



## Do Bewick Wren (*Thryomanes bewickii*) nestlings experience thermal stress in nest-boxes in a tropical urban city?

### ¿Los pollos en el nido del saltapared cola larga (*Thryomanes bewickii*) experimentan estrés térmico en cajas nido en una ciudad tropical?

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#### Abstract

Urban areas present thermal environments that may be challenging for altricial nestlings, particularly in early development. We aimed to evaluate whether altricial Bewick's Wren (*Thryomanes bewickii*) nestlings experienced thermal stress in the nest-box during the early- and late-developmental stages. We determined the thermoregulatory set point (thermoneutral range) of nestlings by measuring cloacal body temperature of five nestlings from three nests, every other day from hatching until they fledged. We also exposed nestlings to a thermal challenge from day 5 to determine the age of endothermy. We used dataloggers to determine the thermal environment inside and outside of three nest-boxes, and obtained the operative temperature for nestlings in the nest-box using 3D-printed models corresponding to early- and late-developmental stages. Ambient temperatures inside the nest box fluctuated throughout the day, being colder at night just before day-break (min range: 10.4°C – 10.8°C), and hotter in the afternoon (max range: 25.8°C - 28.3°C). However, temperatures in the nest-box did not reach the extreme high and low outside temperatures, and were warmer at night. Nestlings reached age of endothermy at 9 ± 2.3 days after hatching, and showed a thermoneutral range of body temperature above ambient temperatures in the nest-box. Operative temperatures of models in the nest-box closely followed ambient temperatures, and were below nestlings' thermoneutral range. Therefore, nestlings could experience thermal stress when parents do not provide additional buffering while off the nest for foraging. Although nest-boxes provide limited thermal benefits for nesting birds in tropical cities, they still provide valuable nesting and roosting sites where natural cavities are limited in availability.

**Keywords:** age of endothermy, altricial birds, nestling developmental temperatures, operative temperature, urban ecology.

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## Resumen

Las áreas urbanas presentan ambientes térmicos que pueden ser un desafío para los pollos altriciales, particularmente durante el desarrollo temprano. Nuestro objetivo fue evaluar si los polluelos del reyezuelo altricial de Bewick (*Thryomanes bewickii*) experimentaron estrés térmico en cajas nido durante las etapas tempranas y tardías de su desarrollo. Determinamos el punto de ajuste termorregulador (rango termoneutral) de los polluelos, midiendo la temperatura corporal cloacal de cinco polluelos de tres nidos, cada dos días desde que eclosionan hasta que abandonaron el nido. También expusimos a los polluelos a un desafío térmico desde el día 5 para determinar la edad de la endotermia. Usamos registradores de datos para determinar el ambiente térmico dentro y fuera de tres cajas nido, y obtuvimos la temperatura operativa para los polluelos en la caja nido usando modelos impresos en 3D de polluelos correspondientes a etapa temprana y tardía de desarrollo. La temperatura ambiente dentro del nido fluctuó a lo largo del día, siendo más fría por la noche justo antes del amanecer (rango mínimo: 10.4 °C – 10.8 °C) y más calientes por la tarde (rango máximo: 25.8 °C – 28.3 °C). Sin embargo, las temperaturas en la caja nido no alcanzaron las temperaturas exteriores extremadamente altas y bajas, y fueron más cálidas durante la noche. Los pollos alcanzaron la edad de endotermia a los 9 ± 2.3 días después de la eclosión y mostraron un rango termoneutral de temperatura corporal por encima de la temperatura ambiente en el nido. La temperatura operativa de los modelos en la caja nido siguieron de cerca las temperaturas ambientales y estuvieron por debajo del rango termoneutral de los pollos. Por lo tanto, los pollos podrían experimentar estrés térmico cuando los padres no les brindan protección adicional mientras están fuera del nido para buscar alimento. Aunque las cajas nido brindan beneficios térmicos limitados para las aves que anidan en las ciudades tropicales, aún proporcionan valiosos sitios de anidación y descanso donde las cavidades naturales tienen una disponibilidad limitada.

**Palabras clave:** aves altriciales, ecología urbana, edad de endotermia, temperatura de crías en desarrollo, temperatura operativa.

## Introduction

Thermal stress occurs when body heat gains or losses are not balanced by energy exchanged with the environment, requiring additional

energy expenditure for animals to maintain their thermoneutral zone (Bohler et al. 2021). This leads to detrimental effects on their health, growth, reproduction, and productivity, impacting their performance and overall fitness (Guthery et al. 2001, Archambault et al. 2014, Mesa et al. 2002, Xie et al. 2017, Pattinson et al. 2020, Bohler et al. 2021, Cunningham 2021, McKechnie et al. 2021, Fathima et al. 2022). Temperature and potential thermal stress can fluctuate spatially and temporally, and may depend on factors such as the time of the day, climate, relative humidity, and animal species (Kingsolver et al. 2013, Santillán et al. 2018, Rodrigues et al. 2022, Sudyka et al. 2023).

Birds are endothermic homeotherms that can regulate their body temperature to maintain a particular temperature range regardless of the ambient temperature (Bicego et al. 2007). However, altricial birds have young that are underdeveloped and highly dependent on parents during the first days after hatching, and lack strategies to maintain body temperature independent of environmental temperature in the early stages of development (Dawson and Evans 1957, Choi and Bakken 1990, Visser 1998, Baarendse et al. 2007). In this sense, young altricial nestlings behave thermally like ectotherms and rely on parental care as an external source of heat (Ricklefs 1984). The age of endothermy, when altricial nestlings have developed thermal strategies enabling them to produce heat and thermoregulate, is species-specific (Morton and Carey 1971, Dunn 1975, Visser and Ricklefs 1993), and develops as nestlings gain mass allowing them to increase their metabolic rate when ambient temperature is below neutral temperature (O'Connor 1975, Visser 1998, Nagy 2005). This implies that prior to the onset of endothermy, adequate thermoregulation in the nest can have implications for nestling fitness as nestlings are unable to regulate their body temperature during early stages of development.

Hence, the thermal environment inside the nest could be key for altricial nestlings at early stages of development when they are more susceptible to thermal stress, for which the use of tree cavities can be beneficial. Predation has been proposed as the main selection force driving the evolution of the use of different nest types (Vanadzina et al. 2024). However, the thermal benefits of cavity nests may outweigh the benefits provided against predation compared to open-nests (Martin et al. 2017). In

particular, cavity nests may buffer temperature fluctuations in extreme environments, and provide a constant thermal environment regardless of outside temperatures (McComb and Noble 1981, Rhodes et al. 2009).

Urban areas represent extreme environments with modified microclimates that produce the heat island effect, where cities are often warmer than adjacent natural areas (Arnfield 2003). The frequency of heatwaves and number of hot days are also increasing in urban areas (Mishra et al. 2015). Nest-boxes are frequently used in cities to compensate for a low availability of natural cavities for birds (Blewett and Marzluff 2005, Harper et al. 2005, LaMontagne et al. 2015). However, nest boxes may be of low thermal quality due to their thinner walls compared to natural cavities (Maziarz et al. 2017, Strain et al. 2020, Sudyka et al. 2023), negatively impacting fitness (Ardia 2013, Sudyka et al. 2022). Given the significant impact of thermal stress on the health and survival of nestlings (Andreasson et al. 2018, Corregidor-Castro and Jones 2021, Arct et al. 2022), understanding the thermal environment of nest-boxes in cities can provide insights on the potential impact of nest provision on thermal stress in urban conditions.

Our understanding of the temperatures experienced by altricial nestlings during early development is limited, particularly for cavity-nesting species in urban environments with limited nesting opportunities, where nest-boxes may frequently be used. Understanding the development of thermoregulation capabilities of nestlings, and how this may fluctuate with diurnal temperature variations can elucidate whether nestlings experience thermal stress during their early development. The Bewick's Wren (*Thryomanes bewickii*) is an altricial passerine bird that is resident from Canada southwards to central Mexico, occupying a variety of natural and urban environments throughout its range (Kennedy and White 2020). Therefore, nestlings of the Bewick's Wren may face a variety of thermal environments that could present thermal challenges, putting nestlings at thermal stress, outside of their thermoneutral zone. We aimed to evaluate the thermal ecology of Bewick's Wren nestlings during the early stages of development to determine whether they were at thermal stress, with body temperature above or below the internal nest temperature, when using nest-boxes in an urban setting.

## Methods

### Study site

We conducted the study in 2019, at three sites in Morelia city, in the state of Michoacán, Mexico. The study sites were located at: 1) Universidad Latina de América (19°41'50.02"N, 101°14'7.86"W) west of the city center; 2) Universidad Michoacana de San Nicolás de Hidalgo (101°14'7.86"W, 101°12'5.87"S) south of the city center; and 3) Instituto Tecnológico del Valle de Morelia (19°44'55.39"N, 101°9'51.64"W) on the northern outskirts of the city. Morelia city has an extension of 67.2 km<sup>2</sup> (IMPLAN 2022), with agriculture and some pasturelands around the outskirts of the city. The average annual temperature is 18.2°C, and mean total annual rainfall is 803.6 mm, where 75% of rainfall occurs from June to September, followed by a drought from October to May (IMPLAN 2022).

We also identified a nest of the species in a cavity of a *Casuarina equisetifolia* tree at Instituto Tecnológico del Valle de Morelia, on the outskirts of the city. We conducted observations at the nest during 12 to 20 February 2024, for a total of 240 mins, to obtain data on the length of time parents were off the nest for food provisioning. When observations were taken, nestlings were about midway through development.

### Nest-boxes

At each of the three study sites, we placed 30 nest-boxes, which were attached with an eyebolt on either a tree branch or the main trunk. Nest-boxes were rectangular, made of 1.5 cm width plywood, and measured 14.5 cm depth x 12 cm width. The front of the nest-box measured 25 cm high, with a 5 cm diameter entrance hole, while the back of the nest-box measured 29 cm high, so that the roof of the nest-box was inclined allowing rainfall runoff and to reduce the likelihood of flooding. We placed nest-boxes on trees from mid-February 2018, so that breeding pairs of the Bewick's Wren could become accustomed to their use. We checked nest-boxes weekly to register the onset of nesting activity, which was determined when nesting material was observed within the nest-box. Once we determined that a nest-box was in use by a nesting pair, we checked the nest every other day to register the laying and hatch-date. Therefore, we could determine the nestling's age within two days of accuracy. The study was conducted under the Mexican Secretary for the Environment permit

### Nestling age of endothermy

To determine nestling' age of endothermy, we exposed 14 nestlings from 8 nests to a thermal challenge at 5 days of age and on each nest visit thereafter (McCafferty et al. 2015). For the thermal challenge, we inserted a thermocouple probe inside the chicks' cloaca, which was attached to an Omega HH501AJK thermometer. Each nestling was then individually placed inside an insulin portable refrigerator for 5 mins at a constant temperature of 7°C. The thermocouple probe allowed us to register the chicks' core temperature at the beginning and at the end of the thermal challenge. We then calculated the endothermy index (H) for each individual (Visser 1998) using:  $H = (T_f - T_c)/(T_s - T_c)$ , where  $T_f$  is the nestling's temperature at the end of the 5 min challenge,  $T_c$  is the refrigerator temperature, and  $T_s$  is the nestling's initial temperature (Ricklefs 1984). The endothermy index provided an estimation of the nestlings' ability to maintain their core temperature within an optimal threshold. A completely endothermic bird would have a value of  $H = 1$ , while values close to 0 would indicate a poikilotherm bird that is unable to regulate body temperature, and has a temperature closer to that of the environment. Following Visser (1998), we considered a nestling to have reached the age of endothermy when it registered  $H \geq 0.8$ .

### Estimation of thermoregulatory set-point range ( $T_{set}$ )

The thermoregulatory set point ( $T_{set}$ ) is defined as the range of preferred temperature along a gradient that enables individuals to maintain a given body temperature, and can also be considered the thermoneutral zone (Hertz et al. 1993). Therefore, to determine the thermoregulatory set point ( $T_{set}$ ) of nestlings, we took the cloacal body temperature ( $T_b$ ) of five nestlings from three nests (2 broods = 1 nestling, 1 brood = 3 nestlings), at each nest inspection from hatching until they fledged. We then obtained  $T_{set}$  25% – 75% as the temperature range that included 50% of all  $T_b$  measurements, corresponding to the second and third quartiles of all nestling body temperatures. In addition, given that thermoregulatory capabilities vary by age in altricial birds, we divided the entire developmental period in two stages: early- (hatching to the day before age of endothermy) and late-nestling (age of endothermy to fledging), and obtained  $T_{set}$  25% –

75% for both developmental phases separately and combined

### Comparison of ambient and operative temperature ( $T_o$ )

We further compared the potential thermal environment offered in the interior and exterior of nest-boxes with that experienced by biophysical models of nestlings. We characterized the potential thermal environment by measuring ambient temperature both inside and outside the nest-box. Temperature readings were stored in a custom-made Arduino nano based datalogger that was programed to register the temperature every 15 mins. This datalogger has several sensors that can register simultaneously readings from independent sources. Two of those sensors were used to obtain simultaneously temperature readings from inside and outside the nest-box.

In addition, we obtained the operative temperature ( $T_o$ ) for the thermal environment experienced by nestlings in nest-boxes. Operative temperature is the temperature of an inanimate object that does not thermoregulate, with zero heat capacity, and has the same size as the animal exposed to the same thermal environment (Bakken 1992). Thereby, operative temperature may be a close representation of the temperature experienced by an ectotherm, and enabled us to take into consideration the potential heat exchange due to radiation, convection, and conduction (Shine and Kearney 2001) of a non-endothermy nestling when parents were off the nest. Using a Ultimaker 2+ 3D printer, we created 3D Acrylonitrile Butadiene Styrene (ABS) biophysical models that imitated the size, shape, posture, and heat capacity of the target organism (Hertz 1992a, b, Shine and Kearney 2001). Although Cooper-made physical models have been used in the past, ABS physical models have proved to be an equally reliable device (Watson and Francis 2015). Biophysical models were 3D printed with a hole in the ventral region, where we inserted an additional sensor of the thermal custom-made datalogger, thereby obtaining operative temperature simultaneously with potential ambient temperature. We built 3D physical models in two sizes according to developmental stage: (a) early-stage model representing individuals that were  $\leq$  the age of endothermy, and (b) late-stage model, with the size of individuals  $\sim$ 14 days, when endothermic nestlings are ready to fledge. We placed 3D physical models in three randomly selected nest-boxes that

had previously been used by Bewick's Wrens: two nest-boxes at Universidad Latina de America, and one at Instituto Tecnológico del Valle de Morelia. Models were placed within nest-boxes on top of the nest-cup after nestlings had fledged as adults of the Bewick Wren usually remove objects from an active nest. We maintained dataloggers and models within nest-boxes for three days to obtain a range of measurements simulating the temperatures experienced by non-endothermic nestlings.

### Thermoregulation indices

We obtained the difference in temperature inside and outside the nest-box ( $\Delta T^\circ$ ) recorded by sensors on dataloggers, and the coefficient of variation (CV). We determined operative temperature for three time periods: a) morning (08:00 to 13:59 hrs), b) afternoon (14:00 to 18:59 hrs), and c) night (19:00 to 07:59 hrs). We also obtained the mean body temperature of nestlings ( $T_b$ ) in both the early- and late-development stages. Based on these indices, we would consider nestlings to be under thermal stress if their thermoneutral zone, estimated from the second and third quartiles of all nestling body temperatures ( $T_{set}$  25% – 75%), was above or below ambient or operative temperatures, indicating additional energy expenditure to maintain the thermoneutral zone. We also determined the period of the day when temperature inside the nest-box was not within the thermoneutral range of the second and third quartiles of nestling body temperatures ( $T_{set}$  25% – 75%), to determine the time of day or night when nestlings could experience thermal stress. Results are expressed as means and standard deviations.

### Statistical analysis

All variables followed a log normal error distribution except for nestling body temperature, which followed a normal error distribution, hence we performed Linear Mixed Models (LMM). We performed five LMM to evaluate the factors influencing ambient and nestling temperatures. To determine whether the type of environment influenced the potential thermal environment available for nestlings, we carried out LMM using ambient temperatures measured by the custom-made dataloggers as the response variable. The fixed effect of type of environment was considered with two levels: i) inside and ii) outside the nest-box. Furthermore, to evaluate whether period of the day could explain variations in ambient temperatures,

we constructed two additional LMM, one with the response variable of temperature inside the nest-box, and the second model considering outside temperature. In both these models, we grouped temperature readings by period of the day, with three levels, i) morning, ii) afternoon, and iii) night, as the fixed effect. To assess the influence of nestling development on body temperature, we constructed a fourth LMM using nestling body temperatures from cloaca measurements. For this model, nestling developmental stage of i) early- and ii) late-stage nestling was considered as a fixed effect. Finally, to determine the influence of nestling developmental stage on operative temperatures in the nest-box, we constructed a fifth LMM with operative temperatures with the 3D-printed models as the response variable. Model developmental stage was considered as the fixed effect, with two levels: small, and large.

To account for the lack of independence in multiple temperature readings taken continuously, we considered the nest-box as the random effect in models, except for the nestling body temperature model, for which nestling identity was used as random effect. We used the nlme package for R (Pinheiro et al. 2015) for normal distribution dataset, and the lme4 package (Bates et al. 2014) for the log normal distribution datasets. Finally, we performed a Pearson's test to evaluate the association between temperature inside the nest-box and the outside temperature.

## RESULTS

### Nestling thermoregulatory set-point range and endothermy age

Although Linear Mixed Models indicated that nestling body temperature did not vary significantly with developmental stage, early-stage nestlings had on average a slightly lower  $35.2 \pm 2.6^\circ\text{C}$  body temperature (range:  $30.1 - 38.3^\circ\text{C}$ ,  $n = 8$ ) than late-stage nestlings ( $36.6 \pm 1.6^\circ\text{C}$ , range:  $32.2 - 38.4^\circ\text{C}$ ,  $n = 8$ ). Nestling body temperature for the entire development period ranged from  $31.1^\circ\text{C}$  to  $40.9^\circ\text{C}$ , with a 50% quartile of nestling body temperatures that ranged from  $35.9^\circ\text{C}$  to  $38.4^\circ\text{C}$  for the entire nestling development period. By comparison, early-stage nestlings had a lower 50% quartile of body temperatures from  $35.1^\circ\text{C}$  to  $37.4^\circ\text{C}$ , while for late-stage nestlings this was between  $36.9^\circ\text{C}$  and  $38.6^\circ\text{C}$ . Nestlings reached age of endothermy at a mean  $9 \pm 2.3$  days after hatching (range: 7 - 13

days,  $n = 7$  nestlings). In the natural cavity, Bewick's Wren parents were off the nest for  $10.4 \pm 6$  mins, and up to 20 mins, foraging for food.

### Ambient temperatures

Linear Mixed Models demonstrated that the type of environment influenced ambient temperature (Estimate =  $0.03 \pm 0.01$  (SE), 95% CI =  $0.02 - 0.04$ ;  $t = 5.25$ ,  $P < 0.001$ ), with temperature inside the nest-box being higher ( $18.3^\circ\text{C} \pm 3.3^\circ\text{C}$ , range:  $12.0 - 25.2^\circ\text{C}$ , CV = 18.1%) than the outside ambient temperature ( $17.8 \pm 3.9^\circ\text{C}$ , range:  $10.4 - 28.3^\circ\text{C}$ , CV = 21.8%). Temperature inside the nest-box was significantly correlated with ambient temperature outside the nest-box ( $r = 0.95$ ,  $P < 0.001$ ), but the inside-outside temperature difference ( $\Delta T^\circ$ ) was on average  $0.5 \pm 1.2^\circ\text{C}$ . This indicates that overall, the nest-box maintained a slightly warmer internal ambient temperature. Nevertheless, the interior of the nest-box was cooler than the exterior at 16:45 hrs ( $\Delta T^\circ = -4^\circ\text{C}$ ), indicating that nest-boxes could provide more benign thermal environments when extreme temperatures occur at key times of the day, such as early afternoon.

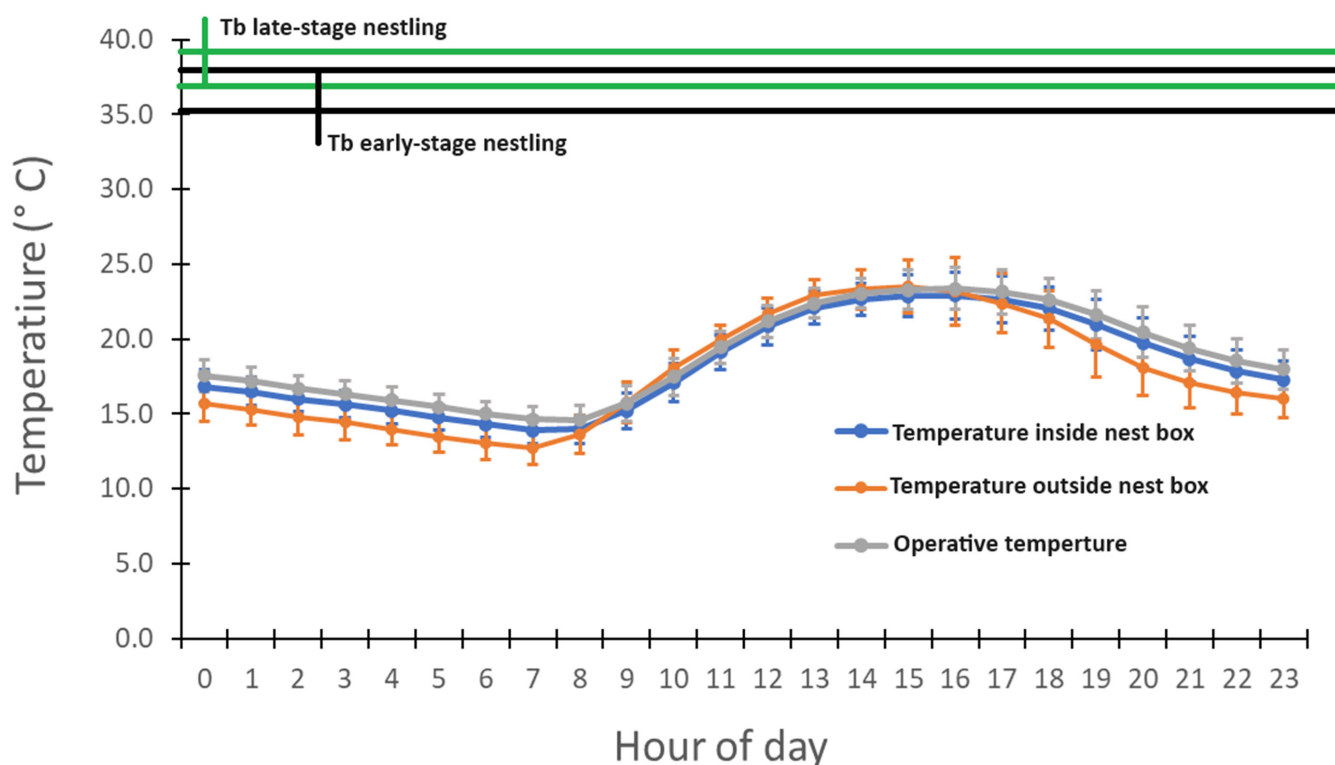
### Timing of extreme temperatures

Extreme high ambient temperatures were recorded

during the afternoon (Fig. 1), with the highest ambient temperature of  $25.0^\circ\text{C} - 25.2^\circ\text{C}$  inside the nest-box between 16:00 and 18:00 hrs, and the highest temperatures of  $25.8^\circ\text{C} - 28.3^\circ\text{C}$  outside the nest-box recorded between 15:00 and 16:45 hrs. The lowest temperatures both inside and outside the nest-box occurred around day-break (Fig. 1). The lowest temperatures of  $12.0^\circ\text{C} - 12.2^\circ\text{C}$  inside the nest-box occurred between 06:45 and 08:15 hrs, while temperatures of  $10.4^\circ\text{C} - 10.8^\circ\text{C}$  outside the nest-box occurred between 06:00 and 07:45 hrs. This indicates that the timing of extreme temperature differs by type of environment, with lowest and highest temperatures occurring earlier in the day outside the nest-box compared to within the nest-box. This also demonstrates that nestlings face a wide range of temperatures in the nest-box during the day, with fluctuations of up to  $13.2^\circ\text{C}$  between the highest and the lowest temperatures recorded.

### Temperatures by day period

LMM analysis indicated that ambient temperatures also varied significantly with period of the day, both inside and outside the nest-box (Table 1). In particular, the environment outside and inside the nest-box in the afternoon was skewed towards high temperatures (Fig. 2a), while the environment



**Figure 1.** Mean ( $\pm$ SD) temperature inside and outside nest-boxes, and operative temperature over 24 hours in Morelia City, Mexico. Second and third interquartile range ( $T_{set}$  25% – 75%) of body temperature is shown for early- (green line) and late-stage (black line) nestlings

**Table 1.** Results of Linear Mixed Models for temperature inside and outside of Bewick's Wren nest-boxes in Morelia City, Mexico. Day period [afternoon] was considered as reference in models.

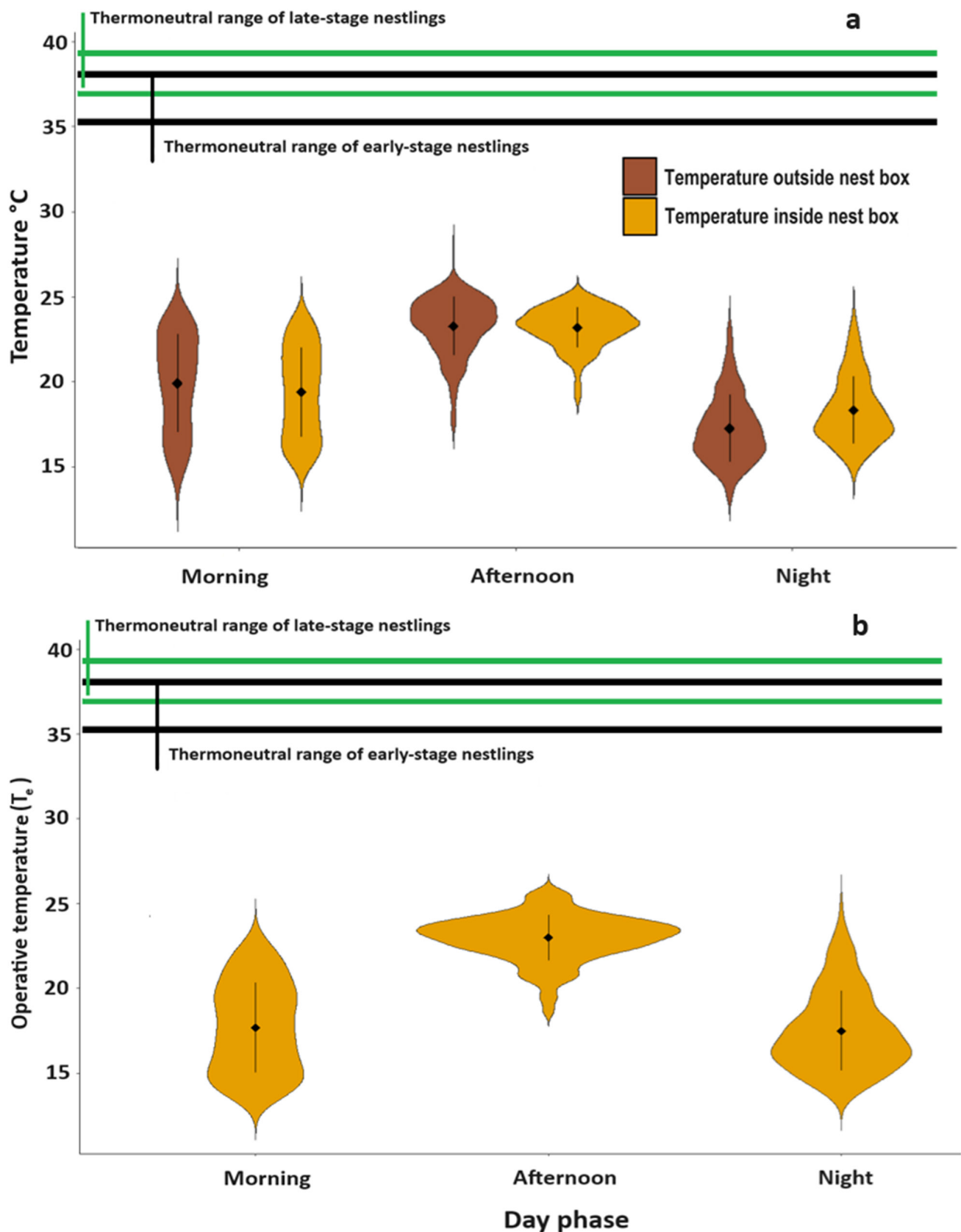
| Predictors                | Temperature inside nest box |                        |                         | Temperature outside nest box |                        |                         |
|---------------------------|-----------------------------|------------------------|-------------------------|------------------------------|------------------------|-------------------------|
|                           | Estimate ± SE               | Test value             | 95% Confidence Interval | Estimate ± SE                | Test value             | 95% Confidence Interval |
| (Intercept)               | 3.12 ± 0.01                 | $t = 336.7, P < 0.001$ | 3.10 – 3.14             | 3.12 ± 0.01                  | $t = 227.9, P < 0.001$ | 3.10 – 3.15             |
| Day phase [Morning]       | -0.23 ± 0.01                | $t = -31.6, P < 0.001$ | -0.24 – -0.21           | -0.2 ± 0.01                  | $t = -26.2, P < 0.001$ | -0.21 – -0.18           |
| Day phase [Night]         | -0.3 ± 0.01                 | $t = -48.9, P < 0.001$ | -0.31 – -0.29           | -0.38 ± 0.01                 | $t = -56.7, P < 0.001$ | -0.40 – -0.37           |
| Random effects $\sigma^2$ | 5.82                        |                        |                         | 6.69                         |                        |                         |
| Observations              | 2469                        |                        |                         | 2469                         |                        |                         |
| Marginal R <sup>2</sup>   | 0.002                       |                        |                         | 0.003                        |                        |                         |

inside and outside the nest-box at night was skewed towards low temperatures (Fig. 2a). The distribution of temperatures was more homogeneous during the morning (Fig. 2). Tukey HSD pairwise comparisons showed that temperatures outside the nest-box differed significantly between all periods of the day (night-morning:  $q = -3.2, P < 0.001$ ; morning-afternoon:  $q = -4.0, P < 0.001$ ; night-afternoon:  $q = -7.2, P < 0.001$ ). Mean temperatures outside the nest-box by day period were lowest at night ( $15.5 \pm 2.4^\circ\text{C}$ ), followed by the morning ( $18.7 \pm 3.4^\circ\text{C}$ ), and higher in the afternoon ( $22.7 \pm 2.0^\circ\text{C}$ ).

Likewise, pairwise comparisons showed that temperatures inside the nest-box differed significantly among periods of the day (night-morning:  $q = -1.3, P < 0.001$ , morning-afternoon:  $q = -4.6, P < 0.001$ , and night-afternoon:  $q = -5.8, P < 0.001$ ). However, average temperatures inside the nest-box showed less variation than the exterior, being only slightly lower at night ( $16.8 \pm 2.4^\circ\text{C}$ ) and in the morning ( $17.2 \pm 2.8^\circ\text{C}$ ), and higher in the afternoon ( $22.5 \pm 1.4^\circ\text{C}$ ).

#### Operative temperatures in nest-boxes

Operative temperatures ( $T_e$ ), representative of those experienced by nestlings in the nest-box environment without parental incubation, fluctuated throughout the day concomitantly with temperature both outside and inside the nest-box (Fig. 1). Likewise, operative temperatures by period of the day followed a similar pattern to that of ambient temperatures, with higher operative temperatures in the afternoon and lower operative temperatures at night and in the morning (Fig. 2b). Operative temperature differed significantly for late-developmental stage models (Estimate =  $0.04 \pm 0.01$  (SE), 95% CI = 0.03 – 0.06;  $t = 5.6, P < 0.001$ ), with a higher mean  $19.2 \pm 2.9^\circ\text{C}$  compared to early-developmental stage models (mean:  $18.8 \pm 3.4^\circ\text{C}$ ). The 50% quartile of operative temperature was  $16.12^\circ\text{C} - 21.9^\circ\text{C}$ , although early-stage models had a slightly lower 50% quartile operative temperature ( $15.6^\circ\text{C} - 21.1^\circ\text{C}$ ) than late-stage models ( $16.5^\circ\text{C} - 22.2^\circ\text{C}$ ). Overall, both early- and late-stage nestlings had higher body temperatures than ambient temperatures inside the nest-box, and the operative temperatures of models in the nest-box (Fig.1). Therefore, as operative temperature of models closely followed internal ambient temperature it is likely that in the absence of brooding adults, nestlings would experience thermal stress.



**Figure 2.** Violin plots depicting the frequency of temperatures registered by dataloggers in three periods of the day for a) ambient temperatures outside and inside the nest-box, and b) operative temperatures experienced by nestlings, as determined by 3D models, in the nest-box. The mean (diamond) with its standard deviation (line) are indicated inside the plot. Second and third interquartile range ( $T_{set}$  25% – 75%) of body temperature is shown for early- (green line) and late-stage (black line) nestlings.

## Discussion

Our results demonstrated that ambient temperatures outside and inside nest-boxes were correlated, and that the interior of nest-boxes were only slightly warmer than the exterior at night and cooler in the day, although they did not reach the same extremes of high and low temperatures as the exterior. This suggests that the thermal benefits to nestlings offered by nest-boxes may be minimal (Maziarz et al. 2017), and require improvement by parental thermal buffering by brooding. Our results also demonstrated that the thermoneutral range of nestlings' body temperature was higher than both the internal thermal environment and operative temperatures of models inside the nest-box, regardless of the developmental stage. Therefore, nestlings could suffer thermal stress when parents do not offer thermal buffering, such as when they are off the nest to forage. This may be critical for altricial birds during the first days after hatching, when nestlings are not fully able to thermoregulate.

We found that the thermoregulatory set-point of Bewick's Wren nestlings was 20.1°C above the average temperature in the nest-box and 22.3°C above the operative temperature of models in the nest-box. This indicates that the interior of the nest-box could potentially be thermally challenging for early-stage nestlings, particularly during parental off-bouts, which may be for up to 20 mins on foraging trips, when chicks might experience thermal stress. Critical periods may occur early in the morning between 06:45 and 08:15 hrs, when nestlings may experience low 12.0°C - 12.2°C temperatures in the nest-box, with the risk of hypothermia. Parent birds may buffer high and low temperatures in the nest by either staying more time in the nest when ambient temperature is low, or reducing the time in the nest when temperature is high (Andreasson et al. 2018, DuRant et al. 2019). Therefore, parental brooding may be crucial in the early- developmental stage when nestlings are not fully endothermic, and hence parent birds may be off the nest for short periods of time at this stage. This suggests that the nest-box may be a thermally challenging environment for nestlings unless brooded by parents.

We found that nest-boxes used by Bewick's Wrens in Morelia City were on average 0.5°C warmer than external temperatures. Nest-boxes in cold weather temperate areas are regularly reported to be warmer than the outside (McComb and Noble 1981, Wachob 1996), although other

studies have recorded larger differences of around 20°C (Corregidor-Castro and Jones 2021). In colder temperate regions, a warmer nest-box not only enables longer foraging trips off the nest by females (Haftorn 1988), but also influences reproductive success (Wachob 1996), as it reduces the thermal stress at low temperatures. However, in a tropical environment we recorded a small temperature difference between the exterior and interior of nest-boxes.

Our results suggest that nest-boxes in a tropical urban setting may provide minimal thermal benefits for secondary cavity nesting birds. This contributes to the ongoing and inconclusive debate on the suitability of nest-boxes as an alternative to natural cavities. The provision of nest-boxes may increase the availability of nesting sites and number of breeding pairs (Corrigan et al. 2011), particularly where there may be limited availability of adequate cavities (Cockle et al. 2010). Nesting pairs may also have greater productivity of eggs and nestlings in nest-boxes (Robertson and Rendell 1990, Libois et al. 2012, Norris et al. 2018), possibly because nest-box design can incorporate dimensions to increase internal space and reduce access to competitors and predators. However, the benefits of nest-boxes for reproductive output may be species-specific (Purcell et al 1997), and they may foster a maladaptive habitat use (Mänd et al. 2005). Moreover, the few studies of thermal constraints on avian reproduction have been conducted in cooler temperate regions (Andreasson et al. 2020). Therefore, our results also shed light on the potential inadvertent consequences of the use of alternative human-made cavities for nesting birds in topical urban areas.

Notably, we have also documented the use of these nest-boxes for roosting at night by birds in Morelia city. Therefore, while nest-boxes may provide limited thermal benefits for breeding, they could provide safe places for roosting in urban areas. Furthermore, the fact that nest-boxes maintain warmer temperatures at night means that they may have a valid utility for roosting activities. Nevertheless, we emphasize the importance of promoting the presence of large, old trees in urban areas that can develop cavities for nesting. These tree-cavities can provide a more stable thermic and humid environment (Sudyka et al. 2023), offering cavity-nesting species with a safe nest-site, and a potentially better environment for raising their young.

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## Literature cited

- Andreasson F, Nilsson JÅ, Nord A. 2020. Avian reproduction in a warming world. *Frontiers in Ecology and Evolution* 8:576331 <https://doi.org/10.3389/fevo.2020.576331>
- Andreasson F, Nord A, Nilsson JÅ. 2018. Experimentally increased nest temperature affects body temperature, growth and apparent survival in Blue Tit nestlings. *Journal of Avian Biology* 49:jav-01620. <https://doi.org/10.1111/jav.01620>
- Ardia DR. 2013. The effects of nestbox thermal environment on fledging success and hematocrit in Tree Swallows. *Avian Biology Research* 6:99-103.
- Archambault JM, Cope WG, Kwak TJ. 2014. Survival and behaviour of juvenile unionid mussels exposed to thermal stress and dewatering in the presence of a sediment temperature gradient. *Freshwater Biology* 59:601-613.
- Arct A, Martyka R, Drobniak SM, Oleś W, Dubiec A, Gustafsson L. 2022. Effects of elevated nest-box temperature on incubation behaviour and offspring fitness-related traits in the Collared Flycatcher *Ficedula albicollis*. *Journal of Ornithology* 163:263-272.
- Arnfield AJ. 2003. Two decades of urban climate research: a review of turbulence, exchanges of energy and water, and the urban heat island. *International Journal of Climatology* 23:1-26.
- Baarendse PJJ, Debonne M, Decuypere E, Kemp B, Van den Brand H. 2007. Ontogeny of avian thermoregulation from a neural point of view. *World's Poultry Science Journal* 63:267-276.
- Bakken GS. 1992. Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist* 32:194-216.
- Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. Available at <http://CRAN.R-project.org/package=lme4>
- Bicego KC, Barros RCH, Branco LGS. 2007. Physiology of temperature regulation: comparative aspects. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 147:616-639.
- Blewett CM, Marzluff JM. 2005. Effects of urban sprawl on snags and the abundance and productivity of cavity-nesting birds. *The Condor* 107:678-693.
- Bohler M, Chowdhury VS, Cline MA, Gilbert ER. 2021. Heat stress responses in birds: a review of the neural components. *Biology* 10:1095-1095. <https://doi.org/10.3390/biology10111095>
- Choi IH, Bakken GS. 1990. Begging response in nestling Red-winged Blackbirds (*Agelaius phoeniceus*): effect of body temperature. *Physiological Zoology* 63:965-986.
- Cockle KL, Martin K, Drever MC. 2010. Supply of tree-holes limits nest density of cavity-nesting birds in primary and logged subtropical Atlantic forest. *Biological Conservation* 143: 2851-2857.
- Corregidor-Castro A, Jones OR. 2021. The effect of nest temperature on growth and survival in juvenile Great Tits *Parus major*. *Ecology and Evolution* 11:7346-7353.
- Corrigan RM, Scrimgeour GJ, Paszkowski C. 2011. Nest boxes facilitate local-scale conservation of common Goldeneye (*Bucephala clangula*) and Bufflehead (*Bucephala albeola*) in Alberta, Canada. *Avian Conservation and Ecology* 6: 1. <http://dx.doi.org/10.5751/ACE-00435-060101>
- Cuningham SJ. 2021. Mortality among birds and bats during an extreme heat event in eastern South Africa. *Austral Ecology* 46:687-691.
- Dawson WR, Evans FC. 1957. Relation of growth and development to temperature regulation in nestling Field and Chipping Sparrows. *Physiological Zoology* 30:315-327.
- Dunn EH. 1975. The timing of endothermy in the development of altricial birds. *The Condor* 77:288-293.

- DuRant SE, Willson JD, Carroll RB. 2019. Parental effects and climate change: will avian incubation behavior shield embryos from increasing environmental temperatures? *Integrative and Comparative Biology* 59:1068-1080.
- Fathima S, Hakeem WGA, Shanmugasundaram R, Selvaraj RK. 2022. Necrotic enteritis in Broiler Chickens: a review on the pathogen, pathogenesis, and prevention. *Microorganisms* 10:1958. <https://doi.org/10.3390/microorganisms10101958>
- Guthery FS, Land CL, Hall BW. 2001. Heat loads on reproducing Bobwhites in the semiarid subtropics. *Journal of Wildlife Management* 65:111-117.
- Haftorn S. 1988. Incubating female passerines do not let the egg temperature fall below the 'physiological zero temperature' during their absences from the nest. *Ornis Scandinavica* 19:97-110.
- Harper MJ, McCarthy MA, Van der Ree R. 2005. The abundance of hollow-bearing trees in urban dry sclerophyll forest and the effect of wind on hollow development. *Biological Conservation* 122:181-192.
- Hertz PE. 1992a. Evaluating thermal resource partitioning by sympatric lizards *Anolis cooki* and *A. cristatellus*: a field test using null hypotheses. *Oecologia* 90:127-136.
- Hertz PE. 1992b. Temperature regulation in Puerto Rican *Anolis* lizards: a field test using null hypotheses. *Ecology* 73:1405-1417.
- Hertz PE, Huey RB, Stevenson RD. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist* 142:796-818.
- IMPLAN 2022. Fichas temáticas Morelia. Retrieved from: <https://implanmorelia.org/site/fichas-tematicas-morelia/> on 15 February 2023.
- Kennedy ED, White DW. 2020. Bewick's Wren (*Thryomanes bewickii*), version 1.0. In *Birds of the World* (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. <https://doi.org/10.2173/bow.bewwre.01>
- Kingsolver JG, Diamond SE, Buckley LB. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology* 27:1415-1423.
- LaMontagne JM, Kilgour RJ, Anderson EC, Magle S. 2015. Tree cavity availability across forest, park, and residential habitats in a highly urban area. *Urban Ecosystem* 18:151-167. <https://doi.org/10.1007/s11252-014-0383-y>
- Libois E, Gimenez O, Oro D, Mínguez E, Pradel R, Sanz-Aguilar A. 2012. Nest boxes: a successful management tool for the conservation of an endangered seabird. *Biological Conservation* 155:39-43.
- Mänd R, Tilgar V, Lõhmus A, Leivits A. 2005. Providing nest boxes for hole-nesting birds - does habitat matter? *Biodiversity and Conservation* 14:1823-1840.
- Martin TE, Boyce AJ, Fierro-Calderón K, Mitchell AE, Armstad CE, Mouton JC, Bin Soudi EE. 2017. Enclosed nests may provide greater thermal than nest predation benefits compared with open nests across latitudes. *Functional Ecology* 31:1231-1240.
- Maziarz M, Broughton RK, Wesołowski T. 2017. Microclimate in tree cavities and nest-boxes: implications for hole-nesting birds. *Forest Ecology and Management* 389:306-313.
- McCafferty DJ, Gallon S, Nord A. 2015. Challenges of measuring body temperatures of free ranging birds and mammals. *Animal Biotelemetry* 3:1-10.
- McComb WC, Noble RE. 1981. Microclimates of nest boxes and natural cavities in bottomland hardwoods. *Journal of Wildlife Management* 45:284-289.
- McKechnie E, Rushworth IA, Myburgh F, Cunningham SJ. 2021. Mortality among birds and bats during an extreme heat event in eastern South Africa. *Austral Ecology* 46:687-691.
- Mesa MG, Weiland LK, Wagner P. 2002. Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall Chinook Salmon. *Northwest Science* 76:118-128.
- Mishra V, Ganguly AR, Nijssen B, Lettenmaier DP. 2015. Changes in observed climate extremes in global urban areas. *Environmental Research Letter* 10 024005.

- Morton ML, Carey C. 1971. Growth and the development of endothermy in the Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*). *Physiological Zoology* 44:177-189.
- Nagy KA. 2005. Field metabolic rate and body size. *Journal of Experimental Biology* 208:1621-1625.
- Norris AR, Aitken KEH, Martin K, Pokorny S. 2018. Nest boxes increase reproductive output for Tree Swallows in a forest grassland matrix in central British Columbia. *PLoS ONE* 13:e0204226. <https://doi.org/10.1371/journal.pone.0204226>
- O'Connor RJ. 1975. The influence of brood size upon metabolic rate and body temperature in nestling Blue Tits *Parus caeruleus* and House Sparrows *Passer domesticus*. *Journal of Zoology* 175:391-403.
- Pattinson NB, Thompson ML, Griego, MS, Russell G, Mitchell NJ, Martin RO, Wolf BO, Smit B, Cunningham SJ, McKechnie AE, Hockey PAR. 2020. Heat dissipation behaviour of birds in seasonally hot arid-zones: are there global patterns? *Journal of Avian Biology* 51:e02350 <https://doi.org/10.1111/jav.02350>
- Pinheiro J, Bates D, DebRoy S, Sarkar D, Heisterkamp S, Van Willigen B, Ranke J. 2015. nlme: linear and nonlinear mixed effects models. R package version 3.1-117. Available at <http://CRAN.R-project.org/package=nlme>
- Purcell KL, Verner J, Oring LW. 1997. A comparison of the breeding ecology of birds nesting in boxes and tree cavities. *The Auk* 114:646-656.
- Rhodes B, O'Donnell C, Jamieson I. 2009. Microclimate of natural cavity nests and its implications for a threatened secondary-cavity-nesting passerine of New Zealand, The South Island Saddleback. *The Condor* 111:462-469.
- Ricklefs RE. 1984. The optimization of growth rate in altricial birds. *Ecology* 65:1602-1616
- Robertson RJ, Rendell WB. 1990. A comparison of the breeding ecology of a secondary cavity nesting bird, the Tree Swallow (*Tachycineta bicolor*), in nest boxes and tree cavities. *Canadian Journal of Zoology* 68:1046-1052.
- Rodrigues LR, McDermott HA, Villanueva I, Djukarić J, Ruf LC, Amcoff M, Snook RR. 2022. Fluctuating heat stress during development exposes reproductive costs and putative benefits. *Journal of Animal Ecology* 91:391-403.
- Santillán V, Quitián M, Tinoco BA, Zárata E, Schleunig M, Böhning-Gaese K, Neuschulz EL. 2018. Spatio-temporal variation in bird assemblages is associated with fluctuations in temperature and precipitation along a tropical elevational gradient. *PLoS ONE* 13:e0196179. <https://doi.org/10.1371/journal.pone.0196179>
- Shine R, Kearney M. 2001. Field studies of reptile thermoregulation: how well do physical models predict operative temperatures? *Functional Ecology* 15:282-288.
- Strain C, Jones CS, Griffiths SR, Clarke RH. 2020. Spout hollow nest boxes provide a drier and less stable microclimate than natural hollows. *Conservation Science and Practice* 3:e416. <https://doi.org/10.1111/csp2.416>
- Sudyka J, Di Lecce I, Wojas L, Rowiński P, Szulkin M. 2022. Nest-boxes alter the reproductive ecology of urban cavity-nesters in a species-dependent way. *Journal of Avian Biology* 2022:e03051. <https://doi.org/10.1111/jav.03051>
- Sudyka J, Di Lecce I, Szulkin M. 2023. Microclimate shifts in nest-boxes and natural cavities throughout reproduction. *Journal of Avian Biology* 2023:e03000. <https://doi.org/10.1111/jav.03000>
- Vanadzina K, Street SE, Sheard C. 2024. The evolution of enclosed nesting in passerines is shaped by competition, energetic costs, and predation threat. *Ornithology* 141:ukad048. <https://doi.org/10.1093/ornithology/ukad048>
- Visser GH, Ricklefs RE. 1993. Development of temperature regulation in shorebirds. *Physiological Zoology* 66:771-792.
- Visser GH. 1998. Development of temperature regulation. In: avian growth and development. Pp. 117-156 In Starck JM, RE Ricklefs (eds). *Evolution within the altricial-precocial spectrum*. Oxford: Oxford University Press.
- Wachob DG. 1996. A microclimate analysis of nest-site selection by Mountain Chickadee. *Journal*

of Field Ornithology 67:525-533.

Watson CM, Francis GR. 2015. Three-dimensional printing as an effective method of producing anatomically accurate models for studies in thermal ecology. *Journal of Thermal Biology* 51:42-46.

Xie S, Romero LM, Htut ZW, McWhorter TJ. 2017. Stress responses to heat exposure in three species of Australian desert birds. *Physiological and Biochemical Zoology* 90:348-358. <https://doi.org/10.1086/690484>