

RESEARCH ARTICLE

Novel approaches for assessing acclimatization in birds reveal seasonal changes in peripheral heat exchange and thermoregulatory behaviors

Juan D. Zuluaga* and Raymond M. Danner

ABSTRACT

Using thermography and behavioral analyses, we found that heat exchange and thermoregulatory behaviors changed seasonally in chipping sparrows (*Spizella passerina*). Studies on seasonal acclimatization in birds have primarily involved metabolic measurements, few of which have investigated behaviors, and none have investigated changes in peripheral heat exchange. We captured chipping sparrows in the winter and summer of 2022 in Wilmington, North Carolina, USA, and we collected thermal images of these birds at 15.0, 27.5 and 40.0°C. We found that heat dissipation through the bill and legs changed seasonally, but surprisingly both were higher in winter than in summer. We found that heat dissipating behaviors were more common in winter, whereas heat conserving behaviors were more common in summer, and that behaviors associated with resource costs (e.g. panting) or predation risk (e.g. bill tucking) showed the most distinct differences between seasons. Meanwhile, low-cost and low-risk postural adjustments (e.g. feather adjustments and tarsus exposure) did not vary as strongly between seasons but followed similar trends. The seasonal adjustments to behaviors suggest that non-acclimatized birds must use costly thermoregulatory behaviors more frequently than acclimatized birds. The use of thermography resulted in the discovery of one completely novel behavior, and the first detection of a known behavior in a new species. Both novel behaviors aided in evaporative heat loss and occurred more commonly in winter, supporting the presence of seasonal acclimatization as evidenced by behavioral adjustments. These results provide novel insights into the process of acclimatization and suggest a role for behavioral adjustments in seasonal acclimatization.

KEY WORDS: Ecophysiology, Thermal imaging, Thermoregulation, Thermovascular physiology, Behavior

INTRODUCTION

Seasonal changes in temperature can create large and predictable thermoregulatory challenges, leading to the evolution of cycles of acclimatization in thermoregulatory traits. This phenomenon has long been studied in the context of cold acclimatization (reviewed in Swanson and Vézina, 2015), providing insights into how birds optimize cold resistance in winter through metabolic upregulation. Seasonal acclimatization has recently been revisited in the context of

acclimatization to heat, and recent research has also primarily focused on changes in metabolic traits. Birds in summer have been found to undergo increases in evaporative water loss or reductions in resting metabolic rate to increase evaporative cooling efficiency (O'Connor et al., 2017; Oswald et al., 2018; Tieleman et al., 2002); such changes may also be coupled with increased heat tolerance and can vary with local aridity (Noakes et al., 2016). In addition to this spatial specificity, seasonal metabolic adjustments can vary among years (Noakes and McKechnie, 2020; Swanson and Olmstead, 1999), highlighting the fact that these traits exhibit phenotypic plasticity and may change facultatively to optimize thermoregulation over the course of a bird's life as environmental conditions change over space and time.

Like metabolism, thermoregulatory behavior has been found to vary with aspects of the thermal environment, but unlike metabolism, no studies to date have examined variation in thermoregulatory behaviors across seasons in the same location and species. We hypothesize that seasonal changes in physiology (e.g. metabolism, evaporative cooling efficiency or heat/cold tolerance) can cause changes in thermoregulatory behavior or, more specifically, the thresholds at which thermoregulatory behaviors are used. We expect this pattern to be most discernible when resource savings can be gained by adjusting behaviors associated with resource costs, such as those that enhance evaporative cooling; we expect other behaviors, such as postural adjustments, to remain relatively constant because of their low resource cost. Behavioral mechanisms of evaporative cooling in birds include panting, gular flutter and urohidrosis (Cabello-Vergel et al., 2021; Dawson, 1982). Southern ground hornbills (*Bucorvus leadbeateri*) have been observed secreting fluid from the nares, also known as nasal watering, which may provide evaporative cooling (Janse van Vuuren et al., 2020; Kemp and Kemp, 1980), but it is unknown whether this behavior exists in other species. While cutaneous evaporation provides an important cooling mechanism for some taxonomic groups (e.g. columbids; McKechnie et al., 2021), it is not currently thought to be associated with a behavior, and as such may be tied more directly to metabolism and/or hydration state than to active behavioral responses to external stimuli. Of the behavioral evaporative cooling pathways, panting is common to passerines and non-passerines, and it requires both water and energy (McKechnie, 2022). Regularly drinking desert birds pant more often (Smit et al., 2016) and have greater evaporative cooling capacity (Czenze et al., 2020) when compared with non-drinking desert birds, underscoring its association with resource costs. If behaviors change seasonally and in tandem with varying resources, then panting may be an effective starting point for investigating seasonal behavioral adjustments. Quantitatively examining the frequency of thermoregulatory behaviors at the same temperature, but across seasons, may reveal behavioral changes that work alongside internal physiological changes to achieve seasonal acclimatization.

Another thermoregulatory mechanism that has been found to vary with aspects of the thermal environment is the use of thermal

University of North Carolina Wilmington Department of Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, NC 28403-5915, USA.

*Author for correspondence (jdz4405@uncw.edu)

 J.D.Z., 0000-0002-9508-3739

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windows, such as the bill and legs (reviewed in Tattersall et al., 2017). Although no studies to date have investigated seasonal changes in heat dissipation through thermal windows per se, Greenberg et al. (2013) found that the bills of two species of saltmarsh-inhabiting song sparrows were larger in summer than in winter, putatively to aid in the dissipation of heat in this thermally challenging habitat. Supporting the notion that bill size can change to increase heat dissipation in summer, Greenberg et al. (2012b) found that the bills of marsh sparrows measured in summer strongly correlated with maximum temperature, whereas the bills of winter birds exhibited little correlation with winter low temperatures. In a previous study, we (Zuluaga and Danner, 2023a) primarily investigated the impact of acute stress on the thermoregulatory role of the bill in song sparrows, and also collected preliminary data from experiments in both seasons (summer $n=11$, winter $n=3$). Our unpublished analyses of season showed that, when held at the same ambient temperature (37°C), the birds in summer consistently had hotter bills, and therefore dissipated more heat than in winter. The seasonal change in surface area in Greenberg et al. (2012b) and/or the seasonal change in surface temperature in our unpublished dataset (from Zuluaga and Danner, 2023a) would provide greater heat dissipation and water savings in summer, and reduced heat loss in winter. It is unknown whether these changes occur in other species, or in other thermal windows such as the legs of birds.

Here, we measured seasonal changes between winter and summer in the surface temperatures of birds during three temperature treatments: 15°C (low temperature treatment), 27.5°C (intermediate temperature treatment) and 40°C (high temperature treatment). To our knowledge, this is the first study to investigate seasonal changes in thermal windows using infrared thermography. We investigated variation in the use of three heat dissipating (panting, exposing legs, feather flattening) and three heat conserving (bill tucking, leg tucking and feather puffing) behaviors across winter and summer. We also investigated a novel thermoregulatory behavior, pant cycling, and detected two instances of an advanced form of nasal wetting behavior, which was previously unknown to occur in passerines. With these data, we tested the overarching hypothesis that seasonal adjustments occur in peripheral heat dissipation and thermoregulatory behaviors. We predicted that (1) thermal windows would dissipate more heat in summer than in winter, (2) costly thermoregulatory behaviors would be used less often if physiological acclimatization is present, and (3) low-cost thermoregulatory behaviors, such as postural changes and adjustments to feathers, would not undergo seasonal changes because they are not resource limited and therefore remain equally viable across seasons.

MATERIALS AND METHODS

Study species and site

The chipping sparrow, *Spizella passerina* (Bechstein 1798), is a small-bodied (11.88 ± 0.18 g, mean \pm s.e.m.; Table S1) passerine with a widespread distribution (Middleton, 2020), and is predicted to experience range reductions along its southern extent and range expansion northward by the year 2080 in eastern North America due in part to heat waves (Wilsey et al., 2019). Birds were captured and tested in Wilmington, NC, USA, from 14 January to 26 February in winter 2022, and from 10 May to 14 July in summer 2022. The mean (\pm s.e.m.) minimum temperatures were $2.32\pm 0.87^{\circ}\text{C}$ and $20.86\pm 0.41^{\circ}\text{C}$ during winter and summer, respectively. The mean (\pm s.e.m.) maximum temperatures were $15.40\pm 0.91^{\circ}\text{C}$ and $31.01\pm 0.37^{\circ}\text{C}$ during winter and summer, respectively (National Weather Service Wilmington; <https://www.weather.gov/ilm/>).

Capture and care

We tested 34 adult chipping sparrows across the winter ($n=15$) and summer ($n=19$) of 2022. Birds were captured with mist nets at the University of North Carolina Wilmington and at a residence near the university. Upon capture of the birds, we confirmed age and sex in the field with the presence of a cloacal protuberance and immediately released females to avoid impacting nesting success; sex was not determined in winter because of the lack of defining characteristics. Birds were immediately transferred individually to a cage ($29\times 41\times 56$ cm) with two perches at uneven heights. The cage was then covered with an opaque cloth and (if necessary) transported to the university, where it was placed indoors in a quiet room with the lights off for 2 h. Birds were not provided with food during this 2 h period to ensure that they were post-absorptive prior to experimentation, which involved respirometry in addition to the thermography reported here. Birds that were captured off campus were provided with *ad libitum* bird seed (Lizzie Mae's Birdseed and Dry Goods Co. Backyard Medley; white millet, sunflower and split peanuts) and water after experimentation during their transportation back to the capture site, whereas birds captured on campus were released immediately upon completion of the experiment and measurements. All 34 birds were safely released at the original site of capture after approximately 7 h in captivity. All capture, care and research described here was approved by the University of North Carolina Wilmington Institutional Animal Care and Use Committee (IACUC #2021-011).

Experiment

Two hours after capture, we collected thermal images of each bird at three ambient temperatures (means \pm s.e.m.): 14.92 ± 0.03 , 27.56 ± 0.02 and $40.02\pm 0.02^{\circ}\text{C}$. We first weighed the test subject using a digital scale with precision of 0.1 g (AWS-600). After recording body mass, we immediately placed the bird inside a respirometry and thermography chamber (henceforth, thermography chamber; $12\times 16\times 24$ cm) equipped with a thermal window (WTS Photonics Co. Ltd, Ge window with diameter 100 mm and thickness 4 mm). The thermography chamber was housed inside a larger temperature-controlled incubator (CARON 7404-10), and air was supplied at a constant rate of 1.4 l min^{-1} via tubing connected to a respirometer (Sable Systems FMS-3) outside the incubator. Before entering the thermography chamber, the air was scrubbed of water by passing through two silica gel columns and one Drierite column, and the tubing was wrapped in a copper coil inside the incubator to ensure that incoming air was at the treatment temperature. Temperature inside the thermography chamber was monitored constantly using a 10 ft (~ 3 m) thermocouple connected to the respirometer (Sable Systems SEN-TC-1). We placed a thermal imaging camera (FLIR T-840, resolution of 464×348 pixels) in front of the thermography chamber, and then closed the door to the incubator for the duration of the experiment to minimize stimulation and stress, which can influence the measurement of surface temperatures (Zuluaga and Danner, 2023a). The inside of the incubator was kept completely dark for the same reasons.

Once the door to the incubator was closed, the bird was given a 30 min habituation period, during which it was monitored at the intermediate temperature (27.5°C). This habituation period was provided to allow the birds to de-stress from being handled and to adjust to the new surroundings, and was therefore not used for data collection. Birds generally remained motionless in the exact location where the researcher placed them for a few minutes. All birds then regained alert posture and subsequently began exploring the chamber within the first 10 min of the habituation period.

After the habituation period, the temperature was set to the first treatment temperature. In winter, 11 birds received 15°C as the first treatment and 4 received 40°C as the first treatment. In summer, 16 birds received 15°C as the first treatment and 3 received 40°C as the first treatment. Once the treatment temperature was reached inside the thermography chamber, the temperature was held constant for 40 min, and we began recording thermal images. The first 20 min allowed birds to adjust physiologically and behaviorally to the new ambient temperature, and only the second 20 min were used for data collection. After the 40 min had elapsed, the above process was repeated with the remaining two temperatures. It took approximately 15 min to ramp up or down between temperatures, and the entire experiment lasted approximately 3 h per bird. Birds were tested individually.

After each experiment, we removed the bird from the chamber and immediately weighed it again using the same digital scale. We then measured tarsus length, tarsus width (medial), tarsus width (lateral), bill length, bill width and bill depth with a precision of 0.01 mm with digital calipers. All bill measurements were taken at the anterior edge of the nares, and all leg measurements excluded toes; therefore, our measurements of surface area and calculations of heat dissipation are likely conservative.

Data extraction

We collected data once per minute in the second half of each temperature treatment, resulting in approximately 20 observations per bird, per temperature treatment. Following methods described in Zuluaga and Danner (2023a), we scanned the video for 10 s, beginning 5 s before each minute mark, to collect behavioral data. We collected binary data for heat dissipating behaviors (panting, exposing the legs and feather flattening) and heat conserving behaviors (bill tucking, leg tucking and feather puffing). Behaviors were considered to be present during the minute if they were observed at all during the 10 s window. We determined that leg exposing behavior was present if the entire tarsometatarsus and ankle joint were visible. We determined that leg tucking behavior was present if the ankle joint was not visible and >50% of the tarsometatarsus was covered by feathers. We considered feather puffing (i.e. full ptiloerection) to be present if the contour feathers of the body were clearly raised and the surface was continuous and smooth. We used the smooth criteria because we were interested in ptiloerection for heat conservation, whereas a raised but jagged or discontinuous plumage surface is indicative of ptiloerection behavior for heat dissipation (Gutiérrez et al., 2023). We considered the feathers to be flattened when they were sleeked down to the body and the bird adopted a tall posture that increased the distance between the belly feathers and the tarsometatarsus. We considered anything between these two clearly distinguishable feather and body postures to be the neutral position, and we present these neutral position data in Fig. S1. The three levels of feather posture were mutually exclusive. After collecting behavioral data, we then selected one frame from within the 10 s window to extract thermographic data. We prioritized frames where the tip of the bird's bill was angled perpendicular to the camera, and frames where the bird was still (i.e. no motion blur present in the frame). We extracted mean, maximum and minimum surface temperatures (T_s) by drawing separate region of interest (ROI) polygons around the bird's bill, eye region and one of the legs (FLIR Thermal Studio Pro). Thermographic data were extracted from a total of 2127 frames across all 34 birds.

We then watched the entire 40 min of the high temperature trials to count the frequency of two novel thermoregulatory behaviors: pant

cycling and nasal wetting. Pant cycling is a previously undescribed pattern of behaviors that occurred between bouts of panting and resulted in the accumulation of liquid in the mouth. Nasal wetting, which may provide a benefit in the form of evaporative cooling, has not been previously observed in the order Passeriformes. As opposed to recording the presence/absence of each behavior at each minute using the instantaneous approach for surface temperature and binary behaviors, we instead collected these data by continuously watching the video and recording every occurrence because of the infrequency of these novel behaviors. Pant cycling occurred frequently during panting behavior, but the resulting wetness of the mouth varied strongly. Because the deeper parts of the mouth tended to regularly have some moisture, this behavior was most clearly distinguished when the tip of the bill was wet upon opening the mouth. Therefore, we only recorded instances of pant cycling when the tip of the bill was wet, which clearly indicated the accumulation of saliva in the mouth. We also recorded every occurrence of the nasal wetting behavior, which was very conspicuous because the bird shakes its head and the upper mandible immediately becomes wet. These continuous behavioral data were collected and analyzed independently of the instantaneous behavioral data.

Calculations

We calculated the bill and leg surface area of each individual using the `areacone()` and `areacylinder()` functions, respectively, in the R package `Thermimage` (<https://CRAN.R-project.org/package=Thermimage>). The bill surface area was calculated as an elliptical cone using the length of the bill as the height of the cone, and the depth and width of the bill as the two axes of the ellipsoid; the base of the cone was excluded. The leg surface area was calculated as a cylinder using tarsus length as the height of the cylinder, and the medial and lateral widths of the tarsus as the two axes of the cylinder; one base of the cylinder was included in the calculation.

After collecting T_s , we exported the data to R version 4.2.2 (<http://www.R-project.org/>) and converted to bill- and leg-mediated heat dissipation (BMHD and LMHD). We present T_s data for the appendages and eye region in Figs S2 and S3, respectively. We first calculated convective (q_{conv}) and radiative (q_{rad}) heat transfer ($W m^{-2}$) using the R package `Thermimage` (<https://CRAN.R-project.org/package=Thermimage>), which follows equations from Blaxter (1989) and Gates (2003). We assumed an emissivity of 0.96 following Zuluaga and Danner (2023a). Kinematic viscosity and conductivity of air were assumed fixed. Ambient temperature and relative humidity were recorded by the respirometer; we extracted these data using `Expedata` (Sable Systems) and we used the average temperature and relative humidity in the chamber for each individual at each temperature in the heat dissipation calculations. The wind speed was measured without a bird in the thermography chamber and was undetectable ($0 m s^{-1}$; Kestrel 5000). We therefore applied the `type="forced"` argument within the `qconv()` function with an estimated wind speed of $0.1 m s^{-1}$ as reasoned in Greenberg et al. (2012b). For surface reflectivity, we used a value of 0.05 (Tattersall et al., 2018). We multiplied q_{conv} and q_{rad} ($W m^{-2}$) by the surface area of the bill and tarsus of each individual (m^2), and then summed the resulting area-specific q_{conv} and q_{rad} to obtain the amount of heat dissipation (W) taking place instantaneously during each frame. We multiplied by 1000 to convert heat dissipation from W to mW, and we multiplied LMHD by 2. Finally, we averaged all response variables by bird and by temperature treatment, resulting in one average for each value of heat dissipation (rate of BMHD and LMHD in $W m^{-2}$ and instantaneous BMHD and LMHD in mW) and one proportion for

each of the six instantaneously sampled behaviors for each 20 min trial. After averaging by temperature treatment and individual, the final sample size was 101 for each response variable.

Data analysis

We analyzed data using the R package lme4 (Bates et al., 2015). We modeled T_s , rate of BMHD and LMHD ($W\ m^{-2}$), and area-specific BMHD and LMHD (mW) using linear mixed effects modeling [lmer()]. We modeled behavioral data using generalized linear mixed effects modeling [glmer()] with a binomial probability distribution and a logit-link function. Repeated measures were accounted for by averaging by treatment per individual prior to modeling. We included the bird's assigned identification number (bird ID) as a random intercept in all models to account for interindividual variation, and we constructed all null models using only the random intercept of bird ID. We built a global model for each response variable that included season, mass and ambient temperature (T_a) as fixed effects, and the random intercept of bird ID. Including the order of temperature treatments did not significantly improve model fit for any of the response variables, and treatment order was therefore not included in the model structure for any analyses. Model support was determined using AICc (Burnham and Anderson, 2002). We created a list of competing models using function dredge() in R package MuMIn (<https://CRAN.R-project.org/package=MuMIn>) and we report all top-ranked and competitive models ($\Delta AICc < 2$). We assessed the fit of the top models by calculating the conditional Nakagawa's R^2 using the function r.squaredGLMM() in the R package MuMIn. If model fit suffered because a behavior was absent or constant during an ambient temperature, the models were re-run on the subset of temperatures where the behavior varied enough to allow modeling. Similarly, we did not model behaviors that were completely absent during one of the seasons because models would not converge and the patterns of raw data were clear. We visually represented findings from our models by plotting model predictions obtained from the package ggeffects (Lüdtke, 2018) along with raw data for each response variable using the package ggplot2 (Wickham, 2016).

RESULTS

Thermal windows

In contrast to our prediction, heat dissipation across the bill and legs was generally higher in winter than in summer (Fig. 1). The top ranked model for the rate of heat dissipation ($W\ m^{-2}$) across the bill included T_a and body mass as interactive fixed effects, and the top ranked model for legs included T_a and season as non-interactive fixed effects (Table 1; $R^2=0.961$ and 0.738 , respectively). The top ranked model for area-specific heat dissipation (mW) for the bill included T_a and body mass as non-interactive fixed effects, while that for the legs included T_a and season as interactive fixed effects (Table 1; $R^2=0.953$ and 0.750 , respectively). Average body mass was 9.66% heavier in winter than in summer (Table S1), and for each measure of heat dissipation, model rankings included body mass in at least one competitive model ($\Delta AICc < 2$) where season was in the top-ranked model, and vice versa. Relative to the bill, the legs had a lower rate of heat loss during the low temperature treatment, and a lower rate of heat gain during the high temperature treatment (Fig. 1A,B). The overall (across seasons) surface area of the bill was $5.26 \times 10^{-2} \pm 7.34 \times 10^{-4}$ mm², while that of the legs was $7.28 \times 10^{-2} \pm 8.79 \times 10^{-4}$ mm² (means \pm s.e.m.) (Table S1). Despite their larger surface area, the legs lost less heat than the bill during the low temperature treatment, but they gained slightly more heat than the bill during the high temperature treatment (Fig. 1C,D).

Thermoregulatory behaviors

Other than one instance during the intermediate control treatment, panting behavior only occurred during the high temperature treatment; panting was therefore only modeled for the high temperature treatment, and the fixed effect of temperature was accordingly removed from its model structure. Panting occurred significantly more frequently in winter than in summer at 40°C (Fig. 2A). The top ranked model for panting behavior included season as the only fixed effect (Table 2; $R^2=0.629$). The top ranked model for leg exposure behavior included T_a and season as interactive fixed effects (Table 2; $R^2=0.893$). Summer birds avoided exposing the legs at low T_a , and exposed the legs at high T_a ; leg exposure in winter birds followed similar trends but exhibited more variation at both temperatures (indicated by larger error bars; Fig. 2C). Birds in both seasons exhibited the most variation in leg exposure during the intermediate temperature treatment.

Bill tucking was only observed in birds during summer; therefore, modeling for this behavior was not possible and not necessary for interpretation. Multiple individuals tucked their bills during the low temperature treatment in summer, two individuals tucked their bills during the intermediate temperature treatment in summer, and no individuals tucked their bills during the high temperature treatment; birds did not tuck their bill during any of the treatment temperatures in winter (Fig. 2B). While birds tucked their legs in both summer and winter, this behavior was notably more consistent in the summer birds at the low T_a (Fig. 2D). Leg tucking behavior was very rare during the high temperature trials, and was only observed in one summer individual and one winter individual. The top ranked model for leg tucking behavior included T_a and season as interactive fixed effects (Table 2; $R^2=0.909$).

The three levels of ptiloerection varied strongly with T_a . Across both seasons, full ptiloerection was most common during low temperature treatments, neutral feather position was most common during intermediate temperature treatments, and feather flattening was most common during high temperature treatments (Fig. 2E,F; Fig. S1). The top model for feather flattening behavior included T_a and season as interactive fixed effects (Table 2; $R^2=0.942$). Feather flattening was used frequently during high temperature trials, and infrequently during low temperature trials; winter birds flattened their feathers more during intermediate temperature trials (Fig. 2E). The top model for feather puffing behavior included T_a and season as interactive fixed effects (Table 2; $R^2=0.912$). Feather puffing during low temperature treatments was observed more consistently during summer than during winter (indicated by smaller error bars); the summer birds also exhibited full ptiloerection considerably more than the winter birds during the intermediate temperature treatment (Fig. 2F). Neutral feather position was most commonly used during intermediate temperature trials, and this behavior was uncommon during low and high temperature trials across both seasons (Fig. S1).

Novel heat dissipation behaviors

In addition to the planned behavioral data collection, we report a completely novel and previously undescribed thermoregulatory behavior. We observed birds performing a suite of behaviors during panting, which consisted of three distinct actions between bouts of panting; first, the bird closed the mouth and fluttered the gular feathers just behind the lower mandible (i.e. likely a result of movement of the hyoid apparatus and tongue to stimulate the salivary glands); second, the bird jittered the mandibles in a manner that suggests movement inside the mouth (i.e. likely the spreading of saliva); and third, the bird opened the bill, revealing an accumulation of saliva inside the mouth. Because the deeper parts of the mouth tended to regularly have some

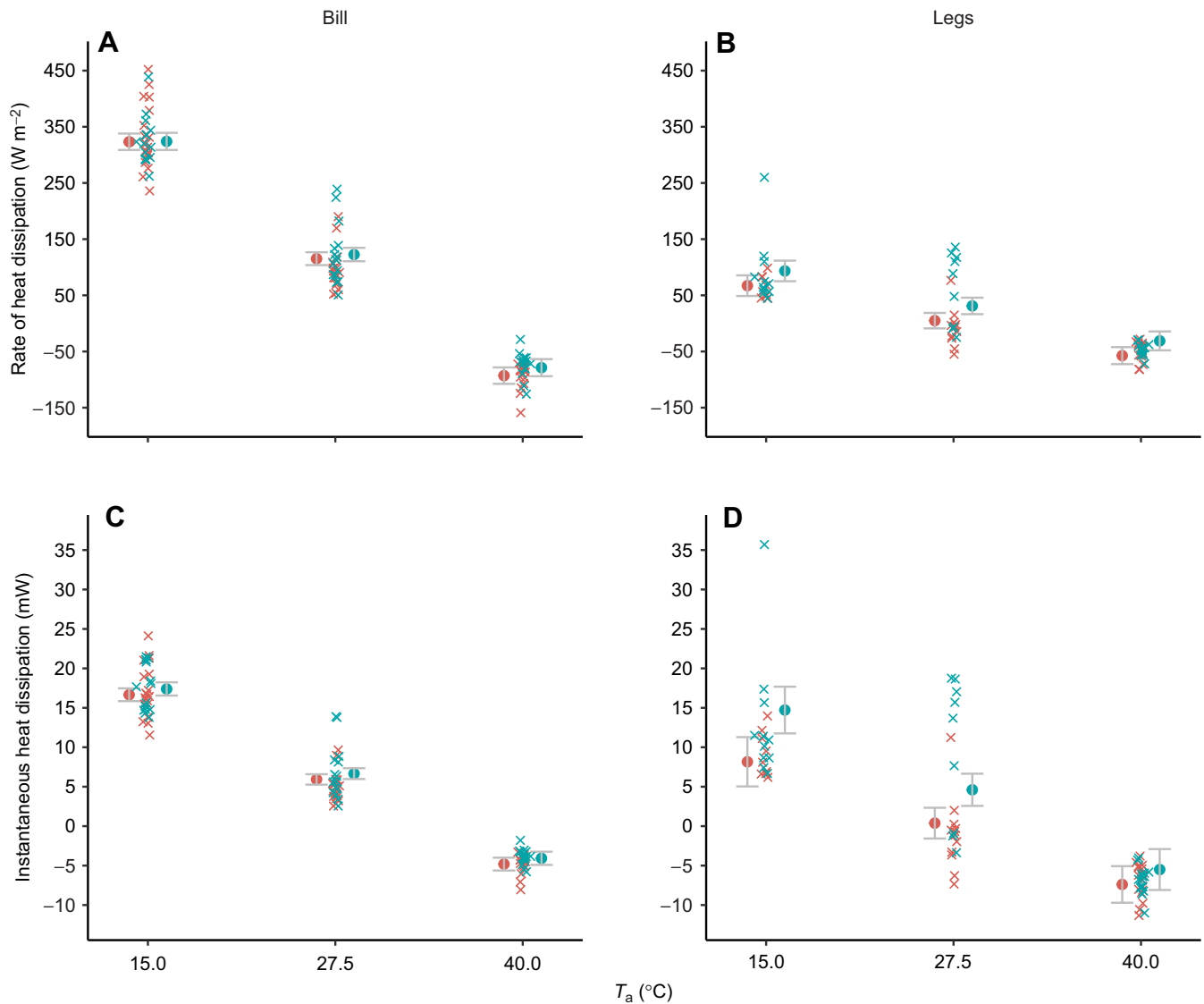


Fig. 1. Rate of heat dissipation and instantaneous heat dissipation across summer and winter. (A,B) The rate of bill-mediated heat dissipation (BMHD) and leg-mediated heat dissipation (LMHD), respectively, versus ambient temperature (T_a). (C,D) The area-specific instantaneous BMHD and LMHD, respectively, versus T_a . Model predictions ($\pm 95\%$ confidence interval, CI) are represented on either side of the raw data. The effect of body mass was calculated for the mean body mass by season for variables where body mass was included in the top-ranked model. Summer data are in red ($n=19$) and winter data are in blue ($n=15$).

moisture, this behavior was most clearly distinguished when the tip(s), and occasionally the outer surface, of the bill were wet upon opening the mouth (Movie 1). After beginning to pant, the tip(s) of the bill warmed back up, indicating the evaporation of saliva from the surface and interior of the bill. Summer birds often lacked the first and second step of the pant cycle, only closing and opening the mouth between bouts of panting. Although birds were regularly observed closing and opening their mouth during panting in both seasons, pant cycling resulting in tip wetting only occurred in one summer bird, whereas it occurred in 11 winter birds (Fig. 3). On average, during the 40 min trials, winter birds performed 8.27 ± 3.13 (mean \pm s.e.m.) pant cycles that ended in tip wetting, whereas only one summer bird performed three pant cycles that ended in tip wetting. This is the first time that pant cycling and its constituent behaviors have been described.

We also observed a thermoregulatory behavior that has not been previously observed in passerines, where birds appeared to sneeze, resulting in a large amount of nasal fluid exiting the nares and

coating the upper mandible of the bill (Movie 2). This behavior is functionally similar to ‘nasal watering’ (Janse van Vuuren et al., 2020; Kemp and Kemp, 1980), but this is the first time it has been reported in a species other than the southern ground hornbill (*Bucorvus leadbeateri*), and it is the first time it has been reported with projectile discharge. This behavior was immediately preceded by a typical pant cycle, and it only occurred twice in winter and not at all in summer.

DISCUSSION

Our results contribute novel insights on how behavioral changes and peripheral heat dissipation contribute to seasonal acclimatization to the heat. Contrary to our first prediction and the pattern observed in our preliminary seasonal analyses (Zuluaga and Danner, 2023a), we found that birds maintained higher surface temperatures, and therefore dissipated more heat, in winter than in summer. We found that summer birds exhibited heat dissipating behaviors less

Table 1. Top ranked and competitive ($\Delta AICc < 2$) linear mixed effects models describing bill-mediated heat dissipation and leg-mediated heat dissipation

Appendage	Model	d.f.	AICc	$\Delta AICc$	AICc weight
BMHD (W m ⁻²)	$T_a \times$ body mass	6	1042.9	0	0.186
	T_a	4	1043.1	0.19	0.170
	T_a +body mass	5	1043.3	0.38	0.154
	T_a +season	5	1044.1	1.14	0.106
	$T_a \times$ season	6	1044.1	1.17	0.104
LMHD (W m ⁻²)	T_a +season	5	766.5	0	0.193
	$T_a \times$ season	6	766.7	0.20	0.175
	T_a +season+body mass	6	767.4	0.85	0.126
	T_a +body mass	5	767.4	0.87	0.125
BMHD (mW)	$T_a \times$ season+body mass	7	767.7	1.22	0.105
	T_a +body mass	5	464.7	0	0.364
LMHD (mW)	T_a +season+body mass	6	466.7	1.99	0.135
	$T_a \times$ season	6	472.7	0	0.215
LMHD (mW)	T_a +season	5	473.3	0.57	0.161
	$T_a \times$ season+body mass	7	473.7	1.02	0.129
	T_a +season+body mass	6	474.1	1.40	0.107
	T_a +body mass	5	474.2	1.46	0.103

BMHD, bill-mediated heat dissipation; LMHD, leg-mediated heat dissipation; AIC, Akaike information criterion; T_a , ambient temperature.

frequently, while winter birds exhibited heat conserving behaviors less frequently, providing support for the prediction that seasonal physiological adjustments allow birds to save resources by reducing the use of thermoregulatory behaviors. That these differences were most distinct in panting behavior provides support for our prediction that seasonal behavioral adjustments occur primarily in costly behaviors. Together, these findings suggest that the chipping sparrows in this study primarily relied on behavioral and metabolic changes to undergo seasonal acclimatization, not changes in peripheral heat dissipation.

Thermal windows

To our surprise, we found that thermal windows dissipated more heat in winter than in summer. We suspect that this occurred because of higher rates of metabolic heat generation in winter to prevent hypothermia in cold weather. Other temperate region birds undergo seasonal changes resulting in greater metabolic heat generation in winter (McKechnie et al., 2015; Swanson, 2010; Swanson and Vézina, 2015), and preliminary data from the metabolic measurements collected during these experiments suggest that chipping sparrows undergo similar metabolic changes (J.D.Z. and R.M.D., unpublished data). Increases in summer heat loss may be unnecessary in chipping sparrows because their small body size likely allows them to dissipate heat efficiently. We also found that birds were 9.66% heavier in winter than in summer, likely contributing to higher metabolic heat generation, which may explain the presence of body mass in highly ranked models of heat dissipation. The inclusion of body mass in competitive models where season is in the top-ranked model, and vice versa, suggests that both body mass and season contribute to heat dissipation and may compete to explain some of the same variation; this trend was not observed in the behavioral models, suggesting that seasonal changes in body mass have a greater influence on thermoregulatory traits associated with peripheral heat dissipation than on behavior.

While the finding that thermal windows dissipated more heat in winter than in summer does not support our first prediction, it does provide support for the hypothesis that seasonal changes can occur in peripheral heat dissipation.

Thermoregulatory behaviors

The same metabolic adjustments that provide increased heat production in winter and more efficient evaporative cooling in summer may allow birds to offset the use of expensive thermoregulatory behaviors. Supporting our prediction that non-acclimatized birds would use expensive thermoregulatory behaviors more frequently than acclimatized birds, winter birds panted 63.79% more of the time than summer birds during the high temperature treatment. The need to pant may have been reduced in the summer birds if they had greater evaporative cooling efficiency or heat tolerance because of metabolic adjustments for acclimatization to heat, as has been reported in other birds (Noakes et al., 2016; O'Connor et al., 2017; Oswald et al., 2018; Tieleman et al., 2002). Alternatively, if the winter birds had higher metabolic heat generation as a result of acclimatization to cold (Swanson and Vézina, 2015), then this may also necessitate more panting to offset greater heat production. Both scenarios would suggest a metabolic origin to the seasonal change in panting behavior observed here.

It is possible that seasonal differences in water availability can contribute to behavioral changes (Smit et al., 2016), and it is unknown whether these behavioral changes arise as direct responses to the availability of water (i.e. saving water during dry time periods) or as a result of physiological adjustments to external conditions (i.e. acclimatization). To ensure that any differences observed were a result of acclimatization, we captured birds at feeders that were positioned near standing water in both seasons. Additionally, we captured birds in the morning to avoid introducing differences in hydration state (i.e. summer birds would likely be more dehydrated than winter birds if captured in the afternoon). Removing these potential sources of bias lessens the likelihood of measuring differences caused by water availability or hydration state, and thus isolates behavioral changes that arise from seasonal acclimatization.

At high temperatures during both seasons, birds commonly exhibited feather flattening and tarsus exposure, and the seasonal change in these behaviors was much less pronounced than that in panting. The difference in seasonal change between these two postural behaviors and panting may be a result of the resource costs associated with panting (McKechnie, 2022), providing support for the idea that birds benefit more from seasonal adjustments to costly thermoregulatory behaviors. Physiological and behavioral adjustment may not be necessary to optimize the use of behaviors that are low in cost, and therefore equally viable throughout the year, such as tarsus exposure or feather flattening. Interestingly, while the seasonal difference was less pronounced in the two low-cost heat dissipating behaviors, summer birds more consistently avoided exposing the legs during low temperature treatments, and they avoided flattening feathers during thermoneutral treatments, whereas winter birds exhibited greater variation in both behaviors at these temperatures, providing subtle evidence that winter birds were more resistant to cold temperatures.

Summer birds were observed using full ptiloerection more frequently and consistently than winter birds during both the low and intermediate temperature treatments. Leg tucking behavior was observed in both seasons, but summer birds exhibited this behavior more consistently during low temperature treatments than winter birds. Bill tucking behavior was only observed in summer,

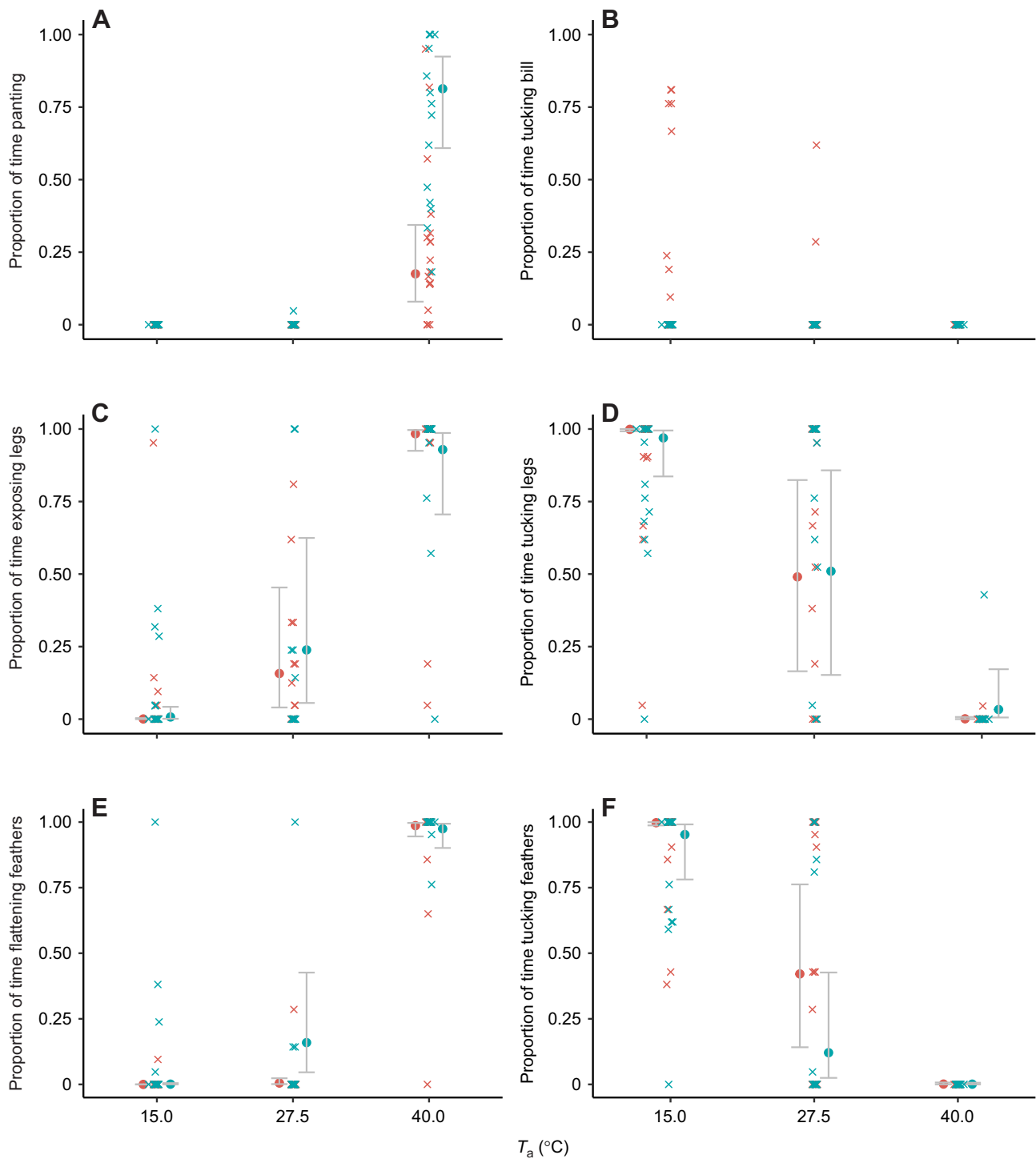


Fig. 2. Proportion of time spent using thermoregulatory behaviors in summer and winter. (A) Panting, (B) bill tucking, (C) leg exposure, (D) leg tucking, (E) feather flattening and (F) feather tucking. Where available, model predictions ($\pm 95\%$ CI) are represented on either side of the raw data. Summer data are in red ($n=19$) and winter data are in blue ($n=15$).

indicating that winter birds did not need to tuck their bill to conserve heat; furthermore, the only two instances of bill tucking during intermediate temperature treatments occurred in summer. Bill tucking showed clear separation between seasons even though it is a postural change that is likely low in resource cost. A possible explanation is that birds may benefit from adjusting thermoregulatory behaviors to reduce risk as well as resource costs. Winter birds likely benefit from avoiding bill tucking because

of the risk it carries in terms of reducing their reaction time to potential predators (Ferretti et al., 2019). The bill lacks adaptations for heat conservation that are present elsewhere, such as plumage and countercurrent heat exchange in the body and legs, respectively. This suggests that the benefits gained by adjusting bill tucking behavior to avoid predation risk outweigh the benefits that would be gained by providing a mechanism of heat retention in the bill during winter.

Table 2. Top ranked and competitive ($\Delta\text{AICc}<2$) generalized linear models describing the use of thermoregulatory behaviors

Behavior	Model	d.f.	AICc	ΔAICc	AICc weight
Heat dissipating					
Panting	Season	3	190.8	0	0.652
Exposing legs	$T_a \times \text{season}$	5	577.0	0	0.626
Flattening feathers	$T_a \times \text{season}$	5	425.3	0	0.562
Heat conserving					
Tucking legs	$T_a \times \text{season}$	5	553.6	0	0.601
Puffing feathers	$T_a \times \text{season}$	5	508.7	0	0.417
	$T_a \times \text{season} + \text{body mass}$	6	510.6	1.88	0.163

Novel heat dissipation behaviors

The use of thermal images allowed us to detect two unexpected behaviors. Pant cycling is a novel and previously undescribed behavior, and nasal wetting was previously unknown to occur in passerines. Supporting our prediction that non-acclimatized birds would use expensive thermoregulatory behaviors more frequently than acclimatized birds, both novel heat dissipating behaviors occurred more frequently in winter and are associated with evaporative cooling. Together, these behaviors shine new light on the function of the bill as a thermal window because they carry functional significance for evaporative cooling, a major thermoregulatory pathway, and the only pathway that allows birds to dissipate heat when ambient temperature exceeds body temperature (McKechnie and Wolf, 2019). In addition to its role in dry heat dissipation and water savings, raising the temperature of the bill at high ambient temperatures can work synergistically with pant cycling and nasal wetting by aiding in the evaporation of fluid from the inside and outside of the bill, respectively.

Pant cycling occurred regularly when panting was observed, suggesting that it is an important component of evaporative cooling. We propose the term ‘pant cycle’ to describe the process by which birds replenish the fluids used during evaporative cooling through panting. The mechanism consists of three distinct actions after the end of a panting bout, and before the beginning of the next panting bout. The first step is the stimulation of the salivary glands, which we hypothesize is achieved by the movement of the hyoid apparatus

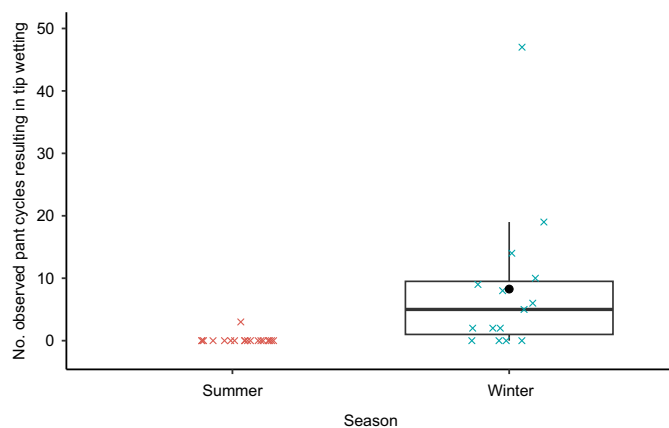


Fig. 3. A box plot of the number of pant cycles resulting in tip wetting observed during high temperature treatments. Box plot shows median and interquartile range for winter; the mean is plotted as a black circle. Counts per individual are in red for summer ($n=19$) and blue for winter ($n=15$). Raw data are jittered for clarity.

and tongue, as indicated by fluttering of the gular feathers near the lower mandible. The second step is the quivering of the upper and lower mandible of the bill, which we hypothesize is indicative of the movement of the tongue as it applies saliva to the inside of the mouth. The third and final step happens when the bird opens its mouth, revealing that the interior of the mouth appears wet, indicated by cooler temperature. The bird then pants until the mouth becomes dry, and the cycle repeats. This finding provides novel insights into the physiology of panting behavior and provides the first description of how fluids are replenished during panting.

The amount of saliva accumulated varied between pant cycles. Some pant cycles resulted in obvious and abundant salivation as indicated by a notable decrease in temperature; in one case, a bird was even observed launching saliva from the mouth in a stream during three separate pant cycles. In other cases, the difference in surface temperatures was undetectable and the only indication that pant cycling was taking place was the presence of its constituent behaviors (closing the bill, gular flutter, bill quivering and opening the bill to pant). Between these two extremes, there were often intermediate changes in temperature and presumably wetness. Additionally, the change in surface temperature was ephemeral because the saliva quickly began to evaporate when the mouth opened. Therefore, we took a conservative approach in describing this behavior by only recording instances of pant cycling that resulted in considerable accumulation of saliva, as indicated by wetness at the tip of the bill. Only one of 19 summer birds exhibited tip wetting, whereas 11 of 15 winter birds exhibited tip wetting. The winter birds also exhibited tip wetting more frequently, with an average of 8.27 pant cycles resulting in tip wetting, compared with the single individual in summer that only exhibited tip wetting 3 times. It is unlikely that these are overestimates of the difference between seasons because of the larger sample size in summer, and because the winter dataset had more missing values in the instantaneously sampled panting column; these missing values indicate times when the bill was not visible because the birds were facing away from the camera or were out of frame. Despite these differences in the two datasets, which would both bias results towards greater detection in summer, fewer instances of tip wetting were observed in summer. Therefore, the difference between seasons is likely underestimated.

Nasal watering has been described before (see Janse van Vuuren et al., 2020), but this is the first time this behavior has been observed in a passerine. This is also the first time this behavior has been observed to result in projectile secretion, for which we propose the term ‘thermal sneeze’ to accurately describe the behavior in this case (Movie 2). It should be noted that the sneeze itself results in a large proportion of the expelled liquid being wasted if it does not land on the bird’s body, which suggests that the thermal benefit received from the sneeze may not be its sole purpose. Further research into the mechanism that triggers a thermal sneeze may reveal whether the purpose is to coat the bill in liquid for evaporative cooling, or whether this occurs as a byproduct of a more typical function of sneezing, such as ridding the bird of over-accumulated saliva that was produced for panting. The thermal sneeze confers a thermal benefit in either case, and it is not the only example of organisms applying liquid to typically dry body surfaces for evaporative cooling. Similar behaviors have been observed in other taxonomic groups, including bats (e.g. Ochoa-Acuña and Kunz, 1999), rodents (e.g. Ramirez et al., 2022), marsupials (e.g. Needham et al., 1974) and ciconiiforms (e.g. Philip Kahl, 1963), though notably the liquid in these cases is not nasal effluent but saliva, or urine and excrement in the case of ciconiiforms. Adding to

these and the previously described occurrence in southern ground hornbills, we provide novel evidence that chipping sparrows, and likely other passerines, also increase evaporative cooling by wetting typically dry body parts with bodily fluids.

Ecophysiological implications

In addition to seasonal acclimatization, the phenotypic plasticity of thermal traits can provide physiological adjustments tailored to sex-specific thermal challenges, such as the development of larger thermal windows for male marsh sparrows that must sing at exposed perches during summer (Greenberg et al., 2013), or the reductions to metabolic rate and water loss developed by female yellow-billed hornbills (*Tockus leucomelas*) during their confinement inside nesting cavities (van Jaarsveld et al., 2021). It is possible that similar changes occur in other species including chipping sparrows, but our lack of female samples during the breeding season precludes their investigation. However, relative to the shadeless and freshwater-lacking habitat of marsh sparrows, the chipping sparrows in this study inhabit a mild habitat characterized by heterogeneous shade, nearby bodies of fresh water and supplemented food availability, which may contribute to higher rates of peripheral heat dissipation (Zuluaga and Danner, 2023a). The uniquely challenging thermal habitat of salt marshes may be an important driver of evolution and seasonal adjustment in thermoregulatory traits, as suggested by behavior (Luther and Danner, 2016) and ecogeographical trends in bill size, particularly in males (Greenberg et al., 2012a). The sex-specific differences in thermal physiology reported in van Jaarsveld et al. (2021) are likely also driven by the challenging thermal conditions experienced by yellow-billed hornbills, and particularly the resource limited conditions experienced by females because of their confinement inside nest cavities during the breeding season; such sex-specific differences in microclimate and resource availability are not present in our study system. Because of the relatively mild thermal conditions and the lack of sex-specific thermal challenges experienced by chipping sparrows, we expect that the birds in this study are less likely to develop sex-specific differences, or at least for these differences to be less pronounced than in Greenberg et al. (2013) or van Jaarsveld et al. (2021). Apart from surface area and metabolic characteristics, it is unknown whether other traits such as surface temperature or behaviors exhibit sex-specific differences, and future research is needed to determine the extent of sex-specific thermal specialization in birds.

The novel descriptions of pant cycling and nasal wetting provide important insights into the function of the bill as a thermoregulatory organ, and showcase its newfound significance for evaporative cooling. That tip and nasal wetting occurred in winter birds, but not in the heat acclimatized summer birds, suggests that these behaviors are only exhibited when a large thermal challenge is encountered. These behaviors may therefore provide an indicator for advanced stages of heat stress, a finding that highlights important considerations for the development of future behavioral studies on thermoregulation during high temperatures. Given the seasonal variability in these behaviors detected here, and if our hypothesis that changing thermal regimes can result in behavioral changes holds true, then we suspect that these behaviors will become more common as climate change advances.

A number of studies hypothesize that heatwaves will increasingly cause mortality (Albright et al., 2017; Conradie et al., 2019, 2020; McKechnie and Wolf, 2010), which can potentially lead to population-level impacts such as those predicted in chipping sparrows (Wilsey et al., 2019). It has been suggested that behavioral indices of heat dissipation can be used to quantify vulnerability to high temperatures (McKechnie,

2019; Pattinson et al., 2020; Smit et al., 2016), and our results suggest that seasonal variation will be an important factor to consider when using such behavioral indices. Furthermore, investigating seasonal variation may provide an altogether novel approach for the assessment of vulnerability to temperature anomalies, such as heat waves. For example, one might expect that a population of birds would exhibit greater use of thermoregulatory behaviors when experiencing the first heatwave of a year when compared with the peak of summer, after the population has had time to acclimatize to high temperatures. One might also expect that species, or populations, that exhibit the greatest behavioral change between the first heatwave and peak summer are the most vulnerable to early heatwaves. Our results suggest that such hypotheses may be testable by measuring seasonal behavioral adjustments. The approach we suggest here may provide an effective way to investigate sensitivity to heat waves. As our understanding of how birds interface with their thermal environment deepens, novel approaches will become available for assessing thermal biology, and as climate change advances, such assessments of susceptibility to heat will become increasingly necessary. We therefore advocate for the consideration, development and subsequent implementation of novel strategies such as the one proposed here to investigate birds' vulnerability to heat.

Conclusion

McKechnie and Wolf (2019) pointed out the lack of information on seasonal acclimatization to heat in avian physiology literature. This study marks the first time that thermography has been used to investigate seasonal changes in the thermal biology of birds. Using thermal imaging and behavioral analyses, we found compelling evidence for the presence of seasonal change in the thermal windows and thermoregulatory behaviors of a small-bodied passerine. The use of thermography also resulted in the discovery of one novel thermoregulatory behavior, and the first detection of a known thermoregulatory behavior in a new taxonomic group. Our results attest to the value of applying such approaches in the young, but quickly developing field of research that investigates seasonal changes in avian heat acclimatization, and more broadly in the ecophysiology of birds.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.D.Z., R.M.D.; Methodology: J.D.Z., R.M.D.; Software: J.D.Z.; Validation: J.D.Z., R.M.D.; Formal analysis: J.D.Z.; Investigation: J.D.Z., R.M.D.; Resources: J.D.Z., R.M.D.; Data curation: J.D.Z.; Writing - original draft: J.D.Z.; Writing - review & editing: J.D.Z., R.M.D.; Visualization: J.D.Z.; Supervision: J.D.Z., R.M.D.; Project administration: J.D.Z., R.M.D.; Funding acquisition: J.D.Z., R.M.D.

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Data availability

Data are available from the Dryad digital repository (Zuluaga and Danner, 2023b): <https://doi.org/10.5061/dryad.n5tb2rc0w>.

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