UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

HACIENDO FRENTE AL CALOR: LÍMITES DE TOLERANCIA TERMAL Y EFICIENCIA DE LOS MECANISMOS DE ENFRIAMIENTO EN AVES

Coping with heat: thermal tolerance limits and efficiency of cooling mechanisms in birds

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“Quien no ha visto un árbol seco
no sabe qu’una calandria contra más calor más canta
contra más canta, más sol”

La Morralla - Carlos Cano

Agradecimientos

Como cantan Belle & Sebastian en '*Sleep the clock around'* (de su enorme tercer LP *The Boy With the Arab Strap*):

"*now the trouble is over,*

everybody got paid,

everybody is happy, they are glad they came"

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Summary

Earth temperature is rising at an unprecedented rate, mainly due to anthropogenic activity (e.g., greenhouse gases emissions). Mean surface temperature has significantly increased since preindustrial values and different climate warming scenarios forecast additional increases by the end of the century. In addition to this increase in mean surface temperature, global warming brings an increase in the number, intensity and duration of climatic extreme events such as heatwaves. These episodes of extreme heat can compromise the physiological capacities of animals, thus resulting in chronic deleterious effects on their fitness (e.g., impeding body mass maintenance or lowering breeding success) or even in mass mortality events. To assess whether and how physiology and behaviour can buffer the impacts of heatwaves and extreme temperatures is one of the major challenges for conservation biologists in the global warming context. Thus, the general objective of this thesis was to evaluate the physiological and behavioural responses to high temperatures by using birds as study model.

The Mediterranean basin has been forecasted as a climate warming hotspot where mean and maximum temperature extremes will experience greater increases than in other regions of the globe. Nonetheless, there is little knowledge about how Mediterranean species, particularly birds, deal with heat extremes. Thus, we studied how heat exposure could compromise the physiological capacities of small Mediterranean songbirds. In Chapter 2, we determined the heat tolerance limits (HTL) and other key thermoregulatory traits – namely body temperature (T_b), resting metabolic rate (RMR), evaporative water loss (EWL), and evaporative cooling efficiency at high temperatures – in small Mediterranean songbirds, and employed these data to estimate their current and future vulnerability to heat exposure. To do this, we assessed both chronic (days above upper critical temperature, T_{uc}) and severe effects (risk of direct mortality though lethal hyperthermia or dehydration) derived from exposure to high temperatures forecasted by climatic models under two climate warming scenarios (RCP4.5 y RCP8.5) across Extremadura, southwestern Spain. We found that Mediterranean songbirds show relatively low heat tolerance limits and limited evaporative cooling efficiencies. Thus, they are currently prone to experience chronic deleterious fitness effects from heat exposition. Future warming scenarios will exacerbate their vulnerabilities, especially for the smallest species, which will experience moderate risk of lethal dehydration (i.e., loss of 15% body mass in < 5 hours) during extremely hot days by the end of the 21st century under the most severe warming scenario. Overall, our results indicate that small Mediterranean songbirds are particularly sensitive to climate warming.

Understanding how physiological thermoregulatory responses to heat vary among populations of a species is crucial to better predict current and future persistence and vulnerability of the species. However, few studies have evaluated how heat tolerance and evaporative cooling efficiency differ among bird populations along a climatic gradient, and none of them has focused on temperate species. In

accordance with the climatic variability hypothesis, individuals from populations that experience more heterogeneous thermal conditions will show greater flexibility in their thermal traits than those from more thermally stable populations. In Chapter 3, we explored seasonal and between-population differences in heat tolerance and evaporative cooling efficiency in two Mediterranean Great tit *Parus major* populations that experience different thermal regimes. Overall, we showed that Great tits from a warmer and more thermally variable lowland site showed greater evaporative cooling efficiency during summer (e.g., greater slopes above inflection point and maximum values) than those from a milder and more thermally stable montane site. Besides, birds from the warmer locality enhanced their HTL and their evaporative cooling capacity during summer through changes in their thermal balance (e.g., by reducing their internal heat loads through diminished T_b and RMR), while those from the milder and more stable site did not show seasonal adjustments in these traits. Broadly these findings therefore conform to the assumptions of the climatic variability hypothesis. Furthermore, birds from the warmer, lowland site displayed a greater seasonal flexibility in their thermoregulatory traits above thermoneutrality than those from the milder montane site. Our results highlight the importance of incorporating between-population variation in thermoregulatory traits when forecasting species vulnerabilities to high temperatures.

Along with physiological responses, birds can cope with heat extremes through behavioural mechanisms. Indeed, heat dissipation behaviours (e.g., ceasing or reducing activity, seeking shade, or changes in body postures) can buffer physiological impacts of high temperatures. Therefore, gaining knowledge about the major determinants of these behaviours and how they affect an animal's heat balance is crucial in the face of climate warming. This is particularly important for species that typically forage and breed in warm, open landscapes where heat stress can be problematic. Storks are renowned for defecating on their legs when heat stressed — a cooling mechanism known as 'urohidrosis'. Despite its potential importance in maintaining heat balance, the major determinants of urohidrosis as well as its energetics have been largely overlooked. In Chapter 4, we identified the major environmental, ecological, morphological, and latitudinal determinants of urohidrosis across all extant stork species. To do so, we combined urohidrosis data from digital sources (photos and videos) with microclimate and ecological data and analysed the data set within a phylogenetic framework. We found that urohidrosis use is mainly triggered by high ambient temperatures, solar radiation and humidity, as well as by low wind speeds. In addition, urohidrosis was more prevalent in open-landscape foraging species than in those which mainly forage in waterbodies. Interestingly, we found substantial interspecific variation in temperature thresholds for urohidrosis use prevalence, which points to different species vulnerabilities to heat. After confirming that urohidrosis is an adaptive response for life in open and warm habitats, we quantified its cooling effect in free-ranging breeding White storks *Ciconia ciconia* by employing thermal imaging (Chapter 5). Thermal images analysis revealed that urohidrosis can lower leg surface temperature by up to 6 ºC (4.4 ± 1.04 ºC), but its cooling effect was of short duration (~2.5 minutes) and decreased with time. Indeed, we recorded individuals performing up to 11 events of urohidrosis in about 1 hour, which could represent a significant amount of heat loss (equivalent to 4% of daily field metabolic rate in adult storks). Overall, our findings indicated that this a priori 'cheap' behavioural response could help prevent overheating in storks and possibly other long-legged birds that frequently engage in this behaviour. Although largely overlooked, behavioural responses must be explicitly integrated into frameworks for predicting the vulnerability of birds.

The results shown in this thesis highlight the importance of considering interspecific and inter-population variation in physiological and behavioural responses when forecasting future persistence and vulnerability of the species or populations under future climate warming scenarios. To date, however, most of predictive models have overlooked the potential variation in this type of responses to heat among species or populations, limiting their predictive power and accuracy. By using recently developed mechanistic modelling tools that allow to integrate behavioural and physiological data into climate-change predictions for individual species, we could obtain finer predictions and better inform future conservation strategies. Along this line, the growing amount of online data sources offers the opportunity to complement traditional data acquisition approaches to gain knowledge about behavioural thermoregulatory responses in wild birds. Finally, we highlight the usefulness of modern, non-invasive techniques to monitor body surface temperatures (such as infrared thermal imaging) and thus obtain insights about the thermoregulatory role of certain bare body parts (like beak and legs), which can act as 'thermal windows' that promote passive heat loss.

Resumen

La temperatura de la Tierra está aumentando a un ritmo sin precedentes, principalmente debido a las actividades antropogénicas (principalmente la emisión de gases de efecto invernadero). La temperatura media superficial se ha incrementado significativamente respecto a valores preindustriales y los diferentes escenarios de calentamiento del clima pronostican incrementos adicionales para finales de siglo. Además de este incremento en las temperaturas medias superficiales, el calentamiento global trae un incremento en el número, intensidad y duración de eventos climáticos extremos como las olas de calor. Estos episodios de calor extremo pueden comprometer las capacidades fisiológicas de los animales, resultando en efectos adversos crónicos sobre su eficacia biológica (por ejemplo, impidiendo el mantenimiento de la condición corporal o reduciendo el éxito reproductor) o incluso en eventos de mortalidad masivos. Determinar sí y de qué forma la fisiología y el comportamiento pueden amortiguar los impactos de las olas de calor y las temperaturas extremas es uno de los principales desafíos para los biólogos conservacionistas en este contexto de calentamiento global. Por tanto, el objetivo general de esta tesis fue evaluar las respuestas fisiológicas y comportamentales a altas temperaturas utilizando aves como modelo de estudio.

La Cuenca Mediterránea se ha predicho como un punto caliente de calentamiento climático, en el cual tanto las temperaturas medias como los extremos de temperaturas máximas experimentarán mayores incrementos que en otras regiones del planeta. Sin embargo, existe poco conocimiento acerca de cómo las especies mediterráneas, particularmente las aves, lidian con los extremos de calor. Así, estudiamos como la exposición al calor podría comprometer las capacidades fisiológicas de pequeños paseriformes mediterráneos. En el Capítulo 2, determinamos los límites de tolerancia al calor (HTL) y otros rasgos termorreguladores clave – como la temperatura corporal (T_b), la tasa metabólica de reposo (RMR), la pérdida de agua por evaporación (EWL) y la eficiencia del enfriamiento por evaporación a altas temperaturas – en pequeños paseriformes mediterráneos, y empleamos estos datos para estimar sus vulnerabilidades actuales y futuras frente a la exposición al calor; para hacer esto, evaluamos tanto los efectos crónicos (días por encima de la temperatura crítica superior, T_{uc}) como severos (riesgo de mortalidad directa por hipertermia o deshidratación letal) derivados de la exposición a altas temperaturas predichas por modelos climáticos considerando dos escenarios de calentamiento climático (RCP4.5 y RCP8.5) a lo largo de Extremadura. Encontramos que los paseriformes mediterráneos muestran límites de tolerancia al calor relativamente bajos y eficiencias de enfriamiento por evaporación limitadas. Por tanto, los pequeños paseriformes mediterráneos residentes están actualmente expuestos a experimentar efectos adversos crónicos sobre su eficacia biológica derivados de la exposición al calor. Los escenarios futuros de calentamiento exacerbarán sus vulnerabilidades, especialmente en el caso de las especies más pequeñas, las cuales experimentarán riesgo moderado de deshidratación letal (pérdida de un 15% de la masa corporal en menos de 5 horas) en días extremadamente calurosos a finales de este siglo bajo el escenario de

calentamiento más severo. En general, nuestros resultados indican que los pequeños paseriformes mediterráneos son particularmente vulnerables al calentamiento del clima.

Comprender cómo las respuestas fisiológicas termorreguladoras al calor pueden variar entre las poblaciones de una especie es crucial para predecir mejor la persistencia y la vulnerabilidad actual y futura de las especies. Sin embargo, pocos estudios han evaluado cómo la tolerancia al calor y la eficiencia de enfriamiento por evaporación difieren entre poblaciones de aves a lo largo de un gradiente climático, y ninguno de ellos se ha centrado en especies de latitudes templadas. De acuerdo con la hipótesis de variabilidad climática, los individuos de poblaciones que experimentan condiciones termales más variables mostrarán una mayor plasticidad fenotípica en sus rasgos termorreguladores que aquellos de poblaciones termalmente más estables. En el Capítulo 3, exploramos las diferencias estacionales e inter-poblacionales en la tolerancia al calor y la eficiencia de enfriamiento por evaporación en dos poblaciones mediterráneas de carbonero común *Parus major* que experimentan diferentes regímenes termales. Globalmente, mostramos que los carboneros de un sitio más caluroso y termalmente heterogéneo mostraron mayor eficiencia de enfriamiento por evaporación (esto es, mayores pendientes por encima del punto de inflexión y mayores valores máximos) que aquellos del sitio de montaña con temperaturas más suaves y mayor estabilidad termal. Además, las aves del sitio más caluroso aumentaron su HTL y su eficiencia de enfriamiento por evaporación durante el verano mediante cambios en su balance termal (disminuyendo sus cargas de calor internas mediante reducciones en su T_b y RMR), mientras que los del sitio más suave y termalmente estable no mostraron un ajuste estacional en estos rasgos. En términos generales, estos hallazgos soportan las asunciones de la hipótesis de variabilidad climática. De acuerdo con esto, las aves del sitio más caluroso mostraron una mayor flexibilidad estacional en sus rasgos termorreguladores por encima de la zona termoneutral que aquellas de del sitio de montaña más suave y termalmente estable. Nuestros resultados subrayan la importancia de incorporar la variación inter-poblacional en los rasgos termorreguladores a la hora de predecir la vulnerabilidad de las especies frente a las altas temperaturas.

Junto con las respuestas fisiológicas, las aves hacen frente a los extremos de calor mediante mecanismos comportamentales. De hecho, los comportamientos de disipación de calor (por ejemplo, el cese o reducción de la actividad, la búsqueda de sombra, o cambios en las posturas corporales) pueden amortiguar los impactos fisiológicos de las temperaturas elevadas. Por tanto, adquirir conocimiento acerca de los principales determinantes de estos comportamientos y cómo afectan al balance de calor de un animal es clave en el contexto del calentamiento climático. Esto es particularmente importante para especies que típicamente se alimentan y crían en paisajes abiertos y cálidos, donde el estrés por calor puede ser problemático. Las cigüeñas son famosas por defecar en sus patas cuando experimentan estrés por calor – un mecanismo de enfriamiento conocido como 'urohidrosis'. A pesar de su potencial importancia en el mantenimiento del balance de calor, los principales determinantes de la urohidrosis, así como su contribución energética han sido en gran parte pasados por alto. En el Capítulo 4, identificamos los principales determinantes ambientales, ecológicos, morfológicos y latitudinales de la urohidrosis en todas las especies de cigüeña. Para hacer esto, combinamos datos de urohidrosis procedentes de fuentes digitales (fotografías y vídeos) con datos microclimáticos y ecológicos y analizamos esta base de datos dentro de un marco filogenético. Encontramos que el empleo de urohidrosis está principalmente desencadenado por altas temperaturas ambientales, radiación solar y humedad, así como por bajas velocidades del viento. Además, el uso de urohidrosis fue más prevalente en especies que se alimentan en paisajes abiertos que en aquellas que se alimentan principalmente en cuerpos de agua. Curiosamente, hallamos una variación interespecífica sustancial en los umbrales de temperatura para la prevalencia del uso de urohidrosis, lo cual apunta hacia diferentes vulnerabilidades al calor entre especies. Tras confirmar que la urohidrosis es una respuesta adaptativa a la vida en hábitat abiertos y calurosos, cuantificamos su efecto de enfriamiento en cigüeñas blancas *Ciconia ciconia* reproductoras en libertad mediante el empleo de imágenes térmicas (Capítulo 5). El análisis de las imágenes térmicas reveló que la urohidrosis puede disminuir la temperatura superficial de la pata hasta 6 ºC (4.4. ± 1.04 ºC), pero su efecto de enfriamiento fue de corta duración (~2.5 minutos) y disminuyó con el tiempo. De hecho, registramos individuos realizando hasta 11 eventos de urohidrosis en alrededor de una hora, lo cual puede representar una cantidad significativa de pérdida de calor (equivalente a un 4% de la tasa metabólica de campo diaria en una cigüeña adulta). En general, nuestros resultados mostraron que esta respuesta comportamental a priori ‘barata’ ayuda a prevenir el sobrecalentamiento en cigüeñas y posiblemente en otras aves zancudas que utilizan este comportamiento con frecuencia. Aunque generalmente se ha pasado por alto, las respuestas comportamentales deben ser explícitamente integradas en los marcos para predecir la vulnerabilidad de las aves.

Los resultados mostrados en esta tesis resaltan la importancia de considerar la variación inter-específica e inter-poblacional en las respuestas fisiológicas y comportamentales al predecir la persistencia y vulnerabilidad futura de especies o poblaciones bajo futuros escenarios de calentamiento climático. Sin embargo, hasta la fecha la mayoría de modelos predictivos han pasado por alto la potencial variation en este tipo de respuestas al calor entre especies o poblaciones, limitando tanto su poder predictivo como su precisión. Mediante el uso de herramientas de modelado mecanicista recientemente desarrolladas que permiten integrar datos comportamentales y fisiológicos en las predicciones de cambio climático para especies individuales, podremos obtener mejore predicciones e informar mejor futuras estrategias de conservación de las especies. En esta línea, la creciente cantidad de fuentes de datos online nos ofrece la oportunidad de complementar las aproximaciones tradicionales para la adquisición de datos para obtener conocimiento acerca de las respuestas termorreguladoras comportamentales en aves salvajes. Para concluir, subrayamos la utilidad de técnicas modernas, no invasivas, para monitorear la temperatura corporal superficial (tales como la fotografía térmica de infrarrojos) y obtener de este modo conocimiento sobre el papel termorregulador de ciertas partes corporales desnudas (como el pico y las patas), las cuales pueden actuar como “ventanas termales” que promueven la pérdida pasiva de calor.

List of abbreviations

BMR – Basal metabolic rate

CI – Confidence interval

EHL – Evaporative heat loss

EHL/MHP – Evaporative cooling efficiency

EWL – Evaporative water loss

GLMM – Generalised linear mixed effects model

HTL – Heat tolerance limit

M_b – Body mass

MHP – Metabolic heat production

PIT – Passive integrated transponder

RMR – Resting metabolic rate

T_a – Air temperature

T_b – Body temperature

T_{lc} – Lower critical temperature

T_{uc} – Upper critical temperature

TNZ – Thermoneutral zone

VH2O – Water production

VIF – Variance inflation factor

VO2 – Oxygen consumption

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1

General introduction

Global climate is warming faster than ever, primary due to human activity. Mean surface temperature has increased by 1.1 ºC since pre-industrial values because of the huge increase in greenhouse gases emissions (IPCC 2021). Besides, projected climate change scenarios (from the most optimistic to the most pessimistic) forecast additional increases in mean surface temperature in the future decades (IPCC 2021) (Figure 1). For instance, even if humanity achieve zero greenhouse gasses emissions to the middle of the century by transitioning to 'clean' green energy (Scenario SSP1-1.9), mean temperature will rise 1.5 ºC further to the end of 21st century (IPCC 2021). The worst-case scenario (SSP5-8.5), which implies no reduction in greenhouse gasses emissions, predicts that by 2100 mean temperature could increase by up to 5 ºC (IPCC 2021).

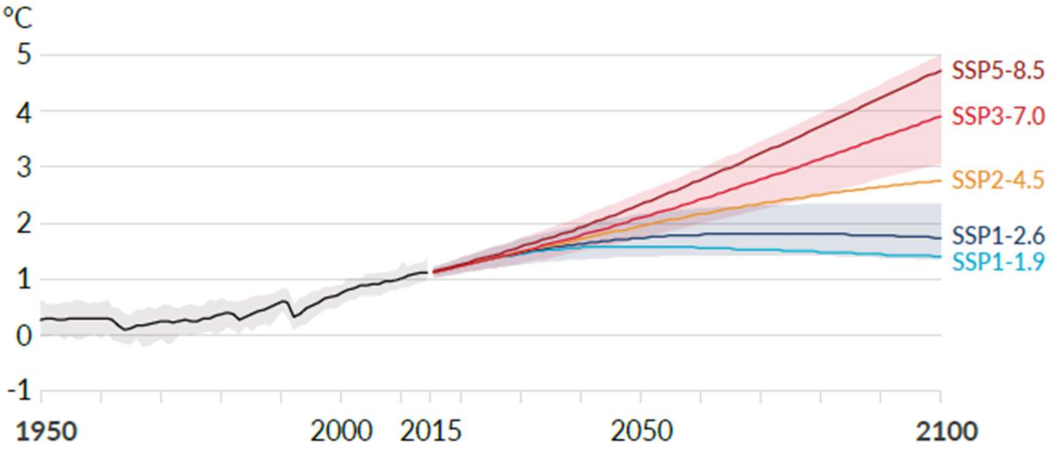


Figure 1. Projected global surface temperature change relative to the reference period (1850-1900) for each climate change scenario (source IPCC 2021).

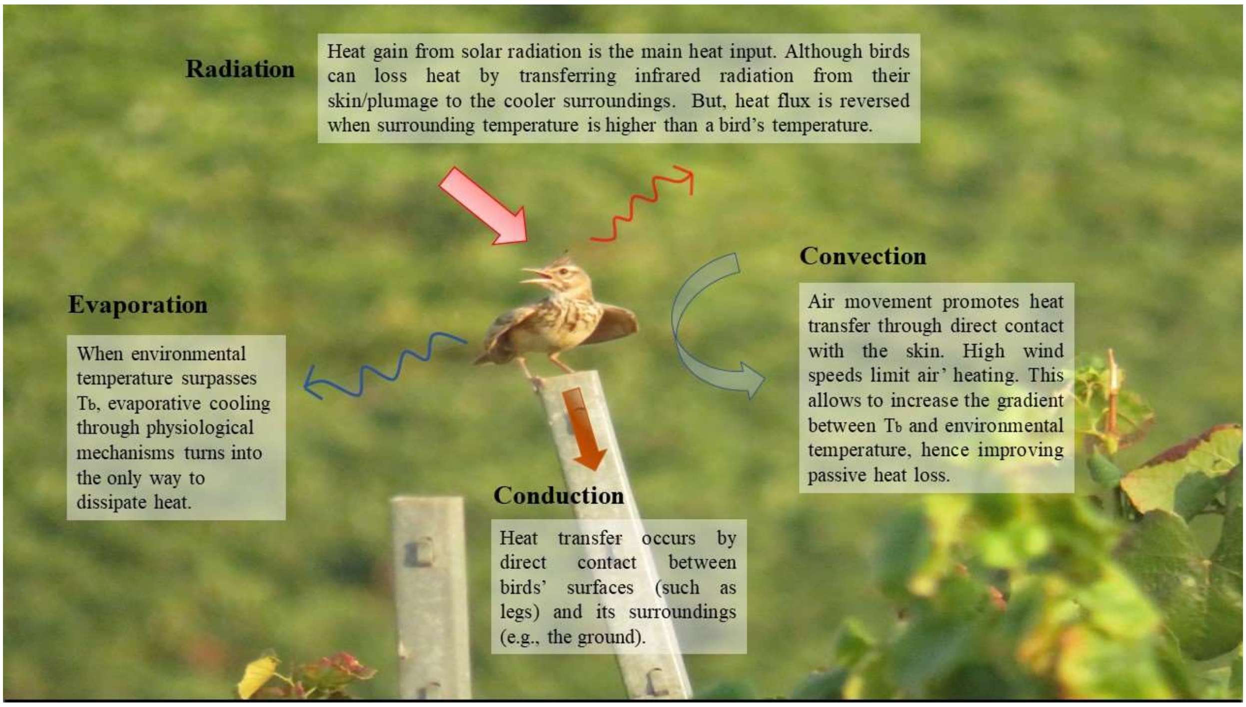
Additionally, global warming brings an increase in the occurrence and magnitude of climatic extreme events like heat waves or heavy droughts, which will be more frequent, intense, and long-lasting (IPCC 2021). This elevated exposition to heat extremes are currently resulting in temperatures that threaten biodiversity, with many animals experiencing heat-related mass mortality events across the globe (Welbergen 2008; McKechnie & Wolf 2010; Catry et al. 2015; Harvell et al. 2019; Genin et al. 2020; McKechnie et al. 2021). As climate change progresses, these events will become one of the greatest impacts of climate change on life (Williams et al. 2016; Stillman 2019).

In this context, understanding how extreme climatic events (such as heatwaves) could impact biodiversity is essential. To date, however, most studies have focused on assessing how increasing exposure to heat affects animal species from deserts and arid-zone regions (i.e., McKechnie & Wolf 2010; Whitfield et al. 2015; Eto, Withers & Cooper 2017; Zhang et al. 2018; Hoole et al. 2019; Czenze et al. 2020; van Jaarsveld et al. 2021; Czenze et al. 2022a, 2022b) as well as from the tropics (i.e., Tweksbury et al. 2008; Huey et al. 2009; Diamond et al. 2012; Czenze & Dunbar 2020; Pollock et al. 2021; Mi et al. 2022). By contrast, few studies have evaluated how rising global temperatures impacts animals from temperate (i.e., Diamond et al. 2012; Catry et al. 2015; Nord & Nilsson 2019; Pollock et al. 2021; Mi et al. 2022) or subpolar regions (Oswald et al. 2008; Molnar et al. 2011; Choy et al. 2021; O'Connor et al. 2021, 2022). This is particularly worrying as both temperate and (sub)arctic ecosystems have been affected in recent years by rapid climate-driven changes at multiple trophic levels. For instance, a recent study indicates that Arctic warming is already disrupting the breeding performance of cold-specialist birds (O'Connor et al. 2022). It is therefore important to determine how exposure to heat extremes can affect species that inhabit regions in which warming is predicted to be greater, such as temperate and polar regions in the Northern Hemisphere (IPCC 2021). Among temperate regions, the Mediterranean Basin has recently emerged as a climate warming hotspot with increasingly more severe, intense and long-lasting heatwaves events (IPCC 2021). Although Mediterranean species have adapted to experience the hottest temperatures during the driest part of the year (Cuttelod et al. 2008), we do not know whether and how these species will deal with rising maximum temperatures and heat stress.

Understanding how physiological and behavioural responses to climate warming vary across taxa is one of the most important challenges for biodiversity conservation, as these are crucial to predict the future persistence and distribution of species (Buckley & Kingsolver 2012; Vasseur et al. 2014; Bozinovic & Pörtner 2015; Moyer-Horner et al. 2015). An increasing number of studies highlights the importance of considering both physiological and behavioural traits when modelling species' sensitivity to warming, as this can increase their accuracy and predictive power (Moyer-Horner et al. 2015; Buckley et al. 2018; Conradie et al. 2020; Enriquez-Urzelai et al. 2020).

Among animals, small endotherms such as birds are particularly susceptible to the effects of high temperatures as their greater surface area-to-volume ratio makes them more prone to accumulate heat faster than larger-sized endotherms (Hill 1980). Besides, unlike most small mammals (which are mostly nocturnal and during the day rest in burrows), their diurnal and non-fossorial habits often prevent birds from switching activity to cooler times of the day and thus reduce heat stress. These features, along with high body temperatures (T_b), metabolic rates and evaporative water loss (EWL) rates, make birds one of the most sensitive taxa to climate warming (Albright et al. 2017).

Therefore, birds are a good model to study physiological and behavioural thermoregulatory responses to warming, including associated increases in maximum temperatures and heat extremes. Stark differences in body sizes, ecology and life history certainly make some taxa more suitable than others to study different types of responses to heat. For instance, relatively small body sizes and high mass-specific metabolic and EWL rates make songbirds an adequate group to study the physiological impacts of heat extremes, as they are a priori more sensitive to high temperatures than other bird groups (McKechnie et al. 2021). On the other hand, larger and thus more easily detectable birds, such as storks, which live and breed in open landscapes where they experience high environmental temperatures are suitable target species to evaluate behavioural thermoregulatory responses. Birds, like other endotherms, respond to temperature in a multitude of ways, and over multiple time scales (Tattersall et al. 2012; McKechnie 2022). Below we summarise the most relevant physiological and behavioural responses to heat in birds.



Radiation

Heat gain from solar radiation is the main heat input. Although birds can loss heat by transferring infrared radiation from their skin/plumage to the cooler surroundings. But, heat flux is reversed when surrounding temperature is higher than a bird's temperature.

Evaporation

When environmental temperature surpasses T_b, evaporative cooling through physiological mechanisms turns into the only way to dissipate heat.

Convection

Air movement promotes heat transfer through direct contact with the skin. High wind speeds limit air' heating. This allows to increase the gradient between T_b and environmental temperature, hence improving passive heat loss.

Conduction

Heat transfer occurs by direct contact between birds' surfaces (such as legs) and its surroundings (e.g., the ground).

Figure 2. Schematic representation of the main ways of heat exchange (inputs and outputs) in a bird. To maintain thermal balance, heat gain must be equal to heat loss. Photograph by Julián Cabello.

Physiological thermoregulatory responses to heat in birds

As endotherms, birds must maintain a relatively stable and safe T_b. This is accomplished through physiological and behavioural adjustments that allow them to maintain their thermal balance, which is governed by heat fluxes between the bird and its environment (Figure 2). Within the thermoneutral zone (TNZ) – the range of air temperatures (T_a) at which an endotherm maintains basal metabolic rate (BMR) and normothermic T_b (40-42 ºC) with minimum energetic costs (McNab 2002) – birds maintain their thermal homeostasis by controlling passive heat loss (e.g., trough convective or conductive ways). TNZ is delimited by the lower critical temperature (T_{lc}) and the upper critical temperature (T_{uc}). When these thresholds are surpassed birds must allocate energetic resources to trigger metabolic heat production (and thus enhance cold tolerance below T_{lc}) or evaporative heat loss (and thus enhance heat tolerance above T_{uc}) (McNab 2002) (Figure 3).

As T_a rises and achieves values near or above normothermic T_b, birds can elude lethal hyperthermia ($T_b \approx$ 46-49 ºC; Dawson 1954; Marder & Arieli 1982; Freeman et al. 2020) through substantial increases in EWL (Calder & Schmidt-Nielsen 1966; Dawson 1982). The main ways by which EWL increases – namely panting, gular fluttering, and cutaneous EWL – differ among bird groups. Panting is the main physiological response to heat in songbirds (order Passeriformes); it promotes EWL through the mucous surfaces of their respiratory apparatus by augmenting respiratory rates as T_a increases (Dawson 1982). By contrast, in birds belonging to the orders Pterocliformes, Charadriiformes, Pelecaniformes, Strigiformes and Caprimulgiformes, heat balance is primarily maintained through gular fluttering, i.e., the resonant movement of the hyoid apparatus (Dawson 1982; O'Connor et al. 2017; Cook et al. 2020; Czenze et al. 2022a). Finally, doves and pigeons (order Columbiformes) primarily rely on cutaneous EWL (Webster & Bernstein 1987; Marder & Arieli 1988), which involves increases in trans-cutaneous evaporation regulated by changes in peripheral microcirculation in the short term (Ophir et al. 2002) or changes in epidermal lipid composition if sustained for long periods (Haugen et al. 2003; Muñoz-García et al. 2008). These three ways of evaporative heat dissipation differ in their metabolic costs, making some bird orders more sensitive to heat than others (McKechnie, Gerson & Wolf 2021). Because of the increased pectoralis muscle activity resulting from augmented respiratory rates, panting notably increases metabolic heat production (Bartholomew et al. 1968; Lasiewski & Seymour 1972; Whitfield et al. 2015; McKechnie et al. 2017; O'Connor et al. 2021). On the other hand, gular fluttering and cutaneous EWL increase total EWL with no or negligible increase in metabolic rate (Calder & Schmidt-Nielsen 1966; Webster & Bernstein 1987; McKechnie et al. 2016a; O'Connor et al. 2017; Czenze et al. 2022a).

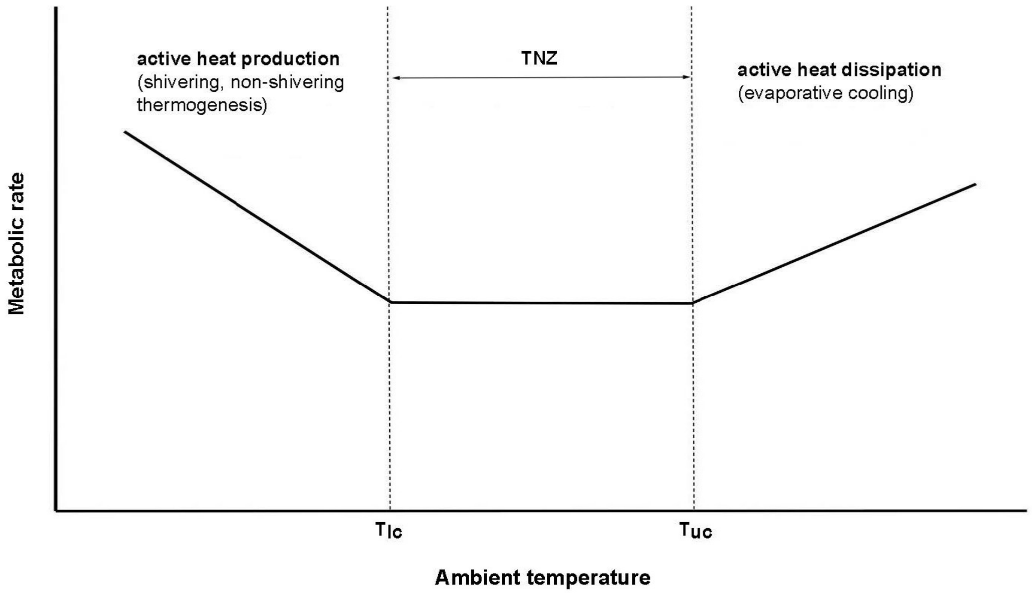


Figure 3. Schematic representation of an endotherm's thermoneutral zone (TNZ). Between upper (T_{uc}) and lower critical temperature (T_{lc}) an endotherm shows neglible thermoregulatory costs.

Another common response to maintain thermal balance at high temperatures (especially among songbirds) is to let T_b rise above normothermic values (2-3 ºC) in a controlled fashion as T_a increases, i.e., facultative hyperthermia (Gerson et al. 2019). In a comparative study comprising 33 arid-zone species from 9 orders, Gerson et al. (2019) found that facultative hyperthermia serves as water conservation strategy, but its benefits vary as a function of species body mass. Specifically, small-sized birds (such as songbirds) benefit from facultative hyperthermia by reducing EWL requirements in two ways as (i) permits to maintain a favourable thermal gradient ($T_b > T_a$), which favours passive heat loss from the body to the environment and (ii) can also be beneficial along unfavourable ($T_a > T_b$) thermal gradients, as it reduces heat gains from the environment by reducing this gradient (i.e., the greater the gradient, the faster the heat transfer) (Tieleman & Williams 1999; Gerson et al. 2019). On the other hand, in larger species (such as sandgrouses or quails) facultative hyperthermia reduces EWL by promoting heat storage; that is, the extra heat can be dissipated when conditions are more favourable (e.g., lower T_a) (Gerson et al. 2019).

In the light of climate warming, assessing how bird species from different regions and taxonomic groups deal with heat is crucial to better understand their current and future vulnerabilities to heat extremes. Over the last decade, several studies employing the same methodology to measure heat tolerance in birds (**see Box 1**) have revealed substantial differences in heat tolerance limits (HTL, the maximum T_a tolerated before the onset of severe heat stress). Typically, desert and arid-zone species show greater HTL and evaporative cooling efficiencies (defined as the ratio between evaporative heat loss and metabolic heat production) than birds from temperate, tropical or subpolar regions (Whitfield et al. 2015; McKechnie et al. 2016; McKechnie et al. 2017; O'Connor et al. 2017; Smith et al. 2017; Smit et al. 2018; Kemp & McKechnie 2019; Czenze et al. 2020; Pollock et al. 2020; Choy et al. 2021; O'Connor et al. 2021; Czenze et al. 2022a). Songbirds generally achieve lower evaporative cooling efficiencies and HTL than other avian orders that rely on more efficient heat dissipation mechanisms such as gular fluttering or cutaneous EWL (reviewed in McKechnie, Gerson and Wolf 2021). Indeed, current evidence suggest that songbirds are among the most sensitive avian taxa to heat.

Despite the growing body of literature about birds' thermoregulation in the heat, most studies have focused on arid-zone and desert species, with only a handful of papers reporting data from temperate, tropical or polar region species (Pollock et al. 2020; Choy et al. 2021; O'Connor et al. 2021, 2022). In this context, special attention should be paid to characterize how species inhabiting climate warming hotspots cope with heat at present to better predict their vulnerability and future persistence across these regions (**Chapter 2**).

Intraspecific variation in thermoregulatory traits

Often, populations within species are exposed to different climatic conditions and thus experience contrasting environmental pressures which, in turn, can vary seasonally (e.g., Cavieres & Sabat 2008; Noakes et al. 2016). This can lead to different physiological adaptations, including those for coping with heat stress (Somero 2010). Some authors have suggested plasticity in physiological traits as pivotal for species or populations persistence under global warming (Boyles et al. 2011). Indeed, differences in thermal physiology among populations could lead to distinct heat tolerance, and thus different population vulnerabilities to current and projected heat extremes. Nonetheless, intraspecific comparisons in responses to heat have been largely overlooked when forecasting future persistence and vulnerability of species;

instead, these studies have commonly assumed that thermoregulatory traits are rather fixed (e.g., the HTL or the T_{uc} of a population represent those of the whole species) (e.g., Kahliq et al. 2014; Conradie et al. 2019; 2020).

Box 1. Standardized protocol to determine Heat Tolerance Limits (HTL)

Earlier studies on bird heat tolerance (e.g., Lasiewski et al. 1966; Hinds & Calder 1973; Wolf & Walsberg 1996a) employed different protocols, making comparisons between species difficult. Several of these studies measured heat tolerance during the resting phase of individuals, and in most of them the temperature profiles (T_a setpoints and their duration) to which birds were exposed were unspecified. Moreover, relative humidity inside metabolic chambers usually varied between 20-60%, which can affect thermoregulatory performance by hampering EWL (see Gerson et al. 2014; Van Dyk et al. 2019). To solve these issues, Whitfield et al. (2015) designed a standardized protocol to measure HTL under controlled-laboratory conditions, which can be summarized as follows:

- heat tolerance trials are performed during the active phase of the birds, and in a post-absorptive state.

- low ambient humidity (< 1 kPa) is kept inside the metabolic chamber by using relatively high flow rates of incoming air.

- metabolic heat production (resting metabolic rate, RMR) and evaporative heat dissipation (EWL) are measured through open-flow respirometry (see photo below) at different T_a, i.e., by exposing birds to a stepped profile of T_a (from values within their TNZ to the highest T_a tolerated). Short et al. (2022) has shown that stepped profiles yield similar results to steady-state profiles where birds are exposed to a single T_a for longer periods (>1–2 h).

- To limit the risk of lethal hyperthermia, T_b is measured throughout the trials by using passive integrated transponders (PIT-tags), as can be seen in the back of the crested lark *Galerida cristata* (see bottom-right inset below):

- HTL is considered the T_a at which a bird show (1) continuous scape behaviour, (2) losing of righting response or balance, (3) sudden decrease in RMR or EWL, or (4) T_b reaching 45 ºC or, if near this value, increasing by more than 0.1 ºC min^{-1}.

There is now ample evidence that birds can seasonally and reversibly adjust their thermoregulatory traits — i.e., they exhibit phenotypic flexibility (sensu Piersma & Drent 2003). During decades, several studies have investigated how temperate and tropical species seasonally adjust their thermoregulatory machinery in response to winter acclimatization (e.g., Liknes et al. 2002; Swanson 2010; McKechnie, Noakes & Smit 2015; Pollock et al. 2019). Generally, temperate birds tend to maximize thermogenic capacity (e.g., through increases in metabolic rates) to enhance cold tolerance (e.g., Liknes et al. 2002; Swanson 2010; Oboikovitz & Swanson 2021), while more variable patterns emerge among tropical birds (e.g., Smit & McKechnie 2010; Pollock et al. 2019; Noakes & McKechnie 2020). However, only a handful of studies have explored how birds seasonally tune their thermal physiology to maximize heat tolerance and evaporative cooling efficiency during summer (Tieleman et al 2002b; Noakes et al. 2016; O'Connor et al. 2017; Oswald, Lee & Smit 2018; Playà-Montmany 2022). Overall, these results have revealed a greater acclimation capacity to heat than previously thought (Glanville et al. 2012).

Despite ongoing climate warming, yet little is known about inter-population variation in thermoregulatory traits in response to heat across species occupying climatic gradients (Trost 1972; Noakes et al. 2016; O'Connor et al. 2017; Noakes & McKechnie 2019). Previous studies showed that populations from hotter and more arid sites display lower EWL and RMR, and achieve higher evaporative cooling efficiencies and HTL than those from milder and mesic sites (Trost 1972; Noakes et al. 2016). This agrees with the climatic variability hypothesis (Stevens 1989), which posits that the thermal tolerance of an organism and its flexibility should match the climatic variability it experiences (Stevens 1989; Somero 2010; Pollock et al. 2019). In support of this expectation, Pollock et al. (2019) found that North American temperate species show a greater seasonal flexibility in their TNZ breadth than tropical species. However, very few studies have determined seasonal and between-population variation in thermoregulatory traits at high temperatures in birds (Noakes et al. 2016; O'Connor et al. 2017; Oswald et al. 2018; Noakes & McKechnie 2019), and these found marked variation in physiological responses to heat. Overall, they found enhanced heat tolerance during summer, with birds' populations from hotter sites dealing better with heat (e.g., displaying greater HTL and evaporative cooling capacities) than birds from milder sites (Trost 1972; Noakes et al. 2016; O'Connor et al. 2017; Oswald et al. 2018).

To our knowledge, only a single study has assessed how heat tolerance and evaporative cooling vary seasonally in a non-arid songbird, the Cape rockjumper *Chaetops frenatus* (a mountain relict species which inhabit a Mediterranean-like climate) (Oswald et al. 2018). Thus, future work is needed to explore how heat tolerance and evaporative cooling efficiency vary among different populations of temperate birds, and how these thermoregulatory traits could be seasonally adjusted among that populations (**Chapter 3**).

Behavioural thermoregulatory responses to heat in birds

In addition to physiological mechanisms, birds exhibit a broad range of behavioural thermoregulatory responses to heat, from postural adjustments to ptilomotor responses, ‘watering’ behaviours, shade seeking or reducing activity levels during the hottest part of the day (Bartholomew 1966; Bryant 1983; Amat & Masero 2004; Du Plessis et al. 2012; Cunningham et al. 2015; Smit et al. 2016; Bladon et al. 2019; Sharpe et al. 2022). These behavioural responses can maximize passive heat dissipation, enhancing convective and conductive heat loss to the environment.

In order to save water and energy, birds likely rely first on behavioural adjustments as they are usually less expensive, only resorting physiological cooling mechanisms when those responses are insufficient to maintain thermal balance (McNab 2002). Furthermore, birds can combine active evaporative heat loss (through panting, gular fluttering, or cutaneous EWL) with heat dissipation behaviours to maximize total heat dissipation (e.g., Wolf & Walsberg 1996; Cook et al. 2020). As postulated by the heat dissipation limit theory (Speakman & Krol 2010), this could have important implications for daily energy budgets since a greater heat dissipation capacity in hot environments means that individuals could spend more energy in other activities different from thermoregulation, such as foraging, mating, or brooding (reviewed in Cunningham et al. 2021). It is important to note, however, that thermoregulatory behaviours sustained for a long period can also impose trade-offs related to opportunity-costs, resulting in reduced fitness (e.g., Du Plessis et al. 2012; Cunningham et al. 2015; Van de Ven, McKechnie & Cunningham 2019; Oswald et al. 2021). For instance, repeated exposure to high ambient temperatures (above 35 ºC) turns into impeded body mass maintenance in Pied babblers *Turdoides bicolor*; this is because increased demands for thermoregulation (e.g., panting, wing-spreading behaviour) result in reduced foraging time (Du Plessis et al. 2012). Likewise, the time spent panting by adult Cape rockjumpers limits their parental effort, resulting in reduced nestling body condition (Oswald et al. 2021).

Storks (Family Ciconiidae) usually forage and breed in warm and open landscapes in which they are often exposed to high operative temperatures (*sensu* Baken 1976; Robinson et al. 1976). Thus, they are prone to experience heat stress. Not surprisingly, this group of birds show one of the wider arrays of behavioural responses to heat. Storks display several wing-spreading postures to increase dry convective heat loss by exposing their thinly feathered underwings to the wind (Kahl 1971; Hancock, Kushlan & Kahl 1992) (Figure 4). Usually, these postures are combined with changes in orientation with respect to the sun (to reduce net radiation loads and thus heat gain) or with ptiloerection (which creates an air layer between the skin and the environment that hamper heat transfer from the hot environment to the body) (Clark 1969; Kahl 1971). Besides, storks – as well as New World vultures, condors, gannets, and boobies – often excrete on their legs when heat exposed, a behaviour known as urohidrosis (Kahl 1963; Hatch 1970; Cooper & Sigfried 1976; Thomas 1984; Hancock, Kushlan & Kahl 1992; Townsend et al. 2002; Finkelstein et al. 2015; Graves 2019). This behaviour is similar to other analogous cooling mechanisms by evaporation of body fluids (such as saliva, mucous, urine or faeces) employed by a number of terrestrial and marine vertebrates to cope with heat stress (Hainsworth, 1967; Gentry, 1973; Sturbaum & Riedesel 1977; Marder et al. 1983; Needham & Dawson 1983; Van Vuuren, Kemp & McKechnie 2020). Unlike mammals, birds lack sweat glands, so urohidrosis can be utilized as an alternative cooling mechanism.

Figure 4. Examples of postural thermoregulatory responses in storks: (A) delta-posture in a Yellow-billed stork *Mycteria ibis*, (B) wing-spreading posture in a Marabou *Leptoptilos crumenifer*, (C) wing-drooping combined with ptiloerection in an African openbill *Anastomus lamelligerus*, (D) full-spread wing posture in an African openbill, and (E) wing-drooping combined with ptiloerection and panting in an Abdim' stork *Ciconia abdimii*. Photos from panel A-E (Pam Rasmussen, ML67889031; Greg Baker, ML257591571; Ron Hess, ML310078031; Niall Perris, ML261573231; Thomas Galewski, ML402584621) were obtained from Macaulay Library at the Cornell Lab of Ornithology.

Storks' legs are well vascularized and possess a simple rete that permit counter-current heat exchange by regulating the blood flow in response to environmental temperature (Figure 5) (Kahl 1963; Steen & Steen 1965). Thus, legs can function as thermal radiators and by defecating on them storks can enhance evaporative heat loss (Kahl 1963). After simulating urohidrosis in captive Wood storks *Mycteria americana* exposed to high temperatures, Kahl (1963) first found that the evaporation of watery excreta resulted in heat dissipation from the skin surface, which can lower T_b and thus prevent hyperthermia. However, little attention has been paid to the topic since Kahl's pioneering experiments. Thus far, the contribution of urohidrosis to heat balance as well as its underlying determinants remain unexplored in the wild. Kahl proposed that urohidrosis is mainly triggered by high environmental temperature but overlooked the potential effect of other variables that influence an animal's heat balance, such as wind speed, solar radiation, or ambient humidity. Moreover, he argued that urohidrosis is an adaptive response to warm and open habitats with regular access to water; and proposed that this behaviour should be more prevalent at low latitudes as storks living in the tropics are more often exposed to heat than those occupying higher latitudes. Nonetheless, no further studies have investigated how environmental variables, latitude, dependency on waterbodies to forage, plumage colour, or body size determine the use of urohidrosis across stork species (**Chapter 4**). Recording and predicting behavioural responses to high temperatures is crucial to understand how and when the species might respond to climate change. Yet, comparative studies of the ecological determinants of behavioural thermoregulation are rare but important for increasing our understanding of the range limitations of species (Smit et al. 2016; Thompson et al. 2018; Pattinson et al. 2020).

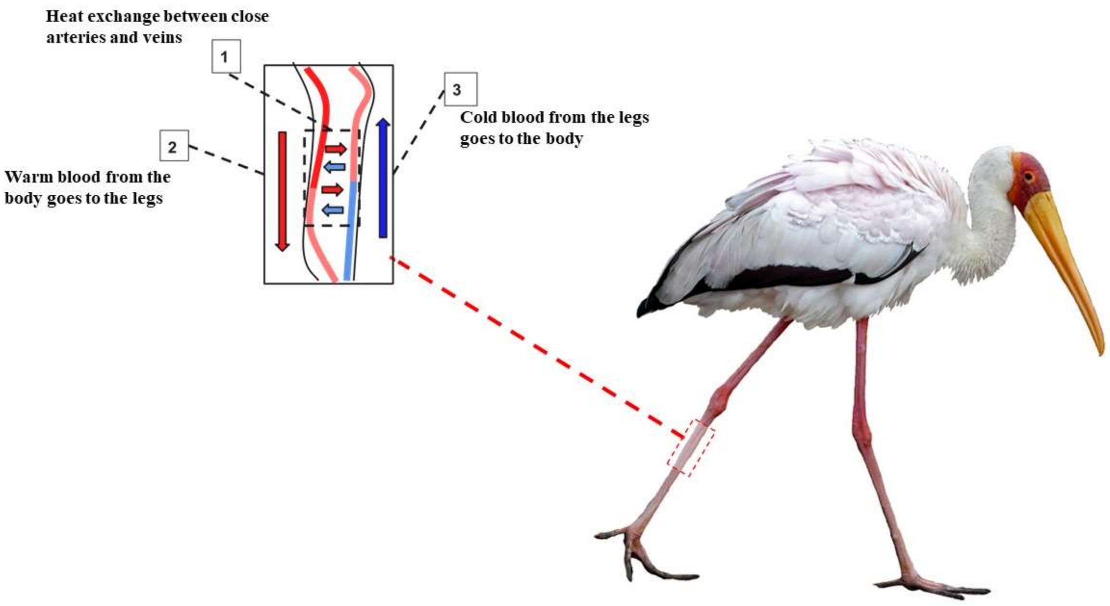


Figure 5. Example of how simple rete works in storks. Painted stork *Mycteria ibis* and schematic representation of the rete were extracted from Wikipedia (license CCBY-4.0).

Furthermore, the thermoregulatory significance of urohidrosis has been only indirectly estimated in captive, immature Wood storks (Kahl 1963). Therefore, it is unknown whether and to what extent urohidrosis lowers legs surface temperature as well as its contribution to heat exchange in free-ranging birds. Additionally, it remains to be tested if the magnitude of the cooling effect of urohidrosis vary between adults and nestlings. The recent development of infrared thermal imaging allows for non-invasive measurements of surface temperature (and thus heat exchange) on conscious, non-instrumented animals exhibiting spontaneous, normal behaviours (e.g., Tattersall et al. 2016; Galván et al. 2017; Eastick et al. 2019; Schraft et al. 2019; van Vuuren et al. 2020). Despite some for methodological considerations (**see Box 2**), thermal imaging therefore provides a powerful, non-invasive tool for quantifying the thermoregulatory significance of urohidrosis and other thermoregulatory behaviours in wild birds (**Chapter 5**).

Box 2. Methodological considerations in thermography

Infrared thermal imaging (or thermography) is a valuable tool to obtain insights into thermoregulation, as allows for the estimation of animal surface temperatures and thus the thermal gradient between an animal's body surface and its surrounding environment. It thus allows us to assess how certain body parts contribute to total heat exchange (e.g., Tattersall et al. 2009; McCafferty et al. 2011). Yet, we must consider the following potential sources of error and uncertainty in the measures (Playà-Montmany & Tattersall 2021):

1. There is a series of features related to the properties of the animal (e.g., physiological and morphological variation in insulation, thermal conductance, or internal body temperature), the environmental conditions (such as ambient temperature, solar radiation or wind speed, which can influence body surface temperature trough convection, conduction, or radiation) or the emissivity of the biological surfaces (commonly assumed equal to 0.95) which are potential sources of error. All of them are difficult to control for in the field.
2. During image capture, we can control source of error such as angle of incidence (as this affects surface emissivity, and hence surface temperature) and distance to the object (as atmospheric humidity absorbs infrared radiation attenuating the emitted radiation by the animal). Thus, we must take pictures with and angle of incidence < 50º (whenever possible with the thermal camera placed perpendicular to the object of interest; see photo below) and at a constant distance which limits the influence of atmospheric water vapour while obtaining enough pixels of the surfaces of interest. These factors can be difficult to control especially in wild animals.

Thesis objectives

The main goal of this thesis is to gain a deeper understanding of the physiological and behavioural thermoregulatory responses used by birds to cope with heat extremes. To do so, this thesis is divided in four specific objectives:

Objective 1: To determine heat tolerance limits and evaporative cooling efficiency in small-size Mediterranean songbirds in South-Western Iberia, as well as to assess their current and future vulnerabilities to heat extremes (Chapter 2).

Objective 2: To evaluate intraspecific differences in heat tolerance limits and seasonal variation in thermoregulatory traits in response to heat in two Mediterranean populations of Great tit *Parus major* that experience different climatic regimes (Chapter 3).

Objective 3: To assess the major climatic, geographical, ecological, and morphological determinants of urohidrosis use across all extant stork species (Chapter 4).

Objective 4: To investigate the thermoregulatory significance of urohidrosis in free-ranging, breeding White storks *Ciconia ciconia* using thermal imaging (Chapter 5).

References

Amat, J.A., Masero, J.A. (2004). How kentish plovers, *Charadrius alexandrinus*, cope with heat stress during incubation. *Behav. Ecol. Sociobiol.* **56**(1), 26–33.

Arad, Z., Marder, J. (1982). Strain differences in heat resistance to acute heat stress, between the bedouin desert fowl, the white leghorn and their crossbreeds. *Comp. Biochem. Physiol. A Physiol.* **72**, 191-193.

Bakken, G.S. (1976). A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *J.Theor. Biol.* **60**, 337-384.

Bartholomew, G.A. (1966). The role of behavior in the temperature regulation of the masked booby. *Condor*. **68**, 523-535. https://doi.org/10.2307/1366261

Bartholomew, G.A., Lasiewski, R.C., Crawford, E.C. (1968). Patterns of panting and gular flutter in cormorants, pelicans, owls and doves. *Condor*, **70**, 31-34.

Bladon, A. J., Donald, P.F., Jones, S.E.I., Collar, N.J., Deng, J., Dadacha, G., Abebe, Y.D., Green, R.E. (2019). Behavioural thermoregulation and climatic range restriction in the globally threatened ethiopian bush-crow *Zavattariornis stresemanni*. *Ibis*, **161**(3), 546–558.

Boyles, J.G., Seebacher, F., Smit, B., McKechnie, A.E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* **51**(5), 676-690.

Bozinovic, F., Pörtner, H.O. (2015). Physiological ecology meets climate change. *Ecol. Evol.* **5**(5), 1025-1030.

Buckley, L.B., Kingsolver, J.G. (2012). Functional and phylogenetic approaches to forecasting species` responses to climate change. *Annual Review of Ecology, Evolution, and Systematics*, **43**.

Buckley, L.B., Cannistra, A.F., John, A. (2018). Leveraging organismal biology to forecast the effects of climate change. *Integr. Comp. Biol.* **58**(1), 38-51.

Bryant, D.M. (1983). Heat stress in tropical birds: Behavioural thermoregulation during flight. *Ibis*, **125**(3), 313–323.

Calder, W.A., Schmidt-Nielsen, K. (1966). Evaporative cooling and respiratory alkalosis in the pigeon. *Proc. Natl. Acad. Sci. U.S.A.* **55**(4),750–756.

Catry, I., Catry, T., Patto, P., Franco, A.M.A., Moreira, F. (2015). Differential heat tolerance in nestlings suggests sympatric species may face different climate change risks. *Clim. Res.* **66**, 13-24.

Cavieres, G., Sabat, P. (2008). Geographic variation in the response to thermal acclimation in rufous collared sparrows: are physiological flexibility and environmental heterogeneity correlated? *Funct. Ecol.* **22**, 509-515.

Choy, E.S., O'Connor, R.S., Gilchrist, H.G., Hargreaves, A.L., Love, O.P., Vézina, F., Elliott, K.H. (2021). Limited heat tolerance in a cold-adapted seabird: implications of a warming Arctic. *J.Exp. Biol.* **224**, jeb242168.

Clark, G.A. (1969). Spread-wing postures in Pelecaniformes, Ciconiiformes, and Falconiformes. *The Auk*. **86**(1), 136-139.

Cook, T.R., Martin, R., Roberts, J., Häkkinen, H., Botha, P., Meyer, C., Sparks, E., Underhill, L.E., Ryan, P.G., Sherley, R.B. (2020). Parenting in a warming world: thermoregulatory responses to heat stress in an endangered seabird. *Conserv.* **8** (1), coz109.

Cooper, J., Siegfried, W.R. (1976). Behavioural responses of young cape gannets *Sula capensis* to high ambient temperatures . *Mar. Behav. Physiol.* **3**(4), 211–220.

Cunningham, S.J., Martin, R.O., Hockey, P.A.R. (2015). Can behaviour buffer the impacts of climate change on an arid-zone bird? *Ostrich*, **86**(1–2), 119–126.

Cunningham, S.J., Gardner, J.L., Martin, R.O. (2021). Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* **19** (5), 300-307.

Cuttelod, A., García, N., Malak, D.A., Temple, H.J., Katariya, V. (2009). The Mediterranean: a biodiversity hotspot under threat. Wildlife in a changing world-an analysis of the 2008 IUCN Red List of Threatened Species, 89.

Czenze, Z.J., Kemp, R., Jaarsveld, B. van, Freeman, M.T., Smit, B., Wolf, B.O., McKechnie, A.E. (2020a). Regularly drinking desert birds have greater evaporative cooling capacity and higher heat tolerance limits than non-drinking species. *Funct. Ecol.* **34**, 1589-1600.

Czenze, Z.J., Dunbar, M. (2020b). Body mass affects short-term heterothermy in Neotropical bats. *Biotropica*, **52**(5), 963-968.

Czenze, Z.J., Freeman, M.T., Kemp, R., van Jaarsveld, B., Wolf, B.O., McKechnie, A.E. (2022a). Efficient evaporative cooling and pronounced heat tolerance in an eagle-owl, a thick-nee and a sandgrouse. *Front. Ecol. Evol.* **9**, 799302.

Czenze, Z.J., Smit, B., van Jaarsveld, B., Freeman, M.T., McKechnie, A.E. (2022b). Caves, crevices and cooling capacity: roost microclimate predicts heat tolerance in bats. *Funct. Ecol.* **36**(1), 38-50.

Dawson, W.R. (1954). Temperature regulation and water requirements of the brown and Abert towhees, *Pipilo fuscus* and *Pipilo aberti*. In *University of California Publications in Zoology* **Vol. 59** (ed. G.A. Bartholomew, F. Crescitelli, T.H., Bullock, W.H., Furgason and A.M. Schechtman), pp. 81-123. Berkeley: University of California Press.

Dawson, W.R. (1982). Evaporative losses of water by birds. *Comp. Biochem. Physiol.* **71** (4), 495-509.

Diamond, S.E., Sorger, D.M., Hulcr, J., Pelini, S.L., del Toro, I., Hirsch, C., Oberg, E., Dunn, R.R. (2012). Who likes it hot? A global analysis of climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Glob. Chang. Biol.* **18**(2), 448-456.

Du Plessis, K.L., Martin, R.O., Hockey, P.A.R., Cunningham, S.J., Ridley, A.R. (2012). The costs of keeping cool in a warming world: implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Glob. Change Biol.* **18**, 3063-3070.

Eastick, D.L., Tattersall, G.J., Watson, S.J., Lesku, J.A., Robert, K.A. (2019). Cassowary casques act as thermal windows. *Sci. Rep.* **9**, 1966.

Enriquez-Urzelai, U., Tingley, R., Kearney, M.R., Sacco, M., Palacio, A.S., Tejedo, M., Nicieza, A. (2020). The roles of acclimation and behaviour in buffering climate change impacts along elevational gradients. *J. Anim. Ecol.* **89**, 1722-1734.

Eto, E.C., Withers, P.C., Cooper, C.E. (2017). Can birds do it to? Evidence for convergence in evaporative water loss regulation for birds and mammals. *Proc. R Soc. B.* **284**, 20171478.

Finkelstein, M., Kuspa, Z., Snyder, N.F. Schmitt, N.J. (2015) California condor (Gymnogyps californianus), version 2.0. In The Birds of North America (P. G. Rodewald, Editor). Cornell Lab of Ornithology.

Freeman, M.T., Czenze, Z.J., Schoeman, K., McKechnie, A.E. (2020). Extreme hyperthermia tolerance in the world's most abundant wild bird. *Sci. Rep.* **10**, 13098.

Galván, I., Palacios, D., Negro, J.J. (2017). The bare head of the Northern bald ibis (*Geronticus eremita*) fulfills a thermoregulatory function. *Front. Zool.* **14**, 15.

Genin, Amatzia, Liraz Levy, G.S., Diamant, A. (2020). Rapid onsets of warming events trigger mass mortality of coral reef fish. *Proc. Nat. Acad. Sci. USA.* **117**(41), 25378-25385.

Gentry, R.L. (1973). Thermoregulatory behavior of eared seals. *Behaviour,* **46**(2), 73-93.

Gerson, A.R., Smith, E.K., Smit, B., McKechnie, A.E., Wolf, B.O. (2014). The impact of humidity on evaporative cooling in small desert birds exposed to high air temperatures. *Physiol. Biochem. Zool.* **87**, 782-795.

Gerson, A.R., McKechnie, A.E., Smit, B., Whitfield, M.C., Smith, E.K., Talbot, W.A., McWorther, T.J., Wolf, B.O. (2019). The functional significance of facultative hyperthermia varies with body size and phylogeny in birds. *Funct. Ecol.* **33**, 597-607.

Glanville, E.J., Murray, S.A., Seebacher, F. (2012). Thermal adaptation in endotherms: climate and phylogeny interact to determine population-level responses in a wild rat. *Funct. Ecol.* **26**, 390-398.

Graves, G. R. (2019). Urohidrosis and tarsal color in *Cathartes* vultures (Aves: *Cathartidae*). *Proc. Biol. Soc. Wash.* **132**(1), 56–64.

Hainsworth, F.R. (1967). Saliva spreading, activity and body temperature regulation in the rat. *Am. J. Physiol.* **212**, 1288-1292.

Hancock, J.A., Kushlan, J.A. Kahl, M.P. (1992). Storks, Ibises and Spoonbills of the World (Academic Press, 1992).

Haugen, M.J., Tieleman, I.B., Williams, J.B. (2003). Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum. *J. Exp. Biol.* **206**, 3581-3588.

Harvell, C., Montecino-Latorre, D., Caldwell, J., Burt, J., Bosley, K., Keller, A., Heron, S., Salomon, A., Lee, L., Pontier, O., Pattengill-Semmens, C., Gaydos, J. (2019). Disease epidemic and a marine heat wave are associated with the continental-scale collapse of a pivotal predator *Pycnopodia helianthoides*. *Sci. Adv.* **5**(1), eaau7042.

Hatch, D.E. (1970). Energy conserving and heat dissipating mechanisms of the turkey vulture. *Auk*. **87**(1), 111-124.

Hill, R.W. (1980). Comparative physiology of animals: an environmental approach. Harper & Row, Publishers, New York.

Hinds, D.S., Calder, W.A. (1973). Temperature regulation of the pyrrhuloxia and the Arizona cardinal. *Physiol. Zool.* **46**, 55-71.

Hoole, C., Czenze, Z.J., Bennett, N.C., McKechnie, A.E. (2019). Thermal physiology of three sympatric small mammals from Southern Africa. *J. Zool.* **307**(1), 28-35.

Huey, R.B., Deutsch, C.A., Tewskbury, J.J., Vitt, L.J., Hertz, P.E., Álvarez-Pérez, H.J., Garland, T. (2009). Why tropical lizards are vulnerable to climate warming? *Proc. R. Soc. B.* **276**(1664), 1939-1948.

IPCC 2021. Summary for policymakers. In: Climate Change 2021: The physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçy, O., Yu, R., Zhou, B. (eds)]. Cambridge University Press, in press.

Kahl, P.M. (1963) Thermoregulation in the wood stork, with special reference to the role of the legs. *Physiol. Zool.* **36**(2), 141-151.

Kahl, P.M. (1971) Spread-wing postures and their possible functions in the Ciconiidae. *Auk*. **88**(4), 715-722.

Kahliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K., Pfenninger, M. (2014). Global variation in thermal tolerances and vulnerability of endotherms to climate change. *Proc. R. Soc. B.* **281**, 20141097.

Kemp, R., McKechnie, A.E. (2019). Thermal physiology of a range-resticted desert lark. *J. Comp. Physiol. B*. **189**, 131-141.

Lasiewski, R.C., Acosta, A.L., Bernstein, M.H. (1966). Evaporative water loss in birds – I. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability. *Comp. Biochem. Physiol.* **19**, 445-457.

Lasiewski, R.C., Seymour, R.S. (1972). Thermoregulatory responses to heat stress in four species of birds weighing approximately 40 grams. *Physiol. Zool.* **45**, 106-118.

Liknes, E.T., Scott, S.M., Swanson, D.L. (2002). Seasonal acclimatization in the American goldfinch revisited: to what extent do metabolic rates vary seasonally? *Condor*. **104**, 548-557.

Marder, J., Porat, I., Raber, P., Adler, J. (1983). Acid-base balance and body temperature regulation of heat stressed *Psammomys obesus* (*Gerbillinae*): The effect of bicarbonate loss via saliva spreading. *Physiol. Zool.* **56**(3), 389-396.

Marder, J., Arieli, U. (1988). Heat balance of acclimated pigeons (*Columba livia*) exposed to temperatures up to 60 ºC T_a. *Comp. Biochem. Physiol.* **91**, 165-170.

McCafferty, D.M., Gilbert, C., Paterson, W., Pomeroy, P.P., Thompson, D., Currie, J.I., Ancel, A. (2011). Estimating metabolic heat loss in birds and mammals by combining infrared thermography with biophysical modelling. *Comp. Biochem Physiol. Part A.* **158**, 337-345.

McKechnie, A.E., Wolf, B.O. (2010). Climate change increases the likelihood of catastrophic avian mortality events during extreme heatwaves. *Biol. Lett.* **6**, 253-256.

McKechnie, A.E., Noakes, M.J., Smit, B. (2015). Global patterns of seasonal acclimatization in avian resting metabolic rates. *J. Ornithol.* **156**(Suppl 1), S367-S376.

McKechnie, A.E., Whitfield, M.C., Smit, B., Gerson, A.R., Smith, E.K., Talbot, W.A., McWhorter, T.J., Wolf, B.O. (2016). Avian thermoregulation in the heat: Efficient evaporative cooling allows for extreme heat tolerance in four southern hemisphere columbids. *J. Exp. Biol.* **219**(14), 2145–2155.

McKechnie, A.E., Gerson, A.R., McWhorter, T.J., Smith, E.K., Talbot, W.A., Wolf, B.O. (2017). Avian thermoregulation in the heat: Evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance. *J. Exp. Biol.* **220**(13), 2436–2444.

McKechnie, A.E., Gerson, A.R., Wolf, B.O. (2021). Thermoregulation in desert birds: scaling and phylogenetic variation in heat tolerance and evaporative cooling. *J. Exp. Biol.* **224**, jeb229211.

McKechnie, A.E., Rushworth, I.A., Myburgh, F., Cunningham, S.J. (2021). Mortality among birds and bats during an extreme heat event in eastern South Africa. *Austral Ecol.* **46**, 687-691.

McKechnie, A.E. (2022). Chapter 45 - Regulation of body temperature: Patterns and proccesses. In C.G. Scanes & S. Dridi (Eds.), Sturkie's Avian Physiology (7th ed., pp. 1231–1264). Academic Press.

McNab, B.K. (2002). The Physiological Ecology of Vertebrates: A View from Energetics. Cornell University Press.

Mi, C., Ma, L., Wang, Y., Wu, D., Du, W., Sun, B. (2022). Temperate and tropical lizards are vulnerable to climate warming due to increased water loss and heat stress. *Proc. R. Soc. B.* **289**, 20221074.

Molnar, P.K., Derocher, A.E., Klanjscek, T., Lewis, M.A. (2011). Predicting climate change impacts on polar bear litter size. *Nat. Commun.* **2**, 186.

Moran, E., Hartig, F., Bell, D.M. (2016). Intraspecific trait variation across scales: implications for understanding global change responses. *Glob. Change Biol.* **22**, 137-150.

Moyer-Horner, L., Mathewson, P.D., Jones, G.M., Kearney, M.R., Porter, W.P. (2015). Modelling behavioural thermoregulation in a climate change sentinel. *Ecol. Evol.* **5**(24), 5810-5822.

Muñoz-García, A., Cox, R.M., Williams, J.B. (2008). Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum in house sparrows (*Passer domesticus*) following acclimation to high and low humidity. *Physiol. Biochem. Zool.* **81**, 87-96.

Needham, A.D., Dawson, T.J. (1983). Influence of environmental temperature un the blood flow distribution in the Red kangaroo *Macropus rufus*. *Therm. Physiol.* 275-278.

Noakes, M.J., Wolf, B.O., McKechnie, A.E. (2016). Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird. *J. Exp. Biol.* **219**, 859-869.

Noakes, M.J., McKechnie, A.E. (2019). Reaction norms for heat tolerance and evaporative cooling capacity do not vary across a climatic gradient in a passerine bird. *Comp. Biochem. Physiol. A Comp. Physiol.* **236**, 110522.

Noakes, M.J., McKechnie, A.E. (2020). Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines. *Physiol. Biochem. Zool.* **93**(2), 140-152.

Nord, A., Nilsson, J. Å. (2019). Heat dissipation rate constrains reproductive investment in a wild bird. *Funct. Ecol.* **33**, 250–259.

Oboikovitz, P., Swanson, D.L. (2021). Seasonal metabolic flexibility is correlated with microclimate variation in horned larks and house sparrows. *Curr. Zool.* **68**(2), 199-210.

O'Connor, R.S., Wolf, B.O., Brigham, R.M., McKechnie, A.E. (2017). Avian thermoregulation in the heat: efficient evaporative cooling in two southern African nightjars. *J. Comp. Physiol. B*. **187**, 477-491.

O'Connor, R.S., Le Pogam, A., Young, K.G., Robitaille, F., Choy, E.S., Love, O.P., Elliot, K.H., Hargreaves, A.L., Berteaux, D., Tam, A., Vézina, F. (2021). Limited heat tolerance in an Arctic passerine: Thermoregulatory implications for cold-specialized birds in a rapidly warming world. *Ecol. Evol.* **11**(4), 1609-1619.

O'Connor, R.S., Le Pogam, A., Young, K.G., Love, O.P., Cox, C.J., Roy, G., Robitaille, F., Elliott, K.H., Hargreaves, A.L., Choy, E.S., Gilchrist, H.G., Berteaux, D., Tam, A., Vezina, F. (2022). Warming in the land of the midnight sun: breeding birds may suffer greater heat stress at high – versus low-Arctic sites. *Proc. R. Soc. B.* **289**, 1981.

Ophir, E., Arieli, Y., Marder, J., Horowitz, M. (2002). Cutaneous blood flow in the pigeon *Columba livia*: its possible relevance to cutaneous water evaporation. *J. Exp. Biol.* **205**, 2627-2636.

Oswald, S.A., Bearhop, S., Furness, R.W., Huntley, B., Hamer, K.C. (2008). Heat stress in a high-latitude seabird: effects of temperature and food supply on bathing and nest attendance of Great skuas *Catharacta skua*. *J. Avian Biol.* **39**, 163-169.

Oswald, K.N., Lee, A.T.K., Smit, B. (2018). Seasonal physiological responses to heat in an alpine range-restricted bird: the Cape rockjumper (*Chaetops frenatus*). *J. Ornithol.* **159**, 1063-1072.

Oswald, K.N., Smit, B., Lee, A.T.K., Peng, C.L., Brock, C., Cunningham, S.J. (2021). Higher temperatures are associated with reduced nestling body condition in a range-restricted mountain bird. *J. Avian Biol.* **52**(8), e02756.

Pattinson, N.B., Thompson, M.L., Griego, M., Russell, G., Mitchell, N.J., Martin, R.O., Wolf, B.O., Smit, B., Cunningham, S.J., McKechnie, A.E., Hockey, P.A.R. (2020). Heat dissipation behaviour of birds in seasonally hot arid-zones: are there global patterns? *J. Avian Biol.* **51**(2), e02350.

Piersma, T., Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends. Ecol.* **18**, 228-233.

Pollock, H.S., Brawn, J.D., Agin, T.J., Cheviron, Z.A. (2019). Differences between temperate and tropical birds in seasonal acclimatization of thermoregulatory traits. *J. Avian Biol.* **50**(4), e02067.

Pollock, H.S., Brawn, J.D., Cheviron, Z.A. (2020). Heat tolerances of temperate and tropical birds and their implications for susceptibility to climate warming. *Funct. Ecol.* **35**, 93-104.

Playà-Montmany, N., Tattersall, G.J. (2021). Spot size, distance and emissivity errors in field applications of infrared thermography. *Methods Ecol. Evol.* **12**, 828-840.

Playà-Montmany, N., González-Medina, E., Cabello-Vergel, J., Parejo, M., Abad-Gómez, J.M., Sánchez-Guzmán, J.M., Villegas, A., Masero, J.A. (2021). The thermoregulatory role of relative bill and leg surface areas in a Mediterranean population of Great tit (Parus major). *Ecol. Evol.* **11** (22), 15936-15946.

Playà-Montmany, N. (2022). Physiological and behavioural strategies underlying the responses of birds to global warming. PhD Thesis, University of Extremadura, Badajoz, Spain.

Robinson, D.E., Campbell, G.S., King, J.R. (1976). An evaluation of heat exchange in small birds. *J.Comp. Physiol.* **105**, 153-166.

Schraft, H.A., Whelan, S., Elliott, K.H. (2019). Huffin' and puffin: seabirds use large bills to dissipate heat from energetically demanding flight. *J.Exp. Biol.* **222**(21), jeb212563

Sharpe, L.L., Prober, S.M., Gardner, J.L. (2022). In the hot seat: behavioural change and old-growth trees underpin an Australian songbird's response to extreme heat. *Front. Ecol. Evol.* **10**, 813567.

Short, J.C., Freeman, M.T., McKechnie, A.E. (2022). Respirometry protocols for avian thermoregulation at high air temperatures: stepped and steady-states profiles yield similar results. *J. Exp. Biol.* **225**(13), jeb244166.

Smith, E.K., O'Neill, J.J., Gerson, A.R., McKechnie, A.E., Wolf, B.O. (2017). Avian thermoregulation in the heat: resting metabolism, evaporative cooling and heat tolerance in Sonoran Desert songbirds. *J. Exp. Biol.* **220**, 3290-3300.

Smit, B., McKechnie, A.E. (2010). Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**, 330-339.

Smit, B., Zietsman, G., Martin, R.O., Cunningham, S.J., McKechnie, A.E., Hockey, P.A.R. (2016). Behavioural responses to heat in desert birds: Implications for predicting vulnerability to climate warming. *Clim. Change Resp.* **3**(1), 1–14.

Smit, B., Whitfiled, M.C., Talbot, W.A., Gerson, A.R., McKechnie, A.E., Wolf, B.O. (2018). Avian thermoregulation in the heat: phylogenetic variation among avian orders in evaporative cooling capacity and heat tolerance. *J. Exp. Biol.* **221**, jeb174870.

Somero, G.N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**(6), 912-920.

Speakman, J.R., Król, E. (2010). Maximal heat dissipation capacity and hyperthermia risk: Neglected key factors in the ecology of endotherms. *J. Anim. Ecol.* **79**(4), 726–746.

Steen, I., Steen, J.B. (1965). The importance of the legs in the thermoregulation of birds. *Acta Physiol. Scand.* **63**(3), 285–291.

Stevens, G.C. (1989). The latitudinal gradient in geographical range: how so many species coexist in the tropics? *Am. Nat.* **133**, 240-256.

Stillman, J.H. (2019). Heat waves, the new normal: summertime temperature extremes will impact animals, ecosystems and human communities. *Physiology*. **34**, 86-100.

Sturbaum, B.A., Riedesel, M.L. (1977). Dissipation of stored body heat by the ornate box turtle, *Terrapene ornata*. *Comp. Biochem. Physiol. Part A Physiol.* **58**(1), 93–97.

Swanson, D.L. (2010). Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* **17**, 75-129.

Tattersall, G.J., Andrade, D.V., Abe, A.S. (2009). Heat exchange from the Toco toucan bill reveals a controllable vascular thermal radiator. *Science.* **325**,468.

Tattersall, G.J., Sinclair, B.J., Whiters, P.C., Fields, P.A., Seebacher, F., Cooper, C.E., Maloney, S.K. (2012). Coping with thermal challenges: physiological adaptations to environmental temperatures. *Compr. Physiol.* **2**(3), 2151-2202.

Tattersall, G.J., Chaves, J.A., Danner, R.M. (2016). Thermoregulatory windows in Darwin's finches. *Funct. Ecol.* **32**, 358-368.

Tewskbury, J.J., Huey, R.B., Deustch, C.A. (2008). Putting the heat on tropical animals. *Science*. **320**(5881), 1296-1297.

Thomas, B. T. (1984) Maguari Stork Nesting: Juvenile Growth and Behavior. *Auk.* **101**, 812–823.

Thompson, M.L., Cunningham, S.J., McKechnie, A.E. (2018). Interspecific variation in avian thermoregulatory patterns and heat dissipation behaviours in a subtropical desert. *Physiol. Behav.* **188**, 311-323.

Tieleman, I.B., Williams, J.B. (1999). The role of hyperthermia in the water economy of desert birds. *Physiol. Biochem. Zool.* **72** (1), 87-100.

Tieleman, B.I., Williams, J.B., LaCroix, F., Paillat, P. (2002b). Physiological responses of houbara bustards to high ambient temperatures. *J. Exp. Biol.* **205**, 503-511.

Townsend, H., Huyvaert, K.P., Hodum, P.J. Anderson, D.J. (2002) Nesting distributions of Galapagos boobies (Aves: Sulidae): an apparent case of amensalism. *Oecologia.* **132**, 419-427.

Trost, C.H. (1972). Adaptations of Horned larks (*Eremophila alpestris*) to hot environments. *Auk.* **89**, 506-527.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol.* **17**, 1351-1364.

Van de Ven, T.M.F.N., McKechnie, A.E., Cunningham, S.J. (2019). The costs of keeping cool: behavioral trade-offs between foraging and thermoregulation are associated with significant mass losses in an arid-zone bird. *Oecologia.* **191**, 205-215.

Van Dik, M., Noakes, M.J., McKechnie, A.E. (2019). Interactions between humidity and evaporative heat dissipation in a passerine bird. *J. Comp. Physiol. B.* **189**, 299-308.

Van Jaarsveld, B., Bennett, N.C., Kemp, R., Czenze, C.J., McKechnie, A.E. (2021). Heat tolerance in desert rodents is correlated with microclimate at inter an intraspecific levels. *J. Comp. Physiol. B.* **191**, 575-588.

Van Vuuren, A.K.J., Kemp, L.V., McKechnie, A.E. (2020). The beak an unfeathered skin as heat radiators in the southern ground-hornbill. *J.Avian Biol.* **51**(6), e02457.

Vasseur, D.A., de Long, J.P., Gilbert, H.S., Harley, C.D., McCann, K.S., Savage, V., Tunney, T.D., O`Connor, M.I. (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. B.* **281**(1779), 1108-1118.

Webster, M.D., Bernstein, M.H. (1987). Ventilated capsule measurements of cutaneous evaporation in mourning doves. *Condor*. **89**, 863-868.

Welbergen, J.A., Klose, S.M., Markus, N., Eby, P. (2008). Climate change and the effects of temperature extremes on Australian flying-foxes. *Proc. R. Soc. B.* **275**, 419-425.

Whitfield, M.C., Smit, B., McKechnie, A.E., Wolf, B.O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol*. **218**, 1705-1714.

Williams, C.M., Buckley, L.B., Sheldon, K.S., Vickers, M., Pörtner, H.O., Dowd, W.W., Gunderson, A.R., Marshall, K.E., Stillman, J.H. (2016). Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. *Integr. Comp. Biol.* **56**, 73-84.

Wolf, B.O., Walsberg, G.E. (1996a). Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. *J. Exp. Biol.* **199**, 451-457.

Zhang, Q., Han, X., Hao, X., Ma, L., Li, S., Wang, Y., Du, W. (2018). A simulated heatwave shortens the telomere length and lifespan of a desert lizard. *J.Therm. Biol.* **72**, 94-100.

2

Low heat tolerance limits and limited cooling efficiencies in Mediterranean songbirds increase their risks of lethal dehydration during hot days

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Abstract

Songbirds are one of the most vulnerable groups to extreme heat events. Although several recent studies have assessed their physiological responses to heat, most of them have focused on arid-zone species solely. We investigated thermoregulatory responses to heat in eight small-sized songbirds occurring in the Mediterranean Basin, where heatwaves are becoming more frequent and intense. Specifically, we determined their heat tolerance limits (HTL) and evaporative cooling efficiency and evaluated their current and future vulnerabilities to heat in southwestern Iberia, a Mediterranean climate warming hotspot. To do this, we exposed birds to an increasing profile of air temperatures (T_a) and measured resting metabolic rate (RMR), evaporative water loss (EWL), evaporative cooling efficiency (the ratio between evaporative heat loss and metabolic heat production) and body temperature (T_b). HTL ranged between 40 and 46ºC across species, and all species showed rapid increases in RMR, EWL and T_b in response to increasing Ta. However, only the crested lark *Galerida cristata* achieved an evaporative cooling efficiency greater than 1. The studied songbirds currently experience summer T_a maxima that surpass their upper critical temperatures of their thermoneutral zone and even their HTL. Our estimates indicated that five of the eight species will experience moderate risk of lethal dehydration by the end of the century. We argue that the limited heat tolerance and evaporative cooling efficiency of small-sized Mediterranean songbirds make them particularly vulnerable to heatwaves, which will be exacerbated under future climate change scenarios.

Keywords: climate warming, heat tolerance, heatwaves, passerines, thermoregulatory traits, thermal physiology

Introduction

Earth's climate is warming at an unprecedented rate, pushing many species toward and beyond the upper temperatures at which they can survive (Quintero and Wiens, 2013; Riddell et al., 2019; IPCC, 2021). Besides global increases in mean surface air temperatures, climate warming is expected to bring more frequent, intense and long-lasting extreme climatic events, such as heatwaves (Coumou and Robinson, 2013; IPCC, 2021). Temperature extremes associated with heatwaves can challenge the physiological capacities of endotherms to maintain their thermal balance, resulting in adverse effects on their fitness (reviewed in Cunningham et al., 2021). These effects can be either sub-lethal (DuPlessis et al., 2012; Cunningham et al., 2013; van de Ven et al., 2019; 2020) or lethal, sometimes resulting in mass mortality events across wide geographical areas, from deserts to circumpolar regions (see Welbergen et al., 2008; Saunders et al., 2011; McKechnie et al., 2012; McKechnie et al., 2021; Quintana et al., 2022).

Among endotherms, small songbirds (order Passeriformes) are especially vulnerable to heatwaves due to their diurnal habits, small body sizes with large area to volume ratios, and high metabolic rates (Albright et al., 2017). Panting is the main physiological response of songbirds to maintain thermal balance and avoid lethal hyperthermia at high ambient temperatures, yet it is expensive in terms of energy and water (Calder and Schmidt-Nielsen, 1966; Dawson, 1982; Wolf and Walsberg, 1996; McNab, 2002). This is one of the reasons why songbirds generally achieve lower evaporative cooling efficiencies — defined as the quotient between evaporative heat loss (EHL) and metabolic heat production (MHP) — than other bird groups that rely on more efficient physiological mechanisms, such as gular fluttering or cutaneous evaporative water loss (reviewed in McKechnie et al., 2021).

In this context, the study of heat tolerance limits (HTL, i.e., maximum air temperature tolerated before the onset of severe heat stress) is essential to determine the vulnerability of species to global warming (Williams et al., 2008). Over the last decade, an increasing number of studies have focused on avian thermoregulation in the heat, particularly in songbirds (e.g., Whitfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Oswald et al., 2018b; Smit et al., 2018; Kemp and McKechnie, 2019; Czenze et al., 2020; Pollock et al., 2020; O’Connor et al., 2021), yet the majority of them have focused on desert and arid-zone species (e.g., Whitfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Kemp and McKechnie, 2019; Czenze et al., 2020). In fact, studies on HTL and evaporative cooling capacity of species from tropical, temperate and subpolar areas are very scarce (Oswald et al., 2018b; Pollock et al., 2020; O’Connor et al., 2021), particularly in the Mediterranean region (Playà-Montmany et al., 2021). These studies have revealed a within-order biogeographic variation in heat tolerance, with desert and arid-zone species dealing better with heat stress by achieving greater HTLs and evaporative cooling efficiencies (see Whitfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Kemp and McKechnie, 2019; Czenze et al., 2020) than those from tropical, temperate, or subpolar regions (Pollock et al., 2020; O'Connor et al., 2021).

The Mediterranean Basin is warming faster than other regions across the globe (IPCC, 2021), having experienced a 1.5 ºC increase in mean annual surface temperature since preindustrial values. Indeed, climate change scenarios predict increments of 1.8 – 3.7 ºC (SSP2-4.5 and SSP5-8.5, respectively) as well as more frequent and intense heatwaves to the end of this century (Gutiérrez et al., 2021). Within the Mediterranean Basin, Southwestern Iberia is predicted to be one of the more sensitive areas to global warming effects, with studies predicting a twofold increase in the number, frequency and severity of heatwaves (Cardoso-Pereira et al., 2017). Understanding how Mediterranean species differ in their HTLs, therefore, is critical for accurately forecasting their vulnerability under future climate change prospects. As HTLs well exceed the upper critical temperature (T_{uc}) of the thermoneutral zone, they are more valuable to inform vulnerability predictive models (see Albright et al., 2017; Conradie et al., 2019; 2020) than T_{uc} (Khaliq et al., 2014; Mitchell et al., 2018).

In this study, we evaluated the HTLs and evaporative cooling efficiency of small-sized (from ~ 10 to ~ 34 g) Mediterranean resident songbirds. Moreover, we investigated current and future vulnerability of the species to heat extremes under different climate warming scenarios, using several proxies: (1) the number of days above T_{uc} (in which birds are forced to actively thermoregulate to maintain thermal balance), (2) the number of days above HTL (days in which birds face direct risk of lethal hyperthermia), and (3) lethal dehydration risks during the hottest days. We predicted that Mediterranean songbirds would display lower HTLs and evaporative cooling efficiencies than arid-zone and desert songbirds previously studied, but in the range found in those from tropical and Northern-temperate climates. Based on previous findings (reviewed by McKechnie et al., 2021), we expected that larger species would deal better with heat than smaller ones. Finally, we also predicted that summer maximum temperatures experienced by the studied species (often exceeding 40 ºC) may currently compromise their physiological thermoregulatory capabilities, which would be exacerbated under future climate warming scenarios.

Methods

Study site

The study took place in Badajoz, southwestern Spain (Supplementary Material, Figure S1), during the summers of 2020 and 2021 (from late June to early September). The climate in the study area is classified as Mediterranean, with mild winters and warm and dry summers. Maximum air temperatures in summer average 31.8 ± 2.1 ºC (mean ± s.d.), with recent records of up to 46 ºC (State Meteorology Agency, https://opendata.aemet.es/). During the last 30 years, this area has presented a significant increase in the number of hot days per summer, currently experiencing up to 50 days in which maximum ambient temperature exceeds 35 ºC (Figure S2).

There are three main types of habitats in our study area: (i) Mediterranean forest, composed of different Quercus species with dense shrub cover under the canopy; (ii) dehesas (transformation of the original Mediterranean forest by clearance and brushwood removal, providing a typical landscape of savannah-like open woodland with scattered trees); and (iii) farmlands, open landscapes dominated by cereal lands, olive groves, vineyards and fallows.

Bird species and capture

We measured HTL and evaporative cooling capacity in eight resident Palearctic songbirds widely distributed across the Mediterranean Basin: crested lark (*Galerida cristata*, Alaudidae; N = 6), house sparrow (*Passer domesticus*, Passeridae; N = 18), Spanish sparrow (*Passer hispaniolensis*, Passeridae; N = 15), chaffinch (*Fringilla coelebs*, Fringillidae; N = 21), greenfinch (*Chloris chloris*, Fringillidae; N = 20), goldfinch (*Carduelis carduelis*, Fringillidae; N = 17), serin (*Serinus serinus*, Fringillidae; N = 11), and great tit (*Parus major*, Paridae; N = 16).

We captured adult and fully developed first calendar year individuals by mist-netting. Adults moulting wings and tail feathers, or showing heavy body moult, were discarded from heat tolerance trials, as moulting is energetically expensive (e.g., Delhey et al. 2020) and could influence metabolic rates (e.g., Klaassen, 1995). All summer juveniles used in measurements were non-moulting individuals. Upon capture, birds were transferred to cloth bags and then moved quickly to large indoor aviaries (320 cm x 260 cm x 255 cm) at the University of Extremadura (Badajoz), where they were kept with food and water ad libitum until heat tolerance trials were performed. Individuals spent a maximum of 24 h in captivity and were always released at the site of capture. All experimental procedures were approved by the bioethical committee of the University of Extremadura (76/2018) and performed under governmental licenses CN0002/20/ACA and CN0002/21/ACA.

Body temperature and gas exchange measures

We used temperature-sensitive passive integrated transponder (PIT) tags (BioTherm13 13 x 2.12 mm, Biomark, USA) to monitor body temperature (T_b) during heat tolerance trials. Tags were inserted under the skin in the interclavicular region, as intraperitoneal implantation has shown adverse effects in small-sized birds (< 25 g) (Oswald et al., 2018a). To prevent PIT tag loss during the trials, we employed approach stitches to seal the wound. T_b readings were recorded every 5 s during heat tolerance trials by a racket antenna (model F201F-ISO, Biomark, USA) connected to an external reader (IS1001 Multiplexing Transceiver System, Biomark).

We measured oxygen consumption (VO_2) and EWL (VH_2O) using an open flow-through respirometry system. Birds were placed individually in a 4.9 L plastic chamber set inside a temperature-controlled cabinet (model F-4, Ibercex) that allowed us to adjust T_a. Within the chamber, a wire mesh platform was placed 4 cm above a 1 cm mineral oil layer to trap excreta and allow birds to perch. T_a inside the chamber was continuously measured using a calibrated thermistor probe (TC-100, Sable Systems).

Ambient dried air was supplied by a compressor (model R-110300, MESTRA), and the air stream was split into two channels: the baseline (an empty chamber) and the metabolic chamber. Flow rates to each channel were controlled by mass flow controllers (MFS5, Sable Systems) or a mass flow generator (Flowkit, Sable Systems) when flow rates greater than 3000 ml min^{-1} were needed. The flow rate to the baseline was always set to 1000 ml min^{-1}, while flow rates to the metabolic chamber were adjusted (2000-9000 ml min^{-1}) during the trials to maintain low ambient humidity values (<1 kPa) at the same time that oxygen depletion rates remained detectable. This permitted birds to stay calm and maintain water vapor gradients that did not potentially preclude EWL rates (see van Dyk et al., 2019). We sequentially switched manually between baseline and metabolic chamber using a multiplexer (RM-8, Sable Systems). Excurrent air was subsampled at a rate of 200 ml min^{-1} (SS3 Subsampler, Sable Systems) and pulled sequentially through a H_2O analyzer (RH300 model, Sable Systems), a Drierite® column and a O_2 analyzer (FC-10 model, Sable Systems). Both analyzers were periodically zeroed and spanned. An analog to digital converter (UI2 model, Sable Systems) was used to digitalize voltage outputs from the analyzers and the thermistor probe, and Expedata software (version 1.9.14, Sable Systems) was employed to record these outputs with a sampling rate of 1 s.

Experimental protocol

Heat tolerance trials took place during the birds' active phase, and a single bird was measured at a time. Food was withdrawn at least two hours before trials started, so we assumed that birds were in post-absorptive state. Gas exchange rates were measured along a stepped profile of increasing T_a: 30, 33, 37, 40 ºC, and 2 °C increments at $T_a > 40$ °C until HTL was reached, following a similar procedure employed in recent studies (e.g., Whitfield et al., 2015). At the beginning of each trial, 10 min of baseline air was subsampled before switching to the metabolic chamber. The first T_a was maintained for approximately 30 min until stable traces of O_2 and H_2O and normothermic T_b values (40-42 ºC) were achieved. Then, the next T_a was sequentially set, until the end of the trial. Between successive target T_a and again at the end, 5 min of baseline were collected. Birds spent a minimum of 10 min at each target T_a, and trials took no longer than 3 h. During measurements, we continuously monitored bird' behaviour with an infrared video camera.

Trials ended when birds reached their HTL, which occurred when birds started to show continuous active escape behaviour (pecking the walls, flapping or jumping around) or signs of severe heat stress, such as loss of righting response or balance, sudden decreases in oxygen consumption or EWL, and/or sustained uncontrolled increases (> 0.1 ºC min^{-1}) in T_b approaching or exceeding 45 ºC (following Whitfield et al. 2015, O'Connor et al. 2021). Following Smit et al. (2017), birds' behaviour was scored from 0-5, with 0 corresponding to a completely calm bird and 5 to a bird showing sustained escape behaviour. For data analyses, highly active birds (scores 4-5) were not considered. When a bird reached its HTL, it was rapidly removed from the chamber and placed in front of a fan. We soaked its legs in alcohol to aid heat dissipation and monitored the bird until its T_b returned to normothermic values. Finally, we hydrated and returned it to the indoor aviaries. PIT tags were removed before releasing the birds.

Data analyses

We corrected for analyzers drift and lag in Expedata software and used equations 10.2 and 10.9 from Lighton (2008) to calculate VO2 and EWL, assuming a respiratory exchange ratio of 0.71 (birds in postabsorptive state during the metabolic trials) (Walsberg and Wolf, 1995). Resting metabolic rate (RMR) and EWL were extracted from the lowest 5 min values at each target T_a to which birds were exposed. RMR was converted to MHP (W) by multiplying with an oxyjoule equivalent [16 + 5.164 (RQ)] (Lighton et al., 1987). EWL was converted to EHL (W) assuming a latent heat of vaporization of 2.406 J mg^{-1} (Tracy et al. 2010). T_b was calculated as the average value during the final 10 min of exposure to each T_a. We reported whole-animal RMR (W) and EWL values (mg h^{-1}), although we also showed mass-specific slopes of both traits to favour interspecific comparisons.

All statistical analyses were performed in R (R Core team 2020). To evaluate HTL and evaporative cooling efficiency, we determined inflection points for RMR, EWL, EHL/MHP and T_b. We fitted broken-stick linear regressions for each species using the package '*segmented*' (Muggeo, 2009). Then, we adjusted generalized linear mixed-effect models using the package '*lme4*' (Bates et al., 2015) to estimate the slopes of each variable above its inflection point in response to T_a, including individual identity as a random factor as we had measurements for each bird at various T_a. Initially, we included M_b as a covariate in all models, but as it did not emerge as significant nor improve model fit, we discarded it from intraspecific analyses.

Additionally, we tested for possible interspecific differences in thermoregulatory traits across Mediterranean songbirds. Initially, we fitted phylogenetic generalized least squares models using the package '*caper*' (Orme et al., 2018) to test for possible phylogenetic relatedness in thermoregulatory traits among our species. However, lambda showed values equal to zero for each trait tested (indicating an absence of phylogenetic effect; see Table S1). Therefore, we fitted generalized linear models with different thermoregulatory traits – mass-specific RMR, mass-specific EWL, EHL/MHP and T_b slopes above inflection points, evaporative scope (the ratio between maximum and minimum EWL), maximum EHL/MHP, and HTL – as response variables and mean M_b of each species (as a proxy of body size) as predictor variable, to test for possible scaling of these thermal traits with body size. In the case of HTL, in addition to mean M_b, we included mean evaporative scope as a predictor, as a previous study found that species with greater evaporative scopes achieve higher HTLs (Czenze et al., 2020).

Vulnerability to high temperatures

We evaluated current and future vulnerability to high temperatures of small Mediterranean resident songbirds across Extremadura, southwestern Spain. Previous work has forecasted particularly severe future increases in heatwaves in this region (Cardoso-Pereira et al., 2017; Viceto et al., 2019). To do so, we employed two metrics: the number of days in which maximum environmental temperature surpasses T_{uc} (i.e., a proxy of possible chronic deleterious effects of heat exposition on individual fitness); and the number of days in which maximum environmental temperature exceeds HTL (i.e., a proxy of lethal hyperthermia risk).

We obtained maximum temperature values on a daily basis from June to September, both at current times (2006-2021) and at the end of the century (2070-2100) under two different climate change scenarios (RCP4.5 and RCP8.5 in the framework of the Fifth Assessment Report of the IPCC; IPCC, 2014). RCP4.5 corresponds to a stabilization scenario that forecasts an additional increase in mean surface temperature of 1.8 ºC, while RCP8.5 represent a 'business as usual' scenario (unmitigated greenhouse gases emissions) and forecasts an additional increase of 3.7 ºC (IPCC, 2014). We obtained climate data projections for Extremadura from the Spanish Climate Change Office' project AdapteCCa (https://adaptecca.es/). This data contained daily maximum temperature predictions from 16 different regional climate models (spatial resolution of 0.11 degrees; EUR-11, ~12.5 km) from EURO-CORDEX (Jacob et al., 2014) (see Supplementary Information). Previous work has shown that these models have similar sensitivity and yield faithful predictions with respect to the reference period 1986-2005 (Barredo et al., 2018). We averaged daily model outputs to obtain the average number of days per summer above T_{uc} and above HTL for each species at different times and scenarios across each grid, and then we mapped these results (as in Conradie et al. 2019).

Furthermore, we calculated current and future lethal dehydration risks of Mediterranean songbirds during heatwaves following a similar approach to Albright et al. (2017). We assumed that birds retreat to shaded microhabitats and cease foraging during the hottest part of the day, thus impeding the replenishment of body water lost through evaporative means. After determining the T_a at which each species started to increase EWL, we used their slopes above the inflection point to calculate hourly EWL rates and hence the time needed to achieve their lethal dehydration thresholds (intended as cumulative EWL surpassing 15% of species M_b; Albright et al., 2017) during extremely hot days (see Supplementary Information for details). We estimated the total evaporative heat losses of species between 10 AM and 8 PM, a period during which T_a is above the EWL inflection point for all the species considered during extremely hot days in the study area. Following Albright et al. (2017), we considered moderate risks of lethal dehydration when birds take ≤ 5 h to lose 15% of their M_b, and severe risk when this threshold was met in ≤ 3 h.

Following previous studies (Albright et al., 2017; Cook et al., 2020), we predicted species-specific current risk of dehydration, by averaging hourly values of environmental temperature from the hottest 10 days during the last 20 years in the study area (State Agency of Meteorology, https://opendata.aemet.es/) (Figure S3). To this hourly profile, we added the predicted temperature increase in mean surface temperature by the two climate change scenarios (RCP4.5 and RCP8.5) in the long term (2070-2100) (Figure S3).

Results

Resting metabolic rate

RMR within the thermoneutral zone ranged between 0.26 W in the serin and 0.51 W in the crested lark (Table 1). T_{uc} varied from 34.16 ºC in the house sparrow to 37.60 ºC in the Spanish sparrow (Table 1). In these species, RMR increased significantly with T_a above T_{uc}, with mass-specific slopes varying from 0.72 mW g^{-1} ºC^{-1} in the house sparrow to 2.39 mW g^{-1} ºC^{-1} in the great tit (Table 1; Figure 1). In the case of the crested lark, we could not find an evident T_{uc}, and RMR appeared to be stable through the T_a profile to which birds were exposed (Table 1; Figure 1).

Maximum to minimum RMR ratios varied across species, from 1.19 in the chaffinch to 1.90 in the Spanish sparrow (Table 1). The interspecific analysis did not show differences across species in mass-specific RMR slope as a function of M_b (estimate = -0.03 ± 0.05; F = 0.28; p = 0.617).

Evaporative water loss

All species showed clear inflection points in EWL, from 34.53 ºC in the crested lark to 38.32 ºC in the goldfinch (Table 1; Figure 2). Minimum EWL varied between 91.06 mg H_2O h^{-1} in the goldfinch and 171.72 mg H_2O h^{-1} in the Spanish sparrow (Table 1). Above inflection points, EWL increased significantly in response to T_a, with mass-specific slopes varying from 2.56 mg H_2O g^{-1} h^{-1} ºC^{-1} in the crested lark to 4.66 mg H_2O g^{-1} h^{-1} ºC^{-1} in the great tit (Table 1; Figure 2). Maximum rates of EWL ranged from 379.81 mg H_2O h^{-1} in the serin to 1051.68 mg H_2O h^{-1} in the Spanish sparrow (Table 1), while evaporative scopes ranged from 3.89 in the great tit to 6.77 in the greenfinch (Table 1).

Among species, M_b had a negative influence on EWL slope (estimate = -0.086; s.e. = 0.018; F = 21.12; p = 0.003), with smaller Mediterranean songbirds showing higher mass-specific slopes than larger ones (Table 1). However, evaporative scope did not differ in response to species M_b (estimate = 0.089; s.e. = 0.05; F = 2.74; p = 0.148).

Evaporative cooling efficiency

EHL/MHP inflection points varied by 3.4 ºC among species, ranging from 33.53 ºC in the Spanish sparrow to 37.13 ºC in the serin (Table 1; Figure 3). Below the inflection points, all species showed minimum EHL/MHP rates of ~0.20 (Table 1), increasing significantly with Ta above these points at rates that varied from 0.05 to 0.08 per ºC (Table 1). Maximum EHL/MHP ranged from 0.62 in the great tit to 1.11 in the crested lark (Table 1).

Interspecific analyses across the eight studied species showed no effect of M_b on the EHL/MHP slope (estimate = 0.001 ± 0.001; F = 1.49; p = 0.267), but larger species achieved greater evaporative cooling efficiencies than smaller ones (estimate = 0.016 ± 0.01; F = 9.81; p = 0.020) (Table 1).

Body temperature and Heat Tolerance Limits

Normothermic T_b differed by 0.8 ºC across species, ranging from 40.85 ºC in the Spanish sparrow to 41.65 ºC in the great tit (Table 1). Segmented regressions revealed clear inflection points in seven of the eight species, varying from 32.67 ºC in the house sparrow to 35.52 ºC in the serin (Table 1; Figure 4). Above inflection points, T_b increased significantly with respect to normothermic values with slopes ranging from 0.26 ºC ºC^{-1} in the Spanish sparrow to 0.37 ºC ºC^{-1} in the serin (Table 1).

Table 1. Mean values (± s.d.) of the physiological thermoregulatory variables studied across a range of air temperatures (T_a) in eight Mediterranean resident songbirds.

Variable	Serin	Goldfinch	Great tit	Chaffinch
Body mass (M_b, g)	10.17 ± 0.52	12.77 ± 1.22	15.50 ± 1.01	18.79 ± 1.55
RMR				
Min RMR (W)	0.26 ± 0.04	0.30 ± 0.04	0.38 ± 0.04	0.42 ± 0.07
Inflection T_a (ºC)	34.76	35.62	35.19	34.86
slope (W ºC^{-1})	0.023	0.016	0.037	0.021
Slope$_{mass\_specific}$ (mW g^{-1} ºC^{-1})	2.26	1.25	2.39	1.12
Max T_{air} (ºC)	42 (6)	42 (5) 44 (2)	40 (15)	42 (6)
Max RMR (W)	0.37 ± 0.05	0.38 ± 0.09 (5) 0.38 ± 0.04 (2)	0.49 ± 0.10	0.50 ± 0.07
Max RMR/min RMR	1.42	1.27	1.29	1.19
EWL				
Min EWL (mg h^{-1})	96.94 ± 34.60	91.06 ± 24.52	115.14 ± 33.36	139.69 ± 38.77
Inflection T_a (ºC)	36.26	38.32	35.99	35.50
Slope (mg ºC^{-1})	45.062	58.884	72.174	74.411
Slope$_{mass\_specific}$ (mg g^{-1} ºC^{-1})	4.43	4.61	4.66	3.96
Max EWL (mg h^{-1})	379.81 ± 66.44	404.10 ± 51.18 (5) 520.52 ± 117.30 (2)	448.22 ± 0.10	622.41 ± 91.69
Max EWL/min EWL	3.92	5.72	3.89	4.46
EHL/MHP				
Min EHL/MHP	0.25 ± 0.11	0.21 ± 0.05	0.20 ± 0.05	0.23 ± 0.05
Inflection T_a (ºC)	37.13	35.96	35.31	35.09
Slope (ºC^{-1})	0.05	0.07	0.07	0.07
Max EHL/MHP	0.68 ± 0.12	0.74 ± 0.17 (5) 0.90 ± 0.10 (2)	0.62 ± 0.17	0.85 ± 0.16
Body temperature (T_b)				
Min T_b (ºC)	41.03 ± 0.99	41.33 ± 0.75	41.65 ± 0.47	41.25 ± 0.50
Inflection T_a (ºC)	35.52	34.76	34.54	33.12
Slope (ºC ºC^{-1})	0.37	0.30	0.29	0.27
Max T_b (ºC)	43.48 ± 0.35	43.22 ± 0.47 (5) 43.06 ± 0.76 (2)	43.22 ± 0.47	43.23 ± 0.44
Max T_b – min T_b (ºC)	2.45	1.90	1.56	1.98

(Table 1 continued)

Variable	Greenfinch	House sparrow	Spanish sparrow	Crested lark
Body mass (M_b, g)	21.66 ± 1.55	22.31 ± 2.65	24.45 ± 1.62	33.55 ± 2.07
RMR				
Min RMR (W)	0.45 ± 0.07	0.40 ± 0.09	0.42 ±0.06	0.51 ± 0.04
Inflection T_a (ºC)	35.64	34.16	37.60	n.a.
slope (W ºC^{-1})	0.042	0.016	0.052	n.a.
Slope$_{\text{mass\_specific}}$ (mW g^{-1} ºC^{-1})	1.94	0.72	2.13	n.a.
Max T_{air} (ºC)	42 (11) 44 (2)	44 (4)	42 (7) 44 (2)	46 (2)
Max RMR (W)	0.69 ± 0.13 (11) 0.61 ± 0.06 (2)	0.54 ± 0.08	0.62 ± 0.08 (7) 0.80 ± 0.16 (2)	0.63 ± 0.25
Max RMR/min RMR	1.53	1.35	1.90	1.24
EWL				
Min EWL (mg h^{-1})	111.62 ± 22.58	112.13 ± 23.29	171.72 ± 59.77	174.86 ± 42.93
Inflection T_a (ºC)	35.46	35.55	36.29	34.53
Slope (mg ºC^{-1})	82.040	73.162	100.148	85.970
Slope$_{\text{mass\_specific}}$ (mg g^{-1} ºC^{-1})	3.79	3.28	4.10	2.56
Max EWL (mg h^{-1})	714.10 ± 147.73 (11) 755.48 ± 14.86 (2)	756.95 ± 79.65	699.11 ± 155.50 (7) 1051.68 ± 198.65 (2)	1015.11 ± 217.32
Max EWL/min EWL	6.77	6.75	6.12	5.81
EHL/MHP				
Min EHL/MHP	0.17 ± 0.04	0.19 ± 0.03	0.28 ± 0.12	0.23 ± 0.06
Inflection T_a (ºC)	33.75	35.57	35.53	34.87
Slope (ºC^{-1})	0.05	0.08	0.08	0.07
Max EHL/MHP	0.69 ± 0.10 (11) 0.83 ± 0.06 (2)	0.94 ± 0.09	0.76 ± 0.18 (7) 0.88 ± 0.01 (2)	1.11 ± 0.21
Body temperature (T_b)				
Min T_b (ºC)	41.46 ± 0.41	41.41 ± 0.45	40.85 ± 0.64	41.17 ± 0.59
Inflection T_a (ºC)	35.40	32.67	33.10	n.a.
Slope (ºC ºC^{-1})	0.29	0.31	0.26	n.a.
Max T_b (ºC)	43.46 ± 0.40 (11) 43.59 ± 0.52 (2)	43.68 ± 0.64	43.48 ± 0.33 (7) 43.63 ± 0.53 (2)	42.67 ± 0.13
Max T_b – min T_b (ºC)	2.132	2.28	2.78	1.50

Crested larks showed a different pattern, and we could not find an inflection point in T_b. Along the range of T_a at which birds were exposed, they showed a steady but not significant increment in T_b (Table 1; Figure 4).

Maximum HTL ranged from 40 ºC in the great tit to 46 ºC in the crested lark, with chaffinch and serin showing HTLs at 42 ºC, and goldfinch, greenfinch, Spanish sparrow, and house sparrow reaching 44 ºC (Table 1).

Most of the trials ended due to sustained escape behaviour, with no birds reaching T_b = 45 ºC. T_b reached at their HTLs differed across species, from 42.67 ºC in the crested lark to 43.63 ºC in the Spanish sparrow (Table 1). Likewise, the gradient between maximum and minimum T_b values ranged from 1.50 ºC in the crested lark to 2.78 ºC in the Spanish sparrow (Table 1).

T_b slope did not differ across species with respect to M_b (estimate = -0.004 ± 0.002; F = 4.82; p = 0.08). Likewise, HTL did not differ among species as a function of their M_b (estimate = 0.095 ± 0.07; F = 6.21; p = 0.240) nor was influenced by their evaporative scopes (estimate = 0.87 ± 0.45; F = 6.21; p = 0.108).

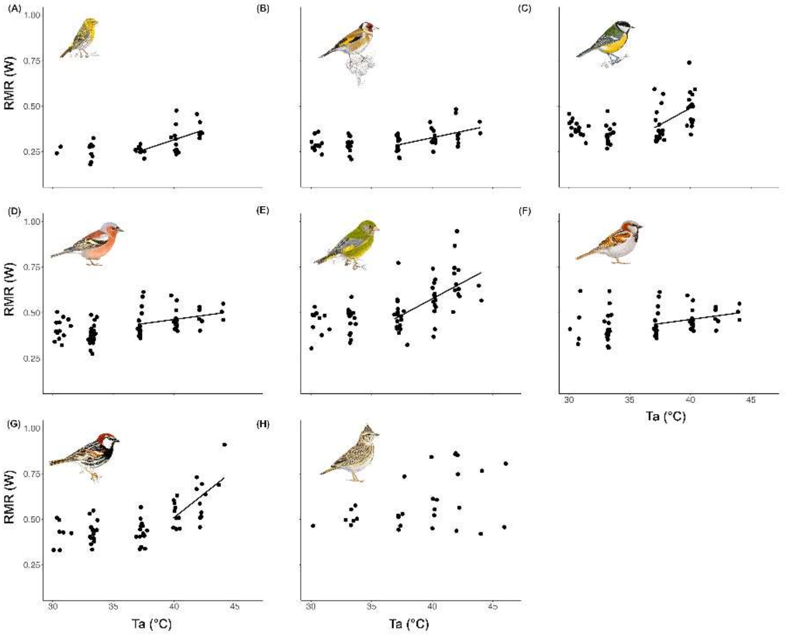

Figure 1. Resting metabolic rate (RMR) as a function of air temperature (T_a) in eight Mediterranean resident songbirds. (A) serin, (B) goldfinch, (C) great tit, (D) chaffinch, (E) greenfinch, (F) house sparrow, (G) Spanish sparrow, and (H) crested lark. RMR was regressed against T_a above the upper critical temperature (T_{uc}; see Table 1), obtaining a significant relationship in seven of the eight species. In the case of crested lark, we did not find a clear T_{uc}. Illustrations are reproduced with the permission of Juan Varela.

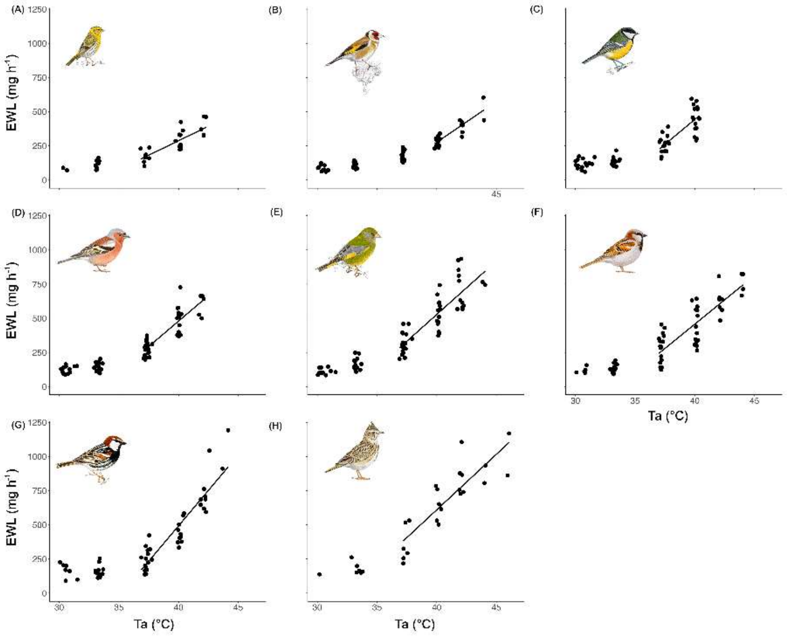

Figure 2. Evaporative water loss (EWL) as a function of air temperature (T_a) in eight Mediterranean resident songbirds. (A) serin, (B) goldfinch, (C) great tit, (D) chaffinch, (E) greenfinch, (F) house sparrow, (G) Spanish sparrow, and (H) crested lark. EWL was regressed against T_a above the upper critical temperature for this variable (see Table 1), obtaining a significant relationship in all species. Illustrations are reproduced with the permission of Juan Varela.

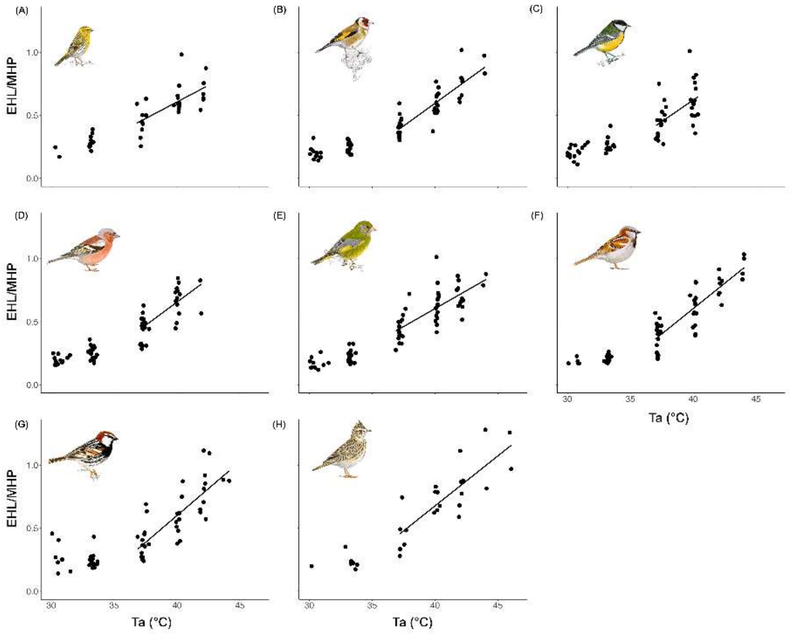

Figure 3. Evaporative cooling efficiency (EHL/MHP) as a function of air temperature (T_a) in eight Mediterranean resident songbirds. (A) serin, (B) goldfinch, (C) great tit, (D) chaffinch, (E) greenfinch, (F) house sparrow, (G) Spanish sparrow, and (H) crested lark. EHL/MHP was regressed against T_a above the upper critical temperature for this variable (see Table 1), obtaining a significant relationship in all species. Illustrations are reproduced with the permission of Juan Varela.

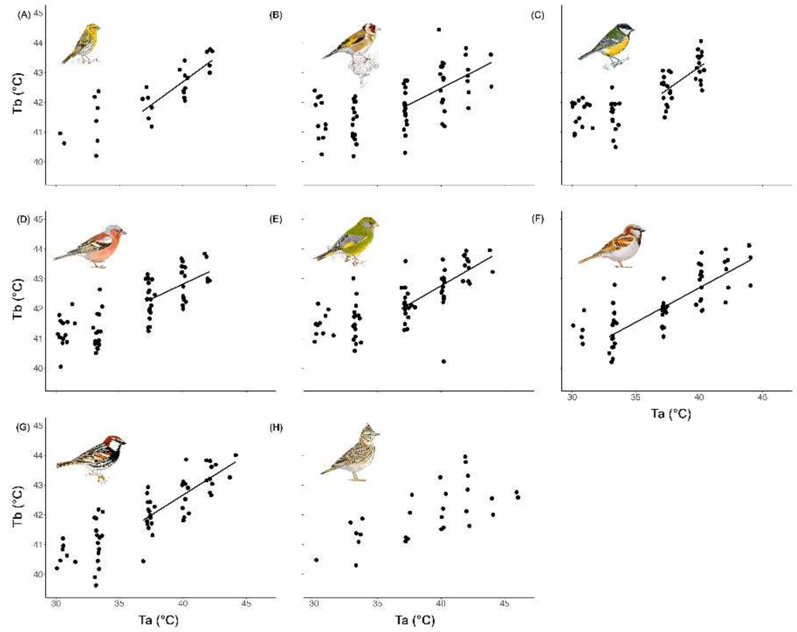

Figure 4. Body temperature (T_b) as a function of air temperature (T_a) in eight Mediterranean resident songbirds. (A) serin, (B) goldfinch, (C) great tit, (D) chaffinch, (E) greenfinch, (F) house sparrow, (G) Spanish sparrow, and (H) crested lark. T_b was regressed against T_a above the upper critical temperature for this variable (see Table 1), showing significant relationships in seven of the eight species. In the case of crested lark, we could not detect an inflection point for T_b. Illustrations are reproduced with the permission of Juan Varela.

Current and future vulnerability to high temperatures

When modelling the mean number of days above T_{uc} across Extremadura, we found an increase for all the species studied but crested lark (for which we could not detect a clear T_{uc}) under projected climate change scenarios with respect to current values (Figure 5; Table S2). At present and under future scenarios, our species showed a higher degree of vulnerability to temperature extremes along the southeastern part of the region (Figure 5). The house sparrow showed the highest number of days per summer above T_{uc} (from 33.07 ± 15.88 days at present to 98.11 ± 8.95 days to 2070-2100 under the RCP8.5 scenario), while the Spanish sparrow showed the lowest (from 0 days at present to 54.89 ± 14.29 days to the end of the century under the RCP8.5 scenario) (Figure 5; Table S2).

According to model predictions, none of the eight studied species are currently experiencing days above their HTLs in Extremadura (Table S2). To the end of the century (2070-2100), only the great tit will experience days above HTL under the RCP8.5 scenario (4.94 ± 11.06 days) (Table S2; Figure S4). Again, the greatest risk of lethal hyperthermia by exposition to maximum temperatures above HTL will be experienced by those populations of great tit occupying the southeast of Extremadura (see Figure S4).

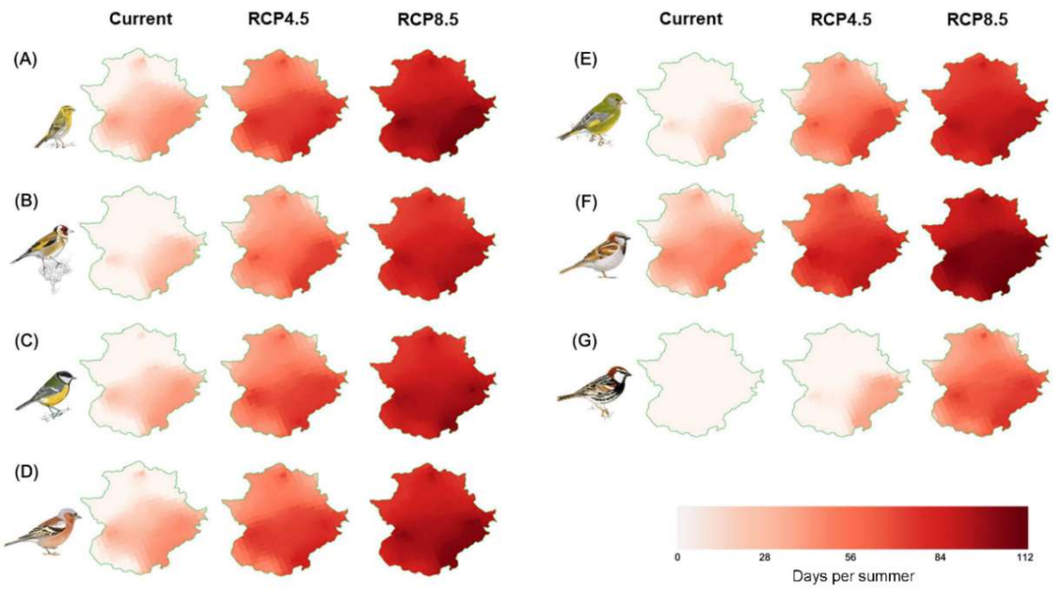

Figure 5. Mean number of days per summer above the T_{uc} (see Table 1) experienced by the studied songbird species across Extremadura under current (2006-2021) and future (2070-2100) projected climate change scenarios (RCP4.5 and RCP8.5, respectively). (A) serin, (B) goldfinch, (C) great tit, (D) chaffinch, (E) greenfinch, (F) house sparrow, and (G) Spanish sparrow. Crested lark could not be mapped as we could not find a clear T_{uc} for this species. Illustrations are reproduced with the permission of Juan Varela.

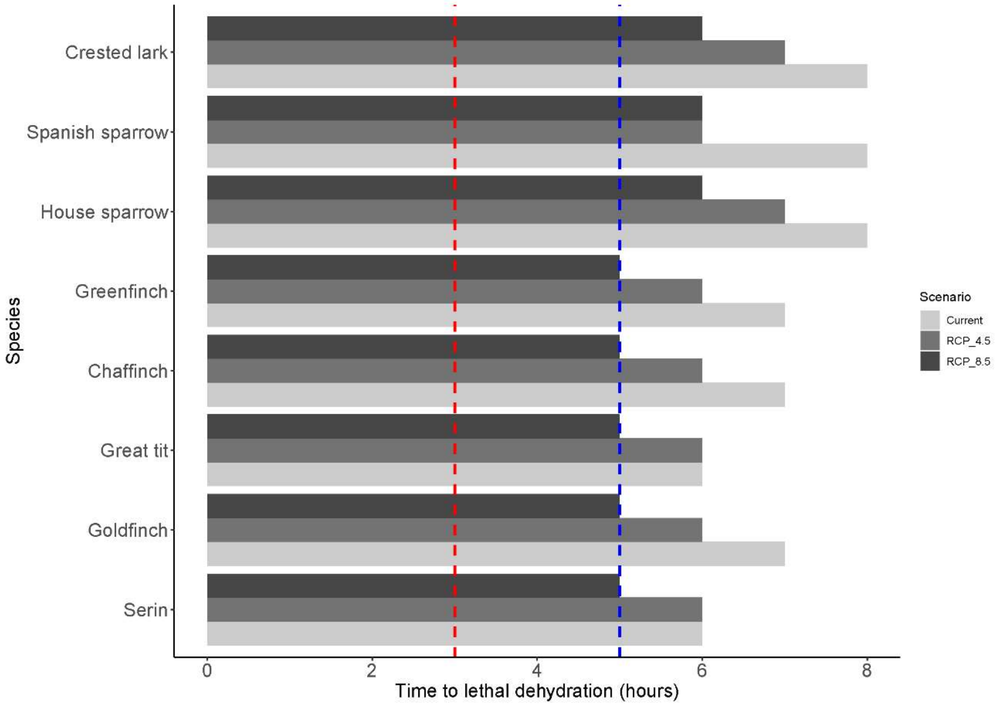

Figure 6. Current (2006-2021) and future (2070-2100) times to lethal dehydration (in h) during an extremely hot day under the RCP4.5 and RCP8.5 climate warming scenarios for each of the Mediterranean songbird species studied. Dotted red line indicates a severe risk of lethal dehydration (i.e., birds losing 15% of M_b through EWL in ≤ 3 h), while dotted blue line indicates a moderate risk of lethal dehydration (i.e., when cumulative EWL surpassed 15% of M_b in ≤ 5 h).

When estimating lethal dehydration risks, none of the species studied showed moderate or severe risk of lethal dehydration during an extremely hot day under current conditions (Figure 6). Current times to lethal dehydration during the hottest days ranged from 6 h in the serin and the great tit to up to 8 h in the house and Spanish sparrows, and in the crested lark (Figure 6). Similarly, times to lethal dehydration varied between 7 h in the Spanish sparrow and the crested lark to 6 h in the rest of our species under the RCP4.5 scenarios, with no species experiencing moderate or severe risk. However, according to the RCP8.5 scenario all the studied species, except the crested lark and the two sparrow species, will experience moderate risk of lethal dehydration during extremely hot days (Figure 6).

Discussion

Overall, we found that small-sized Mediterranean songbirds exhibit relatively low HTL and limited cooling efficiencies, resulting in moderate risks of lethal dehydration under the most severe climate change scenario. The HTLs of our small-sized species ranged from 40 ºC to 46 ºC. These values are among those reported for tropical (39 ºC – 45.5 ºC) and North-American temperate songbirds (39.6 ºC – 45.9 ºC) (Pollock et al., 2020), as well as for an arctic species (43 ºC; O'Connor et al., 2021). As predicted, however, Mediterranean species showed HTLs substantially lower than those reached by similar-sized songbirds from deserts and arid-zones (46 ºC – 54 ºC) (Withfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Smit et al., 2018; Kemp and McKechnie, 2019; Czenze et al., 2020). This limited heat tolerance among our species respect to arid-zone ones could result from greater endogenous heat production (higher mass-specific RMR slopes and T_b slopes) and limited evaporative heat dissipation capacity (lower evaporative scopes), as discuss below.

Mediterranean songbirds showed T_{uc} values similar to those found across songbirds from arid zones (Withfield et al., 2015, McKechnie et al., 2017; Smith et al., 2017; Czenze et al., 2020), as well as from tropical and temperate zones (Pollock et al., 2020). However, the T_{uc} values among the Mediterranean species studied here were above that reported for the Arctic songbird (O'Connor et al., 2021). Our results, therefore, highlight the limitations of using T_{uc} as a proxy of species' thermal tolerance in predictive models of species' vulnerability to climate change (as in Khaliq et al. 2014), as HTLs are well above T_{uc} and there is not a clear relationship between both traits (Mitchell et al. 2018). Among our species, all (but the crested lark) showed a clear T_{uc}, which differed across species. This indicates that some species show an earlier onset of active heat dissipation than others. Most notably, T_{uc} differed greatly between house and Spanish sparrows even though they reached the same HTL. The more urban house sparrows showed a lower T_{uc} value than Spanish sparrows, which live in more open habitats. Furthermore, the absence of a clear T_{uc} in the crested lark points in the same direction. That is, an adaptation of open-landscape species to forage under greater heat loads. This would enable them to postpone the beginning of active heat dissipation and thus extend foraging activity.

Our species displayed 1.4-fold increases in RMR respect to thermoneutral values at their HTLs, a value within the range shown by arid-zone songbirds (Withfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Smit et al., 2018; Kemp and McKechnie, 2019; Czenze et al., 2020) and an Arctic songbird (O'Connor et al. 2021). However, the mass-specific RMR slopes displayed by our songbirds was higher than those reported for arid-zone species (Table S3). This suggests that above T_{uc}, our species generate a greater amount of endogenous heat loads, which could partly explain why they achieved lower HTLs than similar-sized songbirds from deserts and arid-zones. Nevertheless, our species also showed greater mass-specific RMR slopes than the Arctic snow bunting *Plectrophenax nivalis* (O'Connor et al., 2021; Table S3), probably due to the lower T_{uc} shown by the latter. This implies earlier accumulation of metabolic heat, but at a lower rate compared to our species, which can postpone the beginning of panting at higher T_a but results in faster RMR increases.

Above thermoneutrality, our songbirds showed marked increases in EWL starting when the T_b-T_a gradient was still wide enough (~4.34 ºC). Snow bunting showed a similar pattern (O'Connor et al., 2021), while arid-zone songbirds delayed EWL inflection points (~37 to 45 ºC) until T_a approached or exceeded T_b (Withfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Czenze et al., 2020). This could be viewed as an adaptation of the arid-zone species for water conservation, while our species generally have access to drinking water and can thus compensate EWL. Although mass-specific EWL slopes of the studied species were similar to those of arid-zones species (Table S3), we found differences in their evaporative scopes. Mediterranean songbirds achieved lower evaporative scopes than Kalahari (Withfield et al., 2015) and Southern African songbirds (Czenze et al., 2020), although they were similar to those of Australian and Sonoran species (McKechnie et al., 2017; Smith et al., 2017) and greater than snow bunting (O'Connor et al., 2021) (Table S3). This limited evaporative scope among our Mediterranean songbirds compromises their capacity to dissipate endogenous heat loads, thus resulting in limited cooling efficiency and lower HTLs. Besides, among our species, smaller ones showed greater mass-specific EWL slopes, thus resulting in greater risks of lethal dehydration as discuss below.

Indeed, the maximum evaporative cooling efficiencies of Mediterranean songbirds were at the lower end of those reported across the clade. This confirms previous findings showing a limited EHL/MHP in a Mediterranean songbird, the great tit (Playà-Montmany et al., 2021). Among the species studied here, larger-bodied songbirds achieved greater EHL/MHP, supporting the notion that larger-sized species deal better with heat than smaller ones (reviewed by McKechnie et al., 2021). Nonetheless, only the crested lark (the largest species in our dataset) dissipated all the heat metabolically produced to achieve the maximum evaporative cooling efficiency (EHL/MHP = 1.11).

Above inflection points, T_b increased with T_a, suggesting that facultative hyperthermia is a common thermoregulatory response used by Mediterranean songbirds. This increase in T_b allowed birds to maintain a favourable gradient ($T_b > T_a$), thus permitting passive heat dissipation and saving body water by delaying EWL (Tieleman and Williams, 1999; Gerson et al., 2019). In a global context, T_b slopes in response to increasing T_a were similar to those reported for arid-zone and Arctic songbirds (Whitfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Czenze et al., 2020; O'Connor et al., 2021), but lower than those found in North-American temperate and tropical species (Pollock et al., 2020) (Table S3). However, our species started to increase T_b at lower T_a than arid-zone songbirds (Whitfield et al., 2015; McKechnie et al., 2017; Smith et al., 2017; Czenze et al., 2020), resulting in earlier increases in endogenous heat loads. This, together with higher mass-specific RMR slopes, lower evaporative scopes and lower evaporative cooling efficiencies, could explain the lower HTLs achieved by our species compared to arid-zone ones.

Among Mediterranean songbirds, the crested lark — which inhabits open landscapes and usually faces high solar radiation loads and operative temperatures that surpass T_b — achieved the highest HTL (46 ºC), a value identical to that recorded for two arid zone songbirds (the yellow-plummed honeyeater *Lichenostomus ornatus* and the Orange-river white-eye *Zosterops pallidus*) (McKechnie et al., 2017; Czenze et al., 2020). In contrast, the great tit — mainly a forest dweller — only tolerated a maximum of 40 ºC during the trials, suggesting that this species probably resort to behavioural thermoregulation to avoid lethal hyperthermia during heat events, as this temperature is often exceeded every summer in our study area. These differences among species and the relatively low HTLs of Mediterranean songbirds suggest a strong influence of the natural temperature regime experienced by the species on their heat tolerance, with those usually facing environmental temperatures approaching or exceeding T_b reaching greater HTLs (as reviewed in McKechnie and Wolf, 2019; Freeman et al., 2022).

Current and future vulnerability to high temperatures

Regardless the period and the climate change scenario considered, maximum environmental temperatures experienced during summer in Extremadura forced Mediterranean songbirds to engage in physiological thermoregulation. Among species, the mean number of days above T_{uc} differed markedly, pointing to different risks of sublethal chronic effects of heat exposure. Those species mainly foraging in open landscapes such as the Spanish sparrow, the greenfinch or the goldfinch showed a lower number of days per summer in which they have to resort active thermoregulation (Table S2). While urban (the house sparrow) and mainly forest songbirds (the great tit and the chaffinch), as well as our smallest species (the serin) experienced a greater mean number of days above T_{uc} (Table S2). Therefore, the latter species could be more prone to deleterious fitness effects of repeated exposition to environmental temperatures surpassing their T_{uc} (e.g., through trade-offs and constraints that thermoregulatory mechanisms may induce) (Du Plessis et al., 2012; Cunningham et al., 2021). Yet, only the great tit (mainly a forest dweller) will experience direct risk of heat-related mortality across Extremadura. To the end of the century, climatic models forecast that great tits will experience on average 5 days per summer in which T_{max} will surpass its HTL in the southeast of the region under the RCP8.5 warming scenario.

Mediterranean songbirds might reduce lethal dehydration risk by combining the use of thermally-buffered microhabitats during the hottest part of the day (such as shaded places near water streams, shrubs' shade or tree hollows) and regularly drinking to sustain high EWL rates needed to avoid lethal hyperthermia. Despite this, the forecasted temperature increase by the end of the century indicates that all our species (but the crested lark and the house and Spanish sparrows) will experience moderate risk of lethal dehydration under the RCP8.5 warming scenario. This highlighted that across our studied species, smaller ones are more prone to experience dehydration risks as their higher mass-specific EWL slopes result in faster loss of body water, similar than reported for arid-zone species (McKechnie and Wolf, 2010). Furthermore, it is important to note that the EWL rates employed to calculate lethal dehydration times come from resting individuals under laboratory conditions (i.e., overlooking the effect of solar radiation or activity). In the wild, these rates could be substantially higher due to locomotor activity, heat increment of feeding (González-Medina et al. 2020), or higher environmental heat loads. This, combined with the predicted increase in heavy droughts along the Mediterranean Basin (IPCC, 2021), could lead to shorter times to lethal dehydration than predicted among Mediterranean songbirds. In turn, this might result in severe risk of lethal dehydration as forecasted for several arid-zone songbirds (McKechnie and Wolf 2010; Albright et al., 2017; Conradie et al., 2020).

Conclusions

Our findings indicate that Mediterranean songbirds are more sensitive to high-temperature extremes than arid-zone and desert songbirds. Their limited cooling efficiencies are likely due to their relatively low evaporative scopes, which cannot compensate for the endogenous heat loads (RMR and T_b increases) in response to heat. Although the studied species are not currently experiencing lethal dehydration risks, they all are experiencing several summer days in which their thermoregulatory capacities are challenged. This will be exacerbated under predicted future warming scenarios, which will increase the risks of lethal dehydration during heat waves and, ultimately, threaten the persistence of songbird species in southwestern Spain and other Mediterranean regions that are warming rapidly (IPCC 2021).

References

Albright, T.P., Mutiibwa, D., Gerson, A.R., Smith, E.K., Talbot, W.A., O'Neill, J.J., McKechnie, A.E., and Wolf, B.O. (2017). Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proc. Natl. Acad. Sci. U.S.A.* **114 (9)**, 2283-2288.

Barredo, J.I., Mauri, A., Caudullo, G., and Dosio, A. (2018). Assesing shifts of Mediterranean and arid climates under RCP4.5 and RCP8.5 climate projections in Europe. *Pure Appl. Geophys.* **175**, 3955-3971.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* **67(1)**.

Calder, W.A., and Schmidt-Nielsen, K. (1966) Evaporative cooling and respiratory alkalosis in the pigeon. *Proc. Natl. Acad. Sci. U.S.A.* **55(4)**, 750–756.

Cardoso-Pereira, S., Marta-Almeida, M., Carvalho, A.C., and Rocha, A. (2017). Heat wave and cold spell changes in Iberia for a future climate change scenario. *Int. J. Climatol.* **37**, 5192-5205.

Conradie, S.R., Woodborne, S.M., Cunningham, S.J., and McKechnie, A.E. (2019). Chronic, sublethal effects of high temperatures will cause severe declines in southern African arid-zone birds during the 21st century. *Proc. Natl. Acad. Sci. U.S.A.* **116(28)**, 14065-14070.

Conradie, S.R., Woodborne, S.M., Wolf, B.O., Pessato, A., Mariette, M.M., and McKechnie, A.E. (2020). Avian mortality risk during heat waves will increase greatly in arid Australia during the 21st century. *Conserv. Physiol.* **8(1)**, coaa048.

Cook, T.R., Martin, R., Roberts, J., Häkkinen, H., Botha, P., Meyer, C., Sparks, E., Underhill, L.E., Ryan, P.G., and Sherley, R.B. (2020). Parenting in a warming world: thermoregulatory responses to heat stress in an endangered seabird. *Conserv. Physiol.* **8(1)**, coz109.

Coumou, D., and Robinson, A. (2013). Historic and future increase in the global land area affected by monthly heat extremes. *Environ. Res. Lett.* **8**, 034018.

Cunningham, S.J., Martin, R.O., Hojem, C.L., and Hockey, P.A.R. (2013). Temperatures in excess of critical thresholds threaten nestling growth and survival in a rapidly-warming arid savanna: a study of common fiscals. *PloS ONE*. **8(9)**, e74613.

Cunningham, S.J., Gardner, J.L., and Martin, R.O. (2021). Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* **19(5)**, 300-307.

Czenze, Z.J., Kemp, R., Jaarsveld, B. van, Freeman, M.T., Smit, B., Wolf, B.O., and McKechnie, A.E. (2020). Regularly drinking desert birds have greater evaporative cooling capacity and higher heat tolerance limits than non-drinking species. *Funct. Ecol.* **34**, 1589-1600.

Dawson, W.R. (1982). Evaporative losses of water by birds. *Comp. Biochem. Physiol.* **71(4)**, 495-509.

Delhey, K., Guallar, S., Rueda-Hernández, R., Valcu, M., Wang, D., and Kempenaers, B. (2020). Partial or complete? The evolution of post-juvenile moult strategies in passerine birds. *J.Anim. Ecol.* **89(12)**, 2896-2908.

Du Plessis, K.L., Martin, R.O., Hockey, P.A.R., Cunningham, S.J., and Ridley, A.R. (2012). The costs of keeping cool in a warming world: implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Glob. Change Biol.* **18**, 3063-3070.

Freeman, M.T., Czenze, Z.J., Schoeman, K., and McKechnie, A.E. (2020). Extreme hyperthermia tolerance in the world's most abundant wild bird. *Sci. Rep.* **10**, 13098.

Freeman, M.T., Czenze, Z.J., Schoeman, K., and McKechnie, A.E. (2022). Adaptive variation in the upper limits of avian body temperature. *Proc. Natl. Acad. Sci. U.S.A.* **119(26)**, e2116645119.

Gerson, A.R., McKechnie, A.E., Smit, B., Whitfield, M.C., Smith, E.K., Talbot, W.A., McWorther, T.J., and Wolf, B.O. (2019). The functional significance of facultative hyperthermia varies with body size and phylogeny in birds. *Funct. Ecol.* **33**, 597-607.

González-Medina, E., Cabello-Vergel, J., Playà-Montmany, N., Villegas, A., Parejo, M., Abad-Gómez, J.M., Sánchez-Guzmán, J.M., and Masero, J.A. (2020). Going to sleeps with a full belly: thermal substitution by specific dynamic action in shorebirds. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **244**, 110689.

Gutiérrez, J.M., R.G. Jones, G.T. Narisma, L.M. Alves, M. Amjad, I.V. Gorodetskaya, M. Grose, N.A.B. Klutse, S. Krakovska, J. Li, D. Martínez-Castro, L.O. Mearns, S.H. Mernild, T. Ngo-Duc, B. van den Hurk, and J.-H. Yoon. (2021). Atlas. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L.Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K.Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press. In Press. Interactive Atlas available from Available from http://interactive-atlas.ipcc.ch/

IPCC (2021). Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press. In Press.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; Core Writing Team, Pachauri, R.K., Meyer, L.A., Eds.; IPCC: Geneva, Switzerland, 2014; p. 151.

Jacob, D., Petersen, J., Eggert, B., Alias, A., Christensen, O.B., Bouwer, L.M., et al. (2014). EURO-CORDEX: a new high-resolution climate change projections for European impact research. *Reg. Environ.* **14(2)**, 563-568.

Kemp, R., and McKechnie, A.E. (2019). Thermal physiology of a range-restricted desert lark. *J. Comp. Physiol. B.* **189**, 131-141.

Kemp, R., Freeman, M.T., Jaarsveld, B. van, Czenze, Z.J., Conradie, S.R., and McKechnie, A.E. (2020). Sublethal fitness costs of chronic exposure to hot weather vary between sexes in a threatened desert lark. *Emu*. **120(3)**, 216-229.

Khaliq, I., Hof, C., Prinzinger, R., Böhning-Gaese, K., and Pfenninger, M. (2014). Global variation in thermal tolerances and vulnerability of endotherms to climate change. *Proc. R. Soc. B*. **281**, 20141097.

Klaassen, M. (1995). Moult and basal metabolic costs in males of two subspecies of stonechats: the European *Saxicola torquata rubicola* and the East African *S.t. axillaris*. *Oecologia*. **104(4)**, 424-432.

Lighton, J.R.B., Bartholomew, G.A., and Feener, D.H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the Leaf-cutting Ant *Atta colombica Guer*. *Physiol. Zool.* **60**, 524-537.

Lighton, J.R.B. (2008). Measuring metabolic rates: A manual for scientists. Oxford University Press, Oxford.

McNab, B.K. (2002). The physiological ecology of vertebrates: A view from energetics. Cornell University Press, Ithaca, New York.

Mitchell, D., Snelling, E.P., Hetem, R.S., Maloney, S.K., Strauss, W.M., and Fuller, A. (2018). Revisiting concepts of thermal physiology: predicting responses of mammals to climate change. *J. Anim. Ecol.* **00**, 1-18.

Muggeo, V.M.R. (2009). Segmented: a R package to fit regression models with broken-line relationships.

McKechnie, A.E., and Wolf, B.O. (2010). Climate change increases the likelihood of catastrophic avian mortality events during extreme heatwaves. *Biol. Lett.* **6**, 253-256.

McKechnie. A.E., Hockey, P.A.R., and Wolf, B.O. (2012). Feeling the heat: Australian land birds and climate change. *Emu*. **112**, 1-7.

McKechnie, A.E., Gerson, A.R., McWorther, T.J., Smith, E.K., Talbot, W.A., and Wolf, B.O. (2017). Avian thermoregulation in the heat: evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance. *J. Exp. Biol.* **220**, 2436-2444.

McKechnie. A.E., and Wolf, B.O. (2019). The physiology of heat tolerance in small endotherms. *Physiology*. **34**, 302-313.

McKechnie, A.E., Rusworth, I. A., Myburgh, F., and Cunningham, S.J. (2021). Mortality among birds and bats during an extreme heat event in eastern South Africa. *Austral Ecol.* **46**, 687-691.

McKechnie, A.E., Gerson, A.R., and Wolf, B.O. (2021). Thermoregulation in desert birds: scaling and phylogenetic variation in heat tolerance and evaporative cooling. *J. Exp. Biol.* **224**, jeb229211.

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., and Pearse, W. (2018). Caper: comparative analyses of phylogenetics and evolution in R. R package version 1.0.1. https://CRAN.R-project.org/package=caper

Oswald, K.N., Evlambiou, A.A., Ribeiro, A.M., and Smit, B. (2018a). Tag location and risk assessment for passive integrated transponder-tagging passerines. *Ibis*. **160**, 453-457.

Oswald, K.N., Lee, A.T.K., and Smit, B. (2018b). Seasonal physiological responses to heat in an alpine range-restricted bird: the Cape Rockjumper (*Chaetops frenatus*). *J. Ornithol.* **159**, 1063-1072.

O'Connor, R.S., Le Pogam, A., Young, K.G., Robitaille, F., Choy, E.S., Love, O.P., Elliot, K.H., Hargreaves, A.L., Berteaux, D., Tam, A., and Vézina, F. (2021). Limited heat tolerance in an Arctic passerine: Thermoregulatory implications for cold-specialized birds in a rapidly warming world. *Ecol. Evol.* **11(4)**, 1609-1619.

Pollock, H.S., Brawn, J.D., and Cheviron, Z. A. (2020). Heat tolerances of temperate and tropical birds and their implications for susceptibility to climate warming. *Funct. Ecol.* **35**, 93-104.

Playà-Montmany, N., González-Medina, E., Cabello-Vergel, J., Parejo, M., Abad-Gómez, J.M., Sánchez-Guzmán, J.M., Villegas, A., and Masero, J.A. (2021). The thermoregulatory role of relative bill and leg surface areas in a Mediterranean population of Great tit (*Parus major*). *Ecol. Evol.* **11(22)**, 15936-15946.

Quintana, F., Uhart, M.M., Gallo, L., Mattera, M.B., Rimondi, A., and Gómez-Laich, A. (2022). Heat-related massive chick mortality in an Imperial Cormorant *Leucocarbo atriceps* colony from Patagonia, Argentina. *Polar Biol.* **45**, 275-284.

Quintero, I., and Wiens, J.J. (2013). Rates of projected climate change dramatically exceed past rates of climatic niche evolution among vertebrate species. *Ecol. Lett.* **16(8)**, 1095-1103.

Saunders, D.A., Mawson, P., and Dawson, R. (2011). The impact of two extreme weather events and other causes of death on Carnaby's Black Cockatoo: a promise of things to come for a threatened species? *Pac. Conserv. Biol.* **17**, 141-148.

Smith, E.K., O'Neill, J.J., Gerson, A.R., McKechnie, A.E., and Wolf, B.O. (2017). Avian thermoregulation in the heat: resting metabolism, evaporative cooling and heat tolerance in Sonoran Desert songbirds. *J. Exp. Biol.* **220**, 3290-3300.

Smit, B., Whitfield, M.C., Talbot, W.A., Gerson, A.R., McKechnie, A.E., and Wolf, B.O. (2018). Avian thermoregulation in the heat: phylogenetic variation among avian orders in evaporative cooling capacity and heat tolerance. *J. Exp. Biol.* **221**, jeb174870.

Tieleman, I.B., and Williams, J.B. (1999). The role of hyperthermia in the water economy of desert birds. *Physiol. Biochem. Zool.* **72(1)**, 87-100.

Tracy, C.R., Welch, W.R., Pinshow, B., and Porter, W.P. (2010). Properties of air: a manual for use in biophysical ecology, 4th edn. The University of Wisconsin Laboratory for Biophysical Ecology: Technical Report.

Van Dyk, M., Noakes, M.J., and McKechnie, A.E. (2019). Interactions between humidity and evaporative heat dissipation in a passerine bird. *J. Comp. Physiol. B.* **189**, 299-308.

Van de Ven, T.M.F.N., McKechnie, A.E., and Cunningham, S.J. (2019). The costs of keeping cool: behavioural trade-offs between foraging and thermoregulation are associated with significant mass losses in an arid-zone bird. *Oecologia*. **19**, 205-215.

Van de Ven, T.M.F.N., McKechnie, A.E., and Cunningham, S.J. (2020). High temperatures are associated with substantial reductions in breeding success and offspring quality in an arid-zone bird. *Oecologia* **193**, 225-235.

Viceto, C., Pereira, S., and Rocha, A. (2019). Climate change projections of extreme temperatures for the Iberian Peninsula. *Atmosphere*. **10(5)**, 0229.

Walsberg, G.E., and Wolf, B.O. (1995). Variation in the respiratory quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. *J. Exp. Biol.* **198**, 213-219.

Welbergen, J.A., Klose, S.M., Markus, N., and Eby, P. (2008). Climate change and the effects of temperature extremes on Australian flying-foxes. *Proc. R. Soc. B.* **275**, 419-425.

Whitfield, M.C., Smit, B., McKechnie, A.E., and Wolf, B.O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* **218**, 1705-1714.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., and Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLOS Biol.* **6(12)**, e325.

Wolf, B.O., and Walsberg, G.E. (1996a). Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. *J. Exp. Biol.* **199**, 451-457.

Appendix 1: Supplementary Material

Methods

Study area

This study took place in Badajoz (38º51’ N, 6º58’ W), which is part of Extremadura autonomous region, southwestern Spain and is located in the western end of the Mediterranean Basin (Figure S1).

Figure S1. Map showing the Mediterranean Basin, with Extremadura highlighted in black.

Climatological data

During the period 1993-2019, the analysis of climatological data from Badajoz airport weather station (Spanish Meteorology Agency) showed a significant increase in the number of hot days (i.e., those in which maximum ambient temperature reached or surpassed 35 ºC) during summer ($\beta = 1.01$; $p = < 0.001$) (Figure S2).

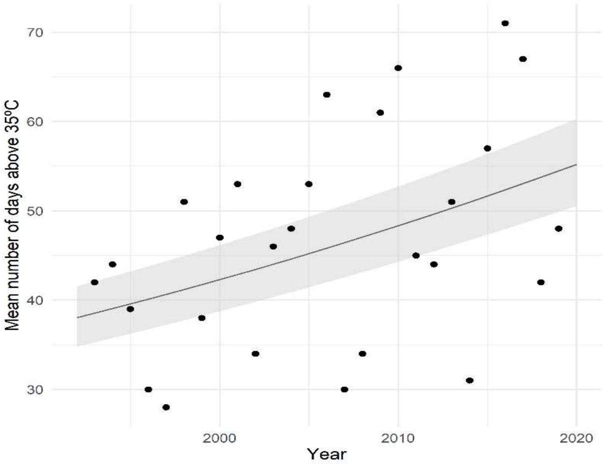

Figure S2. Number of hot days during summer (≥ 35 ºC) along the 1993-2019 period in the study area.

Phylogenetic interspecific comparisons

We tested for possible interspecific differences in thermoregulatory traits among Mediterranean songbirds due to phylogenetic relatedness and body size. To do so, we employed the R package ‘caper’ (Orme et al. 2018) to adjust phylogenetic generalized least squares models to different thermoregulatory traits (mass-specific RMR slope, mass-specific EWL slope, evaporative scope, EHL/MHP slope, maximum EHL/MHP, T_b slope, and HTL), with mean body mass (M_b) of each species as predictor variable. We accounted for phylogeny effect by including a phylogenetic consensus tree, which we derived from 10,000 trees with different topologies obtained from Bird Tree project using ‘Hackett All Species’ as backbone (Jetz et al. 2012). When lambda (λ) = 0 the covariance between species is null, which indicates that a non-phylogenetic regression is more appropriate for data. On the contrary, λ = 1 denote a strong phylogenetic signal in data.

We obtained null phylogenetic signal for each thermoregulatory trait tested, as λ = 0 (Table S1). Therefore, we opted to analyse our data using non-phylogenetic generalized linear models.

Table S1. Statistics from phylogenetic generalized least squares models adjusted to different thermal traits of Mediterranean songbirds. In each model, λ equals to zero and indicates and absence of phylogenetic signal in the data.

Model		β	s.e.	t-value	p-value	λ
RMR slope ~ M_b						0.00
	Intercept	0.005	0.02	0.28	0.793	
	M_b	0.001	0.00	1.32	0.244	
EWL slope ~ M_b						0.00
	Intercept	18.725	10.95	1.71	0.148	
	M_b	2.983	0.59	5.07	0.004	
Evaporative scope ~ M_b						0.00
	Intercept	2.359	1.42	1.67	0.157	
	M_b	0.168	0.08	2.21	0.078	
EHL/MHP slope ~ M_b						0.00
	Intercept	0.047	0.02	2.72	0.042	
	M_b	0.001	0.00	1.21	0.280	
Max EHL/MHP ~ M_b						0.00
	Intercept	0.587	0.15	3.82	0.012	
	M_b	0.013	0.01	1.53	0.186	
T_b slope ~ M_b						0.00
	Intercept	0.383	0.04	9.58	0.001	
	M_b	-0.005	0.00	-2.19	0.079	
HTL ~ M_b + Evaporative scope						0.00
	Intercept	37.111	1.37	27.11	0.001	
	M_b	-0.090	0.08	-1.09	0.337	
	Evaporative scope	1.370	0.35	3.95	0.017	

Future climate warming projections

We downloaded future daily maximum environmental temperature projections from Coupled Model Intercomparison Project 5 (CMIP5) for climate warming scenarios RCP4.5 and RCP8.5 from the Spanish Climate Change Office' project *AdapteCCa* (https://www.adaptecca.es/). This dataset contained projections from the following 16 models from EURO-CORDEX (Jacob et al. 2014): CNRM-CERFACS-CNRM-CM5_r1i1p1_CLMcom-CCLM4-8-17_v1; CNRM-CERFACS-CNRM-CM5_r1i1p1_CNRM-ALADIN53_v1; CNRM-CERFACS-CNRM-CM5_r1i1p1_SMHI-RCA4_v1; ICHEC-EC-EARTH_r12i1p1_CLMcom-CCLM4-8-17_v1; ICHEC-EC-EARTH_r12i1p1_SMHI-RCA4_v1; ICHEC-EC-EARTH_r1i1p1_KNMI-RACMO22E_v1; ICHEC-EC-EARTH_r3i1p1_DMI-HIRHAM5_v1; IPSL-IPSL-CM5A-MR_r1i1p1_IPSL-INERIS-WRF331F_v1; IPSL-IPSL-CM5A-MR_r1i1p1_SMHI-RCA4_v1; MOHC-HadGEM2-ES_r1i1p1_CLMcom-CCLM4-8-17_v1; MOHC-HadGEM2-ES_r1i1p1_KNMI-RACMO22E_v2; MOHC-HadGEM2-ES_r1i1p1_SMHI-RCA4_v1; MPI-M-MPI-ESM-LR_r1i1p1_CLMcom-CCLM4-8-17_v1; MPI-M-MPI-ESM-LR_r1i1p1_SMHI-RCA4_v1a; MPI-M-MPI-ESM-LR_r2i1p1_MPI-CSC-REMO2009_v1; NCC-NorESM1-M_r1i1p1_DMI-HIRHAM5_v2.

Hourly profiles of maximum ambient temperature during heatwaves

We calculated hourly maximum temperature profile during an extremely hot day by averaging hourly values from the hottest 10 days recorded in our study area during the last 20 years (namely 10th of August 2012, 6th of September 2016, 16th and 17th of June 2017, 13th of July 2017, 2nd, 3rd, 4th, 5th and 6th of August 2018) (Figure S3). To this current hourly profile, we added 1.8 and 3.7 ºC respectively to represent climate warming scenarios RCP4.5 and RCP8.5, respectively (Figure S3).

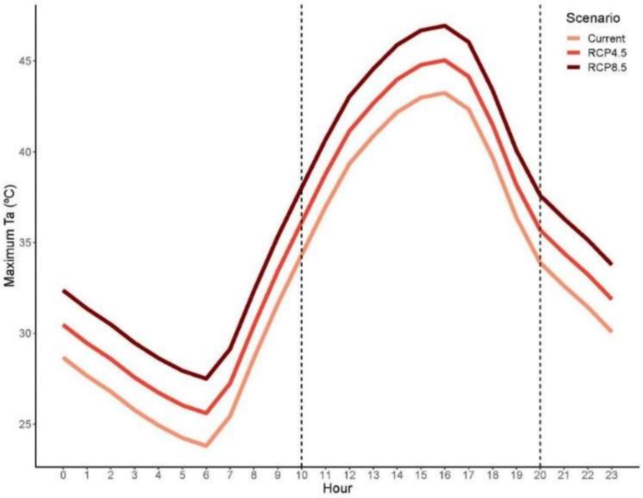

Figure S3. Daily hourly profile of ambient temperature during extremely hot days in the study area (data obtained from Badajoz Airport weather station, Spanish Meteorology Agency) at present and in the future (2070-2100) under two different climate warming scenarios (RCP4.5 and RCP8.5). Dotted lines denoted the time period during which lethal dehydration risk was calculated for the studied Mediterranean songbirds.

Results

Current and future vulnerabilities of Mediterranean songbirds

We calculated the mean number of days in which maximum environmental temperature is above the T_{uc} and heat tolerance limit (HTL) for each species along Extremadura under current and future climate warming scenarios (RCP4.5 and RCP8.5) (Table S2).

Table S2. Mean number of days (± S.D.) per summer (June to September) above the T_{uc} and the HTL for each species at current (2006-2021) and under future (2070-2100) climate warming scenarios along Extremadura. We could not calculate days above T_{uc} for the crested lark as we could not identify a clear T_{uc} for this species.

Species	Days above T_{uc}			Days above HTL		
	Current	**RCP4.5**	**RCP8.5**	**Current**	**RCP4.5**	**RCP8.5**
Serin	20.38 ± 16.9	63.64 ± 13.8	91.38 ± 8.8	0	0	0
Goldfinch	7.18 ± 11.4	47.51 ± 15.3	82.69 ± 7.8	0	0	0
Great tit	13.09 ± 14.9	55.87 ± 14.3	86.78 ± 7.7	0	0	4.94 ± 11.1
Chaffinch	18.55 ± 16.7	61.90 ± 13.9	90.24 ± 8.6	0	0	0
Greenfinch	6.94 ± 11.3	47.13 ± 15.4	82.46 ± 7.4	0	0	0
House sparrow	33.07 ± 15.9	73.70 ± 11.8	98.11 ± 8.9	0	0	0
Spanish sparrow	0	7.63 ± 13.0	54.89 ± 14.3	0	0	0
Crested lark	n.a.	n.a.	n.a.	0	0	0

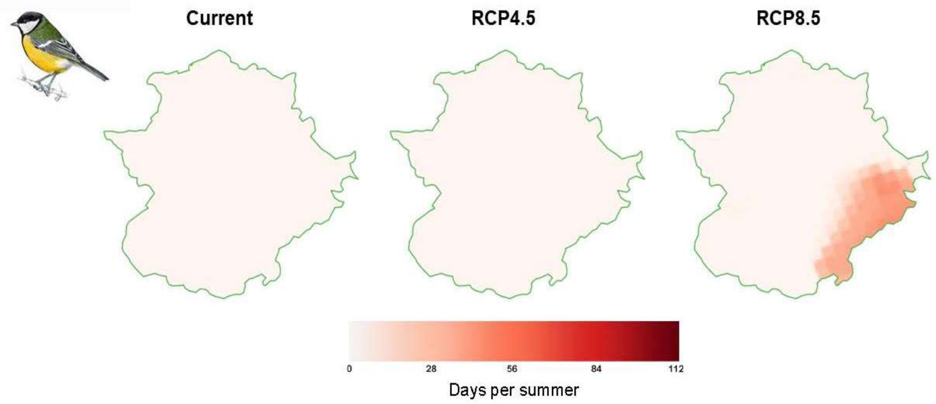

Figure S4. Mean number of days per summer above HTL for the great tit *Parus major* along Extremadura under current (2006-2021) and future (2070-2100) climate warming scenarios (RCP4.5 and RCP8.5). Great tit illustration is reproduced with permission from Juan Varela.

Climate model projections average showed that among our studied species, only the great tit will experience days with maximum environmental temperature above its HTL under future conditions (2070-2100) when RCP8.5 scenario was considered (Figure S4).

Discussion

Comparisons among songbirds from different climatic regions

We compared our results for Mediterranean songbirds to those previously reported for songbirds from different climatic regions across the globe (Whitfield et al. 2015; McKechnie et al. 2017; Smith et al. 20017; Pollock et al. 2020; Czenze et al. 2020; O’Connor et al. 2021). We limited the comparisons to songbirds comprised between a body mass range of ~5 to ~50 g (Table S3).

Table S3. Means (± S.D.) of mass-specific RMR (mW g^{-1} ºC^{-1}) and EWL slopes (mg H_2O g^{-1} h^{-1} ºC^{-1}), evaporative scope and T_b slope (ºC ºC^{-1}) for similar-sized small songbirds from different regions.

Region (*N* species)	**M_b (g)**	**RMR$_{\text{mass-specific}}$**	**EWL$_{\text{mass-specific}}$**	**Evaporative scope**	**T_b slope**	**Source**
SW Iberia (8)	10-34	1.67 ± 0.63	3.92 ± 0.72	5.45 ± 1.18	0.30 ± 0.04	Own
Kalahari (3)	10-40	0.56 ± 0.22	4.53 ± 0.52	15.07 ± 3.88	0.33 ± 0.12	Whitfield et al. 2015
Australia (3)	17-52	0.88 ± 0.35	4.57 ± 0.92	7.56 ± 0.91	0.29 ± 0.06	McKechnie et al. 2017
Sonora (6)	10-42	0.76 ± 0.23	4.48 ± 0.77	6.86 ± 3.02	0.23 ± 0.04	Smith et al. 2017
South Carolina (13)	12-46	Non reported	Non reported	Non reported	0.55 ± 0.36	Pollock et al. 2020
Panama (39)	7-56	Non reported	Non reported	Non reported	0.51 ± 0.15	
Southern Africa (12)	7-46	0.83 ± 0.44	5.79 ± 1.88	9.31 ± 2.75	0.30 ± 0.06	Czenze et al. 2020
Arctic (1)	31	0.452	2.193	2.900	0.299	O’Connor et al. 2021

References

Czenze, Z.J., Kemp, R., Jaarsveld, B. van, Freeman, M.T., Smit, B., Wolf, B.O., and McKechnie, A.E. (2020). Regularly drinking desert birds have greater evaporative cooling capacity and higher heat tolerance limits than non-drinking species. *Funct. Ecol.* **34**, 1589-1600.

Jacob, D., Petersen, J., Eggert, B., Alias, A., Christensen, O.B., Bouwer, L.M., et al. (2014). EURO-CORDEX: a new high-resolution climate change projections for European impact research. *Reg. Environ.* **14**(2), 563-568.

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K., and Mooers, A.O. (2012). The global diversity of birds in space and time. *Nature*. **491**, 444-448.

McKechnie, A.E., Gerson, A.R., McWorther, T.J., Smith, E.K., Talbot, W.A., and Wolf, B.O. (2017). Avian thermoregulation in the heat: evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance. *J. Experim. Biol.* **220**, 2436-2444.

O'Connor, R.S., Le Pogam, A., Young, K.G., Robitaille, F., Choy, E.S., Love, O.P., Elliot, K.H., Hargreaves, A.L., Berteaux, D., Tam, A., and Vézina, F. (2021). Limited heat tolerance in an Arctic passerine: Thermoregulatory implications for cold-specialized birds in a rapidly warming world. *Ecol. Evol.* **11**(4), 1609-1619.

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., and Pearse, W. (2018). caper: comparative analyses of phylogenetics and evolution in R. R package version 1.0.1.

Pollock, H.S., Brawn, J.D., and Cheviron, Z. A. (2020). Heat tolerances of temperate and tropical birds and their implications for susceptibility to climate warming. *Funct. Ecol.* **35**, 93-104.

Smith, E.K., O'Neill, J.J., Gerson, A.R., McKechnie, A.E., and Wolf, B.O. (2017). Avian thermoregulation in the heat: resting metabolism, evaporative cooling and heat tolerance in Sonoran Desert songbirds. *J. Exp. Biol.* **220**, 3290-3300.

Whitfield, M.C., Smit, B., McKechnie, A.E., and Wolf, B.O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol*. **218**, 1705-1714.

3

Seasonal and between-population variation in heat tolerance and cooling efficiency in a Mediterranean songbird

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Manuscript

Abstract

Discrete populations of widely distributed species may inhabit areas with marked differences in climatic conditions across geographic and seasonal scales, which could result in intraspecific variation in thermal physiology reflecting genetic adaptation, phenotypic plasticity, or both. However, few works have studied within and inter-population variation in physiological responses to heat. We evaluated seasonal and inter-population variation in heat tolerance and other key thermoregulatory traits in two Mediterranean populations of Great tit *Parus major* experiencing different thermal environments: a lowland population subject to warmer summers and greater annual thermal amplitude and a montane population subject to milder summer temperatures and lower thermal amplitude. We measured heat tolerance limits (HTL), and body temperature, resting metabolic rate (RMR), evaporative water loss (EWL), and evaporative cooling efficiency within and above the thermoneutral zone (TNZ) during winter and summer. Overall, Great tits enhanced their HTL and evaporative cooling efficiency – the ratio between evaporative heat loss to metabolic heat production – during summer through reduced endogenous heat loads (i.e., lower body temperatures and RMR). Although heat tolerance during summer was similar between populations, birds from the lowland site showed greater evaporative cooling efficiency (e.g., greater slopes and maximum values at their HTL) and displayed greater seasonal flexibility in their thermoregulatory traits above thermoneutrality, thus supporting the climatic variability hypothesis. Our results also support the idea that adaptive thermoregulation in songbirds is more flexible than initially thought and highlight the importance of considering within and inter-population variation in thermoregulatory traits when modelling the future distribution and persistence of species under different climate change scenarios.

Keywords: birds, climate warming, climatic variability hypothesis, heat tolerance, phenotypic flexibility, thermoregulatory traits

Introduction

Thermoregulation is one of the fundamental physiological demands on endothermic vertebrates (McNab 2002), which employ a variety of mechanisms that allow them to cope with climatic extremes (e.g., Trost 1972; Broggi et al. 2004; Bailey et al. 2022). This is particularly true for species whose populations experience contrasting thermal environments across their distribution range (Root 1988) or those that inhabit highly seasonal environments (e.g., Cavieres & Sabat 2008). Thus, intraspecific variation and phenotypic flexibility (*sensu* Piersma & Drent 2003) in thermoregulatory traits can provide important insights into adaptive variation in thermal tolerance (Somero 2010). Indeed, these have been suggested as critical for species persistence in a warming climate (Boyles et al. 2011). However, within and inter-population variation in thermal physiology has been largely overlooked when forecasting species responses to rising temperatures, thus limiting our capacity to predict species vulnerability to climate warming scenarios (Valladares et al. 2014; Moran, Hartig & Bell 2016).

In birds, seasonal adjustments in thermoregulatory traits have been well-studied across temperate and tropical species in response to cold conditions (e.g., McKechnie, Noakes & Smit 2015; Oboikovitz & Swanson 2021; Stager et al. 2016, 2021). Overall, north-temperate birds upregulate their basal (BMR) and summit metabolic rates in winter to maximize cold tolerance and thermogenic capacity (Liknes et al. 2002; Swanson 2010; Petit et al. 2013; Oboikovitz & Swanson 2021), whereas tropical and subtropical birds display more variable patterns (Smit & McKechnie 2010; Noakes, Wolf & McKechnie 2017; Pollock et al. 2019; Noakes & McKechnie 2020). However, there is scarce information on how birds seasonally adjust their thermoregulatory machinery to maximize heat tolerance and cooling efficiency during summer (Tieleman et al 2002b; Noakes et al. 2016; O'Connor et al. 2017; Oswald et al. 2018; Playà-Montmany 2022). These few studies provide evidence for greater individuals' acclimation capacity to heat than previously thought (Araújo et al. 2013).

Panting is one of the main physiological responses to dissipate internal heat loads and avoid lethal hyperthermia in songbirds (McKechnie & Wolf 2019). As panting increases metabolic heat production and evaporative water loss (EWL), we could expect that summer acclimatization to heat would enhance evaporative cooling efficiency (defined as evaporative heat loss to metabolic heat production ratio) and heat tolerance limit (HTL, maximum air temperature tolerated before the onset of severe heat stress). Indeed, this pattern has been shown in species from arid (Noakes et al. 2016; Oswald et al. 2018) and temperate Mediterranean regions (Playà-Montmany 2022). For example, arid-zone songbirds increase their evaporative cooling efficiency in summer through increased EWL and diminished or unchanged resting metabolic rates (RMR) (Noakes et al. 2016; Oswald, Lee & Smit 2018). Besides, Noakes et al. (2016) found that White-browed sparrow-weavers *Plocepasser mahali* reduce their body temperature from winter to summer to reduce their total endogenous heat loads. More recently, Playà-Montmany (2022) showed that Mediterranean resident songbirds decrease BMR during summer and elevate their upper critical temperature (T_{uc}) and EWL inflection points.

Previous interpopulation studies on arid-zone songbird species have found significant intraspecific variability in physiological responses to heat (Trost et al. 1972; Noakes et al. 2016). For instance, Horned larks *Eremophila alpestris* and White-browed sparrow-weavers from hotter and more arid sites exhibited lower EWL rates than populations from milder, mesic sites (Trost 1972; Noakes et al. 2016). This, combined with reduced endogenous heat loads (i.e., lower body temperature and RMR), allows populations from the warmer environments to achieve higher evaporative cooling efficiencies (Trost et al. 1972; Noakes et al. 2016). Furthermore, Noakes et al. (2016) found that White-browed sparrow-weavers from the hotter site showed greater HTL than those from milder ones. Nonetheless, a more recent study showed that reaction norms for both HTL and evaporative cooling efficiency do not vary among populations of this species (Noakes & McKechnie 2019). After acclimating White-browed sparrow-weavers to different temperature conditions (30 ºC, 36 ºC and 42 ºC), Noakes & McKechnie (2019) found that HTL and evaporative cooling efficiency was higher in individuals acclimated to the highest temperature, but the site of capture (hotter vs milder) had no influence on these thermoregulatory traits. Overall, these contrasting results highlight the need to further evaluate intraspecific variability in physiological responses to heat across more species and populations from different regions.

In this paper, we investigated seasonal and inter-population variation in HTL and other key thermoregulatory traits (body temperature, RMR, EWL, and evaporative cooling efficiency) in two Mediterranean populations of Great tit *Parus major* that experience different climatic conditions: a lowland population subject to warmer summers (with maximum temperatures usually exceeding 40 ºC) and greater annual thermal amplitude, and a montane population subject to milder summer temperatures (with maximum temperatures rarely surpassing 35 ºC) and lower thermal amplitude. We hypothesized that HTL and evaporative cooling efficiency during summer would be higher in birds from the lowland population, as they are routinely exposed to environmental temperatures that approach or even exceed normothermic body temperature. The climatic variability hypothesis posits that species experiencing greater climatic variability should display greater phenotypic flexibility in their thermoregulatory traits and thus broader temperature tolerances (Steven 1989; Bozinovic et al. 2011). This pattern has also been described at the population level when comparing the thermogenic capacity of individuals from populations distributed along a thermal gradient (Oboikovitz & Swanson 2021; Stager et al. 2021). Based on this hypothesis, we also predicted that the degree of seasonal variation in thermoregulatory traits would differ between both populations as they are exposed to different annual regimes of temperature variability. Specifically, we predicted: (1) that both Mediterranean populations of Great tits would reduce their body temperature and RMR and increase EWL to achieve greater heat tolerance and evaporative cooling efficiency during summer; and (2) that seasonal flexibility in HTL and in other key thermoregulatory traits would be greater in the lowland population, as they experience greater thermal amplitude and higher maximum environmental temperatures.

Methods

Species and study sites

The Great tit is a small-sized (~15 to 20 g) songbird widespread across the Palearctic (Gosler, Clement & Christie 2020). We compared physiological responses to heat during winter and summer in two Iberian populations of Great tits from different thermal environments: the lowland population is located in an open Mediterranean forest (*dehesa*) dominated by holm *Quercus rotundifolia* and cork oaks *Quercus suber*, in Badajoz (SW Spain; 38º51'N, 6º54'W; 192 m.a.s.l.); while the montane population is located at Piornal (40º07'N, 5º51'W; 1175 m.a.s.l.) and its habitat is composed by deciduous Pyrenean oak *Quercus pyrenaica* forest. During the last 15 years, mean maximum daily temperatures during the hottest month averaged 35.37 ± 3.44 ºC in Badajoz (hereafter lowland site) and 27.91 ± 3.32 ºC in Piornal (hereafter montane site), while mean minimum daily temperatures during the coldest month averaged 4.56 ± 3.87 ºC at the lowland and 0.66 ± 3.28 ºC in the montane sites respectively (State Meteorology Agency, http://www.opendata.aemet.es). Therefore, the lowland population experiences higher annual thermal amplitude (difference between maximum and minimum temperatures = 30.81 ºC) than the montane population (27.25 ºC) (see Supplementary Material Figure S1).

We captured adult Great tits from both populations during the winter of 2021 (lowland population, N = 16; montane population, N = 15), while both adults and fully developed first calendar year individuals were captured during the summers of 2020 and 2021 from the lowland population (N = 18) and during 2021 from the montane population (N = 23). Birds were captured with mist nets during the day, then transferred to cloth bags and transported to the facilities of University of Extremadura (Badajoz), where they were maintained until heat tolerance trials were conducted (as in Chapter 2). Birds spent no more than 48 h in captivity, during which food (insectivorous commercial mix and larvae) and water were supplied *ad libitum*.

All experimental procedures were approved by the bioethical committee of the University of Extremadura (76/2016) and performed under governmental licenses CN0002/20/ACA and CN0002/21/ACA.

Body temperature and gas exchange measurements

Passive integrated temperature-sensitive transponder tags (BioTherm13, Biomark, USA) were implanted subcutaneously in the interscapular region of the birds to monitor their body temperature, following the same procedure as in Chapter 2. Body temperature was recorded throughout the trials every

5 s by using a portable transceiver system (IS1001 Multiplexing Transceiver System, Biomark, USA) connected to a racket antenna (model F201F-ISO, Biomark, USA) placed near the metabolic chamber.

Great tits were placed into a 4.9 L plastic metabolic chamber, inside a temperature-controlled cabinet (model F-4, Ibercex), in which a wire mesh platform permitted birds to perch above a 1 cm mineral oil layer that prevented water evaporation from excreta biased EWL traces. Then, each individual was exposed to a steeped profile of ambient temperatures, measuring traces of O_2 consumption (VO_2) and EWL (VH_2O) at each temperature using an open flow-through respirometry system. The environmental temperature inside the chamber was measured using a calibrated thermistor probe (TC-100, Sable Systems).

Ambient air was supplied by a compressor equipped with an air drier membrane (model R-110300, MESTRA). We separated ambient dry air into two channels (baseline and metabolic chamber). Flow rate to baseline was fixed to 1000 ml min^{-1}, while flow rates to the metabolic chamber were manually adjusted (from 2000 – 5000 ml min^{-1}) as temperature increased. Increased flow rates permitted birds to be calm while maintaining low water vapor pressures (<1 kPa) during measurements, preventing high chamber humidity hampered EWL (see van Dyk et al. 2019). Excurrent air from the baseline and metabolic chamber was sequentially subsampled by using a multiplexer (RM-8, Sable Systems), starting with a 10-min baseline and then switching to the metabolic chamber once each target temperature was achieved and VO_2 and VH_2O traces were stable. Throughout the rest of the trial, 5 min of baseline were collected between successive target temperatures, and again at the end. Subsampled air was sequentially pulled through a H_2O analyzer (RH-300, Sable Systems), a drierite column and an O_2 analyzer (FC-10, Sable Systems). An analogic to digital converter (UI-2, Sable Systems) was used to digitalize voltage output from the analyzers and Expedata software (1.9.14 version, Sable Systems) to record outputs from both analyzers and from the temperature probe every 1 s. All respirometry equipment was calibrated (zeroed and spanned) prior to metabolic measurements.

Experimental protocol

Heat tolerance measurements took place during daylight (active phase of Great tits), and at least two hours after food was removed to generate a post-absorptive state. Only one individual was measured per trial. Each bird was exposed to a profile of increasing air temperatures from 30 to up to 40 ºC in the following sequence: 30 – 33 – 37 – 40 ºC and then increases of 2 ºC until birds reached their HTL. The first temperature was maintained for at least 30 min, until the birds showed stable O_2 and H_2O traces, and normothermic body temperature values (40 – 42 ºC). Then, the following target temperature was set, and this process was repeated until the end of the trial. Each bird spent a minimum of 15 min at each temperature. Bird's behaviour was monitored through metabolic measurements by using an infrared camera, and highly active individuals were discarded from subsequent analyses.

Trials were ended when birds showed (1) sustained scape behaviour (e.g., walking around, jumping or pecking the walls), (2) signs of severe heat distress such as loss of coordination or balance, (3) body temperature reaching 45 ºC or, near this threshold, increasing at a rate > 0.1 ºC min^{-1} indicating the beginning of uncontrolled hyperthermia (as in Whitfield et al. 2015). The air temperature at which any of these instances happened was considered the birds' HTL. Once a bird reached its heat tolerance limit, we rapidly removed it from the chamber, hydrated it and soaked its legs in alcohol to aid body temperature return to normothermic values. After completely recovered, birds were released at the site of capture.

Data analyses

We used the relevant algorithms in Expedata to correct for the analyzers drift and lag. Equations 10.2 and 10.9 from Lighton (2008) were used to calculate VO_2 and EWL, assuming a respiratory exchange ratio of 0.71 (birds in post-absorptive state during the trials) (Walsberg & Wolf 1995). For each bird, RMR and EWL were calculated from the lowest 5 min mean values of VO_2 and VH_2O at each ambient temperature. RMR was converted to metabolic heat production (W) by multiplying VO_2 (ml min^{-1}) with an oxyjoule equivalent [16 + 5.164 (RQ)] (Lighton et al. 1987), while evaporative heat loss (W) was calculated from EWL assuming a latent heat of vaporization of 2.406 J mg^{-1} (Tracy et al. 2010). We calculated evaporative cooling efficiency as the evaporative heat loss to metabolic heat production ratio. Body temperature was calculated as the average value during the final 10 min of exposure to each air temperature.

We conducted all statistical analyses in R 4.1.2 (R Core Team 2021), and all values were reported as means ± s.d. unless specified otherwise. We used the *segmented* package (Muggeo 2009) to determine inflection points in body temperature, RMR, EWL, and evaporative cooling efficiency for each site and season. Then, the data were split based on inflection points for subsequent analyses below and above the upper critical limits of thermoneutrality (as in Whitfield et al. 2015). Linear and linear mixed-effects models were fitted to the data by using the *lme4* package (Bates et al. 2015). We used the *emmeans* package (Lenth 2022) to perform *post-hoc* pairwise contrasts between groups, and visually checked model assumptions in model residuals.

First, to assess seasonal and between-population variation in heat tolerance and thermoregulatory traits, we fitted linear models to HTL and to each thermoregulatory trait (body temperature, RMR, EWL and evaporative cooling efficiency) within (air temperature ~ 30 ºC) and above (air temperature ~ 37 ºC) the thermoneutral zone (TNZ) of Great tits, with body mass, site, season, and the site×season interaction as predictor variables. As only a few birds of each population (one bird from the lowland population and two birds from the montane population) tolerated more than one air temperature above inflection points during winter, mixed-effects models could not be fitted. However, as the inflection points of all variables were below 37 ºC, regardless of the site and season, we were able to assess seasonal variation in thermoregulatory traits above thermoneutrality.

Second, we fitted linear mixed-effects models to evaluate population variation in the slopes of summer body temperature, RMR, EWL and evaporative cooling efficiency against air temperature above thermoneutrality. Initial models included air temperature, body mass, site, and the ambient temperature×site interaction as predictor variables, with bird identity as a random effect, as we had measurements at various air temperatures from the same individuals. When site emerged as a significant predictor, we additionally fitted separate population-specific models to calculate the slopes and y-intercepts of each thermoregulatory trait in response to air temperature.

Results

Body mass

Body mass was significantly affected by site, season, and their interaction (Table 1). Overall, body mass was higher in winter (17.43 ± 0.84 g, N = 31) than in summer (16.02 ± 1.09 g, N = 41) (Table 1). Individuals from the montane population were heavier (16.38 ± 0.99 g, N = 23) than those from the lowland population (15.78 ± 1.17 g, N = 18) during summer, while both populations showed similar body masses in winter (lowland = 17.35 ± 0.87 g, N = 16; montane = 17.30 ± 0.89 g, N = 15) (Table 1).

Table 1. Results from linear models explaining seasonal and between-population variation in body mass, heat tolerance limit (HTL), body temperature (T_b), resting metabolic rate (RMR), evaporative water loss (EWL), and evaporative cooling efficiency (EHL/MHP) of Great tits *Parus major* within (air temperature ~ 30 ºC) and above thermoneutrality (air temperature ~ 37 ºC). Significant explanatory variables ($p < 0.05$) are in bold. (* reference category is Piornal; ** reference category is winter).

Predictor	Body mass	HTL	T_b		RMR		EWL		EHL/MHP	
			30 ºC	37 ºC	30 ºC	37 ºC	30 ºC	37 ºC	30 ºC	37 ºC
Body mass										
β	-	-0.155	0.004	0.011	0.013	0.022	2.423	14.604	-0.004	0.008
s.e.	-	0.16	0.05	0.06	0.01	0.01	3.48	8.26	0.001	0.01
t-value	-	-0.97	0.08	0.16	1.99	1.50	0.70	1.77	-0.65	0.65
p-value	-	0.335	0.934	0.869	0.051	0.139	0.488	0.082	0.517	0.519
Site*										
β	0.962	-0.814	0.237	0.007	0.021	0.004	-5.621	-47.843	-0.019	-0.096
s.e.	0.15	0.39	0.12	0.15	0.02	0.03	9.06	19.92	0.01	0.03
t-value	6.31	-2.10	1.95	0.05	1.20	0.12	-0.62	-2.40	-1.29	-2.97
p-value	**< 0.001**	**0.040**	0.057	0.963	0.235	0.907	0.537	**0.019**	0.201	**0.004**
Season**										
β	1.851	-2.494	0.597	1.059	0.046	0.251	3.685	87.726	-0.014	-0.086
s.e.	0.17	0.49	0.15	0.19	0.02	0.05	11.41	25.54	0.02	0.04
t-value	11.12	-5.05	4.00	5.38	2.12	5.56	0.32	3.43	-0.76	-2.08
p-value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.037**	**< 0.001**	0.748	**0.001**	0.451	**0.043**
Site×season										
β	-0.809	1.393	-0.472	-0.638	-0.049	-0.119	18.667	57.580	0.055	0.178
s.e.	0.24	0.62	0.18	0.25	0.03	0.06	13.20	32.78	0.02	0.05
t-value	-3.44	2.25	-2.67	-2.51	-1.99	-2.05	1.41	1.76	2.61	3.33
p-value	**< 0.001**	**0.029**	**0.009**	**0.015**	0.051	**0.045**	0.162	0.085	**0.011**	**0.002**

Heat tolerance limits

HTL ranged between 37 and 40 ºC, and differed between sites, seasons, and site×season groups (Table 1). Overall, Great tits elevated their heat tolerance from winter (37.6 ± 1.09 ºC) to summer (39.44 ± 1.18 ºC), and HTL were higher in the lowland population (38.61 ± 1.49 ºC) than in the montane one (38.48 ± 1.51 ºC) (Table 1). Although Great tits from the lowland population showed significantly higher HTL during summer (39.80 ± 0.32 ºC) than during winter (37.40 ± 0.33 ºC), birds from the montane population did not show significant seasonal differences (winter = 37.9 ± 0.43 ºC; summer = 39.00 ± 0.23 ºC) (Figure 1).

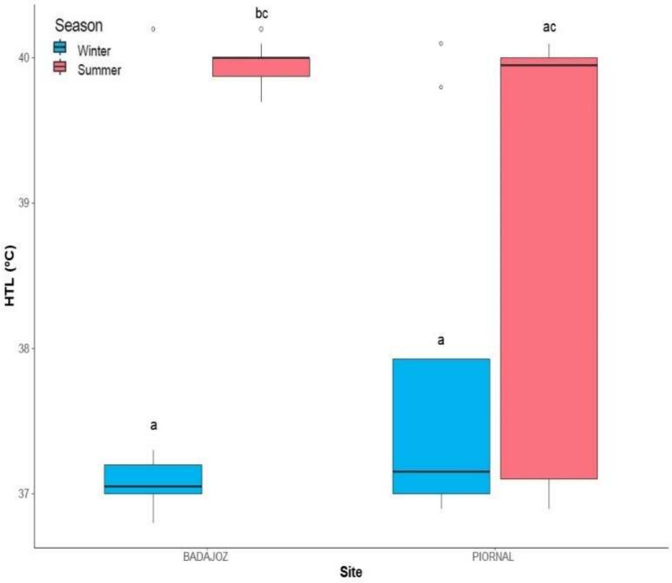

Figure 1. Heat tolerance limits (HTL, ºC) during winter and summer for the two Great tit *Parus major* population studied here. Boxplots show mean values with its 95% confidence intervals. Different letters denote significant differences between groups ($p < 0.05$).

Body temperature

Within thermoneutrality, body temperature was affected by season and the site×season interaction (Table 1). Body temperature was higher in winter (42.15 ± 0.31 ºC) than in summer (41.74 ± 0.45 ºC) (Table 1). Moreover, birds from the lowland site had lower body temperature in summer than in winter, while those from the montane site did not show seasonal differences (Figure 2a).

We detected clear inflection points for body temperature, which did not vary (as indicated by overlapping 95% CI) in Great tits from the lowland site between winter [32.39 ºC (31.03, 33.76)] and summer [34.65 ºC (32.67, 36.63)], nor in birds from the montane population [winter = 33.93 ºC (30.11, 37.75); summer = 35.90 ºC (34.62, 37.19)]. In addition, body temperature inflection points did not significantly differ between sites within seasons.

Above thermoneutrality (air temperature ~ 37 ºC), we also found that season and the site×season interaction significantly affected body temperature (Table 1). Birds lowered their body temperature in summer (42.41 ± 0.45 ºC) with respect to winter (43.26 ± 0.48 ºC) (Table 1). However, *post-hoc* analyses revealed that only birds from the warmer, lowland site showed lower body temperature during summer, while those from the montane site did not adjust seasonally their body temperature above thermoneutrality (Figure 3a).

Moreover, linear mixed-effect models fitted to summer data revealed air temperature, site, and the air temperature×site interaction as significant predictors (Table 2). Birds from the montane site increased their body temperature faster (slope = 0.423 ± 0.03 ºC $ºC^{-1}$) than those from the lowland site (slope = 0.296 ± 0.04 ºC $ºC^{-1}$) (Figure 4a).

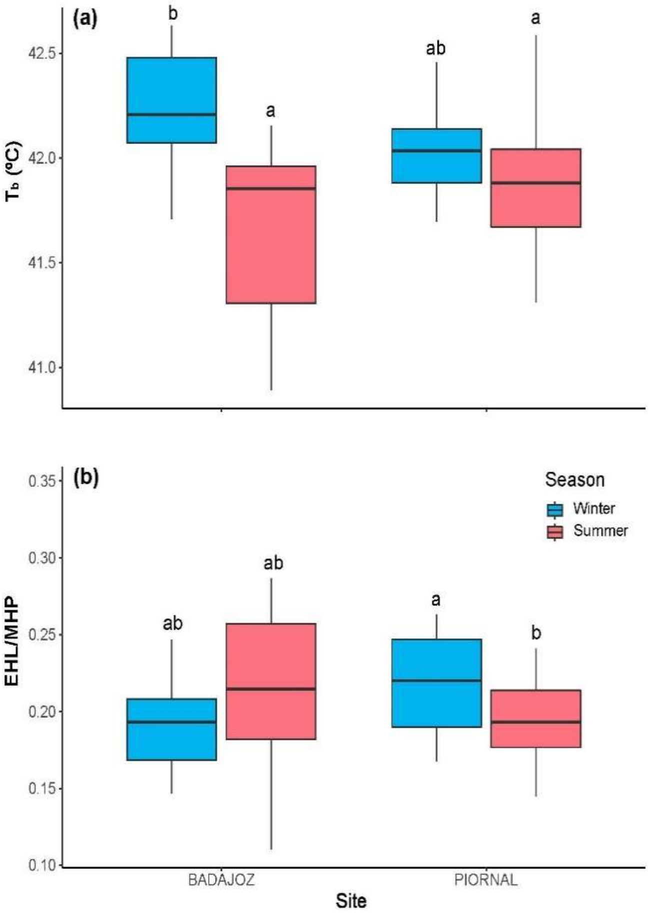

Figure 2. Seasonal and interpopulation comparisons within thermoneutrality (air temperature ~ 30 ºC) for each thermoregulatory trait of Great tit *Parus major* for which the site×season interaction emerged as significant: **(a)** body temperature (T_b, ºC), and **(b)** evaporative cooling efficiency (EHL/MHP). Boxplots denote mean values with its 95% confidence intervals. Different letters indicate significant differences between groups ($p < 0.05$).

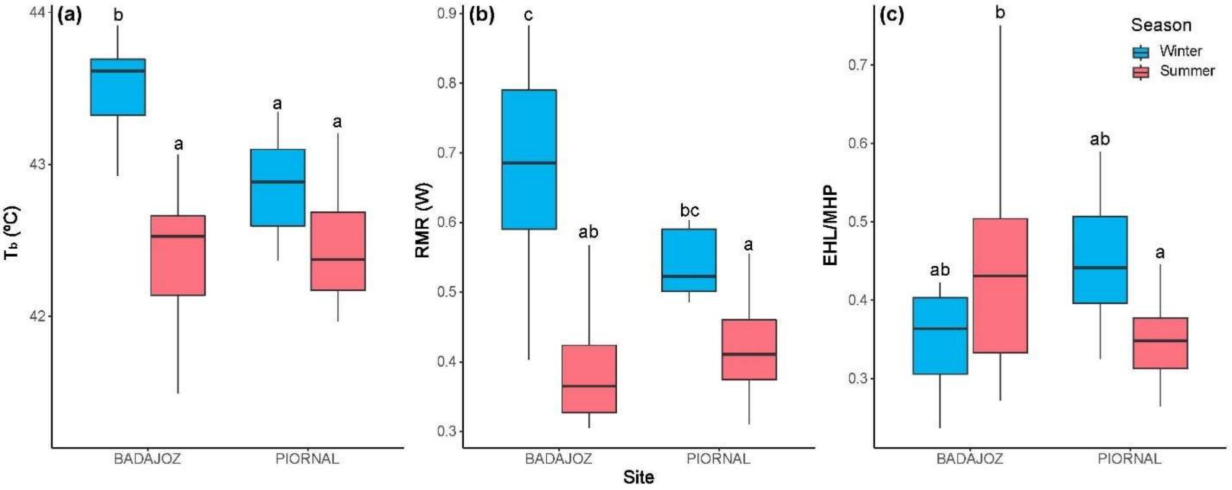

Figure 3. Seasonal and interpopulation comparisons above thermoneutrality (air temperature ~ 37 ºC) for each thermoregulatory trait of Great tit *Parus major* for which site×season interaction had a significant effect: **(a)** body temperature (T_b, ºC), **(b)** resting metabolic rate (RMR, in W) and **(c)** evaporative cooling efficiency (EHL/MHP). Boxplots show mean values with its 95% confidence intervals. Different letters denote significant differences between groups ($p < 0.05$).

Resting metabolic rate

At thermoneutrality, RMR was higher in winter (0.43 ± 0.05 W) than in summer (0.40 ± 0.05 W), but we did not find differences in response to body mass, site, or the site×season interaction (Table 1). We found a clear T_{uc} for each population and season. Birds from the montane site elevated their T_{uc} in summer [35.45 ºC (34.36, 36.54)] with respect to winter [32.80 ºC (31.49, 34.11)], while those from the lowland site did not show seasonal differences [winter = 33.52 ºC (32.22, 34.92); summer = 35.41 ºC (33.38, 37.45)]. Winter and summer T_{uc} values did not significantly differ between populations.

Above thermoneutrality, season and the site×season interaction influenced RMR (Table 1). Linear models showed that RMR was higher in winter (0.64 ± 0.14 W) than in summer (0.41 ± 0.08 W), regardless of the population (Table 1; Figure 3b). Moreover, birds from the lowland population showed larger seasonal differences in BMR than birds from the montane population (Table 1; Figure 3b).

During summer, linear mixed-effects models only revealed a significant effect of air temperature on RMR, which increased at a rate of 0.037 ± 0.01 W $ºC^{-1}$ (Table 2; Figure 4b).

Evaporative water loss

EWL at thermoneutrality was not influenced by any of the predictor variables (Table 1). However, segmented regressions detected clear inflection points for EWL, which varied between populations and seasons. Birds from the lowland site did not significantly adjust EWL inflection points seasonally [winter = 35.72 ºC (34.97, 36.46); summer = 36.03 ºC (34.89, 37.17)], whereas those from the montane site showed higher inflection points in summer [36.27 ºC (35.51, 37.03)] than in winter [32.78 ºC (32.02, 33.54)].

Within a given season, EWL inflection points were significantly lowest in montane birds during winter but did not differ between populations in summer.

Linear models revealed site and season as significant predictors of EWL above thermoneutrality (Table 1). Birds from the montane population had lower EWL rates (254.51 ± 80.37 mg h^{-1}) than those from the lowland population (280.71 ± 93.80 mg h^{-1}) (Table 1). In addition, EWL rates were higher in winter (378.02 ± 78.39 mg h^{-1}) than in summer (230.80 ± 53.07 mg h^{-1}) (Table 1).

In summer, linear mixed-effects models showed a significant and positive effect of air temperature on EWL above inflection point, which increased at a rate of 74.056 ± 7.61 mg h^{-1} $^{o}C^{-1}$ (Table 2; Figure 4c). However, slopes did not differ between populations, as indicated by the non-significant interaction between air temperature and site (Table 2).

Table 2. Results from linear mixed-effect models fitted to body temperature (T_b), resting metabolic rate (RMR, evaporative water loss (EWL) and evaporative cooling efficiency (EHL/MHP) of Great tits *Parus major* above thermoneutrality during summer. Significant explanatory variables are in bold ($p < 0.05$). (*reference category is Piornal).

Predictor	T_b	RMR	EWL	EHL/MHP
Air temperature				
β	0.296	0.037	74.056	0.068
s.e.	0.03	0.01	7.61	0.01
t-value	8.42	5.25	9.73	6.72
p-value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Body Mass				
β	-0.008	0.011	-2.471	-0.012
s.e.	0.75	0.01	10.19	0.02
t-value	-0.11	0.82	-0.24	0.67
p-value	0.916	0.417	0.810	0.507
Site*				
β	-4.772	-0.445	741.536	1.135
s.e.	2.03	0.41	434.88	0.58
t-value	-2.35	-1.09	1.71	1.95
p-value	**0.026**	0.283	0.098	0.062
Air temperature×site				
β	0.127	0.012	-20.561	-0.032
s.e.	0.05	0.01	11.31	0.02
t-value	2.41	1.16	-1.82	-2.13
p-value	**0.023**	0.254	0.079	**0.042**

Evaporative cooling efficiency

Great tits did not significantly change the inflection points of evaporative cooling efficiency seasonally, either at the lowland site [winter = 35.42 ºC (33.32, 37.51); summer = 35.49 ºC (33.02, 37.97)] or at the montane site [winter = 33.26 ºC (31.28, 35.24); summer = 36.57 ºC (34.04, 39.09)]. In addition, we found no significant differences in inflection points for evaporative cooling efficiency between populations and seasons.

Within thermoneutrality, only the site×season interaction emerged as a significant predictor (Table 1). Birds from the montane site showed higher evaporative cooling efficiency in winter than in summer, whereas this trait did not vary seasonally in birds from the lowland site (Table 1; Figure 2b).

Above thermoneutrality, however, evaporative cooling efficiency was significantly influenced by site, season, and the site×season interaction (Table 1). At 37 ºC, birds from the lowland site had greater evaporative cooling efficiency (0.42 ± 0.13) than those from the montane site (0.37 ± 0.07) (Table 1). Overall, evaporative cooling efficiency was higher in winter (0.40 ± 0.12) than in summer (0.38 ± 0.10) (Table 1). *Post-hoc* tests showed, however, that Great tits from the lowland site achieved greater cooling efficiency than those from the montane site during summer. In winter, however, both populations showed similar values (Figure 3c).

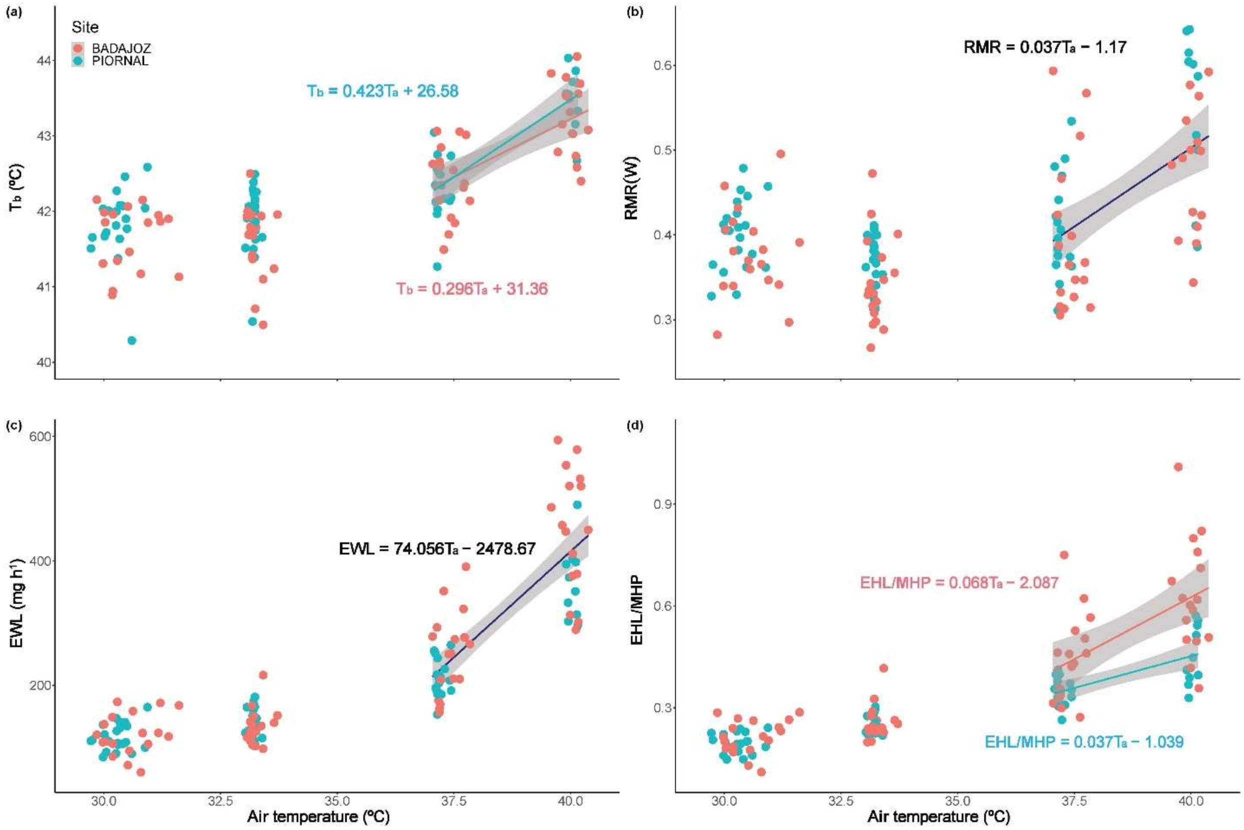

Figure 4. **(a)** Body temperature (T_b, ºC), **(b)** resting metabolic rate (RMR, in W), **(c)** evaporative water loss (EWL, in mg h^{-1}), and **(d)** evaporative cooling efficiency (EHL/MHP) of Great tits *Parus major* from Badajoz (lowland site) and Piornal (montane site) during summer in response to air temperature (ºC). Lines indicate predicted slopes and their 95% confidence intervals from linear mixed-effects models of ambient temperature effect on the different thermoregulatory traits above thermoneutrality. For body temperature and evaporative cooling efficiency site emerged as a significant predictor, so separate regression lines were plotted for each population.

During summer, the significant air temperature×site interaction indicated differences in the rate of increase in evaporative cooling efficiency between populations: birds from the lowland site showed a steeper slope (0.068 ± 0.01 $°C^{-1}$) than those from the montane site (0.037 ± 0.01 $°C^{-1}$) (Table 2; Figure 4d). At their HTL (ambient temperature ~ 40 ºC), birds from the lowland site had a cooling efficiency of 0.63 ± 0.17 (maximum value = 1.01), while those from the montane site achieved a cooling efficiency of 0.47 ± 0.09 (maximum value = 0.57), albeit differences were marginally significant (p = 0.062; Table 2).

Discussion

We found that lowland Great tits facing warmer conditions during summer showed greater evaporative cooling efficiency at high temperatures than their montane counterparts. Yet, summer HTL were similar between populations. Indeed, birds from the lowland, warmer population reduced endogenous heat loads during summer by lowering body temperature and metabolic heat production above thermoneutrality. This allowed them to maximize evaporative cooling efficiency and thus HTL during the summer. On the other hand, birds from the montane population only seasonally adjusted RMR above the TNZ.

This finding is consistent with the climatic variability hypothesis, as birds from the hotter and more thermally variable lowland site showed greater seasonal variation in their thermoregulatory traits above thermoneutrality. This greater seasonal variation translated into enhanced HTL in lowland birds during summer, while those from the montane site did not improve their heat tolerance from winter to summer. Along this line, Noakes et al. (2016) found that White-browed sparrow-weavers from a hotter and more thermally heterogeneous site displayed greater flexibility in key thermoregulatory traits (namely body temperature, and EWL) than those from two milder and more thermally stable sites, resulting into higher HTL in the former population. Similar patterns of flexibility have been reported in response to cold. For instance, the degree of flexibility in thermogenic capacity (e.g., summit metabolic rate) among Dark-eyed juncos *Junco hyemalis* populations correlates with the environmental heterogeneity of their habitats (Stager et al. (2021). Altogether, these results support the idea that more thermally fluctuating environments results in more flexible phenotypes (Schlichting & Pigliuci 1998; Cavieres & Sabat 2008).

Contrary to our expectations, Great tits from the lowland population showed similar HTL to their montane counterparts during summer. We suspect that the lack of differences could be explained by the high interindividual variability in HTL found in the montane population. As predicted, however, our results indicated that Great tits from warmer, lowland population deal better with heat than those from the milder, montane population, as the former showed greater summer evaporative cooling efficiencies above thermoneutrality (e.g., greater slopes and maximum values at their HTL). This points to different vulnerabilities to high temperatures between populations. This result is also in line with that from Trost (1972), who found that Horned larks from a hot site showed greater evaporative cooling efficiency than counterparts from a milder one. Nevertheless, recent studies have found no substantial variation in evaporative cooling efficiency during summer among bird populations along a climatic gradient (Noakes et al. 2016; O'Connor et al. 2017).

Overall, Mediterranean Great tits showed seasonal adjustments to reduce total endogenous heat loads in summer, as previously found in and arid-zone songbird (Noakes et al. 2016). However, Oswald et al. (2018) found no seasonal adjustments in both body temperature and RMR in the Cape rockjumper *Chaetops frenatus* (a mountain relict songbird from the South African Fynbos) which experiences a similar temperature regime (temperature minima -2.6 ºC in winter; temperature maxima 35.4 ºC in summer) to the Great tit populations studied here. The downregulation of body temperature and RMR above TNZ in our birds would permit EWL to compensate for metabolically produced heat, resulting in higher evaporative cooling efficiency and thus greater heat tolerance during summer. Between populations, birds from the lowland site displayed significantly lower body temperature slopes and a tendency to lower RMR at high temperatures, which might explain their greater evaporative cooling capacity during summer compared to their montane counterparts. In line with this, Noakes et al. (2016) found that White-browed sparrow-weavers from a hot site exhibited lower body temperature slopes than those from milder sites.

Along with reduced endogenous heat loads, birds lowered EWL during summer, which is a widespread water conservation strategy found in desert and arid-zone birds (Tieleman et al. 2002b; Noakes et al. 2016; O'Connor et al. 2017). This result is contrary to our expectations of increased EWL during summer, as both populations had access to drinking water. Indeed, Oswald et al. (2018) found that Cape rockjumpers increased their rates of EWL at high temperatures to enhance their cooling capacities during summer, even when water is scarce. We cannot rule out the possibility that the greater EWL rates during winter in Great tits were related to greater heat stress rather than to an adaptation to save water, as they were not acclimatized to high ambient temperatures at the time of measurements. Overall, birds from the montane site showed lower EWL rates above thermoneutrality than those from the lowland site. However, both populations showed similar EWL slopes in summer in response to high air temperatures. This is contrary to earlier findings on an arid-zone songbird, as White-browed sparrow-weavers from a hot site have showed lower EWL than those from milder ones (Noakes et al. 2016). Indeed, limited access to drinking water can exert a strong selective pressure on EWL in arid-zone dwellers (Trost 1972; Williams 1996; Tieleman et al. 2002a; Noakes et al. 2016; O'Connor et al. 2017). However, both Great tit populations had access to water throughout the year (Playà-Montmany et al. 2021) so that they could replenish water reserves to sustain EWL at high temperatures; this could therefore explain the lack of differences in this trait.

Finally, Great tits from the montane site seasonally adjusted their T_{uc} and EWL inflection points (showing higher values in summer), while those from the lowland site did not. We believe that montane birds might show a stronger trade-off between heat-cold tolerance, which could explain seasonal adjustments in these traits. Montane birds might lower their T_{uc} and EWL inflection point during winter to shift their TNZ boundaries downwards and thus enhance cold tolerance as they experience lower minimum environmental temperatures (often below 0 ºC) than those from the lowland site. Yet, both populations showed similar T_{uc} and EWL inflection points during summer, which indicates that both populations strongly resort to physiological thermoregulation at similar ambient temperatures (c. 35 ºC or more) during the hottest part of the year.

In conclusion, we provide experimental evidence that wild Mediterranean Great tits —particularly those from a warmer lowland habitat — increase their heat tolerance and cooling efficiency during the warmer season through adjustments in key thermoregulatory traits. In addition, inter-population differences support the idea that adaptive thermoregulation in small endotherms, such as songbirds, is more flexible than previously thought (Angilletta et al. 2010; Glanville et al. 2012; Smit et al. 2013; Freeman et al. 2022). This flexibility in thermal physiology may buffer against adverse effects of climate change. However, environment-induced thermoregulatory adjustments are probably connected to non-lethal, chronic costs (Cunningham et al. 2021). There is growing evidence that greater thermoregulatory demands interfere with foraging efficiency and reproductive activities (e.g., du Plessis et al. 2012; O'Connor et al. 2022). Hence, these trade-offs will potentially have long-term consequences for survival and fitness. This could be particularly problematic for Great tits and other Mediterranean songbirds with limited cooling efficiencies and HTL (Chapter 2). This makes them sensitive to climate warming, particularly at lowland warm sites such as the one included here, where environmental temperatures can surpass the maximum HTL of Great tits and other songbirds (40 ºC) (Figure S1). This suggests that lowland birds coping with high temperatures must resort to behavioural thermoregulation (e.g., by selecting thermally-buffered microsites and/or reducing activity) to avoid lethal hyperthermia. Finally, the substantial intraspecific variation in thermal physiology observed in Great tits and other birds call for species distribution models including intraspecific variation in thermal phenotype. This could contribute to obtain more accurate and realistic predictions of the future distribution and persistence of species and populations under climate change scenarios (Valladares et al. 2014; Moran, Hartig & Bell 2016; De Marche, Doak & Morris 2018).

References

Angilletta, M.J., Cooper, B.S., Schuler, M.S., Boyles, J.G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci.* **2**(3), 861-881.

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F., Chown, S.L. (2013). Heat freezes niche evolution. *Ecol.* **16**, 1206-1219. https://doi.org/10.1111/ele.12155

Bates, D., Mächler, M., Bolker, B., Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**(1), 1-48. https://doi.org/10.18637/jss.v067.i01

Bailey, L.D., van de Pol, M., Adriaensen, F. et al. (2022). Bird populations most exposed to climate change are less sensitive to climatic variation. *Nat. Comm.* **13**, 2112. https://doi.org/10.1038/s41467-022-29635-4

Boyles, J.G., Seebacher, F., Smit, B., McKechnie, A.E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* **51**(5), 676-690.

Bozinovic, F., Calosi, P., Spicer, J.I. (2011). Physiological correlates of geographic range in animals. *Annu. Rev. Ecol. Evol. Syst*. **42**,155–79. https://doi.org/10.1146/annurev-ecolsys-102710-145055

Broggi, J., Orell, M., Hohtola, E., Nilsson, J.A. (2004). Metabolic response to temperature variation in the great tit: an interpopulation comparison. *J.Anim. Ecol.* **73**, 967-972.

Cavieres, G., Sabat, P. (2008). Geographic variation in the response to thermal acclimation in rufous-collared sparrows: are physiological flexibility and environmental heterogeneity correlated? *Funct. Ecol.* **22**, 509-515.

Cunningham, S.J., Gardner, J.L., Martin, R.O. (2021). Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* **19**(5), 300-307. https://doi.org/10.1002/fee.2324

De Marche, M., Doak, D.F., Morris, W.F. (2018). Incorporating local adaptation into forecast of species' distribution and abundance under climate change. *Glob. Change Biol.* **25**, 775-793. https://doi.org/10.1111/gcb.14562

Du Plessis, K.L., Martin, R.O., Hockey, P.A.R., Cunningham, S.J., Ridley, A.R. (2012). The costs of keeping cool in a warming world: implications of high temperatures for foraging, thermoregulation, and body condition of an arid-zone bird. *Glob. Change Biol.* **18**, 3063-3070.

Freeman, M.T., Czenze, Z.J., Schoeman, K., McKechnie, A.E. (2022). Adaptive variation in the upper limits of avian body temperature. *Proc. Natl. Acad. Sci. U.S.A.* **119**(26), e2116645119

Glanville, E.J., Murray, S.A., Seebacher, F. (2012). Thermal adaptation in endotherms: climate and phylogeny interact to determine population-level responses in a wild rat. *Funct. Ecol.* **26**, 390-398. https://doi.org/5-2435. 2011.01933.x

Gosler, A., Clement, P., Christie, D.A. (2020). Great Tit (*Parus major*), version 1.0. In Birds of the World (Billerman, S.M., Keeney, B.K., Rodewald, P.G., and Schulenberg, T.S., Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.gretit1.01

Lenth, R.V. (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.2. https://CRAN.R-project.org/package=emmeans

Lighton, J.R.B., Bartholomew, G.A., Feener, D.H. (1987). Energetics of locomotion and load carriage and a model of the energy cost of foraging in the Leaf-cutting Ant *Atta colombica. Guer. Physiol. Zool.* **60**, 524-537.

Lighton, J.R.B. (2008). Measuring metabolic rates: A manual for scientists. Oxford University Press, Oxford.

Liknes, E.T., Scott, S.M., Swanson, D.L. (2002). Seasonal acclimatization in the American goldfinch revisited: to what extent do metabolic rates vary seasonally? *Condor.* **104**, 548-557.

McKechnie, A.E., Noakes, M.J., Smit, B. (2015). Global patterns of seasonal acclimatization in avian resting metabolic rates. *J. Ornithol.* **156**(Suppl 1), S367-S376. https://doi.org/10.1007/s10336-015-1186-5

McKechnie, A.E., Wolf, B.O. (2019). The physiology of heat tolerance in small endotherms. *Physiology.* **34**, 302-313. https://doi.org/10.1152/physiol.00011.2019

McNab, B.K. (2002). The physiological ecology of vertebrates: A view from energetics. Cornell University Press, Ithaca, New York.

Moran, E., Hartig, F., Bell, D.M. (2016). Intraspecific trait variation across scales: implications for understanding global change responses. *Glob. Change Biol.* **22**, 137-150.

Muggeo, V.M.R. (2009). Segmented: a R package to fit regression models with broken-line relationships.

Noakes, M.J., Wolf, B.O., McKechnie, A.E. (2016). Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird. *J. Exp. Biol.* **219**, 859-869.

Noakes, M.J., Wolf, B.O., McKechnie, A.E. (2017). Seasonal metabolic acclimatization varies in direction and magnitude among populations of an Afrotropical passerine bird. *Physiol. Biochem. Zool.* **90**(2), 000-000. https://doi.org/10.1086/689030

Noakes, M.J., McKechnie, A.E. (2019). Reaction norms for heat tolerance and evaporative cooling capacity do not vary across a climatic gradient in a passerine bird. *Comp. Biochem. Physiol. Part A.* **236**(2019), 110522.

Noakes, M.J., McKechnie, A.E. (2020). Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines. *Physiol. Biochem. Zool.* **93**(2), 140-152. https://doi.org/10.1086/707679

Oboikovitz, P., Swanson, D.L. (2021). Seasonal metabolic flexibility is correlated with microclimate variation in horned larks and house sparrows. *Curr. Zool.* **68**(2), 199-210.

O'Connor, R.S., Wolf, B.O., Brigham, R.M., McKechnie, A.E. (2017). Avian thermoregulation in the heat: efficient evaporative cooling in two southern African nightjars. *J. Comp. Physiol. B* **187**, 477-491.

O'Connor, R.S., Le Pogam, A., Young, K.E., Love, O.P., Cox, C.J., Roy, G., Robitaille, F., Elliott, K.H., Hargreaves, A.L., Choy, E.S., Gilchrist, G., Berteaux, D., Tam, A., Vezina, F. (2022). Warming in the land of the midnight sun: breeding birds may suffer greater heat stress at high- versus low-Arctic sites. *Proc. R Soc. B.* **289**(1981), 20220300.

Oswald, K.N., Lee, A.T.K., Smit, B. (2018). Seasonal physiological responses to heat in an alpine range-restricted bird: the Cape rockjumper (*Chaetops frenatus*). *J. Ornithol.* **159**, 1063-1072.

Petit, M., Lewden, A., Vezina, F. (2013). Intra-seasonal flexibility in avian metabolic performance highlights the uncoupling of basal metabolic rate and thermogenic capacity. *PLoS ONE*. **8**, e68292.

Piersma, T., Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends. Ecol.* **18**, 228-233.

Playà-Montmany, N., González-Medina, E., Cabello-Vergel, J., Parejo, M., Abad-Gómez, J.M., Sánchez-Guzmán, J.M., Villegas, A., Masero, J.A. (2021). The thermoregulatory role of relative bill and leg surface areas in a Mediterranean population of Great tit (*Parus major*). *Ecol. Evol.* **11**, 15936-15946. https://doi.org/10.1002/ece3.8263

Playà-Montmany, N. (2022). Physiological and behavioural strategies underlying the responses of birds to global warming. PhD Thesis, University of Extremadura, Badajoz, Spain.

Pollock, H.S., Brawn, J.D., Agin, T.J., Cheviron, Z.A. (2019). Differences between temperate and tropical birds in seasonal acclimatization of thermoregulatory traits. *J. Avian Biol.* **50**(4), e02067. https://doi.org/10.1111/jav.02067

Root, T. (1988). Energy constraints on avian distributions and abundances. *Ecology.* **69**, 330-339.

Schlichting, C., Pigliucci, M. (1998). Phenotypic evolution: a reaction norm perspective. Sinauer Associates Inc., Sunderland, Massachusetts.

Smit, B., McKechnie, A.E. (2010). Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**, 330-339.

Smit, B., Harding, C.T., Hockey, P.A.R., McKechnie, A.E. (2013). Adaptive thermoregulation during summer in two populations of an arid-zone passerine. *Ecology*. **94**(5), 1142-1154.

Somero, G.N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**(6), 912-920.

Stager, M., Pollock, H.S., Benham, P.M., Sly, N.D., Brawn, J.D., Cheviron, Z.A. (2016). Disentangling environmental drivers of metabolic flexibility in birds: the importance of temperature extremes versus temperature variability. *Ecography*. **39**(8), 787-795.

Stager, M., Senner, N.R., Swanson, D.L., Carling, M.D., Eddy, D.K., Greives, T.J., Cheviron, Z.A. (2021). Temperature heterogeneity correlates with intraspecific variation in physiological flexibility in a small endotherm. *Nat. Commun.* **12**, 4401. https://doi.org/10.1038/s41467-021-24588-6

Steven, G.C. (1989). The latitudinal gradient in geographical range: how so many species coexist in the tropics? *Am. Nat.* **133**, 240-256.

Swanson, D.L. (2010). Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* **17**, 75-129.

Tieleman, B.I., Williams, J.B., Buschur, M.E. (2002a). Physiological adjustments to arid and mesic environments in larks (*Alaudidae*). *Physiol. Biochem. Zool.* **75**, 305-313.

Tieleman, B.I., Williams, J.B., LaCroix, F., Paillat, P. (2002b). Physiological responses of houbara bustards to high ambient temperatures. *J. Exp. Biol.* **205**, 503-511.

Tracy, C.R., Welch, W.R., Pinshow, B. & Porter, W.P. (2010). Properties of air: a manual for use in biophysical ecology, 4th edn. The University of Wisconsin Laboratory for Biophysical Ecology: Technical Report.

Trost, C.H. (1972). Adaptations of Horned larks (*Eremophila alpestris*) to hot environments. *Auk.* **89**, 506-527.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol.* **17**, 1351-1364. https://doi.org/10.1111/ele.12348

Van Dyk, M., Noakes, M.J., McKechnie, A.E. (2019). Interactions between humidity and evaporative heat dissipation in a passerine bird. *J. Comp. Physiol. B*. **189**, 299-308.

Walsberg, G.E., Wolf, B.O. (1995). Variation in the respiratory quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. *J. Exp. Biol.* **198**, 213-219.

Whitfield, M.C., Smit, B., McKechnie, A.E., Wolf, B.O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* **218,** 1705-1714. https://doi.org/10.1242/jeb.121749

Williams, J.B. (1996). A phylogenetic perspective of evaporative water loss in birds. *Auk.* **113**, 457-472.

Appendix 2: Supplementary Material

Methods

We characterized the thermal environment experienced throughout the year by each population of Great tits *Parus major* studied. To do so, we employed data collected during the last 15 years (2005-2020) from the State Meteorology Agency stations (http://www.opendata.aemet.es) to explore if birds from each population experienced a different regime of annual thermal amplitude.

We found that thermal amplitude is greater in the lowland site (e.g., the difference between summer maximum to winter minimum temperatures) than in the montane site (Figure S1). In the same manner, Great tits from the lowland site are exposed to higher maximum temperatures than those from the montane population, with even some days surpassing their heat tolerance limits (40 ºC) (Figure S1). On the contrary, birds from the montane site experienced lower minimum temperatures than those from the lowland population during winter (Figure S1).

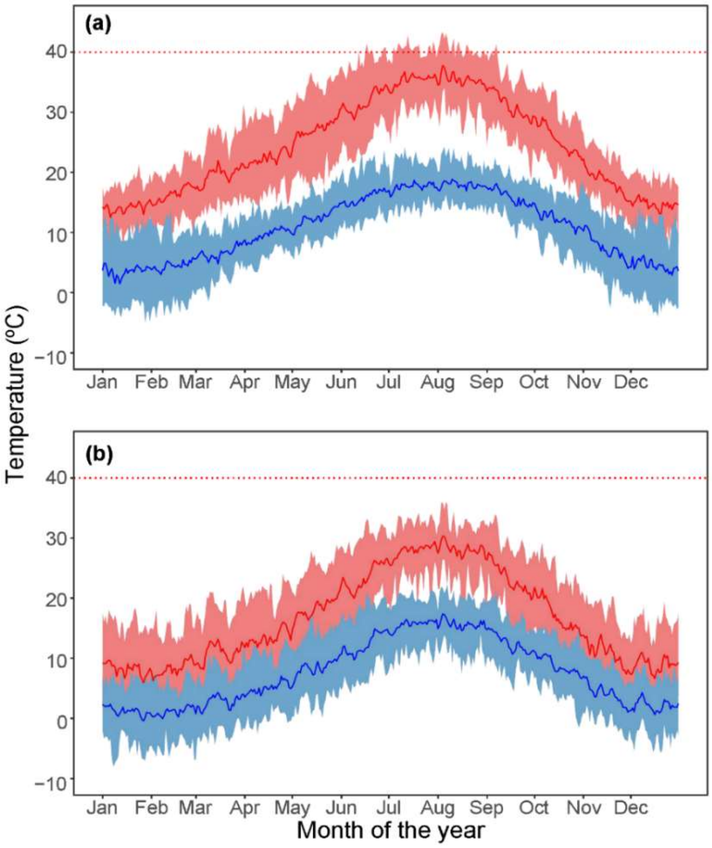

Figure S1. Annual profile of maximum (red line) and minimum temperatures (blue line) experienced by Great tits *Parus major* from lowland site (Badajoz; panel a) and montane site (Piornal; panel b). In both cases, shaded red and shaded blue denote 95% confidence intervals. Dashed red line indicate the maximum heat tolerance limit for each population during summer.

4

Urohidrosis as an overlooked cooling mechanism in long-legged birds

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Abstract

Behavioural thermoregulation could buffer the impacts of climate warming on vertebrates. Specifically, the wetting of body surfaces and the resulting evaporation of body fluids serves as a cooling mechanism in a number of vertebrates coping with heat. Storks (*Ciconiidae*) frequently excrete onto their legs to prevent overheating, a phenomenon known as urohidrosis. Despite the increasingly recognised role of bare and highly vascularised body parts in heat exchange, the ecological and evolutionary determinants of urohidrosis have been largely ignored. We combine urohidrosis data from a scientifically curated media repository with microclimate and ecological data to investigate the determinants of urohidrosis in all extant stork species. Our phylogenetic generalised linear mixed models show that high temperature and solar radiation, and low wind speed, promote the use of urohidrosis across species. Moreover, species that typically forage in open landscapes exhibit a more pronounced use of urohidrosis than those mainly foraging in waterbodies. Substantial interspecific variation in temperature thresholds for urohidrosis prevalence points to different species vulnerabilities to high temperatures. This integrated approach that uses online data sources and methods to model microclimates should provide insight into animal thermoregulation and improve our capacity to make accurate predictions of climate change's impact on biodiversity.

Keywords: birds, climate warming, evaporative cooling, heat tolerance, hot environments, storks, thermoregulatory behaviour

Introduction

Endotherms can deal with heat stress through a series of physiological and behavioural responses in order to maintain their thermal balance. The first response is likely to be behavioural, such as the selection of cooler microclimates or the use of heat-dissipating behaviours (Amat & Masero 2004; DuPlessis et al. 2012; Cunningham, Martin & Hockey 2015; Smit et al. 2016); if those responses are not sufficient, they must perform rapid physiological adjustments (McNabb 2002). Although not without cost (Cunningham, Gardner & Martin 2021), behavioural responses can maximize or postpone the beginning of costly physiological cooling mechanisms, thus contributing to energy and water conservation (Wolf, Wooden & Walsberg 1996; Cook et al. 2020). According to the 'heat dissipation limit theory' — which posits that heat generated during metabolism limits energy intake and, ultimately, reproductive output in endotherms (Speakman & Krol 2010) — behavioural thermoregulation could maximize heat dissipation capacity allowing individuals to allocate energy to other activities different than cooling, such as foraging, mating or brooding (Cunningham, Gardner & Martin 2021). In accordance with this theory, recent studies with free-ranging birds have experimentally supported that constraints on heat dissipation rate could be a key mediator of life-history trade-offs (Nilsson & Nord 2018; Nord & Nilsson 2019; Tapper, Nocera & Burness 2020). Indeed, the potential for behavioural thermoregulation to buffer endotherms against climate warming has been recognized (Cunningham, Martin & Hochey 2021; Buckley, Ehrenberger & Angilletta 2015), and studies on patterns of heat dissipation behaviours have gained attention in recent years (DuPlessis et al. 2012; Cunningham, Martin & Hockey 2015; Edwards, Mitchell & Ridley 2015; Smit et al. 2016; Thompson, Cunningham & McKechnie 2018; Funghi et al. 2019; Kemp et al. 2020; Pattinson et al. 2020). However, studies that incorporate behavioural responses when modelling the impacts of climate warming are still rare (but see Moyer-Horner et al. 2015; Moore, Stow & Kearney 2018; Bladon et al. 2019; Conradie et al. 2019; Enriquez-Urzelai et al. 2020), and the opportunity costs and the response of endotherms to climate warming have been largely overlooked (Cunningham, Gardner & Martin 2021).

Birds are one of the most vulnerable groups against global warming owing to their typically diurnal habits, small body size, relatively high metabolic rates and limited use of thermal refuges (Albright et al. 2017). Panting (sometimes accompanied by gular fluttering) is usually the main thermoregulatory response in birds when environmental temperature exceeds their upper thermoneutral zone limit (Dawson 1982; Wolf & Walsberg 1996). Yet panting is costly because it requires a large expenditure of water and can cause changes in blood chemistry (Calder & Smichdt-Nielsen 1966). Therefore, birds have evolved a series of heat dissipation behaviours that allow them to postpone the beginning of panting to higher temperatures and/or maximize its cooling capacity (e.g., Du Plessis et al. 2012; Smit et al. 2016; Cook et al. 2020). These include postural adjustments, ptilomotor responses, bathing or watering behaviours, shade seeking, or the reduction of activity levels (Amat & Masero 2004; Cunningham, Martin & Hockey 2015; Bladon et al. 2019; Bartholomew 1966; Bryant 1983).

At the same time, studies on avian thermoregulation using infrared thermal imaging have demonstrated that unfeathered and well-vascularized appendages like legs or bills can act as 'thermal radiators' (Tattersall, Andrade & Abe 2009; van de Ven et al. 2016; Van Vuuren, Kemp & McKechnie 2020). This could be particularly important for storks (*Ciconiidae*), as they are long-legged birds that typically inhabit warm, open habitats where heat stress can be problematic (Winkler, Billerman & Lovette 2020). In fact, storks can regulate their blood flow to the legs depending on ambient temperature, promoting vasoconstriction under cold and vasodilation under heat exposition (Kahl 1963; Steen & Steen 1965). Notably, storks can deliberately excrete onto their legs when exposed to increasing environmental temperatures, a phenomenon known as urohidrosis (Kahl 1963) (Figure 1). When overheated, storks repeatedly direct liquid excreta toward their legs (only one leg hit at a time), which usually evaporates before reaching the toes (Kahl 1963). Analogously, the wetting of other body surfaces and the resulting evaporation of body fluids (saliva, mucous, urine or wet faeces) serves as a cooling mechanism in a number of terrestrial, and some marine, vertebrates coping with heat stress (Hainsworth 1967; Gentry 1973; Sturbaum & Riedesel 1977; Marder et al. 1983).

Figure 1. Examples of urohidrosis in different stork species: (a) woolly-necked *Ciconia episcopus*, (b) painted *Mycteria leucocephala* and (c) marabou *Leptoptilos crumenifer* storks. In the first two, the whitish residue produced by urohidrosis covers partially the limbs with feet showing its natural darker colour. In the case of marabou, excreta cover completely the limbs, including the feet. Credit for images: woolly-necked stork – Ossewa (distributed under CC BY-SA 4.0 license; https://creativecommons.org/ licenses/by-sa/4.0/); painted stork – Unni Hariharan (CC BY-SA 4.0); and marabou – Dezidor (CC BY-SA 3.0; https://creativecommons.org/licenses/by/3.0/)

Urohidrosis is relatively rare in birds because it requires regular access to drinking water, but New World vultures, condors, storks, gannets, and boobies engage in this behaviour (Kahl 1963; Hatch 1970; Cooper & Sigfried 1976; Thomas 1984; Hancock, Kushlan & Kahl 1992; Townsend et al. 2002; Finkelstein et al. 2015). Since Kahl's (1963) pioneer work, however, little attention has been paid to the role of urohidrosis for thermoregulation and its underlying mechanisms remain largely unexplored. Kahl reported that urohidrosis is mainly determined by maximum ambient temperature in wood storks *Mycteria americana*; however other environmental variables that also influence heat balance, such as wind speed, solar radiation and humidity, might play a role too. Kahl hypothesized that this behaviour might be more common in tropical species than in temperate species as the latter are less often exposed to heat. He thus proposed urohidrosis to be an adaptive response to life in warm and open habitats with regular access to water. This view is supported by the fact that storks usually, but not always, inhabit landscapes where water sources are fairly accessible (Winkler, Billerman & Lovette 2020). Along this line, a recent study (Czenze et al. 2020) has shown that regularly drinking species have greater cooling capacities and heat tolerance limits than non-drinking species. Hence, interspecific variation in the use of urohidrosis could arise as a function of their dependency on waterbodies.

Here, we combine urohidrosis data from the largest scientifically curated archive of natural history media (photos and videos) with historical microclimate data to investigate the ecological and evolutionary determinants of urohidrosis in all extant stork species. We investigate whether local environmental conditions, latitude, foraging habits, plumage colour, and size (body mass and tarsus length) influence the use of urohidrosis across species. We predict that higher environmental temperatures and a lower dependency on waterbodies promote the use of urohidrosis in order to maintain the heat-water balance. Because of the potentially higher rates of ‘dry’ (non-respiratory) heat loss in species with longer legs (Nudds & Oswald 2007; Symonds & Tattersall 2010), we therefore predict that for species with similar ecological requirements, those with longer legs will use urohidrosis relatively more often to increase heat loss. Likewise, heavier species could exhibit a more pronounced use of urohidrosis due to their relatively larger body water reserves. Finally, we expect darker species to engage in this behaviour more often than lighter ones due to the higher radiation absorptivity of dark plumages (Galván, Rodríguez-Martínez & Carrascal 2018).

Lastly, to assess storks’ vulnerability to high temperatures, we also determined the air temperature at which urohidrosis occurred in 50% of instances for each species separately. Such temperature thresholds are useful in investigating the ecological factors shaping thermal physiological trade-offs, and for understanding ecological and evolutionary determinants of species persistence in hot environments (Smit et al. 2016).

Methods

Data collection

Urohidrosis data

We searched for videos and photos of all 19 extant stork species (Figure 2a) on the Macaulay Library repository (https://www.macaulaylibrary.org; the world's largest scientifically curated archive of natural history media). Since 2020, this online repository also integrates the Internet Bird Collection (https://www.hbw.com/ibc), another repository to videos, photos, and audio recordings from the worldwide community of birdwatchers.

We examined all available images and videos of storks. Of these, we selected 6,112 Macaulay files in which we could determine for each focal individual the presence or absence of urohidrosis with confidence (see Supplementary Information). We recorded the presence/absence of the chalky, whitish residue produced by urohidrosis in each individual (Supplementary Figure S1). We only selected for analyses those photographs or video recordings in which legs were well visible (either in individuals standing or in flight). We discarded files with poor lighting and/or low sharpness. The number of files varied widely across species (range = 38 - 1,534; see Supplementary Information), most likely due either to the rarity or conspicuity of the species in question. We also recorded the date and geographic coordinates in order to associate urohidrosis to environmental conditions (see below).

Morphological and ecological data

For each species, we extracted from the literature data on mean tarsus length (mm; a proxy of leg length) (Hancock, Kushlan & Kahl 1992) and mean body mass (g) (Wilman et al. 2014). Depending on their dependency on waterbodies for foraging activities (Wilman et al. 2014), we classified stork species as: 'wading' (species that mainly feed on fishes and aquatic invertebrates in waterbodies), 'open' (species that mainly feed on terrestrial invertebrates, micromammals and carrion in dry landscapes, such as grassland and savanna habitats) or 'mixed' species (those with mixed diets that forage both on land and in waterbodies).

Plumage scoring

Following Brooke (1998), we scored the plumage colour of the upperparts of each stork species by using colour plates available in Birds of the World (Winkler, Billerman & Lovette 2020). We focused on the upperparts because they are exposed to solar radiation most of the time (either when foraging, resting, flying or breeding). Head, neck, back, wing coverts, primaries and secondaries were scored as 0 when light or as 1 if dark coloured. A total score was obtained by summing up scores in each part, so it ranged from 0 (totally light upperparts) to 6 (totally dark upperparts).

Microclimatic variables

We used the microclima R package (Maclean, Mosedale & Bennie 2019) to obtain microclimatic data for each media file considered in this study. The function 'hourlyNCEP' allows to obtain historical hourly values of several microclimatic variables (maximum air temperature, humidity, pressure, wind speed, wind direction, emissivity, cloud cover and various radiation parameters). We used this function to extract for each focal stork historical microclimate data for the observation date as well as for the previous day, as the presence/absence of urohidrosis can be defined as a 'point event' (i.e., the behaviour has no duration, or its duration is unknown). We considered a conservative time span of two days from the observation as we could not know if the urohidrosis event occurred the day of the observation or earlier. Although urohidrosis residue marks might last even longer, we only included recent events as the inclusion of older, faded marks could have overestimated the use of urohidrosis and thus bias our results (see Discussion).

For the analyses, we selected the following microclimatic variables: mean maximum air temperature (hereafter temperature, ºC), mean solar radiation (radiation, MJ $m^{-2}h^{-1}$), mean wind speed (wind speed, m s^{-1}) and mean specific air humidity (humidity, kg kg^{-1}). All of these were calculated as the average between the values of the day of the observation and the previous day.

Statistical analyses

Interspecific analyses

We modelled the probability of urohidrosis as a function of microclimatic variables and ecological/morphological traits by fitting Bayesian phylogenetic linear mixed models using the package MCMCglmm (Hadfield 2019). We set urohidrosis as a binary (presence/absence) response variable, with temperature, radiation, wind speed, humidity, absolute latitude, mean body mass, mean tarsus length, plumage score, and foraging habit as predictor variables. All continuous variables were scaled in order to facilitate results interpretation. We included media file ID as a random effect to account for possible pseudoreplication derived from the observation of multiple individuals in a given file. To control for phylogenetic effects, we included a consensus tree as random effect. To do this, we first obtained 10,000 trees with different topologies from the Bird Tree project (Jetz et al. 2012) for the 19 extant stork species, using 'Hackett All Species' as backbone. We then derived an ultrametric and rooted consensus tree using the package phytools (Revell 2020). For all analyses, we used weakly informative priors for random effects and ran MCMC chains for 5,000,000 iterations with a burnin of 100,000. We did not detect signs of collinearity between predictors, with all showing variance inflation factors (VIF) values lower than 5. The effect of a given predictor was considered significant if zero was not included in the 95% confidence interval (CI). P-values were calculated automatically by the function based on this assumption and named 'pMCMC' in the package MCMCglmm (Hadfield 2019).

The estimated phylogenetic variance in the model was used to calculate heritability (h^2), a measure of phylogenetic signal equivalent to lambda in phylogenetic generalized least squares models (Freckleton, Harvey & Pagel 2002). h^2 ranges between 0 and 1, with values close to 1 indicating a strong phylogenetic signal in the data and values close to 0 suggesting a negligible phylogenetic signal.

Intraspecific analyses

We constructed similar generalized linear mixed models for each species separately, with urohidrosis as the response variable and microclimatic variables (temperature, radiation, wind speed, and humidity) and absolute latitude as predictors. Again, media file ID was included as a random effect. We used the package lme4 (Bates et al. 2015) to construct mixed effects logistic regression models with a binomial error and logit-link function (fitted by maximum likelihood) (Crawley 2013). First, we included all predictor variables in a global model and then performed model selection using the 'dredge' function in MuMIn package (Barton 2020). We compared among models using an AICc framework, choosing the model with the lowest AICc score as the best supported model. As any of the best supported models had a model weight (wi) greater than 0.90, we used ('full') model averaging (using models with ΔAICc < 2) to identify the most important predictor variables (Grueber et al. 2011; Symonds & Moussalli 2011). We checked each model for overdispersion (values less than 1.5 indicating no issues) and collinearity (all VIF < 5).

When temperature emerged as a significant predictor, we ran separate models to determine the ambient temperature threshold at which urohidrosis is present in the 50% of the observations. Following Smit et al. (2016), this threshold was determined by dividing intercept's absolute value by beta's absolute value.

However, for some species we could not find enough media files that covered a range of latitude or climatic conditions (see Supplementary Table S1) representative of the environmental gradient that they typically experience throughout the year; that was the case for storm's stork *Ciconia stormi* and milky stork *Mycteria cinerea*. Thus, we discarded both species from intraspecific analyses. Similarly, due to model convergence issues, we could not perform analyses for species in which urohidrosis in our dataset is anecdotal; that was the case for saddle-billed *Ephippiorhynchus senegalensis* (instances of urohidrosis: n = 13), black-necked *Ephippiorhynchus asiaticus* (n = 12), maguari *Ciconia maguari* (n = 8), black *Ciconia nigra* (n = 2) and oriental *Ciconia boyciana* (no records of urohidrosis) storks. Therefore, we finally ran intraspecific models for the remaining 12 species for which enough observations of urohidrosis were available over a wide range of latitude and climatic conditions.

All analyses were performed in R (R Core Team 2020). Results are shown as means ± 95 % CI.

Results

Interspecific comparisons

All microclimatic variables emerged as significant predictors (Figure 2b; Supplementary Table S2). Urohidrosis was positively associated with temperature ($\beta = 1.321$, CI = 1.091, 1.591), radiation ($\beta = 0.884$, CI = 0.665, 1.097) and humidity ($\beta = 1.213$, CI = 0.978, 1.474), while was negatively associated with wind speed ($\beta = -0.294$, CI = -0.468, -0.134). Moreover, foraging habit had a significant effect, with open foraging having a significant positive influence on urohidrosis ($\beta = 7.441$, CI = 1.618, 13.804) (Figure 2b). On the other hand, latitude ($\beta = -0.0818$, CI = -0.289, 0.124), plumage score ($\beta = 0.034$, CI = -1.516, 1.606), body mass ($\beta = -2.430$, CI = -6.097, 1.023) and tarsus length ($\beta = 2.982$, CI = -0.091, 6.644) were not significant predictors of urohidrosis, albeit tarsus length had a marginally significant effect (only 7.35 % of its posterior distribution overlapping zero) (Figure 2b).

Overall, data showed a low phylogenetic signal ($h^2 = 0.059$).

Intraspecific comparisons

Model-averaged values (ΔAICc < 2) showed that determinants of urohidrosis varied across the 12 stork species analyzed (Supplementary Table S3). As expected, the most significant predictor across species was temperature, having a positive effect on urohidrosis in six of the 12 species (Supplementary Table S3). The temperature at which 50% of the individuals presented urohidrosis varied across species, ranging from 27.68 ºC in the white stork *Ciconia ciconia* to 33.49 ºC in the woolly-necked stork *Ciconia episcopus* (Figure 3).

Moreover, the use of urohidrosis increased with radiation in the wood stork (Supplementary Table S3), and with humidity in the white stork and in the lesser adjutant *Leptoptilos javanicus* (Supplementary Table S3). Likewise, latitude was positively associated with urohidrosis in the greater adjutant *Leptoptilos dubius* and painted stork *Mycteria leucocephala* but showed a negative effect in the white stork and the jabiru *Jabiru mycteria* (Supplementary Table S3). Nevertheless, the use of urohidrosis was not influenced by any of the studied variables in the African *Anastomus lamelligerus* and Asian *Anastomus oscitans* openbills, the marabou *Leptoptilos crumenifer* and the yellow-billed stork *Mycteria ibis* (Supplementary Table S3).

Finally, wind speed was not a significant predictor of urohidrosis use in any of the species analysed (Supplementary Table S3).

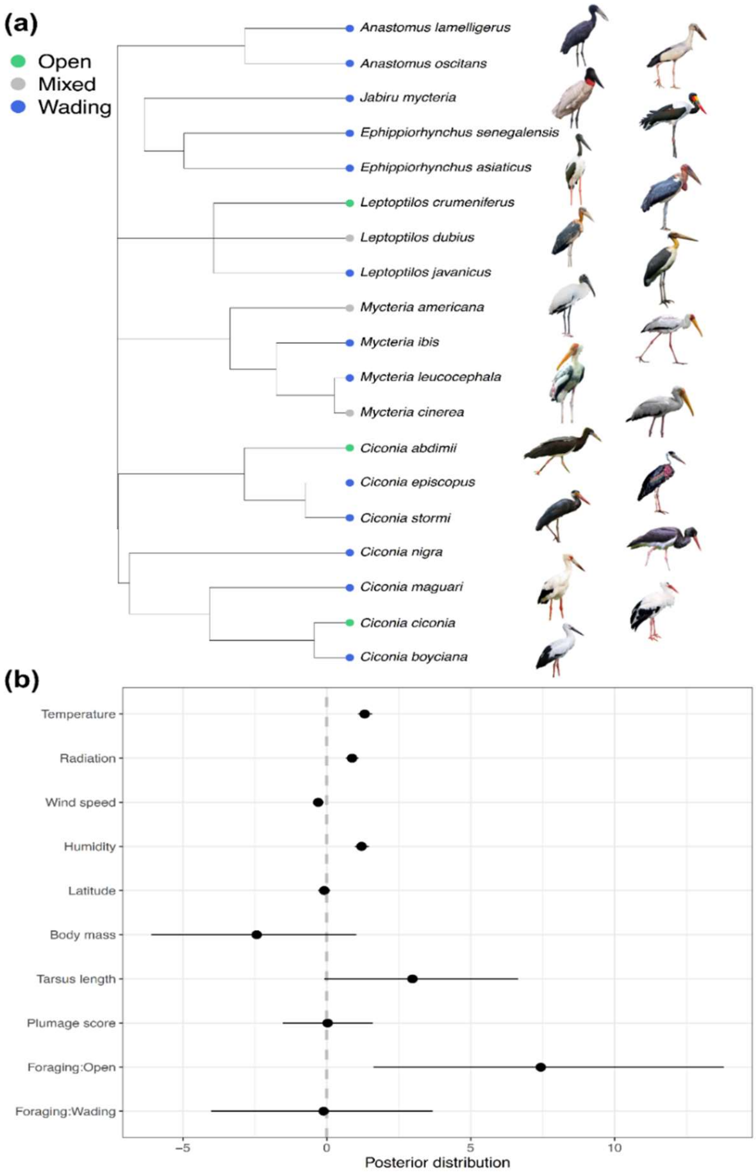

Figure 2. (a) Phylogenetic tree showing the foraging habits of stork species analyzed in this study. **(b)** Posterior distributions (with 95% CI) of predictors estimates from phylogenetic generalized mixed models. Significance is indicated by estimates not crossing zero. Reference level for foraging habit is “mixed”. Species pictures were taken from photographs distributed under CC BY licenses. Credits for pictures: African openbill and maguari stork – Lip Kee Yap (distributed under CC BY 2.0; https://creativecommons.org/licenses/by-sa/2.0/), Asian openbill – Shino J Koottanad (CC BY-SA 4.0; https://creativecommons.org/licenses/by-sa/4.0/), jabiru – Gmmv1980 (CC BY-SA 4.0), saddle-billed, yellow-billed and black storks – Bernard Dupont (CC BY-SA 2.0; https://creativecommons.org/licenses/by-sa/2.0/), black-necked stork – JJ Harrison (CC BY-SA 4.0), marabou and Abdim’s stork – Charles J Sharp (CC BY-SA 4.0), greater adjutant – Yathin S Krishnappa (CC BY 3.0; https://creativecommons.org/licenses/by/3.0/), lesser adjutant – Irothu (CC BY-SA 4.0), wood stork – Kaldari (CC0 1.0; https://creativecommons.org/publicdomain/zero/1.0/), painted stork – Manvendra Banghi (CC BY-SA 2.0), milky stork – Gerifalte Del Sabana (CC BY-SA 4.0), woolly-necked stork – Shantanu Kuveshkar (CC BY-SA 4.0), storm’s stork – Mike Prince (CC BY 2.0), white stork – André Karwath (CC BY-SA 2.5; https://creativecommons.org/licenses/by-sa/2.5/), oriental stork – Alpsdake (CC BY-SA 4.0).

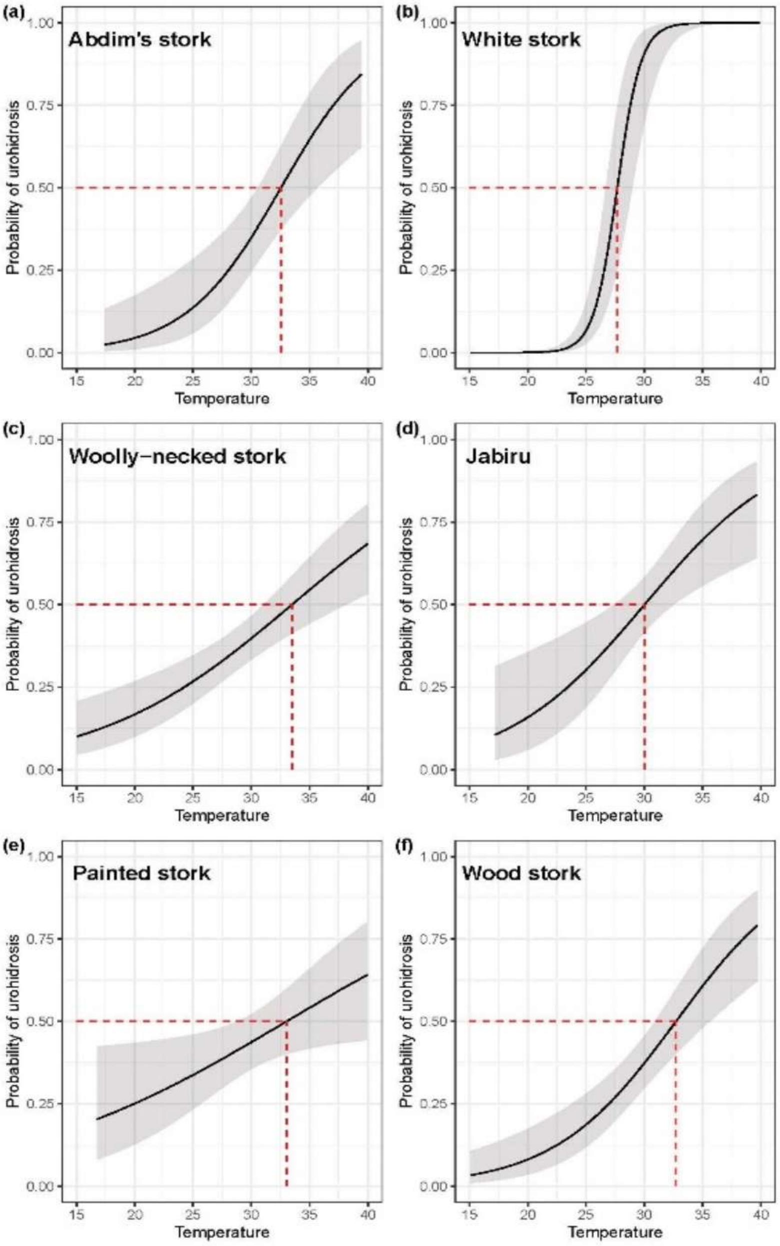

Figure 3. Probability of urohidrosis use in response to air temperature for (a) Abdim's stork *Ciconia abdimii*, (b) white stork *Ciconia ciconia*, (c) woolly-necked stork Ciconia episcopus, (d) jabiru *Jabiru mycteria*, (e) painted stork *Mycteria leucocephala* and (f) wood stork *Mycteria americana*. Dashed red lines indicate the air temperature at which urohidrosis occurred in 50% of instances.

Discussion

Many endotherms inhabiting hot, open environments regularly cope with operative temperatures that approach or exceed their body temperature, thus facing a trade-off between dehydration avoidance and resistance to hyperthermia (Czenze et al. 2020). In storks and other avian clades, urohidrosis is believed to be an important thermoregulatory behavioural response to avoid hyperthermia (Kahl 1963). In this study, we examined the factors driving the use of urohidrosis by storks and its potential implications for thermoregulation in hot environments. After controlling for latitudinal and evolutionary patterns, we found that high environmental temperature, solar radiation and air humidity, as well as low wind speed, promote the use of urohidrosis across species. This finding confirms earlier experiments with wood storks showing that urohidrosis is mainly determined by maximum ambient temperature (Kahl 1963), but also shows that other environmental variables jointly determine the use of this cooling mechanism. This is no surprise as three of these variables (temperature, radiation and wind speed) make up ‘operative temperatures’ that affect an animal’s heat balance (reviewed in Bakken & Angilletta 2014). Besides, increased air humidity can constrain evaporative heat loss through panting or through the skin (Webster, Campbell & King 1985; van Dyk, Noakes & McKechnie 2019) and may force birds to rely more on urohidrosis to increase total heat dissipation.

As predicted, our interspecific analyses also showed that species with a lower dependency on waterbodies (i.e., ‘open’ species typically foraging on land) exhibited a more pronounced use of urohidrosis than those mainly foraging in waterbodies (i.e., ‘wading’ or ‘mixed’ species). Open-foraging species with more limited access to waterbodies rely more on urohidrosis, presumably to maximize heat loss through evaporative cooling. Heat-load problems will occur more frequently in exposed places where heat gain is high (high temperatures and radiation), especially if the heat-loss potential is low (low winds, high humidity) (see Battley et al. 2003). On the other hand, wading species could potentially lose all of their heat production if standing in water, as the thermal conductivity of water is 25 times that of air (Piersma & van Gils 2011). Notably, Fitzpatrick et al. (2015) modelled the thermoregulatory implications of wading in whooping cranes *Grus americana* and showed that the ‘upper critical temperature’ increased substantially when legs were submerged in water. Wading storks could therefore benefit from the higher convective heat transfer of water, increasing net heat loss and diminishing heat stress while foraging. It is possible that wading or ‘mixed’ species rely on urohidrosis while exposed to more stressful thermal conditions, e.g., during reproduction, when parents must balance thermoregulation against breeding activities (Cook et al. 2020). Together, our results support the notion that urohidrosis is an adaptive response for life in open and warm habitats.

However, dark- and light-pigmented storks did not differ in their use of urohidrosis. Although darker colours absorb more radiation than lighter colours (Lustick, Battersby & Kelty 1979; Galván, Rodríguez-Martínez & Carrascal 2018), evidence that darker birds are limited from occupying environments with high temperatures is mixed (Ward et al. 2002; Galván, Rodríguez-Martínez & Carrascal 2018). Melanin-rich, dark feathers and skins are more resistant to abrasion and can protect birds against ultraviolet irradiation (Ward et al. 2002; Nicolaï et al. 2020). In fact, colour itself is not necessarily an important determinant of heat gain and heat loss (Mitchell et al. 2018). The emittance of infrared radiation is hardly affected by colour (Mitchell et al. 2018), while feather structure, plumage thickness and orientation towards the sun have a larger influence on heat transfer (Walsberg, Campbell & King 1978; Lustick, Battersby & Kelty 1979). Also, heat transfer could be modified by behavioural adjustments like ptilomotor responses or postural changes (McFarland & Baher 1968; Walsberg, Campbell & King 1978; Hothola, Rintamäki & Hissa 1980). For instance, Walsberg, Campbell & King (1978) showed how both light- and dark-plumaged pigeons *Columba livia* diminished their radiative heat loads when they made use of ptiloerection, which increased plumage thermal resistance by about 50%. Storks use ptiloerection and other postural adjustments (Kahl 1971) which could contribute to diminish radiative heat load gain through their plumage. This might partly explains the absence of differences in the use of urohidrosis between darker and lighter species.

Likewise, latitude was not associated with the use of urohidrosis across species. This gives no support for the idea that tropical species likely rely more on urohidrosis than temperate species to dissipate heat (Kahl 1963). Nevertheless, our species-specific analyses gave mixed support for this idea: a more pronounced use of urohidrosis near the tropics was found in the white stork and the jabiru, but the opposite was found in the painted stork and the greater adjutant. The white stork winters in the tropics and breeds in temperate latitudes, while the others occupy tropical and subtropical latitudes throughout the year (Winkler, Billerman & Lovette 2020). Therefore, the effect of latitude on urohidrosis in the latter ones could be obscured, with urohidrosis use being associated with local environmental conditions rather than with latitude.

In contrast to our prediction, body mass did not emerge as significant predictor of urohidrosis use, although tarsus length had a marginally significant and positive effect (see Supplementary Table S2). We predicted that larger species (higher body mass and longer legs) would use urohidrosis more frequently due to their larger body water reserves and potentially larger thermal windows. However, all stork species usually have regular access to waterbodies (Winkler, Billerman & Lovette 2020) and thus water should not be a strong limiting factor for maintaining their water balance, as reported for dessert passerines (Czenze et al. 2020). On the other hand, the marginal positive effect of tarsus length supports the idea that species with longer legs use urohidrosis relatively more often to increase heat loss. Although tarsus length varies widely in the studied species (from 121 to 308 mm), its relative length (i.e., tarsus length to height ratio) was rather similar across species (from 3.98 to 6.44). Thus, the relative surface available to dissipate heat through contact of excreta and leg's skin is quite similar across storks. This might explain why tarsus length was not a strong determinant of urohidrosis use.

Furthermore, our data indicates that urohidrosis is a well conserved behaviour across all stork species. This behaviour had also been reported in species from closely related phylogenetic groups like New World Vultures (*Cathartidae*), as well as in unrelated ones like boobies and gannets (Sulidae), suggesting it may be polyphyletic. Notably, species of these groups generally breed and/or forage in open landscapes where heat gain is typically high (Cooper & Siegfried 1976; Hancock, Kushlan & Kahl 1992; Finkelstein et al. 2015). They also have relatively large legs that contribute significantly to the total uninsulated surface area of a bird. In addition to environmental pressures, large and highly vascularized legs — which are able to vasoconstrict and vasodilate in respond to hot or cold environmental conditions (Kahl 1963; Steen & Steen 1965) — can be interpreted as preadaptations favouring the evolution of urohidrosis.

At the intraspecific level, we provided the first temperature thresholds of heat dissipation behaviours for large-bodied wading birds. Compared to other 50% thresholds of heat dissipation behaviours such as panting (from 33.9 to 46.1 ºC) (Smit et al. 2016; Thompson, Cunningham & McKechnie 2018) or wing drooping (from 35.3 to 44.6 ºC) (Smit et al. 2016) in small-sized desert birds, the environmental temperatures at which 50% of birds presented urohidrosis were generally lower (27.7 to 33.5 ºC). These differences could be explained by the fact that urohidrosis is probably cheaper (in terms of metabolic cost and water loss) than panting. In various groups of mammals, the increased output of saliva to provide water for evaporation from the respiratory track in response to heat stress is also utilized for evaporative cooling through saliva-spreading or licking (Hainsworth 1967; Dawson, Robertshaw & Taylor 1974; Marder et al. 1983). Excess production of saliva, droppings – including cloacal evaporation (Hoffman, Walsberg & DeNardo 2007) – and other fluid secretions – e.g., diluted nasal mucous (Van Vuuren, Kemp & McKechnie 2020) – could be seen as cheap means for heat loss by evaporative cooling, especially in birds since they lack sweat glands. This, combined with higher thermal inertia of larger birds, could favour the use of urohidrosis at lower temperatures in order to postpone the onset of physiological responses (e.g., panting, facultative hyperthermia) and/or maximize its cooling capacity.

The pigmentation of legs could play a signaling role besides their function as thermal windows. If so, urohidrosis could potentially result in a trade-off between thermoregulation and social signaling, as hypothesized for New World vultures (Graves 2019). This could be particularly crucial during mating and breeding which usually coincide with the highest annual temperatures. As in other birds, the colour of some storks' legs is pigmented based and might act as an honest signal of mate fitness (Torres & Velando 2005; López-Rull et al. 2016). However, the potential influence of urohidrosis on signaling remains unexplored.

There are several methodological issues that may have impacted the results, although we do not think that they compromise the main inferences. Although scientifically curated repositories such as Macaulay Library are increasingly used to address numerous questions in conservation biology, ecology, and evolution (e.g., Jàric et al. 2020; Gutiérrez & Soriano-Redondo 2021; Vrettos, Reynolds & Amar 2021) we are aware of their limitations. First, we treated urohidrosis as a 'point event' behaviour but we could not ascertain from media files the exact moment in which it occurred. Therefore, we could not match urohidrosis events to hourly estimates of microclimate but had to use daily averaged values instead. This might have resulted in the absence of influence of wind at intraspecific level, as this variable is probably the most changing variable due to local topography or plant cover. Second, our approach prevented the association of urohidrosis with other heat dissipation behaviours and postures (e.g., panting, wing drooping, ptiloerection; Kahl 1971) that have a defined duration and could be potentially ranked into a sequence that reflect different degrees of thermal load. Third, one might argue that the more pronounced use of urohidrosis in 'open' species could be explained by passive washing of urohidrosis marks in 'wading' storks during foraging. Following this reasoning, photographs or videos taken after wading or bathing would show storks with clean legs, missing potential urohidrosis events. However, observations made during late spring in a colony of breeding white storks in southern Spain (unpublished data) proves that urohidrosis marks are quite persistent and usually do not disappear completely after wading or bathing (see Figure S2), lasting for a variable timespan, from some hours to some days (up to 3 days) (J. Cabello-Vergel personal observation). Nonetheless, legs get progressively cleaner after several wading events. Thus, we cannot completely rule out that our indirect approach — based on photographs and videos randomly taken by birdwatchers around the world — has underestimated urohidrosis across all species considered in this study, not only in 'wading' species.

Despite these caveats, our study supports the notion that urohidrosis is correlated with overheating and the foraging ecology of birds. Yet, the energetics associated with this thermoregulatory behaviour (including the potential effect of the higher reflectivity of the leg's surface covered by white excreta) remains to be studied in wild birds. Although Kahl (1963) experimentally showed that the internal body temperature of wood storks equipped with thermistor sensing probes change when increasing or decreasing heat loss from the legs and feet, all attempts to induce urohidrosis in birds wired for temperature readings and harnessed failed. Assuming that 3-kg storks excrete 1-2 cc of urine on their legs about every minute during short periods of heat stress, and that the evaporation of 1 g of water at 40 ºC uses 575 calories, a sizable amount of heat may be dissipated from unfeathered parts of the leg. With modern thermal imaging cameras, it would be possible to quantify the cooling efficiency of urohidrosis along the leg surface and its contribution to overall heat loss (along with other bare parts like the bill).

Finally, this study demonstrates how data accumulated in digital resources combined with novel methods for computing microclimate can provide insight into animal thermoregulation. Future studies on this and similar cooling mechanisms (including the wetting of body surfaces in terrestrial and marine vertebrates; Hainsworth 1967; Gentry 1973; Van Vuuren, Kemp & McKechnie 2020) should significantly contribute to a better understanding of the significance of behavioural thermoregulation in hot environments. We propose that this method could be developed as a proxy for investigating community-level response to heat stress, and that it would be particularly relevant to predict vulnerability to climate warming scenarios.

References

Albright, T. P. et al. (2017) Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 2283–2288.

Amat, J. A. & Masero, J. A. (2004) How Kentish plovers, Charadrius alexandrinus, cope with heat stress during incubation. *Behav. Ecol. Sociobiol.* **56**, 26–33.

Bakken, G. S. & Angilletta, M. J. (2014) How to avoid errors when quantifying thermal environments. *Funct. Ecol.* **28**, 96–107.

Bartholomew, G.A. (1966) The role of behavior in the temperature regulation of the masked booby. *Condor* **68**, 523-535.

Barton, K. (2020) Package MuMin: Multi-model Inference *https://cran.r-project.org/web/packages/MuMIn/index.html.*

Bates, D., Mächler, M., Bolker, B. M. & Walker, S. C. (2015) Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**.

Battley, P. F., Rogers, D. I., Piersma, T. & Koolhaas, A. (2003) Behavioural evidence for heat-load problems in Great Knots in tropical Australia fuelling for long-distance flight. *Emu* **103**, 97–103.

Bladon, A.J. *et al.* (2019) Behavioural thermoregulation and climatic range restriction in the globally threatened ethiopian bush-crow *Zavattariornis stresemanni*. *Ibis*. **161**(3), 546–558.

Brooke, M. D. L. (1998) Ecological factors influencing the occurrence of 'flash marks' in wading birds. *Funct. Ecol.* **12**, 339–346.

Bryant, D. M. (1983) Heat stress in tropical birds: behavioural thermoregulation during flight. *Ibis* **125**, 313–323.

Buckley, L. B., Ehrenberger, J. C. & Angilletta, M. J. (2015) Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Funct. Ecol.* **29**, 1038–1047.

Calder, W. A. & Smichdt-Nielsen, K. (1966) Evaporative cooling and respiratory alkalosis in the pigeon. *Proc. Natl. Acad. Sci. U.S.A.* **55** (4), 750-756.

Conradie, S. R., Woodborne, S. M., Cunningham, S. J. & McKechnie, A. E. (2019) Chronic, sublethal effects of high temperatures will cause severe declines in southern African arid-zone birds during the 21st century. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 14065–14070.

Cook, T. R. *et al.* (2020) Parenting in a warming world: Thermoregulatory responses to heat stress in an endangered seabird. *Conserv. Physiol.* **8**, 1–13.

Cooper, J. & Siegfried, W. R. (1976) Behavioural responses of young cape gannets Sula capensis to high ambient temperatures . *Mar. Behav. Physiol.* **3**, 211–220.

Crawley, M.J. (2013) The R Book (John Wiley & Sons).

Cunningham, S. J., Martin, R. O. & Hockey, P. A. R. (2015) Can behaviour buffer the impacts of climate change on an arid-zone bird? *Ostrich* **86**, 119–126.

Cunningham, S. J., Gardner, J. L. & Martin, R. O. (2021) Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* **19** (5), 300-307.

Czenze, Z. J., Freeman, M.T., Kemp, R., van Jaarsveld, B., Wolf, B.O., McKechnie, A.E. (2020) Regularly drinking desert birds have greater evaporative cooling capacity and higher heat tolerance limits than non-drinking species. *Funct. Ecol.* **34**, 1589–1600.

Dawson, T.J., Robertshaw, D. & Taylor, C.R. (1974) Sweating in the kangaroo: A cooling mechanism during exercise, but not in the heat. *Am. J. Physiol.* **227** (2), 494-498.

Dawson, W. R. (1982) Evaporative losses of water by birds. *Comp. Biochem. Physiol. Part A Physiol.* **71**, 495–509.

du Plessis, K. L., Martin, R. O., Hockey, P. A. R., Cunningham, S. J. & Ridley, A. R. (2012) The costs of keeping cool in a warming world: Implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Glob. Chang. Biol.* **18**, 3063–3070.

Edwards, E. K., Mitchell, N. J. & Ridley, A. R. (2015) The impact of high temperatures on foraging behaviour and body condition in the Western Australian Magpie Cracticus tibicen dorsalis. *Ostrich* **86**, 137–144.

Enriquez-Urzelai, U., Tingley, R., Kearney, M.R., Sacco, M., Palacio, A.S., Tejedo, M., Nicieza, A.G. (2020) The roles of acclimation and behaviour in buffering climate change impacts along elevational gradients. *J. Anim. Ecol.* **89**, 1722–1734.

Finkelstein, M., Kuspa, Z., Snyder, N.F. & Schmitt, N.J. (2015) California condor (Gymnogyps californianus), version 2.0. In The Birds of North America (P. G. Rodewald, Editor). Cornell Lab of Ornithology.

Fitzpatrick, M. J., Mathewson, P. D. & Porter, W. P. (2015) Validation of a mechanistic model for non-invasive study of ecological energetics in an endangered wading bird with counter-current heat exchange in its legs. *PLoS One* **10**, 1–34.

Freckleton, R. P., Harvey, P. H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *Am. Nat.* **160**, 712–726.

Funghi, C., McCowan, L. S. C., Schuett, W. & Griffith, S. C. (2019) High air temperatures induce temporal, spatial and social changes in the foraging behaviour of wild zebra finches. *Anim. Behav.* **149**, 33–43.

Galván, I., Rodríguez-Martínez, S. & Carrascal, L. M. (2018) Dark pigmentation limits thermal niche position in birds. *Funct. Ecol.* **32**, 1531–1540.

Gentry, R.L. (1973) Thermoregulatory behavior of eared seals. *Behaviour* **46**(2), 73-93.

Graves, G. R. (2019) Urohidrosis and tarsal color in Cathartes vultures (Aves: Cathartidae). *Proc. Biol. Soc. Washingt.* **132**, 56–64.

Grueber, C. E., Nakagawa, S., Laws, R. J. & Jamieson, I. G. (2011) Multimodel inference in ecology and evolution: Challenges and solutions. *J. Evol. Biol.* **24**, 699–711.

Gutiérrez, J. S. & Soriano-Redondo, A. (2021) Laterality in foraging phalaropes promotes phenotypically assorted groups. *Behav. Ecol.* **31**, 1429–1435.

Hadfield, A. J. (2019) Package ‘ MCMCglmm ’. https://cran.r-project.org/web/packages/MCMCglmm/

Hainsworth, F.R. (1967) Saliva spreading, activity and body temperature regulation in the rat. *Am. J. Physiol*. **212**, 1288-1292.

Hancock, J.A., Kushlan, J.A. & Kahl, M.P. (1992) Storks, Ibises and Spoonbills of the World (Academic Press, 1992).

Hatch, D.E. (1970) Energy conserving and heat dissipating mechanisms of the turkey vulture. *Auk*. **87**(1), 111-124.

Hoffman, T.C.M., Walsberg, G.E. & DeNardo, D.F. (2007) Cloacal evaporation: an important and previously undescribed mechanism for avian thermoregulation. *J. Exp. Biol.* **210**, 741-749.

Hohtola, E., Rintamäki, H. & Hissa, R. (1980) Shivering and ptiloerection as complementary cold defense responses in the pigeon during sleep and wakefulness. *J. Comp. Physiol*. **136**, 77–81.

Jarić, I. *et al.* (2020) iEcology: Harnessing large online resources to generate ecological insights. *Trends Ecol. Evol.* **35**, 630–639.

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K. & Mooers, A.O. (2012) The global diversity of birds in space and time. *Nature*. **491**(7424), 444–448.

Kahl, P.M. (1963) Thermoregulation in the wood stork , with special reference to the role of the legs. *Physiol. Zool*. **36**(2), 141–151.

Kahl, P.M. (1971) Spread-wing postures and their possible functions in the Ciconiidae. *Auk*. **88**(4), 715-722.

Kemp, R., Freeman, M.T., van Jaarsverld, B., Czenze, Z.J., Conradie, S.R., McKechnie, A.E. (2020) Sublethal fitness costs of chronic exposure to hot weather vary between sexes in a threatened desert lark. *Emu*. **120**, 216–229.

López-Rull, I., Lifshitz, N., Macías Garcia, C., Graves, J. A. & Torres, R. (2016) Females of a polymorphic seabird dislike foreign-looking males. *Anim. Behav.* **113**, 31–38.

Lustick, S., Battersby, B. & Kelty, M. (1979) Effects of insolation on juvenile herring gull energetics and behavior. *Ecologia*. **60**(4), 673-678.

Maclean, I.M.D., Mosedale, J.R. & Bennie, J.J. (2019) Microclima: An r package for modelling meso- and microclimate. *Methods Ecol. Evol.* **10**(2), 280–290.

Marder, J., Porat, I., Raber, P. & Adler, J. (1983) Acid-base balance and body temperature regulation of heat stressed *Psammomys obesus* (*Gerbillinae*): The effect of bicarbonate loss via saliva spreading. *Physiol Zool*. **56**(3), 389-396.

McFarland, D. J. & Baher, E. (1968) Factors affecting feather posture in the barbary dove. *Anim. Behav.* **16**, 171–177.

McNab, B.K. (2002) The Physiological Ecology of Vertebrates: A View from Energetics (Cornell University Press, 2002).

Mitchell, D., Snelling, E.P., Hetem, R.S., Maloney, S.K., Strauss, W.M., & Fuller, A. (2018) Revisiting concepts of thermal physiology: Predicting responses of mammals to climate change. *J. Anim. Ecol.* **87**, 956–973.

Moore, D., Stow, A. & Kearney, M. R. (2018) Under the weather?—The direct effects of climate warming on a threatened desert lizard are mediated by their activity phase and burrow system. *J. Anim. Ecol.* **87**, 660–671.

Moyer-Horner, L., Mathewson, P. D., Jones, G. M., Kearney, M. R. & Porter, W. P. (2015) Modeling behavioral thermoregulation in a climate change sentinel. *Ecol. Evol.* **5**, 5810–5822.

Nicolaï, M. P. J., Shawkey, M. D., Porchetta, S., Claus, R. & D'Alba, L. (2020) Exposure to UV radiance predicts repeated evolution of concealed black skin in birds. *Nat. Commun.* **11**, 1-8.

Nilsson, J. Å. & Nord, A. (2018) Testing the heat dissipation limit theory in a breeding passerine. *Proc. R. Soc. B Biol. Sci.* **285**, 1-7.

Nord, A. & Nilsson, J. Å. (2019) Heat dissipation rate constrains reproductive investment in a wild bird. *Funct. Ecol.* **33**, 250–259.

Nudds, R. L. & Oswald, S. A. (2007) An interspecific test of Allen's rule: Evolutionary implications for endothermic species. *Evolution*. **61**, 2839–2848.

Pattinson, N. B. *et al.* (2020) Heat dissipation behaviour of birds in seasonally hot arid-zones: are there global patterns? *J. Avian Biol.* **51**, 1–11.

Piersma, T. & van Gils, J.A. (2011) The Flexible Phenotype: A Body-Centered Integration of Ecology, Physiology, and Behavior (Oxford University Press, 2011).

R Core Team. (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Revell, M.L.J. (2020) Package ‘ phytools ’ https://cran.r-project.org/web/packages/phytools/

Smit, B., Zietsman, G., Martin, R.O., Cunningham, S.J., McKechnie, A.E., Hockey, P.A.R. (2016) Behavioural responses to heat in desert birds: implications for predicting vulnerability to climate warming. *Clim. Chang. Responses*. **3**, 1–14.

Speakman, J. R. & Król, E. (2010) Maximal heat dissipation capacity and hyperthermia risk: Neglected key factors in the ecology of endotherms. *J. Anim. Ecol.* **79**, 726–746.

Steen, I. & Steen, J. B. (1965) The Importance of the Legs in the Thermoregulation of Birds. *Acta Physiol. Scand.* **63**, 285–291.

Sturbaum, B. A. & Riedesel, M. L. (1977) Dissipation of stored body heat by the ornate box turtle, *Terrapene ornata*. *Comp. Biochem. Physiol. Part A Physiol.* **58**, 93–97.

Symonds, M. R. E. & Tattersall, G. J. (2010) Geographical variation in bill size across bird species provides evidence for Allen’s rule. *Am. Nat.* **176**, 188–197.

Symonds, M. R. E. & Moussalli, A. (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike’s information criterion. *Behav. Ecol. Sociobiol.* **65**, 13–21.

Tapper, S., Nocera, J. J. & Burness, G. (2020) Heat dissipation capacity influences reproductive performance in an aerial insectivore. *J. Exp. Biol.* **223**, 10.

Tattersall, G. J., Andrade, D. V. & Abe, A. S. (2009) Heat exchange from the toucan bill reveals a controllable vascular thermal radiator. *Science*. **325**, 468–470.

Thomas, B. T. (1984) Maguari Stork Nesting: Juvenile Growth and Behavior. *Auk*. **101**, 812–823.

Thompson, M. L., Cunningham, S. J. & McKechnie, A. E. (2018) Interspecific variation in avian thermoregulatory patterns and heat dissipation behaviours in a subtropical desert. *Physiol. Behav.* **188**, 311–323.

Torres, R. & Velando, A. (2005) Male preference for female foot colour in the socially monogamous blue-footed booby, Sula nebouxii. *Anim. Behav.* **69**, 59–65.

Townsend, H., Huyvaert, K.P., Hodum, P.J. & Anderson, D.J. (2002) Nesting distributions of Galapagos boobies (Aves: *Sulidae*): an apparent case of amensalism. *Oecologia*. **132**, 419-427.

Van De Ven, T. M. F. N., Martin, R. O., Vink, T. J. F., McKechnie, A. E. & Cunningham, S. J. (2016) Regulation of heat exchange across the hornbill beak: Functional similarities with toucans? *PLoS ONE*. **11**, 1–14.

Van Dyk, M., Noakes, M.J. & McKechnie, A.E. (2019) Interactions between humidity and evaporative heat dissipation in a passerine bird. *J.Comp. Physiol. B.* **189**, 299-308.

Van Vuuren, A. K., Kemp, L. V. & McKechnie, A. E. (2020) The beak and unfeathered skin as heat radiators in the southern ground-hornbill. *J. Avian Biol.* **51**, 1–7.

Vrettos, M., Reynolds, C., & Amar, A. (2021) Malar stripe size and prominence in peregrine falcons vary positively with solar radiation: support for the solar glare hypothesis. *Biol. Lett.* **17**, 20210116.

Walsberg, G. E., Campbell, G. S. & King, J. R. (1978) Animal coat color and radiative heat gain: A re-evaluation. *J. Comp. Physiol. B*. **126**, 211–222.

Ward, J. M., Blount, J. D., Ruxton, G. D. & Houston, D. C. (2002) The adaptive significance of dark plumage for birds in desert environments. *Ardea*. **90**, 311–323.

Webster, M.D., Campbell, G.S. & King, J.R. (1985) Cutaneous resistance to water-vapor diffusion in pigeons and the role of the plumage. *Physiol. Zool.* **58**(1), 58-70.

Wilman, H., Belmaker, J., Simpson, J., de la Rosa, C., Rivadeneira, M.M., & Jetz, W. (2014) EltonTraits 1.0 : Species-level foraging attributes of the world ’s birds and mammals. *Ecology*. **95**, 2027.

Winkler, D.W., Billerman, S.M. & Lovette, I.J. (2020) Storks (*Ciconiidae*), version 1.0. In Birds of the World (S. M. Billerman, B. K. Keeney, P. G. Rodewald, and T. S. Schulenberg, Editors). Cornell Lab of Ornithology.

Wolf, B.O., Wooden, K.M., Walsberg, G.E. (1966) The use of thermal refugia by two small desert birds. *Condor*. **98**(2), 424-428.

Wolf, B. O. & Walsberg, G. E. (1996) Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. *J. Exp. Biol.* **199**, 451–457.

Appendix 3: Supplementary Material

Methods

Urohidrosis data collection

We looked for urohidrosis across all extant stork photographs and video recordings contained in Macaulay Library (https://www.macaulaylibrary.org; Cornel Lab of Ornithology). After discard those files in which urohidrosis could not be determined with confidence (those in which legs were not well visible or those with low sharpness), we analyzed a total of 6,112 files. The number of files varied considerably across species: African openbill (n = 234), Asian openbill (n = 395), jabiru (n = 202), saddle-billed stork (n = 285), black-necked stork (n = 184), marabou (n = 576), lesser adjutant (n = 216), greater adjutant (n = 78), wood stork (n = 456), yellow-billed stork (n = 388), painted stork (n = 259), milky stork (n = 83), Abdim's stork (n = 174), woolly-necked stork (n = 315), storm's stork (n = 48), black stork (n = 446), maguari stork (n = 201), white stork (n = 1,534), and oriental stork (n = 38).

Figure S1. Example of urohidrosis data collection from a greater adjutant file employed in this study (photograph by Robin Welsh/Macaulay Library at the Cornell Lab of Ornithology, ML146223751). Numbers show the way in which urohidrosis scan in each individual was performed.

When several storks were present in the same file we determined the presence/absence of urohidrosis of each individual in which legs were well visible from top left to bottom right (see Figure S1). Overall, we could extract 9,132 individual observations, distributed between species as follows: African openbill (n = 266), Asian openbill (n = 456), jabiru (n = 226), saddle-billed stork (n = 306), black-necked stork (n = 194), marabou (n = 708), lesser adjutant (n = 231), greater adjutant (n = 160), wood stork (n = 490), yellow-billed stork (n = 540), painted stork (n = 314), milky stork (n = 111), Abdim's stork (n = 235), wooly-necked stork (n = 334), storm's stork (n = 48), black stork (n = 538), maguari stork (n = 210), white stork (n = 3,721) and oriental stork (n = 44).

Besides, during 2021 late spring we performed a study on thermoregulatory behaviour in a white stork's breeding colony placed near to Guadiana River (Mérida, Extremadura). During fieldwork we observed several events of urohidrosis when ambient temperature was high (above 30 ºC). As previously demonstrated, this behaviour is intentional, with storks a) approaching their tibias to the cloaca or b) pointing the cloaca towards the legs in order to assure that the excreta hit their limbs. Once urohidrosis occurred, the white residue marks remained in the legs of known individuals for a variable timespan (from hours to up to 3 days), even after events of wading or bathing. When birds wade in the river in order to take food and/or water to their chicks, urohidrosis marks diluted to some extent and thus faded, thought they did not disappear completely (Figure S2).

Figure S2. Urohidrosis residue marks can persist after wading, as illustrated by a comparison between photographs taken before a stork waded in the river (A) and after that single wading event (B). Photographs taken by the authors (J.C.-V.).

Microclimatic and latitudinal data

From the selected media files we could obtain microclimatic and latitudinal gradients representative of the natural distribution ranges for the majority of the species. That was not the case for storm's and milky storks, in which the low number of observations impeded our ability to obtain a representative gradient of temperature, radiation, wind speed, humidity and/or latitude values (see Table S1).

Table S1. Temperature (ºC), radiation (MJ m^{-2} h^{-1}), wind speed (m s^{-1}), humidity (kg kg^{-1}), and latitude (as absolute) range collected for each species considered in this study.

Species	Temperature	Radiation	Wind speed	Humidity	Latitude
Anastomus lamelligerus	6.8 – 43.1	0.1 – 3.4	0.4 – 6.7	0.00 – 0.02	0.1 – 27.8
Anastomus oscitans	7.4 – 44.3	0.1 – 2.1	0.3 – 8.1	0.00 – 0.03	1.0 – 31.7
Ciconia nigra	-0.8 – 45.3	0.0 – 3.5	0.3 – 9.4	0.00 – 0.03	0.0 – 59.2
Ciconia abdimii	17.4 – 39.4	0.2 – 2.4	0.5 – 7.2	0.00 – 0.02	0.0 – 29.7
Ciconia episcopus	2.9 – 43.9	0.1 – 2.4	0.2 – 10.9	0.00 – 0.03	0.1 – 32.6
Ciconia stormii	25.1 – 32.6	0.2 – 2.0	0.4 – 2.6	0.02 – 0.02	1.0 – 5.7
Ciconia maguari	13.4 – 38.6	0.1 – 2.5	0.9 – 10.5	0.00 – 0.03	1.0 – 40.8
Ciconia ciconia	-6.6 – 46.3	0.2 – 2.8	0.5 – 11.9	0.00 – 0.02	0.0 – 61.8
Ciconia boyciana	-2.9 – 29.2	0.2 – 1.8	1.0 – 12.3	0.00 – 0.02	1.0 – 40.1
Epphipiorhynchus asiaticus	2.6 – 42.9	0.3 – 2.4	0.6 – 9.9	0.00 – 0.02	1.0 – 30.9
Epphipiorhynchus senegalensis	18.0 – 39.3	0.4 – 2.4	0.7 – 7.4	0.00 – 0.02	0.0 – 28.4
Jabiru mycteria	17.2 – 39.7	0.1 – 2.3	0.6 – 7.7	0.00 – 0.03	1.0 – 30.9
Leptoptilos javanicus	5.2 – 43.9	0.1 – 2.4	0.2 – 7.6	0.00 – 0.03	1.0 – 28.5
Leptoptilos dubius	5.2 – 33.5	0.4 – 2.0	0.2 – 5.8	0.01 – 0.02	1.0 – 26.9
Leptoptilos crumenifer	17.4 – 41.1	0.0 – 3.4	0.3 – 6.5	0.00 – 0.02	0.0 – 29.6
Mycteria americana	10.6 – 39.7	0.1 – 2.5	0.4 – 13.1	0.00 – 0.02	0.7 – 41.9
Mycteria cinerea	25.0 – 30.9	0.1 – 1.9	0.3 – 4.7	0.01 – 0.03	1.0 – 14.9
Mycteria ibis	16.1 – 45.5	0.0 – 3.4	0.3 – 6.4	0.00 – 0.02	0.1 – 32.8
Mycteria leucocephala	16.8 – 43.9	0.1 – 2.1	0.4 – 8.1	0.00 – 0.03	1.0 – 31.4

Results

Interspecific comparisons

Table S2. Posterior means of predictors from MCMC interspecific model (with 95% CI). Significant explanatory predictors are in bold. (⁎Reference category is mixed)

	posterior mean	Lower CI	Upper CI	Effective sample	pMCMC
Intercept	- 6.364	-13.037	0.772	1607.1	0.057
Temperature	**1.321**	**1.091**	**1.591**	**756.3**	**<0.001**
Radiation	**0.884**	**0.665**	**1.097**	**736.4**	**<0.001**
Wind speed	**- 0.294**	**- 0.468**	**- 0.134**	**1688.2**	**0.001**
Humidity	**1.213**	**0.978**	**1.474**	**693.3**	**<0.001**
Latitude	- 0.082	- 0.289	0.124	1824.9	0.433
Body mass	- 2.430	- 6.097	1.023	1809.3	0.147
Tarsus length	2.982	- 0.091	6.644	1960.0	0.076
Plumage score	0.0345	- 1.516	1.606	1813.6	0.963
Foraging:Open ⁎	**7.441**	**1.618**	**13.804**	**1713.3**	**0.016**
Foraging:Wading⁎	- 0.102	- 4.005	3.673	1960.0	0.941

Intraspecific analyses

Table S3. Full model-averaged coefficients (ΔAICc < 2) for each stork species considered in intraspecific analyses. Significant explanatory predictors are in bold.

Species	Predictor	Estimate	SE	Adj SE	z value	95% CI
African openbill	**Intercept**	**-11.422**	**2.962**	**2.972**	**3.844**	**-17.246, -5.598**
	Temperature	0.136	0.149	0.149	0.907	-0.137, 0.185
	Radiation	0.049	0.937	0.941	0.052	-0.634, 0.646
	Wind speed	-0.115	0.646	0.649	0.177	-0.464, 0.436
	Humidity	0.378	0.902	0.906	0.417	-0.640, 0.739
	Latitude	-0.049	0.086	0.086	0.096	-0.060, 0.058
Asian openbill	**Intercept**	**-10.791**	**1.755**	**1.759**	**6.136**	**-14.238, -7.344**
	Temperature	0.058	0.106	0.106	0.542	-0.079, 0.095
	Radiation	0.116	1.045	1.047	0.110	-0.702, 0.730
	Wind speed	-0.172	0.479	0.480	0.359	-0.378, 0.333
	Humidity	0.429	0.562	0.563	0.761	-0.477, 0,619
	Latitude	-0.006	0.079	0.080	0.937	-0.055, 0.053
Abdim's stork	**Intercept**	**-8.022**	**2.196**	**2.207**	**3.634**	**-12.349, -3.696**
	Temperature	**0.233**	**0.067**	**0.067**	**3.473**	**0.102, 0.365**
	Radiation	0.541	0.480	0.483	1.121	-0.573, 0.989
	Wind speed	0.169	0.180	0.180	0.938	-0.185, 0.268
	Humidity	-0.330	0.380	0.382	0.865	-0.538, 0.380
	Latitude	-0.001	0.028	0.028	0.037	-0.016, 0.016

(continues)

Species	Predictor	Estimate	SE	Adj SE	z value	95% CI
woolly-necked stork	**Intercept**	**-3.791**	**0.889**	**0.892**	**4.250**	**-5.540, -2.043**
	Temperature	**0.129**	**0.031**	**0.031**	**4.221**	**0.069, 0.189**
	Radiation	-0.516	0.290	0.291	1.771	-1.037, 0.291
	Wind speed	0.039	0.084	0.084	0.466	-0.059, 0.069
	Humidity	0.152	0.150	0.150	1.015	-0.165, 0.254
	Latitude	0.006	0.014	0.014	0.438	-0.010, 0.012
white stork	**Intercept**	**-21.300**	**1.949**	**1.950**	**10.921**	**-25.123, -17.478**
	Temperature	**0.883**	**0.068**	**0.068**	**13.024**	**0.748, 1.015**
	Radiation	1.673	0.914	0.915	1.829	-0.973, 3.279
	Wind speed	-0.064	0.409	0.409	0.157	-0.369, 0.344
	Humidity	**4.263**	**0.421**	**0.421**	**10.117**	**3.437, 5.089**
	Latitude	**-0.119**	**0.027**	**0.027**	**4.424**	**-0.172, -0.066**
marabou stork	**Intercept**	**11.056**	**2.021**	**2.025**	**5.461**	**7.088, 15.024**
	Temperature	0.071	0.145	0.145	0.491	-0.107, 0.127
	Radiation	0.149	0.722	0.723	0.206	-0.495, 0.532
	Wind speed	0.127	0.666	0.667	0.190	-0.456, 0.488
	Humidity	-0.487	0.897	0.898	0.542	-0.817, 0.677
	Latitude	0.015	0.077	0.077	0.191	-0.052, 0.532
greater adjutant	**Intercept**	**-31.435**	**12.158**	**12.233**	**2.570**	**-55.412, -7.458**
	Temperature	0.355	0.353	0.355	1.000	-0.363, 0.526
	Radiation	0.594	2.479	2.498	0.238	-1.768, 1.930
	Wind speed	-0.041	1.132	1.141	0.036	-0.819, 0.808
	Humidity	0.480	1.271	1.281	0.374	-0.935, 1.072
	Latitude	**1.510**	**0.371**	**0.374**	**4.037**	**0.777, 2.243**
lesser adjutant	Intercept	-1.555	0.943	0.947	1.642	-3.410, 0.301
	Temperature	0.051	0.031	0.031	1.623	-0.031, 0.106
	Radiation	0.718	0.413	0.415	1.730	-0.411, 1.456
	Wind speed	0.207	0.163	0.163	1.270	-0.201, 0.381
	Humidity	**0.737**	**0.235**	**0.236**	**3.119**	**0.274, 1.201**
	Latitude	0.006	0.027	0.027	0.205	-0.015, 0.016
yellow-billed stork	**Intercept**	**-13.000**	**3.433**	**3.438**	**3.781**	**-19.737, -6.261**
	Temperature	0.174	0.107	0.108	1.620	-0.095, 0.371
	Radiation	-0.113	0.592	0.593	0.190	-0.421, 0.394
	Wind speed	-0.116	0.445	0.446	0.261	-0.328, 0.299
	Humidity	0.067	0.670	0.671	0.099	-0.461, 0.445
	Latitude	-0.001	0.053	0.053	0.019	-0.036, 0.036
painted stork	**Intercept**	**-3.929**	**1.357**	**1.362**	**2.884**	**-6.599, -1.258**
	Temperature	**0.090**	**0.039**	**0.039**	**2.275**	**0.012, 0.168**
	Radiation	0.092	0.339	0.340	0.271	-0.272, 0.305
	Wind speed	0.016	0.119	0.119	0.137	-0.095, 0.101
	Humidity	-0.089	0.206	0.207	0.432	-0.206, 0.172
	Latitude	**0.059**	**0.025**	**0.025**	**2.307**	**0.009, 0.109**
wood stork	**Intercept**	**-6.341**	**1.447**	**1.451**	**4.370**	**-9.184, -3.497**
	Temperature	**0.149**	**0.044**	**0.044**	**3.362**	**0.062, 0.235**
	Radiation	**1.086**	**0.340**	**0.341**	**3.191**	**0.419,1.754**
	Wind speed	-0.107	0.085	0.085	1.260	-0.193, 0.104
	Humidity	0.212	0.183	0.184	1.156	-0.219, 0.379
	Latitude	-0.019	0.018	0.018	1.101	-0.035, 0.021

(continues)

Species	Predictor	Estimate	SE	Adj SE	z value	95% CI
jabiru	**Intercept**	**-4.009**	**1.620**	**1.629**	**2.462**	**-7.201, -0.817**
	Temperature	**0.201**	**0.057**	**0.058**	**3.475**	**0.088, 0.314**
	Radiation	-0.341	0.390	0.392	0.869	-0.536, 0.382
	Wind speed	0.082	0.109	0.110	0.747	-0.100, 0.134
	Humidity	0.030	0.224	0.225	0.135	-0.170, 0.180
	Latitude	**-0.133**	**0.039**	**0.039**	**3.436**	**-0.209, -0.057**

5

Keeping cool with poop: urohidrosis lowers leg surface temperature by up to 6 ºC in breeding White storks

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Abstract

Storks (*Ciconiidae*) are renowned for defecating on their legs when exposed to high temperatures, a phenomenon known as 'urohidrosis'. Previous work suggested that this behaviour can cool down the blood supply to the legs and thus prevent hyperthermia in captive storks when overheated. However, no study has quantified the magnitude or duration of its evaporative cooling effect in free-ranging birds. Here, we combine urohidrosis data with thermal imaging and microclimate data to investigate the thermoregulatory significance of urohidrosis in White storks *Ciconia ciconia* during the breeding season. We show that urohidrosis can reduce leg surface temperature by up to 6.7 ºC (4.40 ± 1.04 ºC). Yet its cooling effect was of short duration (lasting no more than 2.5 min) and decreased with time since defecation. Thus, for urohidrosis to significantly contribute to heat dissipation, storks must perform it repeatedly when overheated. Indeed, individuals can perform up to 11 urohidrosis events per hour; our estimates indicate that repeated urohidrosis could represent a significant amount of heat loss during short-time spans — equivalent to 4% of daily field metabolic rate for an adult stork. We did not detect differences in the cooling efficiency of urohidrosis between adults and nestlings, probably because all nestlings were recorded during the last phase of the ontogeny of thermoregulation. Besides, during the hottest days adult storks delivered water to their nestlings, which might allow them to replenish body water reserves to sustain urohidrosis and other heat dissipation behaviours such as panting or gular fluttering. Our results indicate that urohidrosis might buffer the impact of high temperatures in breeding storks, preventing overheating and lethal hyperthermia. Gaining knowledge about behavioural thermoregulation in the heat is therefore crucial to better predict the future persistence and vulnerability of species under different climate warming scenarios.

Keywords: birds, evaporative cooling, heat dissipation, thermal imaging, thermoregulation

Main text

Many endotherms living in hot environments are known to wet parts of their body with body fluids — such as saliva, nasal mucous, urine, or faeces — to dissipate heat by evaporation (reviewed in Tattersall et al. 2012). The urination or defecation on the legs to promote evaporation ('urohidrosis', *sensu* Kahl 1963) is an unusual but taxonomically widespread mechanism for enhancing evaporative cooling in birds — including New World vultures, condors, storks, gannets, and boobies (Kahl 1963; Hatch 1970; Cooper & Sigfried 1976; Thomas 1984; Graves 2019). This behaviour is particularly prevalent in storks (*Ciconiidae*) as their long, bare, and well-vascularized legs can act as controllable thermal radiators that facilitate (evaporative and non-evaporative) heat loss in warm weather (Kahl et al. 1963; Mitgärd 1981; Cabello-Vergel et al. 2021). Indeed, there is experimental evidence suggesting that urohidrosis can cool down the blood supply to the legs and thus prevent hyperthermia in captive Wood storks *Mycteria americana* exposed to ambient temperatures over 40 ºC (Kahl 1963).

However, we know very little about the thermoregulatory significance of urohidrosis since the pioneering work of Kahl (1963). Specifically, we do not know whether and to what degree urohidrosis lowers leg temperature as well as its contribution to the total heat exchange in free-ranging birds. Although Kahl argued that repeated urohidrosis could dissipate a sizable amount of heat (when captive storks perform up to 10 successful attempts of urohidrosis in less than 20 min when exposed to 45 ºC or more), he failed to induce urohidrosis in overheated storks harnessed and wired for temperature readings. Instead, he had to simulate the effect of urohidrosis by spraying water on the legs (Kahl 1963). With the development of new non-invasive techniques to monitor surface temperature, such as infrared thermal imaging, it is time to revisit this classic, unresolved topic on the thermoregulatory significance of urohidrosis in free-ranging birds.

While working on the thermoregulation and heat exchange of White storks *Ciconia ciconia* at a nesting colony in southwestern Spain, we recorded 22 urohidrosis events and measured leg surface temperatures to explore heat dissipation areas and calculate heat exchange via urohidrosis using thermal imaging (Figure 1A-B, see Supplementary Material for detailed methods). All the events occurred at midday (mean temperature = 34.10 ± 2.85 ºC [S.D.]; range = 26.1 – 37.1 ºC) during the spring of 2021 (from late March to mid-June). Both adults and nestlings engaged in urohidrosis after panting began. As described earlier (Kahl et al. 1963; Cabello-Vergel et al. 2021), storks downed their tail and approached one leg towards the cloaca, then directing excretion to it — only one leg was hit at a time, subsequently alternating from one leg to the other.

On average, urohidrosis lowered leg surface temperature by 4.40 ± 1.04 ºC (range = 2.80 – 6.70 ºC). These values were higher than those reported by Kahl (1963) when experimentally simulated urohidrosis in Wood stork. After spraying water on its legs, he found that leg temperature dropped about

3 ºC after 6 min and remained 1 ºC below initial temperature after 10 min. Nonetheless, we found that the cooling effect of urohidrosis was shorter in duration, as the temperature gradient between the leg region covered by excreta and the bare skin became negligible after approximately 2.5 min (generalised linear mixed effects models [GLMM]: β = -0.018 ± 0.002 [S.E.]; confidence interval [CI] 95% = -0.022, -0.013; see Figure 1C and Supplementary Material). Perhaps such differences between studies (and species) reflect differences in settings (laboratory vs field) and technologies (thermistor probes vs thermal imaging). Captive birds were confined in a chamber at over 45 ºC (Kahl 1963), whereas wild birds under direct sunlight experienced more environmental variation. It is thus likely that the cooling efficiency of urohidrosis varies with microclimatic variables that influence heat balance, such as wind speed, solar radiation, and humidity (Cabello-Vergel et al. 2021). In any case, single urohidrosis events appears to lower leg and body temperatures for only relatively short time periods.

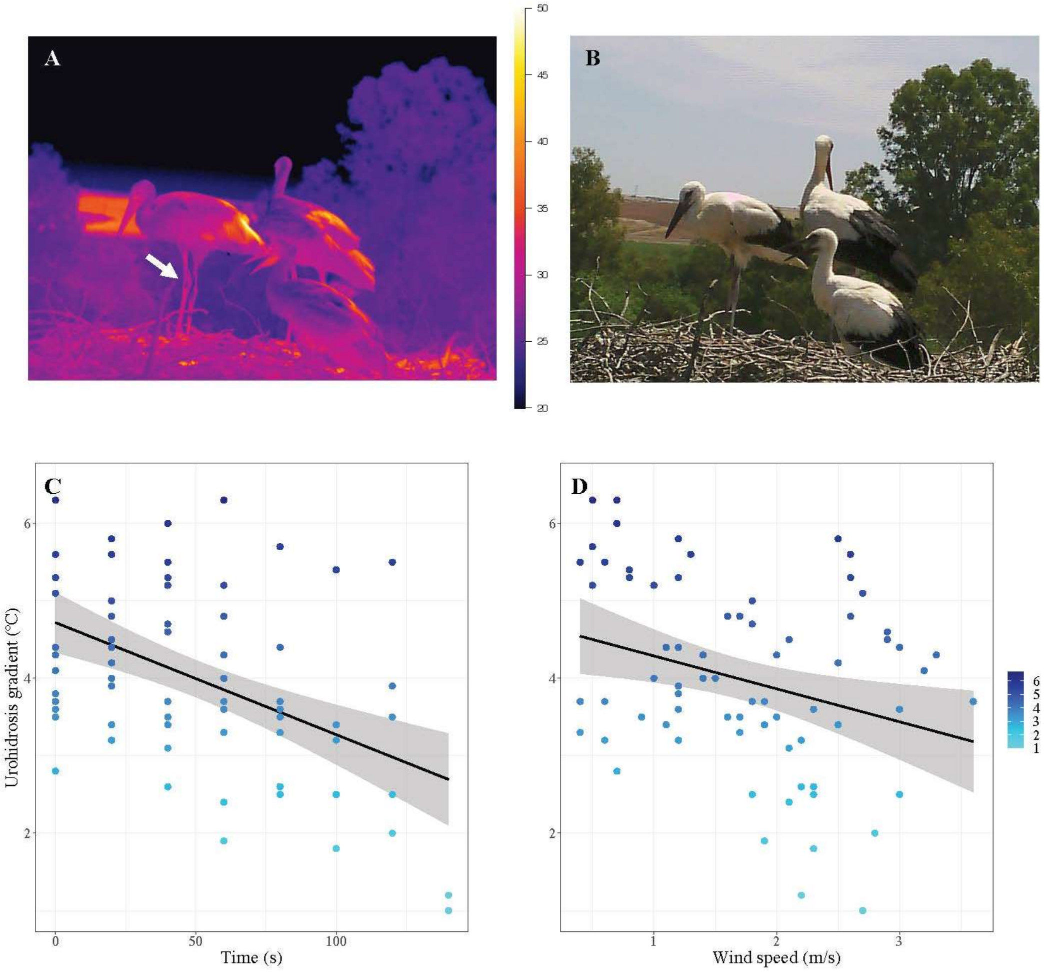

Figure 1. (A) Infrared thermal picture showing the cooling effect of urohidrosis in the nestling stork on the left. Note that the area covered by excreta (denoted by white arrow) shows a darker colour (lower surface temperature) than the surrounding bare skin. (B) Visible picture illustrating the whitish marks produced by urohidrosis on the legs of the nestling on the left. (C) and (D) show the urohidrosis temperature gradient (ºC) as a function of time (s) and windspeed (m s^{-1}), respectively, with darker blue points indicating greater cooling effect and lighter blue points lower cooling effect. Shaded grey denotes 95% confidence intervals.

For urohidrosis to significantly contribute to total heat dissipation, storks must perform it repeatedly when overheated. Around midday at relatively high ambient temperatures (31.5 – 36.2 ºC), we observed a nestling (c. 55 days old) excreting on its legs 11 times in one hour. If storks direct 1-2 cc of liquid excreta on their legs every time (Kahl 1963), this could translate in the dissipation of 590 to 1200 cal (as latent heat of vaporization of water at physiological temperature equals to 570-595 cal g^{-1}; Hill 1980). Then, an individual could dissipate up to 6,600 to 13,200 cal through repeated urohidrosis. Urohidrosis can thus represent a significant amount of heat loss during short-time spans (i.e., equivalent to 4% field metabolic rate, as the daily field metabolic rate of White storks is about 310,505 cal day^{-1}; Mata et al. 2010). However, this could be an overestimation, as we observed that excretion' volume decreased after repeated urohidrosis, as well as seemed to be less and less watery, thus resulting in lower heat dissipation potential.

We did not detect differences in urohidrosis gradient between adults and nestlings (GLMM: β= -0.342 ± 0.337; CI 95% = -0.923, 0.231; see Supplementary Material). This is probably because all nestlings were recorded during the last phase of the ontogeny of thermoregulation in altricial birds (i.e., phase of full-blown homeothermy; Price & Dzialowski 2018). This phase starts at 10-20 days of life and is characterized by complete homeothermy. Therefore, the thermoregulatory system of the recorded young (c. 55 days old) probably was fully functional and comparable to that of adults. This is a novel finding, as Kahl (1963) studied urohidrosis in immature (but fully grown) captive Wood storks.

Additionally, we found that low wind speed enhanced the cooling effect of urohidrosis (GLMM: β = -0.291 ± 0.143; CI 95% = -0.634, -0.048; see Figure 1D and Supplementary Material). This agrees with a previous study showing that low wind speed promote the use of urohidrosis across stork species (Cabello-Vergel et al. 2021). Higher wind speeds promote heat loss by convection but at the same time shorten the cooling effect of urohidrosis as the liquid excreta evaporates faster. On the other hand, we found no effect of air temperature on the urohidrosis gradient (GLMM: β = -0.092 ± 0.111; CI 95% = -0.312, 0.083; see Supplementary Material). Perhaps this is simply because the limited sample size and small variation in microclimate variables, across urohidrosis events (we only recorded one event at ambient temperatures below 30 ºC).

During the hottest days, storks replenished their water reserves by drinking to sustain the elevated evaporative water losses imposed by both panting and urohidrosis. We observed adult storks frequently flying to the nearby Guadiana River (<100 m away from the breeding colony) to drink and bathe. Moreover, parents regularly brought water in their esophagus to the young. This 'watering' behaviour probably allows young to capitalize readily on evaporative cooling mechanisms such as panting and urohidrosis (Kahl 1963; Thomas 1984). To some extent, nestling rely on their parents to engage in heat-reduction behaviours. Indeed, we observed that young resumed urohidrosis with larger volumes of watery excretions after being watered by their parents. Adults not only provided drinking water to their young, but also poured water over the surface of their body where it evaporates resulting in instantaneous cooling (Figure 2; see Supplementary Material for more details). Besides, they provided shade to the nestlings during the hottest part of the day through spread-wing postures, precluding young from direct solar radiation. This behaviour is common across storks and serves to reduce heat stress in young and increases passive heat loss in adults by exposing to air the thinly feathered underwings (Kahl 1971).

Our results confirm the thermoregulatory significance of urohidrosis as a cooling mechanism in free-ranging storks. This could be particularly important for storks and other birds breeding in exposed locations, such as open nests which leads to both rapid heat gain or loss between an individual and its environment due to little shelter from the sun or wind (Cook et al. 2020). At our breeding colony, located at the southern edge of the species' breeding range, storks appear to be physiologically constrained by their time of breeding; thus, they must rely on evaporative cooling (panting and urohidrosis) at relatively low ambient temperatures during incubation and young-rearing stages.

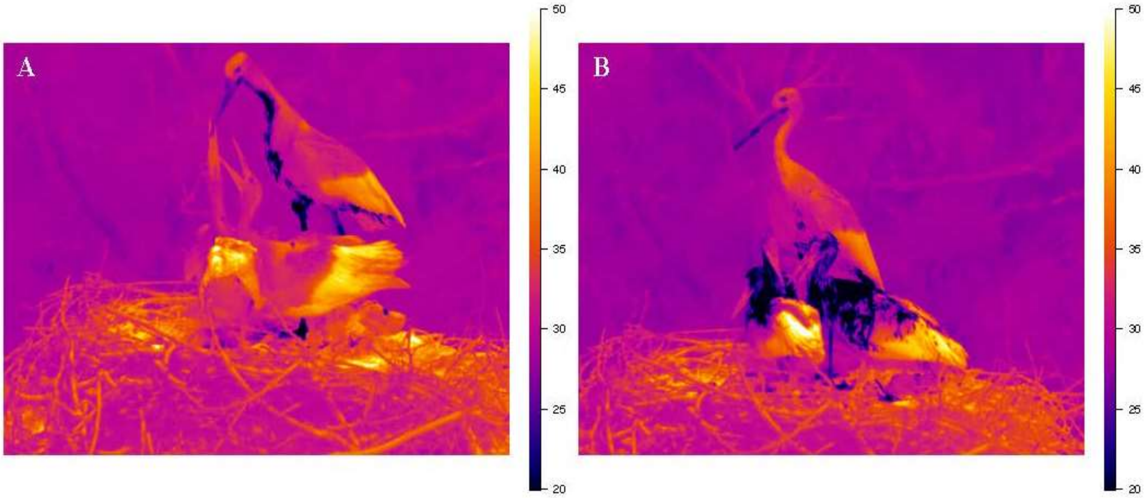

Figure 2. Example of a watering event, in which an adult gives water to its nestlings and spread some on their upper body parts (A). Rapidly (after 20-40 s), this results in an important reduction in surface temperature of juveniles' upper body parts as denote by the darker colour shown by these parts in the thermal image (B).

Conceivably, urohidrosis might buffer the impact of high temperatures on breeding storks, thus preventing overheating and lethal hyperthermia. Future climate change scenarios predict an increase in the intensity and frequency of heatwaves and heavy droughts (IPCC 2021). Climate warming could thus increase the necessity of storks to engage in urohidrosis and other heat dissipation behaviours. Although urohidrosis could be viewed as a metabolically 'cheap' thermoregulatory response, water scarcity could prevent its use and/or increase dehydration risks. Behavioural trade-offs for thermoregulation thus have important implications for fitness in birds (Cunningham et al. 2021). Recent developments in thermal imaging and mechanistic modelling tools provide exciting opportunities to integrate behavioural and physiological data into climate change predictions for species and populations with different heat tolerance limits and evaporative cooling efficiencies (Cunningham et al. 2021).

Finally, some questions about the ecological significance of urohidrosis remain. For instance, could urohidrosis diminish heat gain if the whitish residue covering the legs increases skin reflectance when fully exposed to solar radiation? Or could these urohidrosis marks hamper social signalling based on leg colouration? There is evidence that carotenoid-based colouration of the legs of White stork nestlings is condition-dependent and influences growth rate (Surmacki and Kosicki 2009). Furthermore, the integumentary colouration of storks and New World vultures (*Cathartidae*) may function in inter- and intraspecific signalling (Graves 2019; Rodríguez-Rodríguez and Negro 2021). Further study, involving different social contexts (e.g., mating, rearing, or foraging) across a range of temperature, is thus required to determine if thermoregulation–signalling trade-offs exist.

References

Cabello-Vergel, J., Soriano-Redondo, A., Villegas, A., Masero, J.A., Sánchez Guzmán, J.M., & J.S. Gutiérrez. (2021) Urohidrosis as an overlooked cooling mechanism in long-legged birds. *Sci. Rep.* **11**, 20018. https://doi.org/10.1038/s41598-021-99296-8

Cooper, J., & W.R. Siegfried. (1976) Behavioural responses of young Cape gannets *Sula capensis* at high ambient temperatures. *Mar. Behav. Physiol.* **3**, 211-220.

Cook, T.R., Martin, R., Roberts, J., Häkkinen, H., Botha, P., Meyer, C., Sparks, E., Underhill, L.G., Ryan, P.G., & R.B. Sherle. (2020) Parenting in a warming world: thermoregulatory responses to heat stress in an endangered seabird. *Conserv. Physiol.* **8(1)**, coz109. https://doi.org/10.1093/conphys/coz109

Cunningham, S.J., Gardner, J.L., & R.O. Martin, R.O. (2021) Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* **19(5)**, 300-307.

Graves, G.R. (2019) Urohidrosis and tarsal color in *Cathartes* vultures (Aves: Cathartidae). *Proc. Biol. Soc. Wash.* **132**, 56-64.

Hatch, D.E. (1970) Energy conserving and heat dissipating mechanisms of the turkey vulture. *Auk.* **87(1)**, 111-124. https://doi.org/10.2307/4083662

Hill, R.W. (1980) Comparative physiology of animals: an environmental approach. Harper & Row, New York.

IPCC. (2021) Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (eds.)]. Cambridge University Press. In Press.

Kahl, P.M. (1963) Thermoregulation in the wood stork, with special reference to the role of the legs. *Physiol. Zool.* **36(2)**, 141-151.

Kahl, P.M. (1971) Spread-wing postures and their possible functions in the Ciconiidae. *Auk*. **88(4)**, 715-722.

Mata, A., Massemin-Challet, S., Caloin, M., Michard-Picamelot, D., & Y. Le Maho. (2010) Seasonal variation in energy expenditure and body composition in captive White storks (*Ciconia ciconia*). *Comp. Biochem. Phisiol. Part A Mol. Integr. Physiol.* **155**, 19-24.

Midtgärd, U. (1981) The rete tibiotarsale and arterio-venous association in the hind limbs of birds: a comparative morphological study on counter-current heat exchange systems. *Acta Zool.* **62(2)**, 67-87.

Price, E.R., & E.M. Dzialowski. (2018) Development of endothermy in birds: patterns and mechanisms. *J. Comp. Physiol. B*. **188**, 373-391.

Rodríguez-Rodríguez, E.J., & J.J. Negro. (2021) Integumentary colour allocation in the Stork family (Ciconiidae) reveals short-range visual cues for species recognition. *Birds*. **2**, 138–146. https://doi.org/10.3390/birds2010010

Surmacki, A., & J.Z. Kosicki. (2009) Condition-dependent leg colour of nestling White Storks *Ciconia ciconia*. *Ibis*. **151**, 762–765.

Tattersall, G.J., Sinclair, B.J., Withers, P.C., Fields, P.A., Seebacher, F., Cooper, C.E., & S.K. Maloney. (2012) Coping with thermal challenges: physiological adaptations to environmental temperatures. *Compr. Physiol.* **2**, 2151-2202.

Thomas, B.T. (1984) Maguari stork nesting: juvenile growth and behaviour. *Auk*. **101**, 812-823.

Appendix 4: Supplementary Material

Methods

During the spring of 2021 (from late March to mid-June), we conducted a study on the thermoregulation and heat exchange of White storks *Ciconia ciconia* at a nesting colony in Extremadura, southwestern Spain (38º52’ N; 6º19’W). This colony hosted 12 active nests that were visited periodically to obtain thermal images to study the potential role of different body parts (particularly beaks and legs) as thermal windows along a range of different ambient temperatures. We collected thermal images of each nest throughout the study period, from early incubation to the end of the breeding season.

Figure S1. Example of the method employed to obtain thermal images of White storks. As can be seen on the picture, thermal camera was mounted on a pole at 5-m from focal nest. After few minutes birds were completely habituated to this setup and this did not interfere in their behaviour.

Thermal imaging sessions were performed from 1 h after sunrise until maximum ambient daily temperature was reached, measuring no more than 2 nests per day. We employed a handheld thermal infrared camera (FLIR E95, resolution = 464 x 348 pixels, FLIR Systems, USA), equipped with a 14º lens. The camera was attached to a telescopic 5-m alloy pole, which was placed at a distance of 5 m from the focal nest (Figure S1). We automatically took a thermal image every 20 seconds and simultaneously record local weather data – ambient temperature (ºC); wind speed, (m s^{-1}); and relative humidity (%) – every 30 s using a portable weather station (Kestrel 5400, Kestrel Instruments, USA) mounted on a vane and placed on the colony (at 1.5 m height).

Throughout the study period, we managed to obtain thermal images of 22 urohidrosis events (4 in adults and 18 in nestlings), from 5 different nests and 7 individuals. A total of 173 thermal images of individuals showing urohidrosis were taken (7.86 ± 3.31 per event). Nonetheless, those images in which legs were not well visible or those in which storks' position could compromise accuracy of thermal measures, as incidence angle influences apparent emissivity – with increased angle resulting on declines in apparent emissivity (Playà-Montmany & Tattersall 2021) – were discarded. Then, we selected for the analyses those pictures in which a lateral (or almost lateral) view of the legs could be taken. Eighty-one images met this criterion and were analyzed using the program FLIR ResearchIR (FLIR Systems, USA). For each image, we assumed an emissivity of 0.95 and set ambient temperature, reflected temperature and relative humidity from values obtained from the weather station. Average surface temperature of the leg area covered by excreta was measured using the 'freehand roi' function, while average surface temperature from the adjacent region free of excreta was measured by using the '*line roi*' function. We employed the '*freehand roi*' function to measure urohidrosis due to the irregular shape of excreta marks along the leg. Overall, temperature measures were calculated on areas that had, on average, 20.73 ± 7.97 pixels. We calculated urohidrosis gradient as the difference between the surface temperature of the bare skin leg region and that of the region covered by excreta. When possible, we repeatedly calculated urohidrosis gradient for the same event to estimate its duration and magnitude over time.

Statistical analyses

We fitted a generalized linear mixed effect model using the *lme4* package (Bates et al. 2015) to determine the effect of age (adult vs nestling), ambient temperature, wind speed, humidity and time elapsed since urohidrosis began (T_0, in s) on urohidrosis gradient. We set nest, bird identity, and urohidrosis event as random effects as we had various measurements from same nests, individuals and events (e.g., various values along different times). In each nest, we identified each individual by considering differences on morphological traits, such as size or particular plumage features, as no birds were ringed.

We opted to not estimate urohidrosis energetics through total heat exchange calculations commonly employed when studying the appendage' function as thermal window (e.g., Tattersall, Chaves & Danner 2016; van Vuuren, Kemp & McKechnie 2020), as urohidrosis is *a priori* an evaporative cooling mechanism solely. Equations employed to estimate heat exchange from certain body parts, such as legs, quantify the potential of these regions as thermal radiators. They quantify how a certain region could contribute to heat gain or loss through convective and radiative ways, but not through evaporation. When applying these equations, we obtained greater heat gain in legs covered by excreta, as urohidrosis lowers surface temperature, increasing the gradient between ambient temperature and leg temperature and thus resulting in heat being transferred faster to the legs due to their lower temperature respect environment.

Results

We found that wind speed and time had a significant effect on urohidrosis gradient, while age, ambient temperature and humidity did not (Table S1). Urohidrosis thermal gradient increased with decreasing wind speed and reached its maximum during the first moments after birds defecated on their legs. Urohidrosis is a heat dissipation behaviour that only occurs at relatively high temperatures, which could explain the absence of an effect of ambient temperature on urohidrosis gradient along the narrow temperature range (34.10 ± 2.85 ºC [S.D.]) at which we recorded this behaviour, as we only captured one event at ambient temperature below 30 ºC.

Table S1. Estimates of the effect of each predictor variable on urohidrosis gradient. Significant variables are denoted in bold.

	Estimate	s.e.	t-value	C.I. 95%
Intercept	**8.188**	**4.635**	**1.767**	**0.882, 17.413**
Age	- 0.342	0.337	-1.014	- 0.923, 0.231
Ambient temperature	- 0.092	0.111	-0.828	- 0.312, 0.083
Wind speed	**- 0.291**	**0.143**	**-2.026**	**- 0.634, - 0.048**
Humidity	0.029	0.043	0.674	- 0.051, 0.096
Time	**- 0.018**	**0.002**	**-7.340**	**- 0.022; - 0.013**

Other heat reduction behaviours

Besides of urohidrosis, during the study we recorded other heat-reduction behaviours, such as ‘watering’ (i.e., the delivery of water to nestlings by their parents) and ‘sheltering’ (i.e., parents providing shade to nestling with their bodies) (Kahl 1963; Kahl 1971). Both behaviours are well documented and widespread among *Ciconiidae* (Kahl 1963; Kahl 1971; Thomas 1984). In hot days, adult White storks frequently flew to the nearby river to take some water to their nestlings, as well as cooling themselves by bathing. When arrived in the nest, the adult delivered a part of water directly on the esophagus of their young, while certain amount of water was spread over their upper body parts (head, neck and back) resulting in a reduction in surface temperature as revealed by thermal images. However, due to the low number of watering instances that we could register through thermal images we could not perform an analysis to assess the energetics of this behaviour. Besides, especially during the first weeks of nestlings’ life, parents displayed several postural adjustments (such as wing-spreading) intended to provide shade to the young, thus protecting them from direct solar radiation. This behaviour seems to be crucial to maintain thermal balance in nestlings when they have not yet achieved complete homeothermy (< 20 days old) and thus depend on parents for thermoregulation (Price & Dzialowski 2018). Besides, parents could increment passive convective heat dissipation through these postural adjustments by exposing the thinly feathered underwings to wind.

References

Bates, D., Mächler, M., Bolker, B., & S. Walker. (2015) Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48.

Kahl, P.M. (1963) Thermoregulation in the wood stork, with special reference to the role of the legs. *Physiol. Zool.* **36(2)**, 141-151.

Kahl, P.M. (1971) Spread-wing postures and their possible functions on the Ciconiidae. *Auk*. **88(4)**, 715-722.

Playà-Montmany, N., & G.J. Tattersall. (2021) Spot size, distance and emissivity errors in field applications of infrared thermography. *Methods Ecol. Evol.* **12**, 828-840.

Price, E.R., & E.M. Dzialowski. (2018) Development of endothermy in birds: patterns and mechanisms. *J. Comp. Physiol. B.* **188**, 373-391.

Tattersall, G.J., Chaves, J.A, & R.M. Danner. (2016) Thermoregulatory windows in Darwin’s finches. *Funct. Ecol.* **32**, 358-368.

Thomas, B.T. (1984) Maguari stork nesting: juvenile growth and behaviour. *Auk*. **101**, 812- 823.

Van Vuuren, A.K.J., Kemp, L.V., & A.E. McKechnie. (2020) The beak and unfeathered skin as heat radiators in the southern ground-hornbill. *J. Avian Biol.* **51**, https://doi.org/10.1111/jav.02457.

6

General synthesis

The results presented in this thesis add to an increasing body of literature concerning avian physiological and behavioural mechanisms to deal with heat stress. We determined, for the first time, the heat tolerance limits (HTL) of Mediterranean songbirds, as well as their seasonal and between-population variation at sites with different environmental conditions (i.e., different annual temperature regimes). In doing so, we assessed adjustments in their thermal physiology to enhance heat tolerance during the hottest part of the year. We also shed light on the thermoregulatory significance of urohidrosis in free-ranging storks, an overlooked thermoregulatory behaviour which can significantly contribute to increase heat dissipation at high temperatures. Remarkably, studies characterizing avian thermoregulation in the heat are relatively scarce, particularly in both Mediterranean-resident, small-sized songbirds and widely distributed, large-sized birds such as storks. Therefore, these results are novel and valuable, and could be used to better predict current and future vulnerabilities to heat extremes under predicted climate warming scenarios. Below we discuss our main results in a more general context and propose future work directions.

Mediterranean songbirds in the heat

As expected, our results highlight that small Mediterranean-resident songbirds are less well adapted to deal with heat stress than arid-zone songbirds from South Africa, Australia, or North America (Whitfield et al. 2015; McKechnie et al. 2017; Smit et al. 2017; Smith et al. 2017; Kemp & McKechnie 2019; Czenze et al. 2020). That is, Mediterranean songbirds generally achieve lower HTLs and evaporative cooling efficiencies than their arid-zone counterparts. In fact, the maximum evaporative cooling efficiencies of the songbird species studied in this thesis are among the lowest (from 0.62 to 1.11) reported across passerines (0.75 to 2.35) (Whitfield et al. 2015; McKechnie at al. 2017; Smith et al. 2017; Oswald et al. 2018; Czenze et al. 2020; McKechnie et al. 2021; O'Connor et al. 2021), and other bird groups (1.67 to 5.49; McKechnie et al. 2016; McKechnie et al. 2016b; O'Connor et al. 2017; Smit et al. 2017; Smith et al. 2017; Talbot et al. 2018; Czenze et al. 2022) (Figure 1). Remarkably, our species showed evaporative cooling efficiencies similar to those found in the Arctic Snow bunting *Plectrophenax nivalis* (0.96; O'Connor et al. 2021) and in the Cape rockjumper *Chaetops frenatus* (0.75; Oswald et al. 2018), a mountain relict songbird species that experience a Mediterranean-like climate. Thus, substantial taxonomic and biogeographic variation in heat tolerance and evaporative cooling efficiency points to different adaptations to heat exposure across regions and species (McKechnie & Wolf 2019; Freeman et al. 2022).

It is important to remember that songbirds from Extremadura are currently experiencing up to 33 days per summer in which they are likely to suffer chronic detrimental effects on their fitness from heat exposure — as they are exposed to ambient temperatures surpassing their upper critical temperatures (T_{uc}) which increase both opportunity and energetic costs of behavioural and physiological thermoregulation. In the near future, projected climate warming scenarios will magnify this risk. When considering the most severe climate warming scenario (3.7 ºC increase, RCP8.5) the studied species will experience from 55 to 98 days per summer in which maximum ambient temperature will be above T_{uc}. Such increases in temperature are likely to reduce their body condition and, ultimately, survival prospects. In this vein, recent studies have predicted that the risk of fitness costs of chronic exposure to sustained hot weather will increase dramatically across South-African (Conradie et al. 2019) and Australian arid-zone songbirds (Conradie et al. 2020). Among our species, however, only the Great tit *Parus major* will experience some days (~5 days per summer) in which maximum ambient temperature could surpass its HTL, resulting in risk of direct mortality to the end of this century under the RCP8.5 scenario. This contrasts with the predicted future increase in direct mortality risk by heat exposition across Australian arid-zone birds (Conradie et al. 2020). Overall, our results indicate that current and future maximum temperatures challenge the physiological thermoregulatory capacities of Mediterranean songbirds across Extremadura (and possibly other Mediterranean regions), which could threaten body maintenance and/or breeding in early summer (DuPlessis et al. 2012; Cunningham et al. 2021).

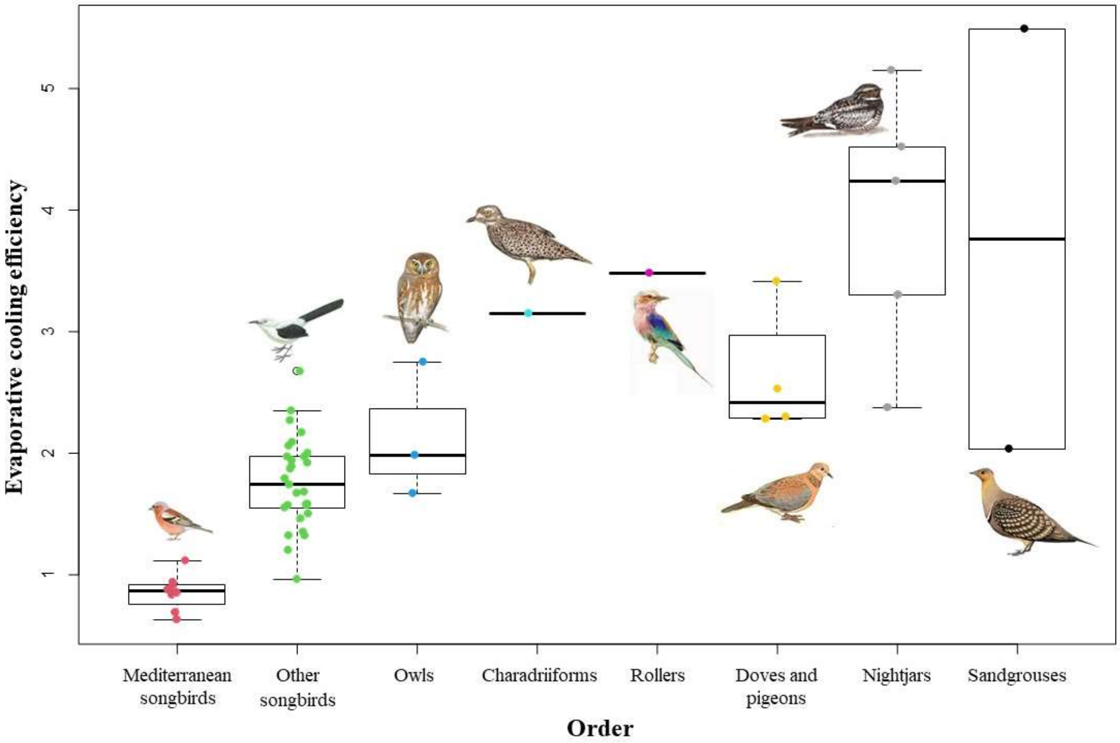

Figure 2. Mean maximum evaporative cooling efficiency (EHL/MHP) for Mediterranean (this study) and other songbirds (data from Whitfield et al. 2015; McKechnie at al. 2017; Smith et al. 2017; Oswald et al. 2018; Czenze et al. 2020; McKechnie et al. 2021; O'Connor et al. 2021), as well as for other birds' orders previously studied (data from McKechnie et al. 2016; McKechnie et al. 2016b; O'Connor et al. 2017; Smit et al. 2017; Talbot et al. 2017; Talbot et al. 2018; Czenze et al. 2022). Boxplots show mean values with 95% confidence intervals. Birds' pictures are credited to Juan Varela (Chaffinch *Fringilla coelebs*), David Allen Sibley (Elf owl *Micrathene whitneyi* and Lesser nighthawk *Chordeiles acutipennis*), Gert Van Niekerk (Spotted thick-nee *Burhinus capensis*), Tim Plowden (Lilac-breasted roller *Coracias caudatus*) and to anonymous Pinterest' contributors (Southern Pied Babbler *Turdoides bicolor*, Laughing dove *Spilopelia senegalensis*, and Namaqua sandgrouse *Pterocles Namaqua*).

Despite the fitness costs of chronic exposition to heat, Mediterranean songbirds will only experience moderate risk of lethal dehydration (15% body mass loss in < 5 hours) during the hottest days by the end of 21st century when RCP8.5 scenario is considered. The current absence of lethal dehydration risk in our studied species is similar to that reported in South-African arid-zone birds (Conradie et al. 2019), and to the low risk across Australian ones (Conradie et al. 2020). This supports the notion that species are adapted to the climate in which they have evolved (reviewed in McKechnie & Wolf 2019; Freeman et al. 2022), although we still do not know if they will be able to adjust their thermal physiology fast enough to deal with forecasted increase in maximum temperatures and heatwaves. Nonetheless, future risks of lethal dehydration differed among Mediterranean species in relation to their body mass: smaller-sized songbirds (< 22 g) are more sensitive than larger ones (22 to 35 g). Thus, larger species – namely the Crested lark *Galerida cristata*, the Spanish sparrow *Passer hispaniolensis*, and the House sparrow *Passer domesticus* – are less likely to experience risk of lethal dehydration, which corroborates previous studies on Australian arid-zone species (Conradie et al. 2020).

Among wide-ranging species, thermal physiology could vary across populations in response to contrasting environmental conditions. Regardless of the ongoing global warming, only a handful of studies have evaluated how thermoregulatory responses to high temperatures vary among populations, with them focusing on arid-zone species (e.g., Trost 1972; Noakes et al. 2016; O'Connor et al. 2017; Noakes et al. 2020). To our knowledge, this thesis explores for the first-time intraspecific differences in HTLs in a Mediterranean species. We find significant seasonal and between-population variation in heat tolerance and other key thermoregulatory traits in two populations of Great tits that experience different thermal environments (Chapter 3). Notably, birds from the hotter (and more thermally variable) lowland site enhance their heat tolerance and achieve greater evaporative cooling efficiency during summer than their counterparts from a milder (and more thermally stable) montane site. These differences point to different physiological capacities to deal with high temperatures between populations and highlight the importance of considering intraspecific variation in thermoregulatory responses to heat when predicting the vulnerability of species spanning wide climatic gradients (Valladares et al. 2014; Moran, Hartig & Bell 2016; De Marche, Doak & Morris 2018).

We acknowledge that not having considered the potential intraspecific variation in HTL (and other key thermoregulatory traits) across different songbird populations from Extremadura could have slightly affected the estimates of their sensitivity to high temperatures (Chapter 2). This limitation is common across predictive models of species vulnerabilities (i.e., McKechnie & Wolf 2010; Albright et al. 2017; Conradie et al. 2019; Conradie et al. 2020), in which the mean value of a given trait (e.g., T_{uc}, EWL slope or HTL) is obtained from individuals from a single site and used to model the species sensitiveness to heat. To increase the accuracy of these models, we encourage future work to characterize thermoregulatory traits across populations from different sites. Besides, further research including common-garden experiments is also needed to determine to what extent these patterns are attributable to phenotypic plasticity and/or local (genotypic) adaptation (e.g., Wikelski et al. 2003; Tieleman et al. 2003; Broggi et al. 2005; Noakes & McKechnie 2019).

We should outline some methodological considerations about heat tolerance trials. Our estimates of HTLs and evaporative cooling efficiencies are collected under standardized laboratory conditions intended to maximize birds evaporative heat loss (i.e., low ambient humidity), with birds at rest and in post-absorptive state. In doing so, we probably overlook the combined effect of environmental variables (such as temperature, wind speed, solar radiation, and humidity), as well as the effect of activity on heat exchange. During the hottest part of the day, free-ranging birds would experience higher solar radiation loads, which combined with low wind speed would result in high operative temperatures (reviewed in Baken & Angilletta 2014). Thus, wild birds may tolerate lower ambient temperatures before the onset of severe heat stress than captive birds during metabolic trials. Additionally, increased ambient humidity would hamper EWL by diminishing the water vapour gradient between a bird's surface and the surrounding environment (Gerson et al. 2014; van Dyk et al. 2019), which would translate in wild birds from humid environments achieving lower HTLs than birds in the laboratory. Wild birds foraging during midday might thus experience high operative temperatures that could result in individuals achieving hyperthermia or dehydration thresholds faster than during heat tolerance trials. Therefore, they might have to cease activity at ambient temperatures as low as ~ 30 ºC, well below the temperature threshold for the beginning of active physiological thermoregulation (e.g., T_{uc}) obtained under laboratory conditions (e.g., Smit et al. 2016; Kemp et al. 2020).

The role of behavioural thermoregulation

To further complicate the matter, wild songbirds could elude maximum ambient temperatures and solar radiation by using thermally-buffered microhabitats (such as riparian forests, tree crevices or tree shade), thus reducing thermal stress during the hottest part of the day (DuPlessis et al. 2012; Carrol et al. 2015; Cunningham, Martin & Hockey 2015; Smit et al. 2016; Sharpe et al. 2022). For instance, a small Australian songbird, the Jacky winter *Microeca fascinans*, experiences temperatures 2 ºC cooler than ambient temperatures when retreating to shaded tree trunks (Sharpe et al. 2022), which leads to reduced evaporative cooling requirements. Likewise, broods of Northern bobwhite (*Colinus virginianus*, a gallinaceous ground-dwelling bird) reduce movement and occupy more moderate microclimates that afford taller vegetation structure during peak diurnal heating (Carrol et al. 2015). Thus, future work assessing the role of microhabitat selection in response to high temperatures across Mediterranean songbirds is needed to refine our predictive models of species vulnerability. This mechanistic approach could be valuable to designate climatic refuges in areas where the impact of predicted warming severely compromises species persistence.

Unlike songbirds, storks usually occupy open and exposed landscapes which, along with its large size, limits their capacity to select cooler microsites to reduce environmental heat load. This is even more exacerbated in nestlings, as they experience high solar radiation loads and operative temperature that could compromise their survival. Therefore, high temperatures can impact more severely the physiological capacity of nestlings, as they often depend on adults to avoid lethal hyperthermia. For this reason, it is common across storks that adults stand with their backs to the sun and spread their wings (wing-spreading posture) to provide shade to their nestlings (Kahl 1971; Thomas 1984). This allows nestlings to maintain their thermal balance, which is to some extent similar to the selection of thermally buffered microsites in songbirds. Nonetheless, when providing shade to offspring and during flights and foraging, adult storks can suffer heat stress that could compromise their thermal balance and increase thermoregulatory needs. As a way to cool off, heat-stressed storks often defecate on their bare, long legs (Chapters 4 and 5). This wetting behaviour, called urohidrosis, enhances their evaporative cooling capacity. By using thermal images, we estimate that urohidrosis can lower leg surface temperature by up to 6.7 ºC in both adult and young White storks *Ciconia ciconia*. Theoretically, we estimate that free-ranging White storks can dissipate up to 4% of the daily field metabolic rate through repeated urohidrosis. These findings demonstrate the thermoregulatory significance of urohidrosis in wild breeding birds.

In addition, adults periodically fly to waterbodies and carry water to their nestling ('watering' behaviour; see also Kahl 1963; Thomas 1984) during the hottest days. This not only allows nestlings to replenish water, but also lower body temperature through evaporative cooling via panting and urohidrosis. In the global warming context, the forecasted increase in heatwaves during late spring (coinciding with breeding season) along with more frequent and long-lasting droughts in the Mediterranean Basin (IPCC 2021) will increase water loss and evaporative cooling demands, while at the same time water supplies will be hampered. This may force adult storks to prioritize survival over breeding, particularly during the initial nestling stage, when shading behaviour is more prevalent and prolonged. A similar trade-off between behavioural thermoregulation and breeding success was recently found in a colonial, endangered seabird (Cook et al. 2020). Cook et al. (2020) found that adult Bank cormorants *Phalacrocorax neglectus* reduced EWL (e.g., less time spent in gular fluttering) by performing postural adjustments, which in turn increased the vulnerability of their eggs and chicks to high environmental temperatures.

All the thermoregulatory behaviours listed above could be employed as proxies of heat stress, and how these responses vary among species or populations used to forecast their sensitiveness to high temperatures. Nonetheless studies on non-arid zone species are scarce (but see Cook et al. 2020; Oswald et al. 2021; Playà-Montmany 2022; this thesis). Most of the studies have characterized how temperature thresholds at which a given behaviour is used (e.g., reduced activity, wing-drooping, or panting) varies across South African or Australian arid-zone birds (e.g., DuPlessis et al. 2012; Smit et al. 2016; Pattinson & Smit 2017; Thompson et al. 2018; Funghi et al. 2019; Kemp et al. 2020). Here, we evaluate the interspecific variation in urohidrosis use across storks globally (Chapter 4), finding substantial variation in the ambient temperature at which 50% of individuals from each species engage in this behaviour. Such urohidrosis temperature thresholds ranged from 27.7 ºC in the White stork to 33.5 ºC in the Woolly-necked stork *Ciconia episcopus*. These thresholds were lower than those reported for other heat dissipation behaviours such as panting (from 33.9 to 46.1 ºC) or wing-drooping (from 35.3 to 46.1 ºC) across arid-zone birds (Smit et al. 2016; Thompson et al. 2018). This could be explained by the higher thermal inertia of large species (due to their smaller surface area to volume ratio), which results in a smaller capacity for passive heat loss through thermal windows (such as unfeathered body parts) (Weathers 1981). Indeed, this is supported by a previous study on arid-zone birds showing that larger species resort to panting and wing-drooping earlier than smaller ones, which results in relatively higher evaporative cooling requirements in larger species that can limit their activity at ambient temperatures as low as 30 ºC (Smit et al. 2016). Collectively, these studies reveal different behavioural responses to heat across species, and thus different sensitivity to heat extremes.

As mentioned above, Mediterranean-resident songbirds are particularly sensitive to climate warming (Chapters 2 and 3). Therefore, we hypothesize that they should strongly rely on a wide array of heat dissipation behaviours (shade seeking, reduced activity during the hottest part of the day, ptiloerection, postural adjustments that promote convective passive heat loss, etc.) to maintain their thermal balance. Hence, gaining knowledge about how species from this and other climate warming hotspots combine physiological and behavioural mechanisms to deal with heat events is crucial to better estimate their vulnerability to future conditions.

Future directions

Towards the integration of physiology and behaviour in predictive models

Species distribution models are one of the most widely used methods to assess future vulnerability of species to forecasted climate warming scenarios (Kearney et al. 2010). For a long time, correlative species distribution models (which considers that a species is constrained in time and space by its climate envelope) were the most common approach for evaluating the effects of climate change in species distributions (e.g., Hannah et al. 2007; Dawson et al. 2011; Thuiller et al. 2013; Urban 2015); yet they overlook how physiology and behaviour interact with the environmental conditions in predicting the response of species to global warming. To solve this limitation, mechanistic species distribution models consider how environmental factors (such as microclimate), body shape or behaviour limit the physiological performance of a species in a given location (Kearney & Porter 2009). Hence, the persistence of a species is unlikely in those regions where fitness is physiologically or ecologically challenged (Kearney & Porter 2009; Gilman et al. 2010). In this way, we can improve inferences on species' range limits and habitat suitability by incorporating behavioural and physiological data (Huey et al. 2012; Sunday et al. 2014; Valladares et al. 2014).

Until now, most studies using mechanistic modelling to predict species' ranges have focused on ectotherms (e.g., Buckley et al. 2010; Buckley et al. 2015; Walker et al. 2015; Enríquez-Urzelai et al. 2019; Kearney et al. 2021a), although endotherms such as birds are gaining attention recently (e.g., Deville et al. 2014; Fitzpatrick et al. 2015; Kearney et al. 2016; Bladon et al. 2018). Recent development of modelling tools like the *endoterm model* implemented in the R package 'NicheMapR' (Kearney et al. 2021b) compute morphological, physiological functional traits (such as metabolic rate, body temperature, EWL, or thermal conductance) and behavioural responses (like changes in body posture, or heat dissipation behaviours) as a function of the *microclimate model* (Kearney & Porter 2017) to make inferences about the consequences of climatic changes on mammals and birds. In this context, the physiological and behavioural responses to high temperatures reported in this thesis are very valuable to explicitly model the distribution of Mediterranean songbirds and storks under current and future climates at finer spatiotemporal scales. Ultimately, this could help conservationists and policymakers define conservation plans that acknowledge fine scale thermal space in climate change scenarios (Porfirio et al. 2014; Carrol et al. 2015).

iEcology: the importance of large online resources

In recent years, a growing amount of online resources has been used to complement traditional ecological data. This approach has been termed iEcology (internet ecology) (Jaric et al. 2020). After scientific curation to guarantee the quality of data contained in these online sources (e.g., text, images, videos, sounds, online activity), iEcology provides novel opportunities to generate insights on species distribution patterns, behaviours, or morphological visible traits (e.g., Jaric et al. 2020; Gutiérrez & Soriano-Redondo 2020; Sbragaglia et al. 2021; Vrettos et al. 2021; Burke et al. 2022; Muller et al. 2022). In this thesis, we highlight the importance of massive online multimedia archives (such as the Macaulay Library) to study the thermoregulatory significance of urohidrosis (Chapter 4). Thus, we believe that this approach can be valuable to evaluate thermal sensitivity in other taxa, especially threatened species whose conservation status precludes invasive ecophysiological studies. For example, ambient temperature thresholds of heat dissipation behaviours can be estimated from digital resources (images and videos) and then used as a proxy of heat stress in the wild. Clearly, iEcology provide valuable support for ongoing research efforts, and represent a toolbox for comparative studies of species thermoregulation.

Assessing heat exchange trough thermal imaging

There is also a growing body of literature evaluating how animals exchange heat with their environments by using thermal imaging (e.g., Tattersall et al. 2009; McCafferty et al. 2011; Goller et al. 2014; Barroso et al. 2020; Schneider et al. 2020). Infrared thermography has been widely used across endotherms, particularly in birds, to obtain insights into heat transfer in response to changing environmental conditions in a non-invasive way (e.g., Tattersall et al. 2009; Symonds & Tattersall 2010;

Burness et al. 2013; Powers et al. 2015; van de Ven et al. 2016; Galván et al. 2017; Jerem et al. 2018; Rogalla et al. 2021; Soravia et al. 2022). Particular attention has been paid to quantify the potential role of bare and well-vascularized appendages (such as bill and legs) as 'thermal windows' that enhance passive dry heat loss by tuning of their surface temperature (e.g., Tattersall et al. 2009; Eastick et al. 2019; van de Ven et al. 2016; van Vuuren et al. 2020). As noted above, breeding in warm environments is especially challenging for birds that nest in locations exposed to high solar radiation and predation risk, including many stork and seabird species. Thermal imaging provides an excellent opportunity to evaluate how these species use their long and bare appendages to maintain heat balance during reproduction. During the rearing period, both adults and young are bound to their nests, which offers a chance to periodically collect thermal pictures from the same individuals over time. This might allow researchers to evaluate the thermal physiological role of bills and legs as well as the early thermal ontogeny (Burness et al. 2013; Stone et al. 2020). For example, Stone et al. (2020) found that juvenile Wandering albatrosses *Diomedea exulans* limit heat loss under cold conditions by reducing bill surface temperature with age.

Finally, recent studies have shown that the temperature of the eye region is a consistent indicator of a bird's physiological condition (e.g., Jerem et al. 2018; Winder et al. 2020). In some bird species, for example, eye temperature correlates with body condition and levels of circulating stress hormones (Jerem et al. 2020). In addition, it has been demonstrated that the unfeathered periorbital skin can contribute to passive heat dissipation (Powers et al. 2015; Robertson et al. 2020; Soravia et al. 2022). In sum, the use of thermal imaging can unveil 'new' cooling mechanisms and help understand the thermal ecology of birds; this is also an tool worthy of attention in future field studies on species that face heat-load problems.

References

Albright, T.P., Mutiibwa, D., Gerson, A.R., Smith, E.K., Talbot, W.A., O'Neill, J.J., McKechnie, A.E., and Wolf, B.O. (2017). Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proc. Natl. Acad. Sci. U.S.A.* **114** (9), 2283-2288.

Bakken, G. S., Angilletta, M. J. (2014). How to avoid errors when quantifying thermal environments. *Funct. Ecol.* **28**, 96–107.

Barroso, F.M., Riaño, G., Sannolo, M., Carretero, M.A., Rato, C. (2020). Evidence form *Tarentola mauritanica* (Gekkota: Phyllodactylidae) helps validate thermography as a tool to infer internal body temperature of lizards. *J. Therm. Biol.* **93**, 102700.

Bladon, A.J., Donald, P.F., Jones, S.E.I., Collar, N.J., Deng, J., Dadacha, G., Abebe, Y.D., Green, R.E. (2018). Behavioural thermoregulation and climatic range restriction in the globally threatened Ethiopian bush-crow *Zavattariornis stresemanni*. *Ibis.* **161**, 546-558.

Broggi, J., Hohtola, E., Orell, M., Nilsson, J.A. (2005). Local adaptation to winter conditions in a passerine spreading north: A common-garden approach. *Evolution*. **59**, 1600–1603.

Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J., Sears, M.W. (2010). Can mechanism inform species' distribution models? *Ecol. Lett.* **13**, 1041-1054.

Buckley, L.B., Ehrenberger, J.C., Angilletta, M.J. (2015). Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Funct. Ecol.* **29**, 1038-1047.

Burke, H.M., Tingley, R., Dorin, A. (2022). Tag frequency difference: rapid estimation of image set relevance for species occurrence data using general-purpose image classifiers. *Ecol. Inform.* **69**, 101598.

Burness, G., Huard, J.R., Malcolm, E., Tattersall, G.J. (2013). Post-hatch heat warms adult beaks: irreversible physiological plasticity in Japanese quail. *Proc. R. Soc. B.* **280**, 20131436.

Carroll, J.M., Davis, C.A., Elmore, R.D., Fuhlendorf, S.D., Thacker, E.T. (2015). Thermal patterns constrain diurnal behavior of a ground-dwelling bird. *Ecosphere*. **6**(11), 1-15.

Conradie, S.R., Woodborne, S.M., Cunningham, S.J., and McKechnie, A.E. (2019). Chronic, sublethal effects of high temperatures will cause severe declines in southern African arid-zone birds during the 21st century. *Proc. Natl. Acad. Sci. U.S.A.* **116**(28), 14065-14070.

Conradie, S.R., Woodborne, S.M., Wolf, B.O., Pessato, A., Mariette, M.M., and McKechnie, A.E. (2020). Avian mortality risk during heat waves will increase greatly in arid Australia during the 21st century. *Conserv. Physiol*. **8**(1), coaa048. http://doi:10.1093/conphys/coaa048

Cook, T.R., Martin, R., Roberts, J., Häkkinen, H., Botha, P., Meyer, C., Sparks, E., Underhill, L.E., Ryan, P.G., and Sherley, R.B. (2020). Parenting in a warming world: thermoregulatory responses to heat stress in an endangered seabird. *Conserv. Physiol.* **8**(1), coz109.

Cunningham, S.J., Gardner, J.L., and Martin, R.O. (2021). Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* **19**(5), 300-307.

Czenze, Z.J., Kemp, R., Jaarsveld, B. van, Freeman, M.T., Smit, B., Wolf, B.O., and McKechnie, A.E. (2020). Regularly drinking desert birds have greater evaporative cooling capacity and higher heat tolerance limits than non-drinking species. *Funct. Ecol.* **34**, 1589-1600.

Czenze, Z.J., Freeman, M.T., Kemp, R., van Jaarsveld, B., Wolf, B.O., McKechnie, A.E. (2022). Efficient evaporative cooling and pronounced heat tolerance in and eagle-owl, a thick-nee and a sandgrouse. *Front. Ecol. Evol.* **9**, 799302.

Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C., Mace, G.M. (2011). Beyond predictions: biodiversity conservation in a changing climate. *Science*. **332**, 53.

De Marche, M., Doak, D.F., Morris, W.F. (2018). Incorporating local adaptation into forecast of species' distribution and abundance under climate change. *Glob. Change Biol.* **25**, 775-793. https://doi.org/10.1111/gcb.14562

Deville, A.S., Labaude, S., Robin, J.P., Bechet, A., Gauthier-Clerc, M., Porter, W., Fitzpatrick, M., Mathewson, P., Gremillet, D. (2014). Impacts of extreme climatic events on the energetics of long-lived vertebrates: the case of the Greater flamingo facing cold spells in the Camargue. *J. Exp. Biol.* **217**(20), 3700-3707.

Du Plessis, K.L., Martin, R.O., Hockey, P.A.R., Cunningham, S.J., and Ridley, A.R. (2012). The costs of keeping cool in a warming world: implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Glob. Change Biol.* **18,** 3063-3070.

Eastick, D.L., Tattersall, G.J., Watson, S.J., Lesku, J.A., Robert, K.A. (2019). Cassowary casques act as thermal windows. *Sci. Rep.* **9**, 1966.

Enriquez-Urzelai, U., Tingley, R., Kearney, M.R., Sacco, M., Palacio, A.S., Tejedo, M., Nicieza, A.G. (2019). The roles of acclimation and behaviour in buffering climate change impacts. *J. Anim. Ecol.* **89**, 1722-1734.

Fitzpatrick, M.J., Mathewson, P.D., Porter, W.P. (2015). Validation of a mechanistic model for non-invasive study of ecological energetics in an endangered wading bird with counter-current heat exchange in its legs. *PLoS ONE*. **10**(8), e0136677.

Freeman, M.T., Czenze, Z.J., Schoeman, K., McKechnie, A.E. (2022). Adaptive variation in the upper limits of avian body temperature. *Proc. Nat. Acad. Of Sci. U.S.A.* **119**(26), e2116645119.

Funghi, C., McCowan, L.S.C., Schuett, W., Griffith, S.C. (2019). High air temperatures induce temporal, spatial and social changes in the foraging behaviour of wild Zebra finches. *Anim. Behav.* **149**, 33-43.

Galván, I., Palacios, D., Negro, J.J. (2017). The bare head of the Northern bald ibis (*Geronticus eremita*) fulfils a thermoregulatory function. *Front. Zool.* **14**, 15.

Gerson, A.R., Smith, E.K., Smit, B., McKechnie, A.E., Wolf, B.O. (2014). The impact of humidity on evaporative cooling in small desert birds exposed to high air temperatures. *Physiol. Biochem. Zool.* **87**(6), 782-795.

Gilman, S.E., Urban, M.C., Tewskbury, J., Gilchrist, G.W., Holt, R.D. (2010). A framework for community interactions under climate change. *Trends Ecol. Evol.* **25**(6), 325-331.

Goller, M., Goller, F., French, S.S. (2014). A heterogeneous thermal environment enables remarkable behavioural thermoregulation in *Uta stansburiana*. *Ecol. Evol.* **17**(4), 3319-3329.

Gutierrez, J.S., Soriano-Redondo, A. (2020). Laterality in foraging phalaropes promotes phenotypically assorted groups. *Behav. Ecol.* **31**(6), 1429-1435.

Hannah, L., Midgley, G., Andelman, S., Araújo, M., Hughes, G., Martinez-Meyer, E., Pearson, R., Williams, P. (2007). Protected area needs in a changing climate. *Front. Ecol. Environ.* **5**(3), 131-138.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 1665-1679.

IPCC (2021). Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press. In Press.

Jaric, I., Correia, R.A., Brook, B.W., Buettel, J.C., Courchamp, F., Di Minin, E., Firth, J.A., Gaston, K.J., Jepson, P., Kalinkat, G., Ladle, R., Soriano-Redondo, A., Souza, A.T., Roll, U. (2020). iEcology: harnessing large online resources to generate ecological insights. *Trends Ecol. Evol.* **35**(7), 630-639.

Jerem, P., Jenni-Eiermann, S., Herborn, K., McKeegan, D., McCafferty, D.J., Nager, R.G. (2018). Eye region surface temperature reflects both energy reserves and circulating glucocorticoids in a wild bird. *Sci. Rep.* **8**(2018), 1907.

Jerem, P., Jenni-Eiermann, S., McKeegan, D., McCafferty, D.J., Nager, R.G. (2020). Eye region surface temperature dynamics during acute stress relate to baseline glucocorticoids independently of environmental conditions. *Physiol. Behav.* **210**, 112627.

Kahl, P.M. (1963). Thermoregulation in the wood stork , with special reference to the role of the legs. *Physiol. Zool*. **36**(2), 141–151.

Kahl, P.M. (1971). Spread-wing postures and their possible functions in the Ciconiidae. *The Auk*. **88**, 715-722.

Kearney, M., Porter, W. (2009). Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol. Lett.* **12**, 334-350.

Kearney, M., Simpson, S.J., Raubenheimer, D., Helmuth, B. (2010). Modelling the ecological niche from functional traits. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **365**(1557), 3469-3483.

Kearney, M.R., Porter, W.P. (2017). NicheMap R – an R package for biophysical modelling: the microclimate model. *Ecography*. **40**, 664-674.

Kearney, M.R., Porter, W.P., Murphy, S.A. (2016). An estimate of the water budget for the endangered night parrot of Australia under recent and future climates. *Clim. Change Resp.* **3**, 14.

Kearney, M.R., Porter, W.P., Huey, R.B. (2021a). Modelling the joint effects of body size and microclimate on heat budgets and foraging opportunities on ectotherms. *Methods Ecol. Evol.* **12**(3), 458-467.

Kearney, M.R., Briscoe, N.J., Mathewson, P.D., Porter, W.P. (2021b). NicheMap R – an R package for biophysical modelling: the endotherm model. *Ecography*. **44**, 1595-1605.

Kemp, R., McKechnie, A.E. (2019). Thermal physiology of a range-restricted desert lark. *J. Comp. Physiol. B.* **189**, 131-141.

Kemp, R., Freeman, M.T., van Jaarsveld, B., Czenze, Z.J., Conradie, S.R., McKechnie, A.E. (2020). Sublethal fitness costs of chronic exposure to hot weather vary between sexes in a threatened desert lark. *Emu*. **120**, 216-229.

McCafferty, D.J., Gilbert, C., Paterson, W., Pomeroy, P.P., Thompson, D., Curriee, J.I., Ancel, A. (2011). Estimating metabolic heat loss in birds and mammals by combining infrared thermography with biophysical modelling. *Comp. Biochem.* **158**(3), 337-345.

McKechnie, A.E., and Wolf, B.O. (2010). Climate change increases the likelihood of catastrophic avian mortality events during extreme heatwaves. *Biol. Lett.* **6**, 253-256.

McKechnie, A.E., Smit, B., Whitfield, M.C., Noakes, M.J., Talbot, W.A., Garcia, M., Gerson, A.R., Wolf, B.O. (2016). Avian thermoregulation in the heat: evaporative cooling capacity in an archetypal desert specialist, Burchell's sandgrouse (*Pterocles burchelli*). *J. Exp. Biol.* **219**, 2137-2144.

McKechnie, A.E., Whitfield, M.C., Smit, B., Gerson, A.R., Smith, E.K., Talbot, W.A., McWorther, T.J., Wolf, B.O. (2016b). Avian thermoregulation in the heat: efficient evaporative cooling allows for extreme heat tolerance in four southern hemisphere columbids. *J.Exp. Biol.* **219**, 2145-2155.

McKechnie, A.E., Gerson, A.R., McWorther, T.J., Smith, E.K., Talbot, W.A., and Wolf, B.O. (2017). Avian thermoregulation in the heat: evaporative cooling in five Australian passerines reveals within-order biogeographic variation in heat tolerance. *J. Exp. Biol.* **220**, 2436-2444. http://doi:10.1242/jeb.155507

McKechnie. A.E., & Wolf, B.O. (2019). The physiology of heat tolerance in small endotherms. *Physiology*. **34**, 302-313. http://doi:10.1152/physiol.00011.2019

McKechnie, A.E., Gerson, A.R., & Wolf, B.O. (2021). Thermoregulation in desert birds: scaling and phylogenetic variation in heat tolerance and evaporative cooling. *J. Exp. Biol.* **224**, jeb229211. http://doi:10.1242/jeb.229211

Moran, E., Hartig, F., Bell, D.M. (2016). Intraspecific trait variation across scales: implications for understanding global change responses. *Glob. Change Biol.* **22**, 137-150.

Muller, J.R., Selier, S.A.J., Drouilly, M., Broadfield, J., Leighton, G.R.M., Amar, A., Naude, V.N. (2022). The hunter and the hunted: using web-sourced imagery to monitor leopard (*Panthera pardus pardus*) trophy hunting. *Conserv. Sci. Pract.* **2022**, e12789.

Noakes, M.J., Wolf, B.O., McKechnie, A.E. (2016). Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird. *J. Exp. Biol.* **219**, 859-869.

Noakes, M.J., McKechnie, A.E. (2019). Reaction norms for heat tolerance and evaporative cooling capacity do not vary across a climatic gradient in a passerine bird. *Comp. Biochem. Physiol. Part A.* **236**, 110522.

Noakes, M.J., McKechnie, A.E. (2020). Seasonal metabolic acclimatization varies in direction and magnitude among years in two arid-zone passerines. *Physiol. Biochem. Zool.* **93**(2), https://doi.org/10.1086/707679

O'Connor, R.S., Wolf, B.O., Brigham, R.M., McKechnie, A.E. (2017). Avian thermoregulation in the heat: efficient evaporative cooling in two southern African nightjars. *J. Comp. Physiol. B.* **187**, 477-491.

O'Connor, R.S., Le Pogam, A., Young, K.G., Robitaille, F., Choy, E.S., Love, O.P., Elliot, K.H., Hargreaves, A.L., Berteaux, D., Tam, A., and Vézina, F. (2021). Limited heat tolerance in an Arctic passerine: Thermoregulatory implications for cold-specialized birds in a rapidly warming world. *Ecol. Evol.* **11**(4), 1609-1619.

Oswald, K.N., Lee, A.T.K., Smit, B. (2018). Seasonal physiological responses to heat in an alpine range-restricted bird: the Cape Rockjumper (Chaetops frenatus). *J. Ornithol.* **159,** 1063-1072. http://doi.org/10.1007/s10336-018-1582-8

Oswald, K.N., Lee, A.T.K., Smit, B. (2021). Seasonal metabolic adjustments in an avian evolutionary relict restricted to mountain habitat. *J. Therm. Biol.* **95**, 102815.

Pattinson, N.B., Smit, B. (2017). Seasonal behavioural responses of an arid-zone passerine in a hot environment. *Physiol. Behav.* **179**, 268-275.

Playà-Montmany, N. (2022). Physiological and behavioural strategies underlying the responses of birds to global warming. PhD Thesis, University of Extremadura, Badajoz, Spain.

Porfirio, L.L., Harris, R.M.B., Lefroy, E.C., Hugh, S., Gould, S.F., Lee, G., Bindoff, N.L., Mackey, B. (2014). Improving the use of species distribution models in conservation planning and management under climate change. *PLoS ONE*. **9**(11), e113749.

Powers, D.R., Tobalske, B.W., Wilson, J.K., Woods, H.A., Corder, K.R. (2015). Heat dissipation during hovering and forward flight in hummingbirds. *R. Soc. Open Sci.* **2**, 150598.

Robertson, J.K., Mastromonaco, G., Burness, G. (2020). Evidence that stress-induced changes in surface temperature serve a thermoregulatory function. *J. Exp. Biol.* **223**, jeb213421.

Rogalla, S., Shawkey, M.D., Vanthournout, B., D'Alba, L. (2021). Thermoregulation and heat exchange in ospreys (*Pandion haliaetus*). *J. Therm. Biol.* **96**(2021), 102857.

Sbrabaglia, V., Coco, S., Correia, R.A., Coll, M., Arlinghaus, R. (2021). Analyzing publicly available videos about recreational fishing reveals key ecological and social insights: a case study about groupers in the Mediterranean Sea. *Sci. Tot. Environ.* **765**, 142672.

Schneider, M., Ziegler, T., Kolter, L. (2020). Thermoregulation in Malayan sun bears (*Helarctos malayanus*) and its consequence for in situ conservation. *J.Therm. Biol.* **91**(2020), 102646.

Sharpe, L.L., Prober, S.M., Gardner, J.L. (2022). In the hot seat: behavioural change and old-growth trees underpin an Australian songbird's response to extreme heat. *Front. Ecol. Evol.* **10**, 813567.

Smith, E.K., O'Neill, J.J., Gerson, A.R., McKechnie, A.E., Wolf, B.O. (2017). Avian thermoregulation in the heat: resting metabolism, evaporative cooling and heat tolerance in Sonoran Desert songbirds. *J. Exp. Biol.* **220**, 3290-3300. http://doi:10.1242/jeb.161141

Smit, B., Zietsman, G., Martin, R.O., Cunningham, S.J., McKechnie, A.E., Hockey, P.A.R. (2016). Behavioural responses to heat in desert birds: implications for predicting vulnerability to climate warming. *Clim. Chang. Responses.* **3**, 1-14.

Smit, B., Whitfiled, M.C., Talbot, W.A., Gerson, A.R., McKechnie, A.E., Wolf, B.O. (2017). Avian thermoregulation in the heat: phylogenetic variation among avian orders in evaporative cooling capacity and heat tolerance. *J. Exp. Biol.* **221**, jeb174870.

Soravia, C., Ashton, B.J., Ridley, A.R. (2022). Periorbital temperatue responses to natural air temperature variation in wild birds. *J. Therm. Biol.* **109**(2022), 103323.

Stone, D.W., Gunn, C., Nord, A., Phillips, R.A., McCafferty, D.M. (2020). Plumage development and environmental factors influence surface temperature gradients and heat loss in wandering albatross chicks. *J. Therm. Biol.* **97**, 102777.

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B. (2014). Thermal-safety margins and the necessity of thermoregulatory behaviour across latitude and elevation. *Proc. Natl. Acad. Sci. U.S.A.* **111**(15), 5610-5615.

Symonds, M.R.E., Tattersall, G.J. (2010). Geographical variation in bill size across bird species provides evidence for Allen's rule. *Am. Nat.* **176**(2), 188-197.

Talbot, W.A., McWorther, T.J., Gerson, A.R., McKechnie, A.E., Wolf, B.O. (2017). Avian thermoregulation in the heat: evaporative cooling capacity of arid-zone Caprimulgiformes from two continents. *J. Exp. Biol.* **220**, 3488-3498.

Talbot, W.A., Gerson, A.R., Smith, E.K., McKechnie, A.E., Wolf, B.O. (2018). Avian thermoregulation in the heat: metabolism, evaporative cooling and gular flutter in two small owls. *J. Exp. Biol.* **221**, jeb171108.

Tattersall, G.J., Andrade, D.V., Abe, A.S. (2009). Heat exchange from the toucan bill reveals a controllable vascular thermal radiator. *Science*. **325**, 468.

Thomas, B.T. (1984). Maguari stork nesting: juvenile growth and behaviour. *The Auk.* **101**, 812-823.

Thompson, M.L., Cunningham, S.J., McKechnie, A.E. (2018). Interspecific variation in avian thermoregulatory patterns and heat dissipation behaviours in a subtropical desert. *Physiol. Behav.* **188**, 311-323.

Thuiller, W., Munkemuller, T., Lavergne, S., Mouillot, D., Mouquet, N., Schiffers, K., Gravel, D. (2013). A road map for integrating eco-evolutionary processes into biodiversity models. *Ecol. Lett.* **16**, 94-105.

Tieleman, I.B., Williams, J.B., Bloomer, P. (2003). Adaptation of metabolism and evaporative water loss along an aridity gradient*.* *Proc. R. Soc. B.* **270**, 207–214.

Trost, C.H. (1972). Adaptations of horned larks (*Eremophila alpestris*) to hot environments. *The Auk.* **89**(3), 506-527.

Urban, M.C., Bocedi, G., Hendry, A.P., Mihoub, J.B., Pe'er, G., Singer, A., Bridle, J.R., Crozier, L.G., de Meester, L., Godsoe, W., Gonzalez, A., Hellmann, J.J., Holt, R.D., Huth, A., Johst, K., Krug, C.B., Leadley, P.W., Palmer, S.C.F., Pantel, J.H., Schmitz, A., Zollner, P.A., Travis, J.M.J. (2015). Improving the forecast for biodiversity under climate change. *Science*. **353**(6304), 1113-1122.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol.* **17**, 1351-1364. https://doi.org/10.1111/ele.12348

Van de Ven, T.M.F.N., Martin, R.O., Vink, T.J.F., McKechnie, A.E., Cunningham, S.J. (2016). Regulation of heat exchange across the hornbill beak: functional similarities to toucans? *PLoS ONE*. **11**(5), e0154768.

Van Dyk, M., Noakes, M.J., McKechnie, A.E. (2019). Interactions between humidity and evaporative heat dissipation in a passerine bird. *J. Comp. Physiol. B*. **189**, 299-308. https://doi.org/10.1007/s00360-019-01210-2

Van Vuuren, A.K.J., Kemp, L.V., McKechnie, A.E. (2020). The beak and unfeathered skin as heat radiators in the southern ground-hornbill. *J.Avian Biol.,* e02457.

Vrettos, M., Reynolds, C., Amar, A. (2021). Malar stripe size and prominence in peregrine falcons vary positively with solar radiation: support for the solar glare hypothesis. *Biol. Lett.* **17**(6), 20210116.

Walker, S., Stuart-Fox, D., Kearney, M.R. (2015). Has contemporary climate change played a role in population declines of the lizard *Ctenophorus decresii* from semi-arid Australia? *J. Thermal Biol.* **54**, 66-77.

Weathers, W.W. (1981). Physiological thermoregulation in heat-stressed birds: consequences of body size. *Physiol. Zool.* **54**(3), 345-361.

Whitfield, M.C., Smit, B., McKechnie, A.E., Wolf, B.O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* **218**, 1705-1714. https://doi.org/10.1242/jeb.121749

Wikelski, M., Spinney, L., Schelsky, W., Scheuerlein, A., Gwinner, E. (2003). Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. *Proc. R. Soc. B*. **270**, 2383–2388.

Winder, L.A., White, S.A., Nord, A., Helm, B., McCafferty, D.M. (2020). Body surface temperature responses to food restriction in wild and captive great tits. *J. Exp. Biol.* **223**, jeb220046.

7

General conclusions

General conclusions

1. We found that Mediterranean small resident songbirds show relatively low evaporative cooling efficiency and heat tolerance. Our results place Mediterranean songbirds in the lowest end of evaporative cooling efficiency among studied songbirds. Only the crested lark (*Galerida cristata*, a cosmopolitan species occurring over a wide range of both mesic and arid habitats) achieved evaporative cooling efficiencies greater than 1 (i.e., all the heat metabolically produced was dissipated through evaporative means). These values are well below those reported among arid-zone passerines (up to 2.5). Likewise, Mediterranean songbirds showed lower heat tolerance limits (40-46 ºC) than arid-zone species worldwide (46-54 ºC), but in the range of tropical, North-American temperate and subarctic songbirds previously studied.

2. Mediterranean songbirds from Extremadura are currently experiencing maximum temperatures that challenge their thermoregulatory capacities, which will be exacerbated under future climate warming scenarios. Besides, we observed marked differences among species in the vulnerability to chronic detrimental fitness effects from heat exposure under current and future conditions. Models showed that under the most severe climate change scenario (RCP8.5), only the Great tit *Parus major* will experience direct risk of mortality by 2070-2100, while all the species except the heaviest ones will experience moderate risk of lethal dehydration (i.e., 15% of body mass loss in less than 5 hours) during extremely hot days by the end of the century.

3. We showed substantial seasonal variation in heat tolerance limits and other key thermoregulatory traits in two Mediterranean Great tits populations which experience different thermal regimes: warmer/higher seasonal amplitude vs milder/lower seasonal amplitude. Birds enhanced heat tolerance during summer by adjusting their upper critical temperature (T_{uc}) and evaporative water loss (EWL) inflection point, and body temperature, resting metabolic rate (RMR) and EWL above thermoneutrality. Together, these adjustments diminished total endogenous heat loads during the hottest part of the year.

4. In accordance with the climatic variability hypothesis, Great tits from a warmer, lowland site enhanced their heat tolerance limits (while those from the milder site did not) and achieved greater evaporative cooling efficiency during summer than those from a milder montane site. Along this line, birds from the warmer, lowland site (which experience greater thermal amplitude through the year) showed a greater seasonal flexibility in their thermoregulatory traits above thermoneutrality than those from the montane and more thermally stable site.

5. By using scientifically curated media file repositories and historical microclimatic data we determined that urohidrosis use in all extant storks is mainly triggered by high ambient temperature, solar radiation,

and air humidity, as well as by low wind speed. Besides, those species which mainly forage in open landscapes rely more on urohidrosis than those usually foraging in waterbodies.

6. We predicted for each stork species the ambient temperature threshold at which the 50% of the individuals would perform urohidrosis. This threshold varied among species (from 27.7 ºC in White stork *Ciconia ciconia* to 33.5 ºC in Woolly-necked stork *Ciconia episcopus*), pointing to different degrees of vulnerability to high temperatures.

7. We used non-invasive thermal imaging to quantify for the first time the thermoregulatory significance of urohidrosis in wild storks. By performing urohidrosis, nest-bound White storks lowered their leg surface temperature by up to 6.7 ºC. Such a cooling effect lasted up to 2.5 minutes. Knowing that both adult and young storks at nest use urohidrosis repeatedly when heat stressed, we estimated that this cooling behaviour could represent a significant amount of heat loss (up to 4% of the daily field metabolic rate of an adult stork), highlighting the importance of this behaviour for dealing with heat stress during reproduction.

Conclusiones generales

1. Encontramos que los paseriformes mediterráneos residentes de pequeño tamaño muestran eficiencias de enfriamiento evaporativo y tolerancia al calor relativamente bajas. Nuestros resultados colocan a los paseriformes mediterráneos en límite más bajo de eficiencia de enfriamiento evaporativo entre todos los paseriformes estudiados. Solo la cogujada común (*Galerida cristata*, una especie cosmopolita que ocurre a lo largo de amplio rango de hábitats tanto mésicos como áridos) alcanzó eficiencias de enfriamiento evaporativo superiores a 1 (es decir, todo el calor producido metabólicamente fue disipado de forma evaporativa). Estos valores están bien por debajo de los reportados entre paseriformes de zonas áridas (hasta 2.5). De igual modo, los paseriformes mediterráneos mostraron límites de tolerancia al calor más bajos (40-46 ºC) que los de especies de zonas áridas a lo largo del planeta (46-54 ºC), pero en el rango de paseriformes tropicales, de zonas templadas de Norte América y de zonas subárticas previamente estudiados.

2. Los paseriformes mediterráneos de Extremadura actualmente experimentan temperaturas máximas que desafían sus capacidades termorreguladoras, lo cual se verá exacerbado bajo futuros escenarios de calentamiento climático. Además, observamos marcadas diferencias entre las especies en su vulnerabilidad a los efectos adversos crónicos sobre su eficacia derivados de la exposición al calor bajo condiciones actuales y futuras. Los modelos mostraron que bajo el escenario de cambio climático más severo (RCP8.5), solo el carbonero común *Parus major* experimentará riesgo de mortalidad directa para 2070-2100, mientras que todas las especies salvo las de mayor tamaño experimentarán riesgo moderado de deshidratación letal (esto es, pérdida de un 15% de masa corporal en menos de 5 horas) durante días extremadamente calurosos para finales de siglo.

3. Mostramos una variación estacional sustancial en los límites de tolerancia al calor y en otros rasgos termorreguladores clave en dos poblaciones mediterráneas de carbonero común que experimentan distintos regímenes termales: caluroso/elevada amplitud estacional vs suave/menor amplitud estacional. Las aves mejoraron la tolerancia al calor durante el verano mediante ajustes en su temperatura crítica superior (T_{uc}), el punto de inflexión de la pérdida de agua evaporative (EWL), y la temperatura corporal, la tasa metabólica de reposo (RMR) y EWL por encima de la termoneutralidad. En conjunto, estos ajustes disminuyeron las cargas totales de calor endógeno durante la parte más calurosa del año.

4. De acuerdo con la hipótesis de variabilidad climática, los carboneros del sitio de vega, más caluroso incrementaron su límite de tolerancia al calor durante el verano (mientras que los del sitio más suave no) y alcanzaron una mayor eficiencia de enfriamiento evaporativo que aquellos del sitio de montaña más suave. En línea con esto, las aves del sitio de vega (las cuales experimentan una mayor amplitud termal a lo largo del año) mostraron una mayor flexibilidad estacional en sus rasgos termorreguladores por encima de la zona termoneutral que aquellas del sitio de montaña más suave y termalmente estable.

5. Utilizando repositorios multimedia científicamente supervisados y datos microclimáticos históricos determinamos que el uso de urohidrosis en cigüeñas está principalmente motivado por alta temperatura ambiental, radiación solar y humedad del aire, así como por bajas velocidades del viento. Además, aquellas especies que principalmente forrajean en paisajes abiertos recurren más a la urohidrosis que aquellas que normalmente forrajean en cuerpos de agua.

6. Predijimos para cada especie de cigüeña el umbral de temperatura ambiente al cual el 50% de los individuos llevarían a cabo urohidrosis. Este umbral varió entre especies (desde 27.7 ºC en la cigüeña blanca *Ciconia ciconia* hasta 33.5 ºC en la cigüeña lanuda *Ciconia episcopus*), apuntando hacia diferentes grados de vulnerabilidad frente a temperaturas elevadas.

7. Utilizamos técnicas no invasivas como la fotografía térmica para cuantificar por primera vez el significado termorregulador de la urohidrosis en cigüeñas salvajes. Al llevar a cabo urohidrosis, las cigüeñas blancas reproductoras disminuyeron la temperatura superficial de sus patas hasta 6.7 ºC. El efecto de este enfriamiento duró hasta 2.5 minutos. Sabiendo que tanto cigüeñas adultas como juveniles utilizan repetidamente la urohidrosis mientras están en el nido cuando sufren estrés por calor, estimamos que este comportamiento de enfriamiento podría suponer una pérdida de calor considerable (hasta un 4% de la tasa metabólica de campo diaria en una cigüeña adulta), resaltando la importancia de este comportamiento para enfrentarse al estrés por calor durante la reproducción.

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