

Body temperature upon mist-netting procedures in three species of migratory songbirds at a stopover site: implications for welfare

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Abstract

Capturing wild birds with mist nets is very common for studying many aspects of avian biology. However, except for adrenocortical reactivity, little is known about other physiological responses to this potentially stressful procedure. Here, we focused on body temperature (T_b) in migratory songbirds, as large numbers are caught for population monitoring and studies of migratory biology. T_b is sensitive to acute stress in other vertebrates and contexts, usually showing an increase (stress-induced hyperthermia). We sampled garden warblers (*Sylvia borin*), whinchats (*Saxicola rubetra*) and barn swallows (*Hirundo rustica*) captured with mist nets using standard protocols at an island stopover site in the central Mediterranean during spring migration. T_b was measured within 3 min (T₀), 30 min (T₃₀) and 180 min (T₁₈₀) from the time the bird hit the net, using an analogue probe inserted into the throat, and a body condition score was calculated for each bird. In the garden warbler, but not the other two species, a slight but significant reduction in T_b occurred after 180 min. In all species, the change in T_b after 3 h of confinement was positively correlated to the change in ambient temperature (T_a) but not to body condition. The mean (\pm SEM) change in T_b over 3 h was $-1.68 (\pm 0.16)^\circ\text{C}$ for garden warblers, therefore within the expected range of normothermy in small birds. Such reduction in T_b is contrary to the expectation of stress-induced hyperthermia; these results suggest that garden warblers are able to modulate their body temperature and that these small changes are influenced by fluctuations in ambient temperature. Therefore, T_b might not be a good indicator of capture stress in small passerine migrants. Our results also indicate that restraint for ringing procedures is unlikely to have adverse effects on T_b regulation of migratory birds, if held within the time-frame typical of ringing operations.

Keywords: animal welfare, bird migration, heterothermy, hypothermia, mist-netting, stress response

Introduction

Every year, hundreds of thousands of wild birds (mostly passerines) are caught by specialised operators with mist nets, handled, and ringed for monitoring, conservation, research (especially on migration) and educational purposes (Anderson & Green 2009; Newton 2010). Each individual is retained for a variable time between the moment of capture and the moment of eventual release. The total time of restraint depends on the time taken to disentangle the bird from the net, and the measurements and experiments that are carried out thereafter. On days with a high capture rate, retention time can exceed 1 h (I Maggini, personal observation 2012). After capture, small birds are usually held individually in cotton bags. Although injury and mortality rates are low (0.59 and 0.23%, respectively; Spotswood *et al* 2012) and below the 1% threshold above which the latter is considered excessive (Ralph *et al* 1993), these procedures have the potential to cause stress, suffering and/or decreased fitness to many thousands of individuals every year (Ralph

et al 1993; Lynn & Porter 2008; Fair *et al* 2010; Spotswood *et al* 2012). However, except for the well-documented activation of the adrenocortical response within an adaptive range as a response to acute capture and handling stress (plasma corticosterone levels: Jenni *et al* 2000; Cockrem & Silverin 2002; Romero & Romero 2002; Cockrem 2007; Adams *et al* 2011; Duarte 2013), accompanied by a change in leucocyte counts (Davis 2005; Davis *et al* 2008), little is known about physiological responses to such a procedure.

The levels of reactive oxygen metabolites and plasma antioxidant capacity did not show any significant variation after 30-min confinement upon mist-netting in a small sample of barn swallows (*Hirundo rustica*) and garden warblers (*Sylvia borin*) (Costantini *et al* 2007). In addition, a 45- to 60-min confinement upon capture by mist-netting did not result in observable negative effects of capture and short-term confinement on blood gas, lactate and haematological parameters in three wild species (Harms *et al* 2016).

Table 1 Mean (\pm SEM) body temperature change (obtained by subtracting T0 from T180) after 180-min confinement upon capture by mist-netting in the four species sampled so far with the same protocol and the same thermometric probe during spring migration at the same stopover site.

Species	ΔT ($^{\circ}C$)	Min ($^{\circ}C$)	Max ($^{\circ}C$)	N	P-value	Year	Reference
Garden warbler	-3.42 (\pm 0.24)	-0.80	-5.80	30	**	2006	Carere <i>et al</i> (2010)
Garden warbler	-1.72 (\pm 0.19)	-0.90	-2.30	6	**	2007	Carere, unpublished
Garden warbler	-1.68 (\pm 0.16)	+0.90	-4.10	63	**	2012	This study
Icterine warbler	-3.40 (\pm 0.49)	-1.40	-6.90	12	**	2006	Carere <i>et al</i> (2010)
Whinchat	-0.73 (\pm 0.57)	+1.10	-4.90	12	ns	2012	This study
Barn swallow	-0.49 (\pm 0.46)	+1.80	-3.00	11	ns	2012	This study

** Significance asterisks ($P < 0.01$) refer to the difference between T0 (within ~ 3 min of capture) and T180 (after 180 min, see *Materials and methods*). Statistical outcome for the 2007 unpublished data in garden warblers: T0 = 40.67 (\pm 0.27); T30 = 40.30 (\pm 0.32); T180 = 38.95 (\pm 0.25); $F_{2,5} = 20.44$; $P = 0.0003$; ns = non significant.

Body temperature (Tb) is an important physiological parameter related to many crucial body functions in vertebrates and a proxy of health and stress state (Prinzinger *et al* 1991; Carere & van Oers 2004; Nord *et al* 2016). Studies have shown that large-sized birds such as hens and eider ducks (*Somateria mollissima*) respond to handling protocols with so-called emotional fever, which is characterised by a fast rise in core Tb and can be blocked by salicylate (Briese & Cabanac 1991; Cabanac & Aizawa 2000; Cabanac & Guillemette 2001). A rise in core Tb is considered a typical component of the emotional stress response of laboratory rodents, which can be suppressed by prior treatment with anxiolytics and is paralleled by elevations in plasma adrenocorticotrophic hormone (ACTH), corticosterone and glucose (Koolhaas *et al* 1997; Dallmann *et al* 2006). The stress-induced rise in Tb tends to be established after approximately 10 min but is predicted to last at least 30–60 min, or even longer in rats (Van der Heyden *et al* 1997; Dallmann *et al* 2006). Such a rise has been documented in other animal species, including birds (Cabanac & Aizawa 2000; Carere *et al* 2001). However, a study on a small bird, the barn swallow, revealed a small but statistically significant decrease of about 0.3 $^{\circ}C$ after 10-min handling (Møller 2010). These contrasting findings in small birds call for further investigation to elucidate the Tb response to stress in more avian species and contexts.

In a migratory stopover context, a decrease in Tb of about 2–4 $^{\circ}C$ after 180 min of confinement has been observed upon mist-netting in garden warblers and icterine warblers (*Hippolais icterina*) (Carere *et al* 2010; see also Table 1). Other studies confining blackcaps (*Sylvia atricapilla*) and garden warblers for more prolonged times and monitoring Tb continuously with loggers suggested that the observed drops in Tb (up to 10 $^{\circ}C$ in one study) could be an adaptive strategy to economise energy and facilitate refueling, at least for individuals in poor condition after prolonged flights (Wojciechowski & Pinshow 2009; Cianchetti Benedetti *et al* 2013).

These observations suggest that Tb variation between and within individuals is common during capture and handling procedures involving migratory birds, and could be facilitated by a predisposition to hypothermic or resting states (Schwilch *et al* 2002) that may occur naturally in such a context as an adaptation to migration. Such variation might be affected by individual condition and ambient temperature (Ta), which are both known to correlate with Tb (Weathers 1981; Briese 1992; Noakes *et al* 2013). Assessing the variation in Tb response to capture and confinement is important to monitor the impact of such procedures on the birds.

Welfare issues related to capture stress are currently the subject of increasing interest by scientific communities due to the changes in the European legislation with the implementation of EU Directive 2010/63/EU (European Parliament 2010). In 2011 alone, at our study site at Ponza ornithological station in central Italy, over 18,000 birds of several different species were caught for the purposes of monitoring the spring migration. Small subsets were kept under license for a variable amount of time (maximum 24 h) for other measurements. Similar sorts of figures are seen in hundreds of other bird-ringing stations worldwide.

Here, we systematically studied the variability of Tb upon capture and restraint at the inter-individual and interspecific level in three species of migratory songbirds upon capture by mist-netting at a stopover site, taking into account Ta and individual condition. We investigated whether any signs of stress-induced hyperthermia were detectable in these birds. Given the contradictory nature of the results of the aforementioned studies, we had no preconceived ideas as to what to expect regarding Tb variations and, here, descriptive data are supplied that will contribute further insight into Tb regulation under capture and confinement in migratory birds, and into the usefulness of Tb as an indicator of stress.

Materials and methods

Study site and species

The study was carried out at the Ponza Research Station (<http://www.unife.it/dipartimento/biologia-evoluzione/progetti/behavioural-biology/ponza-research-station>), located on the island of Ponza approximately 50 km from the Tyrrhenian coast of Italy (40°50' N, 12°58' E, 9.87 km²) between 12 April and 15 May 2012, during the main period of spring migration. Birds were trapped with polyester mesh mist nets with a mesh size of 16 mm. These were suspended between two poles, resulting in the net being virtually invisible. Mist nets have shelves created by horizontally strung lines that create a loose, baggy pocket. When a bird hits the net, it falls into the pocket and remains entangled. For most species, large numbers of arrivals are concentrated into the mid-late morning period (Carere *et al* 2010). We sampled three species (63 garden warblers, 12 whinchats [*Saxicola rubetra*], and eleven barn swallows) that are among the most commonly caught species during migration in Mediterranean bird-ringing stations (eg Spina *et al* 1993; Grattarola *et al* 1999). Garden warblers and whinchats are nocturnal migrants, while swallows travel diurnally.

Sampling protocol and body temperature measurements

Nets were monitored continuously and garden warblers, whinchats, and barn swallows immediately removed on entry to the net. The mean (\pm SD) latency between the bird hitting the net (including its disentanglement) and the beginning of the Tb measurement was 59 (\pm 28) s (range 25–153 s). There were no differences among species in latency (one-way ANOVA: $F_{2,83} = 0.294$; $P = 0.746$). Tb was measured by inserting a 0.5-mm diameter probe 3 cm into the throat of the bird via the beak (Carere *et al* 2010). Probe temperature was measured with an analogue thermometer (Physitemp Instruments Inc Model 12, serial #11187, 0.1°C resolution, Physitemp Instruments Inc, Clifton, NJ, USA), waiting 10–15 s until the value stabilised. This first measurement will be referred to hereafter as T0. Subsequently, the birds were kept individually in cotton bags in a shaded and undisturbed site close to the capture site, and Tb measured again after 30 (T30) and 180 min (T180). Following each measurement, Ta was measured with the same probe in the shaded area where the birds were held in the bag. After T180, the birds were ringed and morphometric measurements taken following Bairlein (1995). The length of the third outermost primary feather was measured as a correction factor for size, as this is considered a measure of structural body size (eg Goymann *et al* 2010). Sex and age were determined from plumage characters where possible (sexing was possible in the whinchat and the barn swallow, ageing only in the whinchat: Svensson 1992; Jenni & Winkler 1994). Fat and muscle scores were determined by visual inspection of the belly, which was exposed by blowing apart the feather coverage. Fat scores ranged from 0 (no fat) to 8 (pectoral muscle completely covered with fat), and muscle scores from 0 (muscle deeply concave) to 3 (muscle concave,

sternum no longer visible) (Bairlein 1995). Birds were weighed with a precision of ± 0.1 g using a Kern-Emb 2200-0 electronic weight scale (Kern & Sohn GmbH, Balingen, Germany). Following these procedures, birds were immediately released. One of the captured birds (a garden warbler) died before Tb at T180 could be measured. Sampling occurred between 0715 and 1706h (T0, median 1209h) on days with no rain or strong wind.

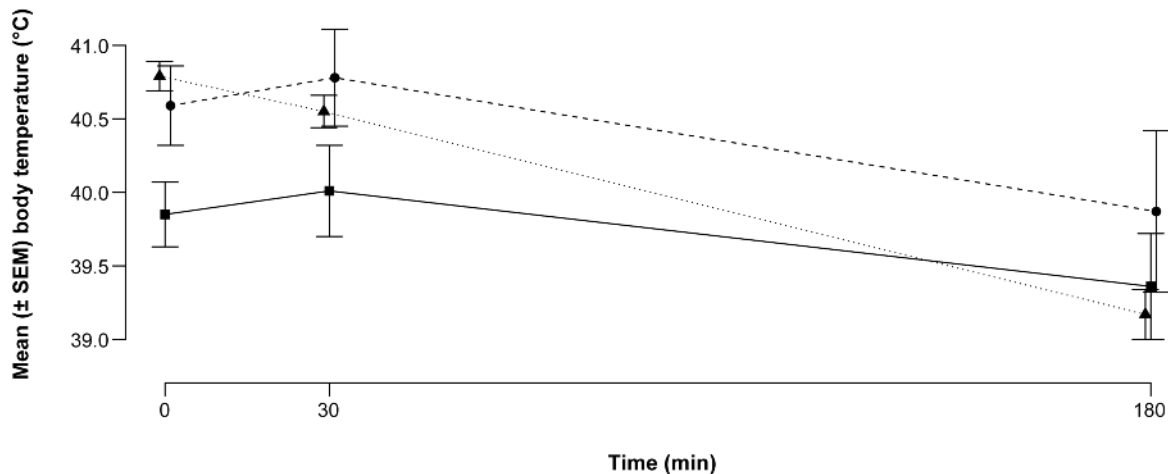
Statistical analysis

We extracted a body condition index called CONDITION separately for each species by applying a Principal Component Analysis (PCA) to size-corrected body mass (body mass divided by the length of the third primary feather, and multiplied by the average length of the third primary for a given species), fat scores and muscle scores (as in Fusani *et al* 2009, 2011). We analysed changes in Tb between T0, T30 and T180 using a linear mixed effect model (LMM), separately for each species. Ta at T0, T30, and T180, respectively, was used as a covariate, as well as the interaction term between Ta and time. The interaction term was removed from final models due to not being significant. Individual was used as random factor. When the effect of time on Tb was significant, Tb was compared at each time-point (T0, T30, and T180) using the glht function with Tukey corrections from the multcomp package in R 3.0.2 (Hothorn *et al* 2008).

The difference in temperature between T0 and T180 was then defined as the delta T (ΔT) to test the relationship between Tb and CONDITION using linear regression models. Here, the difference in Ta (ΔTa) between T0 and T180 was used as a covariate. Latency between capture and Tb measurement (at T0) was added as a covariate in all analyses, but removed from the final analysis because it was not significant in the regression models. This analysis was performed separately for each species, and then repeated, creating a linear regression model including all species and using fat score instead of CONDITION, since the values of CONDITION were generated by independent PCA in each species. Fat score is measured in a standardised way in all species (Kaiser 1993) and is the main variable influencing CONDITION (Fusani *et al* 2009, 2011). We also added two-way interactions (species \times fat score and species \times ΔTa) but removed them from the model when not significant. *Post hoc* pair-wise *t*-tests were performed with Bonferroni corrections in order to compare species.

In all analyses, diurnal changes in Tb were already accounted for through the inclusion of Ta in the models; Ta showed a similar profile to that of Tb across time of day and a strong positive correlation with it (for details, see the supplementary material to papers published in *Animal Welfare* on the UFAW website; <https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material>). All models were checked for normality of residuals and homoscedasticity by plotting residuals against fitted values (Crawley 2007) and all statistical tests were performed using R 3.0.2 (R Core Team 2013).

Figure 1



Mean (\pm SEM) temporal dynamic of body temperature of three species. Barn swallow ($n = 11$): squares/continuous line; whinchat ($n = 12$): circles/dashed line; and garden warbler ($n = 63$): triangles/dotted line. Tb was measured at time T0, T30, and T180. Points are slightly skewed to ease visualisation.

Ethical note

Permission for mist-netting and approval of experimental procedures for 2011 and 2012 was granted by ISPRA and Regione Lazio, Dipartimento Istituzionale e Territorio, Direzione Regionale Ambiente (Permit N A2887 of 06-04-2011). No endangered species were involved in the study. With the exception of one bird that died during measurements, no injury or bleeding occurred and all birds were subsequently released.

Results

The median fat (F) and muscle (M) scores for the three species in question were: swallow ($F = 0$; $M = 1$); whinchat ($F = 1$; $M = 2$); garden warbler ($F = 1$; $M = 2$). For the frequency distribution of these parameters in our sample, see <https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material>.

In all three study species, Ta was significantly correlated with Tb (LMM: garden warbler, $F_{1,121} = 50.32$; $P < 0.001$; whinchat, $F_{1,21} = 13.60$; $P = 0.001$; barn swallow, $F_{1,19} = 9.86$; $P = 0.005$). Tb increased with increasing Ta, irrespective of the time since capture took place.

In the garden warbler, there was a significant effect of time on Tb, after accounting for the effect of Ta (LMM: $F_{2,121} = 97.02$; $P < 0.001$; Figure 1). *Post hoc* pair-wise comparisons indicated a significant difference between T0 and T180 and T30 and T180 (both $P < 0.001$) but not between T0 and T30 ($P = 0.102$). The change in Tb over 3 h of confinement (ΔT) significantly and positively covaried with the change in Ta (linear model: $t = 5.592$; $P < 0.001$) but was not affected by CONDITION ($t = 1.43$; $P = 0.158$). A larger decrease (or smaller increase) in Tb occurred with a larger decrease in Ta (or smaller increase).

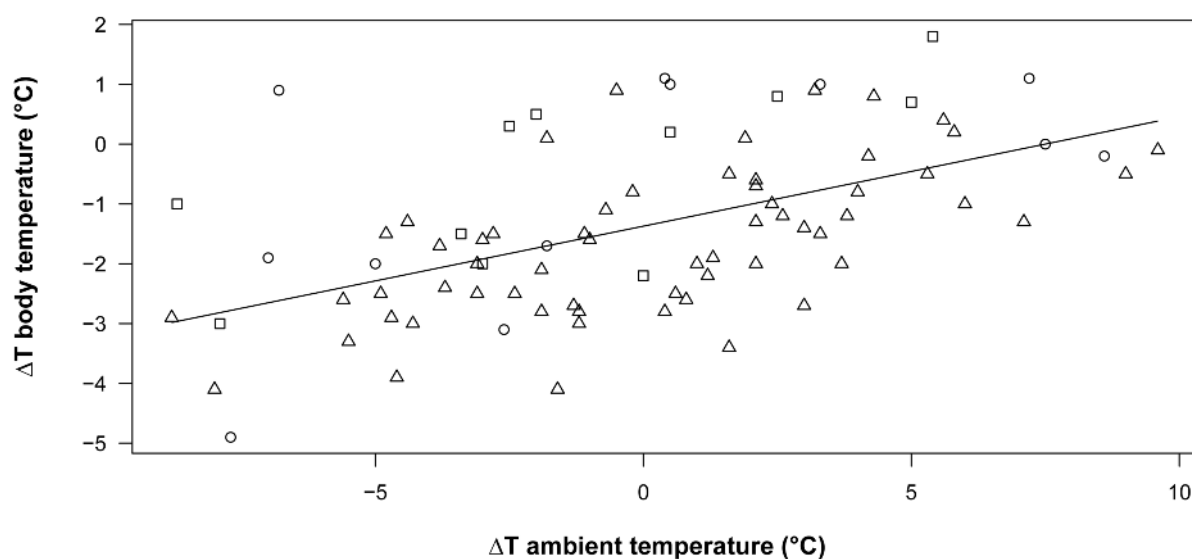
In the whinchat, after accounting for the effect of Ta, there was no significant effect of time on Tb (LMM:

$F_{2,21} = 2.72$; $P = 0.089$; Figure 1). The change in Tb over 3 h of confinement (ΔT) significantly and positively covaried with the change in Ta over the same period (linear model: $t = 2.67$; $P = 0.026$) but not with CONDITION ($t = 2.11$; $P = 0.064$). A larger decrease (or smaller increase) in Tb occurred with a larger decrease in Ta (or smaller increase).

In the barn swallow, there was no change in Tb after accounting for the effect of Ta (repeated measures ANOVA: $F_{2,19} = 1.59$; $P = 0.231$; Figure 1). CONDITION did not affect the change in Tb over 3 h of confinement (ΔT) (linear model: $t = -1.55$; $P = 0.161$). The change in Ta was positively correlated with the change in Tb ($t = 3.14$; $P = 0.014$). A larger decrease (or smaller increase) in Tb occurred with a larger decrease in Ta (or smaller increase).

When pooling all the species into a single model, there was a significant difference among species in ΔT over 3 h (linear model: $F_{2,76} = 8.15$; $P < 0.001$), after accounting for the significant effect of change in Ta over the same period (linear model: $F_{1,76} = 55.34$; $P < 0.001$; Figure 2). *Post hoc* tests indicated that garden warblers reduced their Tb significantly more than barn swallows ($P = 0.033$). There was no difference in ΔT between whinchat and swallow ($P = 0.999$), or between whinchat and garden warbler ($P = 0.101$). In the pooled-species model, no relationship was found between fat score and Tb change (linear model: $F_{4,76} = 1.95$; $P = 0.11$). The change in Ta was positively correlated with ΔT ; larger decreases (or smaller increases) in Tb occurred with larger decreases (or smaller increases) in Ta. All two-way interactions were non-significant, indicating that there was no species-specific effect of fat score or Ta on the change in Tb of birds between T0 and T180.

Figure 2



Correlation between the difference in ambient temperature (T_a) and the difference in body temperature (T_b) in the 3 h of restraint in the three species. Differences are calculated as T_b (respectively T_a) at T180– T_b (T_a) at T0. Symbols represent the species: squares for barn swallow, circles for whinchat and triangles for garden warbler. The regression line refers to all species pooled.

Discussion

Garden warblers were the only species in our study which showed significant changes in T_b when kept restrained during the day. The average direction of the change, namely a decrease of T_b with time, was the opposite of what would be expected if assuming emotional fever. The decrease was only observed in garden warblers after 3 h of confinement, while after 30 min no change in T_b was evident in any species. In both the whinchat and the barn swallow, the latter the only diurnal migrant considered in our study, no decrease in T_b was observed even after 180 min of confinement. No correlation between T_b and individual body condition was noted in any of the study species, however a positive covariation with T_a was found for all.

The observed changes in T_b , considered in relation to T_a , can be regarded as an expression of slight heterothermy, in the range, on average, of 1–3°C, but not as true hypo- or hyperthermia. These changes fall within the documented natural changes between the resting and active phase, and between day and night, a differential estimated at up to 2.84°C in normothermic birds and especially prominent in small birds (Prinzinger *et al* 1991; p 499). In fact, the observed decrease in T_b after 3 h of confinement could also be due to the transition from an active to an inactive state. We do not know whether the temporal dynamics of T_b would have been similar if the birds had not been captured and had continued their normal activity on the island — a technically challenging control — since most individuals are caught after the intense exercise of a long-distance flight and we have no information about birds that were on the island for a prolonged time. A possibility is that nocturnal migrants, such as two of our three study species (excluding the swallow), would tend to be sleepy during the day after prolonged

nocturnal flights, thus lowering T_b towards resting levels (Schwilch *et al* 2002). Therefore, although influenced by manipulation, the patterns of T_b change observed in this study, and especially the reduction observed in the garden warbler, might not be biologically relevant and should not be considered severely harmful in terms of welfare.

By definition, hypothermia entails lowering T_b below the range (between 40 and 41°C core temperature in small passerine birds; Sturkie 1986) set for normal activities reducing some physiological functions (eg cardiac and respiratory), and does not include the normal, slight drop related to rest-phase (Reinertsen 1996). Indeed, previous studies testing whether hypothermia at night could be a strategy to economise energy during migration and involving longer duration of confinements during night-time, reported decreases in T_b of up to 10°C below normothermic levels exclusively at night (blackcap, garden warbler, icterine warbler; Wojciechowski & Pinshow 2009; Carere *et al* 2010), while in our study T_b remained in the normothermic range.

In our study, seven of 63 garden warblers, two of 12 whinchats and one of eleven barn swallows lowered T_b by 3°C or more. This could be considered to be out of the expected range of normothermy (Prinzinger 1991). The lowest T_b values observed in blackcaps stopping over in Israel were described as a “pathological hypothermic response in emaciated or protein-starved birds that do not have large enough energy resources to maintain nocturnal normothermy” (Wojciechowski & Pinshow 2009), which suggests that hypothermic responses might be an emergency response to a life-threatening situation, such as prolonged fasting (Hohtola *et al* 1991; Schleucher 2001; Ben-Hamo *et al* 2010). In contrast, the observations in our

study reflect the pattern of rest-phase reduction described by McKechnie and Lovegrove (2002), which appears to be a common trait in birds. The lack of effect of body condition (as indicated by fat score or by our CONDITION index) on changes in Tb supports this view. Birds might exhibit heterothermy due to their small size and low thermal inertia (Weathers 1981). However, some small species do not exhibit high levels of heterothermy (McKechnie & Lovegrove 2003), suggesting that interspecific differences might be related to particular life-history traits (Noakes *et al* 2013). For example, swallows can exhibit hypothermia or even torpor as an emergency state (Lasiewski & Thompson 1966; Prinzinger & Siedle 1986), but we are not aware of any description of such a state as a common phenomenon during roosting and migration. During our protocol, swallows did not decrease their Tb even after 3 h. We observed that the barn swallows were more active inside the cotton bags during the 3 h, in comparison to the other species (FM Tahamtani, personal observation 2012).

A moderate to high reduction in Tb seems to be common when birds are restrained for a certain duration during day-time since, on migration, they may lower their metabolic costs during periods of inactivity by lowering Tb (Wojciechowski & Pinshow 2009; Cianchetti-Benedetti *et al* 2013). This might entail costs such as increased predation risk associated with reduced flight ability (Carr & Lima 2013), but migrating birds might choose to trade-off safety for energetic gain. Especially during spring, songbirds are known to adopt 'riskier' strategies to reach their breeding grounds early (Dierschke *et al* 2005; Maggini & Bairlein 2010). Due to the small magnitude and, perhaps, small effect size, we do not know whether such a reduction in Tb could lead to an increased risk of predation, delayed departure from the stopover site, or other kinds of harm, but we could hypothesise an energetic cost to recover to normal levels thereafter, which might affect further behaviour and decisions of birds that are stopping and resting for a few hours during their migratory journey. In mourning doves (*Zenaida macroura*), experimentally induced hypothermia with an average Tb reduction of 5.3°C led to an impairment in flight ability that could affect the efficiency of anti-predator behaviour (Carr & Lima 2013). However, this temperature differential was, on average, higher than the slight heterothermy we recorded, except for a few individuals. A different methodological approach (eg tests of flight performance) should be used to test whether such costs arise for the small proportion of birds showing larger decreases in Tb.

As was observed for all three species in this study, a correlation between Ta and Tb in birds has been described in several species (Weathers 1981; Noakes *et al* 2013; Nilsson *et al* 2016). In one mammalian species, Ta has been shown to interact with the stress-induced change in Tb (Briese 1992), but the strong correlation between Tb and Ta observed in the present study in all species does not support a similar interpretation for small birds.

The results obtained suggest that capture stress does not elicit changes in Tb of a magnitude sufficient to raise welfare concerns in birds caught by mist-netting in a migratory stopover context. However, to have stronger conclusions on the sensitivity of this variable, additional species should be tested, including resident ones, and sampling should also be carried out in the non-migratory season. Indeed, a very recent study in wintering chickadees (*Poecile atricapillus*) in which Tb decreased upon handling also suggests that stress hyperthermia is not part of the acute stress response in small songbirds, and that Ta is a key modulator of Tb response in small birds to the point that the authors advised "caution when handling birds in cold winter conditions" (Lewden *et al* 2017).

In Table 1, we summarise the Tb response in the four species tested so far with our protocol at a stopover site after 3 h. So far, only garden warblers show a consistent pattern of Tb response to restraint across different studies and different years ($n = 99$; Table 1). In fact, this study corroborates a similar result obtained for garden warblers and icterine warblers using the same protocol (Carere *et al* 2010) but adds to the latter study by having a larger sample and including of Ta as a significant covariate. Although independent groups measured at different time-points upon capture should be tested to give a definitive answer, it is very unlikely that the procedure of Tb measurement alone triggered the decrease, since 30 min after the first measurement no significant change occurred in both this and the previous study. Notably, after 30 min, the drop in Tb of garden warblers in the previous study was much higher than in this study (Table 1). Those birds were in similar condition and the difference might be due to different Ta (not monitored) or to a day effect. In 2012, garden warblers were sampled across 20 days, while in the other study they were sampled on two consecutive days only (5 and 6 May); different migratory fronts may belong to different populations or could differ because they experienced different conditions during the last migratory trip or in the previous stopover.

Animal welfare implications and conclusion

Guidelines for bird-ringing already mention the importance of keeping the birds restrained for as short a period as possible (Redfern & Clark 2001). Our results indicate that restraint for the time generally used for ringing procedures (usually 10–30 min plus the time spent in the mist net prior to extraction, which can be 0 to 60 min) is unlikely to have adverse effects on body temperature regulation of migratory birds. Core Tb can be easily monitored in birds with simple protocols and equipment such as those used in the present study. Such data would not only provide an immediate evaluation of the individual's thermal condition, but also a physiological database extended to the many different species that are mist-netted in bird-ringing stations.

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References

- Adams NJ, Farnworth MJ, Rickett J, Parker KA and Cockrem JF** 2011 Behavioural and corticosterone responses to capture and confinement of wild blackbirds (*Turdus merula*). *Applied Animal Behaviour Science* 134: 246-255. <https://doi.org/10.1016/j.applanim.2011.07.001>
- Anderson GQA and Green RE** 2009 The value of ringing for bird conservation. *Ringling & Migration* 24: 205-212. <https://doi.org/10.1080/03078698.2009.9674393>
- Bairlein F** 1995 *Manual of Field Methods, Revised Edition*. European Science Foundation: Wilhelmshaven, Germany
- Ben-Hamo M, Pinshow B, McCue MD, McWilliams SR and Bauchinger U** 2010 Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*. *Comparative Biochemistry and Physiology A156*: 84-91. <https://doi.org/10.1016/j.cbpa.2009.12.020>
- Briese E** 1992 Cold increases and warmth diminishes stress-induced rise of colonic temperature in rats. *Physiology and Behavior* 51: 881-883. [https://doi.org/10.1016/0031-9384\(92\)90130-T](https://doi.org/10.1016/0031-9384(92)90130-T)
- Briese E and Cabanac M** 1991 Stress hyperthermia: physiological arguments that it is a fever. *Physiology and Behavior* 49: 1153-1157. [https://doi.org/10.1016/0031-9384\(91\)90343-M](https://doi.org/10.1016/0031-9384(91)90343-M)
- Cabanac AJ and Guillemette M** 2001 Temperature and heart rate as stress indicators of handled common eider. *Physiology and Behavior* 74: 475-479. [https://doi.org/10.1016/S0031-9384\(01\)00586-8](https://doi.org/10.1016/S0031-9384(01)00586-8)
- Cabanac M and Aizawa S** 2000 Fever and tachycardia in a bird (*Gallus domesticus*) after simple handling. *Physiology and Behavior* 69: 541-545. [https://doi.org/10.1016/S0031-9384\(00\)00227-4](https://doi.org/10.1016/S0031-9384(00)00227-4)
- Carere C, Costantini D, Fusani L, Alleve E and Cardinale M** 2010 Hypothermic abilities of migratory songbirds at a stopover site. *Rendiconti Lincei* 21: 323-334. <https://doi.org/10.1007/s12210-010-0094-0>
- Carere C and van Oers K** 2004 Shy and bold great tits (*Parus major*): body temperature and breath rate in response to handling stress. *Physiology and Behavior* 82: 905-912. [https://doi.org/10.1016/S0031-9384\(04\)00312-9](https://doi.org/10.1016/S0031-9384(04)00312-9)
- Carere C, Welink D, Drent PJ, Koolhaas JM and Groothuis TGG** 2001 Effect of social defeat in a territorial bird (*Parus major*) selected for different coping styles. *Physiology and Behavior* 73: 427-433. [https://doi.org/10.1016/S0031-9384\(01\)00492-9](https://doi.org/10.1016/S0031-9384(01)00492-9)
- Carr JM and Lima SL** 2013 Nocturnal hypothermia impairs flight ability in birds: a cost of being cool. *Proceedings of The Royal Society B280*: 20131846. <https://doi.org/10.1098/rspb.2013.1846>
- Cianchetti Benedetti M, Fusani L, Bonanni R, Cardinale M and Carere C** 2013 Condition-dependent nocturnal hypothermia in garden warblers *Sylvia borin* at a spring stopover site. *Ardea* 101: 113-119. <https://doi.org/10.5253/078.101.0206>
- Cockrem JF** 2007 Stress, corticosterone responses and avian personalities. *Journal of Ornithology* 148: 169-178. <https://doi.org/10.1007/s10336-007-0175-8>
- Cockrem JF and Silverin B** 2002 Variation within and between birds in corticosterone responses of great tits (*Parus major*). *General and Comparative Endocrinology* 125: 197-206. <https://doi.org/10.1006/gcen.2001.7750>
- Costantini D, Cardinale M and Carere C** 2007 Oxidative damage and anti-oxidant capacity in two migratory bird species at a stop-over site. *Comparative Biochemistry and Physiology C144*: 363-371. <https://doi.org/10.1016/j.cbpc.2006.11.005>
- Crawley MJ** 2007 *The R Book*. John Wiley & Sons Ltd: Chichester, UK. <https://doi.org/10.1002/9780470515075>
- Dallmann R, Steinlechner S, von Horsten S and Karl T** 2006 Stress-induced hyperthermia in the rat: comparison of classical and novel recording methods. *Laboratory Animals* 40: 186-193. <https://doi.org/10.1258/002367706776319015>
- Davis AK** 2005 Effect of handling time and repeated sampling on avian white blood cell counts. *Journal of Field Ornithology* 76: 334-338. <https://doi.org/10.1648/0273-8570-76.4.334>
- Davis AK, Maney DL and Maerz JC** 2008 The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology* 22: 760-772. <https://doi.org/10.1111/j.1365-2435.2008.01467.x>
- Dierschke V, Mendel B and Schmaljohann H** 2005 Differential timing of spring migration in northern wheatears *Oenanthe oenanthe*: hurried males or weak females? *Behavioral Ecology and Sociobiology* 57: 470-480. <https://doi.org/10.1007/s00265-004-0872-8>
- Duarte LMG** 2013 *Impacts of capture and handling in birds*. PhD Thesis, University of Cardiff, UK
- European Parliament** 2010 Council of the European Union Directive 2010/63/EU of the European Parliament of 22 September 2010 on the Protection of Animals used for Scientific Purposes. *Official Journal of the European Union* 53: 33-79
- Fair JE, Paul E and Jones** 2010 *Guidelines to the use of Wild Birds in Research*. Ornithological Council: Washington DC, USA
- Fusani L, Cardinale M, Carere C and Goymann W** 2009 Stopover decision during migration: physiological conditions predict nocturnal restlessness in wild passerines. *Biology Letters* 5: 302-305. <https://doi.org/10.1098/rsbl.2008.0755>
- Fusani L, Cardinale M, Schwabl I and Goymann W** 2011 Food availability but not melatonin affects nocturnal restlessness in a wild migrating passerine. *Hormones and Behavior* 59: 187-192. <https://doi.org/10.1016/j.yhbeh.2010.11.013>

- Goymann W, Spina F, Ferri A and Fusani L** 2010 Body fat influences departure from stopover sites in migratory birds: evidence from whole-island telemetry. *Biology Letters* 6: 478-481. <https://doi.org/10.1098/rsbl.2009.1028>
- Grattarola A, Spina F and Pilastro A** 1999 Spring migration of the garden warbler (*Sylvia borin*) across the Mediterranean Sea. *Journal of Ornithology* 140: 419-430. <https://doi.org/10.1007/BF01650986>
- Harms CA, Jinks MR and Harms RV** 2016 Blood gas, lactate, and hematology effects of venipuncture timing and location after mist-net capture of mourning doves (*Zenaida macroura*), boat-tailed grackles (*Quiscalus major*), and house sparrow (*Passer domesticus*). *Journal of Wildlife Disease* 52: 54-64. <https://doi.org/10.7589/52.2S.S54>
- Hohtola E, Hissa R, Pyornila A, Rintamaki H and Saarela S** 1991 Nocturnal hypothermia in fasting Japanese quail: the effect of ambient temperature. *Physiology and Behavior* 49: 563-567. [https://doi.org/10.1016/0031-9384\(91\)90281-R](https://doi.org/10.1016/0031-9384(91)90281-R)
- Hothorn T, Bretz F and Westfall P** 2008 Simultaneous inference in general parametric models. *Biometrical Journal* 50: 346-363. <https://doi.org/10.1002/bimj.200810425>
- Jenni L, Jenni-Eiermann SJ, Spina F and Schwabl H** 2000 Regulation of protein breakdown and adrenocortical response to stress in birds during migratory flight. *American Journal of Physiology* 278: 1182-1189. <https://doi.org/10.1152/ajpregu.2000.278.5.R1182>
- Jenni L and Winkler R** 1994 *Moult and Ageing of European Passerines*. Academic Press: London, UK
- Kaiser A** 1993 A new multi-category classification of subcutaneous fat deposits of songbirds. *Journal of Field Ornithology* 64: 246-255
- Koolhaas JM, Meerlo P, de Boer SF, Strubbe JH and Bohus B** 1997 The temporal dynamics of the stress response. *Neuroscience and Biobehavioral Reviews* 21: 775-782. [https://doi.org/10.1016/S0149-7634\(96\)00057-7](https://doi.org/10.1016/S0149-7634(96)00057-7)
- Lasiewski RC and Thompson HJ** 1966 Field observation of torpidity in the violet-green swallow. *Condor* 68: 102-103. <https://doi.org/10.2307/1365178>
- Lewden A, Nord A, Petit M and Vezina F** 2017 Body temperature responses to handling stress in wintering black-capped chickadees (*Poecile atricapillus* L.). *Physiology & Behavior* 179: 49-54. <https://doi.org/10.1016/j.physbeh.2017.05.024>
- Lynn SE and Porter AJ** 2008 Trapping initiates stress response in breeding and non-breeding house sparrows *Passer domesticus*: implications for using unmonitored traps in field studies. *Journal of Avian Biology* 39: 87-94. <https://doi.org/10.1111/j.0908-8857.2008.04204.x>
- Maggini I and Bairlein F** 2010 Endogenous rhythms of seasonal migratory body mass change and nocturnal restlessness in different populations of Northern wheatear *Oenanthe oenanthe*. *Journal of Biological Rhythms* 25: 268-276. <https://doi.org/10.1177/0748730410373442>
- McKechnie AE and Lovegrove BG** 2002 Avian facultative hypothermic responses: a review. *Condor* 104: 705-724. [https://doi.org/10.1650/0010-5422\(2002\)104\[0705:AFH-RAR\]2.0.CO;2](https://doi.org/10.1650/0010-5422(2002)104[0705:AFH-RAR]2.0.CO;2)
- McKechnie AE and Lovegrove BG** 2003 Facultative hypothermic responses in an Afrotropical arid-zone passerine, the red-headed finch (*Amadina erythrocephala*). *Journal of Comparative Physiology B173*: 339-346. <https://doi.org/10.1007/s00360-003-0341-0>
- Møller AP** 2010 Body temperature and fever in a free-living bird. *Comparative Biochemistry and Physiology B156*: 68-74. <https://doi.org/10.1016/j.cbpb.2010.02.006>
- Newton I** 2010 *Bird Migration*. Harper Collins: UK
- Nilsson J-Å, Molokwu MN and Olsson O** 2016 Body temperature regulation in hot environments. *PLoS ONE* 11(8): e0161481. <https://doi.org/10.1371/journal.pone.0161481>
- Noakes MJ, Smit B, Wolf BO and McKechnie AE** 2013 Thermoregulation in African green pigeons (*Treron calvus*) and a re-analysis of insular effects on basal metabolic rate and heterothermy in columbid birds. *Journal of Comparative Physiology B183*: 969-982. <https://doi.org/10.1007/s00360-013-0763-2>
- Nord A, Lehmann M, Ross M, McCafferty DJ, Nager RG, Nilsson J-A and Helm B** 2016 Evaluation of two methods for minimally invasive peripheral body temperature measurement in birds. *Journal of Avian Biology* 47: 417-427. <https://doi.org/10.1111/jav.00845>
- Prinzinger R, Pressmar A and Schleucher E** 1991 Body temperature in birds. *Comparative Biochemistry and Physiology A99*: 499-506. [https://doi.org/10.1016/0300-9629\(91\)90122-S](https://doi.org/10.1016/0300-9629(91)90122-S)
- Prinzinger R and Siedle K** 1986 Experimenteller nachweis von torpor bei jungen mehlschwalben *Delichon urbica*. *Journal of Ornithology* 127: 95-96. [Title translation: Experimental evidence of torpor in juvenile house martins]. <https://doi.org/10.1007/BF01641448>
- Ralph CJ, Geupel GR, Pyle P, Martin TE and DeSante DF** 1993 *Handbook of Field Methods for Monitoring Landbirds*. United States Department of Agriculture Forest Service: Albany, CA, USA. <https://doi.org/10.2737/PSW-GTR-144>
- R Core Team** 2013 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria. <http://www.R-project.org/>
- Redfern CPF and Clark JA** 2001 *Ringers' Manual*. BTO: Thetford, UK
- Reinertsen RE** 1996 Physiological and ecological aspects of hypothermia. In: Carey C (ed) *Avian Energetics and Nutritional Ecology* pp 125-157. Chapman and Hall: New York, USA. https://doi.org/10.1007/978-1-4613-0425-8_5
- Romero L and Romero R** 2002 Corticosterone responses in wild birds: the importance of rapid initial sampling. *Condor* 104: 129-135. [https://doi.org/10.1650/0010-5422\(2002\)104\[0129:CRIVBT\]2.0.CO;2](https://doi.org/10.1650/0010-5422(2002)104[0129:CRIVBT]2.0.CO;2)
- Schleucher E** 2001 Heterothermia in pigeons and doves reduces energetic costs. *Journal of Thermal Biology* 26: 287-293. [https://doi.org/10.1016/S0306-4565\(01\)00032-8](https://doi.org/10.1016/S0306-4565(01)00032-8)
- Schwilch R, Piersma T, Holmgren NMA and Jenni L** 2002 Do migratory birds need a nap after a long nonstop flight? *Ardea* 90: 149-154
- Spina F, Massi A, Montemaggiore A and Baccetti N** 1993 Spring migration across central Mediterranean: General results from the 'Progetto Piccole Isole'. *Vogelwarte* 37: 1-94

- Spotswood EN, Goodman KR, Carlisle J, Kormier RL, Humple DL, Rousseau J, Guers SL and Barton GG** 2012 How safe is mist netting? Evaluating the risk of injury and mortality to birds. *Methods in Ecology and Evolution* 3: 29-38. <https://doi.org/10.1111/j.2041-210X.2011.00123.x>
- Sturkie PD** 1986 *Avian Physiology, Fourth Edition*. Springer Verlag: New York, Berlin, Heidelberg, Tokyo. <https://doi.org/10.1007/978-1-4612-4862-0>
- Svensson L** 1992 *Identification Guide to European Passerines, Fourth Edition*. British Trust for Ornithology: Stockholm, Sweden
- van der Heyden JAM, Zethof TJJ and Olivier B** 1997 Stress-induced hyperthermia in singly housed mice. *Physiology and Behavior* 62: 463-470. [https://doi.org/10.1016/S0031-9384\(97\)00157-1](https://doi.org/10.1016/S0031-9384(97)00157-1)
- Weathers WW** 1981 Physiological thermoregulation in heat-stressed birds: consequences of body size. *Physiological Zoology* 54: 345-361. <https://doi.org/10.1086/physzool.54.3.30159949>
- Wojciechowski MS and Pinshow B** 2009 Heterothermy in small, migrating passerine birds during stopover: use of hypothermia at rest accelerates fuel accumulation. *Journal of Experimental Biology* 212: 3068-3075. <https://doi.org/10.1242/jeb.033001>