Thermoregulatory strategies of songbird nestlings reveal limited capacity for cooling and high risk of dehydration

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Abstract:

Developing young are particularly sensitive to environmental challenges, often with life-long consequences, but the risks of climate warming during this period are not well understood. To quantify the physiological effects of increasing temperatures for developing endotherms, we measured metabolic rate, water loss, and heat dissipation behaviours between 25-45°C in nestlings of a free-living songbird. The thermoneutral zone ranged from 33.1–42.3°C – higher and narrower than adults – and metabolic rate increased sharply above and below this range. Water loss was constant below 33.5°C, above which it increased sharply, when nestlings also drooped their wings to lose heat. Despite nestlings initiating panting (p_{50}) around 40°C, water loss was never sufficient to dissipate metabolically produced heat, indicating poor cooling capabilities. Our data show that while developing young appear relatively tolerant to higher temperatures, they are at a high risk of dehydration and hyperthermia, with limited ability to mitigate these risks of increasing temperatures.

Introduction:

Climate change models predict global increases in maximum air temperature as well as increases in the frequency, severity, and duration of heat waves. Understanding how organisms will respond to rises in temperature throughout their lifespan, including the most sensitive life stages, is crucial for the protection of biodiversity around the world (Kruuk *et al.* 2015; Milne *et al.* 2015; Wiley & Ridley 2016). To improve our ability to forecast the threats of future climates for biodiversity, studies of climate change responses that incorporate physiological mechanisms rather than relying on statistical correlations are urgently needed (Urban *et al.* 2016). However, our current understanding of the physiological heat responses of wild birds pertains almost exclusively to adults (McKechnie & Wolf 2010; Smit *et al.* 2013; McKechnie *et al.* 2016a, 2017, 2021; Smith *et al.* 2017; Oswald *et al.* 2018b). Developing young may be more sensitive to environmental challenges associated with climate warming (Metcalfe & Monaghan 2001; Nord & Giroud 2020) and their strategies to respond to heat may differ substantially from those of adults.

Small endotherms generally are highly sensitive to increasing temperatures (McKechnie & Wolf 2019), and altricial nestlings may be the most at risk. They have limited physiological capacity to regulate their own body temperature, limited opportunity to seek cooler microsites and no access to water sources for drinking (Dunn 1975; Olson 1992). Once they become homeothermic (have developed an ability to thermoregulate), nestlings face a trade-off between the energy costs of rapid growth and development, and energy costs needed to maintain body temperature. This trade-off may have immediate impacts on nestling body condition and important consequences in adulthood (Monaghan 2008). Indeed, long-term

studies have associated increased ambient temperatures during the nestling stage to reduced breeding success and shorter lifespans as adults (Schoech *et al.* 2011; Dupont *et al.* 2019; Kraft *et al.* 2019; Eastwood *et al.* 2022).

Despite the presumed challenges in early life associated with thermoregulating at increasing temperatures, the immediate effects observed in nestlings are often conflicting (Andreasson *et al.* 2018; Sauve *et al.* 2021). Increasing ambient temperatures have been shown to result in nestlings growing slower and being smaller (Ardia *et al.* 2010; Gardner *et al.* 2011; Cunningham *et al.* 2013; Kruuk *et al.* 2015; Rodríguez & Barba 2016; Rodríguez *et al.* 2016; Andrew *et al.* 2017; Andreasson *et al.* 2018; Bourne *et al.* 2021b), growing faster and being larger (Dawson et al., 2005; Ton & Martin, 2016), or to have no effect on growth and size (Nord & Nilsson 2011; Castaño-Vázquez *et al.* 2018; Wheelwright *et al.* 2022). Equally variable results are evident with respect to survival to fledging, with increasing ambient temperatures decreasing success (Ardia, 2013; van de Ven et al., 2020) or having no effect (Dawson et al., 2005; Lloyd & Martin, 2004; Wheelwright et al., 2022). The inconsistent results of increasing ambient temperature presumably arise because the temperature range investigated may or may not impose a thermal challenge on nestlings. This notion is supported by the fact that those studies that detected a physiological stress response, found negative effects on the nestling growth and survival. The observed stress response, such as increased corticosterone, increased heterophil/lymphocyte ratios, or an increase in body temperature, could all be interpreted as indicators of a thermal challenge, and may explain why the increased temperature had a negative effect on nestlings (Ardia 2013; Catry *et al.* 2015; Andreasson *et al.* 2018; Newberry & Swanson 2018). Thus,

an important element that is lacking from our current understanding on the threat of climate change for nestling birds is an assessment of what temperatures are physiologically challenging.

Here we examine the thermoregulatory abilities of nestling superb fairy-wrens (*Malurus cyaneus*), a small free-living songbird from temperate south-eastern Australia with typical fast growing altricial young. This species represents a typical species at a high risk of climate warming; they are sensitive to changes in temperature (Langmore *et al.* 2016), including their nestlings (Kruuk *et al.* 2015), and increases in average temperature and heatwaves are predicted across their range (Clarke *et al.* 2019). In order to determine what constitutes a thermal challenge for the nestlings, we established their physiological and behavioural capabilities to thermoregulate across a range of temperatures. Specifically, we used respirometry to measure (1) metabolic rate, to establish the lower and upper critical temperatures of the thermoneutral zone; and (2) evaporative water loss and cooling efficiency to establish the ability of the nestlings to dissipate heat. Lastly, (3), we examined the temperature profile of the behavioural thermoregulatory responses of the nestlings.

Methods:

Study site and field methods

Superb fairy-wrens were studied at Lysterfield Park, Victoria (37.95°S, 145.30°E) in south-eastern Australia, September to February, 2019-2022. Fieldwork was conducted with Animal Ethics approval (Monash University School of Biological Sciences Animal Ethics Committee #16348) and all relevant permits (Department of Environment Land Water and Planning and Parks Victoria permit no. 10008704;

Australian Bird and Bat Banding Scheme authority no. 2230, 3641). Adult birds were banded with a numbered metal band and a unique combination of one coloured metal and two coloured plastic bands. Nests were found by observations and monitored for egg laying, hatching, and fledging, with typically 3-4 eggs laid for each nest. On day 7 after hatching (hatch day = 1), nestlings were removed from the nest and carried a short distance (<5 mins walk) to a vehicle for banding and respirometry measurements. This is the day when nestlings reach 75% of adult body mass, their growth starts slowing down, feather pins break and the mother ceases brooding; indicating that the nestlings have developed the capacity to thermoregulate (endothermy) (Ton & Martin 2016). From each nest, all nestlings were banded and measured, with one nestling removed from the nest at a time. Any nestlings that were infected with external parasites (botfly larvae) or underweight for their developmental age (<6.0g) were not measured. Each nestling also had a small blood sample taken to determine its sex using PCR (DNA extraction methods described in Eastwood et al., 2018; primers P2 and P8 described in Griffiths et al., 1998).

Respirometry measurements

The rates of O_2 consumption, CO_2 production, and water vapour production of resting nestlings were measured using positive pressure flow-through respirometry, using standard procedures (Lighton 2008). The system is supplied with outside air that is pushed through columns of soda lime and Drierite to remove CO_2 and water vapour, respectively. The system had two channels, one empty reference chamber to assist in baselining, and one chamber for the nestling. Flow rate through each channel was regulated at 100 ml min^{-1} using a mass flow controller (AALBORG GFC17, New York, NY USA). The flow rates were calibrated using a Gilian

Gilibrator-2 NIOSH Primary Standard Air Flow Calibrator with a low-flow cell
(Sensidyne, LP, St. Petersburg, FL USA) before and after each breeding season and
measurements were corrected to standard temperature and pressure (i.e. 101.3 kPa
and 0°C). The excurrent air from the chamber then passed through analysers that
measure the excurrent contents of CO_2 and water vapour (LI-COR, Model Li-840A,
Lincoln, NE, USA) and oxygen (OxZilla, Sable Systems, Las Vegas, NV, USA). CO_2
gas analysers were calibrated with span gases (30.4, 200.1, and 5040 ppm CO_2)
before and after each breeding season and the O_2 gas analyser calibrated to
atmospheric oxygen each day prior to measurements. The system was set up in the
field and powered by a 12V 120A battery.

To standardise for digestive activity, prior to measurement nestlings were fed
mealworms soaked in water. The food was often refused; thus, it was assumed that
nestlings were satiated. To standardised for possible effect of circadian rhythm, all
nestlings were measured between 10:00 and 16:00 h. Surface skin temperature was
monitored via a small, 12mm X 2mm, PIT (passive integrated transponder; Biomark
Biotherm 13 Pit Tag, Boise, ID USA) tag glued to the bare skin on the belly using
eyelash glue. Surface skin temperature was used as a proxy for body temperature
on the basis that there are relatively small difference between skin and core body
temperature and skin varies directly with body temperature in small endotherms
(Torre-Bueno 1976; Mertens 1977; Audet & Thomas 1996; Adelman *et al.* 2010;
McCafferty *et al.* 2015). Nestlings were then placed on a rolled tissue paper nest cup
inside the respirometry chamber (0.2L sealed container) which allowed the nestlings
to settle and rest for the duration of the trial. Both respirometry chambers (reference
and nestling) were placed in a temperature-controlled unit at a constant temperature
between 25°C to 45°C for 1 hour. Temperature in the chamber with the nestling was

monitored for the entire trial using a miniature temperature logger (Thermochron, Maxim Integrated, San Jose, CA USA) on the inside of the chamber lid, which recorded temperature every minute. The average temperature for the last 30 min of the trial was used for analysis. Due to logistical problems, incoming bad weather or issues with battery power, nine trials were ended at 50min. In this case, data from the last 20 min of the trial were included in the analyses. One trial at 44°C was ended at 50min because the nestling appeared distressed (very heavy panting and became restless in the chamber). This individual, and four additional nestlings, also had skin temperatures greater than 43°C for more than 30min of the trial, and low metabolic rates, thus these nestlings were potentially hyperthermic and were excluded from analyses.

Respirometry calculations

The rate of O_2 consumption ($\dot{V}O_2$, ml min^{-1}) and rate of CO_2 production ($\dot{V}CO_2$, ml min^{-1}) were calculated using formulas 10.6 and 10.8, respectively, from Lighton (2008). Rate of evaporative water loss (EWL; g hr^{-1}) was calculated using formula 9.9 from Lighton (2008), assuming a vapour density of 0.803 mg ml^{-1}. The lowest and most stable fractional concentrations of O_2, CO_2, and H_2O were chosen within the last 30 minutes of the trial (when temperature was the most stable) with a minimum time frame of 3 minutes; average time frame used for calculations was 8 minutes. Evaporative heat loss (EHL) was calculated by converting EWL into EHL using the latent heat of vaporisation of water, 2.406 J mg H_2O^{-1} at 40°C (McKechnie *et al.* 2017; O'Connor *et al.* 2021) and metabolic heat production (MHP) was calculated by converting $\dot{V}O_2$ to MHP using the oxycaloric equivalent (19.8 J ml O_2^{-1}) (Walsberg & Wolf 1994; Lighton 2008), given an average RER of 0.7 (see

supplement, Fig. S1). Cooling efficiency was calculated as EHL/MHP and indicates the amount of metabolically produced heat that is being dissipate evaporatively, a higher number indicates a greater evaporative cooling capacity (Lawsiewski 1966). $\dot{V}O_2$ was converted to mass-specific metabolic rate (ml g^{-1} h^{-1}) for each individual to enable direct comparison with adult metabolic rate (Lill *et al.* 2006).

Behavioural observations

While the nestlings were in the metabolic chamber, they were remotely monitored for the duration of the trial using a camera (GoPro Hero7, Calexico, CA USA) and their behaviour recorded every 10 min (6 observations in total per nestling). At each observation, the posture of the nestlings and occurrence (yes/no) of heat dissipation behaviours were recorded: wing-drooping - wings held away from the body; panting - beak is open and the nestling is breathing heavily. The temperature in the chamber at the time of the observation was obtained from the temperature logger inside the chamber. For analysis, we used the last three observations (40, 50 and 60 min) to allow for the nestlings to acclimate to the chamber.

Statistical Analysis

$\dot{V}O_2$ and $\dot{V}CO_2$ were used as proxies for metabolic rate and two inflection points were identified using non-linear mixed effects models in R which included nest identity as a random intercept (Pinheiro et al., 2009) (package: "nlme"). The data for $\dot{V}O_2$ and $\dot{V}CO_2$ were square root-transformed to improve normality and aid in model convergence. The back-transformed coefficients and standard errors are presented in text and figures (Fig 1 and Fig. S3) and raw values in supplement (Table S1). We present $\dot{V}O_2$ in the main results, as measurements of $\dot{V}O_2$ are more robust to

changes in substrate utilisation than $\dot{V}CO_2$; however, calculations based on $\dot{V}O_2$ and $\dot{V}CO_2$ were very similar (compare Fig. 1 with Fig. S3). Non-linear mixed effects models were also used to identify one inflection point for EWL and cooling efficiency with nest identity as a random intercept. The effects of ambient temperature, mass, and nestling sex, on probability of occurrence for wing-drooping and panting (binary variables yes/no per observation point) were determined using general linear mixed models, using the *logit* link-function (Bates *et al.* 2022) (package: "glmer"). Each individual had 4 observations and the random effects of individual and nest identity were included in the models. The 95% confidence intervals for these regressions were predicted using ggpredict, which included the random effects of individual and nest identity in the predicted probabilities (Ludecke et al., 2022). The model predictions were used to determine the temperature at which the behaviour response was present in 50% of observations (*p50*).

Results:

Thermoneutral zone

We obtained reliable metabolic rate measurements from 70 7-day old nestlings from 36 nests and all nestlings rested throughout the entire trial. The average respiratory exchange ratio (RER) was 0.7, indicating that the nestlings were postabsorptive (see supplement, Fig. S1). Metabolic rate was weakly but significantly positively associated with nestling mass (coefficient = 0.07 $\pm$ 0.03 SE, t = 2.31, P < 0.05; see supplement, Fig. S2). However, due to the complexity of the models, we were unable to account for mass in the non-linear mixed effect models when determining the thermoneutral zone. Nonetheless, the range of mass was small (6.0g to 9.0g) and

evenly spread across all temperatures (25–45°C) so that mass did not co-vary with temperature (Pearson's correlation test, r = 0.09 $\pm$ 0.22, P = 0.42).

A clear thermoneutral zone was evident: metabolic rate ($\dot{V}O_2$) as a function of chamber temperature had two significant inflection points, a lower critical limit at 33.1°C ($\pm$ 1.1 SE, t = 29.8, P < 0.001) and an upper critical limit at 42.3°C ($\pm$ 0.6 SE, t = 76.0, P < 0.001) (Fig.1). Below the lower critical limit, metabolic rate significantly decreased with chamber temperature (coefficient = -0.03 $\pm$ 0.01 SE, t = 3.93, P < 0.001) and above the upper critical limit metabolic rate increased more steeply with temperature, but not significantly (coefficient = 0.07 $\pm$ 0.04 SE, t = 1.90, P = 0.07; fewer nestlings were tested above the upper critical limit). The $\dot{V}CO_2$ had similar inflection points (lower limit; 35.1 $\pm$ 0.8, t = 43.8, P < 0.001, upper limit; 41.7 $\pm$ 1.7, t = 24.8, P < 0.001), for details see supplement Fig. S3.

Evaporative water loss and cooling efficiency
EWL had a significant inflection point at 33.5°C ($\pm$ 0.8 SE, t = 42.7, P < 0.001) (Fig.2). Above this point, EWL significantly increased with increasing chamber temperature (coefficient = 0.01 $\pm$ 0.00 SE, t = 10.40, P < 0.001). Cooling efficiency had a significant inflection point at 29.9°C ($\pm$ 1.1 SE, t = 26.4, P < 0.001) and above this point cooling efficiency increased with increasing chamber temperature (coefficient = 0.04 $\pm$ 0.00 SE, t = 11.72, P < 0.001) (Fig. 3). Cooling efficiency did not go to 1.0 or above, indicating that, at all temperatures tested, nestlings were producing more heat metabolically than they were losing evaporatively.

Heat dissipation behaviours

The occurrence of heat dissipation behaviours was monitored in 47 nestlings from 23 nests. The chamber temperature significantly predicted the probability of wing-drooping (t = 89.12, P < 0.001) and panting (t = 103.83, P < 0.001) behaviour and there was no effect of mass or sex on probability of either behaviour (Fig. 4 and Table S3). Wing-drooping p_{50} was at 34°C and panting p_{50} was at 40°C, and by 42°C, all individuals were wing-drooping and panting. During our measurements at temperatures above 42°C, towards the end of the hour, nestlings often started to show heavier and quicker panting. Once they were removed from the chamber, they calmed down rapidly when entering the cooler air.

Discussion:

This study directly measured the thermoneutral zone and determined the physiological and behavioural heat dissipation capacity in small songbird nestlings; a vulnerable life stage to climate change. Exposing nestlings to ecologically relevant temperatures up to 45°C revealed that they are tolerant to high temperatures, however they have limited ability to cool themselves. They are at risk of dehydration at temperatures above 33.1°C, and at temperatures above 42.3°C dehydration risk is doubled and compounded by an increase in metabolic heat production.

High and narrow thermoneutral zone

Determining the thermoneutral zone and the upper critical limit for nestlings is crucial for understanding their vulnerability to increasing environmental temperatures. We found that homeothermic nestlings had a distinct thermoneutral zone between 33-42°C. These temperatures span the average body temperature of small songbirds (38–42°C) (Yahav 2015; Pollock *et al.* 2021), as well as the average nest

temperature for passerines (34–37°C) (Kendeigh 1940; Irving & Krog 1956; Blem 1973). Given that nestlings are relatively inactive and have little insulation, a normal body temperature is thus maintained with minimum thermoregulatory costs at common ambient and nest temperatures. Although there are no comparable data on the thermoneutral zone of nestlings in other species, the range we identified spans temperatures that were previously identified to correspond to lowest energy use (Nager & Wiersma 1996) or presumed to be thermoneutral (Ton & Martin, 2016; Weathers & Sullivan, 1991). A number of studies measured metabolic rate (O_2 consumption or CO_2 production) of nestlings over a range of temperatures to investigate the development of endothermy. From the figures presented in those studies, the lower critical limit of the thermoneutral zone seems to typically fall between 30–35°C (Dawson & Evans, 1957, 1960; Dyer, 1968; Mayer et al., 1982; Nager & Wiersma, 1996; Sirsat et al., 2016), which is consistent with the lower critical limit we identified for superb fairy-wren nestlings. Only one study of developing cattle egret (*Bubulcus ibis*) nestlings studied higher temperatures (up to 45°C) (Hudson *et al.* 1974), which suggested metabolic rate to be minimal from 34°C to 40°C, relatively similar to superb fairy-wren nestlings despite the difference in the body size and ecology of the species. Taken together, the available evidence thus tentatively suggests that potential thermoneutral ranges of nestlings of other species may be comparable to superb fairy-wren nestlings.

The metabolic parameters of nestlings differ substantially from those of adult superb fairy-wrens (Lill *et al.* 2006). At thermoneutral temperatures, the mass-specific metabolic rate of nestlings is 3.95 ml O_2 g^{-1} h^{-1}, which is 1.5 times that of adults (2.65 ml O_2 g^{-1} h^{-1}; Lill et al. 2006). A higher metabolic rate in nestlings is consistent with

other species (Dawson & Evans, 1957; Kendeigh, 1939; Newberry et al., 2021; Weathers & Siegel, 1995) and is presumably related to their rapid growth and development requiring higher energy use (Dunn 1980; Olson 1992). The thermoneutral zone of adult superb fairy-wrens spans a wider temperature range and occurs at much lower temperatures (26°C–35°C; Lill et al., 2006). The narrower thermoneutral zone of nestlings may result from their reduced insulation (body fat and feathering), allowing metabolically produced heat to be dissipated more easily into the environment, whereas adults have limited ability to passively dissipate excess heat (McKechnie & Wolf, 2019). These differences between adults and nestlings may be common, as many adult birds have thermoneutral zones between 25°C and 34°C (Scholander *et al.* 1950; Khaliq *et al.* 2014). A higher and narrower thermoneutral zone in the nestlings compared to the adults indicates that the nestlings are better adapted to warmer temperature but have a greater sensitivity, and this pattern may be general for birds with altricial young.

Limited capacity for cooling

As ambient temperature rises towards body temperature, the scope for heat to be lost passively through the thermal gradient declines and birds become reliant on evaporative water loss for cooling (Smith *et al.* 2017). In the nestlings, the sharp increase in water loss occurs at 33.5°C, around the lower critical limit of the thermoneutral zone, which is in contrast to many adult passerines where water loss increases around the upper critical limit, when panting commences (Albright *et al.* 2017; McKechnie *et al.* 2017; Smith *et al.* 2017). However, this is similar to blue tit (*Cyanistes caeruleus*) nestlings, where water loss also appears to increase above the lower critical limit (30°C; Nager and Wiersma, 1996; no data at higher

temperatures). Given that nestlings are not yet fully feathered, cutaneous evaporation is likely to be occurring at a greater rate with water passively transferred from the skin into the environment (Yahav 2015; McKechnie *et al.* 2016b). Cutaneous evaporation is difficult to control physiologically (Yahav 2015) and water loss is occurring even though body temperature is being maintained with minimum energy usage in the thermoneutral zone. Thus, temperatures in the thermoneutral zone pose a dehydration risk to nestlings, a previously unrecognised risk of climate warming, especially in more arid regions (Albright *et al.* 2017).

Nestlings employed two main heat dissipation behaviours, wing-drooping and panting. The median temperature at which wing-drooping occurs is 34°C, which coincides with the lower critical limit of the thermoneutral zone and the inflection point for water loss. This is in accordance with the notion that wing-drooping encourages passive heat loss through cutaneous evaporation (Pattinson *et al.* 2020), allowing the nestlings to maintain a stable body temperature without increasing metabolic heat production. As temperatures increase, the heat lost through passive dissipation is not enough to prevent a rise in body temperature, thus a more active heat loss strategy is required, through panting, which increases respiratory water loss. The median temperature at which panting was observed occurred at 40°C, and by 42°C all individuals were panting. Panting is often associated with a sharp increase in water loss (Albright *et al.* 2017), however in the nestlings there was no additional inflection point for water loss. A lack of a clear inflection point suggests that nestlings rely more on cutaneous evaporation through wing-drooping for cooling, and that panting does not enhance cooling efficiency.

Nestlings are also not efficient at cooling. Although the cooling efficiency increases as temperature increases, the ratio of heat loss to heat production never exceeded a value of 1 (Fig. 3), indicating that nestlings are unable to dissipate more heat than they produce. Limited data is available on the cooling efficiency of other songbird nestlings or adults, but a similar inability to dissipate their own metabolic heat production was found in blue tit nestlings from a temperate climate (Nager & Wiersma 1996) and in adults of two species from cool/mesic climates (arctic tundra and subtropical mountains (Oswald *et al.* 2018a; O'Connor *et al.* 2021). Conversely, heat-tolerant adult passerines from arid zones are able to dissipate more heat than they produce, indicating more efficient evaporative cooling mechanisms (Whitfield *et al.* 2015; McKechnie *et al.* 2017; no information on nestlings). Birds that regularly inhabit more temperate, mesic regions, such as south-eastern Australia, may depend more on cutaneous evaporation (Yahav 2015), which is not as effective at cooling the body and does not conserve water (McKechnie & Wolf 2019), but can help to control body temperature without an increase in metabolic rate or heat production. Species that rely on cutaneous evaporation are more vulnerable to moderate increases in temperature and our results suggest that this applies to superb fairy-wren nestlings as well.

Ecological implications

Recent and predicted increases in global temperature can negatively affect the physiology and morphology of nestlings which may impact their lifetime fitness (Metcalfe & Monaghan 2001; Gardner *et al.* 2011; Andreasson *et al.* 2018; Eastwood *et al.* 2022). Currently, summers in south-eastern Australia average 8.3 days with a maximum temperature greater than 35°C, and it is predicted by 2050 this will

increase to 14–16 days (Clarke *et al.* 2019). Vulnerability to these increasing temperatures is determined by many different factors, including but not limited to, the physiological thermal tolerances and the physiological and behavioural strategies used to prevent hyperthermia (Pollock *et al.* 2021). Thus, a detailed understanding of the thermal limits and capability of nestlings to prevent hyperthermia is necessary in predicting how individuals and populations will respond to the challenges associated with global warming (Conradie *et al.* 2019; Nord & Giroud 2020; Pollock *et al.* 2021). Nestlings are metabolically more tolerant to warm temperatures than adults (that start showing increased metabolic rate at temperatures > 35°C, Lill *et al.* 2006), suggesting that the immediate risk of hyperthermia for the nestlings is generally lower than adults. However, nestlings incur water loss even within the thermoneutral zone, but are unable to select cooler microclimates and have no access to water, and thus have an associated higher risk of dehydration.

Dehydration is an important threat to nestlings at relatively low temperatures. At 35°C, shortly above the temperature when water loss starts to increase but within the thermoneutral zone, nestlings lose approximately 1% of body mass in water per hour (average mass; 7.5g). At 43°C, above the upper limit of the thermoneutral zone, water loss is doubled and nestlings lose approximately 2% of body mass in water per hour. In the absence of compensatory water intake, after 5 hours at 43°C, nestlings would lose 10% of their body mass in water, which is within the lethal limit for birds (Wolf & Walsberg 1996; Albright *et al.* 2017; Sharpe *et al.* 2019). These physiological responses match the effect of ambient temperature observed in a long-term dataset of superb fairy-wren nestling body mass on day 7 (Kruuk *et al.* 2015): average maximum temperature above 35°C during the two days before measurement was

associated with a decline in nestling body mass of 0.37 g, 5% of body mass (which reduces their fitness; Kruuk et al., 2015). This supports our prediction that for nestlings in the wild, biologically relevant dehydration can occur at temperatures that are within the thermoneutral zone. Provisioning to the nestlings by the parents could mitigate or compensate for the increased water loss and energy requirements at these higher temperatures, e.g. by providing more food, or food with high water content (Wiley & Ridley 2016; van de Ven *et al.* 2019; Bourne *et al.* 2021a), but this would entail substantial thermoregulatory costs for adults themselves (given their upper critical limit of 35°C; Lill et al., 2006).

Once temperatures reach the upper critical limit, the impact of increased water loss is compounded with an increase in metabolic activity when active cooling commences. Although nestlings are panting at this stage, panting is inefficient at substantially increasing their cooling capacity. This situation, when the maximum rate of water loss is insufficient to prevent body temperature from rising, can induce immediate hyperthermia (Mertens 1977), as evident from the fact that at this point skin temperature of the nestlings exceeded air temperature (Fig. 5). Our observations of 5 nestlings at temperatures> 42°C with high body temperature and low metabolic rates support the notion that at these temperatures, nestlings become heat stressed and hyperthermic. Nestlings have little ability to prevent this increase in body temperature and are at a high risk of lethal hyperthermia. Therefore, predicted climate warming, with near doubling in average days above 35°C and increasing risks of extreme temperatures (Clarke *et al.* 2019), poses a high risk of both immediate hyperthermia and long-term hyperthermia and dehydration.

Conclusion

In order to understand how increases in ambient temperature will affect birds, it is necessary to find the temperatures when they face thermal challenge and may become heat stressed. Our results show that when predicting how populations will change with climate, one cannot assume that the thermoregulatory strategies and thermal tolerances of adults can be extended to their nestlings. Additionally, weather conditions, such as humidity, rainfall, wind and solar radiation, and characteristics of the nest (Ricklefs & Hainsworth 1969; Ardia 2013; Welman & Pichegru 2022) as well as brood size, nestling behaviour (huddling or spreading further apart in the nest), and age of the nestlings (Mertens 1977; Andreasson *et al.* 2016; Mitchell *et al.* 2022) can affect their heat load and cooling requirements (Wolf & Walsberg 1996). These conditions can exacerbate ambient conditions, with nestlings incurring additional water loss or energy use maintain body temperature, or help mitigate the thermal environment, reducing water loss and energy requirements. It is therefore important to note that in this study the metabolic and water loss rates were collected on a single resting nestling in a chamber with a constant temperature and very low humidity and caution should be used when extrapolating the results to field conditions. It is thus critical to determine in the field when the environment is thermally stressful for nestlings. The tight correlation between physiological and behavioural responses to increased temperature indicates that both can identify the upper critical temperature equally well. Future studies can thus monitor panting behaviour or skin temperature relative to nest temperature as an accessible approach to measure experienced thermal stress in the field without specialist equipment, with great promise for identifying climate change impacts on birds before these are evident at the population level.

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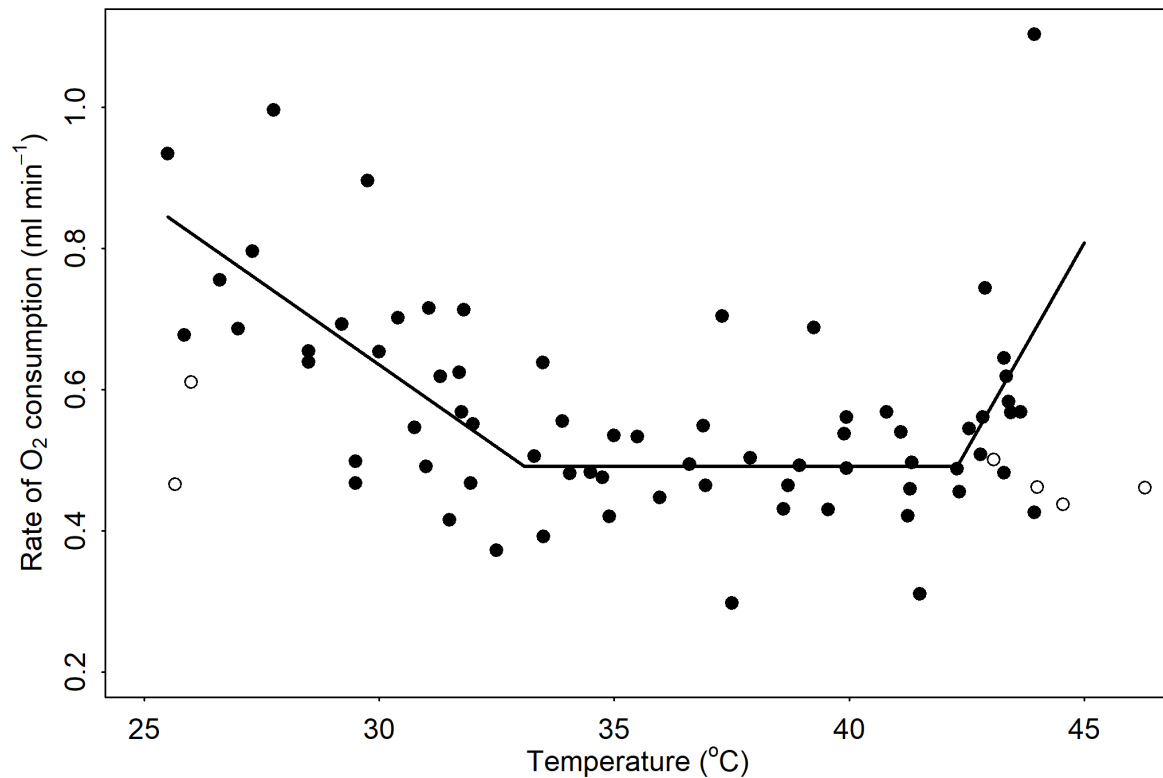
Figures:

Fig. 1. The thermoneutral zone for superb fairy-wren nestlings is between 33.1°C and 42.3°C. Shown is the relationship between chamber temperature and the rate of O_2 consumption ($\dot{V}O_2$) (ml min^{-1}). Open symbols represent nestlings with skin temperatures above 43°C or below 33°C for more than 30 min of the trial; these were excluded from the data analysis due to the probability they were hyper- or hypothermic respectively.
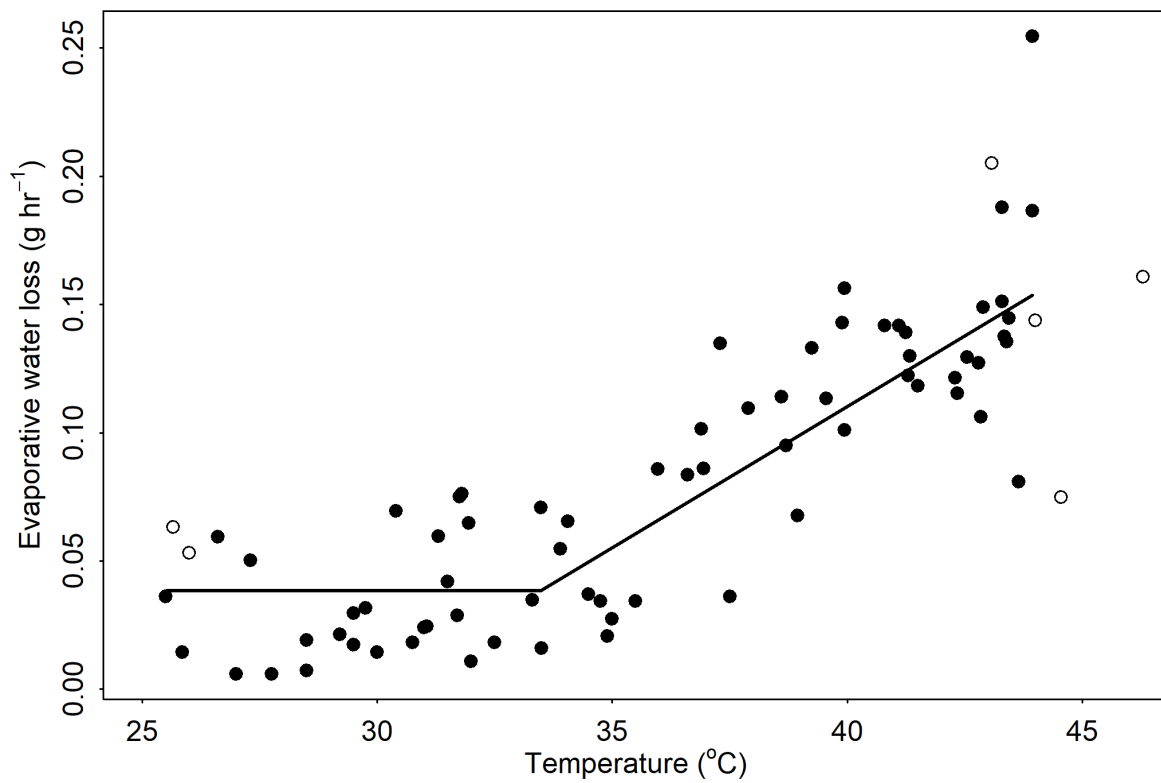
Fig. 2. Evaporative water loss in superb fairy-wren nestlings is constant until an inflection point at 33.5°C, with the rate of water loss ($\dot{V}H_2O$) increasing rapidly with chamber temperature above this point. Open symbols represent nestlings that were excluded from all analyses (details see Fig. 1).
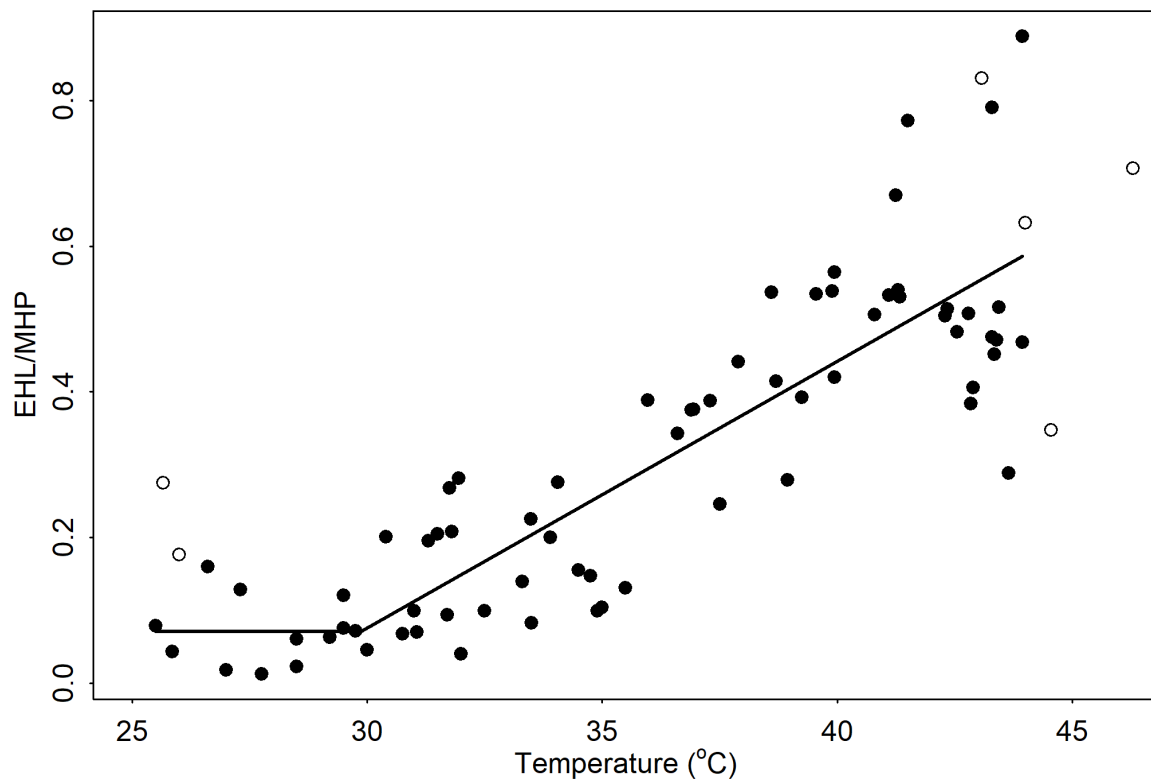
Fig. 3. Cooling efficiency of superb fairy-wren nestlings, the ratio of evaporative heat loss (EHL) to metabolic heat production (MHP), is constant until an inflection point at 29.9°C. Above this point cooling efficiency rapidly increases with increasing chamber temperature. Open symbols represent nestlings that were excluded from all analyses.
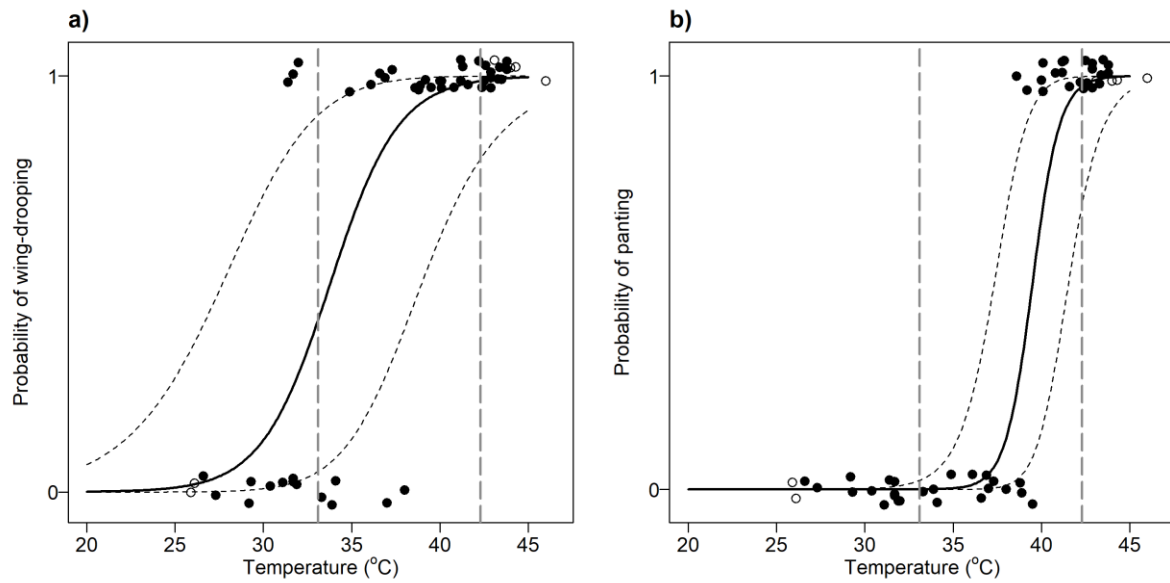
Fig. 4. Thermoregulatory behaviours closely match physiological limits. Shown are the probabilities that heat dissipation behaviours a) wing-drooping and b) panting were observed (1 = yes; 0 = no) with increasing chamber temperature. Each point (jiggered for presentation purposes) represents the last observation of the temperature exposure period; open symbols indicate nestlings that were excluded from all analyses (see Fig. 1 for details). The solid line is the predicted probability and dotted lines the 95% confidence intervals predicted from GLMMs incorporating all observations and accounting for the random effects of nestling and nest identity.
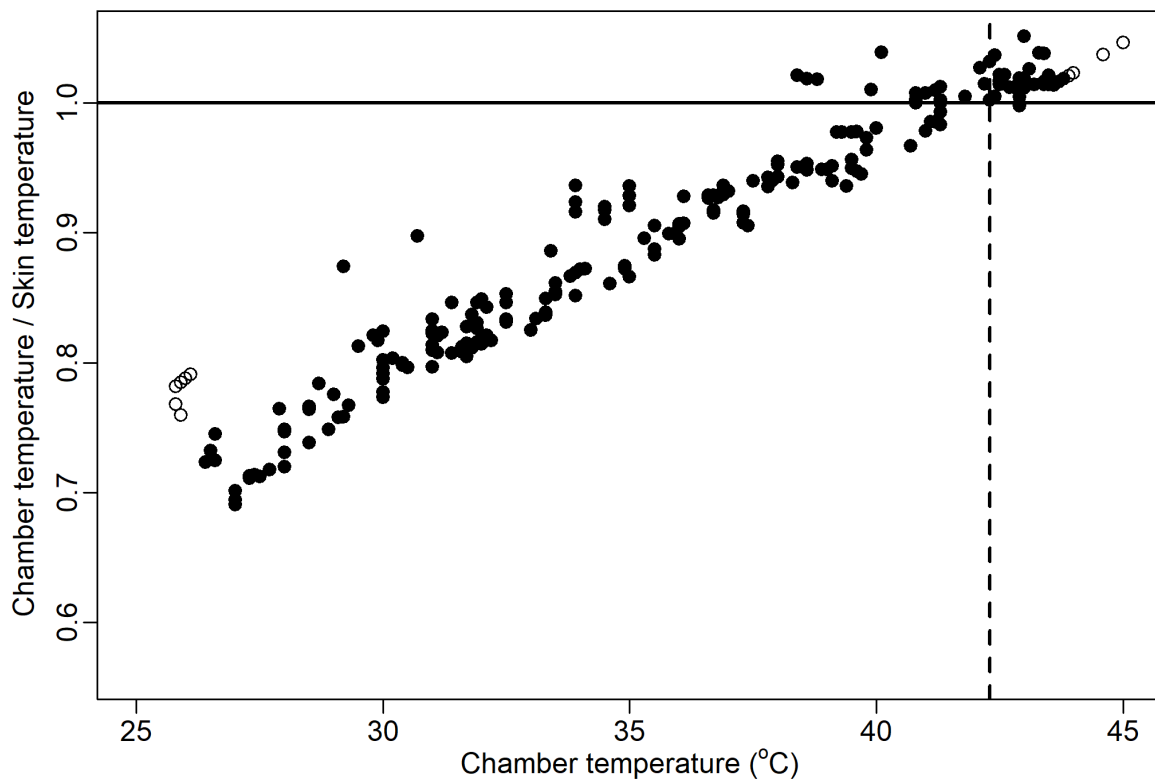
Fig. 5. Nestling skin temperature increases with increasing chamber temperature. Graph shows the ratio of skin temperature to chamber temperature, with the solid line at 1.0 representing the ratio when the nestlings skin temperature is equal to the chamber temperature. Dashed line represents the upper critical limit for the nestlings (see Fig. 1). Open symbols indicate nestling with skin temperature too high or too low to be accurately measured by the PIT tag (range 33-43°C); these are represented by the minimum and maximum, 43°C and 33°C, respectively (although it is likely that the actual temperatures were higher or lower).