1   **Abstract:**

2   Developing young are particularly sensitive to environmental challenges, often with

3   life-long consequences, but the risks of climate warming during this period are not

4   well understood. To quantify the physiological effects of increasing temperatures for

5   developing endotherms, we measured metabolic rate, water loss, and heat

6   dissipation behaviours between 25-45°C in nestlings of a free-living songbird. The

7   thermoneutral zone ranged from 33.1–42.3°C – higher and narrower than adults –

8   and metabolic rate increased sharply above and below this range. Water loss was

9   constant below 33.5°C, above which it increased sharply, when nestlings also

10   drooped their wings to lose heat. Despite nestlings initiating panting (p_{50}) around

11   40°C, water loss was never sufficient to dissipate metabolically produced heat,

12   indicating poor cooling capabilities. Our data show that while developing young

13   appear relatively tolerant to higher temperatures, they are at a high risk of

14   dehydration and hyperthermia, with limited ability to mitigate these risks of increasing

15   temperatures.

16

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

45   studies have associated increased ambient temperatures during the nestling stage to

46   reduced breeding success and shorter lifespans as adults (Schoech *et al.* 2011;

47   Dupont *et al.* 2019; Kraft *et al.* 2019; Eastwood *et al.* 2022).

48

49   Despite the presumed challenges in early life associated with thermoregulating at

50   increasing temperatures, the immediate effects observed in nestlings are often

51   conflicting (Andreasson *et al.* 2018; Sauve *et al.* 2021). Increasing ambient

52   temperatures have been shown to result in nestlings growing slower and being

53   smaller (Ardia *et al.* 2010; Gardner *et al.* 2011; Cunningham *et al.* 2013; Kruuk *et al.*

54   2015; Rodríguez & Barba 2016; Rodríguez *et al.* 2016; Andrew *et al.* 2017;

55   Andreasson *et al.* 2018; Bourne *et al.* 2021b), growing faster and being larger

56   (Dawson et al., 2005; Ton & Martin, 2016), or to have no effect on growth and size

57   (Nord & Nilsson 2011; Castaño-Vázquez *et al.* 2018; Wheelwright *et al.* 2022).

58   Equally variable results are evident with respect to survival to fledging, with

59   increasing ambient temperatures decreasing success (Ardia, 2013; van de Ven et

60   al., 2020) or having no effect (Dawson et al., 2005; Lloyd & Martin, 2004;

61   Wheelwright et al., 2022). The inconsistent results of increasing ambient temperature

62   presumably arise because the temperature range investigated may or may not

63   impose a thermal challenge on nestlings. This notion is supported by the fact that

64   those studies that detected a physiological stress response, found negative effects

65   on the nestling growth and survival. The observed stress response, such as

66   increased corticosterone, increased heterophil/lymphocyte ratios, or an increase in

67   body temperature, could all be interpreted as indicators of a thermal challenge, and

68   may explain why the increased temperature had a negative effect on nestlings (Ardia

69   2013; Catry *et al.* 2015; Andreasson *et al.* 2018; Newberry & Swanson 2018). Thus,

70   an important element that is lacking from our current understanding on the threat of

71   climate change for nestling birds is an assessment of what temperatures are

72   physiologically challenging.

73

74   Here we examine the thermoregulatory abilities of nestling superb fairy-wrens

75   (*Malurus cyaneus*), a small free-living songbird from temperate south-eastern

76   Australia with typical fast growing altricial young. This species represents a typical

77   species at a high risk of climate warming; they are sensitive to changes in

78   temperature (Langmore *et al.* 2016), including their nestlings (Kruuk *et al.* 2015), and

79   increases in average temperature and heatwaves are predicted across their range

80   (Clarke *et al.* 2019). In order to determine what constitutes a thermal challenge for

81   the nestlings, we established their physiological and behavioural capabilities to

82   thermoregulate across a range of temperatures. Specifically, we used respirometry

83   to measure (1) metabolic rate, to establish the lower and upper critical temperatures

84   of the thermoneutral zone; and (2) evaporative water loss and cooling efficiency to

85   establish the ability of the nestlings to dissipate heat. Lastly, (3), we examined the

86   temperature profile of the behavioural thermoregulatory responses of the nestlings.

87

88   **Methods**:

89   *Study site and field methods*

90   Superb fairy-wrens were studied at Lysterfield Park, Victoria (37.95°S, 145.30°E) in

91   south-eastern Australia, September to February, 2019-2022. Fieldwork was

92   conducted with Animal Ethics approval (Monash University School of Biological

93   Sciences Animal Ethics Committee #16348) and all relevant permits (Department of

94   Environment Land Water and Planning and Parks Victoria permit no. 10008704;

95   Australian Bird and Bat Banding Scheme authority no. 2230, 3641). Adult birds were

96   banded with a numbered metal band and a unique combination of one coloured

97   metal and two coloured plastic bands. Nests were found by observations and

98   monitored for egg laying, hatching, and fledging, with typically 3-4 eggs laid for each

99   nest. On day 7 after hatching (hatch day = 1), nestlings were removed from the nest

100 and carried a short distance (<5 mins walk) to a vehicle for banding and respirometry

101 measurements. This is the day when nestlings reach 75% of adult body mass, their

102 growth starts slowing down, feather pins break and the mother ceases brooding;

103 indicating that the nestlings have developed the capacity to thermoregulate

104 (endothermy) (Ton & Martin 2016). From each nest, all nestlings were banded and

105 measured, with one nestling removed from the nest at a time. Any nestlings that

106 were infected with external parasites (botfly larvae) or underweight for their

107 developmental age (<6.0g) were not measured. Each nestling also had a small blood

108 sample taken to determine its sex using PCR (DNA extraction methods described in

109 Eastwood et al., 2018; primers P2 and P8 described in Griffiths et al., 1998).

110

111 *Respirometry measurements*

112 The rates of O_2 consumption, CO_2 production, and water vapour production of

113 resting nestlings were measured using positive pressure flow-through respirometry,

114 using standard procedures (Lighton 2008). The system is supplied with outside air

115 that is pushed through columns of soda lime and Drierite to remove CO_2 and water

116 vapour, respectively. The system had two channels, one empty reference chamber

117 to assist in baselining, and one chamber for the nestling. Flow rate through each

118 channel was regulated at 100 ml min^{-1} using a mass flow controller (AALBORG

119 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

170   supplement, Fig. S1). Cooling efficiency was calculated as EHL/MHP and indicates

171   the amount of metabolically produced heat that is being dissipate evaporatively, a

172   higher number indicates a greater evaporative cooling capacity (Lawsiewski 1966).

173    $\dot{V}O_2$ was converted to mass-specific metabolic rate (ml g^{-1} h^{-1}) for each individual to

174   enable direct comparison with adult metabolic rate (Lill *et al.* 2006).

175

176   *Behavioural observations*

177   While the nestlings were in the metabolic chamber, they were remotely monitored for

178   the duration of the trial using a camera (GoPro Hero7, Calexico, CA USA) and their

179   behaviour recorded every 10 min (6 observations in total per nestling). At each

180   observation, the posture of the nestlings and occurrence (yes/no) of heat dissipation

181   behaviours were recorded: wing-drooping - wings held away from the body; panting -

182   beak is open and the nestling is breathing heavily. The temperature in the chamber

183   at the time of the observation was obtained from the temperature logger inside the

184   chamber. For analysis, we used the last three observations (40, 50 and 60 min) to

185   allow for the nestlings to acclimate to the chamber.

186

187   *Statistical Analysis*

188   Replication Statement table:

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Species (young)	Species (young)	1 species

189

190    $\dot{V}O_2$ and $\dot{V}CO_2$ were used as proxies for metabolic rate and two inflection points

191   were identified using non-linear mixed effects models in R which included nest

192   identity as a random intercept (Pinheiro et al., 2009) (package: "nlme"). The data for

193    $\dot{V}O_2$ and $\dot{V}CO_2$ were square root-transformed to improve normality and aid in model

194   convergence. The back-transformed coefficients and standard errors are presented

195   in text and figures (Fig 1 and Fig. S3) and raw values in supplement (Table S1). We

196   present $\dot{V}O_2$ in the main results, as measurements of $\dot{V}O_2$ are more robust to

197   changes in substrate utilisation than $\dot{V}CO_2$; however, calculations based on $\dot{V}O_2$ and

198    $\dot{V}CO_2$ were very similar (compare Fig. 1 with Fig. S3). Non-linear mixed effects

199   models were also used to identify one inflection point for EWL and cooling efficiency

200   with nest identity as a random intercept. The effects of ambient temperature, mass,

201   and nestling sex, on probability of occurrence for wing-drooping and panting (binary

202   variables yes/no per observation point) were determined using general linear mixed

203   models, using the *logit* link-function (Bates *et al.* 2022) (package: "glmer"). Each

204   individual had 4 observations and the random effects of individual and nest identity

205   were included in the models. The 95% confidence intervals for these regressions

206   were predicted using ggpredict, which included the random effects of individual and

207   nest identity in the predicted probabilities (Ludecke et al., 2022). The model

208   predictions were used to determine the temperature at which the behaviour response

209   was present in 50% of observations (*p50*).

210

211   **Results:**

212   *Thermoneutral zone*

213   We obtained reliable metabolic rate measurements from 70 7-day old nestlings from

214   36 nests and all nestlings rested throughout the entire trial. The average respiratory

215   exchange ratio (RER) was 0.7, indicating that the nestlings were postabsorptive (see

216   supplement, Fig. S1). Metabolic rate was weakly but significantly positively

217   associated with nestling mass (coefficient = 0.07 ± 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

242   go to 1.0 or above, indicating that, at all temperatures tested, nestlings were

243   producing more heat metabolically than they were losing evaporatively.

244

245   *Heat dissipation behaviours*

246   The occurrence of heat dissipation behaviours was monitored in 47 nestlings from 23

247   nests. The chamber temperature significantly predicted the probability of wing-

248   drooping (t = 89.12, P < 0.001) and panting (t = 103.83, P < 0.001) behaviour and

249   there was no effect of mass or sex on probability of either behaviour (Fig. 4 and

250   Table S3). Wing-drooping p_{50} was at 34°C and panting p_{50} was at 40°C, and by

251   42°C, all individuals were wing-drooping and panting. During our measurements at

252   temperatures above 42°C, towards the end of the hour, nestlings often started to

253   show heavier and quicker panting. Once they were removed from the chamber, they

254   calmed down rapidly when entering the cooler air.

255

256   **Discussion:**

257   This study directly measured the thermoneutral zone and determined the

258   physiological and behavioural heat dissipation capacity in small songbird nestlings; a

259   vulnerable life stage to climate change. Exposing nestlings to ecologically relevant

260   temperatures up to 45°C revealed that they are tolerant to high temperatures,

261   however they have limited ability to cool themselves. They are at risk of dehydration

262   at temperatures above 33.1°C, and at temperatures above 42.3°C dehydration risk is

263   doubled and compounded by an increase in metabolic heat production.

264

265   *High and narrow thermoneutral zone*

266 Determining the thermoneutral zone and the upper critical limit for nestlings is crucial

267 for understanding their vulnerability to increasing environmental temperatures. We

268 found that homeothermic nestlings had a distinct thermoneutral zone between 33-

269 42°C. These temperatures span the average body temperature of small songbirds

270 (38–42°C) (Yahav 2015; Pollock *et al.* 2021), as well as the average nest

271 temperature for passerines (34–37°C) (Kendeigh 1940; Irving & Krog 1956; Blem

272 1973). Given that nestlings are relatively inactive and have little insulation, a normal

273 body temperature is thus maintained with minimum thermoregulatory costs at

274 common ambient and nest temperatures. Although there are no comparable data on

275 the thermoneutral zone of nestlings in other species, the range we identified spans

276 temperatures that were previously identified to correspond to lowest energy use

277 (Nager & Wiersma 1996) or presumed to be thermoneutral (Ton & Martin, 2016;

278 Weathers & Sullivan, 1991). A number of studies measured metabolic rate (O_2

279 consumption or CO_2 production) of nestlings over a range of temperatures to

280 investigate the development of endothermy. From the figures presented in those

281 studies, the lower critical limit of the thermoneutral zone seems to typically fall

282 between 30–35°C (Dawson & Evans, 1957, 1960; Dyer, 1968; Mayer et al., 1982;

283 Nager & Wiersma, 1996; Sirsat et al., 2016), which is consistent with the lower

284 critical limit we identified for superb fairy-wren nestlings. Only one study of

285 developing cattle egret (*Bubulcus ibis*) nestlings studied higher temperatures (up to

286 45°C) (Hudson *et al.* 1974), which suggested metabolic rate to be minimal from 34°C

287 to 40°C, relatively similar to superb fairy-wren nestlings despite the difference in the

288 body size and ecology of the species. Taken together, the available evidence thus

289 tentatively suggests that potential thermoneutral ranges of nestlings of other species

290 may be comparable to superb fairy-wren nestlings.

291

292 The metabolic parameters of nestlings differ substantially from those of adult superb

293 fairy-wrens (Lill *et al.* 2006). At thermoneutral temperatures, the mass-specific

294 metabolic rate of nestlings is 3.95 ml O_2 g^{-1} h^{-1}, which is 1.5 times that of adults (2.65

295 ml O_2 g^{-1} h^{-1}; Lill et al. 2006). A higher metabolic rate in nestlings is consistent with

296 other species (Dawson & Evans, 1957; Kendeigh, 1939; Newberry et al., 2021;

297 Weathers & Siegel, 1995) and is presumably related to their rapid growth and

298 development requiring higher energy use (Dunn 1980; Olson 1992). The

299 thermoneutral zone of adult superb fairy-wrens spans a wider temperature range and

300 occurs at much lower temperatures (26°C–35°C; Lill et al., 2006). The narrower

301 thermoneutral zone of nestlings may result from their reduced insulation (body fat

302 and feathering), allowing metabolically produced heat to be dissipated more easily

303 into the environment, whereas adults have limited ability to passively dissipate

304 excess heat (McKechnie & Wolf, 2019). These differences between adults and

305 nestlings may be common, as many adult birds have thermoneutral zones between

306 25°C and 34°C (Scholander *et al.* 1950; Khaliq *et al.* 2014). A higher and narrower

307 thermoneutral zone in the nestlings compared to the adults indicates that the

308 nestlings are better adapted to warmer temperature but have a greater sensitivity,

309 and this pattern may be general for birds with altricial young.

310

311 *Limited capacity for cooling*

312 As ambient temperature rises towards body temperature, the scope for heat to be

313 lost passively through the thermal gradient declines and birds become reliant on

314 evaporative water loss for cooling (Smith *et al.* 2017). In the nestlings, the sharp

315 increase in water loss occurs at 33.5°C, around the lower critical limit of the

316   thermoneutral zone, which is in contrast to many adult passerines where water loss

317   increases around the upper critical limit, when panting commences (Albright *et al.*

318   2017; McKechnie *et al.* 2017; Smith *et al.* 2017). However, this is similar to blue tit

319   (*Cyanistes caeruleus*) nestlings, where water loss also appears to increase above

320   the lower critical limit (30°C; Nager and Wiersma, 1996; no data at higher

321   temperatures). Given that nestlings are not yet fully feathered, cutaneous

322   evaporation is likely to be occurring at a greater rate with water passively transferred

323   from the skin into the environment (Yahav 2015; McKechnie *et al.* 2016b).

324   Cutaneous evaporation is difficult to control physiologically (Yahav 2015) and water

325   loss is occurring even though body temperature is being maintained with minimum

326   energy usage in the thermoneutral zone. Thus, temperatures in the thermoneutral

327   zone pose a dehydration risk to nestlings, a previously unrecognised risk of climate

328   warming, especially in more arid regions (Albright *et al.* 2017).

329

330   Nestlings employed two main heat dissipation behaviours, wing-drooping and

331   panting. The median temperature at which wing-drooping occurs is 34°C, which

332   coincides with the lower critical limit of the thermoneutral zone and the inflection

333   point for water loss. This is in accordance with the notion that wing-drooping

334   encourages passive heat loss through cutaneous evaporation (Pattinson *et al.* 2020),

335   allowing the nestlings to maintain a stable body temperature without increasing

336   metabolic heat production. As temperatures increase, the heat lost through passive

337   dissipation is not enough to prevent a rise in body temperature, thus a more active

338   heat loss strategy is required, through panting, which increases respiratory water

339   loss. The median temperature at which panting was observed occurred at 40°C, and

340   by 42°C all individuals were panting. Panting is often associated with a sharp

341 increase in water loss (Albright *et al.* 2017), however in the nestlings there was no

342 additional inflection point for water loss. A lack of a clear inflection point suggests

343 that nestlings rely more on cutaneous evaporation through wing-drooping for cooling,

344 and that panting does not enhance cooling efficiency.

345

346 Nestlings are also not efficient at cooling. Although the cooling efficiency increases

347 as temperature increases, the ratio of heat loss to heat production never exceeded a

348 value of 1 (Fig. 3), indicating that nestlings are unable to dissipate more heat than

349 they produce. Limited data is available on the cooling efficiency of other songbird

350 nestlings or adults, but a similar inability to dissipate their own metabolic heat

351 production was found in blue tit nestlings from a temperate climate (Nager &

352 Wiersma 1996) and in adults of two species from cool/mesic climates (arctic tundra

353 and subtropical mountains (Oswald *et al.* 2018a; O'Connor *et al.* 2021). Conversely,

354 heat-tolerant adult passerines from arid zones are able to dissipate more heat than

355 they produce, indicating more efficient evaporative cooling mechanisms (Whitfield *et*

356 *al.* 2015; McKechnie *et al.* 2017; no information on nestlings). Birds that regularly

357 inhabit more temperate, mesic regions, such as south-eastern Australia, may

358 depend more on cutaneous evaporation (Yahav 2015), which is not as effective at

359 cooling the body and does not conserve water (McKechnie & Wolf 2019), but can

360 help to control body temperature without an increase in metabolic rate or heat

361 production. Species that rely on cutaneous evaporation are more vulnerable to

362 moderate increases in temperature and our results suggest that this applies to

363 superb fairy-wren nestlings as well.

364

365 *Ecological implications*

366 Recent and predicted increases in global temperature can negatively affect the

367 physiology and morphology of nestlings which may impact their lifetime fitness

368 (Metcalfe & Monaghan 2001; Gardner *et al.* 2011; Andreasson *et al.* 2018; Eastwood

369 *et al.* 2022). Currently, summers in south-eastern Australia average 8.3 days with a

370 maximum temperature greater than 35°C, and it is predicted by 2050 this will

371 increase to 14–16 days (Clarke *et al.* 2019). Vulnerability to these increasing

372 temperatures is determined by many different factors, including but not limited to, the

373 physiological thermal tolerances and the physiological and behavioural strategies

374 used to prevent hyperthermia (Pollock *et al.* 2021). Thus, a detailed understanding of

375 the thermal limits and capability of nestlings to prevent hyperthermia is necessary in

376 predicting how individuals and populations will respond to the challenges associated

377 with global warming (Conradie *et al.* 2019; Nord & Giroud 2020; Pollock *et al.* 2021).

378 Nestlings are metabolically more tolerant to warm temperatures than adults (that

379 start showing increased metabolic rate at temperatures > 35°C, Lill *et al.* 2006),

380 suggesting that the immediate risk of hyperthermia for the nestlings is generally

381 lower than adults. However, nestlings incur water loss even within the thermoneutral

382 zone, but are unable to select cooler microclimates and have no access to water,

383 and thus have an associated higher risk of dehydration.

384

385 Dehydration is an important threat to nestlings at relatively low temperatures. At

386 35°C, shortly above the temperature when water loss starts to increase but within the

387 thermoneutral zone, nestlings lose approximately 1% of body mass in water per hour

388 (average mass; 7.5g). At 43°C, above the upper limit of the thermoneutral zone,

389 water loss is doubled and nestlings lose approximately 2% of body mass in water per

390 hour. In the absence of compensatory water intake, after 5 hours at 43°C, nestlings

391   would lose 10% of their body mass in water, which is within the lethal limit for birds

392   (Wolf & Walsberg 1996; Albright *et al.* 2017; Sharpe *et al.* 2019). These physiological

393   responses match the effect of ambient temperature observed in a long-term dataset

394   of superb fairy-wren nestling body mass on day 7 (Kruuk *et al.* 2015): average

395   maximum temperature above 35°C during the two days before measurement was

396   associated with a decline in nestling body mass of 0.37 g, 5% of body mass (which

397   reduces their fitness; Kruuk et al., 2015). This supports our prediction that for

398   nestlings in the wild, biologically relevant dehydration can occur at temperatures that

399   are within the thermoneutral zone. Provisioning to the nestlings by the parents could

400   mitigate or compensate for the increased water loss and energy requirements at

401   these higher temperatures, e.g. by providing more food, or food with high water

402   content (Wiley & Ridley 2016; van de Ven *et al.* 2019; Bourne *et al.* 2021a), but this

403   would entail substantial thermoregulatory costs for adults themselves (given their

404   upper critical limit of 35°C; Lill et al., 2006).

405

406   Once temperatures reach the upper critical limit, the impact of increased water loss

407   is compounded with an increase in metabolic activity when active cooling

408   commences. Although nestlings are panting at this stage, panting is inefficient at

409   substantially increasing their cooling capacity. This situation, when the maximum

410   rate of water loss is insufficient to prevent body temperature from rising, can induce

411   immediate hyperthermia (Mertens 1977), as evident from the fact that at this point

412   skin temperature of the nestlings exceeded air temperature (Fig. 5). Our

413   observations of 5 nestlings at temperatures> 42°C with high body temperature and

414   low metabolic rates support the notion that at these temperatures, nestlings become

415   heat stressed and hyperthermic. Nestlings have little ability to prevent this increase

416 in body temperature and are at a high risk of lethal hyperthermia. Therefore,

417 predicted climate warming, with near doubling in average days above 35°C and

418 increasing risks of extreme temperatures (Clarke *et al.* 2019), poses a high risk of

419 both immediate hyperthermia and long-term hyperthermia and dehydration.

420

421 *Conclusion*

422 In order to understand how increases in ambient temperature will affect birds, it is

423 necessary to find the temperatures when they face thermal challenge and may

424 become heat stressed. Our results show that when predicting how populations will

425 change with climate, one cannot assume that the thermoregulatory strategies and

426 thermal tolerances of adults can be extended to their nestlings. Additionally, weather

427 conditions, such as humidity, rainfall, wind and solar radiation, and characteristics of

428 the nest (Ricklefs & Hainsworth 1969; Ardia 2013; Welman & Pichegru 2022) as well

429 as brood size, nestling behaviour (huddling or spreading further apart in the nest),

430 and age of the nestlings (Mertens 1977; Andreasson *et al.* 2016; Mitchell *et al.* 2022)

431 can affect their heat load and cooling requirements (Wolf & Walsberg 1996). These

432 conditions can exacerbate ambient conditions, with nestlings incurring additional

433 water loss or energy use maintain body temperature, or help mitigate the thermal

434 environment, reducing water loss and energy requirements. It is therefore important

435 to note that in this study the metabolic and water loss rates were collected on a

436 single resting nestling in a chamber with a constant temperature and very low

437 humidity and caution should be used when extrapolating the results to field

438 conditions. It is thus critical to determine in the field when the environment is

439 thermally stressful for nestlings. The tight correlation between physiological and

440 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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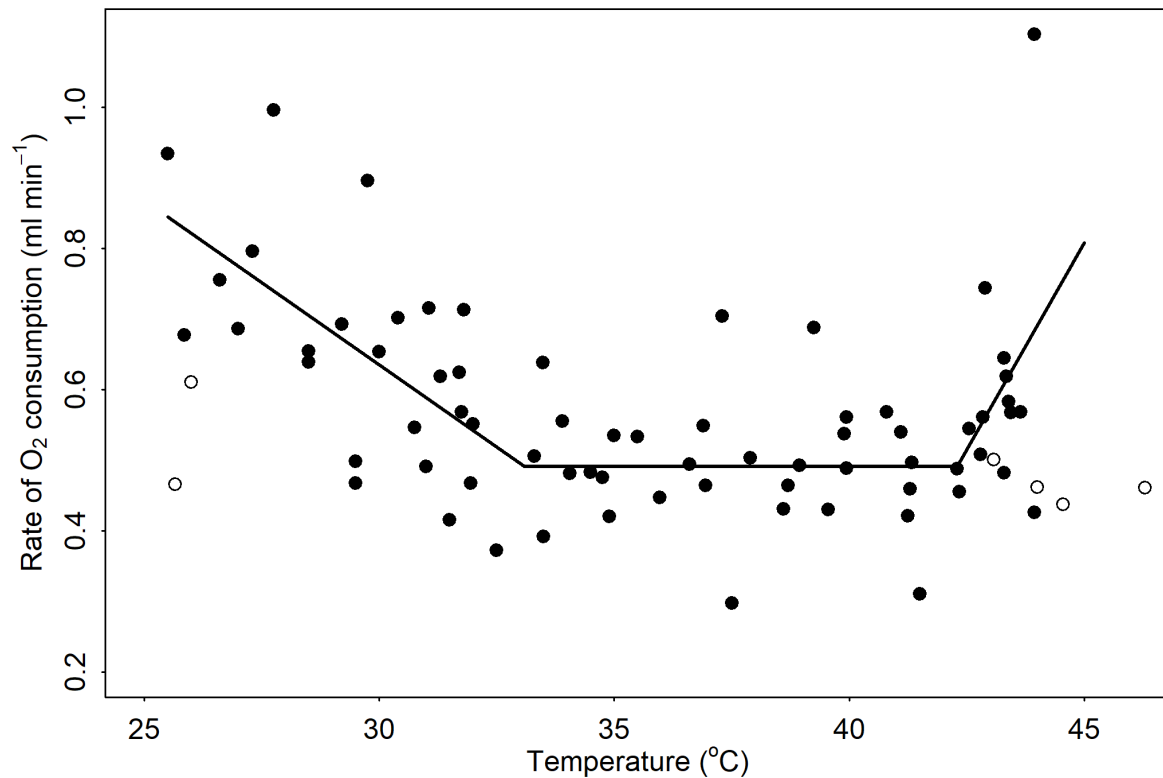582   *Ecol. Evol.*, 16, 254–260.
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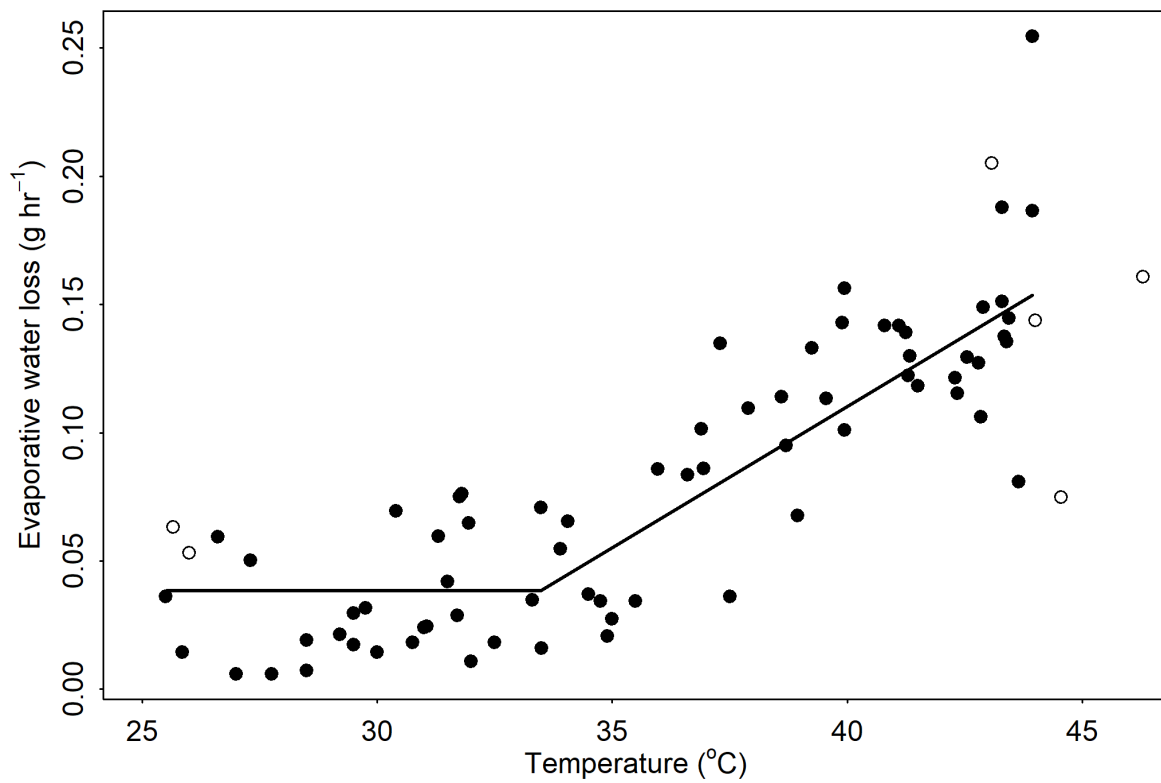677 905.
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680 Figures:

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.

688

689

690   Fig. 2. Evaporative water loss in superb fairy-wren nestlings is constant until an

691   inflection point at 33.5°C, with the rate of water loss ($\dot{V}H_2O$) increasing rapidly with

692   chamber temperature above this point. Open symbols represent nestlings that were
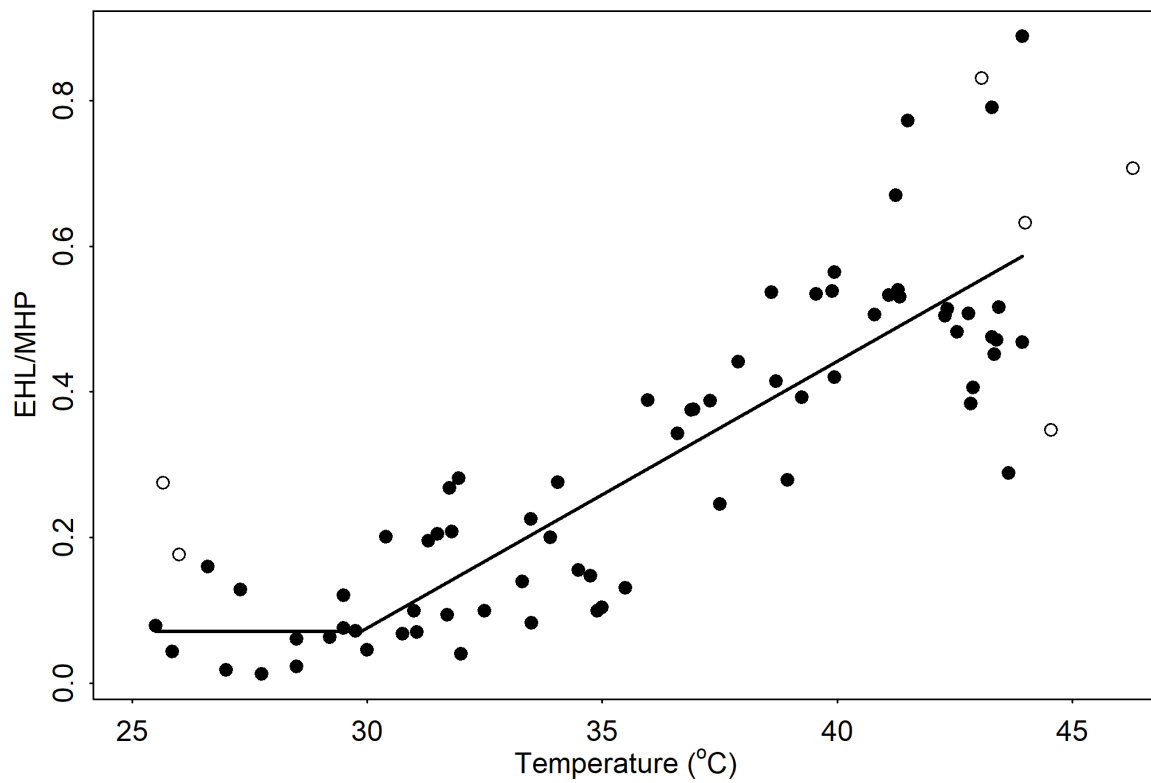
693   excluded from all analyses (details see Fig. 1).

694

695 Fig. 3. Cooling efficiency of superb fairy-wren nestlings, the ratio of evaporative heat

696 loss (EHL) to metabolic heat production (MHP), is constant until an inflection point at

697 29.9°C. Above this point cooling efficiency rapidly increases with increasing chamber

698 temperature. Open symbols represent nestlings that were excluded from all

699 analyses.
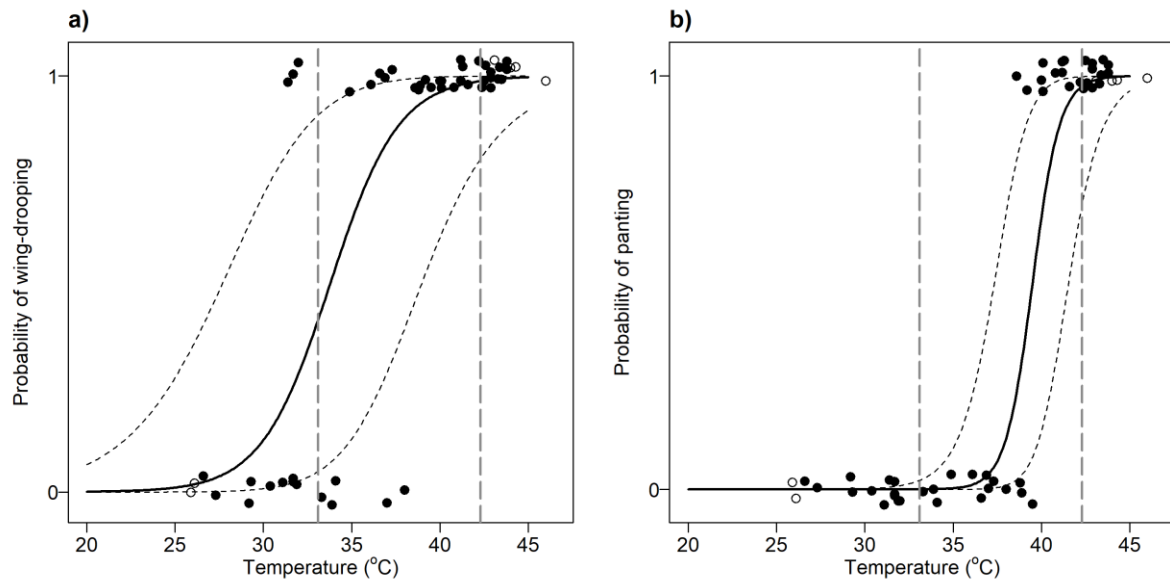
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703   Fig. 4. Thermoregulatory behaviours closely match physiological limits. Shown are

704   the probabilities that heat dissipation behaviours a) wing-drooping and b) panting

705   were observed (1 = yes; 0 = no) with increasing chamber temperature. Each point

706   (jiggered for presentation purposes) represents the last observation of the

707   temperature exposure period; open symbols indicate nestlings that were excluded

708   from all analyses (see Fig. 1 for details). The solid line is the predicted probability

709   and dotted lines the 95% confidence intervals predicted from GLMMs incorporating

710   all observations and accounting for the random effects of nestling and nest identity.
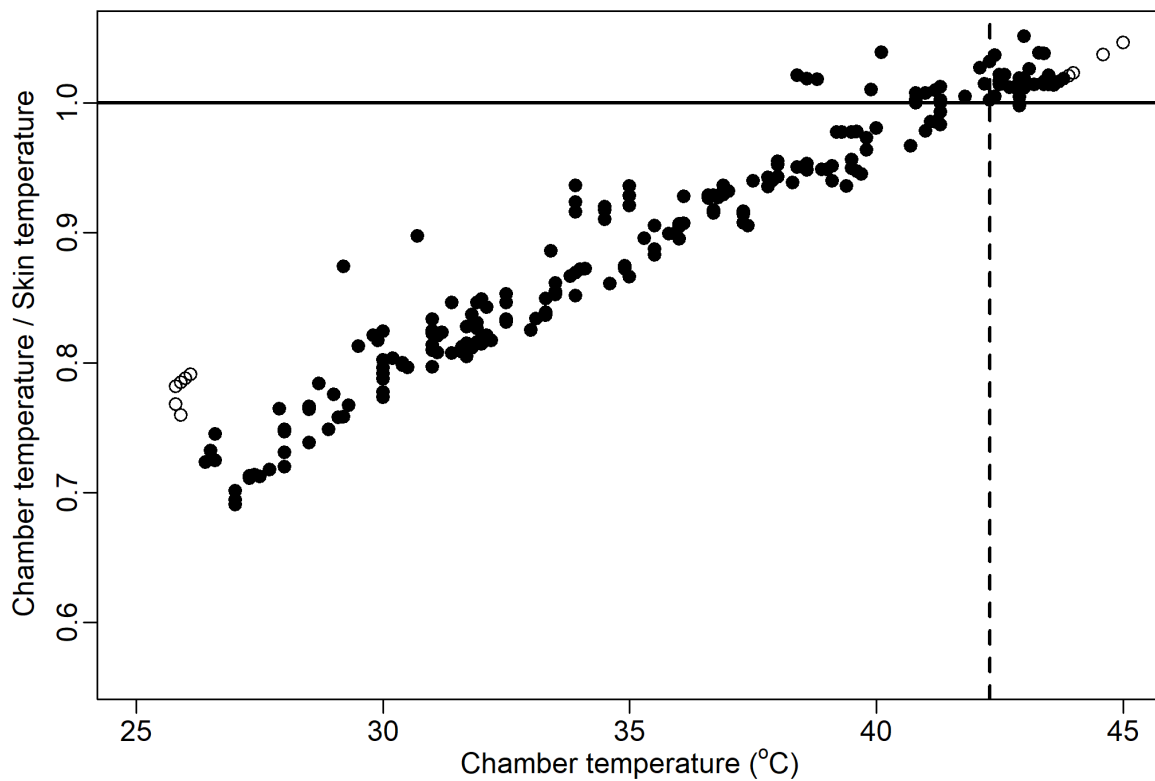
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715 Fig. 5. Nestling skin temperature increases with increasing chamber temperature.

716 Graph shows the ratio of skin temperature to chamber temperature, with the solid

717 line at 1.0 representing the ratio when the nestlings skin temperature is equal to the

718 chamber temperature. Dashed line represents the upper critical limit for the nestlings

719 (see Fig. 1). Open symbols indicate nestling with skin temperature too high or too

720 low to be accurately measured by the PIT tag (range 33-43°C); these are

721 represented by the minimum and maximum, 43°C and 33°C, respectively (although it

722 is likely that the actual temperatures were higher or lower).

723