

Can a behavioural index be used to assess the vulnerability of arid-zone birds to climate change?

by

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Summary

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Accurately predicting species' responses to climate change is complex and requires the integration of multiple determinants of species-specific sensitivity and exposure (Williams *et al.* 2008). The two main approaches currently used to assess vulnerability to climate change are: a) general pattern-based correlative models or b) single-species, mechanistic models. Correlative models have been popular due to their ability to quickly generate predictions for multiple species (Thomas *et al.* 2004). However, detailed species-specific models incorporating comprehensive mechanistic data provide more precise predictions of how individual species will respond to changes in climate (Briscoe *et al.* 2016, Kearney *et al.* 2009). To bridge the gap between predictions provided by these species-specific mechanistic models and more rapid, generalised correlative models, we require novel

modelling approaches to effectively and quickly assess species' vulnerability to rising temperatures.

In this thesis I sought to validate the usefulness of a simple behavioural index, 'pant₅₀', as an indicator of high vulnerability to thermal physiological costs in birds inhabiting hot desert environments. This behavioural index is calculated at a species-specific level and is defined as the air temperature (T_{air}) at which 50 % of observed individuals of a particular bird species are engaged in panting behaviour, which augments rates of evaporative heat loss. To validate the relationship between pant₅₀ and capacity to safely thermoregulate at high air temperatures, I tested predictions relating heat dissipation behaviours to underlying changes in physiology in a range of arid-zone bird species that exhibited wide variation in pant₅₀. These pant₅₀ values represent data collected from either free-living birds or those in semi-natural captivity in the southern Kalahari Desert.

I began by examining whether interspecific variation in panting and other heat dissipation behaviours is functionally linked to body temperature (T_b) regulation. I examined patterns of T_b and behaviour in nine species that differ widely in pant₅₀ (**chapter 1**) and found significant variation in T_b patterns among species at high T_{air} . However, these patterns were not correlated with pant₅₀; instead, species appeared to manage heat loads using species-specific combinations of several behavioural adjustments, of which pant₅₀ is only one. My data also suggest that birds in hot, arid environments may maintain higher T_b than currently thought.

As thermoregulation in the heat is directly coupled with water balance, and panting behaviour (and therefore pant₅₀) is associated with evaporative water loss, I also examined physiological and behavioural consequences of short-term (8 h) water deprivation among species from six families and three orders (**chapter 2**). I found that thermoregulatory responses of species when dehydrated were extremely variable. Most species delayed

panting to higher T_{air} or seldom panted at all when water was restricted, compared to days when water was freely available, while adjustments in other behavioural aspects of thermoregulation with water restriction were highly variable among and within species.

Verifying similarities in behaviour between captive and free-ranging birds is important when findings of studies in captivity are extrapolated to wild counterparts. The work reported in chapters 1 and 2 involved birds kept in large flight aviaries, making it important to ensure thermoregulatory behaviours were comparable to those of free-ranging conspecifics. Therefore, in **chapter 3** I compared behavioural thermoregulatory responses in free-ranging and captive individuals representing six species to examine the extent to which thermoregulatory and behavioural data collected under laboratory conditions can be extrapolated to free-ranging populations in natural environments. While panting behaviour remained unchanged in captivity, unexpectedly, overall activity was reduced in captivity in only three of the six species. Shade-seeking behaviour differed in captivity with species seeking shade at lower T_{air} than wild populations. This suggests that behaviour patterns in captive populations can provide important information about the behaviour of free-ranging populations, but should be interpreted with caution.

Finally, in **chapter 4** I assessed whether the behavioural index, pant_{50} , could be useful to assess species relative vulnerability to rising air temperatures. To do so, I examined whether pant_{50} was functionally linked to underlying physiological changes related to hydration status and hyperthermia, extracted from physiological and behavioural studies of 20 arid-zone bird species from five orders. Unexpectedly, I found no support for my prediction that species-specific changes in thermal physiology during hot weather are reflected by pant_{50} . However, there is potential that pant_{50} may be useful for predicting vulnerability to sublethal fitness costs, which in southern Africa in particular, are predicted to increase substantially during the 21st century.

In summary this thesis provides a thorough investigation into the usefulness of pant_{50} as a tool to quantify species relative vulnerability to increased T_{air} s predicted to accompany climate change in desert environments. The results suggest that pant_{50} as a behavioural index may not be useful in predicting thermal physiological responses in the heat. However, future research should focus on the usefulness of this index to predict sublethal fitness costs and the vulnerability of certain species to these.

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Research Outputs

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Manuscripts in preparation

Thompson M, Cunningham S, & McKechnie AE. Interactions between avian thermoregulation, behaviour and hydration status under semi-natural conditions.

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Thompson ML, Smit B, Bourne AR, van de Ven TMFN, Van Jaarsveld B, Kemp R, Cunningham SJ & McKechnie AE. Can a behavioural index be used to assess species relative vulnerability to rising air temperatures?

Conference presentations

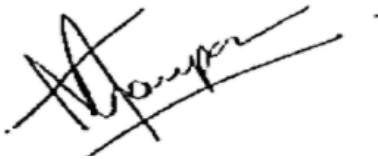
Thompson ML, McKechnie AE, Cunningham SJ. Avian heat dissipation behaviour as an indicator of physiological stress? *Hot Birds Research Project Workshop*. 22-26 July 2019. Gobabeb Research Station. Namibia.

Thompson ML, McKechnie AE, Cunningham SJ. Can avian heat dissipation behaviour indicate underlying physiological stress? *North American Ornithological Conference*. 16-21 August 2016. Washington DC. Washington DC.

Declaration

I, Michelle L. Thompson, declare that the thesis, which I hereby submit for the degree PhD in Zoology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed:



Michelle Thompson

22/11/2021

Date

Ethics Statement

I, Michelle L. Thompson, have obtained the applicable research ethics approval for the research described in this work (University of Pretoria Animal Ethics Committee protocol number: EC-010-15). I declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

I also obtained approval for this research work from the relevant permitting bodies of the Northern Cape (ODB 2815 2013 FAUNA 1088 2013).

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Contents

Summary	1
Research Outputs.....	5
Declaration.....	6
Ethics Statement.....	6
Acknowledgements.....	7
Contents	9
CHAPTER 1 Interspecific variation in avian thermoregulatory patterns and heat dissipation behaviours in a subtropical desert.....	11
1.1 Abstract	11
1.2 Introduction.....	12
1.3 Methods & Materials.....	15
1.4 Results	22
1.5 Discussion	31
1.6 Literature cited.....	37
1.7 Appendix	43
CHAPTER 2 Interactions between avian thermoregulation, behaviour and hydration status under semi-natural conditions.....	45
2.1 Abstract	45
2.2 Introduction.....	46
2.3 Methods & Materials.....	49
2.4 Results	55
2.5 Discussion	63
2.6 Literature cited.....	69
2.7 Appendix	75
CHAPTER 3 Effect of captivity on heat dissipation behaviour and thermoregulation in arid-zone birds	78
3.1 Abstract	78
3.2 Introduction.....	79
3.3 Methods and Materials	82
3.4 Results	85
3.5 Discussion	91

3.6	Literature cited.....	95
3.7	Appendix	103
CHAPTER 4 Can a behavioural index be used to assess species' relative vulnerability to global heating?.....		
4.1	Abstract	104
4.2	Introduction.....	105
4.3	Methods and Materials	109
4.4	Results	114
4.5	Discussion	117
4.6	Literature cited.....	121
4.7	Appendix	129

CHAPTER 1 Interspecific variation in avian thermoregulatory patterns and heat dissipation behaviours in a subtropical desert

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1.1 Abstract

Deserts are physiologically challenging environments for birds, with scarce, unpredictable water resources combined with air temperatures (T_{air}) regularly exceeding avian body temperature (T_{b}). For arid-zone birds, mismatches between water supply and demand are a constant threat, yet interspecific variation in trade-offs between hyperthermia avoidance and dehydration avoidance remain poorly understood, particularly for free-ranging individuals. We examined behavioural and physiological responses to high T_{air} in nine species representing three orders that vary substantially in their heat dissipation thresholds, specifically pant_{50} , the T_{air} at which panting behaviour is present in 50% of observations. Birds housed during mid-summer in large free-flight aviaries in the Kalahari Desert each received a surgically-implanted T_{b} logger, and we quantified shade-seeking, activity and panting behaviours to examine relationships between species-specific pant_{50} and T_{b} regulation. Overall, species setpoint T_{b} values were higher (range: 41.4 ± 0.5 °C to 43.1 ± 0.4 °C) than expected with maximum T_{b} values of 43.4–45.5 °C. Interspecific variation in T_{b} patterns at high T_{air} was substantial, with T_{b} increasing with T_{air} in most species, whereas in others no pattern or a negative relationship between T_{b} and T_{air} was evident. Most species avoided prolonged hyperthermia, with reductions in activity and increased shade-seeking evidently adequate to manage heat load without resorting to hyperthermia in several of our study species. Access to drinking water and food resources in captivity may have affected T_{b} patterns. Our data reveal that thermoregulation varies substantially among species, and

suggest that free-ranging birds in hot, arid environments may maintain higher T_b than currently thought.

Keywords: *hyperthermia, thermal physiology, trade-off, Passeriformes, Columbiformes, Coliiformes.*

1.2 Introduction

In hot, arid environments daily air temperatures can routinely exceed avian normothermic body temperatures. One major physiological challenge facing desert birds, therefore, is the maintenance of stable core body temperature (T_b) and avoidance of lethal hyperthermia (Dawson & Bartholomew 1968, Prinzinger *et al.* 1991, Tieleman & Williams 1999, Møller 2010). Birds respond to increases in T_b via physiological and behavioural adjustments that return T_b to a normothermic range, which often involve costs related to increased water and/or energy demands (Dawson & Bartholomew 1968). During hot weather, when T_{air} approaches or exceeds the normothermic setpoint T_b (T_{bset}), passive heat loss ceases (Wolf 2000, Nilsson *et al.* 2016) and birds need to actively dissipate heat via evaporation to avoid T_b reaching lethal limits ($\sim 46\text{--}48^\circ\text{C}$; Dawson & Schmidt-Nielsen 1964, Tieleman & Williams 1999, Whitfield *et al.* 2014). Birds increase rates of evaporative heat loss via a combination of respiratory and cutaneous evaporative heat loss (REWL and CEWL, respectively). The contribution of these two avenues to evaporative cooling varies among taxa (Wolf & Walsberg 1996, McKechnie *et al.* 2016). Elevated CEWL promotes heat loss without the metabolic heat production associated with the muscle contractions required for panting (REWL), and therefore appears to be the more efficient mechanism of evaporative heat dissipation (Marder & Arieli 1988, McKechnie & Wolf 2004).

Irrespective of the pathways involved, high rates of evaporative cooling are problematic in desert environments where dehydration risk is high and surface water scarce (Dawson 1954, Bartholomew & Cade 1956). For example, at very high T_{air} small birds can lose > 5% of body mass per hour via evaporation (Wolf & Walsberg 1996). To reduce water loss through evaporative cooling in hot environments, birds may show regulated, reversible increases in T_b (facultative hyperthermia; reviewed by Tieleman & Williams 1999). However, the potential benefits of hyperthermia also vary with body mass and among avian taxa (Tieleman & Williams 1999, Whitfield *et al.* 2015, McKechnie *et al.* 2016) and even among populations within species (Smit *et al.* 2013). For instance, a desert population of White-browed Sparrow-Weavers (*Plocepasser mahali*) maintained higher T_{bset} than individuals from milder semi-desert sites, favouring a reduction in the use of hyperthermia (Smit *et al.* 2013).

Birds use behavioural adjustments such as reducing activity and increasing shade-seeking behaviour to reduce physiological costs of thermoregulation at high T_{air} (Calder & King 1974, Wolf 2000, Dawson 1982, Wolf 2000). However, the conflict between actively foraging to maintain energy and water budgets, and hyperthermia avoidance by reducing activity and seeking shade can be expensive in terms of lost opportunity costs (Cunningham *et al.* 2013, 2015). Additionally, high T_{air} can reduce overall fitness, as the aforementioned behaviours coupled with increases in heat dissipation behaviour (HDB, such as panting and wing-drooping) reduce foraging efficiency, causing short-term losses of body condition (Du Plessis *et al.* 2012, van de Ven 2017) and reduced parental care (Cunningham *et al.* 2013, van de Ven 2017). Furthermore, there is considerable interspecific variation in the temperature dependence of HDB as well as activity and shade-seeking behaviours (Smit *et al.* 2016). The latter authors showed that the T_{air} associated with 50 % of a population panting or wing-drooping varies by > 21 °C among

species in the Kalahari. Such interspecific differences in the T_{air} associated with the onset of HDB may provide a basis for using HDB thresholds to assess species vulnerability to high temperatures in hot environments, which consequently could be important for modelling avian responses to climate change (Smit *et al.* 2016). However, it is first necessary to establish whether functional links exist between inter- and intraspecific variation in HDB and T_b regulation (Smit *et al.* 2016).

Most data on avian thermoregulation at high T_{air} originate from laboratory studies in which T_b is measured concomitantly with metabolic rates and evaporative water loss (e.g., Smith *et al.* 2015, Whitfield *et al.* 2015; McKechnie *et al.* 2016). However, laboratory studies may not accurately reflect thermoregulation under natural conditions, as ambient conditions are controlled, and activity restricted (Smit *et al.* 2013). The few studies that have examined diurnal T_b patterns in free-ranging birds during hot weather typically focus on just one or two species (Smit *et al.* 2013, O'Connor *et al.* 2017). These studies confirm that T_b patterns can differ substantially between free-ranging individuals and individuals held in respirometry chambers (O'Connor *et al.* 2017). Yet, despite the importance of thermoregulation and associated trade-offs in the ecology of desert birds, very little is known about interspecific differences in thermoregulatory patterns in free-ranging birds (Smit *et al.* 2013) or how these relate to species traits, including variation in the temperature-dependence of HDB.

Recent years have seen an increasing interest in the notion that, thermal performance of endotherms represents a continuum from thermal specialisation to generalisation, akin to that found in ectotherms (Angilletta *et al.* 2010, Boyles *et al.* 2011a). This view posits that thermoregulation is co-adapted with thermal sensitivity of performance, such that trade-offs occur whereby some species maintain high performance over only a narrow T_b range, responding strongly to changes in T_b (thermal specialists)

whereas others tolerate a wider range of T_b but at lower performance levels and respond less to T_b changes (thermal generalists; Angilletta *et al.* 2010, Boyles *et al.* 2011a). Different environmental pressures likely alter the benefits of being either a thermal specialist or generalist. Yet, little is known about how much variation there is in the thermal performance breadth of specialists and generalist thermoregulators in specific environments, or whether species can shift from being a thermal specialist to a thermal generalist when heat stressed.

Here, we hypothesised that interspecific variation in HDB is functionally linked to body temperature regulation. We tested this hypothesis by examining patterns of T_b and behaviour in nine species that differ widely in the temperature dependence of panting, focussing specifically on panting thresholds, i.e. the T_{air} at which panting behaviour is present in 50 % of observations ($pant_{50}$). Thus, we selected our study species on the basis of $pant_{50}$; which varied by $> 10^\circ\text{C}$ with a range from $34.5 - 45.5^\circ\text{C}$ (Smit *et al.* 2016). We, 1) quantified interspecific variation in T_{bset} , 2) tested the prediction that T_b patterns correspond with $pant_{50}$ in terms of how T_b responds to increasing T_{air} , and 3) quantified hyperthermia use/avoidance across these species. 4.) We also tested the prediction that $pant_{50}$ is related to activity patterns and shade-seeking behaviours at high T_{air} . Due to the onset of evaporative cooling when T_{air} approaches normothermic T_b , we expected $pant_{50}$ to be correlated with T_{bset} , with species which pant at lower T_{air} defending lower T_{bset} and showing a lower degree of heterothermy.

1.3 Methods & Materials

1.3.1 Study animals and housing

We examined thermoregulation in nine bird species resident in the southern Kalahari Desert, representing three orders (Passeriformes, Columbiformes and Coliiformes). These

Table 1: Kalahari desert bird species used to compare variation in body temperature parameters. Each species has a unique abbreviation (Abb.) used for referral in figures and text. Species belong to three orders, passeriformes (PASS), columbiformes (COLU) and Coliiformes (COLI). Summer seasons where species were sampled are indicated by Season (2014 refers to Austral summer: i.e. Nov 2013-March 2014 etc). Species are categorised according to dominant dietary guild; i.e. frugivores (FRU), insectivores (INS), granivores (GRA), omnivores (OMN) using diet information from Hockey *et al.* 2005. Sample sizes for physiological (N_{Tb}) and behavioural data (N_{behav}) included.

Abb.	Order	Species	Season	Diet	N_{Tb} (N_{behav})	Body mass
WBM	COLI	White-backed Mousebird (<i>Colius colius</i>)	2016	FRU	5(10)	34.5 ± 1.7
CGS	PASS	Cape Glossy Starling (<i>Lamprotornis nitens</i>)	2015(2016)	OMN	8(16)	74.5 ± 7.1
REB	PASS	African Red-eyed Bulbul (<i>Pycnonotus nigricans</i>)	2014	FRU	7(10)	29.3 ± 2.2
WBS	PASS	White-browed Sparrow Weaver (<i>Plocepasser mahali</i>)	2015	OMN	6(10)	35.8 ± 2.6
CTD	COLU	Cape Turtle-Dove (<i>Streptopelia capicola</i>)	2015(2016)	GRA	11(16)	129.5 ± 11.3
LAD	COLU	Laughing Dove (<i>Streptopelia senegalensis</i>)	2015	GRA	8(11)	89.6 ± 9.9
SOW	PASS	Sociable Weaver (<i>Philetairus socius</i>)	2014	OMN	8(9)	24.0 ± 1.0
FCL	PASS	Fawn-coloured Lark (<i>Calendulauda africanoides</i>)	2014	OMN	6(8)	23.9 ± 0.7
NAD	COLU	Namaqua Dove (<i>Oena capensis</i>)	2015(2016)	GRA	8(19)	36.9 ± 2.3

species have a ~6-fold range in body mass and represent a variety of foraging guilds (Table 1). Birds were caught using species-appropriate trapping methods including mist-netting, spring traps baited with mealworms and walk-in traps baited with seed. Individual morphometric data including body mass were recorded upon capture, and birds were assigned a unique colour ring combination for individual identification while in captivity. Each species was kept separately for a single two-month period during either of the austral summers of 2014–2016 in one of three large outdoor aviaries (each 3 m high x 3 m wide x 8 m long). The aviaries were erected at Murray Game Ranch (26°59'S, 20°51'E), a private farm ~ 12 km east of the town of Askham in the Northern Cape province of South Africa. Birds were habituated to the aviaries for at least six days before experimentation began. Within the aviaries, birds experienced natural daily ambient temperature cycles. Shade was provided by covering one-third of the aviary roof with sheets of corrugated steel. Perches were placed at various heights and positions inside the aviaries, and a comparable range of perching locations was available in each aviary. Birds were fed

species-specific diets; columbids were fed a diet of wild birdseed, omnivorous passerines were also maintained on a diet of wild birdseed with daily supplements of mealworms and occasional supplements of freshly caught insects (predominantly lacewings and moths) trapped around domestic light sources in the evenings. Frugivorous species were fed seasonal fresh fruit mixed with ProNutro breakfast cereal (Bokomo, Pioneer Foods Groceries (Pty) Ltd, Tygervalley, Western Cape, South Africa) occasionally supplemented with mealworms. All birds received water *ad libitum* during the measurements reported here.

1.3.2 Body and air temperature measurements

Measurements of T_b for the two larger species, Laughing Dove (hereafter LAD) and Cape Turtle-Dove (hereafter CTD), were obtained using miniature temperature loggers (Thermochron iButton, model 1922L, Maxim Dallas Semiconductors, Sunnyvale, California, USA) programmed to record T_b every five minutes. For the remaining seven species (African Red-eyed Bulbul, Cape Glossy Starling, Fawn-coloured Lark, Namaqua Dove, Sociable Weaver, White-browed Sparrow-Weaver, White-backed Mousebird hereafter REB, CGS, FCL, NAD, SOW, WBS and WBM, respectively), we used surgically implanted temperature-sensitive radio transmitters (model BD-2TH, Holohil Systems Ltd., Carp, Ontario, Canada). A data logging station consisting of a VHF radio receiver / data logger (Lotek SRX_400 receiver, Lotek Wireless Inc., Newmarket, Ontario, Canada) was set up outside the aviaries. The logging station included a multiplexor to accommodate three Yagi antennae (one per aviary) with the receiver continuously cycling through transmitter frequencies in such a way that the T_b of individuals was recorded at roughly four-minute intervals. Signal interference in the shaded areas of the aviaries was common and often interrupted the continuous recording of T_b . Individuals with intermittent T_b traces were omitted from the T_b analyses but data from all individuals were included in the

behavioural analyses. The mass of loggers and transmitters mass was $\leq 5\%$ of body mass of all birds, excepting SOW and FCL where mass was $< 7.5\%$ of body mass. Loggers and transmitters were coated with surgical wax before being implanted intraperitoneally into the abdominal cavities of the birds under inhalation anaesthesia by a qualified veterinarian (Dr A Lategan, SAVC reg. no. D90-3031). To allow a full recovery the surgery took place at least a week before data collection. All loggers/transmitters were precalibrated over a temperature range of 0–45°C in a circulating water bath against a mercury-in-glass thermometer with NIST-traceable accuracy. Drift in radio transmitters required T_b traces to be corrected (see Data analysis section).

Air temperature, dew point, rainfall and other weather variables were recorded at five-minute intervals using a portable weather station (Vantage Pro2, Davis Instruments, Haywood, California, USA) placed within 400 m of the aviaries. Air temperature inside the aviaries (T_{aviary}) was also measured using temperature data loggers (TidbiT v2 temperature data logger UTBI-001, Onset Computer Corporation, Bourne, Massachusetts, USA) placed 2 m above the ground at perch level, in the shade at the back of each aviary. Each TidbiT was suspended inside a hollow plastic PVC tube (20 cm long x 5 cm diameter) with holes drilled into the sides for ventilation. TidbiTs were synched with the weather station and recorded T_{aviary} every five min. TidbiTs were also precalibrated following the same procedure as T_b devices.

1.3.3 Behavioural observations

Behavioural data were collected by an observer seated far enough from the aviary that bird behaviour was not influenced by the observer's presence. The distance varied according to observed vigilance and alarm thresholds of the different species but generally ranged between 5-10 m from the aviary. We made instantaneous behavioural observations (Altmann 1974) per individual between 07:00–12:00 h and 14:00–17:30 h

daily. This was done by observing an individual for a short time period (~20 seconds) and recording basic binomial data on panting behaviour (none = 0, panting = 1), shade-seeking (sun = 0, shade = 1) and activity (inactive = 0, active = 1). During these periods, individuals were sampled between 2–5 times depending on the ease of individual identification. Number of Individuals per species included for behavioural data are generally higher than for T_b reflecting the exclusion from the analyses of individuals with poor T_b traces.

1.3.4 Data analysis

All statistical analyses were conducted in the R environment (R Core Team 2016).

Transmitter drift correction

The Holohil temperature-sensitive radio transmitters used in this study have been previously shown to exhibit signal drift (Williams et al. 2009, Cunningham et al. 2017), and we encountered the same issue during the present study. To correct for drift, we examined T_b traces to identify where transmitters began to drift (break point) by running segmented models using the package *segmented* (Muggeo 2008) in R and, following the procedure of Cunningham et al. (2017), corrected each individual T_b trace. Transmitters that did not start drifting immediately typically had break points between 8-15 days after implantation. The magnitude of drift in all transmitters varied anywhere between 0.02 – 0.13 °C/day.

Model fitting and selection

We fitted a global model including all terms that we hypothesised could have an effect on the response variable. The best model was selected by comparing AICc (Akaike's Information Criteria, adjusted for small samples) between all possible models nested within the global model using the dredge function in R package MuMIn (Barton 2010), and selecting the model with the lowest AICc score. All top models had Δ AICc > 2 points lower

than the next best models. For all analyses, we visually inspected residuals of global and final models to ensure model assumptions were met. Statistical significance of the effects of retained predictor variables was inferred if 95% confidence intervals (CIs) excluded zero.

Body temperature analyses

Due to the failure of a number of T_b data loggers in the 2014–15 season, experiments for three species (number of failures: NAD = 7, CTD = 6, CGS = 10) were repeated during the 2015–16 summer (Table 1). For these species, T_b data from different years were pooled.

Analyses focused on T_b patterns across the whole day, hereafter ‘diurnal’ as well as looking at during the hottest period of the day (12:00-19:20), hereafter referred to as ‘hot’. Diurnal (07:00-19:20) variation in T_b was analysed using a simple comparative metric, the Heterothermy Index (HI), which quantifies the extent of heterothermy for each individual bird by examining variation in T_b around the modal diurnal normothermic T_b (Boyles et al. 2011b), and is calculated as:

$$HI = \sqrt{\frac{\sum(T_{bmod} - T_{bi})^2}{n - 1}}$$

where T_{bmod} is the modal normothermic T_b of an individual, T_{bi} is the T_b at time i , and n is the number of times T_b is sampled (Boyles *et al.* 2011b).

In all analyses, we included individual as a random effect to account for repeated measures per individual. Differences in T_{bmod} and HI between species were modelled using linear mixed models (LMM) with fixed factors species and mass run with the package *lme4* (Bates *et al.* 2015). Body mass for each species was calculated as the mean mass of all study individuals. Tukey post-hoc tests using *multcomp* (Hothorn 2008) were used to identify significant differences between species and correlations between

T_{bmod} and HI were tested using Pearson's product-moment correlation. Following Smit (2013) T_{bmod} was determined by pooling all T_b recorded in the early morning (07:00–10:00), when birds were active but unlikely to need to dissipate heat.

For each species, we then modelled diurnal T_b and hot T_b as a function of T_{aviary} , controlling for time of day (diurnal T_b only) and body mass using LMM. Time of day (TOD) was divided into three periods, early: 07:00–10:00 when birds are active but unlikely to need to dissipate heat, midday: 10:00–14:00, and afternoon: 14:00–19:20 where T_{aviary} peaked. Tukey post-hoc tests were used to examine differences in diurnal T_b , T_{bmod} and HI with time of day (early: 07:00–10:00, midday: 10:00–14:00, afternoon: 14:00–19:20).

Behavioural analyses

Individual was included as a random factor in all behavioural analyses. For activity, panting and shade-seeking behaviour, T_{aviary} was the only significant predictor present in top models. We used model predictions for each species to determine the T_{aviary} at which panting behaviour was present in 50 % of observations ($pant_{50}$) following Smit *et al.* (2016). Additionally, following the same methodology, we also calculated the T_{aviary} at which shade-seeking behaviour was present in 50 % of observations ($shade_{50}$) and the T_{aviary} at which species were active in 50 % of observations ($activity_{50}$). $Activity_{50}$ models for CTD and REB did not converge. The removal of a single CTD individual for which no active observations were recorded resolved convergence issues for CTD. For REB, removal of any individual resolved convergence issues, with similar model outputs (all non-significant), regardless of the individual removed. The inability to identify which individual to remove ultimately meant REB model convergence issues remained unresolved. Further, Pearson's product-moment correlations were conducted to establish whether $pant_{50}$ correlated with T_{bset} and mass.

1.4 Results

1.4.1 Body temperature

Diurnal $T_{b\text{mod}}$ varied among the nine species from $41.4 \pm 0.5^\circ\text{C}$ in WBM to $43.1 \pm 0.4^\circ\text{C}$ in REB (Table 2, Figure 1). Variation in diurnal T_b , quantified as HI values, was similar

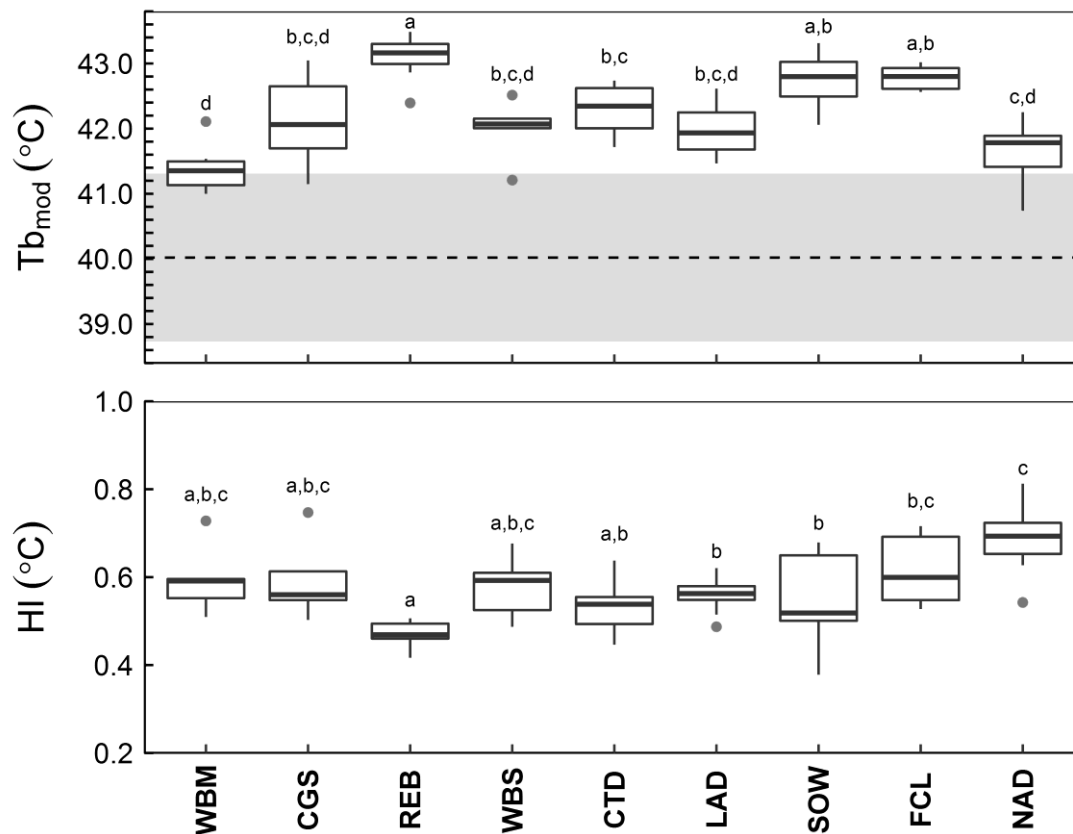


Figure 1: Interspecific differences in $T_{b\text{mod}}$ and heterothermy index (HI) for nine species. Boxes represent means of individual $T_{b\text{mod}}$ and HI. Species are arranged in order of heat dissipation thresholds (pant_{50}), from lowest (left) to highest (right; see Table 1). The dashed line in $T_{b\text{mod}}$ panel indicates the mean (\pm sd = grey ribbon) active phase T_b for 724 bird species taken from Prinzinger *et al.* (1991). Diurnal $T_{b\text{mod}}$ generally fell above mean active phase T_b for all birds. Differences between species are indicated by letters above boxes. If species share a letter, 95% CIs include zero, and values do not differ. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

among most species (Table 2). NAD had higher HI (i.e., T_b was more variable) in comparison to REB, LAD, CTD and SOW. FCL also showed greater variation in T_b than REB (Figure 1). Among species T_{bmod} and HI were not correlated, although REB had the highest T_{bmod} and the lowest HI, whereas NAD had low T_{bmod} and the highest HI (Figure 1). Diurnal T_b was predicted by T_{aviary} in most species, but effect sizes were very small (Table 3; Appendix Figure 8). In most species (CGS, WBS, CTD, SOW and NAD) T_b increased with T_{aviary} . In comparison, the T_b of LAD, FCL and WBM showed small but significant decreases with increases in T_{aviary} (Table 3). The magnitude of the effect of T_{aviary} on T_b was influenced by the interaction of T_{aviary} and mass in CGS and NAD, where smaller bird increased T_b more than larger birds as T_{aviary} increased (Table 3). During the hottest part of the day (12:00-19:20), T_{aviary} was the only significant predictor of T_b for CGS, WBS, SOW,

Table 2: Body temperature (T_b , °C) summaries for species across a range of aviary air temperatures (T_{aviary} , °C). Sample sizes differ for T_b data (N_{Tb}) and behavioural data (N_{behav}) however, the number of days used (N_{days}) is the same for both physiological and behavioural data collection. For the three species that were repeated, N values are presented as Season 1 (Season 2) values. Data for T_{aviary} and T_b variables for these species were pooled. $Pant_{50}$ was calculated using panting observations observed across the range of T_{aviary} . The most frequent T_b during the time of day when birds are active but not thermally stressed, i.e. using all T_b recorded during early morning (7:00-10:00) when T_{aviary} is lower, represents T_{bmod} . T_{bmean} was calculated using T_b recorded throughout diurnal period whilst T_{bmax} was the maximum T_b recorded for the species during the hottest period (12:00-19:20). Heterothermy index ($HI_{diurnal}$) expresses variation in T_b accounting for deviation from T_{bmod} and time spent away from T_{bmod} .

Species	pant ₅₀	N_{Tb} , N_{behav}	N_{days}	T_{aviary} range	Body temperature variables (°C)			
					T_{bmod}	T_{bmean}	T_{bmax}	$HI_{diurnal}$
WBM	36.3	6, 10	7	16.0-41.8	41.4 ± 0.5	41.1 ± 0.7	43.4	0.58 ± 0.16
CGS	36.6	1(4), 11(10)	4(9)	14.6-44.3	42.1 ± 0.7	42.0 ± 0.7	43.9	0.59 ± 0.21
REB	37.4	7, 10	12	17.6-40.5	43.1 ± 0.4	43.0 ± 0.5	45.4	0.47 ± 0.10
WBS	38.3	6, 10	8	17.3-44.3	42.0 ± 0.4	42.1 ± 0.6	44.6	0.59 ± 0.25
CTD	39.1	5(6), 10(6)	8(12)	8.8-41.2	42.4 ± 0.4	42.3 ± 0.7	44.7	0.54 ± 0.12
LAD	43.8	8, 11	12	10.5-40.8	42.0 ± 0.4	42.0 ± 0.6	44.2	0.56 ± 0.12
SOW	44.7	8, 9	13	16.1-40.5	42.8 ± 0.4	42.8 ± 0.6	45.5	0.56 ± 0.19
FCL	46.1	6, 6	12	17.6-40.5	42.8 ± 0.2	42.1 ± 0.5	44.7	0.61 ± 0.16
NAD	–	3(5), 3(5)	8(7)	12.8-41.8	41.6 ± 0.4	41.9 ± 0.8	45.4	0.67 ± 0.22

Table 3: Effect sizes, ([95% lower - upper CIs], t-values) of predictor variables retained in the top model for T_b for each species. Models were fitted to data for the entire diurnal period (07:00-19:20) and for the hottest period of the day (12:00-19:20). Global models tested the effect of aviary temperature (T_{aviary}), mass, time of day (TOD; only for diurnal models) and the interactions between T_{aviary} *mass and T_{aviary} *TOD on T_b . If factors were not present in the top model cells were left blank. Values in bold indicate that 95% CIs do not include zero.

Species	Model	N	T_{aviary} (°C)	Mass (g)	TOD	T_{aviary} *Mass	T_{aviary} *TOD
White-backed Mousebird	diurnal	6	-0.01 [-0.02--0.00] t = 2.29		+		+
	hot	6	0.72 [0.47-0.97] t = 5.63	0.79 [0.43-1.14] t = 4.08		-0.02 [-0.03--0.01] t = -5.72	
Cape Glossy Starling	diurnal	5	0.16 [0.13-0.19] t = 11.10	0.04 [-0.04-0.13] t = 0.99	+	-0.00 [-0.00--0.00] t = -7.8	
	hot	5	0.05 [0.05-0.06] t = 15.36				
African Red-eyed Bulbul	diurnal	7			+		
	hot	7	0.21 [0.14-0.27] t = 6.27	0.23 [0.13-0.34] t = 4.17		-0.01 [-0.01--0.00] t = -5.70	
White-browed Sparrow-Weaver	diurnal	6	0.02 [0.01-0.02] t = 5.39		+		+
	hot	6	0.01 [0.01-0.02] t = 5.13				
Cape Turtle-Dove	diurnal	11	0.02 [0.01-0.02] t = 19.4		+		
	hot	11	0.02 [0.02-0.02] t = 16.66				
Laughing Dove	diurnal	8	-0.01 [-0.02--0.01] t = -6.76		+		+
	hot	8	-0.01 [-0.0--0.00] t = -4.33				
Sociable Weaver	diurnal	8	0.03 [0.02-0.03] t = 9.59		+		+
	hot	8	0.03 [0.03-0.04] t = 13.79				
Fawn-coloured Lark	diurnal	6	-0.02 [-0.03--0.02] t = -7.4		+		+
	hot	6					
Namaqua Dove	diurnal	8	0.16 [0.12-0.20] t = 7.95	0.26 [0.15-0.36] t = 4.64	+	-0.00 [-0.01--0.00] t = -8.97	
	hot	8	0.55 [0.42-0.69] t = 8.42	0.62 [0.47-0.78] t = 7.49		-0.01 [-0.02--0.01] t = -8.45	

Table 4: Differences in T_b between three time of day (TOD) categories (early: 07:00-10:00, mid: 10:00-14:00, aft: 14:00-19:20). Mean $T_b \pm sd$ for the three TOD categories are included. Model outputs* are presented as effect sizes, [95% lower - upper CIs], t-statistic. Values in bold indicate 95% CIs that exclude zero.

Species	mean $T_b \pm sd$			Model output		
	early	mid	aft	early – aft	mid – aft	mid – early
White-backed Mousebird	41.3 ± 0.7	41.2 ± 0.6	41.1 ± 0.6	0.34 [0.27-0.41] t = 10.76	0.06 [0.02-0.10] t = 3.09	-0.28 [-0.33--0.23] t = -10.49
Cape Glossy Starling	42.0 ± 0.7	42.1 ± 0.7	42.2 ± 0.7	0.25 [0.2-0.31] t = 10.82	-0.05 [-0.1--0.01] t = -2.49	-0.31 [-0.36--0.25] t = -13.00
African Red-eyed Bulbul	43.0 ± 0.6	42.8 ± 0.4	43.0 ± 0.4	-0.02 [-0.04-0.01] t = -1.40	-0.20 [-0.27--0.21] t = -17.00	-0.22 [-0.24--0.14] t = -14.50
White-browed Sparrow-Weaver	42.2 ± 0.7	42.0 ± 0.6	42.3 ± 0.5	-0.19 [-0.24--0.13] t = -7.56	-0.33 [-0.38--0.28] t = -14.87	-0.14 [-0.2--0.1] t = -6.06
Cape Turtle-Dove	42.5 ± 0.6	42.1 ± 0.6	42.4 ± 0.7	0.15 [0.12-0.19] t = 11.28	-0.36 [-0.39--0.34] t = -31.23	-0.52 [-0.55--0.49] t = -37.94
Laughing Dove	42.1 ± 0.7	41.7 ± 0.6	42.1 ± 0.6	-0.13 [-0.17--0.9] t = -7.49	-0.42 [-0.46--0.4] t = -29.85	-0.3 [-0.34--0.26] t = -17.91
Sociable Weaver	42.8 ± 0.7	42.5 ± 0.6	42.9 ± 0.6	0.09 [0.04-0.15] t = 3.86	-0.31 [-0.35--0.28] t = -23.3	-0.41 [-0.46--0.36] t = -19.75
Fawn-coloured Lark	42.9 ± 0.6	42.2 ± 0.5	42.7 ± 0.5	-0.32 [-0.38--0.27] t = -13.97	-0.62 [-0.66--0.58] t = -39.41	-0.3 [-0.35--0.25] t = -13.4
Namaqua Dove	41.8 ± 0.8	41.6 ± 0.6	42.3 ± 0.7	-0.68 [-0.73--0.62] t = -28.78	-0.72 [-0.77--0.68] t = -39.1	-0.04 [-0.09-0.01] t = -2.02

* Derived from Tukey post-hoc test using $T_b \sim TOD + T_{aviary}$

CTD and LAD. No factors emerged as good predictors of T_b for FCL during the hottest portion of the day (Table 3). During the hottest part of the day all species increased T_b with increasing T_{aviary} , except LAD, in which T_b decreased with T_{aviary} ; as with the diurnal analyses, the magnitude of effect sizes was small with changes of no > 0.4°C per 10°C increase in T_{aviary} (Table 3). For WBM and NAD mass and the interaction between mass

and T_{aviary} were also important predictors of T_b with smaller birds increasing T_b more than larger birds as T_{aviary} increased. When this interaction term was removed, T_{aviary} and

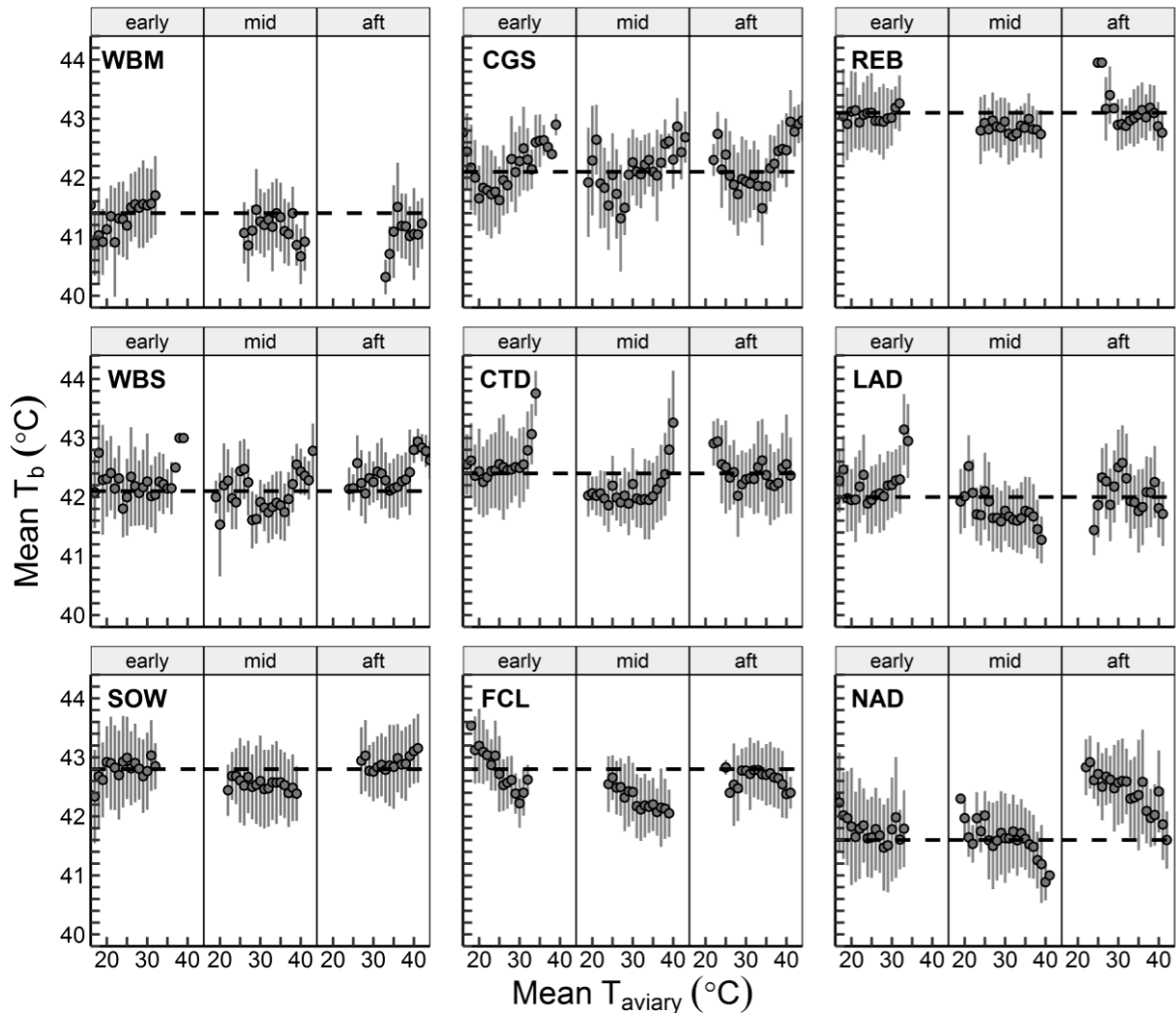


Figure 2: Changes in Mean T_b with increases in mean daily T_{aviary} during 3 times of day (TOD) categories (early: 07:00-10:00, mid: 10:00-14:00, aft: 14:00-19:20). Mean T_b was calculated from all values binned in 1°C intervals of T_{aviary} . Error bars indicate standard deviation. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

mass were no longer significant predictors of T_b . The highest T_b recorded during the hottest part of the day was in NAD (45.4°C) resulting in the greatest deviation of T_b from T_{bmod} ($T_{bmax} - T_{bmod} = 3.8^\circ\text{C}$; Table 2). In all other species $T_{bmax} - T_{bmod} < 2.8^\circ\text{C}$. For all species time of day was a significant predictor of T_b . However, diurnal patterns in T_b were species-specific and the magnitude of the difference in T_b between the three TOD

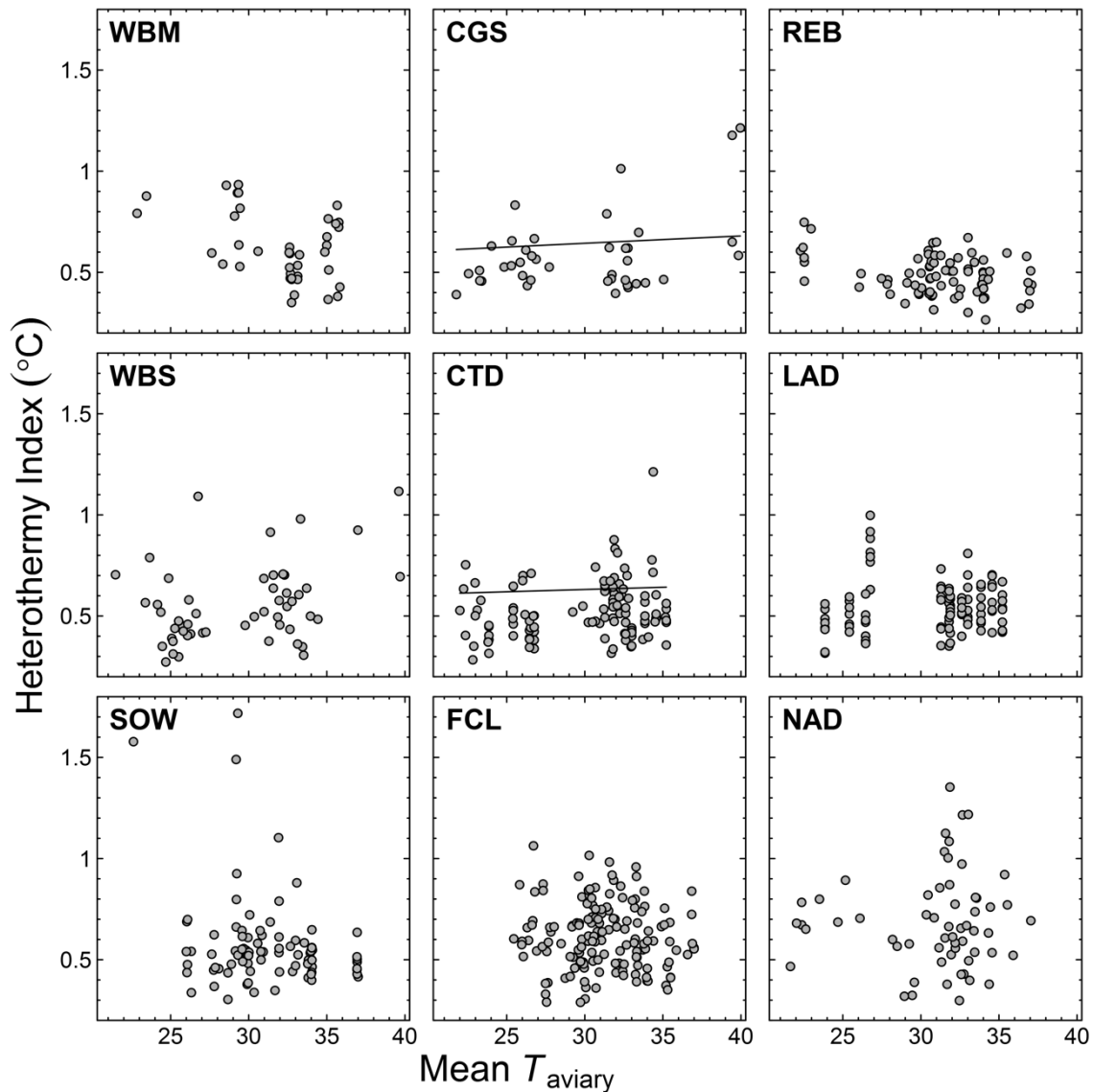


Figure 3: Variation in T_b reflected as heterothermy index (HI) with increases in mean daily T_{aviary} . CGS and CTD were the only species to increase HI with increases in mean T_{aviary} . If the outlier in CTD is removed, the relationship is no longer significant. WBM = White-backed Mousebird, CGS = Cape Glossy, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

categories was small (Table 4). Higher early morning T_{bs} were maintained by WBM compared to the afternoon. During midday T_b decreased below early morning T_b , with small but significant differences in T_b between midday and afternoon periods (Table 4, Figure 2). A decrease in T_b during midday compared to early mornings and the afternoons was observed in CGS, WBS, SOW, LAD, CTD and FCL (Table 4, Figure 2). During the

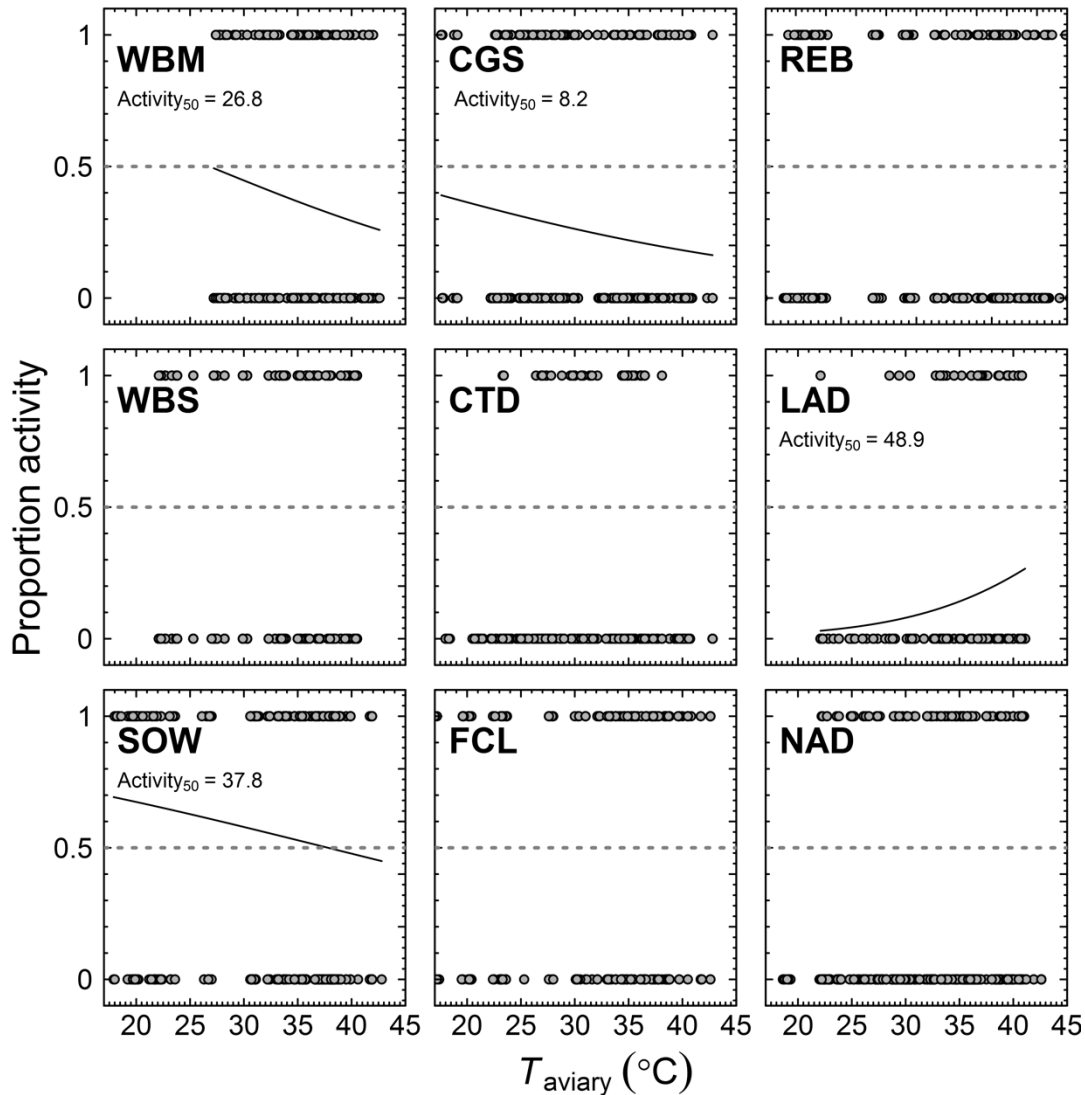


Figure 4: Proportion of time spent active as T_{aviary} increases, where 1 = active and 0 = inactive. Activity₅₀, the T_{aviary} at which 50% of observed individuals are active, is displayed for species where the logistic regression models are significant. Models for REB did not converge. In most species activity is independent of T_{aviary} . Several species (WBM, CGS, SOW) decrease time spent active as T_{aviary} increases while LAD increase activity as T_{aviary} increases. WBM = White-backed Mousebird, CGS = Cape Glossy, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

afternoon, T_b in WBS, LAD and FCL was significantly higher than in the morning, whilst CGS, CTD and SOW had highest T_b early in the morning (Table 4). The T_b of NAD did not differ between early mornings and midday, but T_b was significantly higher ($\sim 1^\circ\text{C}$ higher than $T_{b\text{mod}}$) during the afternoons (Table 4, Figure 2). There was an important interaction between T_{aviary} and TOD in WBM, WBS, SOW, LAD and FCL (Figure 2). WBS and SOW showed increases in T_b with increasing afternoon temperatures, whilst WBM, LAD and

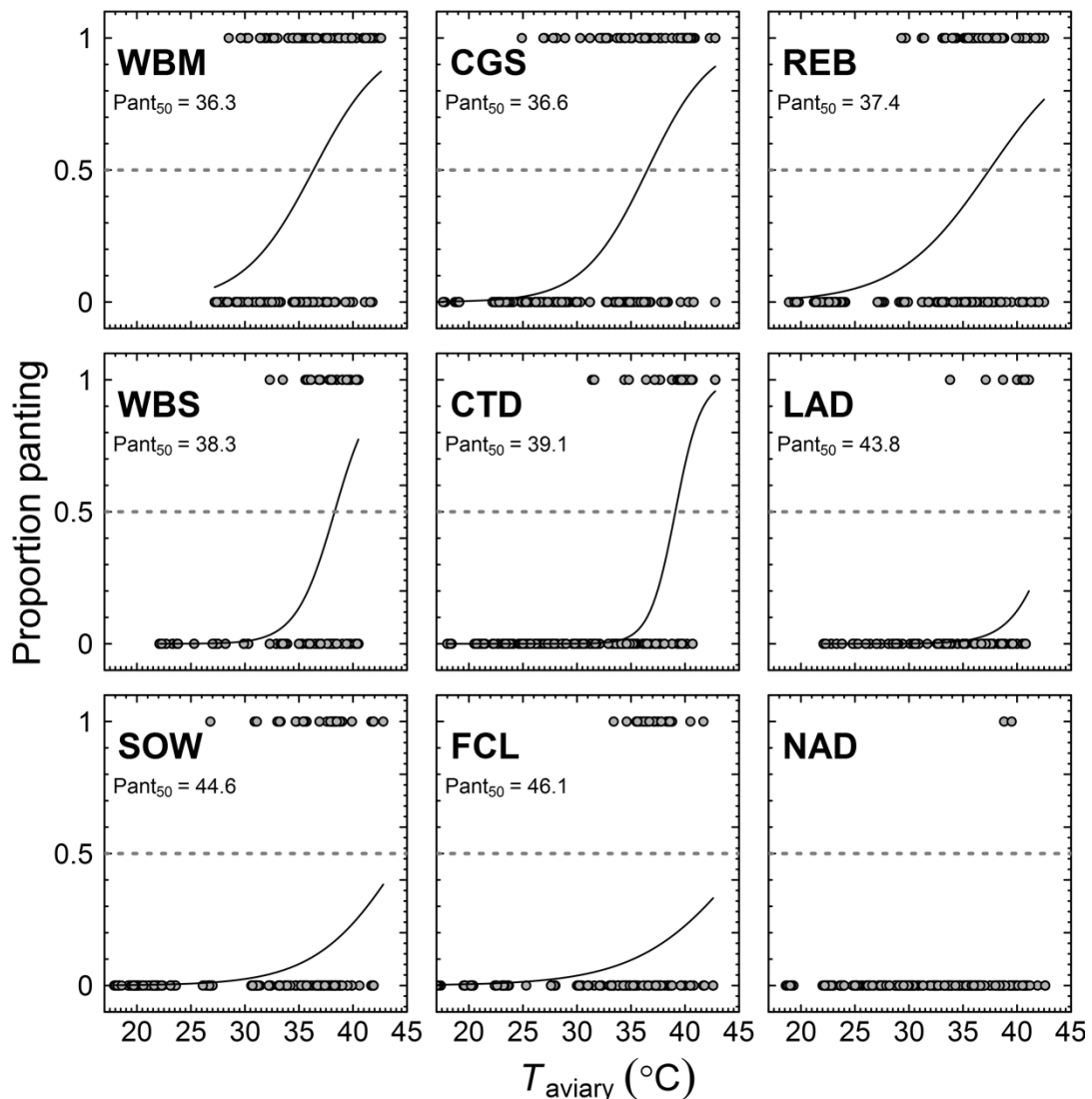


Figure 5: Proportion of time spent heat dissipating with increases in T_{aviary} . $Pant_{50}$ is calculated as the T_{aviary} at which 50% of observed individuals are displaying panting/gular fluttering. Gular fluttering observations in NAD were insufficient to calculate a significant $pant_{50}$. WBM = White-backed Mousebird, CGS = Cape Glossy, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

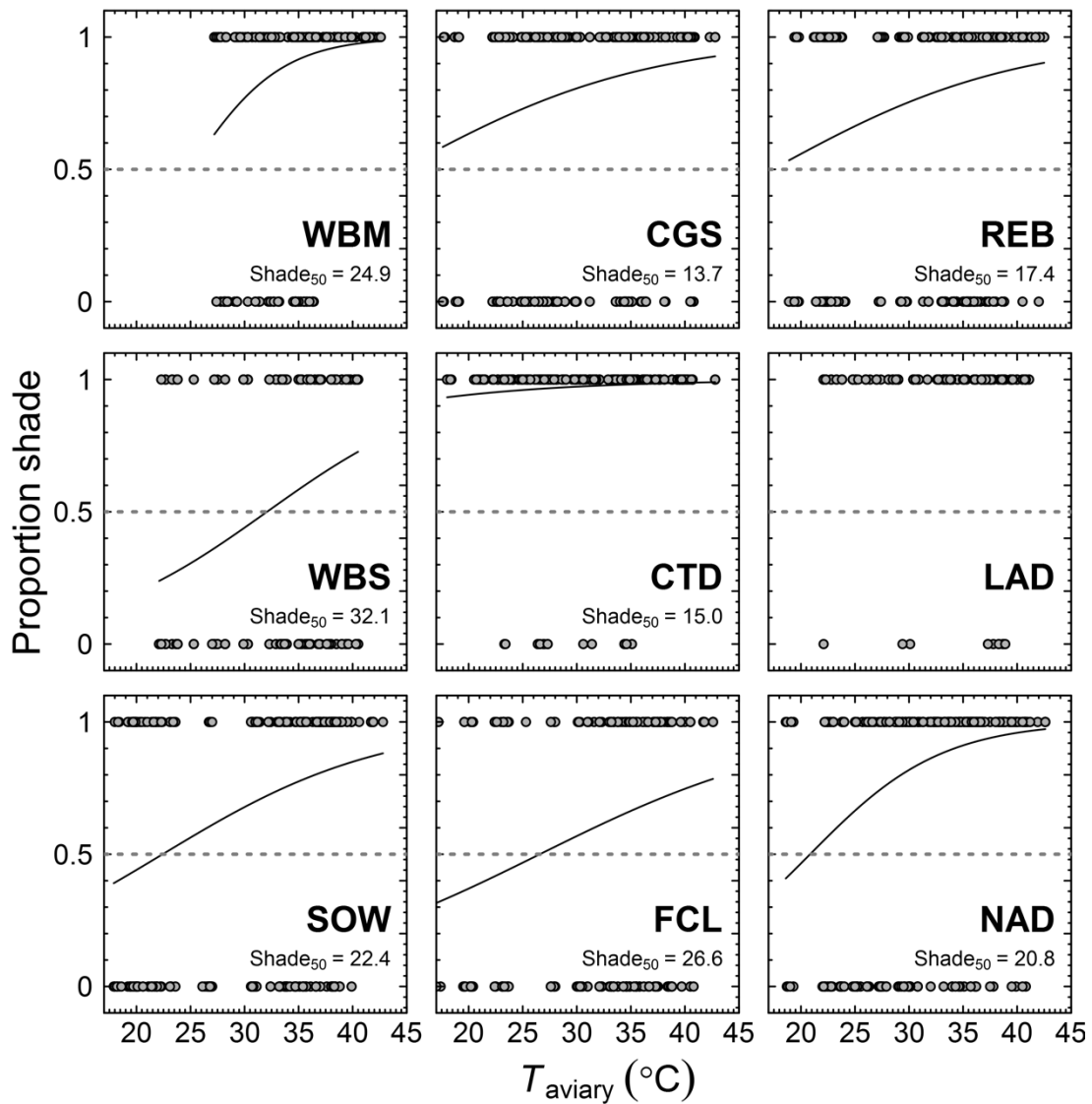


Figure 6: Proportion of time spent in the shade with increases in T_{aviary} , where observations of birds in 1 = dappled or full shade and 0 = sun. Shade use increase with T_{aviary} in all species except CTD and LAD, which spent most of the time in the shade. WBM = White-backed Mousebird, CGS = Cape Glossy, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

FCL decreased T_b with increasing afternoon temperatures (Figure 2). For most species, HI was independent of T_{aviary} (Figure 3). CGS and CTD showed a significant increase in HI with increasing T_{aviary} , however removal of the outlier for CTD resulted in the relationship no longer being significant in this species. In CGS, the magnitude of the effect was small

with only a $\sim 0.2^\circ\text{C}$ change in HI over a 10°C increase in mean T_{aviary} (LMM: 0.02°C , [0.01-0.03], $t = 3.2$; Figure 3).

1.4.2 Behaviour

Unexpectedly, activity was not correlated with T_{aviary} in most species (Figure 4). WBM, CGS and SOW significantly decreased time spent active as T_{aviary} increased, while LAD increased activity as T_{aviary} increased. In contrast, panting behaviour increased with increasing T_{aviary} for most species (Figure 5). Heat dissipation thresholds ranged from $\text{pant}_{50} = 36.3^\circ\text{C}$ for WBM up to $\text{pant}_{50} = 46.1^\circ\text{C}$ in FCL. NAD were only observed panting twice, at $T_{\text{aviary}} > 38.8^\circ\text{C}$. Thus, there were too few panting data to describe the relationship between panting and T_{aviary} for this species (Figure 5). Moreover, neither T_{bmod} nor mass correlated significantly with pant_{50} . Shade-seeking behaviour was strongly temperature-dependent in all species excepting CTD and LAD, which were mostly confined to shady microsites in aviaries regardless of T_{aviary} . However, the inflection points and slopes of the binomial regressions varied widely between species (Figure 6).

1.5 Discussion

Our results reveal that arid-zone birds held under semi-natural conditions show considerable interspecific variation in T_{b} patterns at high T_{air} , with small but significant increases in T_{b} with T_{air} in most species, but some showing the opposite pattern. In addition, although shade-seeking and panting behaviour increased with T_{air} in almost all species, the slopes were variable. Our data suggest that patterns of thermoregulation are strongly species-specific, with each species modulating behavioural and physiological aspects of thermoregulation differently.

Our study species generally had higher T_{bmod} than typical avian active T_{bmean} values (Prinzinger *et al.* 1991, Figure 1). Passerines in general appear to have slightly higher

T_{bmod} than columbids ($42.6 \pm 0.6^\circ\text{C}$ and $42.0 \pm 0.6^\circ\text{C}$, respectively), also consistent with the conclusions of Prinzinger *et al.* (1991). One potential functional basis for the higher T_{bmod} of passerines may concern their basal metabolic rates being 12% higher than those of non-passerine orders (Londoño *et al.* 2015). Of the nine species we investigated, free-ranging T_b patterns during hot weather have been studied previously in only WBS (Smit *et al.* 2013), work demonstrating that T_b patterns may vary both within and between populations under changing environmental conditions. In the present study, WBS showed higher summer T_{bmod} ($42.0 \pm 0.4^\circ\text{C}$) than previously recorded in free-ranging desert ($41.5 \pm 0.2^\circ\text{C}$) and semi-desert ($40.2 \pm 0.3^\circ\text{C}$) populations (Smit *et al.* 2013) despite our study population being only ~60km west from the desert population studied by the latter authors. The higher T_{bmod} value for WBS in the present study could reflect inter-population variation in T_{bset} ; but an alternative explanation is that in our study, captive conditions resulted in stress-induced hyperthermia [SIH; elevated T_b in response to stress (Bakken *et al.* 1999, Carere *et al.* 2001, Keeney *et al.* 2001)] compared to free-ranging populations. Particularly for species that naturally exist solitarily or in pairs, captivity may encourage more frequent SIH due to stressful forced interactions (Cunningham *et al.* 2017). This may explain the higher T_{bmod} in several of our species, not only WBS. Regardless, our T_{bmod} data are still more likely to reflect T_{bset} values of free-ranging birds as they are calculated from continuous T_b records from birds experiencing natural fluctuations in T_{air} , whereas most previous T_b data were collected during laboratory studies.

Across our observed sample of species, thermoregulatory patterns appear to be species-specific. In most species, there were small but significant increases in T_b with increasing T_{air} , but in three species T_b decreased. In most species, T_b responses to increasing T_{air} were also dependent on time of day, but the direction varied among species. This variability, together with the observation that in some species, T_b showed no

obvious response to increasing T_{air} (e.g. FCL) whereas in others T_{b} decreased with increasing T_{air} (e.g. LAD) during the hottest part of the day, leads to several non-mutually exclusive explanations for the large interspecific variation in observed responses.

First, most species did not make prolonged use of facultative hyperthermia (defined here as T_{b} exceeding T_{bmod} by 2°C or more) over the range of T_{air} experienced during this study (Appendix Figure 7). Increasing T_{b} with T_{air} is a common physiological response in birds, thought to reduce evaporative water loss at high T_{air} (Tieleman & Williams 1999). Some species, e.g., SOW, CGS and WBS, did show small increases in T_{b} above T_{bmod} , but only NAD made use of facultative hyperthermia in the afternoons. The high afternoon T_{b} of NAD illustrates the importance of time of day as a predictor of hyperthermia, as in most species there was little overall response in T_{b} to increasing T_{air} (Figure 7, Appendix). The more frequent hyperthermia in NAD is the pattern expected for a species closer to the generalist end of the thermal specialist/generalist continuum, where deviations in T_{b} from T_{bset} are thought to have fewer negative effects on performance. However, this is confounded by hyperthermia shown in NAD being restricted to lower afternoon T_{air} , suggesting an avoidance of high T_{b} during the hottest times when hyperthermia would, theoretically, be most beneficial.

Tolerance of T_{b} values well above T_{bmod} also does not appear to be restricted to species with low T_{bmod} . For example, of the two species with the lowest T_{bmod} values in this study, WBM avoided hyperthermia, but NAD showed facultative hyperthermia during the afternoons. Species in which T_{b} exceeded T_{bmod} also generally had higher HI, but again NAD was the only species to show significantly higher variation in T_{b} compared to other species. However, the distinct lack of response in HI to increasing T_{air} in most species suggests that the range of T_{air} s experienced in this study, or the free availability of water (see below), did not require the species we examined to allow a large variation of T_{b} ,

complicating the process of evaluating links between hyperthermia use and shifts in thermal performance breadth. Despite the small magnitude of effect sizes in T_b patterns, these effects are still likely to be biologically meaningful at very high T_{air} as T_b approaches lethal limits, affecting rates of biochemical reactions and enzymatic structure (Hochochka & Somero 2002, Angilletta *et al.* 2010). The overall variation in T_b patterns between species highlights how complex and poorly understood is the ecological significance of avian facultative hyperthermia.

A second potential explanation for the large interspecific variation in observed responses is that behavioural adjustments accompanying changes in T_b as T_{air} increased were also variable among species, and species differed in their combinations of hyperthermia tolerance and behavioural changes in activity, panting and shade-seeking associated with thermoregulation. At high T_{air} all species sought out shady microsites in the aviaries, however T_{air} values at which shade-seeking took place varied widely (shade₅₀ range: 13.7 – 32.1°C) and were clearly species-specific (Figure 7). Similarly, activity patterns between species differed (activity₅₀ range: 8.2 – 48.9°C). Three species (WBM, CGS and SOW) reduced activity with increasing T_{air} , but most species showed no change in activity levels. Generally, shade-seeking and reductions in activity buffer against physiological costs associated with evaporative cooling and hyperthermia by reducing exposure to higher heat loads (Dawson 1954, Wolf 2000, Angilletta *et al.* 2010; Boyles *et al.* 2011a). During hot weather birds either reduce foraging effort, limiting activity to the shade to avoid lethal hyperthermia, or continue foraging and thereby increase exposure to lethal hyperthermia and dehydration (Williams *et al.* 1999, Cunningham *et al.* 2015). We might therefore expect hyperthermia avoiding species to adjust behaviour to a greater extent at high T_{air} . While this may be true for some species, (i.e. WBM), it is clearly not the case for all. For example, LAD avoided hyperthermia, despite increasing activity with

increasing T_{air} and only initiating panting at $T_{\text{air}} > 37^{\circ}\text{C}$ (Figure 6). One possible explanation for the ability of LAD to maintain T_{b} around T_{bmod} at high T_{air} concerns the mechanism of evaporative cooling. Previous studies suggest that predominance of CEWL may provide a more efficient physiological basis to maintain T_{b} below T_{air} , compared to taxa relying on panting (e.g., passerines) to dissipate heat (Marder & Arieli 1988, McKechnie & Wolf 2004). For example, under laboratory conditions columbids show more gradual increases in T_{b} at high T_{air} (McKechnie *et al.* 2016) than passerines (Whitfield *et al.* 2015). In our study, the lower T_{bmod} during hot weather of the two smaller dove species may reflect the greater efficiency of CEWL compared to panting in the passerines, as more efficient evaporative cooling pathways may result in lower T_{b} s in general. The lack of reduction in activity with T_{air} in most species is, however, supported by previous observations in the Kalahari, where few species have been shown to reduce activity significantly at high T_{air} 39°C compared to milder T_{air} of 29°C , with most species reducing activity by only 10-20% at high T_{air} (Smit 2013).

Finally, freely available drinking water during the course of the study may have ameliorated physiological trade-offs between increasing evaporative cooling to maintain T_{b} below lethal limits and avoiding dehydration by conserving water (Dawson 1954), thereby creating a situation where T_{b} was regulated more precisely than it might have been in free-ranging conspecifics. The *ad libitum* water supply may partially explain the negative or absent T_{b} response to increasing T_{air} in some species and only small increases in T_{b} with T_{air} in the remaining species (Table 3). During the present study, water and food was easily accessible and the energy needed to acquire it presumably a fraction of that under natural conditions for free-ranging birds. Consequently, birds may have drunk more frequently, permitting more evaporative cooling to take place compared to conditions with higher dehydration risk. This notion is supported by the observation that even species

generally relying on preformed water as their main water source were regularly observed drinking water in the aviaries (WBM, WBS, SOW and FCL). Therefore, we argue that the provision of *ad libitum* water likely relaxed the dehydration-thermoregulation trade-off usually faced by free-living arid zone birds. Importantly, this easy access to water also explains the general lack of hyperthermia despite $T_{\text{air}} > 40^{\circ}\text{C}$. We were thus unable to identify any links between pant_{50} and the use of hyperthermia. Species with high pant_{50} did not necessarily show high T_{bmod} or an aversion for tolerating hyperthermia compared to species with low pant_{50} . Previous authors (Smit *et al.* 2016) suggested that larger birds, which have significantly lower pant_{50} , should make more extensive use of evaporative cooling to avoid hyperthermia. However, freely available water is likely to have influenced the relative use of evaporative cooling among species in this study such that the relationship between pant_{50} and body mass was absent. Overall, this suggests that, across the T_{air} range investigated here, pant_{50} may not be a good indicator of physiological heat responses.

In conclusion, the variation in T_{bset} among our study species and the difficulty in drawing any general patterns from behavioural and physiological responses to increasing T_{air} emphasises the need for species-specific studies to understand a species' T_{b} regulation. Many factors influence regulation of T_{b} in free-living birds, including factors we were not able to consider in this study. For example, recent research indicates that social status influences T_{b} patterns, with dominant birds regulating more stable T_{b} than subordinate individuals as T_{air} increases (Cunningham *et al.* 2017). Additionally, the freely available water and food resources birds had access to during this study greatly underestimate the challenges free-ranging desert birds face in terms of balancing water and energy. As such, despite our attempts to make captive conditions as natural as possible, thermoregulatory patterns are likely to vary to an even greater extent

intraspecifically between captive and wild populations. Further, interspecific thermoregulatory patterns will likely also vary more as unequal foraging-dehydration trade-offs persist among wild species due to variation in their dependency on free-standing water and/or preformed dietary water.

Despite the difficulties of extrapolating data from captive birds to free-ranging individuals, captive studies do offer certain benefits including the opportunity for close and continuous behavioural and physiological observations and the practicability to experimentally manipulate resource availability. Little is currently known about how avian thermoregulation is adjusted when water availability is reduced. Captive studies may be the only way to effectively investigate and answer questions such as these and therefore are key to evaluating the usefulness of HDB thresholds to assess species vulnerability to increasing T_{air} , and to further understand the complexities of thermoregulation in hot, arid environments.

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1.7 Appendix

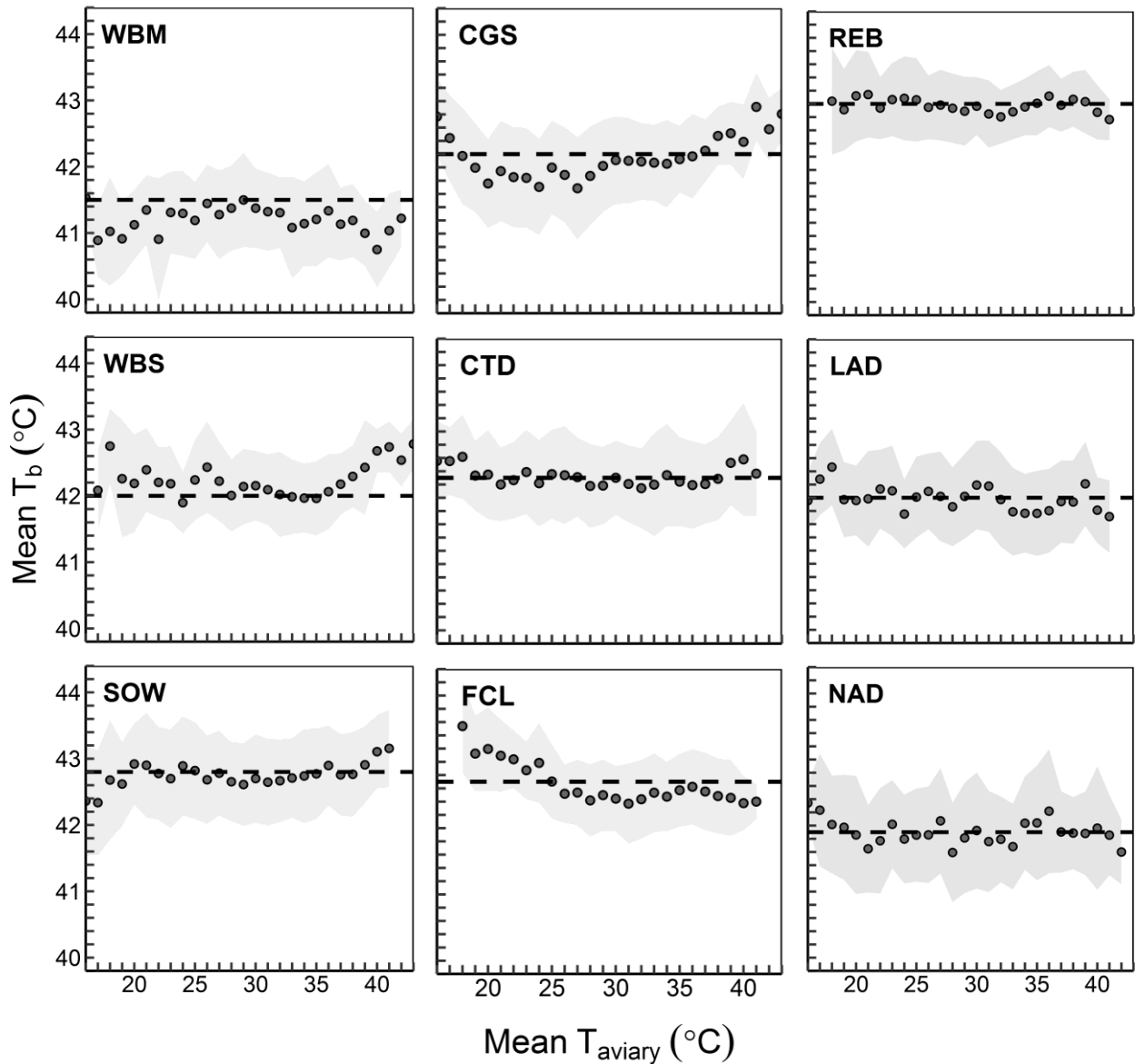


Figure 7: Mean increases in T_b per degree (°C) increase in T_{aviary} for each species observed. White-backed Mousebirds (WBM), Laughing Doves (LAD) and Fawn-coloured Larks (FCL) decreased T_b with increasing T_{aviary} whilst Cape Glossy Starling (CGS), White-browed Sparrow-Weaver (WBS), Cape Turtle-Dove (CTD), Sociable Weaver (SOW) and Namaqua Dove (NAD) increased T_b with increasing T_{aviary} . African Red-eyed Bulbul (REB) did not adjust T_b with increasing T_{aviary} . Grey ribbons represent standard deviation while dashed lines represent species T_{bmod} .

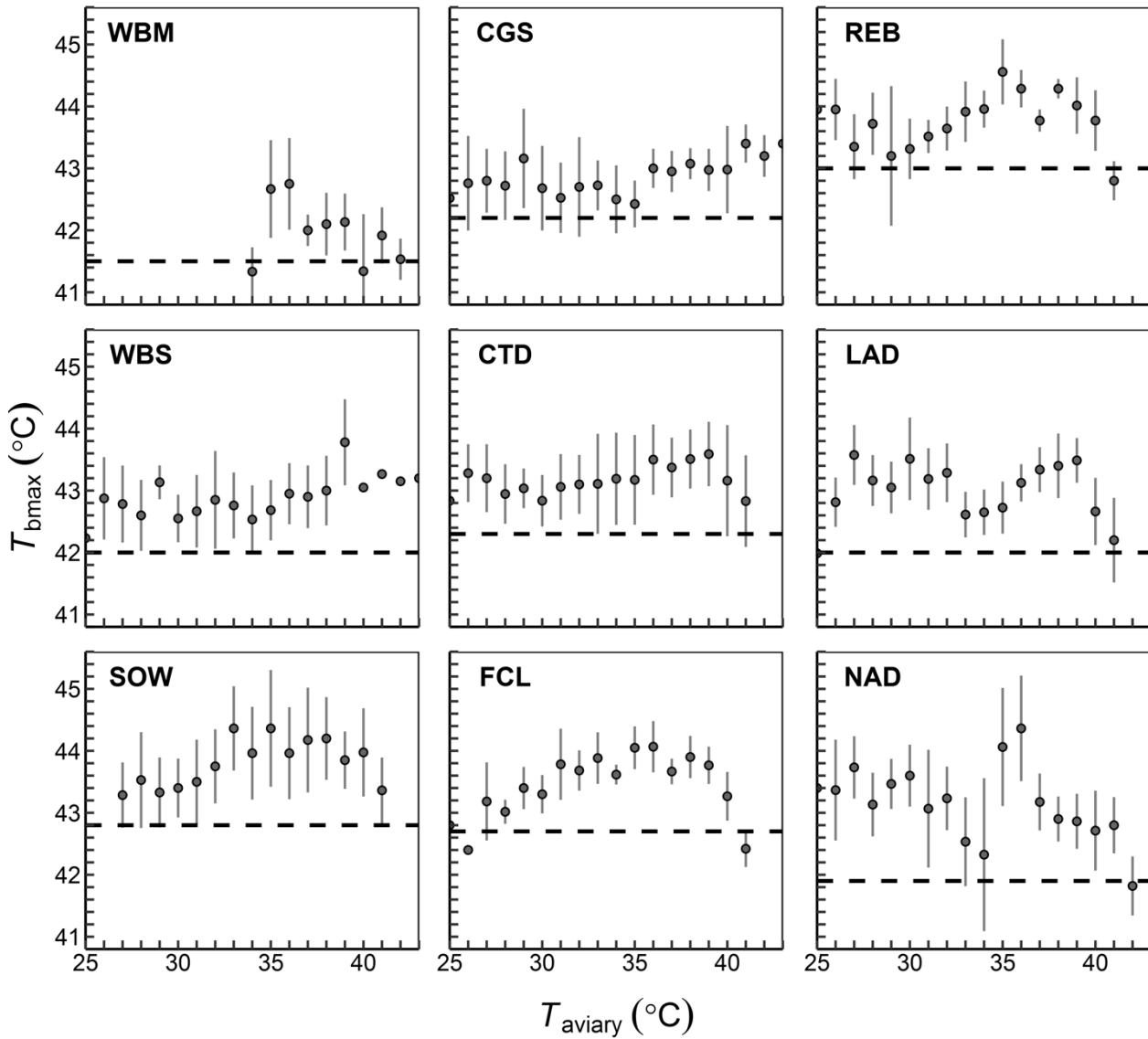


Figure 8: Mean increases in T_{bmax} per degree (°C) increase in T_{aviary} for each species observed. Error bars represent standard deviation while dashed lines represent species T_{bmod} . WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

CHAPTER 2 Interactions between avian thermoregulation, behaviour and hydration status under semi-natural conditions.

2.1 Abstract

In hot environments, heat fluxes between terrestrial organisms and their surroundings are tightly linked to water balance. When environmental air temperature (T_{air}) exceeds body temperature (T_{b}), evaporative water loss (EWL) becomes the only avenue for heat dissipation, and thermoregulation under these conditions is dependent on adequate hydration. When water deprived, birds reduce EWL by reducing panting rates, delaying the onset of panting and/or changing the relative contribution of cutaneous evaporative water loss to EWL. I examined physiological and behavioural consequences of short-term (8 hr) water deprivation among nine species, representing six families and three orders, occurring in the Kalahari Desert of southern Africa. Birds were housed under semi-natural conditions during mid-summer in large free-flight aviaries in the Kalahari Desert. Each individual received a surgically-implanted T_{b} logger, and I quantified shade-seeking, activity and panting behaviours to examine relationships between species-specific heat dissipation behaviours, T_{b} regulation and hydration status. Thermoregulatory responses to dehydration were highly variable among the study species. On water restricted days most species maintained T_{b} at or below their setpoint T_{b} when water was freely available. Significant, but small, reductions in afternoon mean T_{b} (T_{bmean}) by two passerines and small increases in afternoon T_{bmean} by two columbids in response to dehydration were observed. Further, six of nine study species delayed panting to higher T_{air} or seldom panted at all, compared to days when water was freely available. However, adjustments in other behavioural aspects of thermoregulation with water restriction were highly variable among and within species. Taken together, the data

suggest that combinations of behavioural and physiological responses to dehydration are species-specific but typically involve reductions in panting.

Keywords: arid-zone birds, evaporative water loss, panting, dehydration, water restriction, body temperature, behavioural thermoregulation

2.2 Introduction

Heat fluxes between terrestrial organisms and their surroundings are tightly linked to water balance, particularly in hot environments where evaporative heat dissipation is important for defending body temperature (T_b) at sublethal levels. In desert habitats, where water is usually a scarce and unpredictable resource, the functional coupling of thermoregulation to water balance is manifested as a fundamental trade-off between the avoidance of hyperthermia by evaporative cooling and the avoidance of dehydration by conserving body water (Dawson 1982, Tieleman & Williams 1999).

One conceptual framework for adaptive thermoregulation in endotherms proposes that thermal sensitivity of performance is coadapted with thermoregulation and endotherm thermal performance should be viewed as a continuum from thermal specialisation to generalisation (Angilletta *et al.* 2010, Boyles *et al.* 2011a). Therefore, trade-offs between thermal specialisation and generalisation with regards to the defence of a setpoint T_b (T_{bset}) under very hot conditions are expected. At high T_{air} , when water is freely available, individuals are likely to thermoregulate more precisely (maintaining T_b within a narrow range), with thermoregulation becoming less precise (increased fluctuation in T_b) and more frequent hyperthermia when dehydrated. The extent to which thermoregulatory precision decreases when water is unavailable is also likely to vary with the specialist-generalist continuum, with thermal generalists showing much greater fluctuations in T_b compared to thermal specialists.

Arid environments are particularly problematic for endotherms, and this is especially the case for birds. Their predominantly diurnal habits, small size and high mass-specific metabolic rates pose substantial physiological challenges when air temperatures (T_{air}) approach or exceed T_b (Dawson 1954, Dawson & Bartholomew 1968, Wolf 2000). When T_{air} exceeds a bird's T_b , evaporative water loss (EWL) becomes their only means of heat dissipation. The primary avenue of evaporative heat dissipation varies phylogenetically. For example, respiratory evaporative water loss (REWL), facilitated by energetically expensive panting, predominates in passerines, while gular flutter, which provides highly efficient cooling, predominates in caprimulgids (O'Connor *et al.* 2017, O'Connor *et al.* 2018). Cutaneous evaporative water loss (CEWL) makes up the majority of evaporative heat loss in columbids (Wolf & Walsberg 1996, Tieleman & Williams 2002). Small passerines (< 100 g) also rely heavily on hyperthermia to encourage passive heat loss by maintaining $T_b > T_{\text{air}}$, likely to compensate for the inefficiency of panting (versus gular flutter or cutaneous evaporation) as a mechanism of evaporative cooling (Gerson *et al.* 2019). Comparatively, columbids, which effectively dissipate most heat cutaneously, allow T_b to increase only at high T_{air} , which reduces the thermal gradient for heat gain (Gerson *et al.* 2019). These thermoregulatory processes are dependent on adequate hydration, and birds inhabiting hot, arid habitats are thus an ideal model taxon for testing predictions concerning interactions between hydration status and thermoregulation.

In laboratory conditions, birds respond to water deprivation and dehydration physiologically by reducing EWL via a combination of lowered panting rates (Crawford & Schmidt-Nielsen 1967), delays in the onset of panting (Kleinhaus *et al.* 1985, Maloney & Dawson 1998) and/or changes in the relative contribution of CEWL to EWL (Lee & Schmidt-Nielsen 1971, Arad *et al.* 1987, Maloney & Dawson 1998). These reductions in EWL associated with water deprivation are thought to amount to heat storage via facultative

hyperthermia, in order to reduce overall water requirements when dehydrated (e.g., Tieleman & Williams 1999, Gerson *et al.* 2019) and result in smaller thermal safety margins (i.e., permit T_b to more closely approach lethal limits) compared to well-hydrated individuals. It follows that, when dehydrated, birds which frequently make use of hyperthermia will be more efficient at managing water requirements compared to species which avoid hyperthermia. Alternately, species which make regular use of hyperthermia even when fully hydrated may be less able to cope with the pressure of dehydration given the maintenance of smaller thermal safety margins.

Interactions between avian thermoregulation and hydration status under hot conditions are also manifested as behavioural changes that minimize heat gain or promote heat loss (e.g., reduced activity, shade seeking, panting, and wing drooping; Wolf 2000). Temperature-dependence of panting can be quantified using a behavioural index, pant_{50} (the T_{air} associated with 50 % of a population panting), which can vary by > 10 °C among species (Smit *et al.* 2016, Pattinson *et al.* 2020, however see Chapter 1). Similarly, other heat dissipation behaviours such as shade-seeking (shade_{50}) and adjustments in activity (active_{50}) can also be quantified (the T_{air} associated with 50 % of a population seeking shade and remaining active respectively; Smit *et al.* 2016). I expect birds to alter these behaviours when dehydrated, e.g., by delaying panting to higher T_{air} , reducing activity and increasing shade-seeking behaviour in an effort to reduce water loss and minimize heat gain. I also expect some of these adjustments to be more apparent in taxa like passerines that lack gular flutter or the capacity for rapid cutaneous evaporation and rely on panting for the majority of heat dissipation at high T_{air} , and species with low pant_{50} to show greater delays panting when dehydrated than species with higher pant_{50} .

In this chapter, I examined physiological and behavioural consequences of short-term (8 h) water deprivation among nine bird species occurring in the Kalahari Desert of

southern Africa. I hypothesised that thermoregulatory responses to dehydration would vary phylogenetically in a manner correlated with the primary avenue of evaporative cooling, as well as with the temperature dependence of heat dissipation behaviour (e.g., pant_{50}). I predicted that at high T_{air} , when water is unavailable, birds will reduce time spent panting, and increase behavioural responses like shade-seeking to lower EWL and reduce dehydration risk. Further, I predicted reduced thermoregulatory precision and more frequent hyperthermia, which would be evident as upwards shifts in daily mean T_b and pant_{50} . It is uncertain whether these adjustments will be more pronounced in species that generally avoid hyperthermia when adequately hydrated. It should also be considered that there may be differences in these responses as dehydration progresses throughout the day between species which preferentially drink in the mornings compared to the evenings. Finally, I predicted that species with low pant_{50} would experience stronger trade-offs between risks of hyperthermia and dehydration (either by maintaining $T_b > T_{\text{air}}$ for longer periods of time or running the risk of dehydrating earlier in the day) in response to water restriction than species with higher pant_{50} , as these species begin panting at cooler air temperatures.

2.3 Methods & Materials

2.3.1 *Body temperature & general maintenance of birds in captivity*

I examined whether water availability affected thermoregulation in nine bird species resident in the southern Kalahari Desert (Table 1). Body temperature data for the nine species were previously published and bird capture, housing and maintenance protocols follow Chapter 1 [published as Thompson *et al.* (2018)].

Briefly, birds were housed *in situ* in the Kalahari in large outdoor aviaries for approximately two months. Body temperature data were collected using surgically implanted loggers (Thermochron iButton, model 1922L, Maxim Dallas Semiconductors,

Sunnyvale, California, USA) or transmitters (model BD-2TH, Holohil Systems Ltd., Carp, Ontario, Canada), and behavioural data were collected via observational scan samples (Altmann 1974) taken throughout the day across a wide range of T_{air} (13.7 - 43.7°C). An onsite weather station (Vantage Pro2, Davis Instruments, Haywood, California, USA) recorded weather variables (T_{air} , dew point, rainfall etc) at five-minute intervals and air temperature inside the aviaries (T_{aviary}) was also measured using temperature data loggers (TidbiT v2 temperature data logger UTBI-001, Onset Computer Corporation, Bourne, Massachusetts, USA). TidbiTs were suspended inside a hollow plastic PVC tube (20 cm long x 5 cm diameter) with holes drilled into the sides for ventilation and recorded T_{aviary} at five-minute intervals.

As in Thompson *et al.* (2018), the number of individuals per species for which behavioural data were obtained is generally larger than the sample size for T_{b} data. This is due to the exclusion of T_{b} data for individuals for which telemetrically-obtained traces of T_{b} were characterized by a high proportion of missing values.

2.3.2 Experimental manipulation of water availability

I exposed birds to a water restriction treatment to examine the effect on thermoregulatory patterns, particularly on hot days. The experimental protocol for water restricted (WR) days involved removing water from the aviaries at 09:00 and returning water at 17:00, thereby depriving birds of access to water for an 8-h period across a wide range of T_{air} . After a WR day, birds were allowed to recover for a minimum of three days thereafter with water available *ad libitum* (AL days) before the next WR day. However, I excluded from the analyses all AL days occurring within two days following WR days, to ensure birds had adequately recovered from water deprivation. The final numbers of WR days varied between three and seven among species, as result of unforeseen equipment difficulties and

cool periods during the study. The first WR day took place a minimum of six days after the implantation of T_b transmitters/loggers.

2.3.3 Data analysis

All statistical analyses were conducted in the R environment (R Core Team 2017). I fitted a global model including all terms that I hypothesised could influence response variables. The best model was selected by comparing AICc (Akaike's Information Criteria, adjusted for small samples) between all possible models nested within the global model using the *dredge* function in R package *MuMIn* (Barton 2010), and selecting the model with the lowest AICc score. All candidate models with $\Delta AICc < 2$ are presented. For all analyses, I visually inspected residuals of global and final models to ensure model assumptions were met. Statistical significance of the effects of retained predictor variables was inferred if 95% confidence intervals (CIs) excluded zero.

Body temperature analyses

Due to the failure of a number of T_b data loggers in 2014 (number of failures: NAD = 7, CTD = 6, CGS = 10), experiments for these three species were repeated during the 2015–2016 summers (Table 1). For these species, T_b data from different years were pooled. I restricted analyses to the active diurnal period (07:00-19:20) with key heat-related variables analyses restricted to the hottest time of the day. I compared differences in T_b parameters with increasing T_{air} measured within the aviaries (T_{aviary} ; Thompson *et al.* 2018) on water restricted (WR) *versus* ad libitum (AL) water days for each species using linear mixed models (LMM) implemented in the package *lme4* (Bates *et al.* 2015). Individual identity was included as a random factor in all analyses to account for repeated measures per individual. Differences in absolute T_b measurements (corrected for transmitter drift, see Thompson *et*

al. 2018) were modelled as a function of water restriction and T_{aviary} controlling for body mass.

For each species, I also analysed T_b mean and maxima (T_{bmean} and T_{bmax}) and heterothermy index (Boyles *et al.* 2011*b*) as calculated in Thompson *et al.* 2018 ($\text{HI}_{\text{diurnal}}$).

Table 1: Kalahari Desert bird species used to compare variation in body temperature parameters. Each species has a unique abbreviation used for referral in figures and text. Species belong to three orders, Passeriformes, Columbiformes and Coliiformes. Summer seasons during which species were sampled are indicated by Season (2014 refers to Austral summer: i.e., Nov 2013-March 2014 *etc*). Species are categorised according to dominant dietary guild; i.e. frugivores, insectivores, granivores or omnivores using diet information from Hockey *et al.* 2005. Number of individuals for physiological (N_{T_b}) and behavioural data (N_{behav}) included.

Abbreviations	Order	Species	Season	Diet	N_{T_b} (N_{behav})	Body mass (g)
WBM	Coliiformes	White-backed Mousebird (<i>Colius colius</i>)	2016	Frugivore	5(10)	34.5 ± 1.7
CGS	Passeriformes	Cape Glossy Starling (<i>Lamprotornis nitens</i>)	2015(2016)	Omnivore	8(16)	74.5 ± 7.1
REB	Passeriformes	African Red-eyed Bulbul (<i>Pycnonotus nigricans</i>)	2014	Frugivore	7(10)	29.3 ± 2.2
WBS	Passeriformes	White-browed Sparrow Weaver (<i>Plocepasser mahali</i>)	2015	Omnivore	6(10)	35.8 ± 2.6
CTD	Columbiformes	Cape Turtle-Dove (<i>Streptopelia capicola</i>)	2015(2016)	Granivore	11(16)	129.5 ± 11.3
LAD	Columbiformes	Laughing Dove (<i>Streptopelia senegalensis</i>)	2015	Granivore	8(11)	89.6 ± 9.9
SOW	Passeriformes	Sociable Weaver (<i>Philetairus socius</i>)	2014	Omnivore	8(9)	24.0 ± 1.0
FCL	Passeriformes	Fawn-coloured Lark (<i>Calendulauda africanoides</i>)	2014	Omnivore	6(8)	23.9 ± 0.7
NAD	Columbiformes	Namaqua Dove (<i>Oena capensis</i>)	2015(2016)	Granivore	8(19)	36.9 ± 2.3

These variables were analysed as a function of water restriction (AL or WR) and mean T_{aviary} controlling for individual body mass. To visualise the results for T_{bmean} during the hottest time of day (T_b values recorded after 12:00, Afternoon T_{bmean}) and $\text{HI}_{\text{diurnal}}$ I plotted model predictions at 1°C increases in T_{aviary} . Models included water restriction and T_{aviary} as fixed effects and individual identity as a random factor. I used Pearson's product-

moment correlation to identify whether there was a relationship between H_{diurnal} and afternoon T_{bmean} . In addition, I also conducted paired t-tests on individual modal T_{b} (T_{bmod}), T_{bmean} , afternoon T_{bmean} , T_{bmax} and H_{diurnal} on AL and WR days for each species to examine the effect of water restriction on overall T_{b} parameters. I determined T_{bmod} by pooling all T_{bs} recorded in the early morning (07:00–10:00) when birds were active but unlikely to need to dissipate heat, following Smit (2013). Therefore, T_{bmod} is also reflective of the T_{bset} . Model outputs in text are presented as the Model: estimate, [lower and upper 95% confidence intervals] e.g. (LMM: estimate, [L95%CI – U95%CI]).

Behavioural analyses

To examine how activity, panting and shade seeking behaviour were affected by water restriction, I fitted generalised linear mixed models with a binomial error structure (logit link-function). Overdispersion was evaluated by comparing the residual deviance to the residual degrees of freedom and corrections were applied where values exceeded 2.5. I used model predictions for each species to determine the T_{aviary} at which panting behaviour was present in 50 % of observations (pant_{50}) following Smit *et al.* (2016) on both WR and AL days. Similarly, I calculated the T_{aviary} at which shade-seeking behaviour was present in 50 % of observations (shade_{50}) and the T_{aviary} at which species were active in 50 % of observations (activity_{50}) and examined differences with water restriction. For simplicity, in the results the terms ‘panting’ and ‘ pant_{50} ’ are also applied to species which make use of gular fluttering (i.e. columbids and Coliiformes).

Table 2: Diurnal body temperature (T_b , °C) summaries, recorded between 07:00-19:20, for species (Spp.) on days when water was restricted (WR) versus available *ad libitum* (AL). Ranges of aviary air temperatures (T_{aviary} , °C) from the absolute lowest to the absolute highest T_{aviary} for each water restriction category are specified. Number of individuals for which T_b data was collected per species (N_{Tb}) and number of days per water restriction category (N_{day}) are included. T_{bmod} was calculated using most frequent T_b during the time of day when birds are active but not thermally stressed i.e. using all T_b recorded during early morning (7:00-10:00) when T_{aviary} is lower. All T_b and HI variables presented were determined by calculating the respective value per individual and taking the means of all individuals. T_{bmean} was calculated using T_b over the entire diurnal period whilst Afternoon T_{bmean} used values during the hottest time of day (12:00-19:20). Heterothermy index ($HI_{diurnal}$) expresses variation in T_b accounting for deviation from T_{bmod} and time spent away from T_{bmod} (Boyles *et al*, 2011*b*). For the three species that were repeated, values of N and N_{days} are presented as Season 1(Season 2) values. Data for T_{aviary} and T_b variables for these species were pooled. The increase in T_{bmean} on WR days for CTD was very small (mean difference of 0.07°C). WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

Spp, (N_{Tb})	pant ₅₀	Water restriction	N_{day}	T_{aviary} range	Body temperature variables (°C)				
					T_{bmod}	T_{bmean}	Afternoon T_{bmean}	T_{bmax}	$HI_{diurnal}$
WBM, (6)	36.3	AL	7	16.0-41.8	41.4	41.2 ± 0.4	41.1 ± 0.3	43.3 ± 0.3	0.61 ± 0.05
		WR	4	17.6-44.3	41.6	41.2 ± 0.4	41.1 ± 0.4	43.1 ± 0.6	0.62 ± 0.08
CGS, 1(4)	36.5	AL	4(9)	14.6-44.3	42.1	42.2 ± 0.5	42.2 ± 0.4	44.4 ± 0.4	0.58 ± 0.09
		WR	4(3)	9.8-40.4	42.0	42.1 ± 0.5	42.0 ± 0.5	44.1 ± 0.6	0.47 ± 0.02
REB, (7)	37.4	AL	11	17.6-40.5	43.1	43.0 ± 0.2	43.0 ± 0.2	45.0 ± 0.3	0.48 ± 0.06
		WR	4	16.1-40.7	43.0	43.0 ± 0.2	43.0 ± 0.1	44.9 ± 0.4	0.45 ± 0.04
WBS, (6)	38.3	AL	9	17.3-44.3	42.0	42.1 ± 0.4	42.2 ± 0.4	44.2 ± 0.5	0.55 ± 0.06
		WR	3	9.8-41.0	42.1	42.0 ± 0.5	41.9 ± 0.5	43.9 ± 0.7	0.50 ± 0.05
CTD, 5(6)	39.1	AL	8(12)	10.5-41.2	42.3	42.3 ± 0.4	42.2 ± 0.4	44.6 ± 0.7	0.54 ± 0.09
		WR	5(3)	9.8-41.1	42.5	42.3 ± 0.4	42.3 ± 0.4	44.3 ± 0.8	0.54 ± 0.10
LAD, (8)	43.8	AL	12	10.5-40.8	42.2	42.0 ± 0.4	41.9 ± 0.3	44.0 ± 0.3	0.55 ± 0.05
		WR	3	9.8-41.1	42.1	42.0 ± 0.4	41.9 ± 0.4	43.9 ± 0.5	0.56 ± 0.10
SOW, (8)	44.6	AL	12	16.1-40.5	42.8	42.7 ± 0.4	42.7 ± 0.4	44.8 ± 0.6	0.57 ± 0.10
		WR	3	19.1-40.7	42.8	42.8 ± 0.3	42.7 ± 0.3	44.4 ± 0.6	0.52 ± 0.10
FCL, (6)	46.0	AL	12	17.6-40.5	42.8	42.6 ± 0.1	42.5 ± 0.1	45.3 ± 0.5	0.61 ± 0.07
		WR	4	16.1-40.7	42.9	42.6 ± 0.2	42.5 ± 0.2	44.6 ± 0.4	0.59 ± 0.11
NAD, 3(5)	-	AL	8(7)	12.8-41.8	41.8	41.9 ± 0.4	42.0 ± 0.4	44.7 ± 0.7	0.68 ± 0.07
		WR	5(4)	12.2-44.3	42.0	42.1 ± 0.4	42.2 ± 0.4	44.5 ± 0.5	0.83 ± 0.14

* Indicates significant differences, $p < 0.05$, in individual AL and WR values from paired t- tests (see Appendix Table 4) * $p = 0.048$

2.4 Results

Of the nine study species, only CTD showed a significant change in individual T_{bmod} with water restriction, with a slight upward shift on WR days (Table 2). The T_{bmean} values of individuals changed with water restriction in several species, although the directions were variable (Table 2).

Reductions in daily T_{bmean} were observed in CGS, and increases in CTD and NAD, on WR days in comparison to values on AL days (Table 2). However, the increase in T_{bmean}

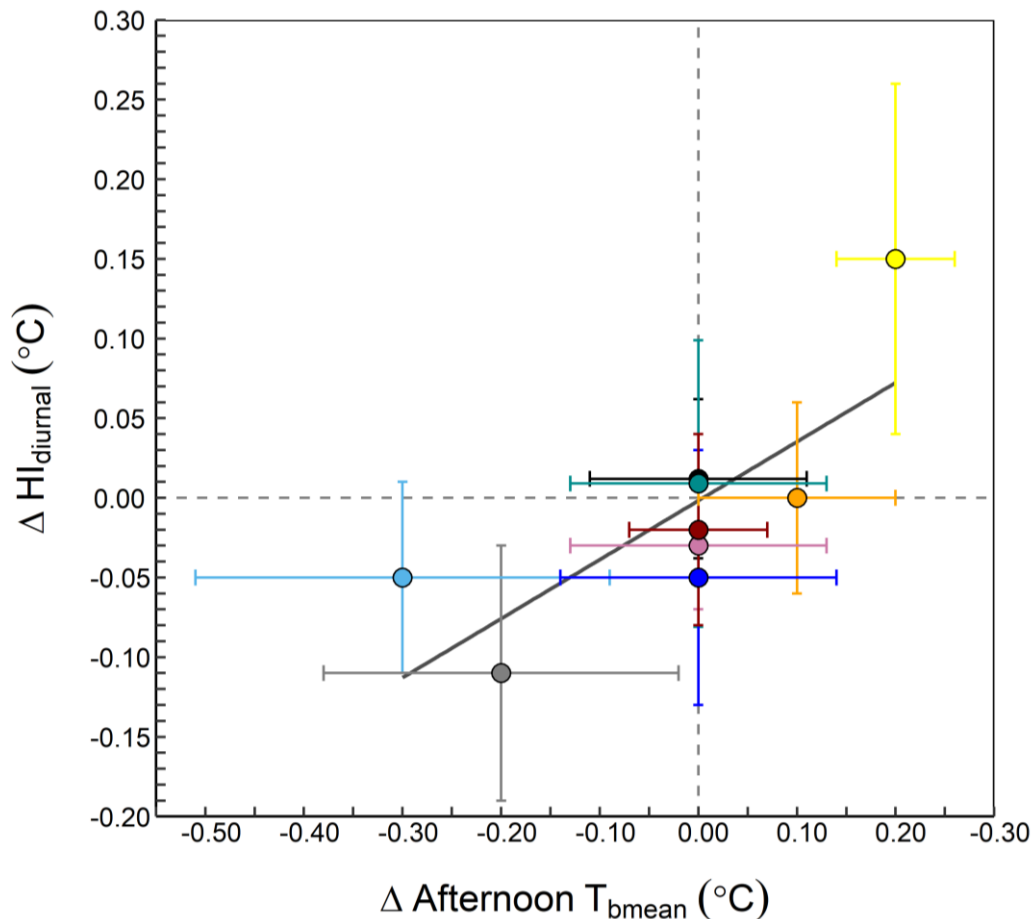


Figure 1: Mean change in individual $HI_{diurnal}$ with changes in individual afternoon T_{bmean} resulting from WR. Negative $\Delta HI_{diurnal}$ and ΔT_{bmean} indicate values were lower on WR days compared to AL days. There was a strong correlation between individual $HI_{diurnal}$ and individual afternoon T_{bmean} where species which increased afternoon T_{bmean} on WR days also showed a greater variation in afternoon T_b . Adjustments of afternoon T_{bmean} were not consistent, with significantly higher afternoon T_{bmean} on AL days in WBS and CGS, and significantly higher afternoon T_{bmean} on WR in CTD and NAD. White-backed Mousebird (black), CGS = Cape Glossy Starling (grey), African Red-eyed Bulbul (pink), WBS = White-browed Sparrow-Weaver (sky blue), CTD = Cape Turtle-Dove (orange), Laughing Dove (cyan), Sociable Weaver (blue), Fawn-coloured Lark (red-brown), Namaqua Dove (yellow).

on WR days for CTD was very small (mean difference of 0.07°C, indistinguishable in Table 2 due to rounding to 1 dp). Variation in T_b , quantified as $H_{I\text{diurnal}}$, was similar on WR and AL days in most species, except NAD (slightly but significantly higher on WR than on AL days; Table 2). Although individual $H_{I\text{diurnal}}$ in CGS was notably lower on WR days, this difference was not statistically significant (Table 2). All species reduced $T_{b\text{max}}$ on WR days compared

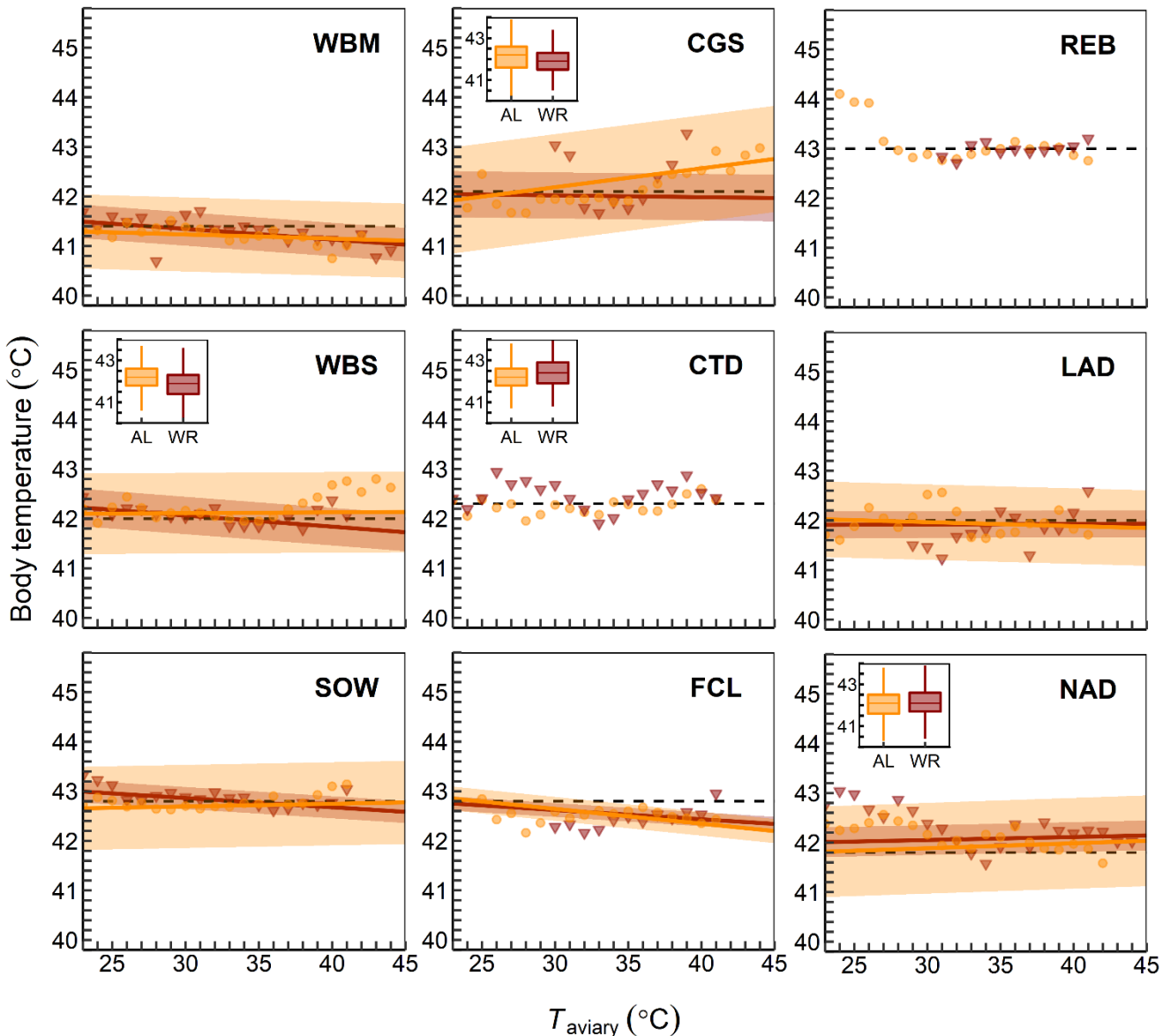


Figure 2: Response of T_b to increasing T_{aviary} on *ad lib* (AL) water days (orange lines \pm 95% CI) and water restricted (WR) days (red-brown lines \pm 95% CI). Model predictions are plotted for all species where T_{aviary} significantly predicted T_b . Plotted points are mean T_b values of individuals at 1°C increments on AL days (orange circles) and WR days (red-brown triangles). Models were fitted to the full range of T_{aviary} data, however plots show only T_{aviary} 23-45°C to focus on trends at higher temperatures. Inset boxplots of T_b indicate species where afternoon $T_{b\text{mean}}$ was significantly different on AL and WR days. Horizontal dashed lines represent the species' AL $T_{b\text{mod}}$. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove

to AL days and these reductions were significant in CGS, CTD, SOW and FCL (Table 2). There was also a strong correlation between changes in T_b during the hottest part of the day (afternoon T_{bmean}) and $HI_{diurnal}$, whereby species which increased afternoon T_{bmean} on WR days also showed greater variation in afternoon T_b compared to AL days. Although effect sizes were small, significantly lower afternoon T_{bmean} on WR days was observed in

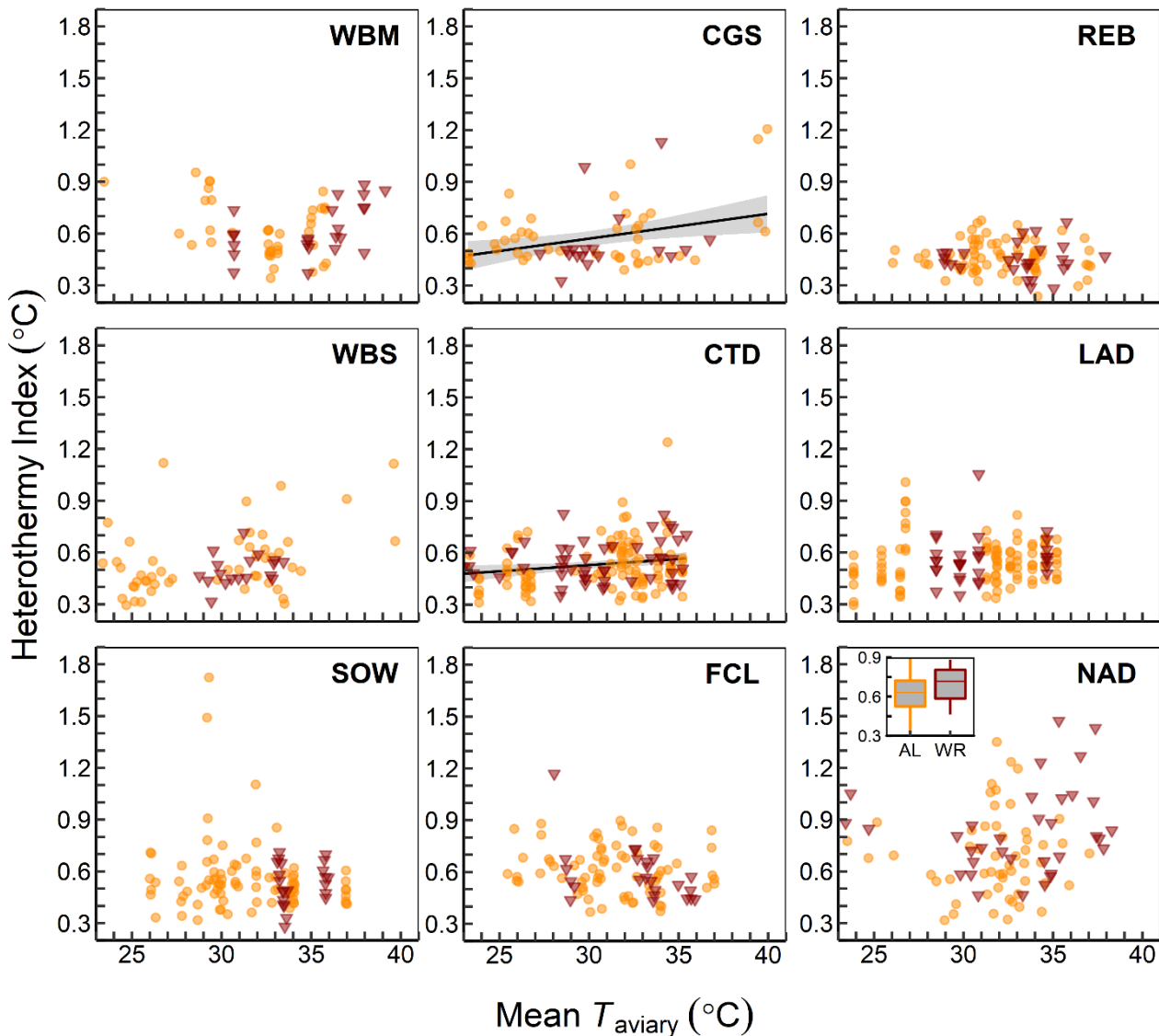


Figure 3: Variation in diurnal T_b , represented as Heterothermy index ($HI_{diurnal}$), with increasing mean diurnal T_{aviary} . The only species with different $HI_{diurnal}$ on ad lib (AL) water days (orange circles) and water restricted (WR) days (red-brown triangles) was NAD: see inset boxplot showing higher $HI_{diurnal}$ on WR than AL days. Models were fitted to the full range of mean T_{aviary} data, however plots show only mean T_{aviary} 23-41°C to focus on trends at higher temperatures. Model regression lines are included for CGS and CTD as mean T_{aviary} was a significant predictor of $HI_{diurnal}$ in these two species. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

Table 3: Outcomes of models (presented as effect sizes, 95% CIs as [LCI, UCI]) for each species (sample sizes = N_{T_b}) using T_b data from the diurnal period (07:00-19:20). Response variables absolute T_b (T_b) and T_b mean and maxima (T_{bmean} and T_{bmax}) were examined. Global models on absolute T_b included the effect of water restriction (Restriction), aviary temperature (T_{aviary}), mass (g) and the interactions between T_{aviary} :Mass, T_{aviary} :Restriction and Restriction:Mass. Global models for T_{bmean} and T_{bmax} were identical, except I included mean T_{aviary} instead of T_{aviary} . Multiple competing models were present in some species and numbered candidate models have been included as well as AICc, $\Delta AICc$ and model weights for comparison. Blank cells indicate predictors were not present in top models. Values for which 95% CIs do not include zero are indicated in bold. Species are ordered by pant₅₀ from lowest to highest. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

Species (N_{T_b})	Response variable	Candidate model	Restriction	T_{aviary}	Body Mass	Restriction: Body Mass	T_{aviary} : Body Mass	T_{aviary} : Restriction	AICc	$\Delta AICc$	Weight
WBM (6)	T_b		0.50 [0.40, 0.60]	-0.01 [-0.01,-0.01]				-0.01 [-0.02,-0.01]	15815.0	0.00	0.80
									50.1	0.00	0.82
	T_{bmax}	1							105.7	0.00	0.39
		2			0.05 [0.02, 0.08]					105.8	0.09
CGS (5)	T_b		0.96 [0.77, 1.15]	0.16 [0.13, 0.19]	0.05 [-0.03, 0.13]		-0.00 [-0.00,-0.00]	-0.04 [-0.05,-0.03]	7056.1	0.00	0.96
									26.1	0.00	0.65
	T_{bmean}	1			0.04 [0.03, 0.06]				27.5	1.45	0.32
		2		-0.14 [-0.29, 0.0]	0.05 [0.03, 0.06]				74.9	0.00	0.45
	T_{bmax}	1			0.04 [0.01, 0.06]				75.0	0.10	0.43
	2										
REB (7)	T_b		0.04 [0.02, 0.06]						11661.0	0.00	0.62
									-55.6	0.00	0.93
	T_{bmax}								131.6	0.00	0.80
WBS (6)	T_b		0.60 [0.40-0.80]	0.00 [-0.00-0.00]				-0.02 [-0.03,-0.02]	7599.8	0.00	0.91
									37.4	0.00	0.81
	T_{bmax}			0.06 [0.03-0.09]					114.9	0.00	0.75
CTD (11)	T_b		-0.54 [-0.75,-0.34]		-0.01 [-0.03-0.02]	0.00 [0.00-0.01]			27234.0	0.00	0.89
									27.1	0.00	0.90
	T_{bmax}	1		0.03 [0.01-0.05]					250.7	0.00	0.56

Table 3: Continued...

Species (N_{Tb})	Response variable	Candidate model	Restriction	T_{aviary}	Body Mass	Restriction: Body Mass	T_{aviary} : Body Mass	T_{aviary} : Restriction	AICc	Δ AICc	Weight
LAD (8)	T_b		-0.32 [-0.43,-0.21]	-0.01 [-0.01,-0.01]				0.01 [0.01-0.01]	17843.0	0.00	0.98
	T_{bmean}								28.0	0.00	0.89
	T_{bmax}		-4.49 [-6.35,-2.65]	0.01 [-0.01-0.03]				0.15 [0.09-0.21]	161.0	0.00	0.97
SOW (8)	T_b		-0.57 [-1.17-0.03]	0.12 [0.08-0.16]	0.08 [-0.23-0.40]	0.06 [0.04-0.09]	-0.00 [-0.01,-0.00]	-0.02 [-0.03,-0.02]	21921.0	0.00	1.00
	T_{bmean}	1							79.6	0.00	0.42
		2		0.03 [0.01-0.05]					79.8	0.21	0.38
	T_{bmax}								141.4	0.00	0.73
FCL (6)	T_b	1	-0.36 [-0.50,-0.23]	-0.03 [-0.03,-0.03]				0.01 [0.01-0.02]	17283.0	0.00	0.66
		2	-0.36 [-0.50,-0.23]	-0.03 [-0.03,-0.03]	0.14 [0.03-0.24]			0.01 [0.01-0.02]	17285.0	1.47	0.31
	T_{bmean}	1		-0.02 [-0.04,-0.01]					-9.0	0.00	0.30
		2							-8.4	0.67	0.22
		3		-0.02 [-0.04,-0.01]	0.14 [0.04, 0.25]				-7.9	1.17	0.17
		4			0.14 [0.04, 0.25]				-7.3	1.72	0.13
	T_{bmax}	1		-0.08 [-0.11, -0.04]	0.28 [0.08, 0.47]				168.3	0.00	0.34
		2		-0.08 [-0.11, -0.04]					168.5	0.23	0.30
NAD (8)	T_b		0.16 [0.13, 0.18]	0.14 [0.11, 0.17]	0.23 [0.12, 0.34]		-0.00 [-0.00,-0.00]		23926.0	0.00	0.97
	T_{bmean}	1	0.19 [0.04, 0.33]						100.5	0.00	0.45
		2							101.3	0.81	0.30
	T_{bmax}	1	0.50 [0.20, 0.80]						225.4	0.00	0.52
		2	0.50 [0.20, 0.80]	0.14 [0.04, 0.23]					226.1	0.71	0.36

WBS and CGS, whilst CTD and NAD had significantly higher afternoon T_{bmean} on WR days compared to AL days (Figure 1, Figure 2). Despite this overall variation, all species except SOW showed small but significant responses in T_b to water restriction (Table 3). However, the significant interaction between T_{aviary} and water restriction affected both direction and magnitude of the response amongst most species, including SOW. Several species (WBM, CGS, WBS, SOW) showed higher T_b at low T_{aviary} and lower or similar T_b at high T_{aviary} on WR days compared to AL days. In contrast, others (LAD, FCL) maintained more stable T_b on WR days but lowered T_b on AL days as T_{aviary} increased, although these effect sizes were small (Table 3, Figure 2).

Although body mass significantly affected T_b response to increasing T_{aviary} (i.e. there was a T_{aviary} :Mass interaction) in CGS, SOW and NAD, with smaller birds showing steeper increases in T_b as T_{aviary} increased, this response was characterised by extremely small effect sizes (Table 3). The only two species where body mass significantly affected T_b response to WR (Restriction:Mass interaction) were CTD and SOW, with larger birds showing higher T_b on WR days than smaller birds, although again the effect size was negligible (Table 3). Therefore, the only species where the water restriction effect on T_b can be interpreted without interaction effects is in NAD, where T_b was higher on WR days. Similarly, despite differences in T_{bmean} on WR and AL days in numerous species (Table 2), WR emerged as a significant predictor of T_{bmean} in NAD alone (Table 3). Further, T_{bmax} was predicted by water restriction in only LAD and NAD however the responses differed. In LAD, T_{bmax} increased with mean T_{aviary} on WR days, but remained stable on AL days regardless of increases in T_{aviary} . In NAD, most variation in T_{bmax} was predicted by water restriction alone (no Restriction: T_{aviary} interaction) with T_{bmax} substantially higher (0.5° C) on WR days (Table 3).

There was no relationship between mean T_{aviary} and $HI_{diurnal}$ in most species, except CGS and CTD, where the only significant predictor of $HI_{diurnal}$ was T_{aviary} (Figure 3). The

only species to adjust $H_{diurnal}$ with water restriction was NAD, where variation in T_b was higher on WR days (LMM: 0.16 °C, [0.06-0.26], Figure 3).

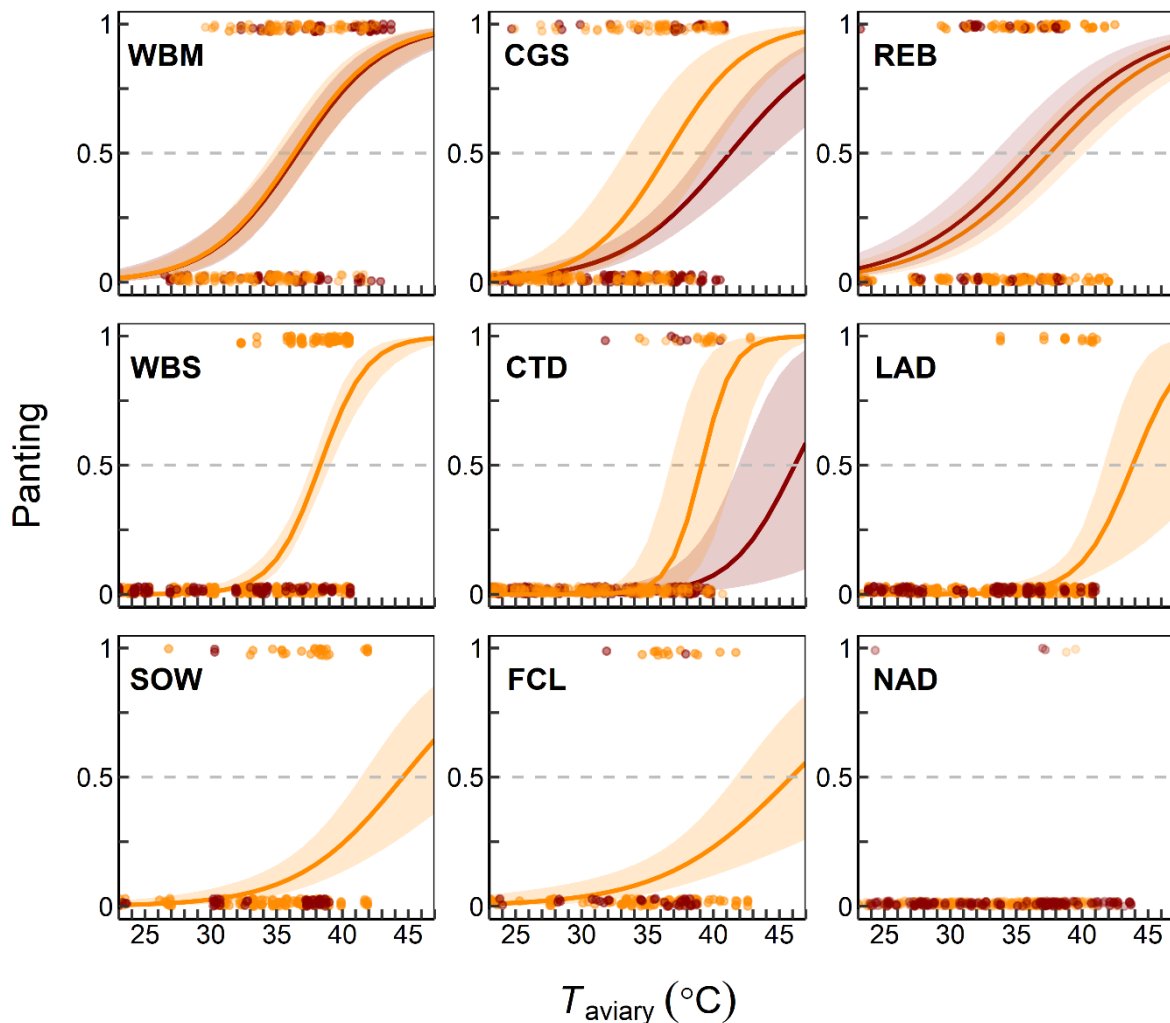


Figure 4: Proportion of time spent panting with increasing T_{aviary} . Binomial regressions were fitted to calculate $pant_{50}$, the T_{aviary} at which 50% of observed individuals are displaying panting/gular flutter, on water restricted days (WR; red-brown circles, 95% CI) and on *ad libitum* water days (AL; orange circles, 95% CI) where panting presence = 1 and panting absence = 0. Models were fitted to the full range of T_{aviary} , however plots show only T_{aviary} 23-47°C to focus on trends at higher temperatures. Several species did not pant at all (WBS, LAD), or panted infrequently (FCL, SOW) on WR days. Higher $pant_{50}$ were observed on WR days in CGS and CTD. Panting thresholds were similar in WBM and REB on AL and WR days. NAD were not often enough observed gular fluttering on AL or WR days to fit binomial regressions. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove

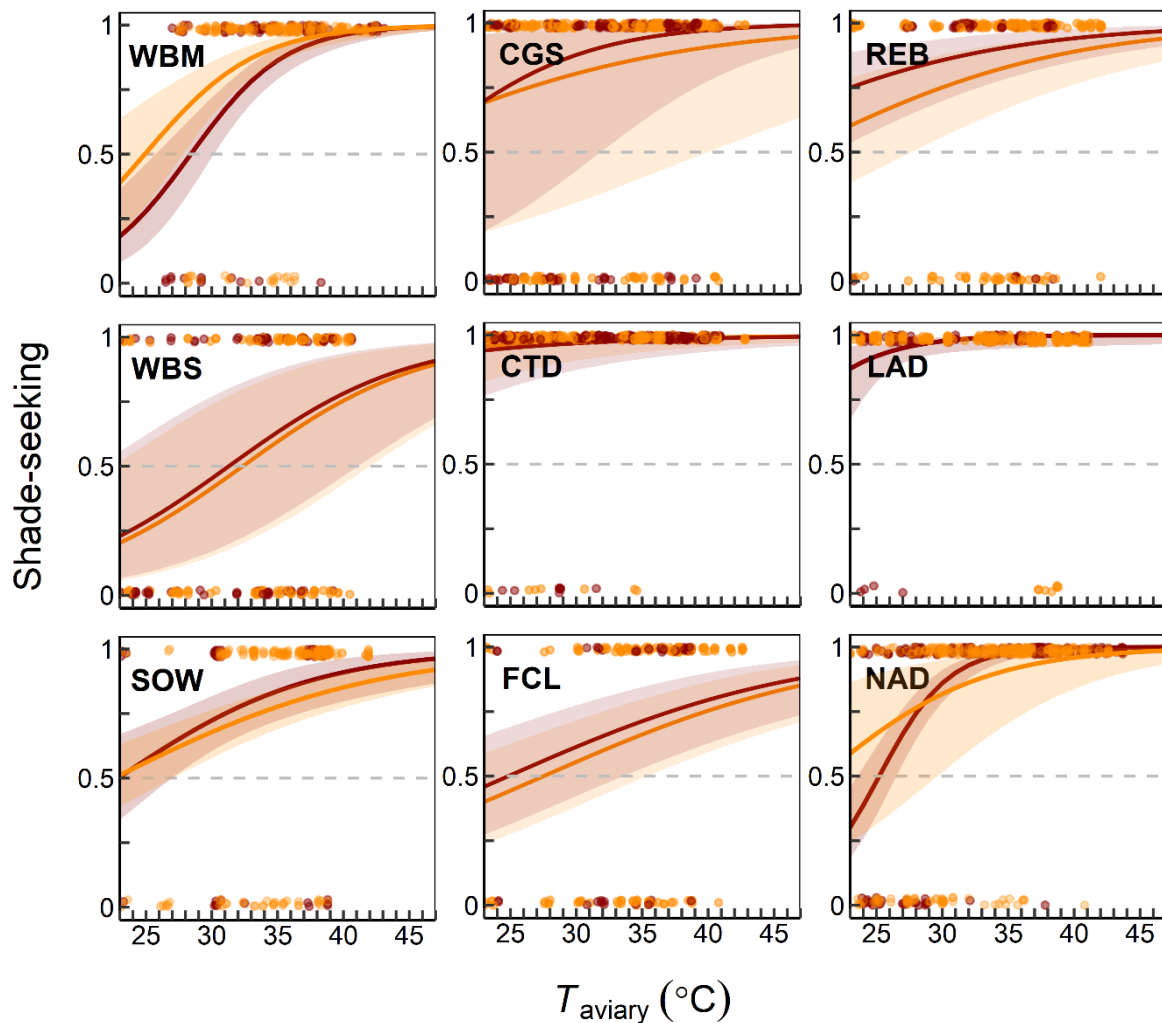


Figure 5: Proportion of time spent shade-seeking with increases in T_{aviary} . Shade_{50} , the T_{aviary} at which 50% of observed individuals are displaying shade-seeking behaviour were calculated by fitting binomial regressions where observations of individuals in shade = 1 and full sun = 0, on water restricted days (WR; red-brown circles, 95% CI) and on *ad libitum* water days (AL; orange circles, 95% CI). Models were fitted to the full range of T_{aviary} , however plots show only T_{aviary} 23-47°C to focus on trends at higher temperatures. Only WBM significantly increased Shade_{50} with WR. REB significantly decreased Shade_{50} with WR. Most species (WBS, SOW, FCL, CTD, CGS, NAD) did not change shade-seeking behaviour in response to water restriction. Shade_{50} on AL days for LAD could not be calculated. WBM = White-backed Mousebird, CGS = Cape Glossy Starling, REB = African Red-eyed Bulbul, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove

Behaviour

Most species (6/9) adjusted panting behaviour with water restriction. Pant_{50} values were significantly higher on WR compared to AL days for two species (CGS and CTD), and four species (WBS, LAD, SOW, FCL) seldom panted at all on WR days. There was no

significant effect of WR on pant_{50} in REB and WBM. NAD were seldom observed panting on either WR or AL days (Figure 4, Table 5 Appendix).

Only two species (WBM, REB) significantly adjusted shade-seeking behaviour as T_{aviary} increased on WR days (Figure 5, Table 5 Appendix). However, the direction of the adjustments differed, with REB seeking shade at lower T_{aviary} and WBM seeking shade at higher T_{aviary} on WR days than AL days. CTD showed drastically reduced shade_{50} on WR days ($\Delta \text{shade}_{50} = 13.4$ °C – below the range of temperatures displayed in Figure 5), but this adjustment was not significant (Table 5 Appendix). Shade-seeking behaviour was initiated at lower T_{aviary} than panting on both AL and WR days in all species for which shade_{50} could be calculated. Most species did not adjust activity with increasing T_{aviary} and WR, and the large reduction in activity_{50} with water restriction in CGS ($\Delta \text{activity}_{50} = 21.8$ °C) was not statistically significant. Surprisingly, WBS exhibited an increase in activity with increasing T_{aviary} (Table 5 Appendix).

2.5 Discussion

My data from arid-zone birds representing six families and three orders reveal that thermoregulatory responses to dehydration are highly variable under semi-natural conditions. Several species, but not all, showed small adjustments in T_b in response to short-term water restriction and six of nine study species delayed panting to higher T_{air} or seldom panted at all compared to days when water was freely available. However, adjustments in all other behavioural aspects of thermoregulation with water restriction varied widely among and within species. Taken together, my data suggest that although changes in T_b tend to be small and panting behaviour is usually reduced in response to dehydration, overall combinations of physiological and behavioural responses to water restriction are highly species-specific.

I did not observe panting in several species (WBS, LAD, SOW) on WR days.

Whether this reflected a physiological response to prevent dehydration by reducing EWL (Taylor 1970, Kleinhaus *et al.* 1985), or reduced observations at high T_{air} on water restricted days (i.e. too few WR days), remains unclear. However, on days with similar T_{air} when water was available, individual WBS, LAD, SOW frequently exhibited panting behaviour, and WR days (although only three WR days for some species) were not consistently cooler than AL days overall. Thus, it is likely the lack of panting during WR days is not due to a lack of observations. Using species-specific EWL data collected previously over a range of T_{air} under laboratory conditions (Whitfield *et al.* 2015, McKechnie *et al.* 2016, Czenze *et al.* 2020) I was able to determine cumulative water loss estimates for each species in the aviaries. Using the maximum T_{aviary} at half hour intervals these calculations suggest that on very hot WR days, doves and passerines would have lost the equivalent of ~7-9% and ~14-23% body mass in water, respectively, if they did not adjust EWL rates with WR. This suggests a significant strain on water budgets on very hot water restricted days. It is not possible to calculate the extent of adjustment of EWL rates achieved by the birds in the study, but the adjustments in panting behaviour observed may have been in response to the inability to replace the significant amount of water lost via these means under normal circumstances. Although I did not observe any obvious signs of hyperthermia (where $T_b > T_{b\text{mod}}$ by more than 2°C; Calder & King 1974) on WR days FCL and LAD did show small increases in T_b with T_{air} , whereas on AL days when adequately hydrated, their T_b was stable at high T_{air} . Therefore, the primary response to dehydration I observed was a reduction in panting behaviour, presumably to maintain water balance.

The inclusion of data from the early morning (between 07:00-09:00), when water was freely available on WR days, is unlikely to have affected these results as EWL rates would have been minimal during this time (T_{air} was below species-specific inflections in T_{air}

for EWL, McKechnie *et al.* 2021). This is further evidenced by the minimal effect of WR on even afternoon T_{bmean} (Table 2). I included these data to be able to calculate T_{bmod} (T_{bset}) on WR days. The absence of hyperthermia can potentially be explained by high T_{bmod} , a consequence of extremely high activity (high heat production) in the morning resulting in high T_b s. The hyperthermic increase in T_b s resulting from increasing T_{air} may not have been detected as T_{bmod} values may have been inflated. Because activity is binary no distinction is made between highly active behaviour (such as flying and intense foraging) and less intense activity (slow foraging during the heat of the day) making this trend difficult to detect.

The frugivorous species in my dataset (REB and WBM) showed no adjustments in $pant_{50}$ or activity with increasing T_{air} . Moreover, they maintained similar afternoon T_{bmean} regardless of water availability, and maintained T_b at or below T_{bset} with increasing T_{air} on water restricted days. These abilities may be related to their consumption of water-rich fruit; which remained available to them on WR days. The fruit and nectar of saguaro cacti, for example, are major contributors to the water budgets of white-winged doves (*Zenaida asiatica*) inhabiting the Sonoran Desert (Wolf *et al.* 2002). Therefore, a water-rich diet could partially buffer the effects of a short-term absence in free drinking water and minimise dehydration. The reduced T_b in response to water restriction I observed in some omnivorous passerines (WBS, CGS) may also be related to a water-rich diet as these species received mealworms, other insects and/or fruit on both WR and AL days. During hot weather, species relying on water in food must increase foraging rates and/or success to increase their dietary water supply, exposing birds to greater dehydration risk and increased heat loads (du Plessis *et al.* 2012). Compared to natural conditions, the aviary conditions inherently reduced foraging effort as food was always readily available. Thus, the trade-offs experienced by wild birds which rely on food for water, i.e. the need to

increase foraging rates and/or success to increase their dietary water supply *versus* the need to reduce foraging activity to reduce metabolic heat production (entailing increased risk of dehydration), were diminished. It is therefore likely that free-ranging birds will show stronger responses to reduced water availability under natural conditions than did my aviary birds.

Perplexingly, at high T_{air} during water restricted days, many of my study species maintained T_{b} at or below their usual (i.e. *ad lib* water) T_{bset} , despite a reduction in panting (Table 3, Figure 2 & 3). During dehydration, the trade-off between hyperthermia avoidance via evaporative cooling and dehydration avoidance via minimising water losses should favour a reduction in EWL and an increase in the frequency and level of hyperthermia. For example, fully hydrated rock doves (*Columba livia*) regulated stable T_{b} during heat-exposure whereas birds dehydrated for 48 h before heat-exposure became significantly hyperthermic, under laboratory conditions (Arad *et al.* 1987). Likewise, dehydrated western mourning doves (*Zenaida macroura marginella*) at $T_{\text{air}} = 39^{\circ}\text{C}$ also displayed higher T_{b} than when fully hydrated (Bartholomew & Dawson 1954). This pattern has also been observed in passerines and, at $T_{\text{air}} > 35^{\circ}\text{C}$ and in the absence of water, zebra finches (*Taeniopygia guttata*) subsisting on a dry diet, maintain $T_{\text{b}} \sim 1^{\circ}\text{C}$ higher than individuals with access to water. Further, zebra finches deprived of water for 30 days or longer significantly reduce EWL over a wide range of T_{air} (15 - 43°C ; Cade *et al.* 1965). At $T_{\text{air}} = 41.5^{\circ}\text{C}$, budgerigars (*Melopsittacus undulatus*) without access to water significantly increase T_{bmean} by 0.7°C compared to drinking birds, and reduced EWL at high T_{air} (Greenwald *et al.* 1967). In the present study, although I did not record an increase in hyperthermia, I consistently recorded a reduction in EWL (reduced panting in 6 of 9 species) when birds were water deprived. As shade-seeking behaviour was also not consistently altered in response to water restriction, the ability of the birds to avoid hyperthermia in this study is very difficult to explain.

Recent insights into the use of hyperthermia among avian taxa suggest that there is variation in how facultative hyperthermia minimises water loss among different orders (Gerson *et al.* 2019). In lab studies, small passerines rely heavily on hyperthermia and should benefit more, in terms of water savings, from hyperthermia than columbids (Gerson *et al.* 2019). Surprisingly, I recorded small but significant reductions in afternoon T_{bmean} and T_{bmod} by two passerines (WBS and CGS) and similarly small increases in afternoon T_{bmean} by two columbids (CTD and NAD; Table 2) with water restriction. Further, when water availability was restricted, species with higher afternoon T_{bmean} exhibited greater variation in T_b (higher $H_{diurnal}$; Figure 1). These shifts suggest a small degree of thermal generalisation among CTD, CGS, NAD and WBS (*sensu* Angilletta *et al.* 2010, Boyles *et al.* 2011a), as their T_b distributions included either a higher (CTD, NAD) or lower (WBS, CGS) range of T_b when water was unavailable. However, the size of these shifts were very small and thus may not be biologically meaningful.

For WBS and CGS, reduced T_b at high T_{air} , and more precise regulation of a lower T_b on water restricted days than on days when water was available, seems counterintuitive. Both species either panted at higher T_{air} (i.e. higher $pant_{50}$) or did not pant at all on water restricted days and did not significantly adjust shade-seeking when dehydrated (Table 5, Appendix). Further, WBS increased activity with increasing T_{air} only on WR days, perhaps as a stress response, but the fact that this did not reflect in increased T_b is perplexing. Generally, as T_{air} increases above thermoneutrality and T_b exceeds optimum, thermal generalists may have an advantage over thermal specialists as less time and energy can be spent thermoregulating (Boyles *et al.* 2011a). Comparatively, lowering T_b at high T_{air} would offer little benefit as an adaptive thermoregulatory mechanism. However, it is noteworthy that these responses manifested after only 8 h of water deprivation, particularly as many of the study species are known to concentrate

drinking to either mornings or evenings under free-ranging conditions. As water was removed only at 09:00, birds likely began WR days fully hydrated most notably species which preferably drink in the mornings. With longer periods of water restriction and no availability of water in the morning, I may have observed larger effects and a clearer indication of the directionality of T_b adjustments. The influence of differences in drinking times (some species only drinking in the morning or evening (Smit 2013)), would merit further investigation in future.

One possible explanation for the lack of clear physiological responses to water deprivation in the study species concerns the high T_b s observed in this study. The study species generally had higher T_b than previously-reported typical avian active phase T_{bmean} measured in the lab (40.02 ± 1.29 °C; Prinzinger *et al.* 1991). Most previous studies quantifying the physiological consequences of water deprivation in birds took place in the laboratory, involving resting birds placed in a darkened metabolic chamber for measurements of gas exchange and quantification of relationships between metabolic rates, EWL, T_b and T_{air} . Under these circumstances, T_b is usually markedly lower than T_b displayed by an active bird at the same T_{air} under natural conditions. The higher T_{bset} values observed here meant that T_{air} rarely exceeded T_b , and study birds resting in the shade may have depended heavily on passive heat dissipation to maintain stable T_b and avoid hyperthermia. Nevertheless, shade-seeking T_{air} thresholds under free-ranging conditions in seven of the nine species were comparable to, or higher than, values observed in captive birds (Smit *et al.* 2016). It is also possible that the high T_{bset} values I observed may reflect stress-induced hyperthermia caused by captive conditions, particularly for species which do not naturally occur in the group sizes I artificially created (Cunningham *et al.* 2017). Thus, there are clearly distinct differences in hyperthermia use for birds under laboratory conditions compared to birds experiencing natural fluctuations in

T_{air} under semi-natural conditions, and most likely further differences under free-ranging conditions. Caution should therefore be exercised in extrapolating T_b responses of captive birds to free-ranging conspecifics.

As far as I am aware, this study represents the first examination of both thermoregulatory and behavioural adjustments in response to water restriction under semi-natural conditions. With subtropical arid regions expected to become drier in decades to come (IPCC 2014), the energy and water demands necessary to maintain T_b will increase (Boyles *et al.* 2011a, McKechnie & Wolf 2010, Noakes *et al.* 2016, Riddell *et al.* 2019). My results confirm that under these circumstances, birds without access to water are likely to reduce the time spent panting during the hottest time of day. The physiological changes associated with these reductions in evaporative cooling are inconsistent amongst avian taxa, with few indications of underlying phylogenetic patterns within my study species. Therefore, understanding how trade-offs between thermoregulation and hydration state manifest will likely always require intensive species-specific studies.

2.6 Literature cited

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2.7 Appendix

Table 4: Paired t-test results for differences in individual T_{bmod} , T_{bmean} , afternoon T_{bmean} (subset T_b recordings between 12:00-19:20) and T_{bmax} on days when water was restricted (WR) and when water was freely available (AL). T_{bmod} was calculated as the most frequent T_b during the time of day when birds are active but not thermally stressed i.e. using all T_b recorded during early morning (7:00-10:00) when air temperatures are lower. T_{bmean} and T_{bmax} were calculated using T_b recorded throughout diurnal period whilst Afternoon T_{bmean} was the maximum T_b recorded for the species during the hottest period (12:00-19:20). Heterothermy index expresses variation in T_b accounting for deviation from T_{bmod} and time spent away from T_{bmod} . For the three species that were repeated, values are presented as Season 1 (Season 2) values. Data for T_{aviary} and T_b variables for these species were pooled.

Species	N	t-test results				
		T_{bmod}	T_{bmean}	Afternoon T_{bmean}	T_{bmax}	HI _{diurnal}
White-backed Mousebird	6	t = -1.8 $p = 0.13$	t = -1.0 $p = 0.36$	t = 0.8 $p = 0.47$	t = 1.2 $p = 0.29$	t = -0.2 $p = 0.98$
Cape Glossy Starling	5	t = 1.1 $p = 0.33$	t = 2.9 $p < 0.05$	t = 2.7 $p < 0.05$	t = 3.6 $p < 0.05$	t = 1.9 $p = 0.13$
African Red-eyed Bulbul	7	t = 1.1 $p = 0.29$	t = -0.7 $p = 0.53$	t = -0.08 $p = 0.94$	t = 0.7 $p = 0.48$	t = 1.3 $p = 0.26$
White-browed Sparrow Weaver	6	t = -0.7 $p = 0.52$	t = 0.8 $p = 0.46$	t = 3.1 $p < 0.05$	t = 1.0 $p = 0.35$	t = 1.9 $p = 0.12$
Cape Turtle-Dove	11	t = -3.2 $p < 0.01$	t = -2.6 $p < 0.05$	t = -2.24 $p < 0.05$	t = 2.6 $p < 0.05$	t = -0.08 $p = 0.93$
Laughing Dove	8	t = 1.3 $p = 0.24$	t = 1.1 $p = 0.31$	t = 0.6 $p = 0.56$	t = 0.4 $p = 0.66$	t = -0.7 $p = 0.51$
Sociable Weaver	8	t = -2.0 $p = 0.08$	t = -2.1 $p = 0.08$	t = 0.4 $p = 0.69$	t = 3.1 $p < 0.05$	t = 1.6 $p = 0.15$
Fawn-coloured Lark	6	t = -0.8 $p = 0.47$	t = 0.8 $p = 0.44$	t = 2.0 $p = 0.10$	t = 4.7 $p < 0.01$	t = 1.3 $p = 0.26$
Namaqua Dove	8	t = -1.1 $p = 0.31$	t = -8.4 $p < 0.001$	t = -6.5 $p < 0.001$	t = 0.8 $p = 0.46$	t = -3.3 $p < 0.01$

Table 5: Differences in pant_{50} , shade_{50} and activity_{50} [calculated as the T_{aviary} at which 50% of the observed individuals of each species were panting, shade-seeking or active, respectively on ad libitum (AL) water days and water restricted (WR) days. Global models* examined panting/shade-seeking/activity observations in response to water restriction and T_{aviary} , with individual included as a random factor. *NO* = *not observed* i.e. no individuals were observed panting/shade-seeking/active and no models could be run. Cells with dashes (-) indicate models were not significant, most likely the result of too few observations.

Species	pant_{50}		Global model output	shade_{50}		Global model output	activity_{50}		Global model output
	AL	WR	[Restriction estimate, 95% CI]	AL	WR	[Restriction estimate, 95% CI]	AL	WR	[Restriction estimate, 95% CI]
WBM	36.3	36.6	[-0.07, -0.46-0.32]	24.9	28.4	[-0.70, -1.20--0.19]	14.5	-	-
CGS	36.5	41.2	[-1.03, -1.45--0.63]	13.7	18.3	[-1.67, -3.58-0.19] ^x	25.3	3.5	[0.33,-0.16-0.83]
REB	37.4	36.3	[0.32, -0.12-0.77]	17.4	15.8	[0.68, 0.19-1.20]	-	-	-
WBS	38.3	<i>NO</i>	-	32.1	31.5	[0.16, -0.25-0.57]	-	50.6	-
CTD	39.1	46.2	[-1.81, -2.65--1.06]	15.0	1.6	[-0.35, -1.06-0.35]	-	-	-
LAD	43.8	<i>NO</i>	-	-	16.0	-	-	-	-
SOW	44.6	<i>NO</i>	-	22.5	22.9	[0.22, -0.29-0.75]	26.3	-	-
FCL	46.0	-	-	27.1	25.7	[0.24, -0.26-0.75]	-	-	-
NAD	-	-	-	20.8	25.2	[0.15, -0.24-0.54]	-	-	-

* Binomial observations of behaviour modelled using GLMM with binomial error structure (logit link function) .

^xOnly species where Restrict: T_{aviary} interaction significant, [0.07 ,0.01-0.14]

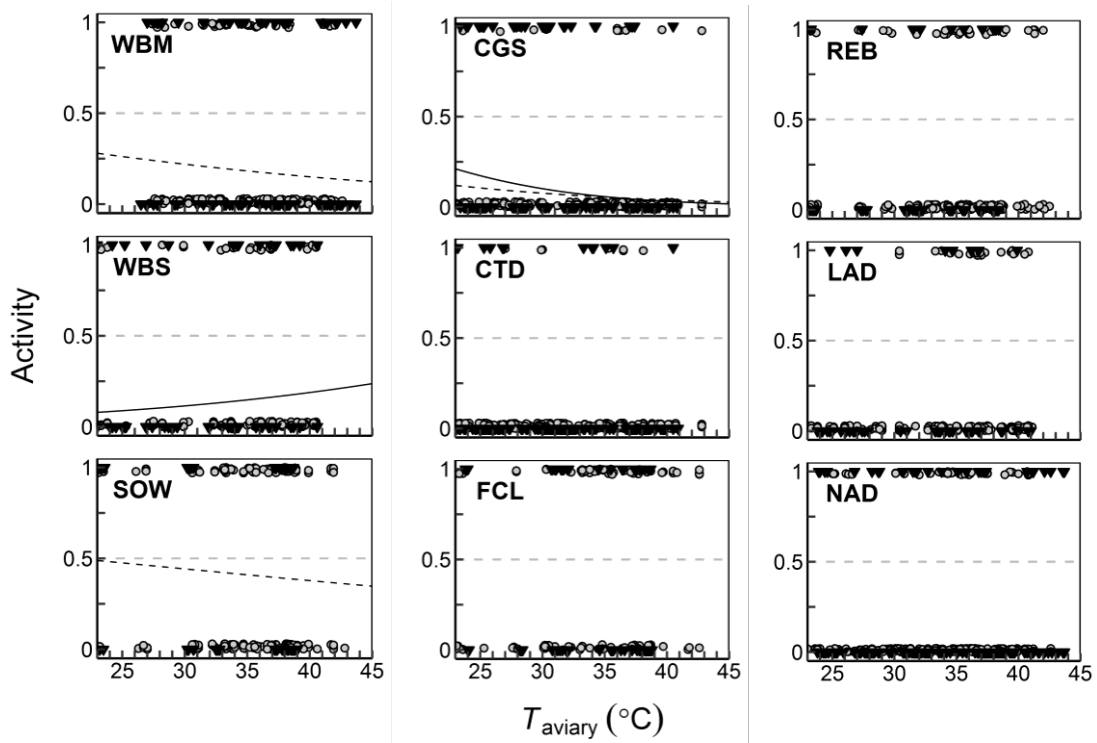


Figure 6: Proportion of time spent active with increases in T_{aviary} . Binomial regressions were fitted to calculate $activity_{50}$, the T_{aviary} at which 50% of observed individuals are active, on water restricted days (WR; solid line) and on *ad libitum* water days (AL; dashed line) where active = 1 and inactive = 0. Most species did not significantly adjust activity with T_{aviary} on either WR or AL days with only CGS reducing activity on WR days compared to AL days. An increase in activity with T_{aviary} was observed in WBS. Species acronyms (sample sizes): WBM = White-backed Mousebird ($N=10$), CGS = Cape Glossy ($N=16$), REB = African Red-eyed Bulbul ($N=10$), WBS = White-browed Sparrow-Weaver ($N=10$), CTD = Cape Turtle-Dove ($N=16$), LAD = Laughing Dove ($N=11$), SOW = Sociable Weaver ($N=9$), FCL = Fawn-coloured Lark ($N=8$), NAD = Namaqua Dove ($N=19$)

CHAPTER 3 Effect of captivity on heat dissipation behaviour and thermoregulation in arid-zone birds

3.1 Abstract

Predicting how avian communities will change as a result of global heating is challenging, as very little is known about inter- or intraspecific variation in thermoregulatory patterns in free-ranging populations. Understanding species-specific thermoregulatory patterns and their likely effectiveness under climate change requires captive studies where food and water resources and T_{air} can be manipulated and species-specific responses recorded. However, the extent to which thermoregulatory and behavioural data collected under laboratory conditions can be extrapolated to free-ranging populations in natural environments remains unclear. I compared behavioural thermoregulation in six Kalahari Desert species held captive *in situ* in aviaries to that of free-ranging conspecifics at the same study site to evaluate whether data from captive birds can be extrapolated to free-ranging populations. I found that, for all six species, the temperature-dependence of panting behaviour did not differ between captive and wild populations. The majority of species sought shade at lower T_{air} in captivity; however, only three of the six species reduced overall activity levels compared to free-ranging conspecifics. This suggests that, whereas interspecific variation in panting behaviour of captive populations can provide important information about free-ranging populations, this needs to be considered alongside other behavioural adjustments.

Keywords: arid-zone birds, captivity, behavioural adjustments, interspecific variation, shade-seeking, activity, panting

3.2 Introduction

For endotherms living in hot, arid environments the defence of a set point body temperature (T_b) in the face of high air temperatures (T_{air}) and intense solar radiation poses considerable thermoregulatory and osmoregulatory challenges. Birds depend on evaporative cooling to avoid potentially lethal hyperthermia, particularly at T_{air} above T_b where evaporative cooling becomes the only mechanism whereby heat can be dissipated to the environment. However, evaporative heat dissipation via panting or gular fluttering (respiratory evaporative water loss [REWL]), or across the skin (cutaneous evaporative water loss [CEWL]), can lead to rapid depletion of body water (Dawson 1982, Tieleman *et al.* 2003). Thus, in arid environments birds face severe trade-offs between hyperthermia avoidance through evaporative cooling and dehydration avoidance through body water conservation (Dawson & Bartholomew 1968, Smit *et al.* 2016).

Birds can occupy extremely hot environments by using physiological (e.g. dehydration tolerance [O'Connor *et al.* 2018] and facultative hyperthermia [Tieleman & Williams 1999]) as well as behavioural adjustments (e.g. reducing activity, shade seeking, panting, and wing drooping; Wolf 2000) to mitigate the severity of dehydration-thermoregulation trade-offs. Interspecific variation in the behavioural and physiological processes involved in thermoregulation gives rise to considerable differences in patterns of T_b regulation (Thompson *et al.* 2018). With the advent of rapid anthropogenic climate change, desert regions are expected to become warmer and drier faster than most terrestrial biomes (IPCC 2014), a trend already observed in southern Africa (Kruger & Sekele 2013, Van Wilgen *et al.* 2016). Although behavioural flexibility may mitigate the costs of climate warming (Kearney *et al.* 2009, Sears *et al.* 2011), lost opportunity costs (i.e. the loss of opportunities to carry out other important behaviours such as foraging, display, or territorial defence [Cunningham *et al.* 2021]) create further challenges. Trade-

offs between maintaining energy and water budgets through active foraging, and hyperthermia avoidance by lowering activity and seeking shaded refugia have potential to impact fitness (Cunningham *et al.* 2015, van de Ven *et al.* 2019). As such, the ability of individuals to maintain T_b and afford lost opportunity costs directly impacts fitness. Consequently, the inter- and intraspecific variation in physiological and behavioural thermoregulatory patterns will likely influence the persistence of species under climate change scenarios.

Studying the ability of birds to thermoregulate in the heat under free-ranging conditions is complex, and very little is known about inter- or intraspecific variation in thermoregulatory patterns in free-ranging populations (Smit *et al.* 2013, 2016). Most data on avian thermoregulation in the heat originate from laboratory-based studies where T_b , EWL and metabolic parameters can be readily quantified (e.g. Smith *et al.* 2015, Whitfield *et al.* 2015; McKechnie *et al.* 2016). Behavioural responses to heat have also been examined in captive individuals; for example, Xie *et al.* (2017) investigated the temperature dependency of time budgets and microsite selection in eight native Australian bird species in captivity at the Adelaide Zoo.

To predict whether species-specific thermoregulatory patterns will change under climate warming, we must understand the implications of increased T_{air} and water stress on T_b . This is easier to examine in captive populations for which resources and T_{air} can be experimentally manipulated. However, the extent to which thermoregulatory and behavioural data collected under laboratory/captive conditions can be extrapolated to free-ranging populations in natural environments remains unclear. There are cases where thermal physiological traits differ between captive and wild populations. For example, avian and mammalian patterns of torpor in laboratory settings can differ substantially from those in the field (Geiser *et al.* 2000). Birds in captivity also show significant differences in

thermal physiological traits (i.e. BMR, EWL, T_b) compared to free-ranging populations (McKechnie *et al.* 2006, Thompson *et al.* 2015, Cooper *et al.* 2008, Tieleman *et al.* 2002). Despite these differences, captive populations held at research facilities, zoos and private aviaries have the potential to shed valuable light on phenomena that have not been observed under free-ranging conditions. Mariette & Buchanan (2016), for example, demonstrated how zebra finch parents acoustically signal high air temperatures to their embryos before hatching which affects nestling begging and growth in response to nest temperature, and further influences the reproductive success and thermal preferences of these individuals as adults. The zebra finches in the aforementioned study were 7th–9th generation wild-derived birds and reproducing such a study under wild conditions is not feasible. Captive studies are therefore important but data collected from captive birds can potentially yield a skewed picture of physiological responses in the field. One component of behaviour particularly likely to differ between captive and wild individuals is overall activity level. Because of their high mobility, birds can travel distances rapidly to take advantage of sparsely distributed food and water resources, especially in arid landscapes (Wolf 2000). In comparison, birds in captivity could reduce their activity levels as movement is restricted, predators are absent and both water and food resources are provided.

Many studies, either implicitly or explicitly, assume that qualitative and quantitative aspects of patterns of thermoregulatory behaviour in captivity can be used as a proxy of corresponding patterns in free-ranging conspecific populations (e.g. Wolf & Walsberg 1996, Schleucher & Withers 2002, McKechnie *et al.* 2006). I tested this assumption using previously published data for captive populations of six species (Thompson *et al.* 2018) and comparing these to behavioural data observed in free-ranging conspecific populations. The latter data were collected at the same study site where captive birds used by

Thompson *et al.* (2018) were captured, from the same free-ranging populations. I predicted that, under captive conditions, birds reduce overall activity levels as flight and mobility is restricted. I also predicted that reductions in activity levels under captive conditions result in increases in species-specific panting thresholds ($pant_{50}$, the T_{air} at which 50% of observed individuals display panting behaviour [Smit *et al.* 2016; Pattinson *et al.* 2020]), on account of reduced rates of metabolic heat gain and the consequent reduced need to dissipate heat associated with lower overall activity levels. Finally, I predicted that the *ad libitum* food resources in both shaded and sunny locations within the aviaries allow captive birds to spend more time in shaded microsites during the heat of the day, thereby avoiding the solar heat gain experienced by free-ranging birds when foraging in sunlit microsites.

3.3 Methods and Materials

3.3.1 Study animals, housing & brief methodology

I compared panting, activity and shade-seeking behaviour between captive and wild birds. Behavioural data for captive birds were obtained from Chapter 1 [published as Thompson *et al.* (2018)], where I examined interspecific variation in behaviour and thermoregulation in nine species resident in the southern Kalahari Desert. Briefly, birds were housed *in situ* in the Kalahari in large outdoor aviaries for approximately two months during the austral summers of 2014/15 and 2015/16. Behavioural data were collected as instantaneous scan samples (Altmann 1974) taken throughout the day across a wide range of T_{air} . An onsite weather station (Vantage Pro2, Davis Instruments, Haywood, California, USA) recorded weather data (T_{air} , dew point, rainfall etc) at five-minute intervals while air temperature inside the aviaries (T_{aviary}) was also measured using temperature data loggers (TidbiT v2 temperature data logger UTBI-001, Onset Computer Corporation, Bourne, Massachusetts,

USA). TidbiTs were suspended inside a hollow plastic PVC tube (20 cm long x 5 cm diameter) with holes drilled into the sides for ventilation and recorded T_{aviary} at five-minute intervals. For the current study, I collected and quantified comparative data on behaviour in free-ranging populations of six of these nine species (Table 1).

3.3.2 Heat dissipation behaviour – free-ranging birds

Data were collected during the austral summers of 2014/15, 2015/16 and 2016/17. Sampling mostly took place between 10:00-16:00, the hottest time of day when birds were most likely to be displaying heat dissipation behaviours. This also allowed data to be collected across a wide range of T_{airS} (range: 12.1 – 41.1°C). Data were collected while walking 14 straight-line transects (each 1.5 km in length) at a slow pace searching for birds. Transects were placed > 500 m apart to minimise the likelihood of repeated sampling of individuals and walked repeatedly in a randomised order on different days,

Table 1: Kalahari Desert bird species used to examine the effect of captivity on panting, activity and shade-seeking. Each species has a unique abbreviation (Abb.) used for referral in figures and text. Species belong to two orders, passeriformes (PASS) and columbiformes (COLU). Summer seasons during which species were sampled in captivity are indicated by Season (2014 refers to Austral summer: i.e. Nov 2013-March 2014 etc). For all species wild observations were taken throughout the summer season of all three years. Sample sizes for behavioural observations on wild (Wild N_{obs}) and captive (Captive N_{obs}) birds included. Total number of individual captive birds are displayed in square brackets [N_{indiv}].

Abb.	Order	Species	Season	Wild N_{obs}	Captive N_{obs} , [N_{indiv}]
CGS	PASS	Cape Glossy Starling (<i>Lamprotornis nitens</i>)	2015(2016)	109	483, [16]
WBS	PASS	White-browed Sparrow Weaver (<i>Plocepasser mahali</i>)	2015	57	326, [10]
CTD	COLU	Cape Turtle-Dove (<i>Streptopelia capicola</i>)	2015(2016)	360	498, [16]
SOW	PASS	Sociable Weaver (<i>Philetairus socius</i>)	2014	415	337, [9]
FCL	PASS	Fawn-coloured Lark (<i>Calendulauda africanoides</i>)	2014	381	197, [8]
NAD	COLU	Namaqua Dove (<i>Oena capensis</i>)	2015(2016)	64	625, [19]

and at different times of day throughout the three-year period. A minimum of three days

elapsed before a particular transect was repeated. Once a bird was located, an instantaneous behavioural observation was recorded (Altmann 1974). Data for each observation included the location of the individual (on the ground/in shrubs or trees), the exposure to sun (in shade or sun), the activity state of the individual (mobile/stationary), what the bird was doing (foraging/resting/scanning/territorial/ calling/breeding related behaviour) and whether heat dissipation behaviour was present (panting/gular fluttering and/or wing-drooping) or not. During the 3-y data collection period, two separate but concurrent studies (Abdu *et al.* 2018, Pistorius 2016) also recorded heat dissipation behaviour in Kalahari Desert birds in the same location, following the same protocol. Instantaneous behavioural observations recorded for the purpose of these two studies were pooled with the data I collected.

3.3.3 Data analysis

All statistical analyses were conducted in the R environment (R Core Team 2017). To compare behavioural thresholds between captive and wild populations of each species, I fitted generalised linear mixed models with a binomial error structure (logit link-function) using *lme4* (Bates *et al.* 2015) following Smit *et al.* (2016) to examine relationships between behaviour and T_{air} in both captive and wild populations, separately. Using model predictions, I calculated the behavioural thresholds (defined as the predicted T_{air} at which panting/gular fluttering [pant₅₀], activity [active₅₀] and shade-seeking [shade₅₀] behaviour was present in 50% of observations) and model slopes for comparison between wild and captive populations. Accordingly, panting was classified as either present = 1 or absent = 0, activity as active = 1 or inactive = 0 and shade-seeking as shaded = 1 or in full sun = 0. For models fitted to data from captive birds, individual was included as a random predictor to account for repeated measures on individual birds (see Thompson *et al.* 2018). In

models fitted to data for free-ranging populations, transect was included as a random predictor to account for possible repeated observations of individuals along transects.

In addition, to assess whether panting, shade-seeking and activity responses to temperature in free-ranging birds were significantly different from those of captive birds of each species, I modelled the response of these behaviours to T_{air} within each species and included wild/captive as a categorical predictor as well as the interaction between T_{air} and wild/captive. I included a combined random effect (for captive birds = individual, wild = transect) to control for nonindependence. Overdispersion was assessed by comparing the residual deviance to the residual degrees of freedom, however as no values exceeded 2.5 no corrections were necessary. Statistical significance was inferred if 95 % confidence intervals (CIs) excluded zero. Measurements of T_{air} recorded by the weather station were used for free-living birds while T_{aviary} , the T_{air} measured inside the aviaries, was used for captive birds. For discussion purposes these are henceforth collectively referred to as T_{air} . I used model selection with AICc (Akaike's Information Criteria, adjusted for small samples) compared between all possible models nested within the global model using the *dredge* function in R package *MuMIn* (Barton 2010). I present top model sets including all candidate models with $\Delta \text{AICc} < 2$. I visually inspected residuals of global and final models, for all analyses, to ensure model assumptions were met.

3.4 Results

3.4.1 Panting

The temperature-dependence of panting did not differ between captive and free-ranging populations for any species for which pant_{50} could be estimated (all species except NAD, for which were observed too few instances of panting behaviour in either wild or captive birds; Figure 1, Figure 3a). Top model sets for several species included Wild/captive as a

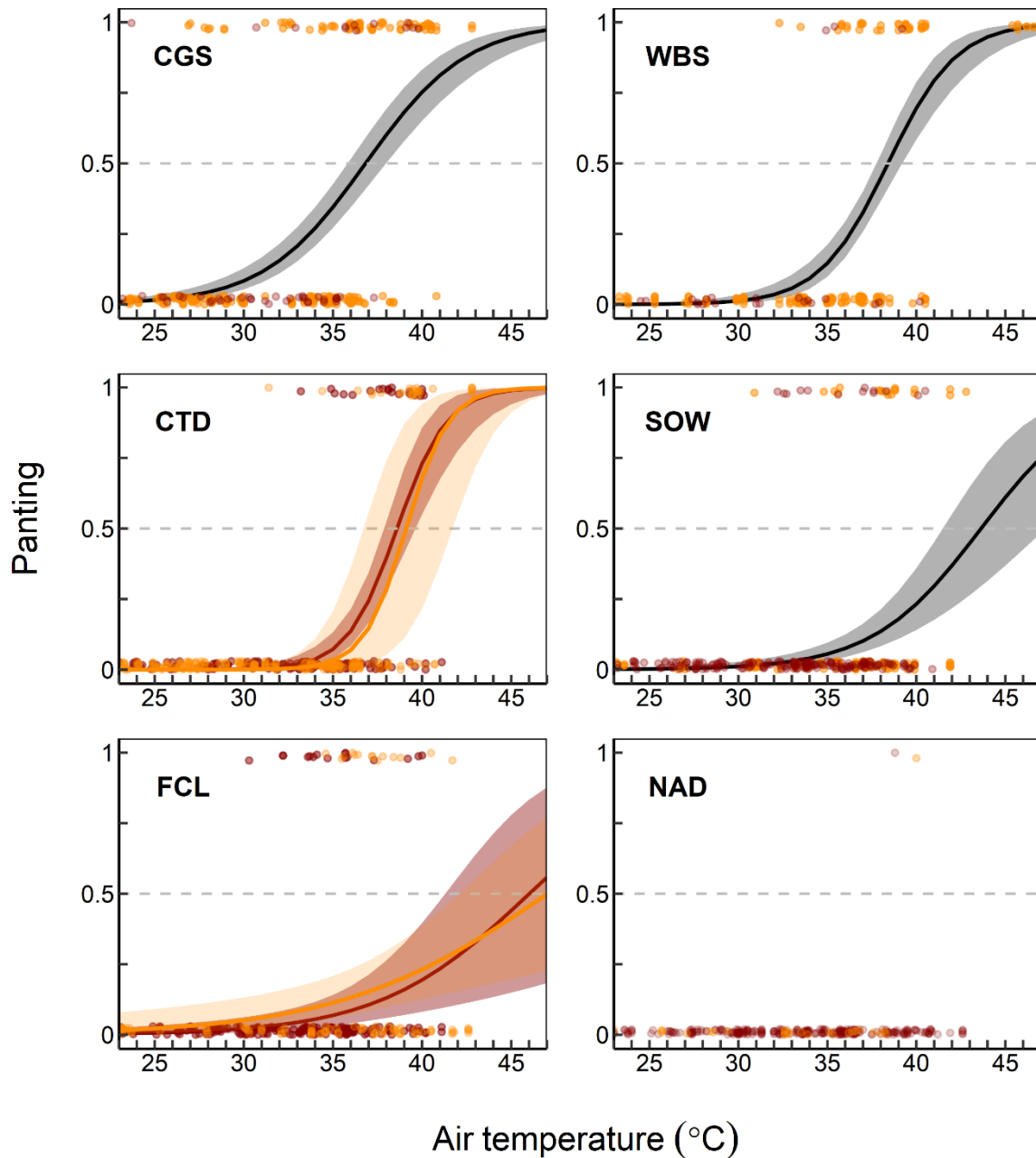


Figure 1: Relationship between panting behaviour and T_{air} . The intersection point of horizontal grey dashed lines with plotted models represents the T_{air} at which 50% of observed individuals are displaying panting/gular fluttering (pant_{50}) for birds in captivity (orange circles) and in the wild (red-brown circles). Points are jittered slightly for clarity. For several species (CGS, WBS, SOW) only T_{air} emerged as a significant predictor of panting behaviour (black line \pm grey 95% CI). Wild/captive emerged as a predictor of panting in top models for CTD and FCL but was not significant (captive: orange lines \pm 95%, wild: red-brown lines \pm 95% CI). Despite large numbers of observations of NAD, gular fluttering was present too rarely to calculate a significant pant_{50} . CGS = Cape Glossy, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, LAD = Laughing Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

predictor of panting behaviour however neither Wild/captive nor the interaction between Wild/captive and T_{air} were significant (Table 2).

Table 2: Outcomes of behavioural models for panting, shade-seeking and activity (presented as effect sizes, [95% lower - higher Cis]) for each species. Global models included predictors whether individuals were wild or in captivity (Wild/Captive), T_{air} and the interactions between T_{air} :Wild/Captive. For several species multiple competing models were present and numbered candidate models have been included as well as AIC, $\Delta AICc$ and model weights for comparison. Blank cells indicate predictors were not present in top models. For values in bold 95% CIs do not include zero. CGS = Cape Glossy Starling, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

Species	Response	Candidate models	T_{air}	Wild/Captive	T_{air} : Wild/Captive	AICc	$\Delta AICc$	Weight
CGS	Pant	1	0.35 [0.29-0.42]			473.5	0.00	0.50
		2	0.35 [0.28-0.42]	-0.47 [-1.31-0.32]		474.2	0.62	0.37
	Shade	1	0.08 [0.05-0.12]	-1.58 [-2.55--0.65]		508.4	0.00	0.54
		2	0.07 [0.03-0.12]	-3.93 [-7.94--0.28]	0.07 [-0.03-0.20]	508.8	0.38	0.45
	Activity	1	-0.04 [-0.07--0.00]	1.09 [0.47-1.71]		564.2	0.00	0.59
		2	-0.04 [-0.08-0.00]	1.41 [-2.21-4.92]	-0.01 [-0.12-0.10]	566.2	1.99	0.22
CTD	Pant	1	0.76 [0.61-0.94]	0.66 [-6.44-15.92]		327.6	0.00	0.43
		2	0.76 [0.61-0.94]			327.9	0.33	0.37
		3	0.81 [0.61-1.05]	4.72 [-6.44-15.92]	-0.11 [-0.40-0.19]	329.1	1.50	0.20
	Shade		-0.04 [-0.13-0.05]	-10.72 [-15.57--6.48]	0.30 [0.16-0.45]	296.8	0.00	1.00
	Activity	1		2.66 [1.96-3.42]		429.0	0.00	0.47
		2	0.04 [-0.04-0.12]	5.71 [2.31-9.28]	-0.09 [-0.20-0.01]	429.6	0.65	0.34
3		-0.01 [-0.06-0.04]	2.67 [1.97-3.45]		430.8	1.81	0.19	
FCL	Pant	1	0.21 [0.12-0.31]	-0.76 [-1.53-0.05]		237.3	0.00	0.46
		2	0.17 [0.08-0.29]	-5.16 [-13.59-2.23]	0.12 [-0.08-0.35]	238.0	0.71	0.32
		3	0.23 [0.14-0.33]			238.7	1.44	0.22
	Shade		0.07 [0.04-0.11]	-4.09 [-6.58--1.76]	0.08 [0.01-0.16]	844.4	0.00	0.86
	Activity	1	0.03 [-0.00-0.05]			925.4	0.00	0.40
		2				926.6	1.01	0.24
3		0.02 [-0.00-0.05]	-0.12 [-1.32--0.66]		927.4	1.96	0.15	

Table 2 continued.

Species	Response	Candidate models	T_{air}	Wild/Captive	T_{air} : Wild/Captive	AICc	$\Delta AICc$	Weight	
WBS	Pant	1	0.52 [0.39-0.66]			269.6	0.00	0.58	
		2	0.52 [0.39-0.66]	-0.16 [-1.27-0.86]		271.5	1.95	0.22	
	Shade	1	0.12 [0.10-0.18]	1.21 [0.29-2.13]		527.7	0.00	0.64	
		2	0.14 [0.10-0.18]	2.42 [-0.169-6.43]	-0.04 [-0.16-0.09]	529.4	1.68	0.28	
	Activity	1				375.2	0.00	0.44	
		2		0.47 [-0.06-1.40]		376.4	1.17	0.25	
		3	0.01 [-0.05-0.03]			377.1	1.86	0.17	
	SOW	Pant	1	0.33 [0.23-0.44]			324.2	0.00	0.45
			2	0.27 [0.17-0.40]	-7.67 [-18.84-1.48]	0.20 [-0.05-0.49]	325.1	0.85	0.29
3			0.32 [0.22-0.43]	-0.45 [-1.49-0.54]		325.4	1.16	0.25	
Shade		1	0.11 [0.08-0.13]	0.26 [-0.07-0.59]		967.6	0.00	0.44	
		2	0.11 [0.08-0.13]			968.0	0.43	0.36	
		3	0.10 [0.07-0.13]	-0.30 [-2.01-1.37]	0.02 [-0.03-0.07]	969.2	1.58	0.20	
Activity		1	-0.03 [-0.06--0.01]			1129.9	0.00	0.33	
		2	-0.03 [-0.06--0.01]	-0.33 [-0.82-0.14]		1129.9	0.08	0.32	
		3	-0.04 [-0.07--0.02]	-1.46 [-3.12-0.18]	0.04 [-0.01-0.09]	1130.0	0.11	0.31	
NAD		Pant	-	-	-	-	-	-	-
		Shade	1	0.20 [0.16-0.25]	-2.11 [-2.84--1.38]		553.2	0.00	0.66
			2	0.21 [0.07-0.13]	-0.11 [-2.01-1.37]	-0.06 [-0.19-0.09]	554.5	1.32	0.34
	Activity	1	-0.03 [-0.06-0.01]	1.11 [0.50-1.71]		805.1	0.00	0.42	
		2		1.11 [0.49-1.71]		805.4	0.28	0.37	
		3	-0.03 [-0.06-0.01]	-0.40 [-4.54-3.52]	0.05 [-0.07-0.17]	806.5	1.44	0.21	

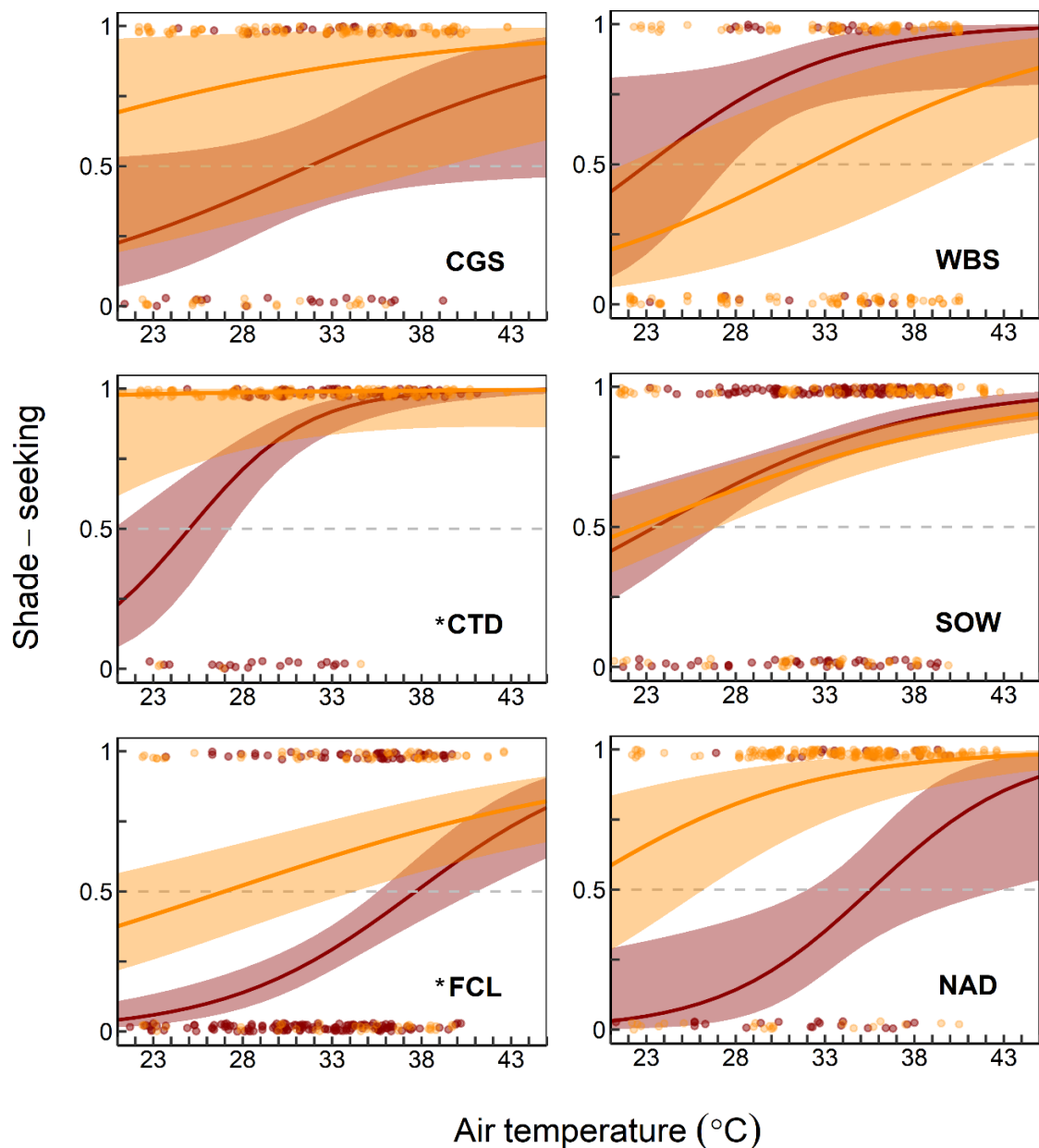


Figure 2: Proportion of observations of birds in shaded refugia with increasing T_{air} in captive (orange circles) and wild (red-brown circles) populations, modelled separately. The intersection point of horizontal grey dashed lines with plotted models represents the T_{air} at which 50% of observed individuals are displaying shade-seeking behaviour. There was no adjustment of shade-seeking with increasing T_{air} in captive CTD. Several species sought shade at higher T_{air} in free-ranging populations compared to in captivity (CGS, FCL, NAD). Only WBS sought shade at higher T_{air} captivity than in the wild. * Species where the interaction between T_{air} and captivity was significant (Table 2). Shade usage in FCL begins at lower T_{air} and increases more slowly in captivity compared to a rapid increase at higher T_{air} in wild conspecifics, CTD are similar except birds remained in the shade throughout the whole day in captivity. CGS = Cape Glossy, WBS = White-browed Sparrow-Weaver, CTD = Cape Turtle-Dove, SOW = Sociable Weaver, FCL = Fawn-coloured Lark, NAD = Namaqua Dove.

3.4.2 Shade-seeking

Shade-seeking behaviour changed in captivity for all species except SOW. Captive birds had a higher probability of being observed in the shade at any given temperature than wild birds, except for WBS, which showed the opposite pattern (Table 2, Figure 2, Figure 3b). In FCL and CTD, rate of increase of shade-use with temperature was different in captivity vs in the wild. CTD remained in the shade over the majority of T_{air} s in captivity but not in the wild and FCL gradually increased shade usage as T_{air} increased in captivity compared to a much more rapid increase in free-ranging birds.

3.4.3 Activity

The only two species to change activity significantly with increasing T_{air} were SOW and CGS (Table 2). However, overall activity differed between free-ranging and captive

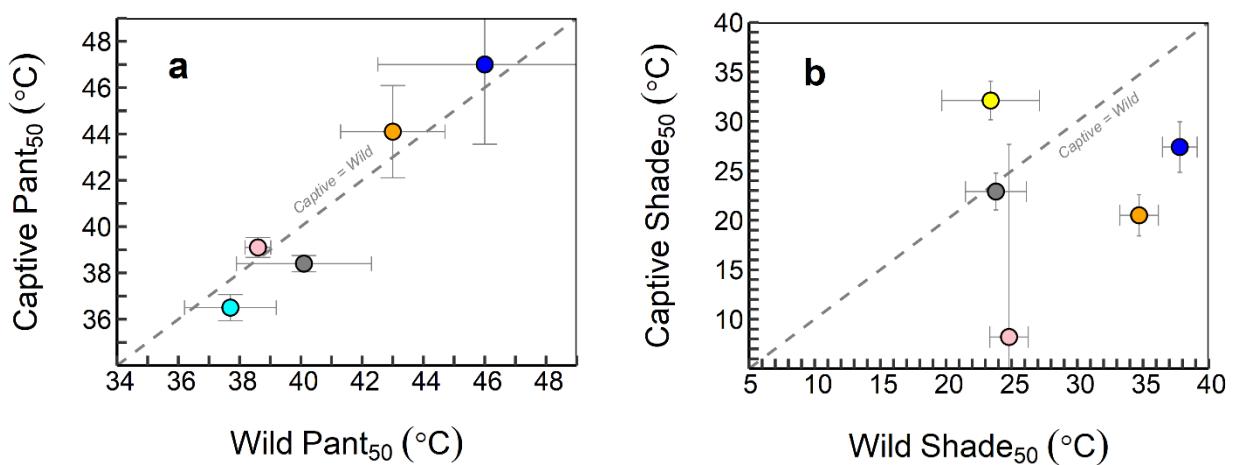


Figure 3: Comparisons of wild and captive pant₅₀ and shade₅₀ with standard error indicated by bars. Values falling on the dashed line indicate values in captivity equalled values in the wild. Models calculating panting thresholds for NAD were not significant due to a lack of gular fluttering observations. Shade-seeking thresholds for CGS in the wild were not significant and could not be compared to those for captive birds. Values available in Table 3 Appendix. CGS = Cape Glossy Starling (cyan), WBS = White-browed Sparrow-Weaver (yellow), CTD = Cape Turtle-Dove (pink), SOW = Sociable Weaver (grey), FCL = Fawn-coloured Lark (blue), NAD = Namaqua Dove (orange).

populations for CGS, CTD and NAD. Captive individuals of these species were less active overall than free-ranging conspecifics (Table 2).

3.5 Discussion

Overall activity was reduced in captivity in only three of six species (CGS, CTD, NAD) relative to wild conspecifics; but only two species (SOW and CGS) showed significant differences between hotter and cooler conditions. Furthermore, none of the six species adjusted panting behaviour in captivity relative to wild conspecifics. Yet, the majority of the study species did adjust shade-seeking behaviour in captivity as predicted, with most seeking shade at lower T_{air} compared to free-ranging conspecifics, and two species showing different rates of increase in shade use with T_{air} in captivity: captive (but not wild) CTD heavily used shade across all T_{air} , and FCL showed a slower rate of increase in shade use with increasing T_{air} in captivity than in the wild.

The reduction in activity by CGS, CTD, NAD in captivity was not surprising, particularly as two of these species were doves. Doves are well known for travelling distances rapidly to obtain food and water (Bartholomew & Dawson 1954, Maclean 1968, Knight 1989, Bucher & Bocco 2009). With these resources freely available in captivity, heat loads gained by actively searching for resources were reduced and activity was noticeably lower. The lower activity of captive CGS also likely reflects the absence of food and water constraints.

However, the remaining three species (all seed-eating omnivorous passerines; FCL, WBS, SOW) did not adjust activity in captivity despite freely available resources. The fact that differences among passerines persist (CGS being a frugivorous omnivore), and that seed eating omnivores specifically appear to not adjust activity with captivity, suggest this may be linked to diet. However, many species inhabiting arid environments face trade-

offs between foraging and thermoregulation (Cunningham *et al.* 2013, 2015; Du Plessis *et al.* 2012, van de Ven *et al.* 2019). When food resources are limited, as during dry periods in arid environments, foraging effort can remain constant as T_{air} increases, though foraging efficiency often declines (Bednekoff & Houston 1994, Du Plessis *et al.* 2012, van de Ven 2019). This is reflected by the fact that only two species reduced activity with increasing T_{air} (SOW, CGS). However, with resources freely available, reductions in the time spent foraging should be expected. For example, Tieleman & Williams (2002*b*) demonstrated a 13-29% decrease in foraging time in food-supplemented free-ranging hoopoe larks (*Alaemon alaudipes*). Thus, the reasons for apparent species-specific differences in reductions, or not, of activity in captivity compared to the wild remain unclear. The possibility that high levels of activity in captivity compared to wild conspecifics were driven by territoriality and defence of feeding points in the aviary cannot be excluded. This could particularly be the case for normally non-social FCL and aggressive WBS.

With the exception of WBS, my study species sought out shade at lower T_{air} in captivity than in the wild. This, again, likely reflects *ad libitum* resource availability in the aviaries and that foraging effort could be reduced or limited to shaded areas to minimize solar heat gain. The higher proportion of observations of WBS in the sun in captivity, in addition to maintained activity, likely reflects elevated stress, as this is a highly territorial species and intraspecific aggression in the aviaries was obvious, meaning individuals were likely excluded from preferred shaded locations. The high intraspecific aggression of this species has been documented in several studies investigating the effects of social instability and territorial intrusion on hormone production and general behaviour in WBS (Colias & Colias 1978, Wingfield *et al.* 1992, Wingfield & Lewis 1993). Telemetric measurements of T_b confirmed that WBS in captivity maintained higher modal T_b (Thompson *et al.* 2018) than free-living conspecifics (Smit *et al.* 2013), most likely due to stress-induced hyperthermia [SIH; elevated T_b in response to stress (Bakken *et al.* 1999,

Carere *et al.* 2001, Keeney *et al.* 2001)] caused by agonistic interactions in captivity (see Cunningham *et al.* 2017). It must also be noted that the thermal environment likely differed between captive and wild environments. Radiative gains and losses as result of differences in habitat structure in the aviaries, and differences in convective heat transfer resulting from materials used in constructing the aviaries would differ to those experienced by wild birds. This could also explain some of the differences in behaviour observed (e.g. higher proportion of observations of WBS in the sun in captivity due to higher radiative heat gain under corrugated steel roof). However, responses are not consistent among species, as all other species sought shade at lower T_{air} in captivity which further complicated interpretation.

My prediction that panting thresholds increase under captive conditions was based on the premise that decreases in activity associated with captive conditions would reduce heat loads and thus evaporative cooling requirements (Dawson 1954, Wolf 2000, Angilletta *et al.* 2010; Boyles *et al.* 2011a, Wolf 2000, Calder *et al.* 1968, Goldstein 1984). The fact that panting behaviour was consistently modulated by T_{air} and did not change in captive compared to wild conspecifics across all species could instead suggest that panting behaviour is mostly dependent on T_{air} , and less on activity. Therefore, the lack of adjustments in panting may suggest that some aspects of behavioural thermoregulation are unaffected by captivity. Unpicking the interactions driving behavioural decisions under free-ranging conditions is notoriously difficult (Tieleman & Williams 2002a). Panting behaviour links directly to avian physiology and is tightly linked to EWL and the trade-off between maintaining T_b and an adequate hydration status. The absence of differences in panting behaviour in captivity compared to the species-specific differences I observed in activity and shade-seeking behaviours is certainly noteworthy. The behavioural adjustments of activity and shade-seeking related to complex trade-offs (associated with,

for example, foraging and social behaviour) are affected in numerous ways by factors that may differ in captive and wild scenarios. Therefore, the distinct lack of differences between captive and wild populations in the temperature-dependence of panting responses has far-reaching consequences for the research potential of captive populations. Panting behaviour is easily observable and data can readily be collected in research facilities. Such data could be particularly useful for modelling water and energy balance in species which cannot be studied in the wild. Further, there are also implications for previous studies conducted in captivity and extrapolating these findings to wild populations.

My study also represents an important step towards better understanding species-specific thermoregulatory patterns, especially under climate warming. Understanding the implications of predicted rises in T_{air} and water stress on T_{b} requires studies where resources and T_{air} can be manipulated (i.e. captive studies). That a behaviour (panting) so tightly linked to underlying physiological processes (EWL) is so consistent among captive and wild populations, compared to other behaviours such as shade-seeking which are impacted by complex behavioural trade-offs (Cunningham *et al.* 2021), has important implications. If physiologically constrained thermoregulatory behaviours are consistent between captive and wild populations, it is possible that adjustments in other physiological thermoregulatory variables observed in captivity persist in free-ranging populations too. This can have broader relevance for other phenomena described in captive populations. For example, parent zebra finches (*Taeniopygia guttata*), which acoustically signal high ambient temperature to their embryos in captivity (Mariette & Buchanan 2016), have also been demonstrated to do so in the wild (Mariette *et al.* 2018). Together with our results this is promising for extrapolating captive findings to wild populations, particularly for physiologically constrained processes less dictated by trade-offs in other behavioural adjustments.

To conclude, I found that panting behaviour did not differ between captive and wild populations of six species occurring in the Kalahari Desert of southern Africa. For behaviours which did differ in captivity (i.e. shade-seeking), these differences were mostly consistent between species. Therefore, interspecific variation in behaviour in captive populations can provide valuable information about free-ranging populations but should be interpreted with caution when clear-cut links to underlying physiology cannot be established.

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3.7 Appendix

Table 3: Behavioural thresholds for panting (Pant₅₀), shade-seeking (Shade₅₀) and activity (Active₅₀) for six bird species (Spp.) in captivity and in the wild, modelled separately. Model slopes are also included for comparison. Species models which were not significant are indicated by a dash (-).

Spp.	Panting						Shade-seeking						Activity					
	Pant ₅₀				Slope		Shade ₅₀				Slope		Active ₅₀				Slope	
	Captive	Captive SE	Wild	Wild SE	Captive	Wild	Captive	Captive SE	Wild	Wild SE	Captive	Wild	Captive	Captive SE	Wild	Wild SE	Captive	Wild
CGS	36.5	0.6	37.3	1.5	0.34	0.39	14.1	5.1	-	-	0.09	-	-	-	-	-	-	-
WBS	38.4	0.3	40.3	2.2	0.56	0.34	32.1	2.0	19.9*	3.7	0.12	0.1	-	-	-	-	-	-
CTD	39.1	0.4	39.0	0.4	0.84	0.71	8.2	19.5	24.2	1.5	0.09	0.15	-	-	-	-	-	-
SOW	44.6	2.0	44.5	1.7	0.27	0.28	22.9	1.9	25.1	2.3	0.11	0.12	27.1	4.7	-	-	-0.04	-
FCL	46.0	3.4	46.3	3.5	0.18	0.24	27.4	2.6	37.1	1.3	0.08	0.15	-	-	-	-	-	-
NAD	-	-	-	-	-	-	20.5	2.1	33.9	1.5	0.16	0.12	-	-	-	-	-	-

* Value approached significance CI: -0.01 - 0.22

CHAPTER 4 Can a behavioural index be used to assess species' relative vulnerability to global heating?

4.1 Abstract

Assessing species vulnerability to global heating is complex and accurate predictions often require the integration of several species-specific factors relating to exposure and sensitivity. I investigated the potential of a behavioural index, pant_{50} (the air temperature at which 50% of individuals within a population will engage in panting behaviour), to predict vulnerability of avian species to climate change on the basis of physiological tolerances. Birds increase evaporative water loss (EWL) at high air temperatures (T_{air}) in order to maintain body temperature (T_b) at sublethal levels. Panting facilitates EWL in birds, making pant_{50} an ideal candidate to link underlying physiological processes to easily observed behaviour. To evaluate whether panting behaviour is correlated with thermoregulatory variables, I examined whether pant_{50} predicted underlying physiological changes related to hydration status and hyperthermia, using data extracted from physiological and behavioural studies of 20 arid-zone bird species from five orders. Unexpectedly, I found no functional links between pant_{50} and any physiological variables linked to thermoregulation and heat tolerance, with null models included in top model sets for all analyses. These findings provide no support for the notion that pant_{50} would be correlated with species-specific changes in thermal physiology during exposure to hot weather. However, as panting behaviour can also correlate with reductions in foraging efficiency, the usefulness of

part₅₀ in predicting vulnerability of species to consequential sublethal fitness costs of rising temperature warrants further investigation.

Keywords: validation, behavioural index, thermal physiology, behaviour, heat tolerance, species vulnerability

4.2 Introduction

Assessing species vulnerability to rising temperatures is complex and requires the integration of factors that determine both the exposure and sensitivity of individual species to climate change (Williams *et al.* 2008). Intrinsic organismal traits (physiology and ecology) determine species sensitivity, whereas exposure is determined by extrinsic factors such as habitat effects and regional climate changes and individual behavioural adjustments. Therefore, data across a wide range of traits and environmental variables must be incorporated to quantify among- and within-species vulnerability (Williams *et al.* 2008).

Models of the impact of climate change on organisms have largely focused on two approaches that represent the ends of a continuum and yield predictions at different scales. Single-species models based on in-depth physiological and behavioural studies provide detailed predictions but for only a focal species (Kearney *et al.* 2008, Wakefield *et al.* 2011, Briscoe *et al.* 2014, Kearney *et al.* 2009), whereas general correlative models can produce predictions for entire communities (Erasmus *et al.* 2002, Thomas *et al.* 2004). Bioclimatic envelope models (BEMs) for example, use species' current climate envelopes and global climate trends to predict large-

scale changes in species distribution to regions where climate will be suitable in future and have gained traction as a tool for predicting climate change responses (Lawler *et al.* 2009, Araújo *et al.* 2006, Thomas *et al.* 2004). In comparison, detailed mechanistic models, which predict niches based on detailed species-specific parameters, incorporate comprehensive mechanistic data concerning ecology, behaviour and physiology. Despite the intensive, time-consuming sampling often required for parameterisation of mechanistic models, these provide more precise predictions about how individual species should respond to changes in climate (Briscoe *et al.* 2016, Kearney *et al.* 2009).

Desert environments are expected to become warmer and drier faster than other terrestrial regions (IPCC 2014) and the capacity for greater heat tolerance to evolve among desert-dwelling taxa to buffer these effects is largely unknown (Blackburn *et al.* 2014). In these environments, high environmental temperatures impose severe constraints on foraging, the maintenance of water and energy balance and keeping body temperature (T_b) at sub-lethal levels (Wolf 2000, Goudie & Piatt 1990). For desert animal communities, examining physiological consequences of residing in hot, arid environments is therefore necessary to accurately predict climate change impacts on the abundance and distributions of species as both are physiologically constrained (Kearney & Porter 2009). This makes hot, arid regions ideal model systems for testing novel ways to predict vulnerability to increasing temperatures.

To effectively and rapidly assess the vulnerability of desert communities to rising temperatures, novel modelling approaches are needed to bridge the gap between predictions provided by species-specific mechanistic models and more generalised correlative models yielding predictions for entire communities. The success of birds

in desert environments as well as their easily observable diurnal behaviour makes them ideal to develop these modelling approaches, hence my focus on this group.

Quantifying microclimates throughout thermal landscapes and obtaining physiological data on heat tolerance is a time-consuming and expensive process. However, observing avian heat dissipation behaviour (e.g. panting/gular fluttering, shade-seeking, wing drooping and reductions in activity) is comparatively simple and requires only minimal equipment. Panting and gular flutter, in particular, increase the rate of evaporative water loss (EWL) through the respiratory tract to maximise heat loss rate and, under very hot conditions ($T_{\text{air}} > T_{\text{b}}$), become the main avenue of heat transfer to maintain T_{b} below lethal limits (Wolf 2000). As such, these easily observed heat dissipation behaviours provide an opportunity to link behavioural responses of birds to underlying physiological processes. This could potentially provide a means to quickly and efficiently assess species vulnerabilities across avian communities using an approach with a strong mechanistic foundation.

Recent investigations of a behavioural index, pant_{50} (the T_{air} at which 50 % of the observed individuals of a given species are panting/gular fluttering to dissipate heat) reveal large systematic variation in this metric among species (Smit *et al.* 2016, Pattinson *et al.* 2020). Studies on southern African desert avifauna have shown that pant_{50} varies predictably with drinking ecology, body mass and activity (Smit *et al.* 2016). Further, thermal physiology can vary with ecological traits. For example, passerines that depend on drinking surface water have a higher evaporative cooling capacity and heat tolerance compared to occasional/non-drinking species, which balance their water budgets using dietary and metabolic water (Czenze *et al.* 2020).

However, more recent work demonstrates that at a global scale, interspecific variation in pant_{50} is poorly predicted by these three organismal traits, as well as

foraging guild and diet (Pattinson *et al.* 2020). Furthermore, in a recent study of nine Kalahari species from three orders, I found considerable interspecific variation in thermal physiological parameters (T_b patterns and hyperthermia use at high T_{air}) that surprisingly did not correlate with $pant_{50}$, suggesting that the behavioural index is not a good indicator of physiological responses to heat (Thompson *et al.* 2018). In addition, for the same limited sample of birds, responses to short-term dehydration also appeared to be species-specific and did not correlate with $pant_{50}$ (Chapter 2). In order to conclusively determine whether $pant_{50}$ is potentially useful as a tool to predict vulnerability to climate change on the basis of physiological tolerances, we therefore need to assess relationships between $pant_{50}$ and a wider range of physiological parameters from a larger number of taxa.

Regardless of any links to underlying thermal physiological parameters, the necessity to offload heat via panting/gular fluttering when T_{air} is high has far reaching consequences that impact reproduction, body condition and survival (Du Plessis *et al.* 2012, Van de Ven *et al.* 2019, 2020). There are strong links between needing to dissipate more heat at higher T_{air} and reduced foraging efficiency or effort, resulting in body mass loss, reductions in nestling growth rates and lowered breeding success above certain T_{air} thresholds. These costs potentially pose a greater threat to arid-zone avian diversity than acute exposure to lethal conditions, at least in southern Africa (Conradie *et al.* 2019). Therefore, it seems clear that species' vulnerability to rising temperatures may not be solely determined by physiological traits.

The aim of this study was to combine $pant_{50}$ data with comprehensive physiological data collected through laboratory studies to determine whether $pant_{50}$ behavioural index provides a reliable indicator of underlying physiological changes and thus, be useful for assessing species' relative vulnerability to rising T_{air} . Further,

in light of the recent emergence of drinking dependency as a strong predictor of evaporative scope and heat tolerance (Czenze *et al.* 2020), taken together with previously identified differences in pant_{50} between drinkers and non-drinkers in the Kalahari (Smit *et al.* 2016, Pattinson *et al.* 2020), I examined whether relationships between pant_{50} and various physiological variables varied with drinking dependency. As panting is tightly linked to evaporative cooling, I predicted that species' pant_{50} is strongly correlated with increases in rates of EWL. *A priori*, one might expect species that begin panting at lower T_{air} (i.e. with low pant_{50}) to be less effective at dissipating heat at very high T_{air} s when heat and dehydration tolerance limits are approached, exhibiting lower evaporative scope and rates of maximum EWL. However, water-dependant species in dry areas have lower pant_{50} s (Orolowitz 2020), but higher heat tolerance limits (HTL) and evaporative scopes than water-independent species (Czenze *et al.* 2020), leading to an alternate prediction of a negative relationship between pant_{50} and these physiological variables. I would also expect species with low pant_{50} to begin increasing T_b at low T_{air} and consequently, when deprived of water to lose a higher percentage of their body mass as EWL. This would likely mean that these species would reach their heat tolerance limits at lower T_{air} if dehydrated. Species which delay the onset of panting to higher T_{air} , with high pant_{50} , are likely to need to dissipate heat rapidly once they begin panting and hence may be expected to show more rapid increases in EWL with increasing T_{air} .

4.3 Methods and Materials

4.3.1 Study species and site

To examine whether avian heat dissipation behaviour is correlated with thermoregulatory variables, I compiled physiological and behavioural data for 20

species from studies conducted in the Kalahari Desert in South Africa over the past decade. Birds inhabiting the Kalahari, which falls into the arid savannah biome, experience low annual rainfall (~ 200 mm) and high summer air temperatures (average maximum summer $T_{\text{air}} \sim 38 \text{ }^{\circ}\text{C}$). As many bird species are resident in the Kalahari year-round, this makes it the ideal site to investigate avian thermoregulation in the heat.

4.3.2 Behavioural data

I investigated specifically whether pant_{50} could be useful in predicting underlying physiological changes related to hydration status and hyperthermia. Therefore, I extracted pant_{50} values from Smit *et al.* (2016) for 17 species, and calculated pant_{50} values for three further species (southern pied babblers (SPB), southern yellow-billed hornbills (SYH) and red larks (REL)) from observational data collected by Bourne 2019, Van de Ven 2017 and Kemp *et al.* (2020), respectively.

Smit *et al.* 2016 ($n = 17$ species) scored panting behaviour as present or absent in instantaneous behavioural observations (Altman 1974). For each species, the inflection T_{air} at which 50% of individuals are panting was determined using binomial logistic regressions (Smit *et al.* 2016). For SPB, instantaneous observations of the presence/absence of panting were taken from known individuals at 20-min intervals while an observer followed habituated individuals (Ridley *et al.* 2008). These data were tested for autocorrelation to ensure data points were independent (AR Bourne, unpublished data). For SYH (Van de Ven 2017) and REL (Kemp *et al.* 2020), data were available from continuous 20-min observations of focal individuals, however observations within each 20-min focal were autocorrelated. Therefore, I randomly selected one observation during each 20-min focal to represent an instantaneous

observation comparable to data collected by Smit *et al.* 2016. For calculations of pant_{50} for SPB, SYH and REL, I included individual as a random factor in binomial regressions. I then examined whether these pant_{50} values were correlated with physiological variables relating to body temperature, evaporative water loss and heat tolerance for the 20 species (obtained from literature listed in Table 1) using simple linear regression models fitted in R (R Core Team 2019).

4.3.3 *Physiological data*

Physiological variables obtained from the literature (Table 1) were all measured by quantifying gas exchange and body temperature using a flow-through respirometry system (for full methodology see literature listed in Table 1). Birds were placed in sealed, plastic chambers through which dry air of known composition was pumped. Birds were then exposed to stepped air temperature profiles whilst activity was monitored on an infra-red video camera. By subsampling excurrent air from the chamber, EWL and carbon dioxide production was measured (Whitfield *et al.* 2015). From these data maximum and minimum thermoneutral resting metabolic rates (**Max RMR**, **Min RMR**) and thus metabolic cost index (**Max RMR/Min RMR**), as well as maximum and minimum thermoneutral EWL (**Max EWL**, **Min EWL**) and evaporative scope (**Max EWL/Min EWL**) could be calculated. The mass-specific EWL slope (**MS EWL slope**) was estimated by fitting a linear model to examine the response of MS EWL to increasing T_{air} .

In these studies body temperature for each individual was measured by injecting a temperature-sensitive passive integrated transponder (PIT) tag (Biomark, Boise, ID, USA) into the abdominal cavity of the bird (Whitfield *et al.* 2015). The T_{air} at which T_{b} began to increase (**T_{air} of T_{b} inflection**) was determined for each

Table 1: Physiological and behavioural data for 20 species collected in the Kalahari Desert of South Africa used for testing whether pant_{50} correlates with species' underlying thermal physiology.

Abb	Species	Order	pant_{50} *	Drink	Mass	T_{air} of T_b inflection	HTL	T_b panting initiated	Max RMR/Min RMR	MS EWL slope	Max EWL resid	Max EWL /Min EWL	EWL as Percentag e Mass	References
			°C		g	°C	°C	°C		g/°C	$\text{g}\cdot\text{g}^{-1}/\text{hr}$		%	
APB	Acacia pied barbet	Piciformes	34.4*	N	32.4	37.5	50.0	42.1	1.83	4.94	0.11	10.14	19.46	Czenze <i>et al.</i> unpubl. data
BUS	Burchell's starling	Passeriformes	37.9*	Y	109.1	34.2	48.8	41.5	1.29	2.11	-0.07	3.97	20.71	Smit <i>et al.</i> 2018
CTD	Cape turtle dove	Columbiformes	40.3*	Y	147.5	-	56.0	-	2.00	1.59	-0.05	11.25	7.54	McKechnie <i>et al.</i> 2016
CFS	Common fiscal	Passeriformes	41.3*	N	37.3	35.8	48.7	42.9	1.73	9.12	0.21	11.69	19.93	Czenze <i>et al.</i> 2020
CBS	Crimson-breasted shrike	Passeriformes	38.4*	N	46.2	35.5	48.0	40.8	1.86	6.06	-0.07	8.30	14.41	Czenze <i>et al.</i> 2020
FCL	Fawn-coloured lark	Passeriformes	46.0*	N	23.0	34.8	48.5	43.2	1.42	3.91	-0.13	6.15	17.29	Czenze <i>et al.</i> 2020
FTD	Fork-tailed drongo	Passeriformes	42.6*	N	43.7	31.3	50.0	39.6	2.11	4.81	0.01	8.50	7.57	Czenze <i>et al.</i> 2020
LAD	Laughing dove	Columbiformes	39.1*	Y	89.4	-	58.0	-	1.98	2.03	-0.03	7.94	9.75	McKechnie <i>et al.</i> 2016
LBR	Lilac-breasted Roller	Coraciiformes	40.4*	N	94.5	36.7	53.4	41.3	1.45	3.57	0.09	5.89	7.20	Smit <i>et al.</i> 2018
MAF	Marico flycatcher	Passeriformes	41.8*	N	21.8	34.7	46.5	42.5	1.31	4.13	-0.15	5.53	13.10	Czenze <i>et al.</i> 2020
NAD	Namaqua dove	Columbiformes	46.0*	Y	37.1	-	60.0	-	1.30	2.59	0.10	15.73	9.23	McKechnie <i>et al.</i> 2016
REL	Red lark	Passeriformes	38.1	N	38.3	36.2	50.0	41.9	1.71	2.09	-0.11	6.17	13.80	Czenze <i>et al.</i> 2020
REB	Red-eyed bulbul	Passeriformes	33.9*	Y	30.1	40.1	52.0	41.6	1.91	5.65	0.22	11.86	25.33	Czenze <i>et al.</i> 2020
SFF	Scaly-feathered finch	Passeriformes	41.4*	N	10.4	-	48.0	-	1.29	4.52	-0.15	10.80	13.60	Whitfield <i>et al.</i> 2015
SOW	Sociable weaver	Passeriformes	42.2*	Y	24.9	-	52.0	-	1.34	5.05	0.07	18.41	16.12	Whitfield <i>et al.</i> 2015
SPB	Southern Pied babbler	Passeriformes	44.1	Y	69.9	41.4	52.0	42.1	1.78	3.00	-0.01	10.43	11.33	Cunningham <i>et al.</i> unpubl. data
SHL	Spike-heeled lark	Passeriformes	35.9*	N	25.0	33.8	50.4	43.6	1.37	4.40	0.00	8.38	12.45	Czenze <i>et al.</i> 2020
WBM	White-backed mousebird	Coliiformes	34.5*	Y	36.2	35.5	48.0	42.0	1.82	3.32	-0.09	9.19	15.70	Czenze <i>et al.</i> unpubl. data
WBS	White-browed sparrow-weaver	Passeriformes	41.7*	Y	39.4	-	54.0	-	1.59	4.02	0.12	15.98	14.86	Whitfield <i>et al.</i> 2015
SYH	Southern Yellow-billed Hornbill	Bucerotidae	38.4	N	218.9	31.9	50.5	40.8	1.92	2.86	-0.08	14.00	4.93	van Jaarsveld <i>et al.</i> 2021

* Pant_{50} values extracted from Smit *et al.* 2016

species by fitting broken-stick regressions to these data. Activities such as the initiation of panting (**T_b panting initiated**) and any signs of stress were noted. Measurements were terminated and birds were removed from the respirometry chamber if they exhibited prolonged escape behaviour (jumping, pecking and/or wing flapping) or if clearly distressed (loss of coordination/balance or a sudden drop in EWL, RMR and/or an uncontrolled increase in T_b). A bird was considered to have reached its upper limit of heat tolerance at the T_{air} associated with the onset of these signs of heat stress and/or if T_b increased uncontrollably. This was considered the heat tolerance limit (**HTL**) of the individual (Whitfield *et al.* 2015).

In addition, I used EWL data extracted from the studies summarised in Table 1 to model water requirements, by predicting EWL over hourly intervals to obtain estimates of cumulative EWL on the hottest day measured during the 2015 Kalahari field season (as had been previously calculated for a subset of nine of the 20 species; Chapter 3). I expressed these cumulative water requirements as a percentage of body mass lost as EWL during an 8-h period during the hottest day experienced (**EWL as Percentage Mass**).

4.3.4 Data analysis

Czenze *et al.* (2020) recently showed that passerines which drink surface water display greater evaporative scope and higher HTLs than those which obtain all their water from food. I therefore including drinking dependence alone and in interaction with each physiological predictor variable in models of pant_{50} . As data were missing for physiological variables related to T_b (T_{air} of T_b inflection, T_b panting initiated) for six species (see Table 1), I fitted two separate candidate model sets, and compared models within them using Akaike's Information Criterion, adjusted for small samples

(Symonds & Moussalli 2011, Harrison *et al.* 2018). Firstly, I omitted the two variables for which data were missing and compared a model set of the null model, drinking dependency, each physiological variable alone and each variable and the interaction with drinking dependency ($n = 20$ species). Secondly, I omitted six species for which data was missing and included all physiological variables and compared outputs of the same model set as mentioned above ($n = 14$ species).

I also examined my dataset for differences in evaporative scope and HTL among the 13 passerines compared to values reported by Czenze *et al.* 2020. I used simple linear models with drinking/non-drinking as a predictor and evaporative scope and HTL as response variables.

I report results in text as (LM: estimate [L95%CI – U95%CI]). Where multiple models were within $\Delta AICc = 2.0$ of the top model, top model sets were averaged using the package MuMin (Barton 2015) and parameter estimates after model averaging were presented for interpretation (Burnham & Anderson 2002, Grueber *et al.* 2011). For all analyses, I visually inspected residuals to ensure model assumptions were met. Statistical significance of the effects of retained predictor variables was inferred if 95% confidence intervals (CIs) excluded zero.

4.4 Results

Pant₅₀ was not correlated with drinking dependency or any of the physiological variables examined (Figure 1), with the null model included in the top model set for the analysis including all 20 species (null model $\Delta AICc = 0.06$; $n = 20$, Table 2A), 2A), and representing the top model for the analysis including the subset of species for which additional T_b variables were available ($n = 14$ species, Table 2B).

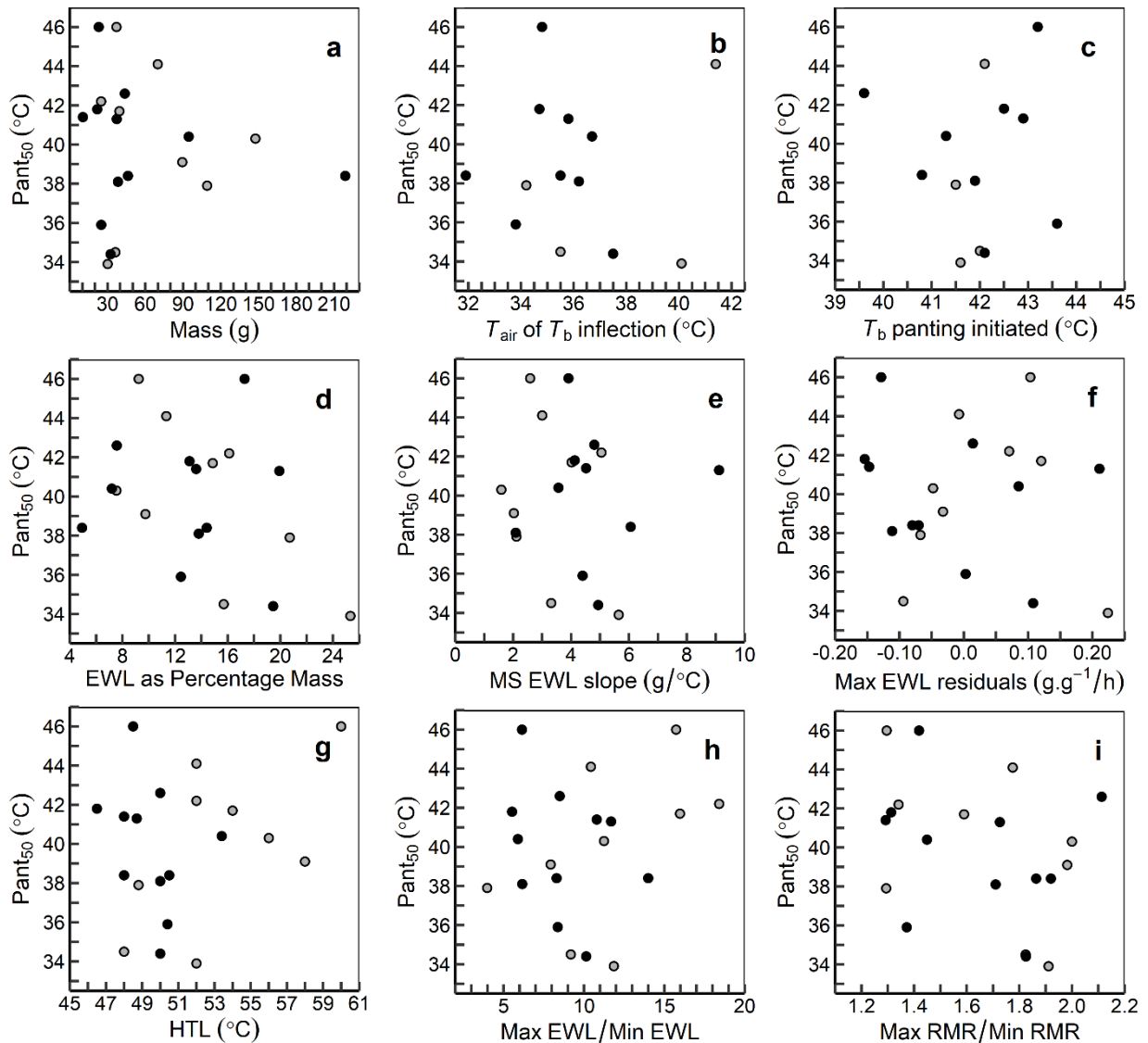


Figure 1: $Pant_{50}$ was not correlated with any of the physiological variables I included in the analysis. I used the residuals of the log linear relationship of Max EWL against body mass (to account for differences in EWL with body mass), in the $Pant_{50}$ analysis of Max EWL. Drinking dependent species are indicated using black filled circles and non-drinking species by grey filled circles.

Cumulative EWL as a percentage of body mass, metabolic cost index (Max RMR/Min RMR) and HTL were included in competing models within $\Delta AICc = 2$ for analysis of all 20 species (Table 2A). However, these variables had no significant relationships with $Pant_{50}$ after model averaging (95% CIs include 0 in all cases; Table

Table 2: Top GLMM models predicting pant₅₀ for all analyses, ranked by AICc. Models within $\Delta AICc < 2$ were considered competing models. Significance is inferred if 95% CIs (LCI-UCI) exclude zero.

A. Candidate model set excluded physiological variables T_{air} of T_b inflection and T_b panting initiated, $n = 20$ species

	AICc	$\Delta AICc$	Weight
pant ₅₀ ~ Percent EWL of Mass	111.09	0.00	0.18
pant ₅₀ ~	111.15	0.06	0.18
pant ₅₀ ~ Max RMR/Min RMR	111.51	0.42	0.15
pant ₅₀ ~ HTL	112.67	1.57	0.08

Effect size of explanatory variables after model averaging

	Estimate	SE	LCI	UCI
Percent EWL of Mass	-0.25	0.16	-0.56	0.07
Max RMR/Min RMR	-4.31	3.02	-10.23	1.62
HTL	0.25	0.25	-0.23	0.73

B. Candidate model set included physiological variables T_{air} of T_b inflection and T_b panting initiated, $n = 14$ species

	AICc	$\Delta AICc$	Weight
pant ₅₀ ~	80.78	0.00	0.25
pant ₅₀ ~ Percent EWL of Mass	82.14	1.37	0.13

Effect size of explanatory variables after model averaging

	Estimate	SE	LCI	UCI
Percent EWL of Mass	-0.24	0.18	-0.61	0.15

Cumulative EWL as a percentage of body mass was also included in a competing model for the analysis of 14 species, but again was not significant after model averaging (Table 2B).

Evaporative scope was significantly higher in drinking passerines compared to non-drinkers (LM: estimate = 5.98 [2.24 - 9.72], $n = 13$) as was HTL (LM: estimate = 3.41 [1.65 – 5.18], $n = 13$).

4.5 Discussion

I found no correlations between pant_{50} and any physiological variables linked to thermoregulation and heat tolerance in arid-zone birds, with null models performing better or competing with top models in all analyses. These findings provide no support for my predictions that pant_{50} could reliably indicate changes in thermal physiology during exposure to hot weather, or heat tolerance limits. There are several potential explanations for this puzzling finding.

First, by including data from orders that vary in their primary avenues of evaporative heat dissipation, I may have obscured within-order patterns. Passerines rely largely on panting for evaporative cooling, a metabolically costly and comparatively inefficient process (Dawson 1982, McKechnie *et al.* 2021a), whereas many non-passerine orders use gular flutter, the rapid movement of the gular membranes driven by pulsation of the hyoid apparatus. Gular flutter provides rapid evaporation at much lower metabolic costs compared to panting (Bartholomew *et al.* 1962). Furthermore, columbiforms also use transdermal cutaneous evaporative water loss to effectively and rapidly dissipate heat at negligible metabolic expense (Dawson & Whittow 2000). To assess whether phylogenetic variation in primary evaporative cooling pathways was driving the results, I repeated analysis on passerines alone. After excluding non-passerines, however, the results remained unchanged (Table S2 & S3). Among southern African passerines, regularly-drinking species that rely on surface water have higher evaporative scope (i.e., maximum

EWL / minimum EWL) and higher heat tolerance limits compared to non-drinking species that balance water budgets using dietary and metabolic water (Czenze *et al.* 2020). Further, in the Kalahari Desert, drinking-dependent species have significantly lower pant_{50} (Smit *et al.* 2016). I therefore predicted that pant_{50} may correlate at an interspecific level with HTL and evaporative scope, with low pant_{50} associated with higher HTL and a greater evaporative scope. The fact that the null models performed better than models including either HTL or evaporative scope and drinking dependency is therefore surprising, particularly within the passerines (Table S2 and S3). My results were consistent with those of Czenze *et al.* 2020 in that drinking passerines exhibited higher HTL and evaporative scope, whereas I found no difference in pant_{50} among drinking and non-drinking passerines. Therefore, while drinking dependence may be functionally related to HTL and evaporative scope among passerines, pant_{50} is apparently not.

Second, although the onset of panting and rapid increases in EWL are widely thought to coincide with the upper critical limit of thermoneutrality (T_{uc}), recent evidence suggests that there may be considerable variation in relationships between T_b , EWL and RMR towards the upper end of the TNZ and at $T_{air} > T_{uc}$. The T_{uc} , the inflection of RMR at the upper boundary of the thermoneutral zone, is thought to represent the energetic cost of heat dissipation mechanisms such as panting (Dawson & Whittow 2000). However, the relatively weak relationship between T_{air} at the onset of panting and the inflection of EWL with T_{uc} among arid-zone passerines from three continents (McKechnie *et al.* 2021) suggests this is not necessarily the case. Further, the inflection T_{air} at which T_b begins to increase is often below the T_{uc} . Therefore, the fact that T_{uc} is a poor indicator of increases in RMR and EWL, and that these variables are only weakly correlated with the T_{air} at the onset of panting

(McKechnie *et al.* 2021) and the T_{air} inflection at which T_{b} increases, suggests that there is considerable variation in the relationship between increases in RMR or EWL and panting behaviour. Given the amount of noise in these relationships, it is unlikely that pant_{50} will accurately reflect physiological heat responses based on these variables.

A third possibility explaining the absence of clear links between pant_{50} and physiological variables arises because I examined pant_{50} independently of other thermoregulatory behaviours. In a subset of nine of the species used in this study, the combination of thermoregulatory behaviours (e.g. reductions in activity, increased shade-seeking and increased panting), and physiological mechanisms (e.g. facultative hyperthermia), birds used to thermoregulate effectively in the heat was clearly species-specific (Thompson *et al.* 2018). Similarly, the order in which these behaviours commenced was also species-specific. Therefore, one would need to understand how each species utilises shade-seeking, wing drooping and reductions in activity to complement pant_{50} in order to accurately use these behaviours to predict when physiological heat dissipation is necessary. In addition, when the captive birds involved in the aforementioned study were subjected to restricted water availability, thermoregulatory responses varied substantially between and even within avian orders, with each species regulating T_{b} using a unique combination of these behavioural and physiological mechanisms to cope with dehydration. As such, although several species delayed panting until higher T_{air} when dehydrated, presumably to conserve water, interpretation of interspecific variation in pant_{50} needs to take place in the context of interactions with other thermoregulatory behaviours, including increased shade-seeking and reductions in activity (Thompson *et al.* 2018) and hydration state (Chapter 2).

Pattinson *et al.* (2020) quantified interspecific variation in heat dissipation behaviour (HDB; including panting, shade-seeking, wing-drooping and activity reduction) across three phylogenetically disparate avian communities inhabiting the Gascoyne Desert in western Australia, the Sonoran Desert in the southern USA and the Kalahari Desert in southern Africa. Although the range of interspecific variation was similar among regions, very little variation in HDB was predicted by organismal traits including body mass, drinking dependency, foraging guild, diet and activity levels. The lack of a relationship between pant_{50} and body mass in the current study is broadly consistent with these findings, as is the absence of any relationship between pant_{50} and drinking dependency. This once again highlights the complexity of interspecific variation in HDB.

In summary, my results here suggest pant_{50} is not suitable as a predictor of avian physiological heat responses or thermal tolerance limits. However, while the risk of exposure of arid-zone avian communities in southern Africa to lethal, acute effects of heat exposure via lethal dehydration or hyperthermia is predicted to remain low in the 21st century (but see McKechnie *et al.*, 2021b), chronic sublethal fitness costs, often associated with missed foraging opportunities due to trade-offs with behavioural thermoregulation at high air temperatures, are predicted to increase dramatically (Conradie *et al.* 2019, Cunningham *et al.* 2021). Sublethal fitness costs include progressive loss of body mass in adults, reduced breeding success, and reduced nestling growth resulting in smaller, lighter fledglings (Du Plessis *et al.* 2012, Cunningham *et al.* 2013, Van de Ven *et al.* 2019, Conradie *et al.* 2019, van de Ven *et al.* 2020). Poor-quality offspring fledged from heat-exposed nesting attempts are likely to be less successful breeders as adults due to the strong correlation between future breeding success and mass at fledging and therefore these effects persist

intergenerationally (Ridley & Raihani 2007, Ghalambor & Martin 2001, Weimerskirch *et al.* 2000). These chronic sublethal costs may be a more pervasive threat to avian diversity in southern Africa's hot, desert environments than acute physiological heat responses and thus, predicting vulnerability to these costs may be more important.

Although less invasive than physiological data collection, collecting data on fitness consequences for species existing in these environments is time-consuming, requiring years of data collected across numerous seasons. $Pant_{50}$ might serve no purpose in predicting vulnerability of avian communities to climate change based on physiological tolerances, but there is a possibility that it may predict vulnerability to the sublethal fitness consequences to rising temperature. Although speculative, preliminary data from Southern Pied Babblers and Southern Yellow-billed hornbills tentatively suggest a correlation between $pant_{50}$ and T_{air} thresholds of diurnal mass gain but many more species will need to be included to elucidate these links.

Therefore, I recommend that future studies examine the value of $pant_{50}$ as predictor of vulnerability to sublethal fitness costs, e.g. via correlations with threshold T_{air} s above which a) food intake is not enough to offset overnight mass loss, b) probability of breeding success drops below 50% and c) impacts on nestling growth and fledging mass are felt, among others. In addition, further research is required to identify T_b thresholds at which panting, shade-seeking and reductions in activity take place, requiring fine scale monitoring of T_b . This could increase the efficiency with which we can predict the impacts of sublethal fitness costs of high T_{air} across avian desert communities, enabling targeted conservation action for the species most vulnerable.

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4.7 Appendix

Table S1: Complete model set outcomes

A. For all species with variables T_{air} of T_b inflection and T_b panting initiated omitted ($n = 20$).

Models	AICc	Δ AICc	Weight
$\text{pant}_{50} \sim \text{Percent EWL of Mb}$	111.09	0.00	0.18
$\text{pant}_{50} \sim$	111.15	0.06	0.18
$\text{pant}_{50} \sim \text{Max RMR/Min RMR}$	111.51	0.42	0.15
$\text{pant}_{50} \sim \text{HTL}$	112.67	1.57	0.08
$\text{pant}_{50} \sim \text{Max EWL/Min Ewl}$	113.22	2.13	0.06
$\text{pant}_{50} \sim \text{Max EWL resid}$	113.72	2.63	0.05
$\text{pant}_{50} \sim \text{Mb}$	113.78	2.69	0.05
$\text{pant}_{50} \sim \text{Drink}$	113.79	2.69	0.05
$\text{pant}_{50} \sim \text{MS EWL slope}$	113.87	2.78	0.05
$\text{pant}_{50} \sim \text{Drink : Max RMR/Min RMR}$	113.90	2.81	0.05
$\text{pant}_{50} \sim \text{Drink : Percent EWL of Mb}$	114.23	3.14	0.04
$\text{pant}_{50} \sim \text{Drink : HTL}$	115.81	4.72	0.02
$\text{pant}_{50} \sim \text{Drink : Max EWL/Min EWL}$	115.99	4.90	0.02
$\text{pant}_{50} \sim \text{Drink : Max EWL resid}$	116.25	5.16	0.01
$\text{pant}_{50} \sim \text{Drink : Mb}$	116.76	5.66	0.01
$\text{pant}_{50} \sim \text{Drink : MS EWL slope}$	117.03	5.94	0.01

B. For all variables including T_{air} of T_b inflection and T_b panting initiated and omitting six data deficient species ($n = 14$).

Models	AICc	Δ AICc	Weight
$\text{pant}_{50} \sim$	80.78	0.00	0.25
$\text{pant}_{50} \sim \text{Percent EWL of Mb}$	82.14	1.37	0.13
$\text{pant}_{50} \sim \text{Max EWL resid}$	82.93	2.15	0.09
$\text{pant}_{50} \sim \text{Max EWL/Min Ewl}$	83.17	2.40	0.08
$\text{pant}_{50} \sim \text{Max RMR/Min RMR}$	83.57	2.79	0.06
$\text{pant}_{50} \sim \text{Tair of Tb inflection}$	83.92	3.14	0.05
$\text{pant}_{50} \sim \text{Drink}$	83.92	3.15	0.05
$\text{pant}_{50} \sim \text{HTL}$	83.97	3.19	0.05
$\text{pant}_{50} \sim \text{Tb panting initiated}$	84.07	3.29	0.05
$\text{pant}_{50} \sim \text{MS EWL slope}$	84.09	3.31	0.05
$\text{pant}_{50} \sim \text{Mb}$	84.09	3.31	0.05

pant ₅₀ ~ Drink : Percent EWL of Mb	85.64	4.86	0.02
pant ₅₀ ~ Drink : Max EWL resid	86.96	6.18	0.01
pant ₅₀ ~ Drink : Max EWL/Min EWL	87.17	6.39	0.01
pant ₅₀ ~ Drink : Max RMR/Min RMR	87.61	6.84	0.01
pant ₅₀ ~ Drink : MS EWL slope	87.78	7.00	0.01
pant ₅₀ ~ Drink : Tair of Tb inflection	87.87	7.10	0.01
pant ₅₀ ~ Drink : HTL	87.92	7.14	0.01
pant ₅₀ ~ Drink : Tb panting initiated	87.95	7.17	0.01
pant ₅₀ ~ Drink : Mb	88.12	7.34	0.01

Table S2: Top GLMM models for predicting pant₅₀ for passerine species, ranked by AICc. Significance is inferred if 95% CIs (LCI-UCI) exclude zero.

A. Excluding physiological variable T_{air} of T_b inflection and T_b panting initiated, n = 13

	AICc	ΔAICc	Weight
Null model	72.7	0	0.44
<i>Top competing models</i>			
pant ₅₀ ~ Percent EWL of Mass [LM: $F_{1,11} = 2.61$, $t = -1.61$, $p = 0.13$]	73.4	0.7	0.31

B. Three species missing data removed, n = 10

	AICc	ΔAICc	Weight
Null model	59.7	0.0	0
<i>No competing models</i>			

Table S3: Complete model set outcomes

A. For all passerines with variables T_{air} of T_b inflection and T_b panting initiated omitted (n = 13).

Models	AICc	ΔAICc
pant ₅₀ ~	72.7	0.0
pant ₅₀ ~ Percent EWL of Mass	73.4	0.7
pant ₅₀ ~ Max EWL resid	75.0	2.3
pant ₅₀ ~ Max RMR/Min RMR	76.0	3.3
pant ₅₀ ~ Max EWL/Min EWL	76.0	3.3
pant ₅₀ ~ Mb	76.0	3.3
pant ₅₀ ~ HTL	76.0	3.4
pant ₅₀ ~ MS EWL Slope	76.1	3.5
pant ₅₀ ~ Drink	76.1	3.5
pant ₅₀ ~ Drink + Percent EWL of Mass	77.7	5.0

pant ₅₀ ~ Drink + Max EWL resid	79.0	6.3
pant ₅₀ ~ Drink + HTL	80.2	7.6
pant ₅₀ ~ Drink + Max RMR/Min RMR	80.3	7.6
pant ₅₀ ~ Drink + Mb	80.3	7.6
pant ₅₀ ~ Drink + Max EWL/Min EWL	80.3	7.6
pant ₅₀ ~ Drink + MS EWL Slope	80.5	7.8

B. For all variables including T_{air} of T_b inflection and T_b panting initiated and omitting three data deficient species (n = 10).

Models	AICc	ΔAICc
pant ₅₀ ~	59.7	0.0
pant ₅₀ ~ Percent EWL of Mass	61.8	2.1
pant ₅₀ ~ Max EWL resid	62.5	2.8
pant ₅₀ ~ HTL	63.1	3.4
pant ₅₀ ~ Max EWL/Min EWL	63.7	4.1
pant ₅₀ ~ T _{air} of T _b inflection	63.8	4.2
pant ₅₀ ~ Drink	63.9	4.2
pant ₅₀ ~ Max RMR/Min RMR	63.9	4.2
pant ₅₀ ~ T _b panting initiated	63.9	4.3
pant ₅₀ ~ Mb	63.9	4.3
pant ₅₀ ~ MS EWL Slope	63.9	4.3
pant ₅₀ ~ Drink : Percent EWL of Mass	67.8	8.1
pant ₅₀ ~ Drink : Max EWL resid	68.4	8.8
pant ₅₀ ~ Drink : HTL	69.0	9.3
pant ₅₀ ~ Drink : Max EWL/Min EWL	69.7	10.0
pant ₅₀ ~ Drink : T _{air} of T _b	69.8	10.1
pant ₅₀ ~ Drink : MS EWL Slope	69.8	10.2
pant ₅₀ ~ Drink : Max RMR/Min RMR	69.9	10.2
pant ₅₀ ~ Drink : Mb	69.9	10.2
pant ₅₀ ~ Drink : T _b panting initiated	69.9	10.2