4}$ 0.017	$3.7_{1,37}$ 0.062	$7.7_{2,36}$ 0.002		$6.0_{3,36}$ **0.006**		$1.7_{1,29}$ 0.206	$25.0_{2,29}$ <**0.001**	
$T_{b\ range\ peaks}$ (mean, °C)	$8.5_{1,6}$ **0.027**	$11.5_{1,39}$ **0.002**	$17.2_{2,39}$ <**0.001**				$11.0_{1,29}$ **0.002**	$21.3_{2,29}$ <**0.001**	
$T_{b\ day}$ (mean, °C)	$2.1_{1,6}$ 0.198	$0.0_{1,38}$ 0.846	$5.5_{2,38}$ **0.008**				$0.2_{1,28}$ 0.674	$7.9_{2,28}$ **0.002**	
$T_{b\ max}$ (mean, °C)	$2.2_{1,6}$ 0.186	$12.7_{1,39}$ **0.001**	$2.7_{2,39}$ 0.080				$11.3_{1,29}$ **0.002**	$4.9_{2,29}$ **0.014**	
$T_{b\ night}$ (mean, °C)	$16.5_{1,6}$ 0.008	$3.4_{1,37}$ 0.074	$9.1_{2,37}$ <0.001		$8.1_{2,37}$ **0.001**		$1.9_{1,29}$ 0.178	$30.5_{2,29}$ <**0.001**	
$T_{b\ min}$ (mean, °C)	$7.9_{1,6}$ 0.031	$5.1_{1,37}$ **0.030**	$9.9_{2,37}$ <0.001		$4.6_{2,37}$ **0.016**		$4.3_{1,29}$ **0.048**	$26.6_{2,29}$ <**0.001**	
Zugunruhe (% of night)	$0.5_{1,6}$ 0.496	$1.3_{1,38}$ 0.269	$9.72_{2,38}$ <0.001		$4.7_{2,38}$ **0.015**	$10.5_{1,38}$ **0.003**	$3.5_{1,31}$ 0.071	$30.1_{2,31}$ <**0.001**	

Bold font is used to highlight significant results for single main effects or alternatively, for significant interactions for main effects.

[a] Test for Day refers to test for differences between stopover day 1, 2, 3 and 4.

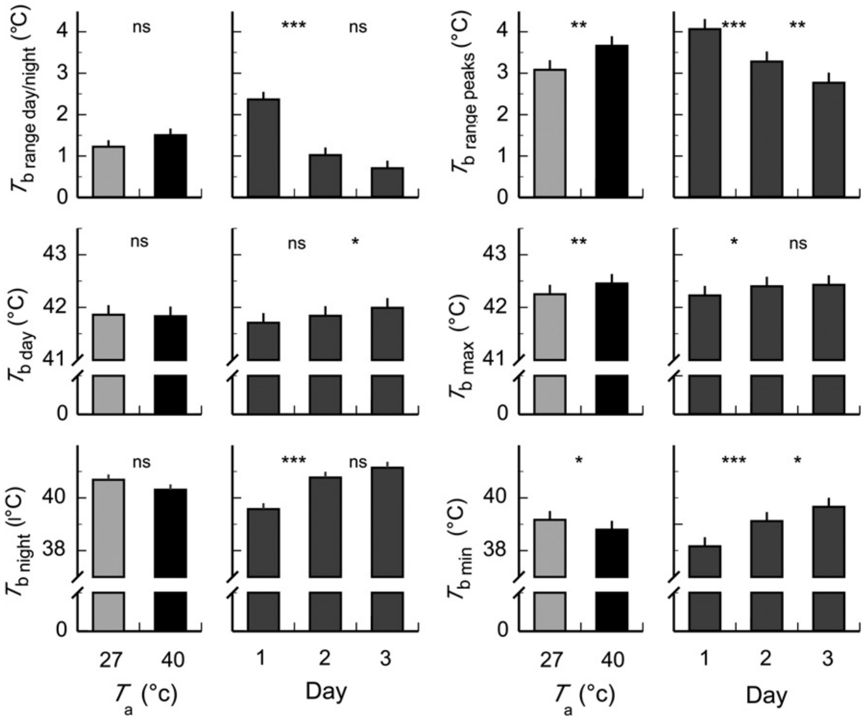

Fig. 4. Least squares means for various T_b measurements for garden warblers of the stopover group only (see text for details) plotted for each T_a regime (1st and 3rd column) and for each stopover day (2nd and 4th column). First row shows $T_{\text{b range day/night}}$ (left) and $T_{\text{b range peaks}}$ (right). Second row shows $T_{\text{b day}}$ (left) and $T_{\text{b max}}$ (right). Third row gives $T_{\text{b night}}$ (left) and $T_{\text{b min}}$ (right). Error bars represent se. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, ns = not significant. Indicated significances are Bonferroni corrected. Light gray bars give means for $T_{\text{a 27/15 °C}}$, black bars give means for $T_{\text{a 40/15 °C}}$, and dark gray bars give means for both T_a regimes combined.

the constant food and stopover group were considered, whereas there was only a difference in $T_{\text{b night}}$ between days and not between T_a when only the stopover group was considered (Table 1). $T_{\text{b night}}$ was lowest on the first day of stopover and increased with stopover day (Fig. 2).

$T_{\text{b max}}$ was significantly higher at $T_{\text{a 40/15 °C}}$ compared to $T_{\text{a 27/15 °C}}$. This applied to both the constant food and stopover groups (full model), or to the stopover considered alone. For the stopover group, $T_{\text{b max}}$ increased significantly after the first stopover day (Fig. 4). $T_{\text{b min}}$ was consistently different between T_as, with a significant interaction between treatment and day (full model) and with a significant difference among days in the model accounting the stopover groups only. $T_{\text{b min}}$ increased with progressive stopover days (Figs. 2 and 4).

3.5. Nocturnal activity (Zugunruhe)

The amount of *Zugunruhe* observed during the night differed between treatment and day ($p<0.05$) and between treatment and T_a ($p<0.01$; full model, Table 1; Fig. 5). In general, the amount of *Zugunruhe* increased significantly with stopover day ($p<0.001$; both models, Table 1). *Zugunruhe* for the stopover group did not change with T_a ($p=0.071$, Table 1).

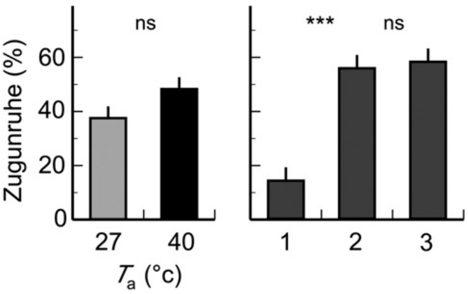

Fig. 5. Least squares means *Zugunruhe* for garden warblers of the stopover group only (see text for details) plotted against T_a (left) and stopover days (right). Error bars are 1 SE. *** indicates $p<0.001$, ns = not significant. Indicated significance is Bonferroni corrected. Light gray bar gives mean for $T_{\text{a 27/15 °C}}$, black bar gives mean for $T_{\text{a 40/15 °C}}$, and dark gray bar give means for both T_a regimes combined.

4. Discussion

Maximum daytime T_b increased in garden warblers exposed to high daytime T_a (40 °C) during a simulated stopover and minimum nighttime T_b decreased compared to when they were exposed to moderate daytime T_a (27 °C; nighttime T_a constant in both cases). These hyperthermic and hypothermic responses could not compensate completely for the increased daytime thermoregulatory requirements, resulting in lower $m_{\text{b change}}$ for birds exposed to higher daytime T_a. Birds that encountered the high daytime T_a also had lower rates of body mass gain, but the compensatory effects associated with altered T_b may still have provided considerable savings.

T_b in small birds (~10 g) typically varies between 38.9 °C and 41.3 °C with a range of 2.5 °C (Prinzinger et al., 1991). We observed a similar range of $T_{\text{b range day/night}}$ between the T_b peaks in garden warblers with mean values of 2.3 °C and 2.9 °C for birds in constant food at $T_{\text{a 27/15 °C}}$ and $T_{\text{a 40/15 °C}}$, respectively. This $T_{\text{b range day/night}}$ was significantly higher (1) during simulated stopover than in constant food, (2) at $T_{\text{a 40/15 °C}}$ compared to $T_{\text{a 27/15 °C}}$ and (3) during the first day of stopover compared to the later days of stopover (Table 1, Figs. 2 and 3).

4.1. Ambient temperature affects body mass gain

Lower body mass increase during stopover in garden warblers exposed to high T_a likely occurred because of increased energy expenditure associated with increased costs of thermoregulation and maintenance of water balance (Calder and King, 1974; Tieleman and Williams, 1999) and changes in protein turnover (Hayashi et al., 1992; Geraert et al., 1996; Yunianto et al. 1997; Temim et al., 1999; Temim et al., 2000). Increased energy expenditure can be a consequence of increased breathing frequency and/or the Q_{10} effect due to increased T_b (Calder and Schmidt-Nielsen, 1964; Calder and King, 1974; Marder and Arad, 1989). The garden warblers were clearly challenged by the 40 °C daytime T_a in that they had lower m_b, lower m_b gain, and higher daytime peak T_b compared to birds exposed to $T_{a\ 27/15\ °C}$. As found in other studies, birds may increase T_b even when T_as are within their thermoneutral zone to reduce energy expenditure associated with thermoregulation by reducing the T_b to T_a temperature gradient (for review see Tieleman and Williams, 1999). Garden warblers in our study were able to increase T_b with increasing T_a, in part because water was provided ad libitum during the simulated stopover, so that evaporation (both respiratory and cutaneous) could dissipate heat when T_a was above the upper critical temperature (Calder and King, 1974; Tieleman and Williams, 1999).

Lower feeding rates during stopover in garden warblers exposed to high T_a may also be related to changes in digestive physiology and altered protein turnover. Nitrogen retention was significantly reduced in broilers maintained for two weeks at high constant T_a of 32 °C vs. T_a of 22 °C, an effect that was explained by reduced nutrient digestibility (Bonnet et al., 1997). Chickens maintained at higher T_a vs. moderate T_a had lower m_b gain and a higher food intake to m_b gain ratio (Dale and Fuller, 1979; Geraert et al., 1992). The range of temperatures typically used in poultry heat stress studies matches the mean $T_{a\ day}$ that we used for garden warblers (31.3 °C ± 0.4, $n = 5$ for $T_{a\ 40/15\ °C}$; 23.9 °C for $T_{a\ 27/15\ °C}$). Furthermore, chickens exposed to constant high T_a of 32 °C vs. T_a of 22 °C had significantly reduced muscle turnover because of reduced protein degradation and protein synthesis, the latter being more effected by heat stress (Temim et al., 1999; Temim et al., 2000). These findings are in agreement with several other studies in young chickens during development that showed decreased protein synthesis (Geraert et al., 1996) or decreased whole-animal protein turnover under higher T_a compared to moderate T_a (Hayashi et al., 1992; Yunianto et al. 1997). The T_a manipulation in the present study may have had similar effects on garden warblers and may in fact be even stronger because these warblers must rebuild digestive tract and other internal organs during stopover.

Under natural conditions, the response of birds to high T_a is typically associated with physiological responses to economize water use (Tieleman and Williams 1998). In our experiment, birds during simulated stopover had ad libitum access to drinking water and food. Stronger effects on m_b and $m_{b\ gain}$ and T_b may occur under conditions when drinking water is not available, or when access to drinking water and food is limited either by quantity or by its temporal availability. During stopover in an oasis blackcaps increased fuel deposition rates when water was supplemented compared to blackcaps that had no access to drinking water (Sapir et al., 2004). However, in the same study, lesser whitethroats (*S. curruca*) did not reveal such differences, indicating potential variance among species in their responses to water availability during stopover. Comparable results were obtained for blackcaps maintained under simulated stopover conditions with or without drinking water on the first day of stopover (Tsurim et al., 2008). Birds without drinking water on day 1 of a simulated stopover had lower food intake and energy intake rates, and less increase in m_b compared to birds that always had access to drinking water.

4.2. Nighttime compensation for increased daytime energy demands

The significantly lower nighttime $T_{b\ min}$ in garden warblers during stopover under $T_{a\ 40/15\ °C}$ compared to $T_{a\ 27/15\ °C}$ suggests that birds compensate for the higher daytime energy expenditure by saving energy at night (Fig. 4). Hypothermic responses may save energy by reducing metabolic costs due to the Q_{10} effect (McKechnie and Lovegrove, 2002; Wojciechowski and Pinshow, 2009). Based on the difference between metabolic rates measured in normothermic and hypothermic blackcaps, Wojciechowski and Pinshow (2009) estimated 30% lower energy expenditure during the night for birds that reduce T_b below normothermic levels. Such savings of energy would be beneficial for fuel deposition of migrating birds during stopover. The lowest T_b for blackcaps was 33–35 °C (Wojciechowski and Pinshow 2009), which is slightly lower than the lowest T_b of garden warblers in the present study (35–36 °C).

The reduction in T_b of garden warblers during the night was not only different between the two T_a regimes, but also changed significantly between the stopover days (Fig. 4). The lowest nighttime $T_{b\ min}$ of garden warblers occurred on the first stopover day and then increased on each of the following two nights. Birds migrating for sustained periods in the wild substantially reduce their digestive organs by up to 50% (Biebach 1998; Battley et al., 2000; Schwilch et al., 2002; Karasov et al., 2004; Bauchinger et al., 2005), a phenomenon that can be simulated by food deprivation in the laboratory (Hume and Biebach, 1996; Karasov and Pinshow, 1998; Battley et al., 2001). Small digestive tract organs upon arrival at a stopover site pose digestive limitations, i.e. reduced food intake rates, assimilation rates and body mass gain (Hume and Biebach, 1996; Karasov and Pinshow, 2000; Karasov et al., 2004; McWilliams et al. 2004, Karasov and McWilliams 2005; McWilliams and Karasov, 2005; Bauchinger et al., 2009). Rebuilding of the digestive tract in Sylvia warblers is a process that requires at least two to three days (Hume and Biebach, 1996; Karasov and Pinshow, 2000). Therefore, the initial strong effect of high daytime T_a on $T_{b\ min}$, and its dampening over time during stopover, may represent the physiological limitations associated with rebuilding of the digestive tract.

Increasing energy stores including rebuilding of the digestive tract, are associated with changes in migratory restlessness of birds during migration (Fusani et al., 2009, Bauchinger et al., 2008). The dampening of the effects on $T_{b\ min}$ in garden warblers during stopover can be explained in part by the increase in migratory restlessness (Fig. 5, Table 2). This increase in nocturnal activity coincided with increasing m_b and T_b values that changed between the stopover days suggesting increasing fuel stores and completed rebuilding of the digestive tract. The amount of nocturnal activity was not different between the two T_a regimes, suggesting that the differences are caused by other factors, rather than nocturnal activity per se. However, due to the low sample size special caution is required for interpretation of non-significant results.

5. Conclusion

Birds that migrate long distances face digestive limitations upon arrival at stopover sites (McWilliams et al., 2004; Karasov and McWilliams, 2005; McWilliams and Karasov, 2005). Reduced digestive organs must be rebuilt before food intake and assimilation rates enable maximum fuel deposition rates (Gannes, 2002; Karasov and Pinshow 2000; Karasov et al., 2004; Bauchinger et al., 2009). The rate of organ renewal may determine the start of the next leg of migration and, thus, speed up the trip (Alerstam and Lindstöm, 1990; Lindström and Alerstam, 1992). Our results indicate that high T_a can impose an additional burden on migratory birds at a stopover site because body mass gain is slower if T_a is close to, or above, the bird's upper critical temperature. High ambient temperature conditions experienced during migratory stopover must be considered to slow down migratory refueling affecting migration speed and subsequently delaying arrival time. Especially in spring, when early arrival at the breeding sites is generally beneficial for individual fitness (Newton, 2006; Newton, 2008) reduced migration speed due to slower refueling at stopover (Alerstam and Lindström, 1990; Lindström and Alerstam, 1992; Hedenström and Alerstam, 1997) likely reduces annual reproductive output.

Climatic extremes often occur in association with heat waves that have been arbitrarily defined as periods of more than 5 days with T_a more than 5 °C above the 1961–1990 normal daily T_{max} (Frich et al., 2002). Frequency of such climatic extremes has increased over the past decades and is predicted to increase even further in the future (Frich et al., 2002; Klein Tank and Können, 2003; Della-Marta et al., 2007; Easterling et al., 2000). Such extreme maximum T_as, in association with heat waves have been documented with increasing frequency in Europe (Kyselý, 2008; Fischer and Schär, 2009; Kyselý, 2010; Kuglitsch et al., 2010) with sites around the Mediterranean having the most pronounced increases in the frequency and intensity of heat waves (Della-Marta et al., 2007; Kyselý, 2008; Fischer et al. 2010; Kuglitsch et al., 2010). High spring T_as are typical for the region north of the Sahara desert belt. The generally higher T_a in those areas more frequently exceeds the upper critical temperature of many bird species, which typically ranges between 36 and 38 °C (Tieleman and Williams, 1999; Burton and Weathers, 2003). It is predicted that the frequency of heat waves will increase most in northern Africa, southern Spain, and Middle Eastern Countries like Jordan and southern Turkey (Giannakopoulos et al., 2009), all important areas for refueling of birds after the spring migration across the Sahara desert belt. Increasing T_a and more frequent heat waves represent major environmental challenges for wildlife, especially for those animals that live in, or stopover at, areas with generally warmer climates. The present study demonstrates the importance of investigating extreme environmental factors such as heat waves on animal performance. Recent research on global change indicates the need to investigate specific events rather than trends, and extremes rather than means (Easterling et al., 2000; Jentsch et al., 2007; Frich et al., 2002). Catastrophic events of mass mortality in association with extreme heat waves are documented for birds (McKechnie and Wolf, 2010) and call for a better understanding of pathological effects of temperature extremes as well as the effects of non-lethal temperature extremes on avian physiology and performance. Knowledge of physiological limits is a key factor for understanding the potential impacts of global change on animal performance and evolution (Travis et al., 1999).

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