

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

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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 poikilothermic to endothermic after hatching. Telomeres of ectothermic and endothermic organisms respond differently to environmental temperature, but investigations within species that transition from ectothermy to endothermy are lacking. Also, ambient temperature influences parental brooding behavior, 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 correspond to three different thermoregulatory stages (ectothermic, poikilothermic and endothermic respectively); 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 (poikilothermic stage) compared to controls. Additionally, parents of heated broods reduced the time they spent brooding offspring (at five days old) compared to controls. Lower brooding effort was associated with shorter telomeres in 12 day old nestlings. 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 behavior during growth.

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 (Pachauri *et al.* 2014; Meehl & Tebaldi 2004). While some organisms may plastically adjust or adapt to rising temperatures over time (Somero 2010; Franks & Hoffmann 2012), heat waves are thought to be harder to buffer 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). These physiological modifications can be detrimental 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 species (Hausmann *et al.* 2005; Wilbourn *et al.* 2018; Tricola *et al.* 2018) independently from age (Bize *et al.* 2010). 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 (Metcalf & Alonso Alvarez 2010; Entringer *et al.* 2011; Boonekamp *et al.* 2014; Atema *et al.* 2015; Monaghan & Ozanne 2018; Eastwood *et al.* 2019) 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 (Halliwell & Gutteridge 2015; Burraco *et al.* 2020). 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 (Reichert & Stier 2017; Olsson *et al.* 2018), 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 adult.

Altricial birds are particularly interesting in this regard as they transition from being ectothermic to poikilothermic to endothermic 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. Parental care is known to be important for telomere dynamics (Blaze *et al.* 2015, Viblanc *et al.* 2020, Quque *et al.* 2021), 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 behavior 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 altricial species 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 behavior.

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 (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, we demonstrated that heat waves had no effect on growth or whole body metabolic and hygric physiology of zebra finch at different ages (Ton *et al.* 2021a). We also previously found that heated nestlings had lower body temperature during the poikilothermic 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 *et al.* 2021a). 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, higher leak respiration is also known to help 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 beneficial for telomeres as predicated under the *uncoupling to survive hypothesis* (Brand 2000), or detrimental depending on the thermoregulatory conditions. While telomeres were not considered in the aforementioned studies, those findings (Sheldon *et al* 2020, Sheldon *et al* 2021, Ton *et al* 2021a) provide testable predictions about the likely effects of the experimental treatment on telomere length.

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) that provide samples from each of the three different thermoregulatory states (ectothermic, poikilothermic and endothermic) respectively. We expected heat waves to cause higher attrition and shorter telomeres during the endothermic stage due to physiological conditions being closer to the threshold for heat stress. Conversely, we expect telomere loss to be lower during the ectothermic and poikilothermic parts of growth when offspring produce minimal heat and the thermal balance of the brood can be buffered by changes in parental care. Therefore, we also videotaped adult activity at the nest during the ectothermic stage to understand the effects of heat waves on brooding rates, and the possible downstream consequences for telomeres.

MATERIALS AND METHODS

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 (22×12×15cm) located 175cm 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 S1-4 for a detailed description of the sampling protocol). Our set up allowed us to control for food availability (Hall *et al.*2004) and prevented all sources of predation risk (Noguera & Velando 2019) that are also factors known to have reflections on telomere dynamics.

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 six hours 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 seconds 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 (ver. 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 ($T_a=21.5 \pm 1.84^\circ\text{C}$, $t_{1,11} = 19.99$, $p < 0.001$; $T_{br}=33.7 \pm 1.33^\circ\text{C}$, $t_{1,11} = 2.49$, $p = 0.01$) (See Ton *et al.* 2021a for further details).

Bleeding protocol and telomere measurements

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

We extracted genomic DNA (gDNA) from blood samples using the NucleoSpin blood kit (Macherey-Nagel) with some minor modifications. 2 μl of whole blood were removed from the sample tube, allowed to air-dry evaporating the ethanol, and subsequently added to 198 μl of PBS. From this stage, we followed the manufacturer's protocol, with the gDNA eluted into 35 μl 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; $A_{260/280}$ [?] 1.8, $A_{260/230}$ [?] 1.9 (Thermo Fisher).

We assayed rTL using the qPCR method (Crisuolo 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 (6 serial dilutions of zebra finch gDNA from 40 to 1.25ng/well) was also included on each plate and all samples (ran in triplicate) fell within standard curve boundaries. 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: $((1 + E_{\text{telomere}})^{\Delta\text{Cq}_{\text{telomere}} (\text{control} - \text{sample})} / (1 + E_{\text{GAPDH}})^{\Delta\text{Cq}_{\text{GAPDH}} (\text{control} - \text{sample})})$.

Measurements of adult brooding behavior

We videotaped nests for about 6 hours 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, Tokyo, Japan) 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 minutes 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 behavior is higher during the early nestling stage and often ceases as young get older and approach endothermy.

Growth rates and other life history measurements

As the expected hatch date was approaching, we intensified nest monitoring to multiple 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 (Sofaer *et al.*, 2013; Remesê and Martin, 2002; Ricklefs, 1968). 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 (Vedder *et al.* 2017; Monaghan & Ozanne 2018), 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 (Reichert *et al.* 2014; Costanzo *et al.* 2016; Noguera *et al.* 2016). 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).

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 six hours of daily treatment (see above). For each nest we compared growth rates (K) for nestling mass using nonlinear 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 lmerTest (Bates *et al.* 2014; Kuznetsova *et al.* 2017) with telomere length as the dependent variable, body mass, growth rate, as 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). To ensure that we were not over-fitting the data by including the interaction between treatment and age, we also performed a model comparison test using Akaike Information Criterion (AIC) (Akaike 1987) and Bayesian Information Criterion (BIC) (Schwarz 1978). This test indicated that our model 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 adjustment 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 as a covariate. This part of the analysis however needs to be interpreted with extreme caution (see sections 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 influential observations (S6.3.3). All models assumptions and performances (homogeneity of variance, normality of residuals, normality of random effects, collinearity, and influential observations) were checked (see Supplemental Materials). All analyses were performed with

R version 4.1.2 for Mac (R Core Team 2021).

RESULTS

We found that growth rate ($p = 0.893$), brood size ($p = 0.600$), hatching order ($p = 0.919$), and sex ($p = 0.749$) had no influence on telomere dynamics (Table S3.1.2). Telomere length showed a negative correlation with mass index independent of age and treatment ($p = 0.044$; Fig 1, Table S3.2.2). Average telomere length showed an overall decline in our control birds over time (Fig 2). Telomeres at d5 were significantly longer compared to age d12 and 80 ($p = 0.009$ and $p < 0.001$ respectively), similarly to what we found for telomeres at age d12 compared to age d80 ($p < 0.001$; Table S3.2.2). Subjects exposed to the heat treatment showed no decline in telomere length between d 5 and d12, and between age d5 and d80 ($p = 0.391$), but had significantly shorter telomeres at day 80 compared to day 12 ($p = 0.006$; Fig 2; Table S3.2.2). Overall the heat treatment had no effect on telomere length ($p = 0.278$), but showed a significant interaction with age in determining telomere attrition ($p < 0.001$; Table S3.2.2). In particular between age 5 and 12, when the temperature treatment was taking place, telomere length decreased in our control, but in contrast actually showed an increase in the birds involved in the heat treatment ($p = 0.031$; Fig 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 spent brooding by our control birds ($p < 0.001$; Fig 3, Table S5.2.2). We found a significant three-way interaction among the covariates showing higher brooding effort to be associated with longer nestling telomeres at 12 days of age for our heated birds ($p = 0.016$; Fig 4; Table S6.3.2).

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 chicks 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 (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/poikilothermic nestlings.

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 *et al.* (2021a) found no changes in metabolic rates or growth caused by heat, and detected a lower body temperature during the poikilothermic stage. 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 coupling activity during proton leak as found in our previous study (Ton *et al.* 2021a) 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 *et al.* 2021a). 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 (Zhang *et al.* 2018; Axelsson *et al.* 2020), 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 much less severe heat treatment, since we kept birds within ranges that reflect current temperatures experienced in nature during their breeding season (Griffith *et al.* 2016).

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 42C (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 a study that demonstrated telomere elongation in lizards kept at warmer temperatures (Fitzpatrick *et al.* 2019) and longer telomeres in ectothermic organisms in the heat (Burraco *et al.* 2020). This might also involve a higher expression of telomerase, as predicted for ectotherms growing in warmer environments (Olsson *et al.* 2018). Telomerase functions as a repair mechanism for telomeres and shows higher activity during the post-natal stage in ectothermic than in endothermic species (Olsson, Wapstra, & Friesen, 2018). Our within species test show that experimental heat waves reduced telomere attrition on chicks of zebra finch during their ectothermic stage. However, post-natal stages of songbirds that grow in enclosed or cavity nests are ectothermic only for 8-16 days in their life (Cheng & Martin 2012) and there may be disagreement on whether they should be considered as 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 (Fitzpatrick *et al.* 2019; Burraco *et al.* 2020). 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. 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 behavior as a potential causal agent was not tested. The lower rate of brooding we demonstrated for our heated nests have been suggested to reflect energy benefits for the parents (Yom-Tov & Hilborn 1981; Bryan & Bryant 1999), but tests during incubation show that, as temperature increases, these savings appear to be negligible (Ton *et al.* 2021b, 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, we found 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, but encourages 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 (Jensen *et al.* 2003; Boonekamp *et al.* 2020), and may influence bird fitness (Ringsby *et al.* 2015). Similar results were previously generated in a selection experiment aimed at manipulating tarsus 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 (Pipoly *et al.* 2013; Liu *et al.* 2022) but the consequences for parental care and its potential contribution to the thermal environment

and to telomere dynamics and outcomes for individuals over the life course is an avenue of research still in its infancy that deserves further investigation.

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REFERENCES

- Akaike H (1987) Factor analysis and AIC. In: *Selected papers of hirotugu akaike* pp. 371-386 Springer, .
- Ardia DR, Pérez JH, Chad EK, Voss MA, Clotfelter ED (2009) Temperature and life history: experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour. *Journal of Animal Ecology*, **78** , 4-13.
- Atema E, Mulder E, Dugdale HL, Briga M, van Noordwijk AJ, Verhulst S (2015) Heritability of telomere length in the Zebra Finch. *Journal of Ornithology*, **156** , 1113-1123.
- Axelsson J, Wapstra E, Miller E, Rollings N, Olsson M (2020) Contrasting seasonal patterns of telomere dynamics in response to environmental conditions in the ectothermic sand lizard, *Lacerta agilis*. *Scientific Reports*, **10** , 1-9.
- Bates D, Maechler M, Bolker B, Walker S (2014) *lme4: Linear mixed-effects models using Eigen and S4.R package version 1.1-7*, .
- Bize P, Criscuolo F, Metcalfe NB, Nasir L, Monaghan P (2009) Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **276** , 1679-1683.
- Blaze J, Asok A, Roth TL (2015) The long-term impact of adverse caregiving environments on epigenetic modifications and telomeres. *Frontiers in behavioral neuroscience*, **9** , 79.
- Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., & Verhulst, S. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society of London B: Biological Sciences*, 281, 20133287.
- Boonekamp J, Rodriguez-Munoz R, Hopwood P, et al (2020) Telomere length is highly heritable and independent of growth rate manipulated by temperature in field crickets. *bioRxiv*, .
- Brand M (2000) Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Experimental gerontology*, **35** , 811-820.
- Bryan S, Bryant D (1999) Heating nest-boxes reveals an energetic constraint on incubation behaviour in great tits, *Parus major*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **266** , 157-162.
- Burraco P, Orizaola G, Monaghan P, Metcalfe NB (2020) Climate change and ageing in ectotherms. *Global Change Biology*, **26** , 5371-5381.
- Cheng Y, Martin TE, (2012) Nest predation risk and growth strategies of passerine species: grow fast or develop traits to escape risk? *American Naturalist*, **180** 285-295.
- Coe BH, Beck ML, Chin SY, Jachowski CM, Hopkins WA (2015) Local variation in weather conditions influences incubation behavior and temperature in a passerine bird. *Journal of Avian Biology*, **46** , 385-394.
- Costanzo A, Parolini M, Bazzi G, et al (2016) Brood size, telomere length, and parent-offspring color signaling in barn swallows. *Behavioral Ecology*, , arw147.
- Criscuolo F, Bize P, Nasir L, et al (2009) Real-time quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology*, **40** , 342-347.

- Dezetter M, Le Galliard J, Leroux-Coyau M, Brischoux F, Angelier F, Lourdais O (2022) Two stressors are worse than one: combined heatwave and drought affect hydration state and glucocorticoid levels in a temperate ectotherm. *Journal of Experimental Biology*, **225** , jeb243777.
- Donelson J, Munday P, McCormick M, Pankhurst N, Pankhurst P (2010) Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish. *Marine Ecology Progress Series*, **401** , 233-243.
- Eastwood JR, Hall ML, Teunissen N, et al (2019) Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular ecology*, **28** , 1127-1137.
- Entringer S, Epel ES, Kumsta R, et al (2011) Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, **108** , E513-8.
- Fitzpatrick L, Olsson M, Parsley L, et al (2019) Temperature and telomeres: thermal treatment influences telomere dynamics through a complex interplay of cellular processes in a cold-climate skink. *Oecologia*, **191** , 767-776.
- Franks SJ, Hoffmann AA (2012) Genetics of climate change adaptation. *Annual Review of Genetics*, **46** , 185-208.
- Friesen CR, Wapstra E, Olsson M (2021) Of telomeres and temperature: Measuring thermal effects on telomeres in ectothermic animals. *Molecular ecology*, .
- Fuller A, Hetem RS, Maloney SK, Mitchell D (2014) Adaptation to heat and water shortage in large, arid-zone mammals. *Physiology*, **29** , 159-167.
- Fuller A, Maloney SK, Blache D, Cooper C (2020) Endocrine and metabolic consequences of climate change for terrestrial mammals. *Current Opinion in Endocrine and Metabolic Research*, **11** , 9-14.
- Griffith SC, Mainwaring MC, Sorato E, Beckmann C (2016) High atmospheric temperatures and ‘ambient incubation’ drive embryonic development and lead to earlier hatching in a passerine bird. *Royal Society Open Science*, **3** , 150371.
- Hall ME, Nasir L, Daunt F, et al (2004) Telomere loss in relation to age and early environment in long-lived birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **271** , 1571-1576.
- Halliwell B, Gutteridge JM (2015) *Free radicals in biology and medicine* Oxford University Press, USA.
- Hausmann MF, Winkler DW, Vleck CM (2005) Longer telomeres associated with higher survival in birds. *Biology letters*, **1** , 212-214.
- Hausmann MF, Winkler DW, O’Reilly K, Huntington CE, Nibet ICT, Vleck CM (2003) Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society B* **270** , 1387–1392.
- Jensen H, Saether B, Ringsby TH, Tufto J, Griffith SC, Ellegren H (2003) Sexual variation in heritability and genetic correlations of morphological traits in house sparrow (*Passer domesticus*). *Journal of Evolutionary Biology*, **16** , 1296-1307.
- Kuznetsova A, Brockhoff PB, Christensen RH (2017) lmerTest package: tests in linear mixed effects models. *Journal of statistical software*, **82** , 1-26.
- Lenth R, Singmann H, Love J, Buerkner P, Herve M (2019) *Estimated marginal means, aka least-squares means. R package version 1.3.2*, .
- Liu W, Liu P, Cui L, et al (2022) Moderate climate warming scenarios during embryonic and post-embryonic stages benefit a cold-climate lizard. *Functional Ecology*, .

- Lowe WH, Martin TE, Skelly DK, Woods HA (2021) Metamorphosis in an era of increasing climate variability. *Trends in Ecology & Evolution*, **36** , 360-375.
- McKechnie AE, Wolf BO (2019) The physiology of heat tolerance in small endotherms. *Physiology*, **34** , 302-313.
- McKechnie AE, Wolf BO (2010) Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. *Biology letters*, **6** , 253-256.
- Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science (New York, N. Y.)*, **305** , 994-997.
- Metcalfe NB, Alonso-Alvarez C (2010) Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, **24** , 984-996.
- Mitchell A, Boersma J, Anthony A, Kitayama K, Martin TE (2020) Experimental amelioration of harsh weather speeds growth and development in a tropical montane songbird.
- Monaghan P, Ozanne SE (2018) Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **373** , 20160446.
- Monaghan P (2014) Organismal stress, telomeres and life histories. *The Journal of experimental biology*, **217** , 57-66.
- Noguera JC, Metcalfe NB, Boner W, Monaghan P (2015) Sex-dependent effects of nutrition on telomere dynamics in zebra finches (*Taeniopygia guttata*). *Biology Letters*, **11** , 20140938.
- Noguera JC, Metcalfe NB, Reichert S, Monaghan P (2016) Embryonic and postnatal telomere length decrease with ovulation order within clutches. *Scientific reports*, **6** , 1-8.
- Noguera JC, Velando A (2019) Reduced telomere length in embryos exposed to predator cues. *The Journal of experimental biology*, **222** , 10.1242/jeb.216176.
- Olsson M, Wapstra E, Friesen C (2018a) Ectothermic telomeres: it's time they came in from the cold. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **373** , 20160449.
- Olsson M, Wapstra E, Friesen CR (2018b) Evolutionary ecology of telomeres: a review. *Annals of the New York Academy of Sciences*, **1422** , 5-28.
- Pachauri RK, Allen MR, Barros VR, et al (2014) *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change* Ipcc.
- Paital B, Panda SK, Hati AK, et al (2016) Longevity of animals under reactive oxygen species stress and disease susceptibility due to global warming. *World journal of biological chemistry*, **7** , 110-127.
- Pinheiro J, Bates D, DebRoy S, et al (2017) Package 'nlme'. *Linear and nonlinear mixed effects models, version*, **3** .
- Pipoly I, Bokony V, Seress G, Szabo K, Liker A (2013) Effects of extreme weather on reproductive success in a temperate-breeding songbird. *PLoS One*, **8** , e80033.
- Quque M, Paquet M, Zahn S, et al (2021) Contrasting associations between nestling telomere length and pre and postnatal helpers' presence in a cooperatively breeding bird. *Oecologia*, **196** , 37-51.
- Reichert S, Stier A (2017) Does oxidative stress shorten telomeres in vivo? A review. *Biology Letters*, **13** , 20170463.
- Reichert S, Stier A, Zahn S, et al (2014) Increased brood size leads to persistent eroded telomeres. *Frontiers in Ecology and Evolution*, **2** , 9.

- Remeš V, Martin TE (2002) Environmental influences on the evolution of growth and developmental rates in passerines. *Evolution*, **56** , 2505-2518.
- Ricklefs RE (1968) Patterns of growth in birds. *Ibis*, **110** , 419-451.
- Ringsby TH, Jensen H, Pärn H, et al (2015) On being the right size: increased body size is associated with reduced telomere length under natural conditions. *Proceedings of the Royal Society B: Biological Sciences*, **282** , 20152331.
- Schwarz G (1978) Estimating the dimension of a model. *The annals of statistics*, , 461-464.
- Sheldon EL, Schrey AW, Hurley LL, Griffith SC (2020) Dynamic changes in DNA methylation during postnatal development in zebra finches *Taeniopygia guttata* exposed to different temperatures. *Journal of Avian Biology*, **51** .
- Sheldon EL, Ton R, Boner W, et al (2021) Associations between DNA methylation and telomere length during early life: insight from wild zebra finches (*Taeniopygia guttata*). *Molecular ecology*, .
- Simide R, Angelier F, Gaillard S, Stier A (2016) Age and heat stress as determinants of telomere length in a long-lived fish, the Siberian sturgeon. *Physiological and Biochemical Zoology*, **89** , 441-447.
- Sirsat SKG, Sirsat TS, Crossley JL, Sotherland PR, Dzialowski EM (2016) The 12-day thermoregulatory metamorphosis of Red-winged Blackbirds (*Agelaius phoeniceus*). *Journal of Comparative Physiology B*, **186** , 651-663.
- Sofaer HR, Chapman PL, Sillett TS, Ghalambor CK (2013) Advantages of nonlinear mixed models for fitting avian growth curves. *Journal of Avian Biology*, **44** , 469-478.
- Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *The Journal of experimental biology*, **213** , 912-920.
- Stahlschmidt Z, French S, Ahn A, Webb A, Butler MW (2017) A simulated heat wave has diverse effects on immune function and oxidative physiology in the corn snake (*Pantherophis guttatus*). *Physiological and Biochemical Zoology*, **90** , 434-444.
- Stillman JH (2019) Heat waves, the new normal: summertime temperature extremes will impact animals, ecosystems, and human communities. *Physiology*, **34** , 86-100.
- Telemeco RS, Addis EA (2014) Temperature has species-specific effects on corticosterone in alligator lizards. *General and comparative endocrinology*, **206** , 184-192.
- Thornton PK, Erickson PJ, Herrero M, Challinor AJ (2014) Climate variability and vulnerability to climate change: a review. *Global Change Biology*, **20** , 3313-3328.
- Ton R, Stier A, Cooper CE, Griffith SC (2021a) Effects of heat waves during post-natal development on mitochondrial and whole body physiology: an experimental study in zebra finches. *Frontiers in physiology*, **12** , 554.
- Ton R, Hurley LL, Griffith SC (2021b) Higher experimental ambient temperature decreases female incubation attentiveness in Zebra Finches (*Taeniopygia guttata*) and lower effort yields negligible energy savings. *Ibis*, **163** , 1045-1055.
- Ton R, Martin TE (2017) Proximate effects of temperature versus evolved intrinsic constraints for embryonic development times among temperate and tropical songbirds. *Scientific Reports*, **7** , 895.
- Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D. C., . . . Haussmann, M. F. (2018). The rate of telomere loss is related to maximum lifespan in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* , *373* (1741), 20160445. <https://doi.org/10.1098/rstb.2016.0445>.

Vedder O, Verhulst S, Bauch C, Bouwhuis S (2017) Telomere attrition and growth: a life-history framework and case study in common terns. *Journal of Evolutionary Biology*, **30** , 1409-1419.

Viblanc VA, Schull Q, Stier A, et al (2020) Foster rather than biological parental telomere length predicts offspring survival and telomere length in king penguins. *Molecular ecology*, .

Vleck CM (1981) Energetic cost of incubation in the zebra finch. *The Condor*, **83** , 229-237.

Whittemore K, Vera E, Martinez-Nevaldo E, Sanpera C, Blasco MA (2019) Telomere shortening rate predicts species life span. *Proceedings of the National Academy of Sciences of the United States of America*, **116** , 15122-15127.

Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences* , *373* , 20160447.

Wojciechowski MS, Kowalczywska A, Colominas-Ciuro R, Jefimow M (2021) Phenotypic flexibility in heat production and heat loss in response to thermal and hydric acclimation in the zebra finch, a small arid-zone passerine. *Journal of Comparative Physiology B*, **191** , 225-239.

Yom-Tov Y, Hilborn R (1981) Energetic constraints on clutch size and time of breeding in temperate zone birds. *Oecologia*, **48** , 234-243.

Zhang Q, Han X, Hao X, et al (2018) A simulated heat wave shortens the telomere length and lifespan of a desert lizard. *Journal of thermal biology*, **72** , 94-100.

DATA ACCESSIBILITY

Authors have archived their data in the publicly accessible repository FigShare. DOI:

STATEMENT OF CONFLICTING INTEREST

The authors declare no conflict of interest.

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 assay. R.T. conducted the statistical analyses. The manuscript was written by R.T. and commented on and edited by all authors. The study was supported by funding from the Australian Research Council to SCG and PM.

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Figure 1 : Plot of the relationship between nestling mass index and telomere length. Each dash on the x axis represents a sample.

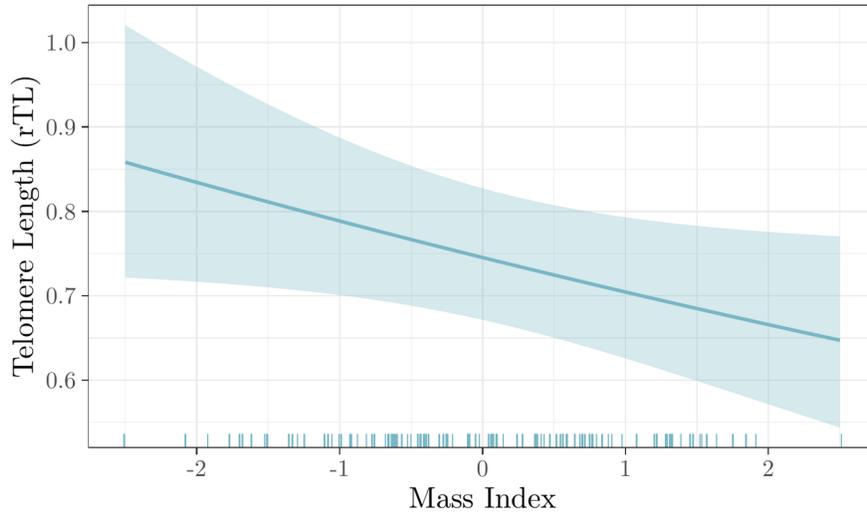


Figure 2 . Mean \pm SE of telomere length for our treatment (from hatch to day 18 of nestling) and control birds. Lines connecting dots describe changes in telomere length across ages.

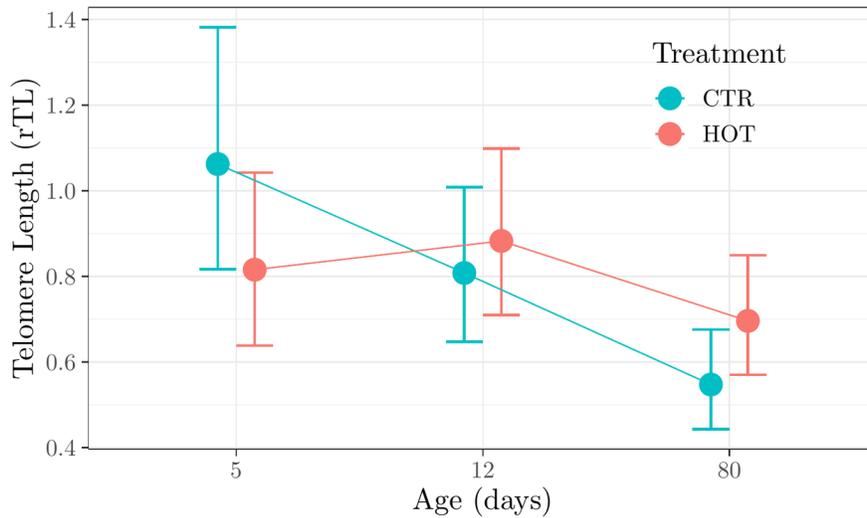


Figure 3 . Mean \pm SE of treatment effect on parental brooding behavior measured for six hours between 8:50 and 15:00 at day 4 or 5 of the nestling stage.

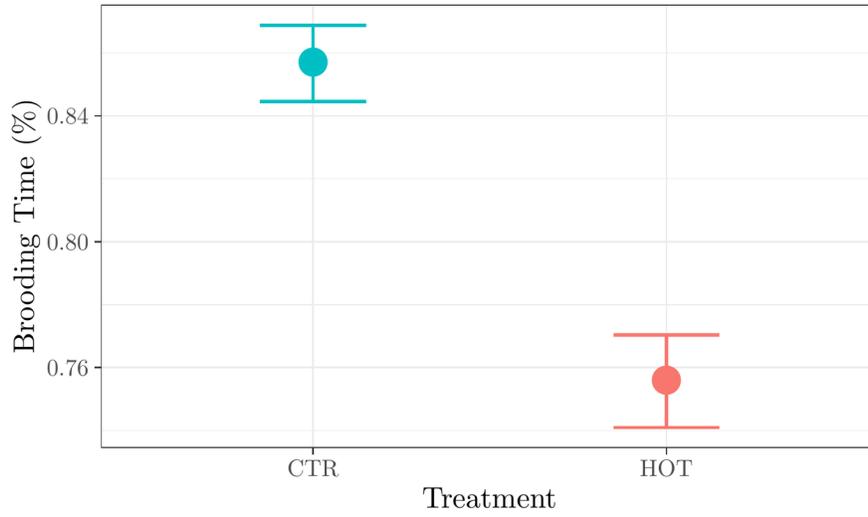


Figure 4 . Relationships between telomere length and parental brooding behavior for treatment and control birds.

