



Behavioural and physiological responses to experimental temperature changes in a long-billed and long-legged bird: a role for relative appendage size?

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Abstract

Maintaining homeothermy may be a major challenge when species are confronted with ambient temperatures beyond their thermoneutral zone. Bird species occupying open landscapes are inherently exposed to acute heat loss and heat gain, which force them to adopt a suite of behavioural and physiological strategies to maintain homeothermy. Both types of responses could be influenced by their relative bill and leg sizes, but experimental data are lacking. Here, we evaluated how variation in body postural adjustments, panting, and locomotor activity in the dunlin *Calidris alpina* can be explained by experimental ambient temperature and relative bill and leg sizes. Additionally, we estimated resting metabolic rate and evaporative water loss to assess potential links between both physiological traits and relative bill and leg sizes. Temperatures below the thermoneutral zone were counteracted by enhancing metabolic heat production through increased locomotor activity, while body postural adjustments were used less than expected. Within the thermoneutral zone, back rest (tucking the bill under body feathers) and unipedal (standing on one leg) were preferred by dunlins, probably as being more comfortable for resting. At temperatures above the thermoneutral zone, dunlins were inactive and increased the time of bill exposure and wet-sitting and ultimately panting when challenged with temperatures above 37 °C. Interestingly, above the thermoneutral zone, but below their body temperature, birds with relatively longer bills and legs spent more time exposing them, probably to increase dry heat transfer into the environment. Our findings also highlighted the importance of the availability of wet substrates for minimising heat stress in wetland species.

Significance statement

Recent correlational field studies found support for a relationship between relative bill and leg sizes and thermoregulatory behaviour in birds inhabiting open landscapes. However, experimental data are lacking, and the mechanisms underlying this relationship remain poorly understood. Here, we performed an experiment to model behavioural and physiological responses to ambient temperature change and relative bill and leg sizes in the dunlin *Calidris alpina*, a long-billed and long-legged shorebird. Additionally, we also examined potential links among metabolic rates, evaporative water loss and relative appendage sizes. Our findings showed a strong experimental relationship between behavioural and physiological responses and ambient temperature, as well as a link between appendage size and resting body postures. Our findings also have a conservation message by highlighting that the type of substrate available for roosting is important for minimising heat stress in wetland species.

Keywords Bill size · Evaporative water loss · Leg size · Metabolic rate · Shorebirds · Thermal behaviour

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Introduction

Maintaining a high body temperature (T_b) within a very narrow range may be a major challenge for endothermic vertebrates when they are confronted with environmental

temperatures beyond their thermoneutral zone, i.e., range of ambient temperatures within which endotherms are able to maintain their T_b without increasing metabolic rate and water loss (Boyles et al. 2011; McKechnie et al. 2017). Many birds live in open landscapes where they are regularly exposed to acute external cold and heat loads and as a result, these have evolved specific behavioural and physiological responses to cope with these thermal challenges (Luskick et al. 1978; Brodsky and Weatherhead 1984; Tieleman et al. 2002; Amat and Masero 2004; Gutiérrez et al. 2015; González-Medina et al. 2020). Groups such as shorebirds, waterfowl and gulls rely on exposed intertidal areas for foraging. However, at high tide, they are forced to move to alternative sites, which are usually open, non-vegetated beaches or mudflats near the tide line (e.g., Rogers et al. 2006), where they rest for long periods of time while waiting for the next low-tide period (e.g., Rosa et al. 2006). To minimise the high metabolic costs associated with resting in these open habitats and adjust the pattern of heat transfer between their bodies and the environment, they can alter both the unfeathered appendage area exposed to the surrounding air and their surface-to-volume ratio (Brodsky and Weatherhead 1984; Wiersma and Piersma 1994; Ryeland et al. 2017, 2019, 2021). Other (non-mutually exclusive) behaviours include selecting microhabitats that maximise metabolic efficiency at high temperatures, such as wet substrates (Walsberg 1993; Wolf and Walsberg 1996; van de Ven et al. 2019; Ryeland et al. 2021). In addition to these behavioural adjustments, these species may rely on metabolically costly processes such as shivering, panting or gular fluttering to regulate their thermal balance in challenging environmental temperatures (Bartholomew et al. 1968; Amat and Masero 2004; du Plessis et al. 2012).

Unfeathered bills, legs and feet are bird appendages that are highly vascularized, and their potential roles in regulating thermal balance are well recognised (e.g., Steen and Steen 1965; Hagan and Heath 1980; Tattersall et al. 2009, 2017; Carr and Lima 2012; Cabello-Vergel et al. 2021; Playà-Montmany et al. 2021). Appendage size may vary among populations of the same bird species according to the thermal environment, and it has been suggested, for example, that a larger bill surface area could be an adaptation to increase dry heat loss under hot climatic conditions (Greenberg et al. 2012). However, these appendages may also be a source of heat gain for birds living in extremely hot environments, where the environmental temperature can approach or surpass the T_b (Greenberg and Danner 2012; Gardner et al. 2016; Ryeland et al. 2017), or of heat loss when exposed to cold (Hagan and Heath 1980; Tattersall et al. 2009; Greenberg and Danner 2012; Gardner et al. 2016), although these aspects of bird thermoregulation are less-known (Gardner et al. 2016; van de Ven et al. 2016;

Ryeland et al. 2017, 2019; Benham and Bowie 2021; Playà-Montmany et al. 2021).

In the case of shorebirds (Charadrii), the role of these appendages as ‘thermal windows’ for dry heat exchange may be particularly important, since these birds generally have large relative bill and leg sizes. Thus, for example, shorebirds with smaller legs expend less energy to maintain homeothermy in cold environments than individuals with longer leg lengths (Cartar and Guy Morrison 2005). Within this context, Ryeland et al. (2017, 2019, 2021) found that resting body postures of shorebirds can be mediated by bill and leg sizes. These studies supported back rest (bill tucked within the back plumage) and unipedal (standing on one leg) postures at high tide as effective strategies for reducing heat loss at low temperatures, while sitting in wet substrates favouring body heat loss in hot conditions. They also found evidence for the use of back rest as a strategy to minimise heat gain through the bill at the examined upper-temperature limits (Ryeland et al. 2017, 2019). In contrast, they did not find support for the use of unipedal posture as a strategy to insulate against heat gain, although its use at extremely high environmental temperature values cannot be disregarded (Ryeland et al. 2017, 2019). In this framework, experimental data are needed to test the correlations that predict bird postural adjustments according to ambient temperature (T_a).

Here, we experimentally exposed a migratory shorebird with a relatively large bill, and leg size, the dunlin *Calidris alpina*, to a wide range of T_a values, in order to model behavioural and physiological responses to environmental temperature change in relation to relative bill and leg size while resting. As birds adjust body posture, they adopt heat-dissipating/conserving behaviours to maintain thermal homeostasis (Ryeland et al. 2017, 2019, 2021; Pavlovic et al. 2019). We predicted that (i) dunlins would reduce the exposed unfeathered area to minimise body heat loss and body heat gain under cold and extremely hot conditions (where T_a approaches T_b), respectively; and (ii) dunlins would initiate metabolically and hygric costly processes, such as panting, only in extremely hot environments. Furthermore, it is well established that one of the strategies used to dissipate body heat and minimise evaporative water loss (EWL) in endotherms is to reduce locomotor activity (Wolf 2000; Thompson et al. 2018). We also predicted, therefore, that (iii) dunlins would decrease their locomotor activity as T_a increased to decrease body heat production. Last, we measured the resting metabolic rate (RMR) and EWL of dunlins at different T_a values to assess the potential role of relative bill and tarsi sizes in explaining variations in both rates. EWL is a metabolically costly process, so if the appendage surface area can be used as a radiator to expel “dry” heat under thermally stressful conditions (Greenberg et al. 2012), we expected that individuals with larger relative bill and tarsi sizes would consume less oxygen to maintain

homeothermy when T_a exceeds their thermoneutral zone but below their T_b (Playà-Montmany et al. 2021).

Materials and methods

Bird capture and maintenance

Wintering adult dunlins were caught using mist nets in Extremadura's rice fields, in southwest Spain (39°01'N, 5°58'W), which is a key area for many nonbreeding waterbirds (Masero et al. 2011; Navedo et al. 2012). After capture, birds were individually colour-ringed to facilitate further identification and were weighed (± 0.01 g). The bill length, width and depth (both measures taken at the anterior edge of the nostrils), tarsus length, width and depth (both taken at the middle of the tarsus) were measured with a digital calliper (± 0.01 mm), while the wing length (flattened and straightened length) was measured using a wing ruler (± 0.5 mm). The 11 individuals showing the highest variability in appendage size (among the 40 individuals captured) were selected for this study (bill length CV = 8.29%; tarsus length CV = 4.27%) and transported to the bird facilities at the University of Extremadura (e.g., Gutiérrez et al. 2011). There, the birds were housed in an environmentally controlled room (320 cm \times 260 cm \times 255 cm), where they were exposed to fluorescent lighting in a 14 h light:10 h dark cycle starting at 7:00 AM. The daylight period was divided into high-tide (from 2:30 to 7:30 PM) and low-tide (the remaining light hours) periods simulating a diurnal tide through food availability. Live fly larvae *Protophormia terraenovae* and commercial pellets (Dibaq-Diproteq) were available *ad libitum* in several trays during the "low tide" period, and all food was removed during the "high tide" period (see details below).

The room temperature was set at 10 °C and 8 °C during the day and night, respectively, throughout the acclimation period (5 weeks), based on the average environmental temperature in the Extremadura's rice fields during catching. A large pool (80 cm \times 260 cm, 3 cm deep) provided fresh water *ad libitum* for bathing and drinking, and the rest of the ground surface was covered with wet sand. The room was equipped with a one-way window to allow vision through the window only by the observer.

All birds completed the experiment, and all of them were released in good health in the area of capture.

Metabolic measurement protocol

Following the acclimation period, the RMR and EWL were measured at 10 °C, 25 °C and 35 °C during the "high tide" simulation period after 4 h of fasting to ensure that the birds were in a postabsorptive state (see González-Medina et al.

2020). The dunlins were weighed (± 0.1 g) before and after the metabolic measurements, and the mean body mass (M_b) was used in the analyses. T_b was measured prior to and following metabolic trials by using a calibrated laboratory thermometer (P700, Dostmann electronic GmbH, Wertheim, Germany) attached to a copper–constantan thermocouple inserted 1 cm into the cloaca. A maximum of six individuals were measured at a time. Individuals were randomly exposed to 10 °C or 25 °C using a flow rate of 1000 ml min⁻¹ during three trials of 110 min each. Birds were exposed at 35 °C during two trials of 65 min each, and the flow rate was increased up to 3000 ml min⁻¹ to ensure that individuals remained calm under the hot conditions (Whitfield et al. 2015), which was verified by the use of infrared cameras.

Gas exchange measurements

Oxygen consumption (VO_2) and EWL were measured using an open flow-through respirometry system (Sable Systems) following González-Medina et al. (2020). Briefly, each post-absorptive individual was placed in a polypropylene metabolic chamber (effective volume = 3.9 L) inside a compressor-cooled incubator (Memmert GmbH, Germany). An identical, but empty, chamber was used for baseline measurements. The floor of each chamber was covered with a 1 cm mineral oil layer to avoid evaporation from excreta and equipped with a wire mesh platform suspended 3 cm above this oil layer to allow individuals to rest without touching the oil. Dry air (< 1 kPa WVP) was pumped into the metabolic chamber at a rate of 1000 or 3000 ml min⁻¹ using a mass flow controller (MFS, Sable Systems International) (95% washout time: 4–12 min). Low humidity levels were maintained during trials (0.7–0.9 kPa at the highest T_a). The water vapour density ($\mu\text{g ml}^{-1}$) and O_2 concentration (%) in the excurrent airstream were analysed using an H_2O analyser (RH300, Sable Systems) and an O_2 analyser (FC-10 Oxygen Analyser, Sable Systems), respectively. Before each trial, both gas analysers were zeroed using nitrogen and spanned using the O_2 dilution technique for the H_2O analyser (Lighton 2008) and incoming air (20.95% O_2) for the O_2 analyser. The RMR and EWL were estimated as the 2 min the most stable values at each temperature using Eqs. 10.2 and 10.9, respectively, from Lighton (2008) with a respiratory quotient of 0.70, assuming lipid and protein catabolism (Kvist and Lindström 2001). Expedata software (version 1.9.14, Sable Systems, Las Vegas, NV, USA) was used to control the multiplexer, record data and process data for analyses.

Experimental temperature treatment

Behavioural observations were conducted 2 weeks after the respirometry measurements to ensure that individuals

were re-habituated to captivity and stayed calm. Birds were exposed to each experimental T_a (10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 38 °C) during a single daylight period and on consecutive days (a steady 5–3 °C in the last step–stepwise increase of experimental T_a starting at 10 °C and ending at 38 °C). Each experimental T_a was set at 7:00 AM, i.e., 7.5 h before the resting period, and ended at 9:00 PM. The room temperature at night was kept 5 °C lower than the experimental temperature tested the following daylight period. There were variations between the experimental temperature set for the room and the temperature that the dunlins experienced near the ground surface. Thus, as an approximation of the temperature experienced by birds on the ground, we measured the air temperature at dunlin height, i.e., 2 cm above water and 5 cm above ground level, every 2 min using HOBO Micro Station Data Loggers H21-002 (Onset Computer Corporation, Bourne, Massachusetts, USA). We also recorded the water temperature (°C; 1 cm upper layer) and wet sand temperature (°C; 1 cm upper layer). The mean T_a above water and wet sand differed by 0.57 ± 0.15 °C (mean \pm SE) on average; thus, the mean value was used in successive analyses (see below). The relative humidity ranged from 75 to 90% throughout the observational trials, and the birds did not experience air movement.

Behavioural data collection

Focal behavioural observations were conducted during the high-tide period. These observations started at 3:30 PM, i.e., 1 h after the food was removed, to allow the birds time to recover from the disturbance caused by entering the room. Each individual was observed continuously during a period of 5 min every 20 min. This led to a total of four observation bouts of 5 min each per individual at each T_a , and the total proportion of each observation bout representing each body posture was calculated. Observations were conducted by a single researcher (NPM) through the one-way window using 10×42 binoculars. The order of observation of focal individuals was randomly chosen at the beginning of the observation period and was maintained during the following observations. It was not possible to record data blind because our study involved following a focal individual.

Although shorebirds usually rest during the high-tide period (no food available), individuals can be active, showing locomotion (walking) and preening behaviours. We considered that an individual was resting once it remained inactive for > 60 s. In each resting posture, we noted whether the bird was standing on one leg, standing on both legs or sitting. The bill position was noted as back rest or not. We also noted panting behaviour (defined as gaping when breathing; Smit et al. 2016) (bill open for \geq 5 s, see Oswald et al. 2019), as well as the substrate on which the dunlins were resting (wet sand or water). We quantified the times that

each individual was engaged in each behaviour and located on each substrate.

Data analysis

Bill and tarsus measurements were converted to surface area estimates following Greenberg et al. (2012). We used equations to approximate the bill surface area as an elliptical cone and the tarsi surface area as an elliptical cylinder (see equations in Greenberg et al. 2012). The coefficients of variation for bill surface area and tarsi surface area were 13.13% and 6.32%, respectively.

We used the proportion of time per observation period spent resting on each body posture as the response variables. Factors influencing these response variables were assessed by fitting generalised linear mixed models (GLMMs) with a binomial response and logit-link function (fitted by the maximum likelihood with Laplace approximation). This calculation treated each minute of observation as a “presence” (for example, a bird in a back rest posture) or “absence” (a bird with the bill exposed) (see Ryeland et al. 2017, 2019, 2021). We modelled each resting body posture by including T_a as a covariate, appendage size, and the interaction between T_a and appendage size as fixed factors, body mass (M_b) to control for body size across individuals, and bird identity as a random factor to account for repeat observations of birds. The interaction term was included in the models to assess whether dunlins with different appendage size might show different behavioural responses across the range of temperatures. The quadratic term of T_a was also included in models where preliminary analyses and previous studies (Ferns 1992; Ryeland et al. 2021) showed a quadratic relation between T_a and body posture. To limit model overfitting, and for comparative purposes with previous studies (Ryeland et al. 2017, 2019, 2021), the back rest model only included as appendage size the bill surface area, whereas unipedal and sitting models only included tarsi surface area. Appendage sizes and M_b were log-10 transformed and all explanatory variables were standardised prior to analysis.

We checked model assumptions using the DHARMA package in R (Hartig 2020). Observation bout was also included in the models as a random factor to absorb additional variance in the response variable (Harrison 2014), as there was some evidence of overdispersion (dispersion parameter > 1.5).

The influence of T_a on the proportion of time that birds were active during the high tide period was also examined by performing a GLMM with a binomial response and logit-link function. We included the same predictors, excluding appendage surface areas. M_b was included in this analysis because previous studies showed that it can influence behavioural thermoregulation (Ryeland et al. 2017, 2019, 2021; Pattinson et al. 2020). Panting was exclusively performed

at 38 °C and occurred rarely (see results), so this behaviour was not modelled.

The potential relationships between physiological thermoregulatory and morphological traits at 10 °C, 25 °C and 35 °C were analysed by fitting GLMMs, where RMR (ml O₂ min⁻¹) or EWL (mg h⁻¹) was the response variables, and T_a, appendage size and the interaction between T_a and appendage size were included as covariates. Individual identity was included as a random factor and M_b as a fixed factor. We ran separately (for the reasons stated above) a model with bill surface area and a model with tarsi surface area as predictors.

Potential collinearity among predictor variables in the global models was evaluated by calculating the variance inflation factor (VIF) using the “car” package (Fox and Weisberg 2019). Variables were only removed if VIF were > 5 (Zuur et al. 2010). We first built global models using the ‘lme4’ package (Bates et al. 2014) for each analysis. Model selection was performed based on the Akaike information criterion for small sample sizes (AIC_c) to identify the top model(s) (all models with ΔAIC_c < 2) and AICc weights (w_i) to further distinguish between the top models (Burnham and Anderson 2002). We used the “dredge” function in the R package MuMIn (Barton 2018) for this procedure. For cases in which more than one model had ΔAICc < 2 but w_i < 0.9 (Burnham and Anderson 2002), we performed model averaging of parameter estimates (Grueber et al. 2011). A predictor was considered significant when the 95% confidence interval (CI) for the estimated coefficient did not overlap zero. Statistical analyses were conducted in R 4.0.2 (R Core Team 2014), and all figures were produced

using the R package “ggplot2” (Wickham 2016). Data are presented as mean ± SE.

Results

Overall, T_a significantly predicted all behaviours (Table 1). Birds increased their time spent in back rest and standing on one leg with increasing T_a values until they were exposed from 23 to 26 °C. From this temperature range upwards, the use of both resting body postures decreased (Fig. 1). Back rest and unipedal behaviours across the range of temperatures also were influenced by bill surface area and tarsi surface area, respectively (Tables 1, 2). Individuals with relatively smaller bill sizes displayed back rest behaviour more frequently than those with larger bill sizes at high T_a, whereas dunlins with smaller tarsi sizes, relative to body size, tended to display a greater use of unipedal posture than individuals with larger tarsi sizes at such high T_a (Fig. 1).

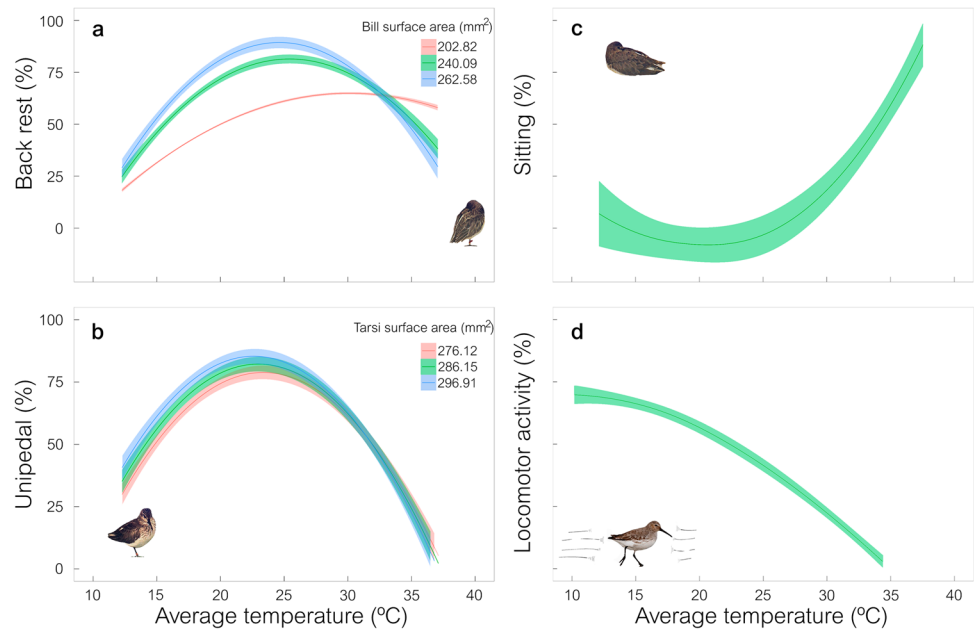
Sitting and locomotor activity were only predicted by both the linear and quadratic terms of T_a (Tables 1, 2). Sitting was mainly used above 30 °C (30.67 ± 0.03 °C at the ground level), after which point its use increased linearly, achieving 100% at 37 °C (36.52 ± 0.23 °C at the ground level) (Fig. 1). Locomotor activity during the resting period strongly decreased from 20 °C (22.03 ± 0.04 °C at the ground level) upwards (Fig. 1), with birds remaining active less than 5% of time above 35 °C (34.81 ± 0.14 °C at the ground level). Panting occurred rarely (it was only observed in a few individuals and in a few occasions) and only when the room temperature was set to the maximum

Table 1 Top-ranked candidate models explaining thermoregulatory behaviours and physiological traits in dunlins at high tide (resting period). Models were selected using the Akaike Information Criterion with a correction for small samples (AICc). Only models with

ΔAICc < 2 are shown. For each model, the number of parameters (df), log-likelihood (logLik), AICc weights (w_i) and R-squared (R²) are shown

Model	df	logLik	AICc	ΔAICc	w _i	R ²
Back rest						
T _a + T _a ² + M _b + Bill surface area + bill surface area:T _a + bill surface area:T _a ²	9	-677.28	1373.2	0.00	0.86	0.51
Unipedal						
T _a + T _a ² + M _b + Tarsi surface area + Tarsi surface area:T _a + Tarsi surface area:T _a ²	9	-608.86	1236.4	0.00	0.59	0.66
T _a + T _a ² + M _b + Tarsi surface area + Tarsi surface area:T _a	8	-610.41	1237.4	0.96	0.37	0.65
Sitting						
T _a + T _a ² + M _b	6	-232.79	477.9	0.00	0.74	0.97
Locomotor activity						
T _a + T _a ²	5	-488.13	986.50	0.00	0.62	0.79
T _a + T _a ² + M _b	6	-487.56	987.40	0.95	0.38	0.79
RMR						
T _a + M _b	5	37.08	-61.90	0.00	0.99	0.79
EWL						
M _b	4	18.95	-28.5	0.00	0.85	0.02

Fig. 1 Model predictions for the percentage (%) of the resting time (simulated high tide) that dunlins spent in **a** back rest (tucking the bill under body feathers), **b** unipedal (standing on one leg), **c** sitting in wet sand and **d** locomotor activity in relation to environmental temperatures ($^{\circ}\text{C}$). The shaded areas show the 95% confidence interval (CI) around the predicted values. Bill and tarsi data were back-log transformed, and grouped according to quartiles (lower 25%, median 50%, and upper 25%) for representation purposes only



temperature (37°C ; $36.52 \pm 0.23^{\circ}\text{C}$ at the ground level) and while sitting.

Variations in RMR and EWL were not explained by the bill or tarsi surface area. The best model for RMR included T_a and M_b , and both predictors had a significant effect on RMR (Tables 1, 2). The best-fitting model explaining EWL variation only included M_b (Table 1), but it was not a significant predictor (Table 2).

Discussion

Thermoregulation plays a major role in organism homeostasis. When exposed to extreme T_a , endotherms adjust body heat production and heat interchange through behavioural and physiological mechanisms. Among the behavioural strategies used to dissipate body heat at high T_a , the experimental data showed that dunlins dealt with high T_a values during the daily resting period by using specific body postures. Our study also suggested that dunlins minimised body heat production at high T_a values by decreasing locomotor activity. However, during cold exposure, dunlins only seemed to rely on heat production by maintaining high levels of locomotor activity (our model predicted dunlins to spend $>60\%$ of time active in cold conditions). We also found a role of unfeathered appendage size in explaining body posture adjustments, but not in RMR and EWL variation across temperatures.

Dunlins, like other birds inhabiting open habitats at temperate latitudes, experience cold winters with commonly high wind speeds, the latter of which greatly contributes to increased body heat loss by forced convection (Wiersma

and Piersma 1994). Wintering in temperate latitudes should, therefore, impose the need to implement behavioural and physiological strategies to maintain T_b (e.g., Wolf 2000; Ryeland et al. 2017, 2019, 2021; Cooper et al. 2019). Shorebirds have previously been shown to consistently display body postural adjustments to minimise body heat loss at low T_a values (see Ryeland et al. 2017, 2019). However, we found that dunlins used back rest and unipedal resting less than expected when exposed to temperatures below the lower critical value of the thermoneutral zone (19.8°C according to Kelly and Weathers 2002). In the absence of wind near the ground level, captive dunlins did not experience heat loss by forced convection (wind), and they seemed to be able to cope with lower temperatures (12°C on average) mainly by increasing heat production through locomotor activity. Most shorebirds roost communally in flocks ranging from tens to thousands of birds, and in windy cold environments they can reduce heat loss by flocking and facing the wind (Wiersma and Piersma 1994). However, it has also been shown that shorebirds such as the red knot *Calidris canutus* can compensate for thermoregulation costs at low temperatures by using heat generated as a by-product of walking or running (Bruinzeel and Piersma 1998), and presumably, flight would have a similar or stronger effect (Rogers et al. 2006). Our findings confirmed this large compensation to maintaining homeothermy by using locomotor activity in cold and windless environments. Last, when remaining inactive in cold conditions, dunlins barely used unipedal resting or sitting, suggesting bipedal standing as the main body posture adopted under cold conditions.

At temperatures within thermoneutrality (e.g., from 20 to 30°C), no additional energy or water is allocated for

Table 2 Model-averaged parameter estimates (\pm SE) for predictors and 95% confidence intervals (CI) of thermoregulatory behaviours and physiological traits in dunlins at high tide (resting period)

Model	Estimate	CI (95%)	SE
Back rest			
Intercept	1.11	0.59, 1.64	0.25
T_a	0.49	0.34, 0.65	0.08
T_a^2	-0.88	-1.07, -0.69	0.10
M_b	-0.13	-0.58, 0.33	0.21
Bill surface area	0.82	0.33, 1.32	0.24
Bill surface area: T_a	-0.20	-0.36, -0.04	0.08
Bill surface area: T_a^2	-0.50	-0.70, -0.30	0.10
Unipedal			
Intercept	1.24	0.85, 1.63	0.20
T_a	-0.15	-0.31, 0.00	0.08
T_a^2	-1.40	-1.60, -1.19	0.10
M_b	-0.23	-0.75, 0.30	0.27
Tarsi surface area	0.32	-0.25, 0.89	0.29
Tarsi surface area: T_a	-0.24	-0.40, -0.09	0.08
Tarsi surface area: T_a^2	-0.11	-0.33, 0.12	0.11
Sitting			
Intercept	-8.15	-10.08, -6.53	0.88
T_a	4.27	3.32, 5.88	0.60
T_a^2	2.80	1.96, 3.48	0.36
M_b	0.58	-0.65, 1.85	0.57
Locomotor activity			
Intercept	-1.21	-1.81, -0.61	0.31
T_a	-1.79	-2.05, -1.54	0.13
T_a^2	-0.67	-0.87, -0.47	0.10
M_b	0.10	-0.30, 0.51	0.20
RMR			
Intercept	0.29	0.26, 0.31	0.01
T_a	-0.11	-0.13, -0.09	0.01
M_b	0.05	0.03, 0.08	0.01
EWL			
Intercept	2.14	2.10, 2.18	0.02
M_b	0.02	-0.02, 0.06	0.02

T_b maintenance (Wiersma and Piersma 1994); thus, non-thermoregulatory responses should be expected within this range of T_a . We found dunlins standing on one leg and tucking the bill while exposed to thermoneutral conditions. Back rest is commonly adopted by several shorebird species, including dunlins, while resting or sleeping at high tide (e.g., Klaassen 1990; Dominguez 2003). Previous studies conducted with the flamingo *Phoenicopterus ruber*, for example, showed unipedal resting to be a less energetically demanding posture that favours muscle relaxation and body balance (see Chang and Ting 2017). Thus, within the thermoneutral zone, a back rest posture and unipedal resting could have been selected by dunlins as being

more comfortable for resting (Klaassen 1990; Dominguez 2003; Chang and Ting 2017).

At high T_a values (> 30 °C), dunlins increased the time of bill exposure, as this may favour body heat dissipation by radiation. This result is analogous to that found by Ryeland et al. (2017), in which shorebirds reduced their back rest behaviour at high T_a values to favour heat flux into the environment. At hot conditions, but below their T_b (39–40 °C; see also González-Medina et al. 2020), we also found that the proportion of time spent resting on back rest, and to lesser extent on unipedal, was related to the relative size of bill and legs, respectively. Dunlins with the largest relative bill sizes clearly spent more time with their bills exposed compared to dunlins with the smallest relative bills, supporting dunlins adjusted their bill postures to increase dry heat transfer into the environment at high temperatures.

In addition to adjusting their bill position, as T_a increased, dunlins remained sitting in wet sand most of the time, which is a previously observed behaviour known as ‘wet-sitting’ (see Ryeland et al. 2021). Previous studies with wild shorebirds highlighted the importance of sitting in wet substrates under heat stress, as this is an effective mechanism to avoid hyperthermia (Battley et al. 2003; Rogers et al. 2006; Ryeland et al. 2021). Ryeland et al. (2021), for example, showed that several coastal shorebird species spent more time wet-sitting at high tide when roosting at ~ 30 °C. By selecting this microhabitat and body posture, shorebirds may favour body heat loss by conduction across the leg surface and the lower body, as both surfaces directly contact the wet substrate (e.g., Ferns 1992; Ryeland et al. 2021). Sitting can also be less energetically expensive than standing, contributing to the balance of energy budgets under these conditions (Tickle et al. 2012; Pavlovic et al. 2019). The maximum temperature registered on the surface of the wet sand inside the room was ~ 37 °C, which was lower than the core T_b (~ 39 °C) measured in dunlins when exposed to 35 °C inside the metabolic chambers. Therefore, the thermal gradient ($T_a - T_b$) between the wet substrate and the body would allow dry heat transfer from the body to the wet sand when sitting, reducing the body heat burden of the birds (Rogers et al. 2006; Ryeland et al. 2021). At the highest T_a value, this gradient should have been minimised, and a few dunlins additionally dissipated heat by using panting (forced evaporative water loss) (Amat and Masero 2004; McKechnie and Wolf 2019) while wet-sitting. Models predicting panting/gular flutter behaviours in arid-zone bird species found that panting (behavioural response present in 50% of observations) occurred at ~ 37 °C and ~ 41 °C in drinking and nondrinking species, respectively (Smit et al. 2016). Our experimental birds had access to freshwater *ad libitum*, and they started to use this avian respiratory heat dissipation behaviour at ~ 37 °C, supporting model predictions for drinking species. Finally, we did not observe heat-avoidance

behaviours such as ptiloerection (raising of the ‘back’ feathers) and wing-dropping (defined as holding the wings away from the body), which have both been described in small-sized wild shorebirds (Battley et al. 2003; Amat and Masero 2004). Feather erecting may increase convective or evaporative cooling (Battley et al. 2003), while wing-dropping could be a mechanism to increase the surface area of the body to enhance radiative and convective dry heat loss by exposing thermal windows under the wing (Smit et al. 2016). The absence of ground-level wind inside the room could explain why dunlins did not use both heat-avoidance behaviours.

Inactivity is a response shared among a large fraction of the world’s avifauna to minimise the impacts of heat stress (e.g., Davies 1982; Wolf 2000; Silva et al. 2015; Cooper et al. 2019), which we know mainly from studies performed in passerine species from arid habitats and Mediterranean climates (Kelly et al. 2004; Thompson et al. 2018; Cooper et al. 2019). However, although this thermoregulatory response to thermal stress has been largely overlooked in non-passerine species such as shorebirds, our results indicated that changes in locomotor activity are a common response used to overcome thermoregulatory challenges, as dunlins adjust locomotor activity to increase or minimise metabolic heat production at low and high T_a values, respectively. Inevitably, some of these thermoregulatory behaviours involve trade-offs with other fitness-improving activities, such as antipredator behaviours or foraging (du Plessis et al. 2012). For example, the time allocated to sitting cannot be spent being alert to predators (Ryeland et al. 2017, 2019; Timmis et al. 2022), which makes sitting individuals much more vulnerable (Ryeland et al. 2017, 2019; Timmis et al. 2022). These constraints associated with thermal behaviours can be accentuated by the current global warming, which forces birds to spend longer periods of time adopting behavioural strategies to alleviate thermal stress.

We found no significant influence of relative appendage sizes on either RMR or EWL. The low intraspecific variation in bill and tarsi sizes might explain why they did not influence both physiological traits. The studies that found large effects on bill and tarsus lengths on shorebirds thermoregulation involved several species that displayed enormous variation in morphological trait sizes (Ryeland et al. 2017, 2019, 2021). For example, the CV obtained by Ryeland et al. (2019) for tarsus length using many shorebird species was approximately 53%, while in our dunlin sample it was ~6%. Therefore, this circumstance could explain the lack of explanatory power of the appendage sizes in explaining variation in RMR and EWL.

In conclusion, we provided a better understanding of the physiological and behavioural responses to thermal stress in a small bird species inhabiting open landscapes.

Our findings indicated that captive dunlins adopted for behavioural strategies that included body postural adjustments and locomotor activity to defend T_b under challenging T_a values. In hot environmental conditions, dunlins used thermoregulatory behaviours that strongly favoured conductive (sitting) and radiative (bill exposition) dry heat transfer from the body to the surroundings, while in cold environments, individuals remained active for longer periods of time to favour metabolic heat production. Our study showed that panting was only performed for a few individuals at the highest T_a values, supporting that the birds favoured behavioural responses over this costly physiological response. Lastly, previous studies (Ryeland et al. 2021) supported the importance of wetland margin substrate in the thermoregulation of several shorebird species, as the time spent sitting was significantly mediated by the substrate upon which they roosted. Our experimental findings support this positive role of the substrate type (in our case wet sand) in minimising heat stress in shorebirds, which is remarkably relevant to shorebirds, as they face a reduction in wetland habitat availability along with increasing T_a values due to global warming (Ryeland et al. 2021).

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Author contribution NPM, AV and JAM conceived the idea and designed the study. JMAG, MP and JAM captured the dunlins. NPM and EGM collected the data, and NPM analysed the data. NPM led the writing of the manuscript with the support of JAM and AV. All authors contributed to the revisions and approved the final version of the manuscript.

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Data availability Data are available as supplementary material.

Declarations

Ethical approval All procedures were approved by the bioethical committee of the University of Extremadura, Spain (108/2016), and were conducted under the governmental license CN0032/18/ACA. All applicable national and institutional guidelines for the use of animals were followed.

Conflict of interest The authors declare no competing interests.

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