Heat loss in sleeping garden warblers (Sylvia borin) during migration

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ABSTRACT

For small songbirds, energy is often a limiting factor during migration and, for this reason, they are forced to alternate nocturnal flights with stopovers to rest and replenish energy stores. Stopover duration has a key role for a successful migration and may have an important impact on fitness. Thus, migrants need to optimize their energy consumption at this stage to reduce their permanence at the site. A recent study has shown that lean individuals reduce their metabolic rate when tucking the head in the feathers during sleep. The underlying mechanism is very likely a reduction in conductance, but the thermoregulatory benefit of the increased insulation has never been quantified yet. Here, we compared heat loss in individual migratory birds while sleeping in different postures. Using a thermal camera and a within-individual approach, we estimated that Garden Warblers can reduce their rate of heat loss by 54% by sleeping with the head tucked in the feathers. This energy saving has a relevant impact on the individual's energy balance because it can account for up to 8.69% of daily energy expenditure during stopover. Our study provides novel and important information to understand the fundamental role of thermoregulatory strategies on bird's energy management.

1. Introduction

Among several phyla, all species studied so far have been found to engage in some form of sleep or sleep-like behavior (Dewasmes et al., 1985; Hartse, 2009; Rattenborg and Amlaner, 2002; Rattenborg, 2006; Shapiro and Hepburn, 1976; Shaw et al., 2000; Tobler and Neuner-Jehle, 1992; Zhdanova et al., 2001). Because of its widespread presence among species and its occurrence even under risky circumstances (Lima et al., 2005), sleep appears to serve an important function for a multitude of organisms. Although in the last decades several hypotheses have been formulated, the actual functions of sleep are still debated and poorly understood (Rechtschaffen, 1998). A longstanding theory posits that sleep reduces energy consumption to lower levels than quiet wakefulness, allowing a more effective energy conservation (Berger and Phillips, 1995). This hypothesis is based on the observation that, during sleep, metabolic rate decreases to 15% of the metabolic rate during quiet wakefulness (Jung et al., 2011; Markwald et al., 2013; White et al., 1985). For this reason, sleep may play an important role when energy management becomes a constraint for the individual, i.e. during high energy demanding life-history stages, acute shortage of food

resources, or low ambient temperature (Walker and Berger, 1980). In these contexts, animals can save energy by minimizing metabolic rate during inactive periods (resting metabolic rate, RMR). A reduction of heat loss can contribute to minimize RMR, since it allows to decrease the amount of energy invested in thermogenesis to maintain body temperature. This mechanism is particularly relevant for small animals because of the high ratio between exposed surface and body mass, which leads to a high heat dissipation.

From a behavioral perspective, sleeping animals display some universal features: they become motionless (behavioral quiescence), show a higher threshold for arousal in response to stimulation, can reverse to wakefulness fast (Flanigan, 1972; Piéron, 1913), and display species-specific postures. Adopting a particular posture can be an effective way to regulate body insulation, thus controlling heat dissipation. This seems to be particularly important in birds (Ferretti et al., 2020; Ferretti et al., 2019b; Pavlovic et al., 2019). Birds display essentially two postures during sleep: the head is either pulled back towards the body and faces forward (hereafter untucked), or is turned backwards with the bill tucked between the scapular feathers (hereafter tucked) (Amlaner Jr. and Ball, 1983). It has been observed in different species

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that the posture displayed during sleep is often associated to different environmental conditions. In particular, a relationship between sleep postures and outdoor temperature has been observed in several bird species, where animals responded to a decrease in ambient temperature by tucking the head and the bill into the feathers (Midtgård, 1978; Reebs, 1986; Wellmann and Downs, 2009). A role for sleep posture in energy management has been recently shown for birds during migration (Ferretti et al., 2019b). Due to the large amount of energy required to sustain endurance migratory flights, birds need to make refueling stopovers along their migratory route (Schmaljohann et al., 2007). During the stopover phase, birds must manage their energy reserves to minimize the overall time of migration (Hedenström and Alerstam, 1997), which can increase their reproductive success (Smith and Moore, 2005). As shown by a recent study, migratory Garden Warblers (*Sylvia borin*) modulate their sleep pattern depending on the extent of their energy reserves. Moreover, the extent of energy reserves is associated with the proportion of time spent in each posture during sleep, with lean birds sleeping mostly in the tucked posture (Ferretti et al., 2019b). The same study showed that most heat was dissipated through the head, although we did not quantify the benefit of the tucked posture in terms of lowered heat dissipation. Feathers are very good insulators (Scholander et al., 1950; Veghte and Herreid, 1965), therefore birds can reduce the exposure of non-insulated surfaces, and consequently their thermal conductance, by tucking the featherless parts of their body - i.e. the eye region and the bill – under the scapular feathers. Consequently, the tucked sleep posture has been often assumed to function to reduce heat loss (Bouchard and Anderson, 2011; Fortin and Gauthier, 2000; Pavlovic et al., 2019; Wellmann and Downs, 2009), yet its energetic benefits have not been demonstrated and/or quantified. Indeed, the use of this posture as a behavioral thermoregulatory strategy is more frequent in bird species with large featherless body parts, such as bill and legs (Pavlovic et al., 2019).

Here, we aimed to quantify the reduction in heat dissipation and estimate the amount of energy saved in Garden Warblers when sleeping tucked. Through the use of infra-red thermographic imaging and a within-individual approach, we compared the heat dissipation (here, rate of heat transfer, or Q_{total}) of birds imaged over their profile in individuals caught at a Mediterranean stopover site during spring migration which displayed both sleep postures. In addition, we examined the results of this study in relation to the difference in metabolic rate between sleeping postures which we measured by means of respirometry in recent work (Ferretti et al., 2019b), discussing the thermal benefits of increased insulation in the frame of energy balance during stopover.

2. Material and methods

2.1. Study site and experimental setup

This study was carried out on the island of Ponza in the Tyrrhenian Sea (40°55′ N, 12°58' E). The island is an important stopover site for many European-African migratory birds, particularly during spring migration.

During spring 2019, we took thermal images of the flat profile of four Garden Warblers sleeping at night between 22:00 and 1:30. In order to reduce potential variability linked to body condition, we used only birds with similar amounts of energy reserves (fat score = 2, muscle score = 2; Bairlein, 1995). We took multiple images of each individual displaying both postures using an infra-red thermographic camera (FLIR SC325; Accuracy: ±2 °C or ±2% of reading, IR resolution: 320 × 240 pixels, FLIR System) connected to a laptop running ThermaCAM Researcher 2.10 (FLIR System). The final sample size was of 11 tucked (3 ± 1 image/bird) and 11 untucked sleeping birds (3 ± 1 image/bird). The thermographic camera was kept at 0.5 m from a custom-made cage in which one of the walls was substituted with a polyethylene film (CAP-T, manufactured by IRISS, Terrance Bradenton, Florida, USA; for the transmittance curve see Supplementary Fig. 1). We accounted for the

presence of the polymer by setting the long-wave infra-red transmittance at 45%. This value for transmittance was assessed empirically using all the collected thermal images and ThermaCAM Researcher 2.10. We modified the transmittance value until the temperature of the tip of the tail in each thermal image matched with the ambient temperature. We decided to use the tip of the tail as a reference because is the only body part of the bird with known emissivity (0.96) and, given the length of tail feathers, is not affected by body temperature (as visible in Fig. 1). Indeed, the precision of this correction was confirmed by the match between the ambient temperature and the tip of the tail in each image measured using this setting. The cage was placed in a recording room (Ta: 20.52 ± 1.56 °C, RH: 76.91 ± 5.12%) illuminated by a warm light LED bulb. Illumination was not direct to the cage but redirected and reduced (<1 lux) using aluminum foil. We collected thermal images exclusively when the bird was visible from a lateral view to standardize the orientation of the main body axis in relation to the camera. Because birds tucked their head also on the side that did not face the thermal camera, animals were also filmed using an infrared-sensitive video-camera to determine the position of the head during tucked sleep. Thermal images were collected exclusively when the head was tucked on the side facing the infra-red thermographic camera. The infrared-sensitive camera was also used to determine whether untucked birds were asleep or not. A bird was considered asleep when its eyes were closed, it showed immobility for longer than 5 s and increased feather volume. The 5 s rule is based on the fact that EEG signs of slow-wave sleep occur within a few seconds after onset of immobility in a sleep posture in a variety of avian species, including songbirds (see figures in Lesku et al., 2012; Rattenborg et al., 2004; Scriba et al., 2013; Tisdale et al., 2018; Tisdale et al., 2017).

2.2. Estimate of the rate of heat dissipation

Based on raw infra-red radiance recorded by the infra-red thermographic camera, we compared heat dissipation from the body, the bill, and the head, which includes the eye region (Fig. 1). Surface temperatures were extracted from the manually drawn Regions Of Interest (ROIs) in the thermal image using ThermaCAM Researcher 2.10 (for ROIs see Supplementary Table 1). For these areas, we calculated the average temperature (mean of temperatures recorded in each ROI) and estimated the rate of heat exchange (W/m^2). For each image, we assumed an emissivity of 0.96 (Tattersall, 2016). The rate of heat exchange (loss = negative, gain = positive) was calculated separately for each body part as the sum of the convective and radiative heat exchange (Tattersall et al., 2018):
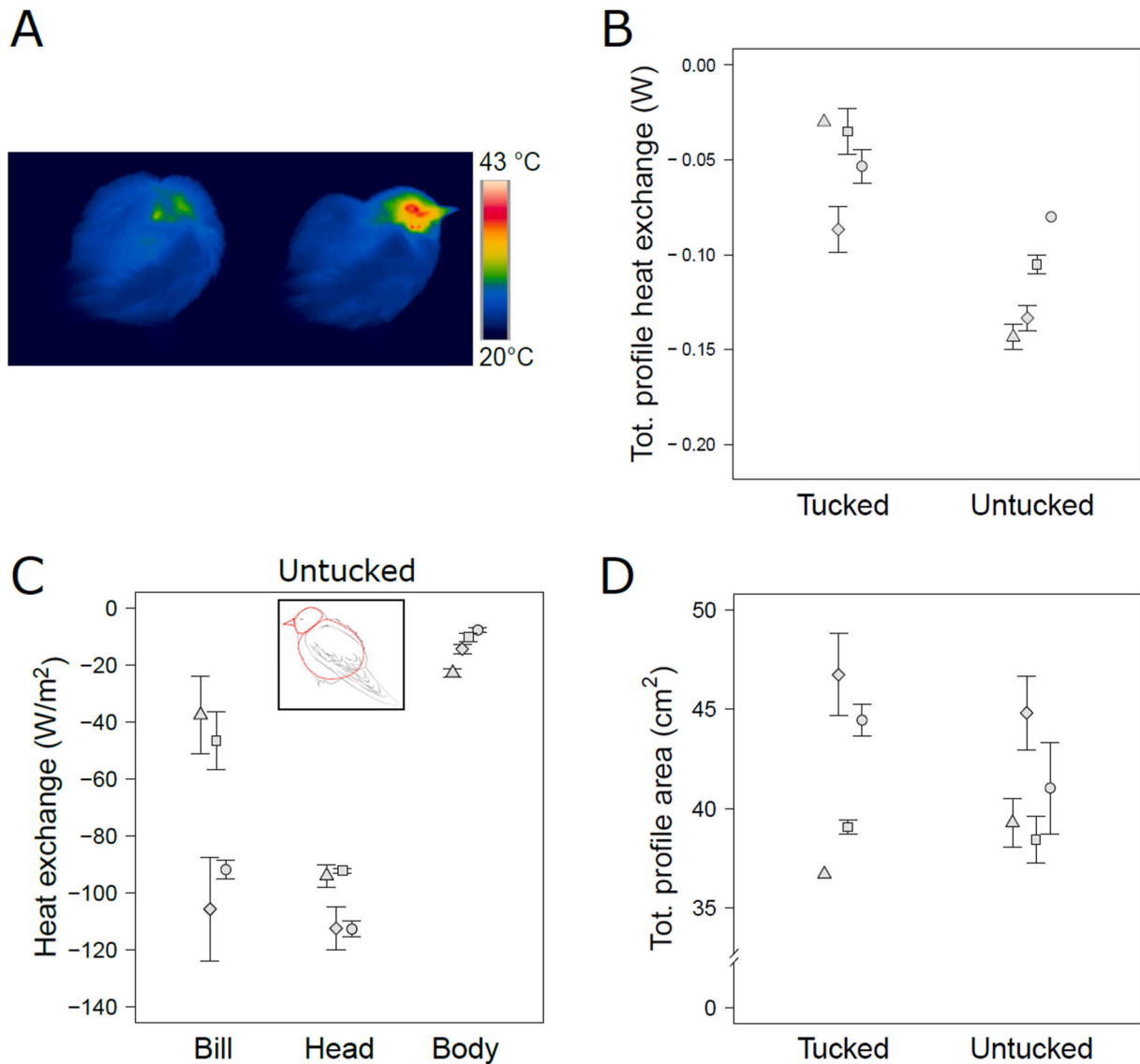
Heat Exchange = Q_c + Q_r

Convective (Q_c) and radiative (Q_r) rate of heat exchange were calculated using the Thermimage package in R (Tattersall, 2016; Tattersall, 2017). For further details about equations on which these functions are based, see Tattersall (2016). Because the aim of this study was to compare heat exchange between sleep postures, we did not consider energy transfer between the bird and the perch because it is independent of the posture displayed. In the computation of Q_c and Q_r we incorporated independent measurements of ambient temperature (from an external temperature logger), and relative humidity. In the computation of Q_c, we considered different shapes depending on the posture and on the body part (see Supplementary Table 2). We obtained Relative humidity readings from the reference measurement of a respirometry system (HIH9131, Accuracy: ± 1.7% RH, Honeywell International Inc. Morristown, New Jersey, USA) that was placed in the same testing room for a different experiment. Because our images were collected indoors and in low-intense indirect light condition, we assumed null surface reflectance, zero solar energy (rho = 0; SE = 0) and a negligible indoor air speed (V = 0.1 m/s).

For each image, we estimated heat loss from the head, the bill and
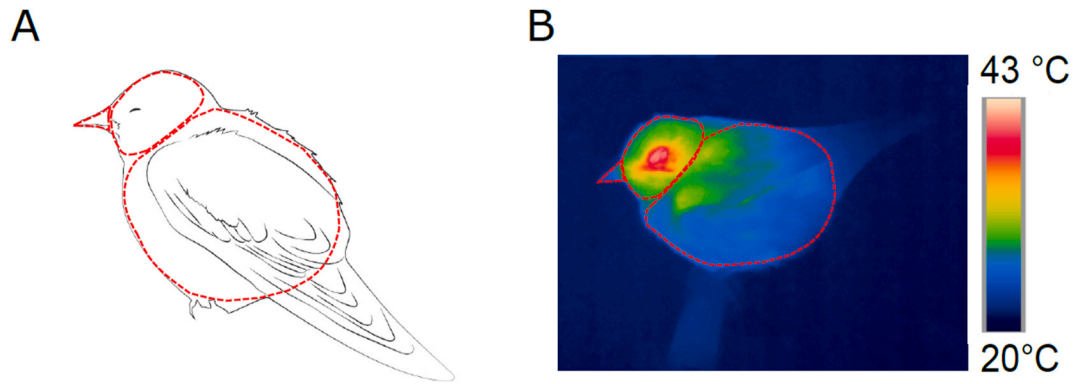
Fig. 1. Estimation of heat loss rate through different body parts in untucked Garden Warblers. Drawing and thermal image showing the delineation of body parts: body, head with a patch of higher temperature around the eye, and bill. Thermal imaging showed noticeable differences in the surface temperature between the body and the featherless area of the head.

Fig. 2. Rate of heat exchange in sleeping Garden Warblers. A) Thermal profile of Garden Warblers sleeping tucked and untucked. B) Tucked birds showed a significantly lower rate of heat loss. C) In untucked sleeping birds, the estimated rate of heat exchange was significantly larger for the tucked body part (head and bill) than in the rest of the body. D) When sleeping tucked, Garden Warblers reduce their exposed surface of 3%. Average values and standard errors are represented by different symbols for each individual (N = 4).

the body separately as the sum of energy exchange from each body part multiplied by its surface area (m^2):

$$Q_{bill} = (Qc \times Area_{bill}) + (Qr \times Area_{bill})$$

$$Q_{head} = (Qc \times Area_{head}) + (Qr \times Area_{head})$$

$$Q_{body} = (Qc \times Area_{body}) + (Qr \times Area_{body})$$

As only the body is clearly visible in tucked birds, we considered the whole profile of the bird in this posture as 'body'. The cumulative rate of energy exchanged (W) by the whole-body profile was calculated as:

$$Q_{total} = Q_{bill} + Q_{head} + Q_{body}$$

In order to compare heat saved by tucking in the head and the bill, we computed the rate of energy exchange of a standard area (3.00 cm^2) centered around the point with the highest temperature measured within the body profile, which corresponds to the position of the eye under the feathers (Fig. 2A). Finally, we estimated the proportional reduction of the surface area in birds sleeping tucked compared to untucked ones.

2.3. Estimation of object dimension

All dimensions that were used in the estimation of heat loss, including surface areas, were estimated from thermal images using an image processing software (ImageJ, Schneider et al., 2012). We first manually drew ROIs and saved the thermal image as JPEG file using ThermaCAM Researcher 2.10. Then, we estimated the area from each JPEG file with ImageJ, using an object in the image with known dimensions as a reference. Given that the distance between the focal individual and the thermal camera may slightly change depending on where the bird perches, we used the perch width measured near the animal as a reference to calibrate the surface area estimations of each thermal image.

2.4. Statistical analysis

We ran three separate Linear Mixed-effects Models (LMMs, 'lmer' function in 'lme4' R package; Bates et al., 2015) using the rate of heat exchange from a standard area and the total profile, as well as from different body parts, as response variables. For each model, we adjusted for repeated measurements by computing random intercepts for each bird ID and entering posture as a fixed effect. We did not include ambient temperature in the model because it was included in the algorithm used to calculate heat exchange and it remained relatively constant across the experiment (see Supplementary Table 3). In each model normality of residuals was assessed by Shapiro test. Using Breusch-Pagan test, we confirmed homoscedasticity in our models. Statistical analysis was conducted with R (R Core Team, 2018) (version 3.5.1), using a significance level of α < 0.05. P-values were provided by the package 'LmerTest' (Kuznetsova et al., 2017) using Satterthwaite's method.

3. Results

The head was the body part with the highest rate of heat dissipation (Pairwise post-hoc test; Bill Vs Head: Estimate = 31.238, SE = 8.227, p-value < 0.001; Head Vs Body: Estimate = −89.699, SE = 8.227, p-value < 0.001; Bill Vs Body: Estimate = −58.461, SE = 8.227, p-value < 0.001; Fig. 2C). When we analyzed total rate of energy exchange in the same individual displaying both sleep postures, we found that by sleeping tucked-in migrating Garden Warblers significantly reduced the amount of dissipated energy in comparison to untucked sleep (LMM; Estimate = −0.057; SE = 0.009; t-value = −6.158, p-value < 0.001; Random effect residual σ < 0.001; Fig. 2B). These findings were further supported by the investigation of the area around the highest

temperature (LMM; Estimate = −0.031; SE = 0.003; t-value = −10.450, p-value < 0.001; Random effect residual σ < 0.001; Fig. 2D).

4. Discussion

As endotherms, birds invest a large proportion of their daily energy expenditure into the maintenance of a high and relatively constant body temperature (King and Farner, 1961). For this reason, several physiological, morphological and behavioral adaptations evolved to cope with environmental temperature fluctuations (Pavlovic et al., 2019; Tattersall et al., 2012). An example of these behavioral strategy is huddling (Chaplin, 1982; Putaala et al., 1995). This strategy has been described mainly in colonial bird species (Brown and Brown, 1999; Brown and Brown, 2000; Gilbert et al., 2006; Møller, 1994), suggesting that it requires some form of social structure. For this reason, it is unlikely that this strategy is adopted by migrating passerine species, for which there is no evidence of social aggregations during stopover. So far, huddling has been reported in migratory passerines only in caged birds exposed to low ambient temperature (Wojciechowski et al., 2011) and/or after 12 h of fasting at ambient temperatures similar to those at our study site (Wojciechowski et al., 2008). Thus, migrating songbirds may use individual strategies to cope with fluctuating environmental temperature. For example, when the environmental temperature is high, birds can look for favorable cooler microhabitats (Cunningham et al., 2015; Wolf and Walsberg, 1996) and/or display yawning or panting (Gallup et al., 2009). On the contrary, when ambient temperature decreases or energy resources are scarce, birds display thermoregulatory behaviors that involve postural changes aimed to conserve heat, i.e. tucking the head under the feathers (Pavlovic et al., 2019). These postural changes often involve the bill, a body part that plays an important role for thermoregulation (Tattersall et al., 2009; Tattersall et al., 2017). In addition to this, birds may also downregulate their body temperature (Carere et al., 2010; Cianchetti Benedetti et al., 2013; Maggini et al., 2018; Wojciechowski and Pinshow, 2009). However, heat-conserving postures have also a cost and their use depends on a trade-off between the energy advantage and the risk of being predated. Indeed, several species have shown a slower reaction to threats while displaying heat-conserving postures (Carr and Lima, 2012; Ferretti et al., 2019b; Yorzinski et al., 2018) that may increase their predation risk in the wild.

Our results confirm that birds can significantly reduce the rate at which their heat is dissipated by placing the head and the bill under the feathers during sleep. Indeed, the total amount of lost energy decreases by almost 54% when birds tuck their head in because of the insulation provided by feathers. The proportion of the body surface that is reduced in the tucked compared to the untucked posture is only 3%, therefore the amount of heat saved by reducing the total body surface appears to be negligible in comparison to the energy saved by increasing insulation of featherless body parts, in particular of the eye region. In previous work, we showed that Garden Warblers spend 62% of the night sleeping (i.e. 5 hrs between 20:00 and 4:00; Ferretti et al., 2020). Assuming that both profiles have the same rate of heat loss (untucked: −0,12 W, SD: 0.03, SE: 0.01; tucked: −0,05 W, SD: 0.03, SE: 0.01), birds that display exclusively a tucked posture would save approximately 2258 J/night from thermoregulation in comparison with those sleeping exclusively untucked. This rough calculation of energy loss is probably underestimated for two reasons: 1) the rate of heat loss in the tucked posture is not symmetric in both profiles, that is, the side where the head is tucked-in is likely to dissipate more heat; 2) it is likely that birds may experience lower ambient temperature at stopover sites than those measured in the current study, which would result in a larger amount of energy saved by sleeping tucked in. Estimating the daily energy expenditure of a free living bird as 3 times its basal metabolic rate (BMR, Nagy, 1987) and assuming an average BMR of Garden Warbler during fattening of 26 KJ/day (Klaassen and Biebach, 1994), our estimated amount of saved energy represents 8,69% of the daily energy expenditure during the stopover. Although it may seem trivial, this amount can

be pivotal in the perspective of energy management during stopover. In Wheatears, long-distance migratory songbirds with a body size similar to Garden Warblers, the energetic cost of flight has been estimated at 0.44 kJ/km (Schmaljohann et al., 2012). Assuming a similar energy expenditure for flying Garden Warblers, the amount of energy saved by sleeping exclusively tucked would allow to fuel a 5.1 km flight. Moreover, this amount of energy saved may become particularly relevant for refueling individuals. Considering the energy equivalent of fat as 37.6 kJ/g (Jenni and Jenni-Eiermann, 1998), tucked birds can save 0.06 g of fat reserves by reducing heat loss. During spring migration, Garden Warblers that increase their body mass on Ponza have an average fuel deposition rate (FDR) of 0.18 g/day (0.007 g/h; Ferretti et al. unpublished data), which is very similar to the FDR of congeneric species caught at a spring stopover site in the desert (*Sylvia hortensis*: 0.17 g/day; *Sylvia communis*: 0.13 g/day) (Jenni-Eiermann et al., 2011). Therefore, the accumulation of the amount of energy saved by the increased insulation in tucked birds would require 9 h of refueling on Ponza. Although these estimates may not be very accurate due to the low sample size and should be considered with caution, they provide a straightforward explanation for why the tucked posture is common among passerine species during migration (Ferretti et al., 2020), despite being associated with an increased predation risk (Ferretti et al., 2019b).

Migratory songbirds need to optimize their energy expenditure during the stopover phase to ensure a fast recovery of their depleted energy reserves (Schaub et al., 2008) and, consequently, minimize their permanence at the site (Fusani et al., 2009; Goymann et al., 2010). Due to physiological factors – i.e. initial low efficiency of the digestive system after endurance flight and/or the need to recover protein reserves prior energy reserves (Bauchinger et al., 2005; Ferretti et al., 2019a; McWilliams and Karasov, 2001) -, energy storage at staging sites is slower than energy consumption during flight (Alerstam, 2011; Alerstam and Lindström, 1990; Hedenström and Alerstam, 1997). As a consequence, migrants can spend up to 80% of the migratory period at the stopover site (Hedenström and Alerstam, 1997), which results in a total amount of energy consumption that is twice the energy invested in migratory flights (Wikelski et al., 2003). During stopover, songbirds might adopt different strategies to optimize their energy consumption. For example, birds can reduce locomotor activity, which requires a high energy investment (Ferretti et al., 2019b), when large food resources are available (Cohen et al., 2012; Ferretti et al., 2019a; Klinner et al., 2020). Another important strategy to optimize energy expenditure is modulation of sleep. The low metabolic rate experienced during sleep can be related to a small decrease in body temperature, facilitated by physiological mechanisms (i.e. vasoconstriction and piloerection) that reduce the body's thermal conductance (Berger and Phillips, 1995). Besides such physiological mechanisms, migrating songbirds can further decrease their energy consumption through the modulation of the posture displayed during sleep. Tucked sleep allows saving about 14% of energy compared to the untucked posture (Ferretti et al., 2019b). Although preliminary and based on different individuals, the comparison between thermography and respirometry data suggests that the amount of dissipated energy saved by sleeping tucked is larger than the difference in metabolic rate between sleep postures. However, we must consider that our measured rate of heat loss refers only to one profile of the bird. Thus, the difference in rate of heat loss between the two postures may be even higher if we consider the total body surface. Although we are aware that this difference might be due to differences in the methodology – i.e. the two techniques provide measurements that reflect different physiological processes, and heat loss is an estimation whereas respirometry is a direct measurement – and/or to minor undetected differences in environmental conditions between the two datasets, our results are suggestive of an energy allocation function of sleep (Schmidt, 2014). If during sleep animals optimize energy investment in some fundamental physiological processes (Mackiewicz et al., 2007; Nakanishi et al., 1997; Xie et al., 2013), a reduced conductance may allow them not only to reduce the global energy consumption but also to increase investment in

the latter processes. Further studies are required to investigate this hypothesis.

5. Conclusions

In the current study, we provide evidence for an energy saving function of one of the oldest known avian behaviors—sleeping with the head tucked – by comparing heat dissipation between the two sleep postures, tucked and untucked. Previous studies have assumed the existence of a relationship between sleep posture and energy consumption based on the rationale that tucked birds cover with feathers parts of their body that have high conductivity (Midtgård, 1978; Reebs, 1986), however this relationship has been demonstrated only recently (Ferretti et al., 2019b) and the difference in heat loss between postures had not been quantified. This was achieved in the current study by the use of a thermal-camera and a within-individual approach. Our estimation of the energetic advantage offered by the choice of the tucked sleep posture allows to better understand how stopover behavior modulates energy management during this migratory stage. Therefore, sleep patterns should be taken in account in future investigations aimed at modelling stopover behavior in relation to the energy budget.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102772.

Declaration of interest

The authors declare that they have no conflict of interest.

Authors contributions

AF, IM and LF conceived the study; AF and MC conducted the experimental work; AF and IM analyzed the data; AF, LF and IM wrote the paper.

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