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Cool, dry nights and short heatwaves during growth result in longer telomeres in temperate songbird nestlings

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Abstract

Exposure to rising sublethal temperatures can affect development and somatic condition, and thereby Darwinian fitness. In the context of climate warming, these changes could have implications for population viability, but they can be subtle and consequently difficult to quantify. Using telomere length (TL) as a known biomarker of somatic condition in early life, we investigated the impact of pre-hatching and nestling climate on six cohorts of wild nestling superb fairy wrens (*Malurus cyaneus*) in temperate south-eastern Australia. Models incorporating only climate information from the nestling phase were best supported compared to those including the (pre-)laying to incubation phase (previously shown to affect mass) or both phases combined. This implies that nestling TL is most sensitive to ambient climate in the nestling phase. The top model showed a negative relationship between early-life TL and nestling mean daily minimum temperature when rainfall was low which gradually became positive with increasing rainfall. In addition, there was a positive relationship between TL and the frequency of hot days (daily maximum temperature ≥35°C), although these temperatures were rare and short-term. Including other pre-hatching and nestling period, climate variables (e.g., mean daily maximum temperature and mean diurnal temperature variability) did not improve the prediction of nestling TL. Overall, our results suggest that cooler nights when conditions are dry and short-term temperature spikes above 35°C during development are conducive for somatic maintenance. While these findings indicate a potential pathway for climate warming to impact wildlife fitness, they emphasize the need to elucidate the mechanisms underlying these complex associations.

KEYWORDS
climate change, ecology, heat wave, juvenile, life history, young

1 | INTRODUCTION

Predicting the impact of climate warming on wildlife is reliant on our knowledge of how temperature and other environmental conditions interact to influence Darwinian fitness and subsequently population viability. While temperature extremes can cause mass mortality events (McKechnie & Wolf, 2009), sublethal temperatures have the capacity to reduce population fitness by affecting life-history processes through effects on health (Conradie et al., 2019) and these may be particularly pronounced during sensitive early-life stages (Eastwood et al., 2022). While species are likely to vary in their early-life response to rising temperatures, altricial birds are particularly vulnerable due to their inability to thermoregulate efficiently as nestlings.

Developing birds are ectothermic during the egg and early hatchling phase but transition to full endothermy prior to fledging (Price & Dzialowski, 2018). Throughout this thermoregulatory transition, nestling homeothermy is susceptible to ambient temperature fluctuations, and although parental brooding can buffer cold weather (Katzenberger et al., 2015), nestlings have high metabolic and water loss rates (Diehl et al., 2023), which increases their risk of physiological dysregulation, hyperthermia or dehydration during heatwaves. In addition, altricial birds have a limited capacity to evade heat exposure as they are confined to the nest, but may also be susceptible to malnutrition because parental food provisioning can be reduced under hot conditions (Albright et al., 2017; Bourne, Ridley, Spottiswoode, & Cunningham, 2021; Plessis et al., 2012). Furthermore, resources for growth and development can be re-directed under thermal exposure to optimize thermoregulatory processes which can have permanent consequences for body mass (Gardner et al., 2011; Ryding et al., 2021), development and mortality risk (Briga & Verhulst, 2015; Conradie et al., 2020). However, sometimes these subtle early-life impacts are difficult to quantify, and there is little understanding of the effects of climate warming between species that differ in their environment or life history.

Telomeres, DNA protein complexes at the distal ends of linear chromosomes, may be a biomarker of early-life climate on somatic condition because they shorten in response to growth and physiological stress—both of which relate to ambient temperature in birds (Fragueira et al., 2019). However, because telomeres change with age (Remot et al., 2022) and covary with lifespan and lifetime reproductive success (Eastwood et al., 2019; Sheldon, Eastwood, et al., 2022; Wilbourn et al., 2018), their damage could also indicate the impact of climate warming on population health and viability (Dupoué et al., 2022; Eastwood et al., 2022). Telomeres shorten during cell replication because DNA polymerases do not completely replicate linear chromosomes. Thus, telomere shortening rates increase with growth rate and cellular renewal. Telomere shortening can also be expected to accelerate due to thermoregulatory responses to elevated temperatures. For instance, birds are known to increase metabolism, glucocorticoid and heat shock protein levels when air temperatures are above a species thermoneutral zone (McKechnie & Wolf, 2019), which could subsequently influence telomere attrition (Angelier et al., 2018; Armstrong & Boonekamp, 2023; Friesen et al., 2022). However, the relationship between thermal conditions and telomere dynamics can also be indirect. For example, reduced parental provisioning effort (e.g., Plessis et al., 2012) or shifts in peak food availability in the environment (e.g., Hidalgo Aranzamendi et al., 2019; Visser & Gienapp, 2019; Woinarski & Cullen, 1984) could lead to poor nutrition and shorter telomeres (Cram et al., 2017). The thermal environment may also have intergenerational effects on nestling telomere length (TL) through parental genetic (Chik et al., 2022) or epigenetic-like inheritance (Bauch et al., 2019). Therefore, due to the absence of telomeric repair (i.e., telomerase is downregulated in somatic cells), early-life TL could indicate the net physiological cost of experiencing suboptimal thermal conditions during growth and the inherited effects of climatic conditions prior to fertilization.

Experimental manipulation of egg incubation temperature indicates that increased or variable incubation temperature leads to shorter nestling TL (Hope et al., 2023; Stier et al., 2020; Vedder et al., 2018). Although parental brooding can buffer environmental conditions during incubation (ectothermic phase of avian development), climate conditions during the incubation phase do predict nestling TL in wild house sparrows (*Passer domesticus*; longest telomeres at intermediate North Atlantic Oscillation index; Pepke et al., 2022). Similarly, the effects of climate on offspring TL may vary during the metabolic transition phase of nestlings (ectothermic to endothermic). For example, mean daily temperature was not associated with TL in wild zebra finch nestlings from a hot arid zone (*Taeniopygia guttata*; Sheldon, Ton, et al., 2022), while experimental heatwaves reduced TL decline during the early (ectothermic) nestling phase (Ton et al., 2023). Also, there was no impact on nestling TL after experimental cooling of wild eastern bluebird (*Sialia sialis*) nestlings (Lynn et al., 2022). On the other hand, artificially increasing temperatures of wild great tit (*Parus major*) nestlings decreased juvenile TL (Stier et al., 2021), and a recent observational study in nestling purple-crowned fairy wrens (*Malurus coronatus*) in tropical NW Australia found that nestlings exposed to hot conditions had shorter telomeres (Eastwood et al., 2022). However, the latter effect of heat in fairy wrens was ameliorated during the wet season and when there had been recent rainfall events. Similar interactions among temperature, season or environmental factors on early-life TL have also been found in mammals (Seeker et al., 2021; van Lieshout et al., 2022). It then seems plausible that temperature-related telomere shortening in nestlings may be dependent on environmental ‘harshness’ disrupting their ability to thermoregulate. However, a few studies have considered how climate conditions (e.g., precipitation, temperature extremes, variability, and heatwaves) interact to influence early-life TL.

In this study, we investigated the effects of ambient climate on TL in a small (8–11 g) cooperatively breeding passerine found in temperate regions of south-eastern Australia, the superb fairy wren (*Malurus cyaneus*), a common species that is declining across much of its range (Harrisson et al., 2013). This species breeds during the spring and summer months (September to February) when temperatures range from below zero to over 40°C. Breeding peaks in

December but can end earlier under drought and heatwave conditions (Lv et al., 2020). Breeding groups are territorial, and there can be as many as five subordinate helper males on the territory (Cockburn et al., 2016). The sole female in the group builds the domed nest and incubates the clutch of modal three eggs that hatch synchronously. Although the whole group provides nestling care, the presence of helpers does not increase reproductive success, instead improving survival of female breeders (Cockburn et al., 2008). Previous research identified both short- and long-term effects of climate on nestling mass (which predicts recruitment): warmer conditions in the 2–8 weeks before hatching resulted in greater nestling mass (Kruuk et al., 2015), but this positive effect was reduced with increasing rainfall and vice versa. In contrast, body mass was reduced if nestlings had experienced a heatwave early during the nestling period. These contrasting climate effects acting in different directions make predictions of longer-term trends due to climate change difficult (Kruuk et al., 2015).

Therefore, our study aimed to better understand the effect of climate in early life by using TL as a biomarker of somatic condition, testing the previously identified climate variables that impact superb fairy wren mass in the same population (Kruuk et al., 2015) as well as those affecting TL in a closely related species (Eastwood et al., 2022). Specifically, our study: (1) investigated contrasting relationships between climate and TL across pre-hatching and nestling periods; (2) tested for a relationship between TL and climate, including mean temperature (daily minimum and maximum), temperature variability, temperature extremes and precipitation; while (3) controlling for pertinent individual, ecological and social impacts on TL.

2 | MATERIALS AND METHODS

2.1 | Study species and field site

Fieldwork was conducted at the Australian National Botanic Gardens in Canberra, Australia (35°16′ S, 149°06′ E), as part of a long-term study operating since 1988. To ensure accurate hatch dates for each nest, all known breeding pair territories were searched for nesting activity every 3 days (hatch day accuracy ±1 day). The species is multi-brooded and females lay one egg on each consecutive day until clutch completion with a dominant modal brood size of three (Lv et al., 2020). The number of successful broods depends on environmental conditions as well as a number of failed attempts (predominately via predation during the nestling period; Cockburn et al., 2016). Incubation begins after the clutch is complete (females lay one egg per day) and lasts approximately 13 days when nestlings hatch synchronously. Only the dominant female incubates, but all males (dominant plus 0–4 subordinates) contribute to nestling care (Cockburn et al., 2016). The presence of helpers does not increase reproductive success because of load lightening by the dominants. Instead, better parents and territories are more likely to cause helper number to increase. However, load lightening causes improved survival of female breeders (Cockburn et al., 2008), which suggests that social groups may be able to buffer suboptimal conditions as has been observed in other cooperative breeders (Bourne et al., 2020; Guindre-Parker & Rubenstein, 2020). After hatching, nestlings were banded at between 6 and 8 days of age (nestling period 11–12 days), with a unique identification band (Australian Bird and Bat Banding Scheme) and a combination of colour bands for observing identity from a distance. At the same time as banding, a blood sample was taken from the brachial vein using a heparinized capillary tube and frozen at −20°C. Mass, brood size and the number of social helpers in the territory were recorded. The sex of birds that did not reach adulthood, and parentage of all individuals was determined genetically (using the *hiphop* package, Cockburn et al., 2021). The latter was necessary because breeding pairs are territorial and known to be highly promiscuous, with approximately 60% of a brood sired by a male other than their social father (Hajduk et al., 2021; Mulder et al., 1994). For this study, we used archived samples from cohorts hatched in 1996–2001. The rationale for including these cohorts was based on the availability of TL data from previous work which required cohorts with complete life-history data (Eastwood, Dupoué, Delhey, et al., 2023).

2.2 | Climate variables

Temperature (daily maximum and minimum, °C) and rainfall (daily total precipitation, mm) data were accessed from a nearby weather station approximately 8 km from the field site (Canberra Airport weather station 70014; 35.30° S 149.20° E) using the Australian Bureau of Meteorology's online repository (http://www.bom.gov.au/climate/data). Important climate variables influencing condition were determined a priori from existing literature. Pre-hatching climate variables were defined based on the results of a previous study of climate effects on nestling body mass in the same population (Kruuk et al., 2015). For the post-hatching climate variables, we used the nestling period, as that makes sense biologically (this is also the approach we followed in a sister species; Eastwood et al., 2022). For assessing the impacts of pre-hatching temperatures on TL, we calculated mean daily maximum temperature ($T_{\text{max-pre}}$) over a 19-day period between 25 and 6 days prior to telomere measurement (TL measured in 6- to 8-day-old nestlings). Thus, $T_{\text{max-pre}}$ mostly overlaps egg formation in the days prior to and during laying (typically 3 days) and incubation (typically 13 days). Kruuk et al. (2015) identified this period as a critical temperature window affecting nestling body mass, with higher temperatures resulting in heavier nestlings. Similarly, we summed daily total rainfall ($\text{Rainfall}_{\text{pre}}$) over a 44-day period between 57 and 13 days prior to telomere measurement. Kruuk et al. (2015) identified this period as a critical rainfall window affecting nestling body mass, with more rain resulting in heavier nestlings. Kruuk et al. (2015) also determined the effect of 2-day heat waves, calculated as mean maximum daily temperature of the 2 days prior to measurement >35°C (yes/no). However, in our data subset, only four nestlings (two nests) experienced those conditions which led us to exclude this variable from our analysis (in Kruuk et al., 2015, only 2.4% of chicks experienced these conditions). Finally, Kruuk et al. (2015) identified mean daily maximum temperature over the previous autumn (previous February/March) as a predictor of body mass. However, this measure was not informative in the current data subset because there was insufficient variation across the six cohorts. Therefore, we did not include long-term climate variables in our analyses.

In a closely related species, exposure to hot and dry conditions during the nestling phase is associated with shorter telomeres (Eastwood et al., 2022). Therefore, we included such nestling phase climate measures calculated between hatch date and date of telomere measurement. These included mean daily maximum temperature ($T_{\text{max-nestling}}$), mean daily minimum temperature ($T_{\text{min-nestling}}$) and total rainfall ($\text{Rainfall}_{\text{nestling}}$). Because diurnal temperature variability can be high and may also affect growth and survival (Briga & Verhulst, 2015), we calculated the mean difference between daily maximum and minimum temperatures over the nestling period ($T_{\text{var-nestling}}$). In superb fairy wrens, nestlings start to lose water and perform behaviours that maximize heat dissipation when temperatures are between 33 and 40°C (Diehl et al., 2023). Therefore, and because short-term periods above 35°C were found to reduce nestling mass (Kruuk et al., 2015), we calculated the frequency of days ≥35°C ($T_{\text{35-nestling}}$) during the nestling phase (between hatching and telomere measurement) to understand the effect of heat extremes on nestling TL.

2.3 | Telomere measurement

Telomere length was measured following a previously optimized qPCR technique in superb fairy wrens (Eastwood, Dupoué, Delhey, et al., 2023). In brief, DNA was extracted using an ammonium acetate protocol, and its concentration and purity were assessed using a NanoDrop ND-1000 (1.6-2260/280 ratio and 1.8-3.0260/230 ratio). DNA integrity was assessed on a 1.5% agarose gel. The telomere and normalizing control gene (glyceraldehyde-3-phoshate dehydrogenase; GAPDH) were run separately with each sample in duplicate. The final reaction volume was 15 μL and included 7.5 μL of SYBR Green I (Roche), 18–20 ng of DNA, 300 nM of both GAPDH primers (Integrated DNA Technologies; GT2-GAPDH-forward 5′-CCATCACAGCCACACAGAAG-3′ and GT2-GAPDH-reverse 5′-TTTTCCCACAGCCTTAGCAG-3′) (Atema et al., 2013) or 500 nM of both telomere primers (Tel1b 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′ and Tel2b 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′). qPCR reactions were pipetted using an EpMotion 5075 (Eppendorf) to reduce pipetting error. LightCycler 480 (Roche) cycling conditions were 95°C for 15 min, followed by 25 cycles of 15 s at 95°C, 30 s at 56°C, 30 s at 72°C for the telomere assay, and 95°C for 15 min, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, 30 s at 72°C for GAPDH. A melt-curve analysis followed both reactions to ensure that the correct product was amplified. Relative TL (rTL) was calculated following Eastwood et al. (2018). Assay repeatability (repeatability = 0.85, se = 0.07) and efficiencies [mean (standard deviation) efficiency telomere 94.13 (3.37)%, GAPDH 97.34 (2.72)%] were high.

2.4 | Statistical analysis

This study included $N = 343$ nestlings from 180 nests, 149 genetic sires and 117 mothers. Statistical analyses were conducted in R version 4.0.5 (R Core Team, 2016), and the results were presented using the *ggplot2* package (Wickham, 2016). To analyse the climate predictors of nestling TL, we used a linear mixed effect model approach implemented using the function lmer (*lme4* package, version 1.1-28; Bates et al., 2015) fitted using maximum likelihood estimation (Zuur et al., 2009). Nestling TL was standardized to have a mean of zero and standard deviation of 1 to allow between study comparisons of effect sizes (Verhulst, 2020). All continuous variables were mean centred, and we confirmed that regression assumptions were met for each model. Initially, all models contained five random terms: cohort (September to February in the following year) to account for non-independence of sampling during the breeding season, qPCR ID to account for variation in telomere measurement batches, sire ID to account for multiple offspring from each genetic male, mother ID to account for multiple maternal offspring, and nest ID nested within mother to account for multiple individuals within a nest and social father effects. However, qPCR ID and cohort explained less than 1% of the variance and were therefore excluded. Each model also contained the same set of biological covariates: nestling sex (factor 2 levels male/female), nestling age (factor with 3 levels) to account for age at sampling differences between nestlings (6–8 days), nestling body mass at sampling (continuous; 3.7–9.5 g), brood size (continuous; 1–4 nestlings) to account for differences in sibling competition and the presence/absence of helpers (factor, 2 levels) to account for differences in nestling provisioning. The breeding season spans the spring (October and November) and summer (December, January and February) months with peak activity in November and December (Cockburn et al., 2016). As broader environmental conditions independent of climate or population demographics might vary across the breeding season (e.g., over the breeding season predation threat and food availability increase and decrease, respectively), we included hatch month in each model as a fixed factor with four levels.

We performed a series of models exploring the impact of climate variables identified in Kruuk et al. (2015), hereby referred to as pre-hatching variables, those during the nestling phase (identified in Eastwood et al., 2022), and a combination of both periods on early-life TL.

Across the study period, average pre-hatching maximum temperature (19-day period between 25 and 6 days prior to telomere measurement) and nestling phase maximum temperatures were highly correlated ($r = .82$; Figure S1) and their ranges overlapped (mean $T_{\text{max-pre}}$ across the 180 nests = 24.2°C, SD = 3.8, range = 17.8–31.3°C; mean $T_{\text{max-nestling}} = 25.4$°C, SD = 4.0, range = 17.0–34.5°C). Pre-hatching rainfall (44-day period between 57 and 13 days prior to telomere measurement) ranged between 17 and 177 (mm), while nestling phase rainfall (over a shorter timespan) ranged between 0 and 94 (mm) with 29 nests (19.7%) without any rain. During the nestling phase, the number of days warmer than 35.0°C was highly skewed towards zero with 33 out of 147 nests having at least 1 day above 35.0°C. $T_{\text{min-nestling}}$ ranged between 3.8 and 18.0°C (mean = 10.5°C, SD = 2.8) and mean $T_{\text{var-nestling}}$ was 14.9 (SD 2.5) and ranged between 6.7 and 20.13. Among the nestling phase climate variables, $T_{\text{min-nestling}}$ and $T_{\text{max-nestling}}$ were highly correlated with each other ($r = .78$; Figure S1) and moderately correlated with $T_{\text{35-nestling}}$ ($r < .57$; Figure S1). $T_{\text{max-nestling}}$ was also moderately correlated with Rainfall$_{\text{nestling}}$ ($r = -.44$; Figure S1) and highly correlated with $T_{\text{var-nestling}}$ ($r = .71$; Figure S1), whereas $T_{\text{min-nestling}}$ was correlated with neither ($r < .12$; Figure S1). The presence of highly correlated climate variables necessitated a series of models excluding such variables together in the same statistical model ($r > .6$) to avoid collinearity issues causing biased or unstable estimates (Schielzeth et al., 2020). Each climate variable was then independently assessed to check that estimates were stable when included together in the same model. See Table 1 models 2–4 for variables included in each model. We took this approach, instead of, for example, a principal component analysis, as it allows the inclusion of interactions between temperature and rainfall which have been previously identified to impact TL in a closely related species (Eastwood et al., 2022).

2.4.1 | Pre-hatching period (model 1)

In this model, we included climate variables identified in Kruuk et al. (2015) which include the period during egg formation and the incubation period (see *climate variables* above) to estimate the pre-hatching effects of climate conditions on TL. The variables added were $T_{\text{max-pre}}$, Rainfall$_{\text{pre}}$ and their pairwise interaction. We also included $T_{\text{max-pre}}$ squared, following Kruuk et al. (2015). However, the interaction and quadratic term were not significant and therefore removed from the final model.

2.4.2 | Nestling period (models 2–4)

In these models, we included climate variables calculated from hatching to telomere measurement to estimate the effects of climate conditions in the nestling phase on TL. Pairwise interactions between each temperature variable and rainfall were included in each model. We also included an interaction between average temperature (either $T_{\text{max-nestling}}$ or $T_{\text{min-nestling}}$) and $T_{\text{35-nestling}}$ to explore the possibility that the extreme temperatures may have an increased impact on TL when nestlings are exposed to chronically high environmental temperatures. However, we excluded $T_{\text{var-nestling}}$ interactions with other variables because we had no a priori predictions for any relationship with TL. Non-significant interactions were removed.

2.4.3 | Combined model (model 5)

In this model, we included pre-hatching and nestling period climate variables that were not highly correlated ($T_{\text{max-pre}}$, $T_{\text{35-nestling}}$, Rainfall$_{\text{pre}}$, Rainfall$_{\text{nestling}}$) and their pairwise interactions in the same statistical model and again removed non-significant interactions.

2.4.4 | Model selection

To determine the model with the highest statistical support, we calculated the relative weight for each models 1–5 (described in, e.g., Symonds & Moussalli, 2010) and the null model which included only biological covariates and hatch month (i.e., excluding climate variables, *model 6*). Akaike information criterion corrected estimates (AIC$_c$) were used to adjust for a small sample bias. The model weight is constrained between 0 and 1 (a value of 1 can be interpreted as a 100% chance that the model is 'best' given the other models considered). Model selection was performed using the *MuMIn* package (Bartoń, 2022).

3 | RESULTS

3.1 | Model selection

Akaike weights indicated a 73% probability that model 2 was the most likely model of the six candidate models (Table 1). The top model included the climate variables $T_{\text{35-nestling}}$ and $T_{\text{min-nestling}}$ and an interaction between Rainfall$_{\text{nestling}}$ and $T_{\text{min-nestling}}$. The second-best

TABLE 1 Full candidate set of models exploring the impact of climate parameters on nestling telomere length.

Model	Climate parameters	df	AIC$_c$	ΔAIC$_c$	Akaike weight
2	$T_{\text{min-nestling}} + T_{\text{35-nestling}} +$ Rainfall$_{\text{nestling}} + T_{\text{min-nestling}}$*Rainfall$_{\text{nestling}}$	18	945.2	0	0.73
4	$T_{\text{var-nestling}} + T_{\text{min-nestling}} + T_{\text{35-nestling}}$	17	947.5	2.3	0.23
5	$T_{\text{max-pre}} + T_{\text{35-nestling}} +$ Rainfall$_{\text{pre}} +$ Rainfall$_{\text{nestling}} + T_{\text{max-pre}}$*$T_{\text{35-nestling}}$	19	952.8	7.7	0.02
3	$T_{\text{max-nestling}} + T_{\text{35-nestling}} +$ Rainfall$_{\text{nestling}}$	17	953.4	8.2	0.01
6	Null model	14	953.7	8.5	0.01
1	$T_{\text{max-pre}} +$ Rainfall$_{\text{pre}}$	16	957.8	12.6	0.001

Note: Models are ranked in descending order from the best approximating model (Table 2). For full candidate model details, refer to Tables S1–S5.

TABLE 2 The top linear mixed effects model after model selection predicting nestling relative telomere length (rTL; *z*-standardized) shows contrasting impacts of climate during the nestling phase on relative telomere length in superb fairy wren nestlings.

Fixed effects	Parameter estimate	SE	*t*-statistic	*p*-value
Intercept	0.88	0.22	4.01	<.001
$T_{\text{min-nestling}}$	−0.09	0.03	−2.96	.004
$\text{Rainfall}_{\text{nestling}}$	−0.0002	0.004	−0.05	.96
$T_{\text{min-nestling}} \times \text{Rainfall}_{\text{nestling}}$	0.003	0.001	2.17	.03
$T_{\text{35-nestling}}$	0.29	0.10	2.95	.004
Hatch month (relative to January)				
October	−0.71	0.29	−2.43	.02
November	−0.68	0.20	−3.36	<.001
December	−0.33	0.18	−1.85	.07
Chick age (relative to 6 days)				
7 days	−0.59	0.18	−3.39	<.001
8 days	−0.64	0.24	−2.64	.009
Sex male (relative to female)	0.16	0.10	1.59	.11
Brood size	−0.05	0.09	−0.54	.59
Body mass	0.04	0.07	0.51	.61
Helpers yes (relative to no)	−0.08	0.11	−0.67	.50
Random effects		**Variance**		**SD**
Nest identity		0.08		0.28
Mother		0.07		0.27
True sire		0.04		0.21
Residual		0.66		0.81

Note: $\text{AIC}_c = 945.19$; including fixed predictors only marginal $R^2 = .15$; including random and fixed predictors conditional $R^2 = .34$.

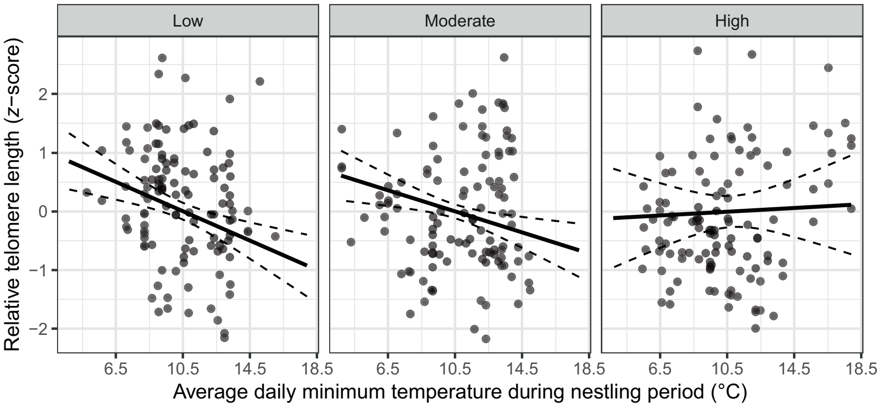

FIGURE 1 Mean minimum temperature ($T_{\text{min-nestling}}$) and total rainfall ($\text{Rainfall}_{\text{nestling}}$) during the nestling period interact to predict relative telomere length (rTL *z*-score; Table 2). To illustrate the interaction between $T_{\text{min-nestling}}$ and rainfall, we partitioned the $\text{Rainfall}_{\text{nestling}}$ data into terciles low (0–2 mL), moderate (2–12 mL) and high (12–94.6 mL) and plotted model predicted lines based on three estimates: zero rainfall, mean rainfall (11.8 mm) and maximum rainfall (47 mm, after excluding outliers). Dashed lines refer to 95% confidence intervals, solid lines model predicted values (Table 2), and the points refer to the unmanipulated data.

model (model 4) had substantially less support (23% probability this is the best model) and included $T_{\text{var-nestling}}$, $T_{\text{min-nestling}}$ and $T_{\text{35-nestling}}$, with the effect of $T_{\text{var-nestling}}$ not being statistically significant (Table S1). Despite a high correlation between $T_{\text{max-nestling}}$ and $T_{\text{min-nestling}}$, the model including $T_{\text{max-nestling}}$ (model 3) had lower support than the top model including $T_{\text{min-nestling}}$ (Table 1). This suggests that night temperatures are more important for nestling telomere maintenance than day temperatures. However, another possibility could be that $T_{\text{min-nestling}}$ is a better representation of nest-climate conditions. The null, pre-hatching climate and both pre-hatching and nestling period climate models had far lower AIC_c compared to the top model (Akaike weight ≤0.02; Table 1).

3.2 | Contrasting effects of nestling period climate conditions on nestling TL

The top model (model 2) showed that both $T_{\text{min-nestling}}$ and $T_{\text{35-nestling}}$ predicted nestling TL but in complex and contrasting ways (Table 2). $T_{\text{min-nestling}}$ had a negative impact on nestling TL but became increasing positive with increasing $\text{Rainfall}_{\text{nestling}}$ and vice versa. This finding suggests that warmer and drier conditions are associated with shorter telomeres, but that relationship is reversed under wetter conditions (becoming positive with high rainfall; Figure 1). In contrast, nestlings exposed to a greater number of days above 35°C had longer telomeres (Figure 2). For each additional day of 35°C during the nestling period, TL increased by 0.29 standard deviations (Table 2). However, because few nests that experienced >1 day above 35°C, the relationship may not extend beyond a single day (Figure 2). Indeed, replacing $T_{\text{35-nestling}}$ with a factor with two levels (≥35°C yes or no) also showed that nestling TL was higher if they experienced at least 1 day over 35 ($T_{\text{35 (y/n)-nestling}}=0.56$, $\text{SE}=0.18$, $p=.002$) and was a slightly better fit to the data (AIC_c was 1.24 lower than model 2). There was no evidence for an interaction between $T_{\text{35-nestling}}$ and rainfall.

In addition to climate effects on nestling TL, we found statistically significant variation in nestling TL over the breeding season (Table 2). Nestling TL was higher on average towards the end of the breeding season (Figure 3) compared to the beginning (October) and peak periods (November and December) which indicates a seasonal component predicting nestling TL independent of climate. However, because climate is correlated with month at our study site (Lv et al., 2023), we removed hatch month from the top model to determine the robustness of the climate variable coefficients. This did not change our conclusions, as the interaction between $T_{\text{min-nestling}}$ and $\text{Rainfall}_{\text{nestling}}$ and the main effect of $T_{\text{35-nestling}}$ remained (Table S6). Similarly, the effect sizes for month were qualitatively and quantitatively comparable when including or excluding climate predictor variables (Table S5). This confirms the suggestion of independent climate and other seasonal effects.

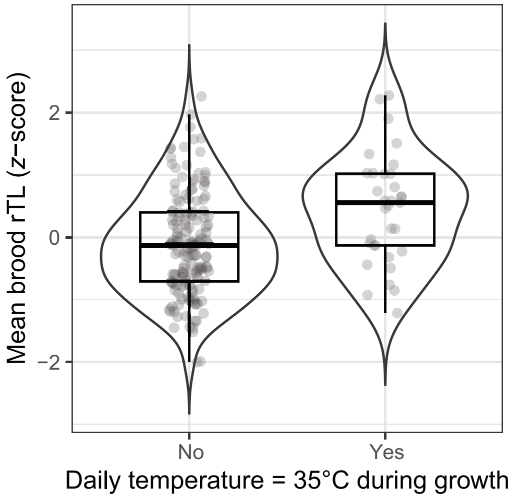

FIGURE 2 Nestlings experiencing at least 1 day of 35°C and over (x-axis) had longer relative telomeres ($T_{\text{35 (y/n)-nestling}}$ $p=.002$). The y-axis shows mean relative telomere length (rTL) for each brood to account for pseudo replication of individuals. Note that modelling heat waves as the frequency of days ≥35°C as a continuous variable ($T_{\text{35-nestling}}$) were also significant ($p=.004$).

3.3 | Biological effects on nestling TL

Nestling TL varied between 6 and 8 days of age (Figure S2) and was as considerably shorter at 7 and 8 days of age (Table 2; Figure S2). We found no evidence to indicate that sex, body mass, brood size or the presence of helpers significantly predicted nestling TL.

4 | DISCUSSION

Our study shows that rainfall and temperature experienced during early life can have implications for superb fairy wren TL in complex and contrasting ways. We found clear evidence that climate over the nestling phase was more influential in predicting nestling TL compared to pre-hatching climate or both stages combined. We also observed a strong seasonal effect of hatch month that was independent of temperature and rainfall, suggesting a large environmental or timing of breeding influence not accounted for by local climate. The top model explaining nestling TL revealed that the relationship between mean minimum temperature and TL was negative when rainfall levels were average or below, but minimum temperature appeared unimportant with high rainfall (Figure 1). By contrast, nestlings exposed to at least 1 day with maximum temperature above 35°C had longer telomeres. Overall, these findings suggest that cooler, drier nights relative to warm and dry, and short-term heat waves during the nestling phase may be important for somatic maintenance as expressed in telomere dynamics.

4.1 | Nestlings more sensitive to climate than eggs

Experimental manipulation of egg incubation temperatures has been shown to affect nestling TL in other birds (Hope et al., 2023; Stier et al., 2020; Vedder et al., 2018). Contrary to these studies, we observed that pre-hatching climate conditions, which overlap with egg formation, laying and incubation period, did not predict nestling TL. This might be because ectothermic eggs are buffered from ambient climate due to parental regulation of temperature, for example, by modifying nest position, nest structure or their own incubation behaviour (DuRant et al., 2019). However, parental or nest identity explained only a small proportion of the variance in TL. Additionally, environmental temperatures that exceed optimal incubation temperatures (typically ≥35°C: Webb, 1987) were rare in this study. Therefore, the observed negative impacts of experimentally manipulated incubation temperatures on TL are potentially unimportant in this and many other temperate climate species. Instead, our

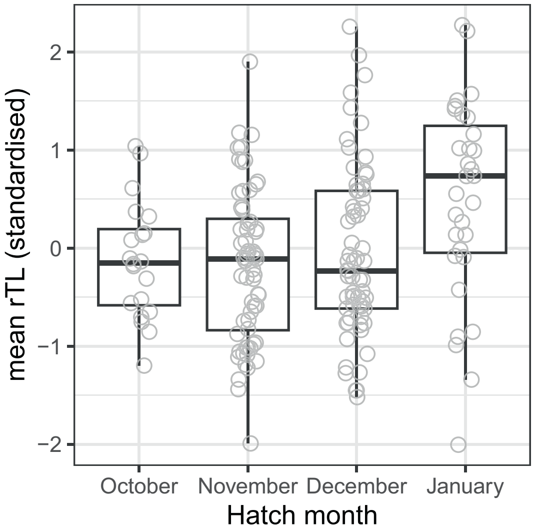

FIGURE 3 Nests hatched in January had longer mean relative telomere length (standardized rTL) compared to earlier in the breeding season. For graphical purposes, we plotted (grey circles) mean telomere length for each nest.

study revealed that climate variation over the early nestling phase predicted TL, perhaps because nestlings are still highly vulnerable during the poikilothermic phase when TL attrition is greatest (Roast et al., 2022; Sheldon, Eastwood, et al., 2022) and parental ability to buffer temperature is restricted by requirements for food provisioning to fuel rapid nestling growth (e.g., Bjørnstad & Lifjeld, 1996; Moreno, 1987).

4.2 | Contrasting patterns between nestling climate and TL: potential mechanisms?

While the specific mechanisms explaining climate effects on nestling TL are evidently complex and currently unresolvable without experimentation, our findings highlight several research priorities. Nestlings have a limited capacity to produce heat early after hatching (ectothermic phase) leaving them sensitive to cold weather. Therefore, it is possible that nestlings exposed to one or more hot days (≥35°C and maximum of 39.9°C in this study) could have longer telomeres because the environment is within their thermoneutral zone (reduced metabolic activity between 33 and 42°C; Diehl et al., 2023). However, nestling evaporative water loss starts increasing at 33°C and they start performing heat dissipation behaviours at 34°C (50% of nestlings pant at 40°C and 100% at 42°C) which increases their risk of dehydration (Diehl et al., 2023). Furthermore, warmer night temperatures under dry conditions predict shorter telomeres despite daily minimum temperatures being relatively low ($T_{\text{min-nestling}}$ mean = 10.5°C, SD = 2.8). This contradiction suggests that different mechanisms may be involved which potentially interact with each other. For instance, climate effects on TL may also derive from trade-offs between resources allocated towards development or telomere maintenance (Monaghan & Ozanne, 2018), which is feasible given that climate predicts nestling body mass in this population (Kruuk et al., 2015). In addition, extreme conditions (the warmest and driest conditions experienced naturally) could influence TL indirectly through reduced quantity or quality of arthropod food available (Chown et al., 2011; Razeng & Watson, 2015), decreased parental provisioning (Bourne, Ridley, McKechnie, et al., 2021; Plessis et al., 2012) or other processes causing physiological stress (Mentesana & Hau, 2022). It is probable that a combination of nestling physiology, parental behaviour and environmental quality shape how early-life TL reacts to climate conditions, and we encourage further research on these aspects.

4.3 | Telomere length as an indicator of early-life climate and beyond?

Climate 'harshness' in combination with species thermal tolerance limits is likely to determine the direction and strength of any association between temperature and TL. We observed some similarities to climate effects on TL in a closely related species, the purple-crowned fairy wren (Eastwood et al., 2022) that lives in the monsoonal tropics where temperatures frequently exceed 35°C. Despite contrasting thermal regimes and water availability between locations, in both species, the effects of average temperature on nestling telomeres varied with water availability conditions (wet season or rainfall), highlighting the importance of considering water availability when investigating or forecasting the effects of climate warming on birds generally (beyond arid zone birds; e.g., Riddell et al., 2019).

More broadly, climate impacts on early-life TL also exist beyond birds in both endotherm (Seeker et al., 2021; van Lieshout et al., 2022) and ectotherm vertebrates (Debes et al., 2016; McLennan et al., 2018; Noreikiene et al., 2017; Rollings et al., 2014; Zhang et al., 2023). For the most part, these studies support our suggestion that season and interspecific thermal capacity are important considerations in understanding how TL is impacted by climate (Friesen et al., 2022; Seeker et al., 2021; van Lieshout et al., 2022). However, inference from this disparate collection of studies warrants caution because telomere dynamics can vary between taxa (e.g., TL repair mechanisms appear active throughout life in reptiles but not birds; Olsson et al., 2018) and what constitutes a climate stressor for different species (e.g., ectothermic, poikilothermic or endothermic) is often unknown except in extreme cases. Despite these complications, early-life TL represents a promising biomarker of climate variation in vertebrates that is worth pursuing.

Exposure to suboptimal conditions during development can have negative consequences for survival or reproductive success (Metcalfe & Monaghan, 2001). Across vertebrates, longer early-life TL has been associated with reduced mortality risk (Eastwood, Dupoué, Delhey, et al., 2023) which, in context of our results, implies that the climatic conditions experienced early in life may influence fitness. In a warming climate, where developing terrestrial animals are increasingly exposed to warmer and drier conditions, the negative impacts on TL may have implications for population viability (Dupoué et al., 2022; Eastwood et al., 2022). However, in this superb fairy wren population, early-life TL does not appear to predict mortality (Eastwood, Dupoué, Delhey, et al., 2023).

5 | CONCLUSIONS

A major aim of contemporary wildlife research is to discern the effects of climate warming on fitness. However, identifying the consequences of sublethal thermal conditions and their underlying mechanisms remains a formidable challenge. Here, we used TL as a biologically meaningful indicator of climate impacts in early life, a critical period with lasting consequences in many species, to identify contrasting impacts of climate variation. Our results highlight the importance of considering the interactions among climate variables, the developmental phase and interspecies differences in driving relationships between climate and early-life TL. We encourage further research into the mechanisms underpinning telomere sensitivity to climate while incorporating potential cofounding variables such as growth, environment and parental behaviour into any study design.

AUTHOR CONTRIBUTIONS

J.E and A.P. were involved in study design; A.C. conducted the fieldwork and collected samples; A.D. performed the lab work; J.E. performed the statistical analysis with input from A.C., S.V. and A.P.; all authors contributed to writing the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data required to replicate the statistical analyses in this paper are available from the Dryad repository (Eastwood, Dupoué, Verhulst, et al., 2023): https://doi.org/10.5061/dryad.cc2fqz6c2.

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