Warm and cool temperatures decrease early-life telomere length in wild pied flycatchers

Furic C[1,2§], Marciau C[1,3], Hsu B-Y[1,4], Cossin-Sevrin N[1,5], Fleitz J[1], Reichert S[1,2], Ruuskanen S[1,6*] & Stier A[1,2§*]

[1]Department of Biology, University of Turku, Finland

[2]Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

[3]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Australia

[4]Department of Life Science, Tunghai University, Taichung, Taiwan

[5]Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, Finland

[6]Department of Biological and Environmental Science, University of Jyväskylä, Finland

§ corresponding author: cmfuri@utu.fi; antoine.stier@iphc.cnrs.fr

* co-last authors

Abstract

Climate change represents a major challenge for avian species. It is characterized by an increase in average ambient temperatures, but also in occurrence of extreme weather events, such as heat weaves and cold snaps. These abrupt temperature changes can modify the immediate and long-term survival prospects of nestling birds, when their thermoregulatory capacities are still not fully developed. While immediate nestling survival can easily be measured, long-term survival is more challenging to evaluate. Early-life telomere length has been suggested as a potential biomarker of future fitness prospects. To evaluate the potential impact of changes in early-life temperature, we thus experimentally increased (*ca.* +2.8°C) and decreased (*ca.* -1.7°C) average nestbox temperatures in wild pied flycatchers (*Ficedula hypoleuca*) during nestling postnatal growth, and measured nestling telomere length before fledging. Shorter telomeres were observed in individuals exposed both to an experimental heating or cooling during growth. Our results suggest that long-term survival prospects or long-term performances of individuals exposed to abrupt changes in early-life temperature may be decreased.

Keywords: endotherm, passerine, telomere, thermoregulation, cellular ageing

Introduction

Ongoing climate change involves not only rising global mean temperatures but also an increase in the occurrence of extreme weather events such as cold snaps and heat waves (IPCC 2014). These abrupt temperature changes can cause direct mortality (McKechnie & Wolf 2009, McKechnie *et al.* 2021) but also sub-lethal consequences that may impact fitness because of delayed or long-lasting effects (Conradie *et al.* 2019). These extreme weather events can represent a major challenge for avian species, especially during early development when altricial nestlings are transitioning from an ectothermic stage to a fully endothermic stage prior to fledging (Price & Dzialowski 2018). As individuals do not fully regulate body temperature during these early-life stages (Boyles *et al.* 2011), their homeothermy depends on ambient temperature fluctuations and parental brooding throughout this thermoregulatory transition (Katzenberger *et al.* 2015). This temperature-sensitivity exposes individuals to short-term mortality risks caused by temperature variation (Hepp & Kennamer 2012, Moreno *et al.* 2015, Andreasson *et al.* 2018, Ospina *et al.* 2018) but also potential long-lasting or delayed effects on their health or reproduction (Metcalfe & Monaghan 2001, Preston *et al.* 2018) that may affect fitness (Greño *et al.* 2008, Hepp & Kennamer 2012, Berntsen & Bech 2016, Nord & Nilsson 2016, Andreasson *et al.* 2018). Previous studies have demonstrated the effect of heatwaves (Greño *et al.* 2008, Nord & Nilsson 2016, Andreasson *et al.* 2018) or cold snaps (Moreno *et al.* 2015, Ospina *et al.* 2018) events during pre- or postnatal stages on growth and parental brooding behavior (Rodríguez & Barba 2016). However, the potential long-term survival effects of such abrupt changes in temperature can be more difficult to characterize, because long-term recaptures rates are often very low for passerine birds (Andreasson *et al.* 2018, Stier *et al.* 2025). Heat waves or cold snap may have direct causal effects, but also indirect effects since changes in temperature are known to influence numerous biotic factors (*e.g.* food availability, parasite abundance; Schöll *et al.* 2016, Mennerat *et al.* 2021). Thus, testing for a causal effect of temperature requires experimental approaches, such as nest heating (*e.g.* Rodríguez & Barba 2016a, Andreasson *et al.* 2018) or cooling (*e.g.* Rodríguez & Barba 2016b, Corregidor-Castro *et al.* 2023).

To elucidate these potential long-term or delayed effects of early-life thermal environment, telomere length may be used as a molecular biomarker for future fitness prospects (Eastwood et al. 2019). Located at chromosome ends, telomeres serve as a protective role by preserving genome integrity and ensuring proper cellular division (Lange *et al.* 2006). It has been shown that telomeres progressively shorten with age (Remot *et al.* 2022), and that telomere length predicts survival prospects in some species at least (Wilbourn *et al.* 2018). Their rate of shortening can be accentuated by environmental challenges, particularly those encountered during early-life development, when

72 telomere shortening is the most pronounced (Chatelain *et al.* 2019). For instance, telomere length
73 may be affected by early-life temperature conditions, with experimental elevation of egg incubation
74 temperatures leading to shorter telomeres (Vedder *et al.* 2018, Stier *et al.* 2020a, Hope *et al.* 2023).
75 Yet, the effect of heatwaves and cold snaps on telomere length during postnatal stages have been less
76 characterized. There is some evidence of adverse effects of natural or experimental hot conditions on
77 telomere length in the wild (from postnatal d7 to d14: Stier *et al.* 2025; from postnatal d0 to d7:
78 Eastwood *et al.* 2022), but no effect of experimental heatwaves on telomere length in captivity (from
79 hatching to postnatal d12: Ton *et al*. 2023). To the best of our knowledge, the effect of postnatal
80 coldsnap on telomere length has never been investigated, but two studies simulating parental neglects
81 (*i.e.* cooling down nestling for up to 1 hour per day for *ca*. the first week post-hatching) found no
82 significant effect on telomere length (Lynn *et al.* 2022, Ghimire *et al.* 2025). Accelerated telomere
83 attrition can be linked to fast growth and rapid cellular proliferation (Monaghan & Ozanne 2018),
84 oxidative damage (Reichert & Stier 2017; Armstrong & Boonekamp 2023), or disruptions in metabolic
85 balance (Casagrande & Hau 2019). All these mechanisms may underlie potential effects of early-life
86 temperature on telomere length.

87 In this study, we investigated the effects of experimental increase and decrease in nest
88 temperature during early postnatal development on telomere length in wild pied flycatchers (*Ficedula*
89 *hypoleuca*). As study years also differed in terms of ambient thermal conditions (see results), we also
90 used this opportunity to investigate the potential relationship between yearly ambient temperature
91 and telomere length. Age- and stress-related telomere shortening have been previously demonstrated
92 in this species and its close relative, the collared flycatcher (*Ficedula albicollis)* (Kärkkäinen *et al.* 2019,
93 2020, 2021, 2022, Stier *et al.* 2020b). Investigating experimentally the effects of heatwave and
94 coldsnap on postnatal telomere length in a free-living species should help to determine the potential
95 long-term or delayed effects of extreme weather events on fitness. We predict that being exposed to
96 early-life hot environmental conditions should lead to shorter telomeres, as found in Stier *et al*. (2025)
97 and Eastwood *et al.* (2022). However, experiencing a coldsnap may lead either to shorter telomeres,
98 if the coldsnap triggers an increase in metabolic rate or energetic imbalance (Casagrande & Hau 2019,
99 Casagrande *et al.* 2020), or to longer telomeres, if the coldsnap reduces the metabolic rate of partly
100 ectothermic nestlings (Friesen *et al.* 2022).

Material and methods

General field procedures:

Two nest temperature manipulations were conducted in a nestbox population of pied flycatchers located in the island of Ruissalo (Turku, Finland, location: 60°25'N, 22°10'E). In 2018, a heating experiment was performed, (data partly published in Hsu *et al.* (2020)), and in 2019 a cooling experiment was performed. Nestbox temperature was recorded every 3 minutes with an Ibutton thermochron (0.0625°C accuracy) installed *ca.* 5 cm above the rim of the nest. On the second day after hatching (d2), each individual was identified by clipping nails and half of the nestlings (minimum 2 individuals) with the same age and weight were swapped with another nest for other experimental purposes (*i.e.* cross-fostering to disentangle environmental and genetic effects).

Heating experiment (2018):

In 2018, nestbox temperature was increased for half of the boxes (hereafter, 'heated nests') between postnatal d2 and d8. Heating pads (UniHeat 72h, USA) were installed under the ceiling and replaced every 2nd day in the morning. The other half was considered as controls (hereafter, 'non-heated nests'), non-functional heating pads were used and nests were visited every 2nd day as well to standardize disturbance. At d8 after hatching, individuals were identified and ringed, and at postnatal d13 one blood sample of *ca.* 40µl was collected from the brachial vein using heparinized capillaries. A total of 97 nestlings from 41 nests were analyzed, specifically 42 individuals from 21 heated nests and 55 individuals from 20 non-heated ones. When possible, the nestlings selected for telomere analysis included (1) both a female and male nestling from each nest (2) one of genetic origin and one foster nestling, and otherwise were randomly selected. Since females can actively brood nestlings until postnatal d6 or d7 (Lundberg & Alatalo 1992), they could potentially change their behavior depending on the heating treatment, but no evidence was found in this experiment (previously published in Hsu *et al.* (2020)).

Cooling experiment (2019):

In 2019, nestbox temperature was decreased for half of the boxes (hereafter, 'cooled nests') between d7 and d13 after hatching of the nestlings. The timing of the experiment was chosen to avoid any potential confounding effects of brooding behavior by the female, and knowing that pied flycatchers are not yet fully capable of thermoregulation at d7 (Lundberg & Alatalo 1992). Two frozen (-20°C) cooling pads (VWR 26.7*14*3.2*cm, 909g) were attached on each outer side of the nestbox (protected on the outside with insulating material) and replaced every morning. The other half

134 (hereafter, 'non-cooled nests') was equipped with the same pads, but unfrozen, and visited every day
135 too. Before the treatment, at d7 after hatching, individuals were identified and ringed. Morphological
136 measurements (body mass and wing length) were conducted at postnatal d13, and one blood sample
137 of *ca.* 40µl was collected from the brachial vein using heparinized capillaries. A total of 56 nestlings
138 from 48 nests were analyzed, including 25 individuals from 23 cooled nests and 31 individuals from 25
139 non-cooled nests. The nestlings were selected for telomere analysis by trying to balance the number
140 of female and male nestlings, and having one of genetic origin and one foster nestling.

141 Relative telomere length analysis:

142 Blood samples were stored in a cooler (*ca.* 4°C) during the day of collection in the field, and
143 were transferred to a -80°C freezer at the end of the day. We measured relative telomere length using
144 a real-time quantitative PCR (qPCR) assay previously used and validated in pied flycatcher using the
145 TRF method (Kärkkäinen *et al.* 2019, 2021). DNA was extracted from whole blood samples using the
146 standard salt extraction alcohol precipitation method (Aljanabi & Martinez 1997). DNA concentration
147 and purity were assessed spectrophotometrically (ND-1000-Spectrophotometer, Thermo Fisher
148 Scientific, Waltham, MA, USA), and the DNA extract was considered good if purity (260/280 > 1.8 and
149 260/230 > 2.0) and quantity ([DNA] > 40 $ng.\mu L^{-1}$) criteria were met. DNA integrity was checked using
150 gel electrophoresis (50 ng of DNA, 0.8% agarose gel at 100 mV for 60 min, Midori Green staining
151 (NIPPON Genetics Europe, Düren, Germany). Each sample was then diluted to 1.2 $ng.\mu l^{-1}$, and the final
152 reaction contained 6 ng of DNA, 300nM of forward and reverse primers and SensiFAST SYBR Lo-ROX
153 master mix (Bioline, London, UK) for a total volume of 12 µl. Tel1b was used as a forward telomere
154 primer (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'), Tel2b as the reverse one (5'-
155 GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3') and RAG1 as a single copy gene (forward
156 primer 5'-GCAGATGAACTGGAGGCTATAA-3' and reverse primer 5'-CAGCTGAGAAACGTGTTGATTC-3')
157 following Kärkkäinen *et al.* (2021). The qPCR conditions started with an initial denaturation (3 min at
158 95°C), then 40 cycles with first step of 10s at 95°C, second step of 15s at 58°C and third step of 10s at
159 72°C, with melting curve analysis in the end performed by QuantStudio™ 12K Flex Real-Time PCR
160 System (Thermo Fisher Scientific, Waltham, MA, USA) using 384-well plates. Each plate contained
161 negative control and three internal standards. Samples from the different experimental treatments
162 were distributed evenly across all plates. To determine the baseline fluorescence, the quantification
163 cycle values and the qPCR efficiencies, we used LinRegPCR (Ruijter *et al.* 2009). Relative telomere
164 length was calculated based on plate-specific efficiencies (average ± SE and [range] for telomere and
165 RAG1 reactions were, respectively 95.1 ± 1.6% [90.1 – 103.3%] and 101.7 ± 1.8% [94.4 – 105.8%]) using
166 the mathematical model presented in Pfaffl (2001). Technical repeatability based on triplicate

167 measurements of telomere length was 0.74 (95% CI [0.69, 0.79], $p < 0.001$), and inter-plate
168 repeatability (calculated by using the average of triplicates) based on 37 samples measured on more
169 than one plate was 0.80 (95% CI [0.67, 0.93], $p < 0.001$). Samples have been analyzed over 16 qPCR
170 plates in total. Molecular sexing was conducted using qPCR (adapted from Ellegren & Fridolfsson 1997,
171 Chang *et al.* 2008; see details in Hsu *et al.* (2020)).

172 Statistical analysis:

173 Statistical analysis was carried out using R software version 4.3.2 (R Core Team 2014).
174 Normality and homogeneity of residual variances were checked using the R package DHARMa (Hartig
175 2022).

176 *Effect of the experimental manipulations and year on nestbox temperature:*

177 For each year, to evaluate nestbox temperature (*i.e.* hourly average) differences among
178 groups, we used linear mixed-effects models (LMMs - implemented in the lme4 package (Bates *et al.*
179 2015), Satterthwaite's degrees of freedom), with group (2018: heated vs. non-heated; 2019; cooled
180 vs. non-cooled) and time of day (*i.e.* treated as a categorical variable) treated as fixed effects. We
181 included random intercepts for date (to account for temporal autocorrelation) and rearing nestbox
182 (to control for repeated measurements).

183 To investigate natural variation in ambient and nestbox temperatures between 2018 and 2019
184 (for each year, between June 4th to July 12th), LMMs were used on hourly air temperature (provided
185 by the Finnish Meteorological Institute and collected by Artukainen weather station in Turku, 2 to 4
186 km from the study sites) and nestbox temperature (*i.e.* only non-heated and non-cooled), with year
187 and time of day included as fixed effects, and date modeled as a random effect to account for repeated
188 measurements within days. Model parameters were estimated using restricted maximum likelihood
189 (REML).

190 *Effect of the experimental manipulations of temperature on body mass:*

191 The effect of heating treatment on growth was analysed previously and no effect was
192 detected at any age for body mass (Hsu *et al.* 2020). We conducted similar analyses for the cooling
193 experiment, and no significant effects of the treatment were detected for body mass ($t = 0.39$, $P =$
194 0.70).

195 *Effect of the experimental manipulations of temperature on telomere length:*

A LMM on relative telomere length for each dataset was conducted separately to assess the effects of the treatment (heated vs. non-heated nests; cooled vs. non-cooled nests) and other traits of interest (*i.e* sex, cross fostering, hatching date, brood size and body mass). Nest of origin, nest of rearing and the qPCR plate identities were integrated in the model as random intercepts. No significant sex-dependent effects of temperature treatments on telomere length were found ($P > 0.12$), and thus interaction~~s~~ between sex and treatment were removed from final models to enable proper interpretation of the main effects.

Relationships between nestbox temperature and telomere length:

Although analyzing experimental data with the above-mentioned treatment *vs.* control comparisons is the most appropriate way to test for a causal effect of temperature on telomere length, we also ran correlative analyses on both experiments, replacing experimental treatment by the temperature measured inside each nestbox. We ran 3 models per experiment, one for mean temperature, one for minimum daily temperature (averaged over the 7 days of treatment), and one for maximum daily temperature (averaged over the 7 days of treatment). These models had the exact same structure as the ones described above, except for the replacement of treatment by nestbox temperature (mean, minimum or maximum).

Year effect on telomere length:

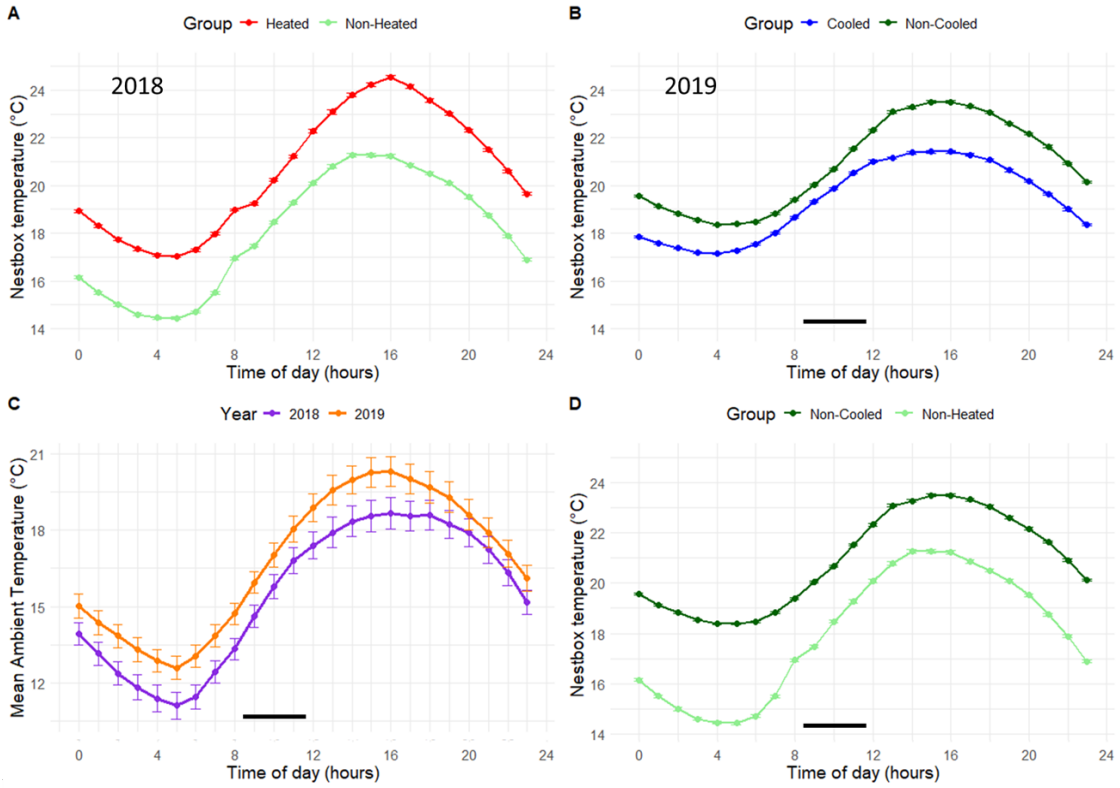
As experimental manipulations differed markedly between years (*e.g.* timing of temperature manipulation), we cannot directly compare the two experiments. Yet, environmental conditions (including ambient temperature, see results) differed between the two study years, and thus comparing telomere length of control individuals between years (*i.e.* non-heated from 2018 and non-cooled from 2019) may provide information regarding the response to longer-term natural variation in temperature. This was conducted using a linear mixed model relatively similar to the ones described above, with telomere length as a response variable, the different traits of interest (*i.e* year, sex, cross fostering, hatching date, brood size and body mass) as fixed effects and three random intercepts (*i.e* nest of origin, nest of rearing and qPCR plate identities). Only 2 adults bred both years, which is insufficient to include the identity of the parents as a random intercept.

Results

Nestbox temperature: experimental effects and natural variation between years

226 In 2018, the temperature in heated nest boxes was on average higher than in non-heated ones
227 (estimate: +2.83°C, 95% CI: [1.73 ; 3.53], t = 6.09, p < 0.001, Fig 1A). In 2019, the temperature in cooled
228 nestboxes was on average lower than in non-cooled nestboxes (estimate: -1.72°C, 95% CI: [-1.56 ; -
229 0.45], t = -3.52, p < 0.01, Fig 1 B). Yet, there were in both cases strong interactions between time of
230 day and experimental treatments (2018: $F_{23.1}$=31.2, p<0.001; 2019: $F_{23.1}$=103.7, p<0.001), with
231 treatment effect being more pronounced during mid-day (Fig. 1A and 1B). For instance, nestbox
232 temperature at 16:00 was *ca.* 3.0°C higher in heated nests (Fig. 1A) and *ca.* 2.0°C lower in cooled nests
233 (Fig. 1B).

234 During the experiments (June-July), ambient temperature was significantly higher in 2019
235 than 2018 (estimate: +1.54°C, 95% CI: [0.93 ; 2.15], t = 4.98, p < 0.001, Fig 1 C). This temperature
236 difference was also observed in nestbox temperatures between non-heated and non-cooled groups
237 with higher temperature in 2019 than 2018 (estimate: 2.16°C, 95% CI: [1.08 ; 3.24], t = 3.92, p < 0.01,
238 Fig 1 D).

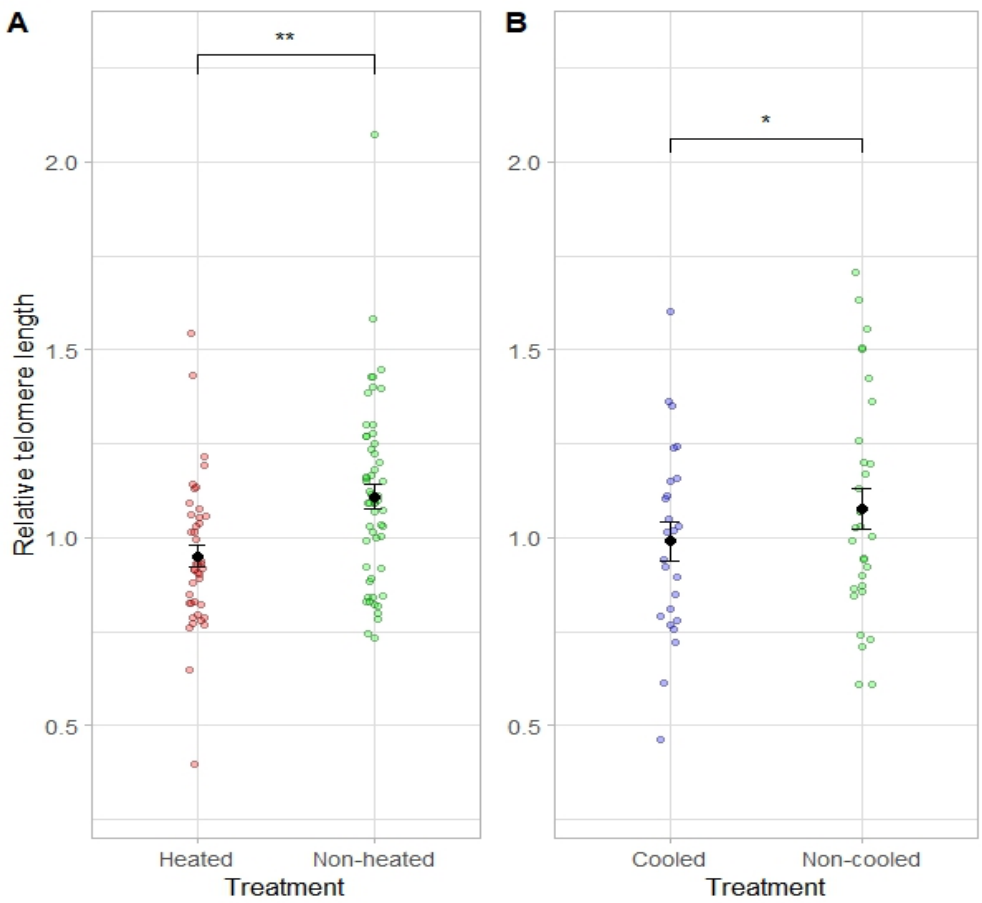


239

240 **Fig 1: Experimental and natural variation in nestbox and ambient temperatures: effect of**
241 **experimental heating (A) and cooling (B) on nestbox temperature; natural variation between years**
242 **in ambient (C) and nestbox (D) temperatures**. The black bar represents the time of cooling or heating
243 pads replacement. Hourly mean ± SE are reported for 41 nests in 2018 (21 heated vs; 20 non-heated)
244 and 48 nests in 2019 (23 cooled vs. 25 non-cooled).

245 *Effect of experimental manipulations and natural variation in nestbox temperature on telomere length*

246 Nestlings from heated nests had significantly shorter telomeres than non-heated ones (LMM:
247 $t_{26.39}$ = 2.85, P = 0.008; Fig.2A; Table 1A) and nestlings from cooled nests had shorter telomeres
248 compared to the ones from non-cooled nests (LMM: $t_{35.26}$ = 2.23, P = 0.032; Fig. 2B; Table 1B).
249 Replacing heating treatment by the actual nestbox temperature revealed no significant relationship
250 between telomere length and mean ($\beta \pm$ SE = -0.012 $\pm$ 0.015, P = 0.43, Table S1A) or minimum
251 temperature ($\beta \pm$ SE = -0.005 $\pm$ 0.015, P = 0.72, Table S2A), but there was a non-significant trend for
252 higher maximum temperature to be associated with shorter telomeres ($\beta \pm$ SE = -0.017 $\pm$ 0.009, P =
253 0.073, Table S3A). Replacing cooling treatment by the actual nestbox temperature revealed that
254 cooler mean temperature was associated with shorter telomeres ($\beta \pm$ SE = 0.068 $\pm$ 0.032, P = 0.043,
255 Table S1B), but no significant relationship was found with minimum ($\beta \pm$ SE = 0.046 $\pm$ 0.032, P = 0.17,
256 Table S2B) nor maximum temperature ($\beta \pm$ SE = 0.016 $\pm$ 0.017, P = 0.36, Table S3B).



257

258 **Fig. 2: Effect of experimental heating (A) and cooling (B) on relative telomere length in pied**
259 **flycatcher nestlings at day 13 after hatching.** Individual raw data points are presented along with
260 estimated marginal means ± SE from statistical models described in Table 1.

261 **Table 1: Summary of the linear mixed models testing the effects of experimental temperature**
262 **manipulation ((A) heating treatment in 2018, (B) cooling treatment in 2019) and relevant covariates**
263 **on relative telomere length of pied flycatcher nestlings at day 13 after hatching**. Non-significant
264 interactions between treatment and sex were removed from final models, but are presented in the
265 table for completeness. Singular fit partly occurred for the nest of rearing and the identity of qPCR
266 plate for the heating experiment, and nest of origin for the cooling experiment, but they were kept as
267 random intercepts as they were part of the experimental design.

(A) Experimental heating (2018, N = 97 nestlings)

Random effects:		**Variance**
Nest of origin (N=26)	Intercept	0.0028
Nest of rearing (N=41)	Intercept	0
Plate qPCR (N=6)	Intercept	0
Residual		0.0474

Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	1.120	0.346	3.24	63.65	0.002
Heating (Non-heated)	**0.141**	**0.049**	**2.85**	**26.39**	**0.008**
Sex (Male)	-0.054	0.046	-1.18	88.13	0.24

Cross-fostering (Yes)	0.054	0.050	1.08	59.94	0.29
Hatching date	0.002	0.004	0.62	46.89	0.54
Brood size	0.013	0.023	0.57	39.26	0.57
Body mass	-0.018	0.018	-0.99	66.03	0.33
Heating (Non-heated) × Sex (Male)	-0.145	0.093	-1.54	88.25	0.12

Sample size: N = 55 nestlings from non-heated nest, N = 42 nestlings from heated nest

(B) Experimental cooling (2019, N = 56 nestlings)

Random effects:		**Variance**
Nest of origin (N=29)	Intercept	0
Nest of rearing (N=48)	Intercept	0.005
Plate qPCR (N=10)	Intercept	0.019
Residual		0.051

Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**

Intercept	1.078	0.572	1.89	39.53	0.069
Cooling (Non-cooled)	**0.156**	**0.070**	**2.23**	**35.26**	**0.032**
Sex (Male)	0.020	0.072	0.27	44.48	0.79
Cross-fostering (Yes)	-0.019	0.070	-0.27	46.68	0.79
Hatching date	0.013	0.008	1.61	14.77	0.129
Brood size	0.033	0.030	1.11	30.21	0.28
Body mass	-0.035	0.031	-1.11	42.97	0.27
Cooling (Non-cooled) × Sex (Male)	-0.034	0.148	-0.23	44.98	0.82

Sample size: N = 31 nestlings from non-cooled nest, N = 25 nestlings from cooled nest

Natural variation in telomere length and body mass between years

We observed no significant association between year and telomere length for control individuals (LMM: $t_{5.00}$ = -0.31, P = 0.77; Fig. 3, Table 2), as well as no significant year effect on nestling body mass (LMM: t = 0.30, P = 0.76).

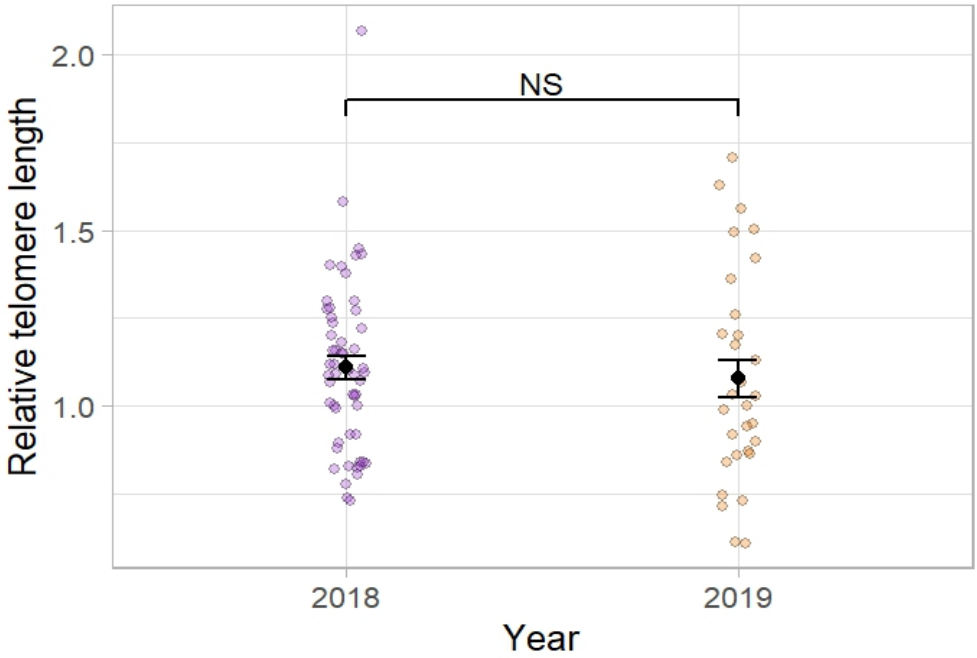


Fig. 3: Effect of year on relative telomere length in pied flycatcher nestlings at day 13 after hatching from control nests. Individual raw data points are presented along with estimated marginal means ± SE from statistical models described in Table 2.

Table 2: Summary of the linear mixed model testing the effects of the year (2018 vs. 2019) and relevant covariates on relative telomere length of pied flycatcher nestlings at day 13 after hatching from control groups (non-heated from 2018 and non-cooled from 2019). Non-significant interaction between year and sex was removed from the final model, but is presented in the table for completeness. Boundary singular fit occurred for the nest of rearing and nest of origin, but they were kept as a random intercept since it was part of the experimental design.

Control groups treatment (2018 and 2019, N = 86 nestlings)		
Random effects:		**Variance**
Nest of origin (N=35)	Intercept	0

Nest of rearing (N=40)	Intercept	0
Plate qPCR (N=16)	Intercept	0.008
Residual		0.061

Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	1.413	0.339	4.17	77.19	< 0.001
Year (2019)	-0.023	0.075	-0.31	5.00	0.77
Sex (Male)	-0.050	0.058	-0.86	80.00	0.39
Cross-fostering (Yes)	-0.061	0.059	-1.04	79.23	0.30
Brood size	0.034	0.029	1.19	61.19	0.24
Body mass	-0.031	0.019	-1.62	79.90	0.11
Year (2019) × Sex (Male)	0.193	0.124	1.56	77.51	0.12

Sample size: N = 55 nestlings from 2018, N = 31 nestlings from 2019

284

285 **Discussion**

286 In this study, we demonstrated that exposure to experimental changes in environmental
287 temperature (warmer or cooler) during early-life can significantly shorten telomeres in pied flycatcher
288 nestlings. This suggests that the relationship between ambient temperature and telomere length in
289 correlative analyses could be expected to be non-linear, following an inverted U-shape, as already
290 suggested in ectotherms (Axelsson *et al.* 2020, Friesen et *al.* 2022) and to a lesser extent in birds
291 (Pepke *et al.* 2022). Since avian altricial nestlings transition from ectothermy to endothermy during
292 development (Price & Dzialowski 2018), these results echo the effects of experimental increase of
293 temperature on telomere lengths in ectotherms, such as fishes (Siberian sturgeon (Simide *et al.* 2016),
294 Atlantic Salmon at the embryo stage (McLennan *et al.* 2018)) or squamates (Desert Agama (Zhang *et*
295 *al.* 2018)). To the best of our knowledge, only one cooling experiment has been conducted in
296 ectotherms, showing no significant effect of a transition from 30°C to 20°C (Eastern mosquitofish
297 (Rollings *et al.* 2014). Yet, it has been recently shown in amphibians that cooler temperature, for a
298 similar growth rate, is associated with shorter telomeres (Burraco *et al.* 2023). So, our observations of
299 shorter telomeres in response to experimental heating and cooling seem consistent with an
300 ectothermic-like phenotype. Indeed, high temperature has also been shown to accelerate telomere
301 shortening in ectothermic or partly ectothermic avian embryo and nestlings (Stier *et al.* 2020a, Stier
302 *et al.* 2025, Eastwood *et al.* 2022), but also in endotherms such as humans (Ni *et al.* 2022) and domestic
303 chicken (Musa *et al.* 2025). Future studies investigating temperature effects in fully endothermic avian
304 models (*e.g.* after fledging) are needed to establish the potential role of (partial) ectothermy on the
305 effects of temperature on telomere shortening.

306 In contrast with the results stemming from our experimental manipulation, we observed that
307 while the mean ambient temperature of 2019 was 1.5°C higher than in 2018 (and nestbox
308 temperature 2.2°C higher), there was no significant year effect on telomere length when comparing
309 the data from control broods. This suggests that long-term variations in temperature may have less
310 effect on telomere length than short-term changes mimicked by our experimental conditions,
311 although this remains to be experimentally demonstrated. Indeed, an alternative explanation could
312 be that the higher temperature in 2019 contributed to accelerate telomere shortening, but that higher
313 food availability, usually resulting from warmer environmental temperatures (Avery & Krebs 1984,
314 Veistola *et al.* 1997), compensated for any temperature-related shortening. It has for instance been
315 shown that better nutritional conditions can limit telomere loss in great tit nestlings (Casagrande *et*
316 *al.* 2023).

The temperature-related telomere shortening we observed in this study in response to experimentally increased or decreased temperature may be explained by several potential mechanisms. First, telomere shortening has been shown to be accentuated by fast growth and rapid cellular proliferation (Monaghan & Ozanne 2018). This seems unlikely to explain the effects we observed on telomeres since experimental heating (Hsu *et al.* 2020) or cooling (present study) had no detectable effects on body mass. Second, telomere shortening can be accelerated by increased oxidative stress levels (Reichert & Stier 2017), and acute heat or cold exposure have been shown to potentially increase oxidative stress levels (Costantini *et al.* 2012, Stier *et al.* 2014). Yet, this seems unlikely to explain the effects we observed on telomeres since experimental heating had no detectable effects on oxidative stress levels (Hsu *et al.* 2020). Third, telomere shortening can be accelerated by energetic imbalance, because of potential trade-offs between immediate and long-term survival during challenging environmental conditions (Casagrande & Hau 2019). This has been suggested to be mediated by glucocorticoid hormone (*i.e.* "stress hormone") effects on cellular signaling via the mTOR pathway (Casagrande & Hau 2019). Since abrupt changes in temperature can increase glucocorticoid levels in altricial nestlings (Jessop *et al.* 2016, De Bruijn & Romero 2018), it is possible that the telomere shortening could be explained by this mechanism, as already suggested in an experimental heating study in great tit (Stier *et al.* 2025). Changes in ambient temperature can potentially influence body temperature, as for instance found in an experimental heating study in blue tits (Andreasson *et al.* 2018), with such effect being of higher magnitude at earlier (*i.e.* more ectothermic) postnatal stages. Changes in body temperature could be linked to energetic imbalance and glucocorticoid secretion in explaining temperature-dependent telomere shortening. Hypothetically, changes in body temperature could also be linked to telomere attrition via effects in telomerase (enzyme responsible for telomere elongation), since telomerase activity has been suggested to be temperature-sensitive (Friesen *et al.* 2022). Measuring glucocorticoid levels, mTOR signaling, body temperature and telomerase activity would be required to test these hypotheses.

To conclude, we have demonstrated that experimental increase or decrease in early-life temperature can shorten telomeres, which may impact long-term survival and fitness. Yet, directly measuring effects of experimental temperature manipulations on fitness would be needed to confirm this hypothesis, which is challenging in small passerine species having very low survival rates after fledging. Consequently, investigating those effects in long-lived species with high recruitment rate and known relationship between telomere length and fitness related traits (*e.g.* Alpine swift, Bize *et al.* 2009) would seem promising to better understand the effects of heatwaves and cold-snaps during early-life on telomeres and fitness.

Author contribution:

SRu, AS, CM and BYH designed the studies and conducted fieldwork. CM, AS and NCS conducted laboratory work. CF, JF and AS analyzed the data. CF, JF & AS wrote the manuscript, with input from SRu, SRe, NCS & CM. SRu and SRe acquired funding. SRu, SRe and AS supervised early-career researchers involved in this study.

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Data accessibility

All data are accessible at https://figshare.com/s/eb091d4259185e8646a6 (doi: 10.6084/m9.figshare.28844615)

Ethical permits

All procedures were approved by the Animal Experiment Committee of the State Provincial Office of Southern Finland (license numbers ESAVI/2902/2018 & ESAVI/5718/2019) and by the Environmental Center of Southwestern Finland (license numbers VARELY/549/2018 & VARELY/924/2019) granted to SRu.

Conflict of interest

The authors declare no competing or financial interests.

Significance statement:

Rapid environmental change is exposing wildlife to increasingly frequent and intense weather extremes, including heatwaves and cold snaps, which are particularly likely to impact sensitive early-life stages. Our results in pied flycatcher nestlings demonstrate that both experimentally elevated and reduced nest temperature during early life significantly shorten telomeres (i.e. a biomarker of long-

term survival and fitness prospects). This study offers novel and ecologically meaningful insights into how environmental stress linked to climate change may shape avian life-history trajectories.

References

Aljanabi, S. M. & Martinez, I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25: 4692–4693. doi:10.1093/nar/25.22.4692

Andreasson, F., Nord, A., Nilsson, J. A. 2018. Experimentally increased nest temperature affects body temperature, growth and apparent survival in blue tit nestlings. Journal of Avian Biology. 1–14. doi:10.1111/jav

Armstrong, E. & Boonekamp, J. 2023. Does oxidative stress shorten telomeres in vivo? A meta-analysis. Ageing Res Rev. 85:101854. doi: 10.1016/j.arr.2023.101854.

Avery, M. & Krebs, J. R. 1984. Temperature and foraging success of great tits Parus major hunting for spiders. Ibis. 126(1):33–38. https://doi.org/10.1111/j.1474-919X.1984.tb03661.x

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. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Berntsen, H. H., & Bech, C. 2016. Incubation temperature influences survival in a small passerine bird. J Avian Biol. 47: 141-145. https://doi.org/10.1111/jav.00688

Bize, P., Criscuolo, F., Metcalfe, N., 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. https://doi.org/10.1098/rspb.2008.1817

Boyles, J. G., Seebacher, F., Smit, B., McKechnie, A. E. 2011. Adaptive Thermoregulation in Endotherms May Alter Responses to Climate Change. Integrative and Comparative Biology. 51: 676–690. doi:10.1093/icb/icr053

Burraco, P., Hernandez-Gonzalez, M., Metcalfe, N. B., Monaghan, P. 2023. Ageing across the great divide: tissue transformation, organismal growth and temperature shape telomere dynamics through the metamorphic transition. Proc Royal Soc B 290, 20222448. doi: 10.1098/rspb.2022.2448.

Casagrande, S. & Hau, M. 2019. Telomere attrition: metabolic regulation and signalling function? Biology Letters. 15: 20180885–11. doi:10.1098/rsbl.2018.0885

414 Casagrande, S., Stier, A., Monaghan, P., Loveland, J. L., Boner, W., Lupi, S., Trevisi, R., Hau, M. 2020.
415 Increased glucocorticoid concentrations in early life cause mitochondrial inefficiency and short
416 telomeres. Journal Of Experimental Biology. 223, jeb222513-14. doi: 10.1242/jeb.222513.

417 Casagrande, S., Loveland, J. L., Oefele, M., Boner, W., Lupi, S., Stier, A., Hau, M. 2023. Dietary
418 nucleotides can prevent glucocorticoid-induced telomere attrition in a fast-growing wild vertebrate.
419 Mol. Ecol. 32, 5429–5447. https://doi.org/10.1111/mec.17114

420 Chang, H. W., Gu, D. L., Su, S. H., Chang, C. C., Cheng, C. A., Huang, H. W., Yao, C.T., Chou, T. C., Chuang,
421 L. Y., Cheng, C. C. 2008. High-throughput gender identification of Accipitridae eagles with real-time
422 PCR using TaqMan probes. Theriogenology. 70(1):83-90. doi: 10.1016/j.theriogenology.2008.02.011

423 Chatelain, M., Drobniak, S. M., Szulkin, M. 2019. The association between stressors and telomeres in
424 non-human vertebrates: a meta-analysis. Ecology Letters. 99, 21–18.
425 https://doi.org/10.1111/ele.13426

426 Conradie, S. R., Woodborne, S. M., Cunningham, S. J., McKechnie, A. E. 2019. Chronic, sublethal effects
427 of high temperatures will cause severe declines in southern African arid-zone birds during the 21st
428 century. Proceedings Of The National Academy Of Sciences Of The United States Of America. 116:
429 14065–14070. doi:10.1073/pnas.1821312116

430 Corregidor-Castro, A., Morinay, J., McKinlay, S. E., Ramellini, S., Assandri, G., Bazzi, G., Glavaschi, A.,
431 Capua, E. L. D., Grapputo, A., Romano, A., *et al.* 2023. Experimental nest cooling reveals dramatic
432 effects of heatwaves on reproduction in a Mediterranean bird of prey. Glob. Chang. Biol. 29(19),
433 5552–5567. doi: 10.1111/gcb.16888

434 Costantini, D., Monaghan, P., Metcalfe, N. B. 2012. Early life experience primes resistance to oxidative
435 stress. Journal Of Experimental Biology. 215, 2820–2826. doi: 10.1242/jeb.072231.

436 De Bruijn, R. & Romero, L. M. 2018. The role of glucocorticoids in the vertebrate response to weather.
437 Gen Comp Endocrinol. 269:11-32. doi: 10.1016/j.ygcen.2018.07.007.

438 Eastwood, J. R., Hall, M. L., Teunissen, N., Kingma, S. A., Aranzamendi, N. H., Fan, M., Roast, M.,
439 Verhulst, S. and Peters, A. 2019. Early-life telomere length predicts lifespan and lifetime reproductive
440 success in a wild bird. Molecular Ecology 28:1127–1137.
441

442 Eastwood, J. R., Connallon, T., Delhey, K., Hall, M. L., Teunissen, N., Kingma, S. A., La Porte, A. M.,
443 Verhulst, S., Peters, A. 2022. Hot and dry conditions predict shorter nestling telomeres in an
444 endangered songbird: Implications for population persistence, Proc. Natl. Acad. Sci. U.S.A. 119 (25)
445 e2122944119, https://doi.org/10.1073/pnas.2122944119
446

447 Ellegren, H. & Fridolfsson, A. K. 1997. Male–driven evolution of DNA sequences in birds. Nat Genet.
448 17, 182–184. https://doi.org/10.1038/ng1097-182
449

450 Friesen, C. R., Wapstra, E., Olsson, M. 2022. Of telomeres and temperature: Measuring thermal effects
451 on telomeres in ectothermic animals. Mol. Ecol. 31, 6069–6086. doi: 10.1111/mec.16154.

Ghimire, A., Young, R. C., Westneat, D. F., Heidinger, B. J. 2025. Repeated Experimental Cold Exposure During Early Life Affects Several Metrics of Success but not Telomeres in a Common Songbird. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. https://doi.org/10.1002/jez.2927

Greño, J. L., Belda, E. J., Barba, E. 2008. Influence of temperatures during the nestling period on post-fledging survival of great tit Parus major in a Mediterranean habitat. J Avian Biol. 39: 41–49. doi:10.1111/j.0908-8857.2008.04120.x

Hartig, F. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. R package version 0.4.6, https://CRAN.R-project.org/package=DHARMa

Hepp, G. R. & Kennamer, R. A. 2012. Warm is better: incubation temperature influences apparent survival and recruitment of wood ducks (Aix sponsa). PLoS ONE. 7: e47777. doi:10.1371/journal.pone.0047777

Hope, S. F., Angelier, F., Ribout, C., Groffen, J., Kennamer, R. A., Hopkins, W. A. 2023. Warmer incubation temperatures and later lay-orders lead to shorter telomere lengths in wood duck (Aix sponsa) ducklings. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. 339(1), 101–111. https://doi. org/10.1002/jez.2659

Hsu, B. Y., Sarraude, T., Cossin-Sevrin, N., Crombecque, M., Stier, A., Ruuskanen, S. 2020. Testing for context-dependent effects of prenatal thyroid hormones on offspring survival and physiology: an experimental temperature manipulation. Sci Rep. 10, 14563. https://doi.org/10.1038/s41598-020-71511-y

Jessop, T. S., Lane, M. L., Teasdale, L., Stuart-Fox, D., Wilson, R. S., Careau, V., Moore, I. T. 2016. Multiscale Evaluation of Thermal Dependence in the Glucocorticoid Response of Vertebrates. The American Naturalist. 188(3), 342–356. https://www.jstor.org/stable/26519175

Kärkkäinen, T., Teerikorpi, P., Panda, B., Helle, S., Stier, A., Laaksonen, T. 2019. Impact of continuous predator threat on telomere dynamics in parent and nestling pied flycatchers. Oecologia. 191, 757-766. doi:10.1007/s00442-019- 04529-3

Kärkkäinen, T., Bize, P., Stier, A. 2020. Correlation in telomere lengths between feathers and blood cells in pied flycatchers. Journal of Avian Biology. 51(4), Article e02300. https://doi.org/10.1111/jav.02300

Kärkkäinen, T., Teerikorpi, P., Schuett, W., Stier, A., Laaksonen, T. 2021. Interplays between pre- and post-natal environments affect early-life mortality, body mass and telomere dynamics in the wild. J Exp Biol. 224(Pt 1):jeb231290. doi: 10.1242/jeb.231290.

Kärkkäinen, T., Laaksonen, T., Burgess, M., Cantarero, A., Martínez-Padilla, J., Potti, J., Moreno, J., Thomson, R. L., Tilgar, V., Stier, A. 2022. Population differences in the length and early-life dynamics of telomeres among European pied flycatchers. Mol Ecol. 31(23):5966-5978. doi: 10.1111/mec.16312.

Katzenberger, J., Tate, G., Koeslag, A., Amar, A. 2015. Black Sparrowhawk brooding behaviour in relation to chick age and weather variation in the recently colonised cape peninsula, South Africa. Journal of Ornithology. 156(4), 903–913. https://doi.org/10.1007/s1033 6-015-1199-0

Lange, T. D., Lundblad, V., Blackburn, E. H. 2006. Telomeres. Cold Spring Harbor Laboratory Press, New York 576.

Lundberg, A. & Alatalo, R. 1992. The Pied Flycatcher (Poyser, London)

Lynn S. E., Kern M. D., Serrurier B., Sirman A., Heidinger B. J. 2022. Chill out: Environmentally relevant cooling challenge does not increase telomere loss during early life. Gen Comp Endocrinol. 329:114108. doi: 10.1016/j.ygcen.2022.114108.

McLennan, D., Armstrong, J. D., Stewart, D. C., Mckelvey, S., Boner, W., Monaghan, P., Metcalfe, N. B. 2018. Telomere elongation during early development is independent of environmental temperatures in Atlantic salmon. J Exp Biol. 221(Pt 11):jeb178616. doi: 10.1242/jeb.178616.

McKechnie, A. E. & Wolf, B. O. 2009. Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. Biology Letters. 6(2), 253–256. https://doi.org/10.1098/rsbl.2009.0702

McKechnie, A. E., Rushworth, I. A., Myburgh, F., Cunningham, S. J. 2021. Mortality among birds and bats during an extreme heat event in eastern South Africa. Austral Ecology. doi:10.1111/aec.13025

Mennerat, A., Charmantier, A., Perret, P., Hurtrez-Boussès, S., Lambrechts, M. M. 2021. Parasite intensity is driven by temperature in a wild bird. Peer Community J. 1: e60. doi:10.24072/pcjournal.65

Metcalfe, N. & Monaghan, P. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution. 16(5):254-260. doi: 10.1016/s0169-5347(01)02124-3.

Monaghan, P. & Ozanne, S. E. 2018. Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. Philos Trans R Soc Lond B Biol Sci. 373(1741):20160446. doi: 10.1098/rstb.2016.0446.

Moreno, J., González-Braojos, S., Ruiz-de-Castañeda, R. 2015. A spring cold snap is followed by an extreme reproductive failure event in a mountain population of Pied Flycatchers Ficedula hypoleuca. Bird Study. 62(4), 466–473. https://doi.org/10.1080/00063657.2015.1073680

Musa, A. A., Mamat-Hamidi, K., Idrus, Z., Chung, E. L. T., Samat, N., Kassim, N. A. 2025. Effects of heat stress on growth metrics, carcass characteristics, telomere length, and gene expression in chickens. Poult. Sci. 104, 105698.

Ni, W., Wolf, K., Breitner, S., Zhang, S., Nikolaou, N., Ward-Caviness, C. K., Waldenberger, M., Gieger, C., Peters, A., Schneider, A. 2022. Higher Daily Air Temperature Is Associated with Shorter Leukocyte Telomere Length: KORA F3 and KORA F4. Environ. Sci. Technol. 56, 17815–17824. doi: 10.1021/acs.est.2c04486.

Nord, A. & Nilsson, J. A. 2016. Long-term consequences of high incubation temperature in a wild bird population. Biology Letters. 1–4. doi:10.1098/rsbl.2016.0087&domain=pdf&date_stamp=2016-04-05

Ospina, E. A., Merrill, L., Benson, T. J. 2018. Incubation temperature impacts nestling growth and survival in an open-cup nesting passerine. Ecol Evol. 8(6):3270-3279. doi: 10.1002/ece3.3911.

Pepke, M. L., Kvalnes, T., Ranke, P. S., Araya-Ajoy, Y. G., Wright, J., Sæther, B., Jensen, H., Ringsby, T. H. 2022. Causes and consequences of variation in early-life telomere length in a bird metapopulation. Ecol. Evol. 12, e9144.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT–PCR. Nucl. Acids Res. 29(9):e45. doi: 10.1093/nar/29.9.e45.

Preston, J. D., Reynolds, L. J., Pearson, K. J. 2018. Developmental Origins of Health Span and Life Span: A Mini-Review. Gerontology. 64: 237–245. doi:10.1159/000485506

Price, E. R. & Dzialowski, E. M. 2018. Development of endothermy in birds: Patterns and mechanisms. Journal of Comparative Physiology B. 188(3), 373–391. https://doi.org/10.1007/s0036 0-017-1135-0

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Reichert, S. & Stier, A. 2017. Does oxidative stress shorten telomeres in vivo? A review. Biol Lett. 13(12):20170463. doi: 10.1098/rsbl.2017.0463.

Remot, F., Ronget, V., Froy, H., Rey, B., Gaillard, J.-M., Nussey, D. H., Lemaitre, J.-F. 2022. Decline in telomere length with increasing age across nonhuman vertebrates: A meta-analysis. Molecular Ecology. 31(23), 5917–5932. https://doi.org/10.1111/mec.16145

Rodríguez, S. & Barba, E. 2016a. Nestling Growth is Impaired by Heat Stress: an Experimental Study in a Mediterranean Great Tit Population. Zoological studies. 55: e40. doi:10.6620/zs.2016.55-40

Rodríguez, S. & Barba, E. 2016b. Effects of Cool Nest Microclimates on Nestling Development: An Experimental Study with Mediterranean Great Tits Parus major. Ardeola. 63, 251–260. doi: 10.13157/arla.63.2.2016.ra2

Rollings, N., Miller, E., Olsson, M. 2014. Telomeric attrition with age and temperature in Eastern mosquitofish (Gambusia holbrooki). Naturwissenschaften. 101(3):241-4. doi: 10.1007/s00114-014-1142-x.

Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bak-ker, O., van den Hoff, M. J., Moorman, A. F. M. 2009. Amplification efficiency: linking baseline and bias in the analy-sis of quantitative PCR data. Nucl. Acids Res. 37: e45–e45. doi: 10.1093/nar/gkp045.

Schöll, E. M., Ohm, J., Hoffmann, K. F. and Hille, S. M. 2016. Caterpillar biomass depends on temperature and precipitation, but does not affect bird reproduction. Acta Oecologica. 74, 28–36. doi: 10.1016/j.actao.2016.06.004

558 Simide, R., Angelier, F., Gaillard, S., Stier, A. 2016. Age and Heat Stress as Determinants of Telomere
559 Length in a Long-Lived Fish, the Siberian Sturgeon. Physiol Biochem Zool. 89(5):441-7. doi:
560 10.1086/687378.

561 Stier, A., Massemin, S., Criscuolo, F. 2014. Chronic mitochondrial uncoupling treatment prevents acute
562 cold-induced oxidative stress in birds. Journal Of Comparative Physiology B-Biochemical Systemic And
563 Environmental Physiology. 184, 1021–1029. doi: 10.1007/s00360-014-0856-6.

564 Stier, A., Metcalfe, N. B., Monaghan, P. 2020a. Pace and stability of embryonic development affect
565 telomere dynamics: An experimental study in a precocial bird model. Proceedings of the Royal Society
566 B: Biological Sciences. 287(1933), 20201378. https://doi.org/10.1098/rspb.2020.1378

567 Stier, A., Hsu, B.-Y., Marciau, C., Doligez, B., Gustafsson, L., Bize, P., Ruuskanen, S. 2020b. Born to be
568 young? Prenatal thyroid hormones increase early-life telomere length in wild collared flycatchers.
569 Biology Letters. 16, 20200364–4. doi: 10.1098/rsbl.2020.0364.

570 Stier, A., Hsu, B.-Y., Cossin-Sevrin, N., Garcin, N., Ruuskanen, S. 2021. From climate warming to
571 accelerated cellular ageing: An experimental study in wild birds. bioRxiv.
572 https://doi.org/10.1101/2021.12.21.473625

573 Ton, R., Boner, W., Raveh, S., Monaghan, P., Griffith, S. C. 2023. Effects of heat waves on telomere
574 dynamics and parental brooding effort in nestlings of the zebra finch (Taeniopygia castanotis)
575 transitioning from ectothermy to endothermy. Mol Ecol. 32(17):4911-4920. doi: 10.1111/mec.17064.

576 Vedder, O., Verhulst, S., Zuidersma, E., Bouwhuis, S. 2018. Embryonic growth rate affects telomere
577 attrition: an experiment in a wild bird. J Exp Biol. 221(Pt 15):jeb181586. doi: 10.1242/jeb.181586.

578 Veistola, S., Lehikoinen, E., Eeva, T. 1997. Weather and breeding success at high latitudes - The Pied
579 Flycatcher Ficedula hypoleuca and the Siberian Tit Parus cinctus. Ornis Fennica. 74. 89-98.

580 Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., Boonekamp, J.J. 2018. The
581 relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-
582 analysis. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 373:
583 20160447–9. doi:10.1098/rstb.2016.0447

584 Zhang, Q., Han, X., Hao, X., Ma, L., Li, S., Wang, Y., Du, W. 2018. A simulated heat wave shortens the
585 telomere length and lifespan of a desert lizard. J Therm Biol. 72:94-100. doi:
586 10.1016/j.jtherbio.2018.01.004.

Supplementary Table S1: Summary of the linear mixed models testing the relationship between telomere length of nestlings at day 13 after hatching and mean nestbox temperature ((A) 2018 heating experiment, (B) 2019 cooling experiment) while controlling for other variables.

(A) 2018 experimental heating: Day-13 Telomere length (N = 97 nestlings)					
Random effects:		**Variance**			
Nest of origin (N=26)	Intercept	0			
Nest of rearing (N=41)	Intercept	0.009			
Plate qPCR (N=6)	Intercept	0.001			
Residual		0.045			
Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	1.395	0.451	3.09	58.33	0.003
Mean temperature	-0.012	0.015	-0.80	31.32	0.43
Sex (Male)	-0.056	0.048	-1.18	89.45	0.24
Cross-fostering (Yes)	0.055	0.054	1.02	63.59	0.31
Hatching date	0.003	0.004	0.67	48.81	0.50
Brood size	0.021	0.026	0.81	46.44	0.42
Body mass	-0.018	0.019	-0.93	78.26	0.36
Sample size: N = 55 nestlings from non-heated nest, N = 42 nestlings from heated nest					
(B) 2019 experimental cooling: Day-13 Telomere length (N = 54 nestlings)					
Random effects:		**Variance**			
Nest of origin (N=2**8**)	Intercept	0			
Nest of rearing (N=4**6**)	Intercept	0.0002			
Plate qPCR (N=10)	Intercept	0.023			
Residual		0.053			
Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	-0.189	0.875	-0.22	29.77	0.83
Mean temperature	**0.068**	**0.032**	**2.12**	**29.29**	**0.043**
Sex (Male)	0.015	0.070	0.22	41.03	0.83
Cross-fostering (Yes)	-0.014	0.071	-0.19	45.49	0.84
Hatching date	**0.027**	**0.010**	**2.69**	**16.76**	**0.016**
Brood size	-0.001	0.032	-0.04	24.80	0.97

Body mass	-0.040	0.031	-1.30	40.86	0.20

Sample size: N = 30 nestlings from non-cooled nest, N = 24 nestlings from cooled nest

Supplementary Table S2: Summary of the linear mixed models testing the relationship between telomere length of nestlings at day 13 after hatching and minimum nestbox temperature ((A) 2018 heating experiment, (B) 2019 cooling experiment) while controlling for other variables.

(A) 2018 experimental heating: Day-13 Telomere length (N = 97 nestlings)

Random effects:		**Variance**			
Nest of origin (N=26)	Intercept	0			
Nest of rearing (N=41)	Intercept	0.011			
Plate qPCR (N=6)	Intercept	0			
Residual		0.045			
Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	1.139	0.385	2.96	67.69	0.004
Minimum temperature	0.005	0.015	0.36	43.10	0.72
Sex (Male)	-0.055	0.048	-1.16	89.96	0.25
Cross-fostering (Yes)	0.053	0.054	0.98	68.40	0.33
Hatching date	0.001	0.005	0.23	58.61	0.82
Brood size	0.022	0.026	0.84	49.40	0.41
Body mass	-0.019	0.019	-1.00	80.70	0.32

Sample size: N = 55 nestlings from non-heated nest, N = 42 nestlings from heated nest

(B) 2019 experimental cooling: Day-13 Telomere length (N = 54 nestlings)

Random effects:		**Variance**			
Nest of origin (N=2**8**)	Intercept	0			
Nest of rearing (N=4**6**)	Intercept	0.073			
Plate qPCR (N=10)	Intercept	0.129			
Residual		0.052			
Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	0.083	0.660	1.26	30.19	0.22
Minimum temperature	0.046	0.032	1.41	32.75	0.17

Sex (Male)	0.016	0.073	0.23	42.02	0.82
Cross-fostering (Yes)	-0.031	0.072	-0.43	45.47	0.66
Hatching date	**0.019**	**0.009**	**2.17**	**14.82**	**0.047**
Brood size	0.002	0.035	0.07	33.37	0.95
Body mass	-0.058	0.033	-1.74	42.54	0.090

Sample size: N = 30 nestlings from non-cooled nest, N = 24 nestlings from cooled nest

Supplementary Table S3: Summary of the linear mixed models testing the relationship between telomere length of nestlings at day 13 after hatching and maximum nestbox temperature ((A) 2018 heating experiment, (B) 2019 cooling experiment) while controlling for other variables.

(A) 2018 heating experiment: Day-13 Telomere length (N = 97 nestlings)

Random effects:		**Variance**			
Nest of origin (N=26)	Intercept	0			
Nest of rearing (N=41)	Intercept	0.001			
Plate qPCR (N=6)	Intercept	0.001			
Residual		0.046			
Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	1.698	0.462	3.66	58.70	0.001
Maximum temperature	**-0.017**	**0.009**	**-1.84**	**37.01**	**0.073**
Sex (Male)	-0.059	0.048	-1.25	89.41	0.21
Cross-fostering (Yes)	0.042	0.052	0.80	64.28	0.43
Hatching date	0.003	0.004	0.63	45.33	0.53
Brood size	0.020	0.025	0.82	43.60	0.41
Body mass	-0.020	0.019	-1.09	74.13	0.29

Sample size: N = 55 nestlings from non-heated nest, N = 42 nestlings from heated nest

(B) 2019 cooling experiment: Day-13 Telomere length (N = 54 nestlings)

Random effects:		**Variance**
Nest of origin (N=2**8**)	Intercept	0
Nest of rearing (N=4**6**)	Intercept	0.103
Plate qPCR (N=10)	Intercept	0.118

Residual		0.223			
Fixed effects:	**Estimate**	**Std. Error**	**t**	**df**	**P (>|t|)**
Intercept	0.692	0.902	0.77	43.25	0.45
Maximum temperature	0.016	0.017	0.92	36.87	0.36
Sex (Male)	0.013	0.074	0.18	43.06	0.86
Cross-fostering (Yes)	-0.010	0.073	-0.13	45.62	0.90
Hatching date	**0.019**	**0.010**	**1.95**	**18.46**	**0.067**
Brood size	0.030	0.031	0.97	31.57	0.34
Body mass	-0.038	0.033	-1.15	42.77	0.26

Sample size: N = 3**0** nestlings from non-cooled nest, N = 2**4** nestlings from cooled nest