



Effects of heat waves on *telomere* dynamics and parental brooding effort in nestlings of the zebra finch (*Taeniopygia castanotis*) transitioning from ectothermy to endothermy

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Abstract

Heat waves are predicted to be detrimental for organismal physiology with costs for survival that could be reflected in markers of biological state such as telomeres. Changes in early life telomere dynamics driven by thermal stress are of particular interest during the early post-natal stages of altricial birds because nestlings quickly shift from being ectothermic to endothermic after hatching. Telomeres of ectothermic and endothermic organisms respond differently to environmental temperature, but few investigations within species that transition from ectothermy to endothermy are available. Also, ambient temperature influences parental brooding behaviour, which will alter the temperature experienced by offspring and thereby, potentially, their telomeres. We exposed zebra finch nestlings to experimental heat waves and compared their telomere dynamics to that of a control group at 5, 12 and 80 days of age that encapsulate the transition from the ectothermic to the endothermic thermoregulatory stage; we also recorded parental brooding, offspring sex, mass, growth rates, brood size and hatch order. Nestling mass showed an inverse relationship with telomere length, and nestlings exposed to heat waves showed lower telomere attrition during their first 12 days of life (ectothermic stage) compared to controls. Additionally, parents of heated broods reduced the time they spent brooding offspring (at 5 days old) compared to controls. Our results indicate that the effect of heat waves on telomere dynamics likely varies depending on age and thermoregulatory stage of the offspring in combination with parental brooding behaviour during growth.

KEYWORDS

birds, brooding, ectotherms, parental care, temperature, thermoregulation

1 | INTRODUCTION

Global warming is predicted to increase average ambient temperature as well as the frequency, intensity and duration of extreme

weather events such as heat waves (Meehl & Tebaldi, 2004; Pachauri et al., 2014). While some organisms may plastically adjust or adapt to rising temperatures over time (Franks & Hoffmann, 2012; Somero, 2010), heat waves are thought to be harder to buffer

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and may represent an immediate threat to survival (McKechnie & Wolf, 2010; Stillman, 2019). Reduced survival can be caused by high temperatures acting on extrinsic factors such as food, water availability (Donelson et al., 2010; Fuller et al., 2014), or predation (Lowe et al., 2021), as well as on intrinsic mechanisms like metabolic and endocrine pathways (Fuller et al., 2020), stress responses (Telemeco & Addis, 2014) and the production of reactive oxygen species (ROS; Paital et al., 2016). Physiological modifications caused by increased temperatures can be detrimental, but have also been found to be advantageous under some conditions (Liu et al., 2022), and may influence important processes linked to biological age like rates of DNA methylation (Sheldon et al., 2020) and telomere attrition (Whittemore et al., 2019). Telomeres are protective structures found at the end of chromosomes that can be affected by high ambient temperatures (Fitzpatrick et al., 2019). Reduced telomere length and increased attrition are associated with lower survival within and across some species (Hausmann et al., 2005; Tricola et al., 2018; Wilbourn et al., 2018) independently from age (Bize et al., 2009). Therefore, heat waves may impact telomere dynamics and fitness (Zhang et al., 2018), but experimental studies testing this possibility are limited and the magnitude and direction of results appear to vary with temperature (Liu et al., 2022).

Temperature effects on telomeres are known to be especially important during the sensitive early life stages of growth and development (Atema et al., 2015; Boonekamp et al., 2014; Eastwood et al., 2019; Entringer et al., 2011; Metcalfe & Alonso-Alvarez, 2010; Monaghan & Ozanne, 2018) and can have very marked effects in ectothermic organisms (Burraco et al., 2020; Dezetter et al., 2022; Liu et al., 2022). Heat waves may increase somatic growth and metabolic activity in ectotherms leading to higher ROS production (Stahlschmidt et al., 2017) and associated damage with detrimental consequences for telomere dynamics (Burraco et al., 2020; Halliwell & Gutteridge, 2015). Alternatively, telomere length in ectotherms could benefit from warmer temperatures if these push organismal physiology closer to the optimal thermal conditions for development (Friesen et al., 2021). Growth in endotherms is typically influenced less by ambient temperature (Olsson et al., 2018a, 2018b; Reichert & Stier, 2017), but telomeres may be shorter and show higher attrition in response to increased growth and organismal stress due to deflection from homeostasis depending on species-specific levels of heat tolerance (Monaghan, 2014; Simide et al., 2016). Interspecific variation between ectotherms and endotherms in the effects of temperature-induced stress on telomere dynamics might also be rooted in other aspects of the evolutionary history of the species. Our current understanding of this interspecific variation does not allow us to draw conclusions on the consequences of heat waves for telomere dynamics in the ectothermic and endothermic stages of an organism that encompasses both thermoregulatory regimes. A test performed within a species would therefore be very informative to gather critical evidence about the presence of developmental intervals with different sensitivity to physiological and molecular effects of environmental temperature on phenotypic development. This information is particularly important to acquire because embryos

and offspring of many species are unable to produce heat and therefore physiologically ectothermic for a period of their existence, independently of whether they ultimately become endothermic as adults.

Nestlings of songbirds are particularly interesting in this regard as they are ectothermic at birth and transition to endothermy within a few days of hatching (Sirsat et al., 2016). This transition occurs during a period when the temperature exposure of the chicks is influenced both by the external conditions and by the amount of heat provided by brooding adults (Mitchell et al., 2020). Parental conditions and levels of care are known to be important for telomere dynamics (Blaze et al., 2015; Quque et al., 2021; Viblanc et al., 2020; Young et al., 2022), and parental brooding effort in songbirds is responsive to environmental temperature during the incubation and the nestling stage (Ardia et al., 2009; Coe et al., 2015; Mitchell et al., 2020). This means that variation in adult brooding behaviour, and how this is influenced by environmental temperature, will have downstream effects on offspring telomere length during these early stages of development. Therefore, nestlings of songbirds provide unique opportunities to test the effects of heat waves on telomere dynamics at different thermoregulatory capabilities within the context of possible additional influences from parental care behaviour.

To date our understanding of parental care and ambient temperature in explaining post-natal telomere dynamics at different thermoregulatory stages is limited. Descriptive studies of wild zebra finches *Taeniopygia castanotis* exposed to natural variation in ambient conditions have revealed positive correlations between ambient temperatures and another biomarker of aging, the DNA methylation rate (He et al., 2021; Sheldon et al., 2020), and of the latter with rates of telomere shortening (Sheldon et al., 2021). These correlative results suggest that thermal conditions during early life influence telomeres, but since temperature was not manipulated in that study (Sheldon et al., 2021), other factors may affect the results such as seasonal variation in reproductive investment, parental condition, or food availability. In an earlier study focused on the same experimental offspring that we use in the current research, we demonstrated that heat waves had no effect on growth or whole body metabolic and hygric physiology of zebra finch at different ages (Ton, Stier, et al., 2021). We also found that nestlings experiencing experimental heat had lower body temperature during the ectothermic stage (day 12), which may help in reducing heat stress and telomere loss by keeping the organism further from thermal upper limits. However, these same birds showed increased mitochondrial metabolism for leak respiration as endothermic adults (Ton, Stier, et al., 2021). On one hand leak respiration is the process responsible for heat production and its increase requires higher heat dissipation with a greater risk of heat stress and telomere attrition. On the other hand, the *uncoupling to survive hypothesis* (Brand, 2000) postulates that higher leak respiration helps buffering damage from ROS, a major mechanism underpinning telomere erosion and reduced intrinsic survival (Halliwell & Gutteridge, 2015). When rate of uncoupling increases, the amount of ROS is expected to decrease, but more

heat is released. So, if the organism experiences high environmental temperatures, this might change the outcome of the trade-off between mitochondrial efficiency and exposure to ROS damage. Therefore, the physiological adjustments triggered by heat in our previous experiment may be detrimental or beneficial for telomeres depending on the thermoregulatory conditions.

To investigate the effect of environmental temperature during early development on telomere dynamics, we exposed newly hatched zebra finches to experimental heat waves within the nest chamber and measured telomeres at three ages (day 5, 12 and 80 after hatch day) that provide samples from each of the ectothermic and endothermic thermoregulatory states. We expected heat waves to cause higher attrition and shorter telomeres during the endothermic stage. This prediction is grounded on the idea that our experiment will challenge the nestling ability to offset their heat load when associated with higher levels of active heat produced during endothermy. These physiological conditions will push nestlings closer to the threshold for heat stress and lead to telomere erosion. Conversely, we expect telomere loss to be lower during the ectothermic parts of growth. This is because when endothermic, offspring produce minimal heat and therefore our experiment would keep nestlings closer to their physiologically optimal temperature. However, parental brooding provides a critical contribution to the thermal environment of nestlings during growth. Thus, the consequences of our experiment on telomere dynamics will also depend on the reactions of the parents to the treatment. We videotaped parental activity at the nest during the ectothermic stage expecting to replicate the reduction in brooding associated to higher temperatures found in other studies (Mitchell et al., 2020; Ton, Hurley, & Griffith, 2021). We also proceeded further and tested the possible downstream consequences of changes in parental brooding behaviours for telomeres.

2 | MATERIALS AND METHODS

2.1 | Species and housing

Between July and September 2018, we established 45 pairs of zebra finches from a pool of wild-derived captive birds available at Macquarie University. Randomly chosen groups of three males and three females were placed in aviaries 230×175×410 cm (height×width×depth), containing four nest boxes (22×12×15 cm) located 175 cm from the ground and shaded from direct sunlight. Birds were fed dry finch seed mix and water ad libitum, with a daily supplement of hardboiled eggs, cuttlebone and green seeds. After an initial week of acclimation, birds were provided with November grass, cotton strings and Emu feathers to allow nest construction. Clutches were monitored occasionally during the first part of the incubation and checked multiple times a day when approaching hatch date. When exact hatching date was ascertained, we experimentally heated 13 nests with a minimum brood size of three and simulated the same experimental procedure for 12 control nests

(See Tables S1–S4 for a detailed description of the sampling protocol). Working with captive birds allowed us to avoid variation in food availability and to remove all sources of predation risk that are ecological factors known to affect telomere dynamics (Hall et al., 2004; Noguera & Velando, 2019).

2.2 | Heating experiment and temperature measurements

Nests were heated starting on the day after hatch (day 1 of the nestling stage) until the first hatched nestling was 18 days old. The treatment was suspended at this age to provide a consistent amount of heat to all our samples within and among nests, as all birds fledged at days 19 or 20 of age. A Kapton flexible heating device (Omega Engineering KHA-404(10)-P), powered by a DC regulated Powertech power supply (HW1200R-12), was connected to a Vemer digital heat regulator (HT NIPT-1P3A VM628500) set at $40 \pm 0.2^\circ\text{C}$ and to a digital time switch (Vemer MICRO-D) that automatically started and ended the daily treatment. The heat output was regulated on the base of the nest temperature recorded by a Vemer VE122800 double injection IP68 probe placed in the nest. Whenever the air temperature in the nest was higher than the set point (40°C), the circuit was automatically turned off. Vice versa when nest temperature was lower, power was activated and heating resumed. This temperature value was chosen to replicate daily maximal T_a conditions recorded in the natural habitat for this species during a substantial part of their breeding season (Griffith et al., 2016). Also, 40°C represents an ideal compromise between challenging the nestlings to maintain homeostasis (Wojciechowski et al., 2021) and yet moderating exposure to harmful temperatures. The heat treatment was applied for 6 h a day from 9:00 until 15:00. In this way, we simulated the natural pattern of heat waves experienced in the field, while also allowing parental care and measurements to be taken by us before and after this time interval. Control nests were exposed to the same type of disturbance as heated nests with the difference that sham devices substituted batteries, thermostats, heaters and wires.

Two distinct temperature values were measured every 24 s at two separate positions inside each nest box between day one and day eight post-hatch. One probe was suspended 3 cm below the box roof to record ambient temperature T_a . A second probe was placed on the bottom of the nest cup in contact with the nestlings to record brood temperature T_{br} . Both probes were wired to a Gemini Tinytag Plus 2 data logger. Temperature was recorded only during this first ectothermic half of the post-natal period because measurements of later treatment effects on T_{br} become unreliable due to the increased mobility of nestlings at older ages that often leads them to cluster in corners. Temperature traces were analysed and processed using TINYTAG EXPLORER (version 4.7), and during the experiment, both were significantly higher in treatment ($T_a = 37.3 \pm 1.33^\circ\text{C}$; $T_{br} = 35.4 \pm 1.84^\circ\text{C}$) compared to control boxes ($T_a = 21.5 \pm 1.84^\circ\text{C}$, $t_{1,11} = 19.99$, $p < .001$; $T_{br} = 33.7 \pm 1.33^\circ\text{C}$, $t_{1,11} = 2.49$, $p = .01$; See Ton, Stier, et al., 2021 for further details).

2.3 | Bleeding protocol and telomere measurements

We collected blood (10–30 μ L) at age 5 (from the jugular vein) and at days 12 and 80 days (from the brachial vein) from 75 nestlings (36 controls and 39 heated) using a hypodermic needle and capillary tube. Blood was taken from all individuals of each brood and preserved in 1 mL of 95% ethanol, stored at room temperature in an Eppendorf tube and labelled with a unique alphanumeric code.

We extracted genomic DNA (gDNA) from blood samples using the NucleoSpin blood kit (Macherey-Nagel) with some minor modifications. Two microlitre of whole blood was removed from the sample tube, allowed to air-dry evaporating the ethanol and subsequently added to 198 μ L of PBS. From this stage, we followed the manufacturer's protocol, with the gDNA eluted into 35 μ L of BE buffer and stored at -20°C awaiting telomere length analysis. The quantity and purity of the gDNA was measured on a Nanodrop 8000 and all samples were within the accepted parameters; $A_{260/280} \geq 1.8$, $A_{260/230} \geq 1.9$ (Thermo Fisher).

We assayed rTL (relative telomere length) using the qPCR method (Criscuolo et al., 2009). Briefly, the rTL of each sample was measured by determining the ratio (T:S) of telomere repeat copy number (T) to a single copy or non-variant control gene (S), relative to a pooled DNA reference sample from five zebra finches at age 12 days that was run on all plates. We used Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the control gene. A standard curve (six serial dilutions of zebra finch gDNA from 40 to 1.25 ng/well) and a no template control compromising all the qPCR reagents except DNA were also included on each plate and all samples (ran in triplicate) fell within standard curve boundaries. All assays were run on the Stratagene MX3005p and utilized the MXPRO software. Mean reaction efficiencies were within the acceptable range for the telomere and the control gene (mean \pm SE, TEL: $87.14 \pm 1.30\%$; GAPDH: $106.39 \pm 1.12\%$). Average inter-plate variation of the Ct values was 1.58 for the telomere assay and 0.42 for the GAPDH assay. Intra-plate coefficient of variation for the telomere and GAPDH assays for the raw Ct values were 13.59 and 24.31, respectively. The average intra-plate variation of the Ct values was 0.8 for the telomere assay and 0.41 for the GAPDH assay.

rTL measurements were calculated using the method by Pfaffl (2001). The mean values were used to calculate the rTL (T:S ratio) using the formula: $((1 + E_{\text{telomere}})^{\Delta Cq_{\text{telomere}} (\text{control} - \text{sample})} / (1 + E_{\text{GAPDH}})^{\Delta Cq_{\text{GAPDH}} (\text{control} - \text{sample})})$.

2.4 | Measurements of adult brooding behaviour

We videotaped nests for about 6 h starting at 8:50 and ending at 15:00 to collect information on the rate of parental brooding during the heating experiment. Video cameras (Sony Corporation) were concealed in a corner of the aviary about 3 m away from the nest and were left unattended. Brooding effort was calculated as percentage

of time spent by at least one of the parents inside the nest box after excluding the first 10 min of recordings to allow adults to resume their normal activity after the initial disturbance caused by positioning the camera. Video recordings were made during the ectothermic phase of growth on day four or five of the nestling stage because brooding behaviour is higher during the early nestling stage and often ceases as young get older and approach endothermy.

2.5 | Growth rates and other life history measurements

As the expected hatch date was approaching, we intensified nest monitoring between three and five times a day in order to accurately identify what is conventionally defined as day 0 of the nestling stage. Once the nestlings emerged from the egg, we painted their tarsi using an individual combination of non-toxic markers to allow measurement of individual growth trajectories. On the same day, we also started weighing each nestling using a portable electronic scale (Nuweigh; ± 0.01 g). Measurements proceeded every second day at the same time ($16:00 \pm 1$ h) until fledging. We estimated the nestling growth rate constant (K) using logistic regressions (Remeš & Martin, 2002; Ricklefs, 1968; Sofaer et al., 2013). This method allows us to obtain asymptotic body mass at fledge date and to calculate the slope of the line tangent to the growth curve at inflection point (K). This is a dimensionless and mass-independent metric of growth speed that is not confounded by variation among individuals in size at hatch. These measurements allowed us to test whether fast rates of somatic growth (Monaghan & Ozanne, 2018; Vedder et al., 2017), and offspring mass (Ringsby et al., 2015) affect telomere length and attrition.

We also recorded brood size and kept track of hatching order, which are both known to influence telomere dynamics (Costanzo et al., 2016; Noguera et al., 2016; Reichert et al., 2014). Finally, when the offspring produced in our experiment reached 60 days of age, we recorded their sex, on the basis of plumage, to account for gender differences in telomeres (Noguera et al., 2015). All tests and measurements performed in our study were reviewed and approved by the Macquarie University Animal Ethics Committee (ARA 2017/024).

2.6 | Statistical analysis

We used t -tests to compare the two nest box temperature measurements T_a and T_{br} of our control and heated nests during the 6 h of daily treatment (see above). For each nest, we estimated growth rates (K) for nestling mass using non-linear mixed models (package NLME Pinheiro et al., 2017) following the methodology of Sofaer et al. (2013). This approach uses age and treatment as fixed effects and nest and nestling identity as random effects to account for the lack of independence among siblings of the same brood and for repeated measures of the same individual over time.

We initially tested the effects of simulated heat waves by fitting linear mixed models using the packages LME4 and LMTEST (Bates et al., 2014; Kuznetsova et al., 2017) with telomere length as the dependent variable, body mass, growth rate, as continuous covariates, and brood size, hatching order, treatment, sex and age plus their interactions as categorical fixed factors. Due to the strong correlation between offspring mass and age during post-natal growth, we created a variable called mass index that encapsulated the standardized differences in mass according to the age class of each subject, with a high mass index meaning that individuals are relatively heavy for their age and vice versa (see more details in S3.1). We included random slopes for individuals to account for repeated measures of subjects belonging to the same brood at different times. From this initial model, we dropped the non-significant variables and fitted our best model that included only treatment, age, mass index and the interaction between treatment and age, as well as individual identity as random factor (see Section S3.2). We also performed a model comparison test using Akaike Information Criterion (AIC; Akaike, 1987) and Bayesian Information Criterion (BIC; Schwarz, 1978). The AIC approach is more exploratory and favours models that maximize predictive accuracy while BIC penalizes complexity and aims to identify the exact model to falsify hypotheses (Aho et al., 2014). When both criteria converge on the selection of the same model, we get strong indications about the goodness of fit. These tests allowed us to ensure that we were not over-fitting the data and indicated that our model including the interaction was preferred by both approaches (AICweights=99%; BICweights=80%) to a model with an additive effect of treatment and age and to another with no interaction at all (see Tables S3.2.3). We conducted post hoc pairwise comparisons between groups using the emmeans package (Lenth et al., 2019), which applies Tukey test and Kenward-Roger degree of freedom adjustments. We also built a General Linear Model with time spent brooding as a dependent variable and treatment as a fixed factor to test the effects of heat waves on time spent brooding by the parents. Since this latter model indicated an effect of treatment on brooding effort, we attempted a test of the effects that variation in brooding rate has on telomere length and attrition by including in our initial model brooding and its interaction with age and treatment among the predictors. This part of the analysis however needs to be interpreted with extreme caution (see Section S6). Indeed, AIC suggested the best model to be the one including the three-way interaction while BIC gave opposite indications; also results were highly sensitive to some data points that exerted a meaningful effect on the regression coefficients (i.e. influential observations; Figure S23). All model assumptions and performances (homogeneity of variance, normality of residuals, normality of random effects, collinearity and influential observations) were checked (see Figures S9–S16, S18, S21, S22). All descriptive statistics for our variables and simple correlations between telomere length and the predictors are available on the supplemental materials (Tables S1–S8, Figures S1–S8). All analyses were performed with R version 4.1.2 for Mac (R Core Team, 2021, see Section S7 for full information on the packages used in the analysis).

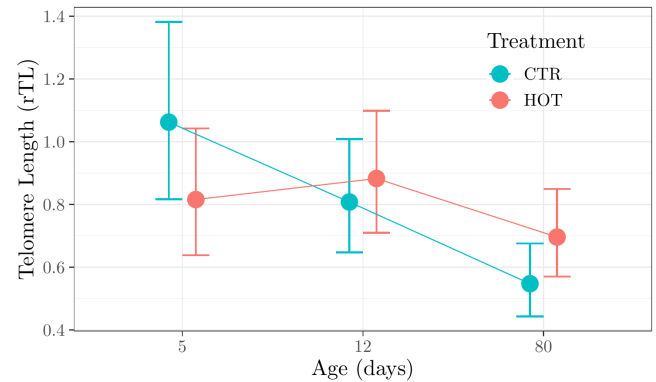


FIGURE 1 Mean \pm SE of relative telomere length for our experimentally heated (HOT) and control (CTR) groups. Lines connecting dots describe changes in telomere length across the three ages sampled.

3 | RESULTS

3.1 | Telomeres within treatments

Average telomere length was influenced by nestling age ($\chi^2=65.459$, $df=2$, $p<.001$) and showed an overall decline within our control group of birds over time (Figure 1), as expected in descriptive cross-sectional analyses (Hall et al., 2004). In the control group, telomeres at d5 were significantly longer compared to those at age d12 and 80 ($z=3.408$, $SE=0.105$, $p=.009$ and $z=7.812$, $SE=0.164$, $p<.001$, respectively), similarly to what we found for telomeres at age d12 compared to age d80 ($z=6.191$, $SE=0.092$, $p<.001$; Table S3.2.2). Subjects exposed to the heat treatment showed no difference in telomere length between d5 and d12 ($z=-0.937$, $SE=0.077$, $p=.938$) or between age d5 and d80 ($z=1.928$, $SE=0.096$, $p=.392$), but had significantly shorter telomeres at day 80 compared to day 12 ($z=3.518$, $SE=0.085$, $p=.006$; Figure 1; Table S3.2.2).

3.2 | Telomeres and parental brooding between treatments

Our experiment had no effect on absolute telomere length ($\chi^2=1.176$, $df=1$, $p=.278$), thus providing no confirmation for our predictions about the consequences of higher temperatures on this trait. However, we found support for our initial expectation of a beneficial effect of the experiment during the ectothermic phase by the detection of a significant interaction between treatment and age in determining telomere attrition ($\chi^2=18.201$, $df=2$, $p<.001$; Table S3.2.2). Indeed, between age 5 and 12, when the temperature treatment was taking place, telomere length showed a negative slope indicating telomere shortening in our control, but in contrast actually showed a positive slope associated to telomere elongation in the birds involved in the heat treatment ($z=3.033$, $SE=0.165$, $p=.031$; Figure 2). We also found that growth rate ($\chi^2=0.018$, $df=1$, $p=.893$), brood size ($\chi^2=0.273$, $df=1$, $p=.600$), hatching order

($\chi^2=0.010$, $df=1$, $p=.919$) and sex ($\chi^2=0.102$, $df=1$, $p=.749$) had no influence on telomere dynamics (Table S3.1.2). Telomere length showed a negative correlation with mass index independent of age and treatment ($\chi^2=4.046$, $df=1$, $p=.044$; Figure 2; Table S3.2.2).

Parents of heated nests spent a mean \pm SE 75% \pm 0.007 of their time brooding offspring, significantly less than the 85% \pm 0.006 of time spend brooding by our control birds ($\chi^2=106.52$, $df=1$, $p<.001$; Figure 3; Table S5.2.2; Figure S17) in line with our predictions. When testing a possible effect of parental brooding on telomeres, we found a significant three-way interaction showing higher brooding effort to be associated with longer nestling telomeres at 12 days of age for our heated birds ($\chi^2=8.216$, $df=2$, $p=.016$; Figure 4; Table S6.3.2; Figure S20). Similarly to what found in the previous model, nestling mass was negatively associated to telomere length ($\chi^2=4.447$, $df=1$, $p=.035$; Figure S24).

4 | DISCUSSION

In the last decade, there has been an increasing interest in understanding the ecological and physiological pathways affected by extreme weather events and their consequences for fitness (Thornton et al., 2014). If there are critical windows in which telomere dynamics are affected in a way that has lifelong effects, the timing of these extreme weather events becomes very important. Our experimental increase in nest temperature caused no overall change in telomere length but does suggest that the pattern of consistent decline in telomere length is altered in the nestlings in the heat-treated nests. While telomeres of the control nestlings had a reduction, the experimental group showed no changes during the first 12 days of life. This is particularly important because diminished telomere shortening has been highlighted as predictor of increased lifespan in many species (Haussmann et al., 2003) and may reflect lower exposure to damage from ROS. Therefore, our results match with our predictions and suggest that a warmer environment during post-natal growth may produce transient benefits in 12-day-old ectothermic nestlings.

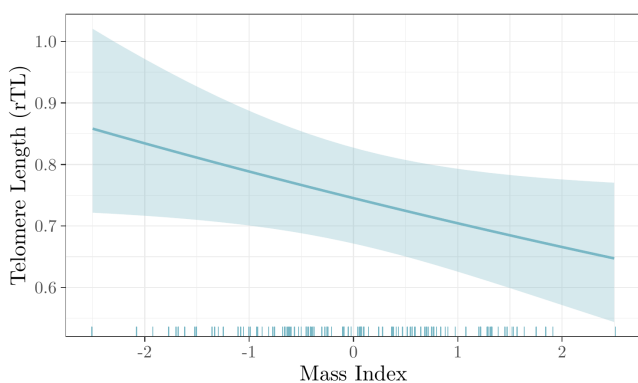


FIGURE 2 Plot of the relationship between nestling mass index and telomere length measured at age 5, 12 and 80 days. The shaded area describes 95% confidence intervals. Each dash on the x axis represents a sample.

The telomere dynamic we found between age 5 and 12 for heated nestlings is consistent with other information generated in the same experimental trial used in this study. Ton, Stier, et al. (2021) found no changes in metabolic rates or growth caused by heat and detected a lower body temperature during the first 12 days of life. The absence of acceleration in growth and metabolic rates associated with lower body temperature is expected to have no adverse consequences for stress and ROS production. Indeed, an increase in uncoupling activity during proton leak as found in our previous study (Ton, Stier, et al., 2021) is hypothesized to reduce costs from ROS (Brand, 2000) that may be triggered independently from variation in metabolic or growth rates; this could potentially prevent oxidative damage to telomeres. Therefore, the absence of detrimental treatment effects for telomeres that we detected here matches with theoretical predictions generated from previous tests on the same subjects (Ton, Stier, et al., 2021). Additionally, a recent study (in which telomeres were not measured) indicated that moderate warming above current temperature averages experienced during ontogeny and growth can be beneficial by increasing the survival of ectothermic organisms, (Liu et al., 2022). Conversely other studies documented detrimental effects of heat waves (Axelsson et al., 2020; Zhang et al., 2018), where lizards were exposed to stressful temperatures that exceeded the field average by 6°C had shorter telomeres compared to those kept at cooler thermal conditions (Zhang et al., 2018). Our experiment involved a much less severe heat treatment that avoided the significant telomere erosion detected in the aforementioned study (Zhang et al., 2018). We kept birds within ranges that reflect current temperatures experienced in nature during their breeding season (Griffith et al., 2016) and therefore elicited telomere responses that are within the evolved norm of reaction for the species.

The effects of warming for telomere dynamics during some part of nestling growth but not at other ages may be explained by the thermoregulatory context. Nestlings are ectothermic initially after hatching and gradually switch to endothermy (Sirsat et al., 2016), when they can reach operational temperatures approaching 42°C (McKechnie & Wolf, 2019). Our experiment suggests that bringing ambient temperatures as close as possible to the physiological optima of ectothermic nestlings (Friesen et al., 2021) provides some resistance to the causes of telomere attrition. Of course it is possible that other age-associated physiological changes may be involved, but these findings are consistent with studies that demonstrated telomere elongation in lizards (Fitzpatrick et al., 2019) and longer telomeres in ectothermic organisms exposed to warmer temperatures (Burraco et al., 2020). This might also involve a higher expression of telomerase, as predicted for ectotherms growing in warmer environments (Olsson et al., 2018a, 2018b). Telomerase functions as a repair mechanism for telomeres and shows higher activity during the post-natal stage in ectothermic than in endothermic species (Olsson et al., 2018a, 2018b). Our within species test shows that experimental heat waves did not cause telomere attrition on chicks of zebra finch, while they transition from the ectothermic to their final endothermic stage (i.e., the first 12 days of life). However, post-natal stages of songbirds that grow in enclosed or cavity nests are ectothermic

only for 6–16 days in their life (Cheng & Martin, 2012), and there may be disagreement on whether their telomeres should respond to temperature to the same extent as those of a true ectotherm during this short window. Still, the relationship between telomere dynamics and heat waves during the first days after hatch aligns with that found for true ectothermic species (Burraco et al., 2020; Fitzpatrick et al., 2019). This result is particularly meaningful for birds because, in contrast to other endothermic vertebrates, their embryos grow outside of the stable thermal environment of the mother. Indeed, early life stages of birds are subjected to considerable temperature fluctuations due to variation in parental care (Martin et al., 2007), and this variation can have important downstream consequences for development and metabolism (Ton & Martin, 2017).

Here we also investigated the presence of complex strategies whereby parent birds may buffer offspring to a degree in early life.

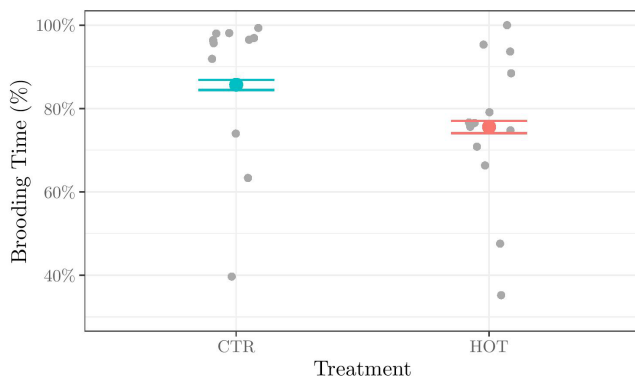


FIGURE 3 Mean \pm SE brooding behaviour for the experimentally heated (HOT) and control (CTR) groups. Parental brooding behaviour (% of time spent on the nest) was measured for 6 h between 9:00 and 15:00 at day 4 or 5 of the nestling stage.

We found that our heat treatment significantly reduced brooding effort at the nest. This is not a novel finding (Mitchell et al., 2020), but it is important in the present context because a cross-fostering experiment found parental care to drive telomere dynamics independently of genetic background (Viblanco et al., 2020), and in that study, the role of brooding behaviour as a potential causal agent was not tested. The lower rate of brooding we demonstrated for our heated nests has been suggested to reflect energy benefits for the parents (Bryan & Bryant, 1999; Yom-Tov & Hilborn, 1981), but tests during incubation show that, as temperature increases, these savings appear to be negligible (Ton, Hurley, & Griffith, 2021; Vleck, 1981). Instead, lowering brooding at high temperatures may be a strategy aimed at buffering potential risks of thermal stress for the adults and for their offspring. However, the significant interaction we found between heat treatment, age, and telomere length indicated shorter telomeres associated to lower brooding effort for the heated nestlings at age 12, which may reflect differences in parental quality and heat tolerance. As already stated, the apparent effect of brooding on telomere length that we detected needs to be interpreted with caution because of the small sample size and the opposite indications provided by our selection criteria (see S6.3.4 for further details). Still, our result should encourage future studies to elucidate if lowering brooding effort during extreme heat may yield higher chances of surviving and therefore future reproduction for the parents, while also reducing telomere length and future survival for the young.

Finally, we also found a negative correlation between telomere length and body mass independently of treatment and age, and this is important because both telomere and body size have been shown to be heritable (Boonekamp et al., 2020; Jensen et al., 2003), and may influence bird fitness (Ringsby et al., 2015). Similar results were previously generated in a selection experiment aimed at manipulating tarsus

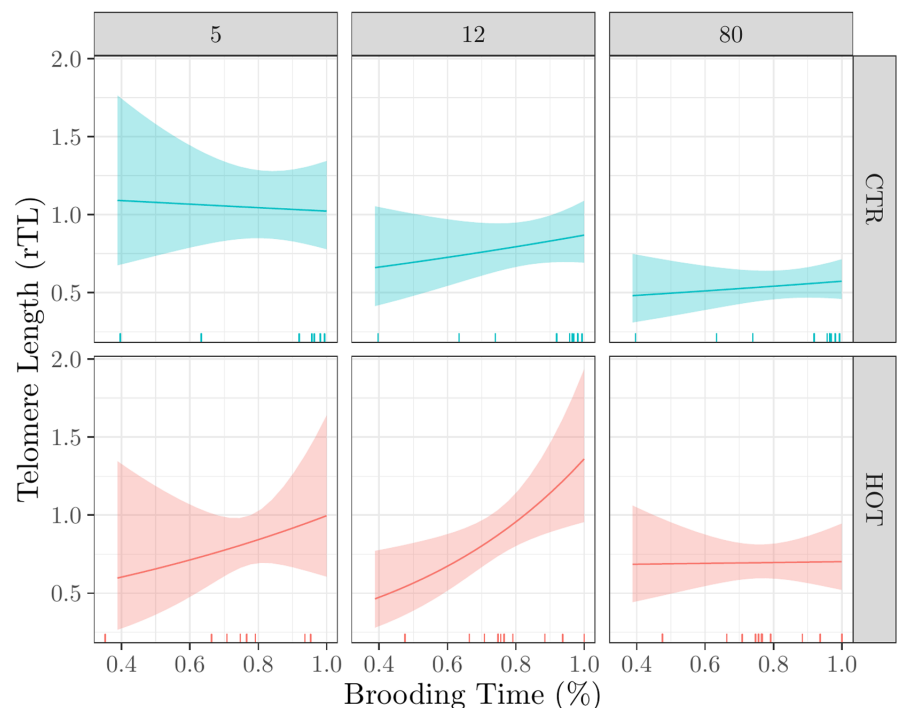


FIGURE 4 Relationships between telomere length (rTL) and parental brooding behaviour for experimentally heated (HOT) and control (CTR) birds for each of the three ages sampled (Day 5, 12 or 80). The shaded area describes 95% confidence intervals. Each dash on the x axis represents a sample.

length which is related to structural size (Ringsby et al., 2015) and in a descriptive study on the same species used here (Sheldon et al., 2021). Similar to the findings of another study (Boonekamp et al., 2020), variation in telomere length in our analysis did not appear to be associated with differences in rates of growth. This may be because in captivity with food available ad libitum, all chicks were growing at their optimal rate and did not experience the accelerated growth that is associated with higher telomere attrition (Monaghan & Ozanne, 2018).

Our study highlights the complexity of the mechanisms underlying variation in telomere length by finding amelioration of telomere attrition caused by heat during the ectothermic stage of an endothermic species. This kind of effect has been found in other correlative and experimental studies (Liu et al., 2022; Pipoly et al., 2013). Here we also highlight the consequences for parental care and its potential contribution to the thermal environment and to telomere dynamics. The outcomes of these interactions for individuals over their life course are an avenue of research still in its infancy that deserve further investigation.

AUTHOR CONTRIBUTIONS

R.T., P.M. and S.C.G. conceived the study. R.T. collected the data. W.B., R.T., S.R. and P.M. performed the qPCR assays. R.T. conducted the statistical analyses. The manuscript was written by R.T. and commented on and edited by all authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Authors have archived their data in the publicly accessible repository Open Scientific Framework DOI: 10.17605/OSF.IO/WMNH6.

BENEFIT SHARING

Benefits generated: Benefits from this research accrue from the sharing of our data and results on public databases as described above. The present work strongly benefitted from a collaboration with scientists at University of Glasgow that contributed to the conceptual and methodological aspects of the study related to telomere measurements.

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SUPPORTING INFORMATION

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