



HAL
open science

From climate warming to accelerated cellular ageing: an experimental study in wild birds

Antoine Stier, Bin-Yan Hsu, Nina Cossin-Sevrin, Natacha Garcin, Suvi Ruuskanen

► To cite this version:

Antoine Stier, Bin-Yan Hsu, Nina Cossin-Sevrin, Natacha Garcin, Suvi Ruuskanen. From climate warming to accelerated cellular ageing: an experimental study in wild birds. 2024. hal-04424676

HAL Id: hal-04424676

<https://hal.science/hal-04424676>

Preprint submitted on 29 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 From climate warming to accelerated cellular ageing: an 2 experimental study in wild birds

3 Antoine Stier ^{a,b*§}, Bin-Yan Hsu ^{a§}, Nina Cossin-Sevrin ^{a,c}, Natacha Garcin ^{a,b} & Suvi Ruuskanen ^{a,d}

4
5 ^a Department of Biology, University of Turku, Turku, Finland

6 ^b Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622, Villeurbanne,
7 France

8 ^c Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

9 ^d Department of Biological and Environmental Science, University of Jyväskylä

10
11 §: equal contribution

12 *Corresponding author: antoine.stier@gmail.com ; amstie@utu.fi

13 Antoine Stier: <https://orcid.org/0000-0002-5445-5524>

14 Bin-Yan Hsu: <https://orcid.org/0000-0002-3799-0509>

15 Nina Cossin-Sevrin: <https://orcid.org/0000-0002-8174-8357>

16 Suvi Ruuskanen: <https://orcid.org/0000-0001-5582-9455>

17
18 **Author contribution:** AS, BYH & SR designed the study, conducted the fieldwork and analyzed data.
19 BYH conducted the final statistical analyses. AS, NCS, NG & SR conducted laboratory work. AS, BYH &
20 SR wrote the manuscript, with input from NCS & NG.

21
22 **Competing interest statement:** the authors declare having no competing interests

23
24 **This file includes:** Main Text, Figures 1-3

25 **Word count:** 5191, **Reference count:** 46

26 **Preprint server:** *bioRxiv*

27
28 **Keywords:** climate change, early-life, telomere, mitochondria, thyroid hormone, survival,
29 bird

30 **Abstract**

31 Climate change is increasing both the average ambient temperature and the frequency
32 and severity of heat waves. While direct mortality induced by heat waves is increasingly
33 reported, sub-lethal effects are also likely to impact wild populations. We hypothesized that
34 accelerated ageing could be a cost of being exposed to higher ambient temperature, especially
35 in early-life when thermoregulatory capacities are not fully developed. We tested this
36 hypothesis in wild great tit (*Parus major*) by experimentally increasing nest box temperature
37 by *ca.* 2°C during postnatal growth and measuring telomere length, a biomarker of cellular
38 ageing predictive of survival prospects in many bird species. While increasing early-life
39 temperature does not affect growth or survival to fledging, it accelerates telomere shortening
40 and reduces medium-term survival from 34% to 19%. Heat-induced telomere shortening was
41 not explained by oxidative stress, but more likely by an increase in energy demand (*i.e.* higher
42 thyroid hormones levels, increased expression of glucocorticoid receptor, increased
43 mitochondrial density) leading to a reduction in telomere maintenance mechanisms (*i.e.*
44 decrease in the gene expression of telomerase and protective shelterin). Our results thus
45 suggest that climate warming can affect ageing rate in wild birds, with potential impact on
46 population dynamics and persistence.

47

48 **Significance statement**

49 Stressful environmental conditions are known to accelerate cellular ageing, especially when
50 experienced early in life. One unexplored avenue through which climate warming might affect
51 wild animal populations is accelerated ageing. Here we show that increasing nest temperature
52 by *ca.* 2°C during postnatal growth in a wild bird species can impact numerous physiological
53 pathways and medium-term survival. Notably, artificially warming nests accelerates the
54 shortening of telomeres, which are the protective end-caps of chromosomes considered as a
55 hallmark of ageing. We thus suggest that warm ambient temperatures might accelerate
56 ageing in wild animals, which can potentially impact population dynamics and extinction risk
57 in the face of climate change.

58

59

60 Introduction

61 Climate change is increasing both the average ambient temperature and the frequency
62 and severity of heat waves (IPCC 2014). While direct mortality induced by heat waves is
63 increasingly reported (*e.g.* [1]), sub-lethal effects are also likely to impact population dynamics
64 and persistence [2]. Despite regulating their body temperature, endotherms can also be
65 sensitive to (even small) changes in temperature, especially during early-life stages when their
66 thermoregulation is not yet fully developed [3]. Given that early-life experiences are known
67 to have long-lasting effects on health, reproduction and even longevity (*e.g.* [4,5]), changes in
68 early-life thermal environment associated with climate change are predicted to impact
69 offspring phenotype and survival. Accordingly, some studies demonstrated associations
70 between pre- or postnatal temperatures and survival in wild endotherm populations (*e.g.* [6–
71 10]), and even longevity in humans [11]. Yet, the underlying physiological mechanisms remain
72 poorly understood.

73 The physiological mechanisms of heat stress have been rather well characterized in
74 laboratory endotherms. (i) Acute heat stress is known to increase the levels of key
75 thermoregulatory and metabolic hormones, thyroid hormones (THs) [12,13]. (ii) Heat stress
76 influences mitochondria, the cell powerhouse, as it reduces its efficiency to convert nutrients
77 to ATP and increases its number [14] and (iii) increases the production of reactive oxygen
78 species [15] contributing to oxidative stress and cellular ageing [16]. Yet, extrapolating
79 findings from laboratory studies to wild populations in the context of climate change is
80 challenging at best, partly because the range of temperature manipulations often exceeds
81 temperature changes experienced in natural populations. A few studies in wild populations
82 have experimentally increased early-life postnatal temperatures in endotherms [10,17,18],
83 and report alterations of growth and body temperature. However, the potential mid to long-
84 term consequences of such effects have not been characterized.

85 A key challenge, especially in relatively long-lived animals and wild populations, is to
86 quantify the potential long-term deleterious effects of early-life conditions. Using the length
87 of telomeres (*i.e.* the protective structure located at the end of chromosomes that
88 progressively shorten with age), a key hallmark of ageing, as a molecular biomarker may help
89 to overcome this challenge. Indeed, telomere length has been shown to predict survival (*i.e.*
90 meta-analysis in [19]), and even lifetime reproductive success [20], and most of the telomere
91 shortening is known to occur early in life (*e.g.* [21]). Various early-life environmental stressors,

92 including high incubation temperature in birds (*e.g.* [21]), have been shown to accelerate
93 telomere shortening [22]. Telomere shortening is accelerated by oxidative stress [23],
94 mitochondrial dysfunction [24] and changes in metabolic demand [25], which are predicted
95 to increase in response to thermal stress (see above). While thermal environment has been
96 shown to affect telomere shortening in ectotherms (*e.g.* [26,27]), we critically lack data from
97 endotherm species.

98 In this study, we comprehensively assessed the effects of an experimental increase in
99 temperature during early postnatal development on growth, short and medium-term survival
100 (*i.e.* to fledging and to the next autumn-winter), as well as on key physiological and ageing
101 markers (thyroid hormones, mitochondrial density, oxidative stress, telomere length and gene
102 expression) in a wild great tit (*Parus major*) population. The temperature elevation mimicked
103 the predicted ~ 2°C temperature increase (IPCC), and was applied during a vulnerable period
104 of postnatal growth, *i.e.* when offspring are not protected by their mother, but still not fully
105 capable of thermoregulation. We predicted that increasing nest temperature would lead to (i)
106 reduced growth due to possible energetic costs of heat dissipation and/or lowered
107 mitochondrial efficiency, (ii) reduced survival prospects, (iii) increased THs, (iv) increased
108 oxidative stress, (v) increased mitochondrial density and (vi) shorter telomeres due to either
109 oxidative stress-induced shortening or a decrease in telomere maintenance mechanisms.

110 **Material & methods**

111 **Experimental design**

112 The experiment was conducted in a nest box population of great tits on the island of
113 Ruissalo ('60°26.055 N,'22°10.391 E) in Finland. Half of the nestlings of each nest (total N =32
114 nests) were swapped between nests two days after hatching to account for the effects of
115 genetic background and rearing environment. To increase nest box temperature during
116 growth of *ca.* 2°C, one heating pad (Uniheat Shipping warmer®, USA) was installed under the
117 ceiling in half of the nest boxes (N = 17) between d7 and d14 (hereafter, “heated” nests), *i.e.*
118 second half of postnatal development. For the other half (N= 15), a control pad (identical to
119 the heating pads but not producing heat) was installed (hereafter “control” nests). Heating
120 pads were checked and replaced every second day, and control nests were also visited to
121 standardize human disturbance between the experimental groups. The actual nest box
122 temperature was recorded with a thermo-logger (iButton thermochron, measuring at 3min
123 intervals, 0.0625°C accuracy) and an average temperature over the course of heating
124 treatment was calculated for each nest box (see ESM).

125 Nestling body mass and tarsus length were measured prior to the treatment on d7 and
126 post-treatment on d14 after hatching. Blood samples were collected from the brachial vein at
127 d14 (*ca.* 70µl) using heparinized capillaries. Whole-blood samples for oxidative stress and
128 DNA/RNA extraction were immediately snap-frozen in liquid nitrogen, and an aliquot of
129 whole-blood was stored on ice pack until centrifugation in the laboratory at the end of the
130 day to assess plasma thyroid hormone levels.

131 To study potential long-term and delayed effects of early-life heating treatment, we
132 recaptured juvenile great tits during the following autumn-winter using mist-nets at seven
133 feeding stations located across the field site (total of 126 hours of mist-netting). Mass and
134 wing length of the juveniles were recorded and a blood sample (*ca.* 80µl) was collected and
135 stored as explained above. Our method of recapture provides an estimate of post-fledging
136 survival (*i.e.* apparent survival), but could be slightly biased by dispersal.

137

138 ***Plasma thyroid hormones and oxidative stress***

139 Plasma thyroid hormones (T3 and T4, expressed as pg/µL) were measured from d14
140 nestlings with nano-LC-MS/MS following [28]. Total glutathione (tGSH), the most abundant
141 intra-cellular antioxidant, was measured from whole-blood samples with the ThioStar®

142 Glutathione Fluorescent Detection Kit (K005-FI, Arbor Assays, USA; technical repeatability: R
143 = 0.97 (95% C.I. [0.96-0.98])). As a measure of oxidative damage, we assessed blood lipid
144 peroxidation (malonaldehyde, MDA) using the TBARS-assay following [29] (technical
145 repeatability: R = 0.92 (95% C.I. [0.88-0.94])).

146

147 **Mitochondrial density, telomere length and molecular sexing**

148 Relative telomere length (rTL) and mitochondrial DNA copy number ($mtDNA_{cn}$, an index
149 of mitochondrial density) were quantified on DNA extracted from blood cells using real-time
150 quantitative PCR (qPCR) assays, following [30] (see details in ESM). This technique estimates
151 relative telomere length by determining the ratio (T/S) of telomere repeat copy number (T) to a
152 single copy gene (SCG), and the relative $mtDNA_{cn}$ as the ratio between one mitochondrial gene
153 and the same single copy gene. Birds were molecularly sexed using a qPCR approach adapted from
154 [31,32] (see details in ESM).

155

156 **Gene expression analysis**

157 We used RT-qPCR to quantify the relative expression levels of 6 genes of interest from RNA
158 extracted from blood cells (see ESM for details on methods). We quantified the expression of
159 genes related to (i) cellular stress response: the glucocorticoid receptor (GCR) *nr3c1*, two heat
160 shock proteins of the HSP70 (*i.e.* HSPA2) and HSP90 (*i.e.* HSP90B1) families, as well as the nuclear
161 factor erythroid 2-related factor 2 NRF2 (an oxidative-stress-induced regulator of several
162 antioxidants and cellular protective genes); and to (ii) telomere maintenance processes: the
163 telomeric repeat binding factor 2 TERF2 (a shelterin protein helping in protecting telomeres) and
164 the telomerase reverse transcriptase TERT (catalytic subunit of the enzyme responsible for
165 telomere elongation).

166

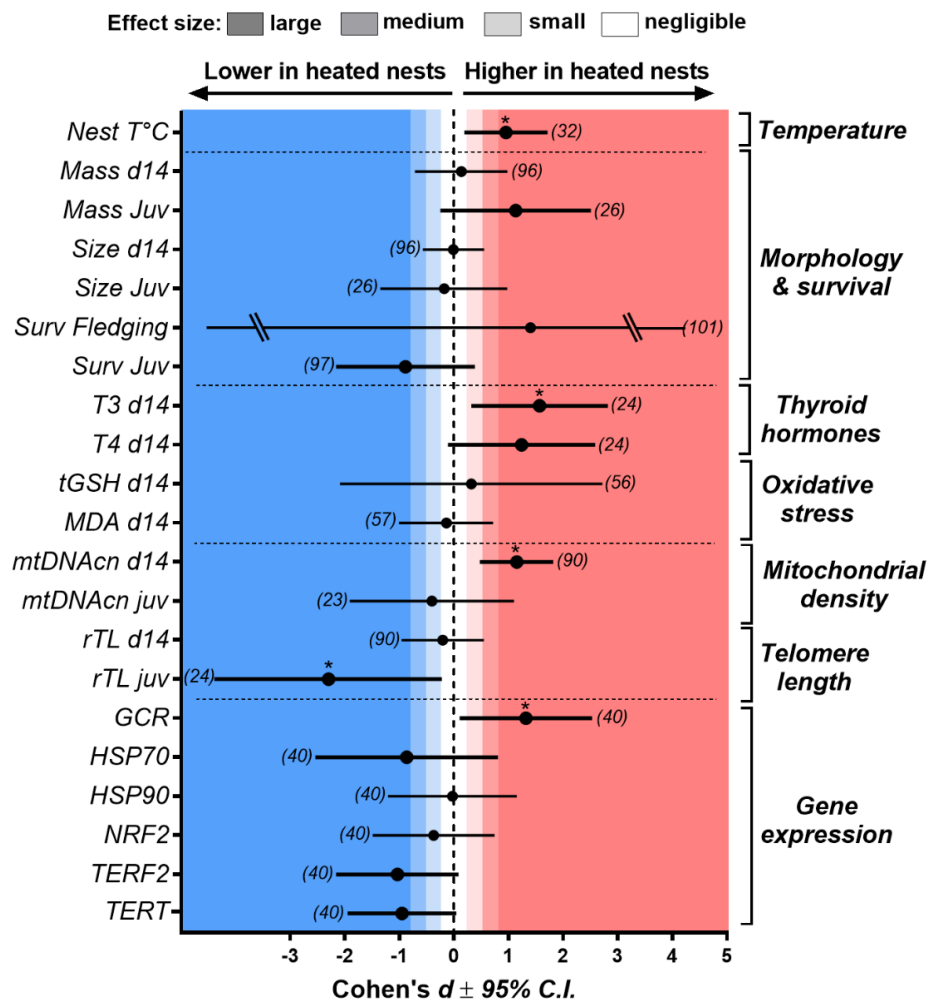
167 **Statistical analysis**

168 For each trait of interest (see Fig. 1), we sequentially fitted three generalized linear mixed
169 models (GLMMs, R package *lme4*): Model I assessed the effects of heating treatment, Model II
170 the association with actual nest box temperature, and Model III their interaction. Maximum
171 sample size was $N = 32$ nests, $n = 98$ nestlings d14 and $n = 26$ juveniles, but the final sample size
172 is smaller and varies between physiological parameters according to sample availability and
173 success of laboratory analyses (see specific sample size in ESM tables 2-21 and Fig. 1). In all

174 models, the identity of nest of origin (*i.e.* where a nestling was born) and the nest of rearing (*i.e.*
 175 where a nestling grew up after cross-fostering at day 2) were treated as random factors. Other
 176 random factors and fixed-effect covariates are included when relevant for the trait in question
 177 (see details in ESM). We report standardized effect size for each trait as Cohen's *d* and 95%
 178 confidence interval using the *emmeans* package in *R*. For survival analyses, In Odds Ratios were
 179 transformed to Cohen's *d* using the formula: $d = \ln(\text{OddsRatio}) \times (\sqrt{3}/\pi)$ following [33]

180

181 Results



195

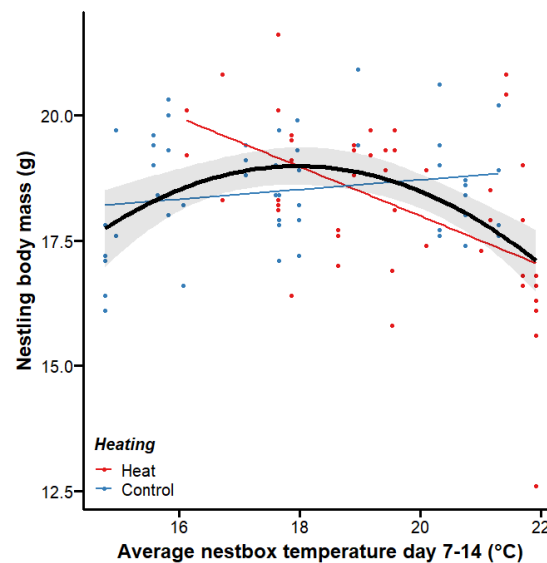
197 **Fig. 1: Effects of nest heating treatment on key life-history and physiological traits in wild great tits.**
 198 Effects are presented as standardized effect sizes (Cohen's *d*, or transformed to Cohen's *d*) and 95%
 199 confidence intervals. d14: nestlings being 14 days old, Juv: juveniles (*i.e.* captured during the
 200 autumn/winter following their fledging); surv: survival; T3 and T4: plasma thyroid hormones; MDA:
 201 biomarker of oxidative damage to lipids measured in blood, tGSH: total glutathione (*i.e.* intra-cellular
 202 antioxidant molecule) content in blood cells; mtDNAcn: mtDNA copy number, a proxy for
 203 mitochondrial density measured in blood cells; rTL: relative telomere length (*i.e.* a biomarker of ageing)
 204 of blood cells; GCR: gene expression of the glucocorticoid receptor, HSP70 and HSP90: gene expression
 205 of heat shock proteins, NRF2: gene expression of an oxidative-stress-induced regulator of several
 206 antioxidants and cellular protective genes, TERF2: gene expression of a shelterin protein helping in

207 protecting telomeres, TERT: gene expression of the catalytic subunit of the enzyme responsible for
208 telomere elongation. Detailed information on statistics is available in ESM Tables S2-S21. Significant
209 effects according to full statistical models are presented with * symbols, and large effects presented
210 in bold. The standard Cohen's *d* scale for negligible <0.2, small <0.5, medium <0.8 or large >0.8 effect
211 size is presented with a colour scale. Sample size for each trait is presented between brackets.
212

213 We confirmed that our heating treatment was effective in increasing average nest
214 temperature by *ca.* 1.84°C (large effect size; $t_{27.5} = 2.66$, $p = 0.013$; Fig. 1) over the heating
215 period (*i.e.* day 7 to day 14). Nestlings from heated nests were not significantly lighter or
216 smaller than control ones at day 14 (negligible and small effect size respectively; $p = 0.748$ and
217 0.969; Fig. 1, Tables S2-S3). Yet, we found a significant quadratic effect of actual nest
218 temperature on body mass at day 14 (nestlings being heaviest at mid-temperatures, $p = 0.050$;
219 Fig. 2, Table S2), that might be driven by an interaction (although non-significant; Table S2)
220 between heating treatment and nest temperature (Fig. 2). Juveniles from heated nests were
221 non-significantly heavier (large effect size; $p = 0.078$) but not larger (small effect size; $p =$
222 0.723) than control ones when recaptured the following autumn/winter (Fig. 1, Tables S4-S5).

223

Fig. 2: Relationship between nestling (*i.e.* day 14) body mass, actual nest temperature and heating treatment. The quadratic relationship (yellow line \pm 95% confidence interval) between nestbox temperature and nesting body mass is significant ($p = 0.050$), while the interaction between heating treatment and temperature is not ($p = 0.261$). See Table S1 for full details on statistical models.



233

234 Survival to fledging was very high overall and not significantly influenced by the heating
235 treatment (control: 94% vs. heated: 98%; $p = 0.82$; Fig. 1, Table S6). Birds from heated nests
236 tended (although not significantly so, $p = 0.223$) to be less likely to be recaptured as juveniles
237 (control: 34% vs. heated: 19%; large effect size; Fig. 1, Table S7).

238 Nestlings from heated nests had higher circulating thyroid hormones levels than
239 controls, significantly so for T3 (large effect size; $p = 0.032$) but not for T4 (large effect size; p
240 = 0.090; Fig. 1, Tables S8-S9). Heating treatment did not significantly alter oxidative stress

241 levels (glutathione: small effect size, $p = 0.347$; oxidative damage to lipids: negligible effect
242 size, $p = 0.682$; Fig. 1, Tables S10-11). However, total glutathione levels were positively related
243 to actual nest temperature ($\beta = 0.061$, $p = 0.039$; Table S11).

244

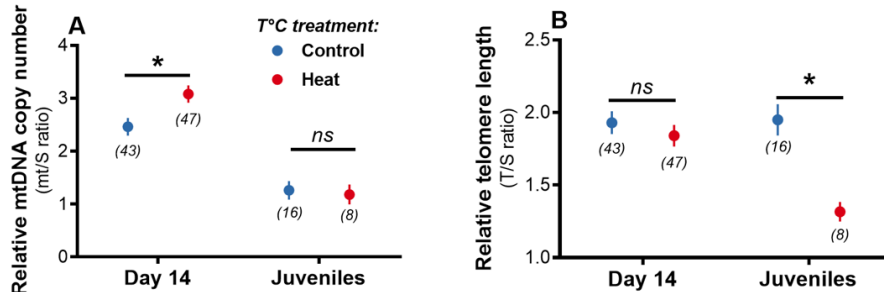
245

246

247

248

249



250 **Fig. 3: Effects of age and nest heating treatment on: (A) mtDNA copy number as a proxy of**
251 **mitochondrial density and cellular energetics, and (B) relative telomere length as a biomarker of**
252 **ageing.** Means are plotted \pm SE and full statistical models are presented in Tables S12-S15.
253

254

255

256

257

258

259

260

261

262

263

264

265

266

267

268

269

270

271

272

Mitochondrial density in blood cells decreased sharply during postnatal development (Fig. 3A), and nestlings from heated nests had a higher mitochondrial density at the end of the heating treatment (large effect size; $p = 0.002$; Fig. 1 & 3A, Table S10), but not when recaptured as juveniles (negligible effect size; $p = 0.554$; Fig. 1 & 3A, Table S11). Mitochondrial density at day 14 was also positively related to actual nest temperature ($\beta = 0.15$, $p = 0.050$, Table S10). Heating treatment did not significantly influence nestlings' telomere length (negligible effect size; $p = 0.580$; Fig. 1 & 3B, Table S12), but juveniles from heated nests had markedly shorter telomeres than controls (large effect size; $p = 0.033$; Fig. 1 & 3B, Table S13).

Gene expression was partly altered by nest heating treatment, with nestlings from heated nest showing significantly higher expression levels of the glucocorticoid receptor (GCR: large effect size; $p = 0.045$; Fig. 1, Table S16), and although non-significantly, lower expression levels of the telomere-related genes TERF2 (large effect size; $p = 0.088$; Fig. 1, Table S17) and TERT (large effect size; $p = 0.085$; Fig. 1, Table S18). There was however no clear effect on the expression of heat shock proteins (HSP70: medium effect size, $p = 0.298$; HSP90: negligible effect size, $p = 0.985$; Fig. 1, Tables S19-20) or the regulator of antioxidant protection NRF2 (small effect size, $p = 0.504$; Fig. 1, Table S21). Actual nest temperature was positively related to GCR expression ($\beta = 0.16$, $p = 0.074$, Table S20), and negatively to TERF2 ($\beta = -0.25$, $p = 0.010$, Table S20), TERT ($\beta = -0.27$, $p = 0.002$, Table S20), HSP70 ($\beta = -0.22$, $p = 0.020$, Table S20) and NRF2 ($\beta = -0.18$, $p = 0.032$, Table S21).

273 Discussion

274 By experimentally manipulating early postnatal temperature of *ca.* 2°C (in line with
275 predictions of climate changes), we demonstrated that exposure to higher ambient
276 temperature during early-life could affect several physiological pathways (*i.e.* thyroid
277 hormones, mitochondrial biogenesis, glucocorticoid signalling) and ultimately lead to
278 accelerated cellular ageing (*i.e.* shorter telomeres) through a potential deregulation of
279 telomere maintenance processes (*i.e.* shelterin protein and telomerase). While immediate
280 survival was not impacted by the experimental increase in nest temperature, birds from
281 heated nests were less likely to be seen alive the following autumn-winter.

282 Growth rate is known to be influenced in a complex manner by weather conditions
283 [34], and previous experimental studies increasing nest temperature have reported
284 contrasted results (positive effect: [17]; negative effects: [10,18]). Our experimental
285 manipulation did not affect body size or mass during postnatal growth, but our results suggest
286 that intermediate nest temperature might be optimal in our great tit population (*i.e.*
287 significant quadratic effect of actual nest temperature).

288 Apparent survival to autumn-winter was lower for birds originating from heated nests
289 (19% vs. 34%, large effect size), but not significantly so. Obtaining large enough sample size to
290 detect significant effects on survival in experimental studies on wild animals is unfortunately
291 challenging. Yet, our results are in accordance with observational data from a 12-year
292 monitoring study of great tit survival in Spain, showing a negative association between
293 ambient temperature during postnatal growth and post-fledging survival [6].

294 While there is some evidence from laboratory acute heat stress studies that thyroid
295 hormones could be up-regulated when facing increased ambient temperatures [12,13], we
296 show here that even a small increase in early-life temperature can increase thyroid hormones
297 levels. Importantly, high thyroid hormone levels have been linked to increased mortality risks
298 in adult humans [35] and increased susceptibility to free radicals in birds [36]. Yet, we found
299 no significant effect of nest heating on two oxidative stress biomarkers (glutathione and
300 oxidative damage to lipids), nor on NRF2 gene expression (an oxidative-stress-induced regulator
301 of several antioxidants). Similarly, the gene expression of two heat shock proteins (HSP70 and
302 HSP90 families) was not clearly impacted by nest heating. This suggests that a *ca.* 2°C increase
303 in temperature might be too low to induce oxidative stress or a heat shock response
304 (compared to heat stress experiments in the lab that often use > +10°C; *e.g.* [37]). Yet, the

305 gene expression of the glucocorticoid receptor *nr3c1* was increased in heated nests,
306 suggesting either a response to a stressful stimulus (*e.g.* [38]) or potentially an increase in
307 metabolic demand [39].

308 Both the rise in thyroid hormones and the observed increase in mitochondrial density
309 at the end of the heating period would support the hypothesis of an increase in metabolic
310 demand. In line with our results on mitochondrial density, mild heat stress has been shown to
311 increase mitochondrial biogenesis *in vitro* [14] and *in vivo* [40]. This could be a way to
312 compensate the typical decrease in mitochondrial coupling efficiency observed at higher body
313 temperature (*e.g.* [41]), but measuring both body temperature (*i.e.* [10] showed that nest
314 heating induced hyperthermia) and mitochondrial coupling efficiency [42] would be needed
315 here to test this hypothesis.

316 While telomeres were not immediately impacted by the heating treatment (*i.e.* no
317 effect at the end of the heating period on day 14), we discovered some important delayed
318 effect since juveniles from heated nests had markedly shorter telomeres than controls
319 individuals. Sample size of juveniles was relatively limited, and the lower apparent post-
320 fledging survival of birds from heated nests could lead to bias associated with selective
321 disappearance. Yet, this is unlikely to bias our results since we would expect individuals with
322 shorter telomeres to disappear earlier from the population (and not the opposite), as this has
323 previously been shown in our model species [43]. Additionally, the trend towards a decreased
324 expression of genes related to telomere maintenance (TERF2 and TERT) observed at day 14 in
325 nestlings from heated nests support the results observed on telomere length in juveniles. Our
326 results are in accordance with recent findings in humans showing a negative effect of warm
327 temperature and a positive effect of cold temperature during gestation on newborn telomere
328 length [44]. While oxidative stress-induced telomere shortening [23] is unlikely to explain our
329 results (see above), the ‘metabolic telomere attrition hypothesis’ [25] could be a good
330 candidate. Indeed, this theory stipulates that increased metabolic demand mediated by
331 increased glucocorticoid signalling (*i.e.* increased *nr3c1* expression observed here) can
332 decrease investment in telomere maintenance processes (*i.e.* decreased expression of TERF2
333 and TERT observed here) and be associated with mitochondrial dysfunction [45].

334 To conclude, our study provides the first evidence that a moderate increase in ambient
335 temperature during early-life can accelerate cellular ageing in a wild endotherm species.
336 While the exact consequences of such increase in early-life temperature on individual fitness

337 and population dynamics remain to be determined, previous evidence at the inter-population
338 level has shown that shorter telomeres might precede population extinction and be used as
339 an early warning sign [46].

340

341 **Funding**

342 This study was financially supported by the Academy of Finland (#286278 to SR). NCS
343 acknowledges support from the EDUFI Fellowship and Maupertuis Grant. B-Y.H work was
344 supported by a grant from the Ella and Georg Ehrnrooth Foundation. AS was supported by a
345 ‘Turku Collegium for Science and Medicine’ Fellowship and a Marie Skłodowska-Curie
346 Postdoctoral Fellowship (#894963) at the time of writing. All authors declare no conflict of
347 interest.

348

349 **Acknowledgements**

350 We are grateful to Jorma Nurmi, Lucas Bousseau, Thomas Rossille, Hsiao-Yin Liu and
351 Päivi Kotitalo for their help in the field. The laboratory facilities were supported by Biocenter
352 Finland

353

354 **Data accessibility**

355 All data will be archived publicly in FigShare upon acceptance of the manuscript.

356

357 **Ethical permits**

358 All procedures were approved by the Animal Experiment Committee of the State Provincial
359 Office of Southern Finland (license number ESAVI/2902/2018) and by the Environmental
360 Center of Southwestern Finland (license number VARELY549/2018) granted to SR.

361 **References**

362

363 1. McKechnie AE, Rushworth IA, Myburgh F, Cunningham SJ. Mortality among birds and bats during
364 an extreme heat event in eastern South Africa. *Austral Ecology*. 2021. doi:10.1111/aec.13025

365 2. Conradie SR, Woodborne SM, Cunningham SJ, McKechnie AE. Chronic, sublethal effects of high
366 temperatures will cause severe declines in southern African arid-zone birds during the 21st century.
367 *Proceedings Of The National Academy Of Sciences Of The United States Of America*. 2019;116:
368 14065–14070. doi:10.1073/pnas.1821312116

369 3. Boyles JG, Seebacher F, Smit B, McKechnie AE. Adaptive Thermoregulation in Endotherms May
370 Alter Responses to Climate Change. *Integrative and Comparative Biology*. 2011;51: 676–690.
371 doi:10.1093/icb/icr053

372 4. Metcalfe N, Monaghan P. Compensation for a bad start: grow now, pay later? *Trends in Ecology &*
373 *Evolution*. 2001;16: 254–260. Available:
374 [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9069966856375812607related:_8HYFs3-3nOJ)
375 [_uids=9069966856375812607related:_8HYFs3-3nOJ](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9069966856375812607related:_8HYFs3-3nOJ)

376 5. Preston JD, Reynolds LJ, Pearson KJ. Developmental Origins of Health Span and Life Span: A Mini-
377 Review. *Gerontology*. 2018;64: 237–245. doi:10.1159/000485506

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

381 7. Hepp GR, Kenamer RA. Warm is better: incubation temperature influences apparent survival and
382 recruitment of wood ducks (*Aix sponsa*). *PLoS ONE*. 2012;7: e47777.
383 doi:10.1371/journal.pone.0047777

384 8. Berntsen HH, Bech C. Incubation temperature influences survival in a small passerine bird. *Journal*
385 *of Avian Biology*. 2015; n/a-n/a. doi:10.1111/jav.00688

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

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

392 11. Catalano R, Bruckner T, Smith KR. Ambient temperature predicts sex ratios and male longevity.
393 *Proc National Acad Sci*. 2008;105: 2244–2247. doi:10.1073/pnas.0710711104

394 12. BOWEN SJ, WASHBURN KW. Thyroid and Adrenal Response to Heat Stress in Chickens and Quail
395 Differing in Heat Tolerance1. *Poultry science*. 1985;64: 149–154. doi:10.3382/ps.0640149

- 396 13. Iqbal A, Decuypere E, Azim AAE. Pre-and post-hatch high temperature exposure affects the
397 thyroid hormones and corticosterone response to acute heat stress in growing chicken (*Gallus*
398 *domesticus*). *Journal of Thermal Biology*. 1990;15: 149–153. doi:10.1016/0306-4565(90)90032-d
- 399 14. Salgado RM, Sheard AC, Vaughan RA, Parker DL, Schneider SM, Kenefick RW, et al. Mitochondrial
400 efficiency and exercise economy following heat stress: a potential role of uncoupling protein 3.
401 *Physiological Reports*. 2017;5: e13054. doi:10.14814/phy2.13054
- 402 15. Kikusato M, Ramsey JJ, Amo T, Toyomizu M. Application of modular kinetic analysis to
403 mitochondrial oxidative phosphorylation in skeletal muscle of birds exposed to acute heat stress.
404 *FEBS Letters*. 2010;584: 3143–3148. doi:10.1016/j.febslet.2010.05.057
- 405 16. Balaban RS, Nemoto S, Finkel T. Mitochondria, Oxidants, and Aging. *Cell*. 2005;120: 483–495.
406 doi:10.1016/j.cell.2005.02.001
- 407 17. Dawson RD, Lawrie CC, O’Brien EL. The importance of microclimate variation in determining size,
408 growth and survival of avian offspring: experimental evidence from a cavity nesting passerine.
409 *Oecologia*. 2005;144: 499–507. doi:10.1007/s00442-005-0075-7
- 410 18. Rodríguez S, Barba E. Nestling Growth is Impaired by Heat Stress: an Experimental Study in a
411 Mediterranean Great Tit Population. *Zoological studies*. 2016;55: e40. doi:10.6620/zs.2016.55-40
- 412 19. Wilbourn RV, Moatt JP, Froy H, Walling CA, Nussey DH, Boonekamp JJ. The relationship between
413 telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical*
414 *transactions of the Royal Society of London Series B, Biological sciences*. 2018;373: 20160447–9.
415 doi:10.1098/rstb.2016.0447
- 416 20. Eastwood JR, Hall ML, Teunissen N, Kingma SA, Aranzamendi NH, Fan M, et al. Early-life telomere
417 length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular Ecology*. 2019;28:
418 1127–1137. doi:10.1111/mec.15002
- 419 21. Stier A, Metcalfe NB, Monaghan P. Pace and stability of embryonic development affect telomere
420 dynamics: an experimental study in a precocial bird model. *Proceedings of the Royal Society of*
421 *London Series B: Biological Sciences*. 2020;287: 20201378–9. doi:10.1098/rspb.2020.1378
- 422 22. Chatelain M, Drobniak SM, Szulkin M. The association between stressors and telomeres in non-
423 human vertebrates: a meta-analysis. Cote J, editor. *Ecology Letters*. 2019;99: 21–18.
424 doi:10.1111/ele.13426
- 425 23. Reichert S, Stier A. Does oxidative stress shorten telomeres in vivo? A review. *Biology Letters*.
426 2017;13: 20170463–7. doi:10.1098/rsbl.2017.0463
- 427 24. Liu L, Trimarchi JR, Smith PJS, Keefe DL. Mitochondrial dysfunction leads to telomere attrition and
428 genomic instability. *Aging Cell*. 2002;1: 40–46. Available:
429 [http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12882352&retmode=ref](http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12882352&retmode=ref&cmd=prlinks)
430 [&cmd=prlinks](http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12882352&retmode=ref&cmd=prlinks)
- 431 25. Casagrande S, Hau M. Telomere attrition: metabolic regulation and signalling function? *Biology*
432 *Letters*. 2019;15: 20180885–11. doi:10.1098/rsbl.2018.0885

- 433 26. Simide R, Angelier F, Gaillard S, Stier A. Age and Heat Stress as Determinants of Telomere Length
434 in a Long-Lived Fish, the Siberian Sturgeon. *Physiological And Biochemical Zoology*. 2016;89: 441–
435 447. doi:10.1086/687378
- 436 27. Debes PV, Visse M, Panda B, Ilmonen P, Vasemägi A. Is telomere length a molecular marker of
437 past thermal stress in wild fish? *Molecular Ecology*. 2016. doi:10.1111/mec.13856
- 438 28. Ruuskanen S, Espín S, Sánchez-Virosta P, Sarraude T, Hsu B-Y, Pajunen P, et al. Transgenerational
439 endocrine disruption: Does elemental pollution affect egg or nestling thyroid hormone levels in a
440 wild songbird? *Environ Pollut*. 2019;247: 725–735. doi:10.1016/j.envpol.2019.01.088
- 441 29. Espin, Sanchez-Virosta, AJ G-F, T E. A microplate adaptation of the thiobarbituric acid reactive
442 substances assay to determine lipid peroxidation fluorometrically in sm all sample volumes. *Revista*
443 *de Toxicología*. 2017;34: 94–98.
- 444 30. Stier A, Hsu B-Y, Marciau C, Doligez B, Gustafsson L, Bize P, et al. Born to be young? Prenatal
445 thyroid hormones increase early-life telomere length in wild collared flycatchers. *Biology Letters*.
446 2020;16: 20200364–4. doi:10.1098/rsbl.2020.0364
- 447 31. Ellegren H, Fridolfsson AK. Male-driven evolution of DNA sequences in birds. *Nature genetics*.
448 1997;17: 182–184. doi:10.1038/ng1097-182
- 449 32. Chang H-W, Cheng C-A, Gu D-L, Chang C-C, Su S-H, Wen C-H, et al. High-throughput avian
450 molecular sexing by SYBR green-based real-time PCR combined with melting curve analysis. *BMC*
451 *Biotechnology*. 2008;8: 12–8. doi:10.1186/1472-6750-8-12
- 452 33. Hasselblad V, Hedges LV. Meta-Analysis of Screening and Diagnostic Tests. *Psychol Bull*.
453 1995;117: 167–178. doi:10.1037/0033-2909.117.1.167
- 454 34. Sauve D, Friesen VL, Charmantier A. The Effects of Weather on Avian Growth and Implications for
455 Adaptation to Climate Change. *Frontiers in Ecology and Evolution*. 2021;9: 5.
456 doi:10.3389/fevo.2021.569741
- 457 35. Bano A, Dhana K, Chaker L, Kavousi M, Ikram MA, Mattace-Raso FUS, et al. Association of Thyroid
458 Function With Life Expectancy With and Without Cardiovascular Disease. *JAMA Internal Medicine*.
459 2017;177: 1650–8. doi:10.1001/jamainternmed.2017.4836
- 460 36. REY B, Romestaing C, Bodennec J, Dumet A, Fongy A, Duchamp C, et al. Thyroid status affects
461 membranes susceptibility to free radicals and oxidative balance in skeletal muscle of Muscovy
462 ducklings (*Cairina moschata*). *Journal of Experimental Zoology Part A: Ecological Genetics and*
463 *Physiology*. 2014; n/a-n/a. doi:10.1002/jez.1872
- 464 37. Xie S, Tearle R, McWhorter TJ. Heat Shock Protein Expression is Upregulated after Acute Heat
465 Exposure in Three Species of Australian Desert Birds. *Avian Biology Research*. 2019;11: 263–273.
466 doi:10.3184/175815618x15366607700458
- 467 38. Lattin CR, Romero LM. Chronic stress alters concentrations of corticosterone receptors in a
468 tissue-specific manner in wild house sparrows (*Passer domesticus*). *J Exp Biol*. 2014;217: 2601–2608.
469 doi:10.1242/jeb.103788

- 470 39. Pradhan DS, Ness RV, Jalabert C, Hamden JE, Austin SH, Soma KK, et al. Phenotypic flexibility of
471 glucocorticoid signaling in skeletal muscles of a songbird preparing to migrate. *Horm Behav.*
472 2019;116: 104586. doi:10.1016/j.yhbeh.2019.104586
- 473 40. Hafen PS, Abbott K, Bowden J, Lopiano R, Hancock CR, Hyldahl RD. Daily heat treatment
474 maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to
475 immobilization. *Journal of applied physiology (Bethesda, Md : 1985).* 2019;127: 47–57.
476 doi:10.1152/jappphysiol.01098.2018
- 477 41. Fiorenza M, Lemminger AK, Marker M, Eibye K, Iaia FM, Bangsbo J, et al. High-intensity exercise
478 training enhances mitochondrial oxidative phosphorylation efficiency in a temperature-dependent
479 manner in human skeletal muscle: implications for exercise performance. *Faseb J.* 2019;33: 8976–
480 8989. doi:10.1096/fj.201900106rrr
- 481 42. Stier A, Bize P, Hsu B-Y, Ruuskanen S. Plastic but repeatable: rapid adjustments of mitochondrial
482 function and density during reproduction in a wild bird species. *Biology Letters.* 2019;15: 20190536.
483 doi:10.1098/rsbl.2019.0536
- 484 43. Salmón P, Nilsson JF, Watson H, Bensch S, Isaksson C. Selective disappearance of great tits with
485 short telomeres in urban areas. *Proceedings of the Royal Society of London Series B: Biological
486 Sciences.* 2017;284: 20171349–8. doi:10.1098/rspb.2017.1349
- 487 44. Martens DS, Plusquin M, Cox B, Nawrot TS. Early Biological Aging and Fetal Exposure to High and
488 Low Ambient Temperature: A Birth Cohort Study. *Environmental Health Perspectives.* 2019;127:
489 117001–10. doi:10.1289/ehp5153
- 490 45. Casagrande S, Stier A, Monaghan P, Loveland JL, Boner W, Lupi S, et al. Increased glucocorticoid
491 concentrations in early life cause mitochondrial inefficiency and short telomeres. *Journal Of
492 Experimental Biology.* 2020;223: jeb222513-14. doi:10.1242/jeb.222513
- 493 46. Dupoué A, Marciau C, Miles D, Meylan S. Shorter telomeres precede population extinction in wild
494 lizards. *Scientific Reports.* 2017;7: 1–9. doi:10.1038/s41598-017-17323-z
- 495

ESM of 'From climate warming to accelerated cellular ageing: an experimental study in wild birds'

Antoine Stier^{a,b,*§}, Bin-Yan Hsu^{a§}, Nina Cossin-Sevrin^{a,c}, Natacha Garcin^{a,b} & Suvi Ruuskanen^{a,d}

qPCR assays for telomere length, mtDNA copy number and molecular sexing

We extracted DNA from blood cells using a standard salt extraction alcohol precipitation method ([1]). Extracted DNA was diluted in elution buffer BE for DNA preservation. DNA concentration and purity ($260/280 > 1.80$ and $260/230 > 2.00$) were checked with a ND-1000-Spectrophotometer (NanoDrop Technologies, Wilmington, USA). DNA integrity was verified in 24 samples chosen randomly using gel electrophoresis (50 ng of DNA, 0.8 % agarose gel at 100 mV for 60 min) and DNA staining with Midori Green. Each sample was then diluted to a concentration of $1.2 \text{ ng}\cdot\mu\text{L}^{-1}$ for subsequent qPCR analysis.

Relative telomere length (*rTL*) and mitochondrial DNA copy number (*mtDNAcn*, an index of mitochondrial density) were quantified using qPCR. Here, we used recombination activating gene 1 RAG1 as a single copy gene (verified as single copy using a BLAST analysis on the great tit genome) and cytochrome oxidase subunit 1 (COI1) as a mitochondrial gene (verified as non-duplicated in the nuclear genome using a BLAST analysis). The qPCR reactions were performed on a 384-QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher), in a total volume of $12\mu\text{L}$ including 6ng of DNA, primers at a final concentration of 300nM and $6\mu\text{L}$ of SensiFAST™ SYBR lo-ROX (Bioline). Telomere, RAG1 and COI2 reactions were performed in triplicates on the same plates (10 plates in total); the qPCR conditions were: 3min at 95°C , followed by 35 cycles of 10 s at 95°C , 15 s at 58°C and 10s at 72°C . A DNA sample being a pool of DNA from 10 adult individuals was used as a reference sample and was included in triplicate on every plate. The efficiency of each amplicon was estimated from a standard curve of the reference sample ranging from 1.5 to 24ng. The primer sequences, as well as qPCR efficiencies and technical precision estimates (coefficient of variation and technical repeatability) are provided in Table S1. The relative telomere length and *mtDNAcn* of each sample were calculated as $(1+E_{f_{\text{Tel or COI2}}})^{\Delta\text{Cq}_{\text{Tel or COI2}}}$

$COI2 / (1 + Ef_{RAG1})^{\Delta Cq_{RAG1}}$; Ef being the amplicon efficiency, and ΔCq the difference in Cq-values between the reference sample and the focal sample.

The use of mtDNAcn as an index of mitochondrial density has been questioned in human [2], but we have previously shown good correlations between mtDNAcn and mitochondrial respiration rates in pied flycatcher [3] and great tit (Cossin-Sevrin et al. *in revision*). Great tits have quite peculiar telomeres, characterized notably by some ultra-long telomeres that do not seem to shorten with age in adults [4]. Since qPCR only provides an estimate of overall telomere length, it could be suboptimal for this study species. Yet, relative telomere length (*i.e.* measured using qPCR) in this species has been shown to shorten during the nestling stage [5,6], to respond to environmental factors (e.g. hatching asynchrony: [5]; altitude: [6]; urbanization: [7]) and to predict adult survival [8]. Within-individual repeatability of telomere length has recently been suggested to be an important factor to evaluate the pertinence of telomere length data in a given study/species [9], and the biological repeatability in our dataset was $R = 0.44$ [0.25-0.60], which is close to the average reported by qPCR studies (*i.e.* $R = 0.47$), and well within the range of what has been reported for great tits [9].

Birds were molecularly sexed using a qPCR approach adapted from [10,11]. Forward and reverse sexing primers were 5'- CACTACAGGGAAAACGTAC-3' (2987F) and 5'- CCCCTTCAGGTTCTTTAAAA -3' (3112R), respectively. qPCR reactions were performed in a total volume of 12 μ L including 6ng of DNA, primers at a final concentration of 800nM and 6 μ L of SensiFAST™ SYBR® Lo-ROX Kit (Bioline). qPCR conditions were: 3 min at 95°C, followed by 40 cycles of 45 s at 95°C, 60 s at 52°C and 60s at 72°C, then followed by a melting curve analysis (95°C 60s, 45°C 50s, increase to 95°C at 0.1°C/s, 95°C 30s). Samples were run in duplicates in a single plate and 6 adults of known sex were included as positive controls. Sex was determined by looking at the dissociation curve, with two peaks indicating the presence of a Z and W chromosome (female), and one peak indicating the presence of only the Z chromosomes (male).

RT-qPCR assays for evaluating gene expression

We used RT-qPCR to quantify the expression levels of 6 genes of interest in d14 nestlings (Table S1). First, RNA was extracted from 10 μ L of blood (immediately after the first thawing following -80°C storage) using Nucleospin RNA Plus extraction kit (Macherey-Nagel) following manufacturer instructions. Second, RNA concentration and purity were quantified using optical density. Samples not meeting quality criteria (i.e. RNA concentration $> 25 \text{ ng}/\mu\text{L}$, $260/280$ and $260/230 > 1.80$) were excluded for further analysis. RNA integrity was checked using E-Gel 2% electrophoresis system (Invitrogen), and the ribosomal RNA 18S vs. 28S bands intensity, and deemed satisfactory. Samples were stored at -80°C for 2 weeks before cDNA synthesis. 600ng of RNA were used for cDNA synthesis using the SensiFAST™ cDNA Synthesis kit (Bioline) following manufacturer instructions. cDNA was diluted at a final concentration of $1.2 \text{ ng}/\mu\text{L}$ for qPCR analysis. No-RT control samples were prepared following the same protocol, but without reverse transcriptase enzyme.

We assessed the expression genes related to 1) cellular stress response: the glucocorticoid receptor (GCR) *nr3c1*, two heat shock proteins of the HSP70 (i.e. *HSPA2*) and HSP90 (i.e. *HSP90B1*) families, as well as the nuclear factor erythroid 2-related factor 2 NRF2 (an oxidative-stress-induced regulator of several antioxidants and cellular protective genes); and 2) telomere maintenance processes: the telomeric repeat binding factor 2 TERF2 (a shelterin protein helping in protecting telomeres) and the telomerase reverse transcriptase TERT (catalytic subunit of the enzyme responsible for telomere elongation). qPCR was performed in a total volume of $12\mu\text{L}$ containing $5\mu\text{L}$ of each diluted cDNA sample (i.e. $1.2\text{ng}/\mu\text{L}$) and $7\mu\text{L}$ of reaction mix containing primers (forward and reverse) at a final concentration of 300nM and Sensifast SYBR®No-ROX Mix (Bioline). The succinate dehydrogenase complex subunit A (SDHA; [12]) and the ribosomal protein L13 (RPL13, [13]) were used as reference genes. SDHA and RPL13 were identified as the most stable reference genes using geNorm software (geNorm M < 0.7 , geNorm V < 0.15) and the geometric mean of these two genes was thus used as our reference gene. Primers (Table S1) have been designed whenever possible on exon-exon junction using NCBI primer designing tool using the *Parus major* reference genome. Specificity has

been checked using BLAST analysis and confirmed by a single narrow peak in melting curve analyses and the presence of a single PCR product of the expected size on agarose gel. Amplification in no-RT controls never occurred before at least 5 cycles after the lower Cq sample (> 8 Cq for all genes but TERT), and thus contamination by genomic DNA could not interfere with our results.

We ran gene-specific qPCR plates and each sample was analyzed in duplicate. Experimental groups were always balanced within each plate. We used a cDNA reference samples (*i.e.* ratio = 1) being a pool of 5 different individuals on every plate. One inter-plate standard sample was also run on every plate. qPCR assays were performed on a Mic qPCR instrument (Bio Molecular Systems) and included a two-step cycling with the following conditions: 2 minutes at 95°C; then 40 cycles of 5s at 95°C followed by 20s at 60°C (fluorescence reading) for all reactions. The expression of each gene was calculated as $(1+E_{f_{\text{Target}}})^{\Delta Cq(\text{Target})} / \text{geometric mean} [(1+E_{f_{\text{SDHA}}})^{\Delta Cq_{\text{SDHA}}}; (1+E_{f_{\text{RPL13}}})^{\Delta Cq_{\text{RPL13}}}]$, E_f being the amplification's efficiency and ΔCq being the difference between the Cq-values of the reference sample and the sample of interest.

Nest box temperature recording and data process

On day 7 upon the installation of the heating pad, a thermo-logger (iButton thermochron) was installed on the backboard inside the nest box, approximately 5 cm above the nest rim. Each iButton was programmed to record temperature data at a 3-min interval to the accuracy of 0.0625 °C and the time stamp was synced by the 1-Wire software (Maxim integrated) to one computer at the University of Turku. After the retrieval of iButtons on day 14, temperature data were exported. We calculated the daily (00:00 to 23:59) average temperature for each nest box, and then averaged the daily temperatures across the heating duration as the nest box temperature we used in the data analyses.

Statistics: Model specification

For each trait of interest, we sequentially fitted three generalized linear mixed models (GLMMs, R package *lme4* [15]): Model I assessed the effects of heating treatment, Model II the association with actual nest box temperature, and Model III their interaction (*i.e.* to test for potential accentuated effects of heating already warmer nests). In Model II, nest box temperature was first centered to the grand mean and both linear and quadratic effects of nest box temperature were tested. When the quadratic effect of nest box temperature was not significant, we dropped the quadratic term and performed the significance test on the linear effect of nest box temperature. In Model III, nest box temperature was mean-centered within each heating treatment to avoid collinearity, to properly test the interaction between nest temperature and the heating treatment. We assumed Gaussian error distribution for all traits, except for nestling and juvenile survival. Physiological markers (tGSH, MDA, plasma T3 and T4 concentrations and mtDNAcn), telomere length and gene expression data were all natural-log transformed before model fitting. Visual inspection on the residuals of all models confirmed no clear violation of such assumption. Because rTL, mtDNAcn and gene expression levels are relative values, we performed z-transformation to allow across-study comparison on the results (Verhulst 2020). Kenward-Roger approximation (package *pbkrtest* [16]) was used for significance testing. For nestling and juvenile survival, we fitted binomial GLMMs with Laplace

approximation. Nestlings that died before day 7 (*i.e.* before the heating treatment started, n=20) or day 9 (n=5) were all excluded. Sex-specific effects of heating treatment (Model I) and nest temperature (Model II) were tested but always came back non-significant (see Table S2-S21). Results of the main factors and covariates in Model I and II were therefore reported from the models without interactions.

The identities of nest of origin (*i.e.* where a nestling was born) and nest or rearing (*i.e.* where a nestling was reared after cross-fostering) were always considered random factors. However, in many cases, we observed very low estimate on the variance of the random factor in question. In such cases, we are able to exclude the common reasons that can cause singularity because our random factors contain more than 5 levels and in most cases the sample size per group is ≥ 2 . Secondly, we observed that the singular fit occurred on different random factors (the nest of origin or the nest of rearing) for different traits. This suggests that the natal condition such as genetics or prenatal maternal effects explain the variation better for some traits, while the postnatal rearing condition accounts for more variation in other traits. Therefore, the singularity in our models more likely reflects very low but true between-nest variance and should not be considered model failure. In support of the argument, in many cases where singularity occurred, we still observed that the variance for the random factor in question could be estimated, only it was extremely low (to the magnitude of 10^{-5} and often even lower). This again indicates that the singular fit reflects very low between-group variance and high within-group variance. Therefore, we believe that our data points are effectively independent and should not inflate type I error. Nevertheless, to fully reveal the information for readers' own judgement, we marked all the low random-effect variance in red to highlight singularity in the supplementary tables.

Due to missing data, sample sizes varied by traits and models, and are specified along with Table S2-S15.

Table S2: Results of linear mixed models for nestling body mass

Day-14 body mass (n=96)							
Model I. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.3767	0.6138				
Nest of origin (n=21)	Intercept	0.2964	0.5444				
Residual		0.5562	0.7458				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		1314.750	516.246	2.547			
Heating (No-heat)		-0.100	0.302	-0.332	1, 17.95	0.106	0.748
Brood size		-0.272	0.098	-2.772	1, 26.90	6.984	0.014
Day-7 body mass		0.394	0.067	5.870	1, 81.40	32.251	<0.001
Date		-0.073	0.029	-2.518	1, 36.22	6.252	0.017
Sex (male)		0.436	0.190	2.299	1, 74.67	5.083	0.027
Heating × sex		-0.495	0.374	-1.321	1, 74.76	1.667	0.201
Model II. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.2533	0.5033				
Nest of origin (n=21)	Intercept	0.3115	0.5581				
Residual		0.5661	0.7524				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		1267.548	482.647	2.626			
Brood size		-0.277	0.087	-3.163			
Day-7 body mass		0.386	0.066	5.814			
Date		-0.071	0.027	-2.595			
Sex (male)		0.401	0.189	2.118			
Nest temperature		-0.047	0.079	-0.604			
(Nest temperature)²		-0.073	0.034	-2.158	1, 22.65	4.292	0.050
Sex × (nest temperature) ²		0.043	0.041	1.055	1, 77.53	1.058	0.307
Model IIIa. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.4020	0.6340				
Nest of origin (n=21)	Intercept	0.2715	0.5210				
Residual		0.5591	0.7477				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		1114.376	547.111	2.037			
Heating (No-heat)		-0.021	0.316	-0.068			
Brood size		-0.249	0.103	-2.417			
Day-7 body mass		0.393	0.067	5.848			
Date		-0.062	0.031	-2.010			
Sex (male)		0.414	0.192	2.162			
Nest temperature (mean-centered within treatment)		-0.146	0.140	-1.046			
Heating × temperature		0.204	0.171	1.193	1, 18.33	1.348	0.261

Table S3: Results of linear mixed models for nestling body size

Day-14 body size (tarsus length, n=96)							
Model Ib. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	<0.0001	<0.0001				
Nest of origin (n=21)	Intercept	0.1960	0.4428				
	Residual	0.2155	0.4643				
Fixed effects:		Estimate	Std. Error	t	df	F	p
	Intercept	231.400	304.800	0.759			
	Heating (No-heat)	0.005	0.123	0.041	1, 15.09	0.002	0.969
	Brood size	<0.001	0.044	0.012	1, 14.68	0.001	0.991
	Day-7 tarsus length	0.216	0.053	4.067	1, 83.42	15.594	<0.001
	Date	-0.012	0.017	-0.699	1, 34.07	0.480	0.493
	Sex (male)	0.623	0.112	5.585	1, 75.39	29.349	<0.001
	Measurer B	0.149	0.179	0.832	1, 25.89	0.962	0.395
	Measurer C	0.272	0.185	1.474			
	Heating × sex	-0.431	0.225	-1.920	1, 79.02	3.452	0.067
Model IIb. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	<0.0001	<0.0001				
Nest of origin (n=21)	Intercept	0.2012	0.4485				
	Residual	0.2139	0.4625				
Fixed effects:		Estimate	Std. Error	t	df	F	p
	Intercept	236.415	302.383	0.782			
	Brood size	-0.003	0.043	-0.070			
	Day-7 tarsus length	0.224	0.052	4.295			
	Date	-0.012	0.017	-0.722			
	Sex (male)	0.621	0.113	5.515			
	Measurer B	0.149	0.177	0.839			
	Measurer C	0.322	0.188	1.716			
	Nest temperature	0.013	0.045	0.283	1, 26.57	0.458	0.504
	(Nest temperature) ²	-0.014	0.017	-0.824	1, 22.39	0.619	0.440
	Sex × (nest temperature) ²	-0.039	0.024	-1.602	1, 76.45	2.396	0.126
Model IIIb. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.4023	0.6342				
Nest of origin (n=21)	Intercept	0.5150	0.7176				
	Residual	0.6524	0.8077				
Fixed effects:		Estimate	Std. Error	t	df	F	p
	Intercept	1340.607	648.956	2.066			
	Heating (No-heat)	0.050	0.334	0.149			
	Brood size	-0.320	0.116	-2.754			
	Day-7 tarsus length	0.385	0.096	4.030			
	Date	-0.075	0.037	-2.046			
	Sex (male)	0.489	0.208	2.347			
	Measurer B	0.102	0.447	0.229			
	Measurer C	0.145	0.406	0.357			
	Nest temperature (mean-centered within treatment)	-0.161	0.158	-1.022			
	Heating × temperature	0.240	0.187	1.286	1, 18.33	1.556	0.228

Table S4: Results of linear mixed models for juvenile body mass

Juvenile body mass (n=25)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	0.0125	0.1118			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.8796	0.9379			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	13.911	3.985	3.491			
Heating (No-heat)	-1.057	0.418	-2.532	1, 8.44	4.013	0.078
Brood size	0.141	0.134	1.051	1, 6.45	0.751	0.417
Sex (male)	0.408	0.419	0.973	1, 15.55	0.650	0.432
Day-14 body mass	0.225	0.204	1.104	1, 18.46	0.853	0.368
Heating × sex	0.051	0.956	0.053	1, 11.32	0.002	0.968
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	0.2858	0.5346			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9387	0.9689			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	12.630	4.584	2.755			
Brood size	0.080	0.163	0.493			
Sex (male)	0.312	0.497	0.629			
Day-14 body mass	0.275	0.234	1.176			
Nest temperature	0.079	0.108	0.729	1, 9.47	0.426	0.530
(Nest temperature) ²	<0.001	0.061	0.007	1, 8.97	<0.001	0.996
Sex × (nest temperature) ²	0.119	0.145	0.822	1, 11.33	0.526	0.483
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9371	0.9680			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	13.422	4.150	3.234			
Heating (No-heat)	-1.050	0.428	-2.456			
Brood size	0.131	0.141	0.927			
Sex (male)	0.570	0.457	1.246			
Day-14 body mass	0.248	0.213	1.162			
Nest temperature (mean-centered within treatment)	0.116	0.220	0.527			
Heating × temperature	-0.218	0.251	-0.870	1, 6.36	0.450	0.526

Table S5: Results of linear mixed models for juvenile body size

Juvenile body size (wing length, n=26)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=17)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
	Residual	2.9310	1.7120			
Fixed effects:	Estimate	Std. Error	t	df	F	p
	Intercept	80.931	7.178			
	Heating (No-heat)	0.315	0.726	0.433	1, 8.86	0.134
	Brood size	0.314	0.241	1.307	1, 7.23	1.261
	Sex (male)	1.664	0.735	2.264	1, 18.76	3.488
	Day-14 body mass	-0.365	0.366	-0.999	1, 17.95	0.720
	Heating × sex	0.169	1.625	0.104	1, 12.86	0.008
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=17)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.1316	0.3628			
	Residual	2.7276	1.6516			
Fixed effects:	Estimate	Std. Error	t	df	F	p
	Intercept	80.937	7.012	11.543		
	Brood size	0.352	0.240	1.468		
	Sex (male)	1.612	0.735	2.192		
	Day-14 body mass	-0.336	0.358	-0.940		
	Nest temperature	0.116	0.154	0.752	1, 9.99	0.455
	(Nest temperature) ²	-0.099	0.087	-1.145	1, 8.24	0.883
	Sex × (nest temperature) ²	-0.178	0.211	-0.847	1, 12.80	0.545
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
	Residual	2.7970	1.6720			
Fixed effects:	Estimate	Std. Error	t	df	F	p
	Intercept	82.177	7.140	11.509		
	Heating (No-heat)	0.346	0.711	0.486		
	Brood size	0.311	0.242	1.285		
	Sex (male)	1.233	0.761	1.622		
	Day-14 body mass	-0.417	0.366	-1.141		
	Nest temperature (mean-centered within treatment)	-0.190	0.379	-0.503		
	Heating × temperature	0.530	0.431	1.230	1, 9.44	1.034

Table S6: Results of generalized linear mixed models for nestling survival

Nestling survival from day 7 to fledging (n=101)					
Model I. Heating treatment					
Random effects:		Variance	Std. Dev.		
Nest of rearing (n=34)	Intercept	397.8000	19.9500		
Nest of origin (n=21)	Intercept	<0.0001	<0.0001		
Fixed effects:		Estimate	Std. Error	z	p
Intercept		12.574	6.237	2.016	
Heating (No-heat)		-1.399	6.175	-0.227	0.821
Model II. Nest box temperature					
Random effects:		Variance	Std. Dev.		
Nest of rearing (n=34)	Intercept	372.0000	19.2900		
Nest of origin (n=21)	Intercept	<0.0001	<0.0001		
Fixed effects:		Estimate	Std. Error	z	p
Intercept		12.207	5.032	2.426	
Nest temperature		0.364	1.485	0.245	0.807
(Nest temperature) ²		-0.066	0.591	-0.112	0.911
Model III. Heating treatment × Nest box temperature					
Random effects:		Variance	Std. Dev.		
Nest of rearing (n=34)	Intercept	366.9000	19.1600		
Nest of origin (n=21)	Intercept	<0.0001	<0.0001		
Fixed effects:		Estimate	Std. Error	z	p
Intercept		12.426	6.050	2.054	
Heating (No-heat)		-0.945	6.675	-0.142	0.887
Nest temperature (mean-centered within treatment)		0.027	3.055	0.009	0.993
Heating × temperature		0.503	3.613	0.139	0.889

For survival, binomial GLMMs were fitted with maximum likelihood by Laplace approximation. As there were only 4 day-7 nestlings that failed to fledge, other covariates (sex and day-7 body mass) were excluded in these models.

Table S7: Results of generalized linear mixed models for juvenile survival

Post-fledging survival (n=97)					
Model I. Heating treatment					
Random effects:		Variance	Std. Dev.		
Nest of rearing (n=32)	Intercept	1.0590	1.0293		
Nest of origin (n=21)	Intercept	<0.0001	0.0014		
Fixed effects:		Estimate	Std. Error	z	p
Intercept		-9.411	4.840	-1.944	
Heating (No-heat)		0.837	0.687	1.218	0.223
Day-14 body mass		0.399	0.257	1.553	0.120
Sex (male)		0.812	0.629	1.291	0.197
Heating × sex		-0.174	1.157	-0.151	0.880
Model II. Nest box temperature					
Random effects:		Variance	Std. Dev.		
Nest of rearing (n=32)	Intercept	1.0576	1.0284		
Nest of origin (n=21)	Intercept	0.1757	0.4191		
Fixed effects:		Estimate	Std. Error	z	p
Intercept		-10.867	5.282	-2.058	
Day-14 body mass		0.471	0.273	1.724	0.085
Nest temperature		-0.001	0.167	-0.009	0.993
(Nest temperature) ²		0.107	0.091	1.177	0.239
Sex (male)		0.922	0.650	1.419	0.156
Sex × (nest temperature) ²		0.005	0.167	0.030	0.976
Model III. Heating treatment × Nest box temperature					
Random effects:		Variance	Std. Dev.		
Nest of rearing (n=32)	Intercept	0.4695	0.6852		
Nest of origin (n=21)	Intercept	0.3719	0.6098		
Fixed effects:		Estimate	Std. Error	z	p
Intercept		-11.861	5.038	-2.354	
Heating (No-heat)		0.709	0.641	1.107	0.268
Day-14 body mass		0.534	0.267	2.000	0.046
Nest temperature (mean-centered within treatment)		0.485	0.316	1.532	0.126
Sex (male)		0.856	0.630	1.359	0.174
Heating × temperature		-0.661	0.387	-1.708	0.088

Table S8: Results of linear mixed models for nestling plasma T3 levels

Day-14 nestling blood T3 level (n=24)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.0942	0.3069			
	Residual	0.0952	0.3085			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.458	1.294	1.127			
Heating (No-heat)	-0.484	0.151	-3.204	1, 6.19	7.559	0.032
Sex (male)	-0.078	0.157	-0.497	1, 10.80	0.140	0.716
Day-14 body mass	-0.070	0.071	-0.987	1, 13.41	0.660	0.431
Heating × sex	0.631	0.336	1.880	1, 18.81	2.564	0.126
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	0.0111	0.1052			
Nest of origin (n=13)	Intercept	0.0228	0.1509			
	Residual	0.1477	0.3843			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.138	1.549	-0.089			
Sex (male)	0.086	0.192	0.448			
Day-14 body mass	0.008	0.083	0.093			
Nest temperature	0.124	0.058	2.138	1, 17.32	2.649	0.122
(Nest temperature) ²	-0.043	0.026	-1.649	1, 10.51	1.947	0.192
Sex × (nest temperature) ²	0.042	0.074	0.568	1, 12.09	0.189	0.671
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.0893	0.2989			
	Residual	0.1091	0.3303			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.114	1.514	0.736			
Heating (No-heat)	-0.478	0.161	-2.965			
Sex (male)	-0.025	0.183	-0.139			
Day-14 body mass	-0.053	0.082	-0.648			
Nest temperature (mean-centered within treatment)	0.035	0.100	0.346			
Heating × temperature	0.023	0.111	0.209	1, 13.02	0.034	0.857

Table S9: Results of linear mixed models for nestling plasma T4 levels

Day-14 nestling blood T4 level (n=24)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.0934	0.3056			
	Residual	0.0525	0.2291			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	2.423	1.004	2.413			
Heating (No-heat)	-0.283	0.118	-2.403	1, 5.23	4.314	0.090
Sex (male)	0.233	0.122	1.905	1, 8.99	1.998	0.191
Day-14 body mass	-0.033	0.055	-0.599	1, 11.027	0.243	0.632
Heating × sex	0.293	0.288	1.019	1, 17.02	0.763	0.395
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	0.0032	0.0566			
Nest of origin (n=13)	Intercept	0.0827	0.2876			
	Residual	0.0753	0.2744			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.928	1.232	1.565			
Sex (male)	0.223	0.155	-0.142			
Day-14 body mass	-0.009	0.066	-0.142			
Nest temperature	0.040	0.052	0.767	1, 12.31	0.523	0.483
(Nest temperature) ²	-0.019	0.022	-0.898	1, 8.66	0.644	0.444
Sex × (nest temperature) ²	0.085	0.065	1.308	1, 9.94	1.165	0.306
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.1076	0.3280			
	Residual	0.0489	0.2210			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	2.961	1.089	2.719			
Heating (No-heat)	-0.246	0.120	-2.051			
Sex (male)	0.180	0.135	1.328			
Day-14 body mass	-0.061	0.059	-1.045			
Nest temperature (mean-centered within treatment)	-0.094	0.085	-1.109			
Heating × temperature	0.116	0.090	1.282	1, 12.91	1.211	0.291

T4 and T3 were natural-log transformed.

Table S10: Results of linear mixed models for nestling blood oxidative damage to lipids (MDA) levels

Day-14 nestling blood MDA level (n=57)							
Model I. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=26)	Intercept	0.0005	0.0220				
Nest of origin (n=18)	Intercept	<0.0001	<0.0001				
Batch of TBARS (n=6)	Intercept	0.0994	0.3152				
Residual		0.0519	0.2278				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-2.991	0.530	-5.642			
Heating (No-heat)		0.033	0.074	0.447	1, 15.72	0.174	0.682
Sex (male)		0.017	0.071	0.240	1, 47.59	0.050	0.825
Day-14 body mass		-0.018	0.028	-0.629	1, 37.22	0.327	0.571
Heating × sex		0.027	0.146	0.183	1, 45.71	0.028	0.867
Model II. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=26)	Intercept	<0.0001	<0.0001				
Nest of origin (n=18)	Intercept	<0.0001	<0.0001				
Batch of TBARS (n=6)	Intercept	0.1126	0.3356				
Residual		0.0502	0.2241				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-3.069	0.525	-5.847			
Sex (male)		0.017	0.069	0.239			
Day-14 body mass		-0.010	0.028	-0.350			
Nest temperature		-0.005	0.019	-0.284	1, 11.51	0.017	0.898
(Nest temperature) ²		-0.015	0.009	-1.608	1, 11.62	1.715	0.216
Sex × (nest temperature) ²		-0.032	0.017	-1.905	1, 33.96	2.634	0.114
Model III. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=26)	Intercept	0.0006	0.0241				
Nest of origin (n=18)	Intercept	<0.0001	<0.0001				
Batch of TBARS (n=6)	Intercept	0.0890	0.2984				
Residual		0.0521	0.2282				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-2.930	0.533	-5.501			
Heating (No-heat)		0.050	0.074	0.672			
Sex (male)		0.003	0.073	0.042			
Day-14 body mass		-0.021	0.028	-0.742			
Nest temperature (mean-centered within treatment)		-0.027	0.030	-0.892			
Heating × temperature		0.053	0.036	1.488	1, 10.96	1.835	0.203

Table S11: Results of linear mixed models for nestling blood total glutathione (tGSH) levels

Day-14 nestling blood tGSH level (n=56)							
Model I. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=27)	Intercept	<0.0001	<0.0001				
Nest of origin (n=18)	Intercept	0.0237	0.1539				
Batch of GSH (n=2)	Intercept	0.0207	0.1438				
Residual		0.0936	0.3060				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-0.226	0.773	-0.292			
Heating (No-heat)		-0.098	0.095	-1.030	1, 14.62	0.944	0.347
Sex (male)		-0.007	0.097	-0.067	1, 49.43	0.004	0.951
Day-14 body mass		-0.056	0.042	-1.351	1, 47.08	1.558	0.218
Heating × sex		0.247	0.194	1.277	1, 44.97	1.360	0.250
Model II. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=27)	Intercept	0.0063	0.0791				
Nest of origin (n=18)	Intercept	0.0058	0.0761				
Batch of GSH (n=2)	Intercept	0.0148	0.1217				
Residual		0.0949	0.3080				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-0.485	0.751	-0.646			
Sex (male)		-0.000	0.091	-0.001			
Day-14 body mass		-0.046	0.040	-1.154			
Nest temperature		0.061	0.025	2.438	1, 15.48	5.099	0.039
(Nest temperature) ²		0.006	0.013	0.491	1, 17.84	0.184	0.673
Sex × nest temperature		0.013	0.045	0.296	1, 48.24	0.072	0.790
Sex × (nest temperature) ²		0.008	0.024	0.307	1, 40.72	0.073	0.789
Model III. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=27)	Intercept	0.0122	0.1105				
Nest of origin (n=18)	Intercept	<0.0001	<0.0001				
Batch of GSH (n=2)	Intercept	0.0064	0.0797				
Residual		0.0927	0.3044				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-0.389	0.736	-0.529			
Heating (No-heat)		-0.095	0.099	-0.964			
Sex (male)		-0.018	0.095	-0.184			
Day-14 body mass		-0.047	0.040	-1.185			
Nest temperature (mean-centered within treatment)		0.009	0.039	0.226			
Heating × temperature		0.084	0.051	1.640	1, 13.84	2.180	0.162

Table S12: Results of linear mixed models for nestling blood cell mtDNA copy number

Day-14 nestling mtDNA copy number (n=90)							
Model I. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.1105	0.3224				
Nest of origin (n=21)	Intercept	0.3423	0.5851				
Residual		0.5128	0.7161				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		0.028	1.431	0.019			
Heating (No-heat)		-0.821	0.220	-3.735	1, 16.85	13.112	0.002
Sex (male)		0.127	0.193	0.655	1, 79.06	0.400	0.529
Day-14 body mass		0.018	0.078	0.224	1, 84.38	0.046	0.831
Heating × sex		-0.174	0.374	-0.466	1, 77.93	0.203	0.653
Model II. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.3526	0.5938				
Nest of origin (n=21)	Intercept	0.1323	0.3637				
Residual		0.5271	0.7261				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-0.204	1.551	-0.132			
Sex (male)		0.102	0.200	0.511			
Day-14 body mass		0.013	0.083	0.157			
Nest temperature		0.153	0.077	1.983	1, 29.95	4.163	0.050
(Nest temperature) ²		-0.004	0.036	-0.103	1, 28.97	0.010	0.922
Sex × nest temperature		0.014	0.094	0.146	1, 79.05	0.020	0.888
Sex × (nest temperature) ²		0.024	0.053	0.452	1, 83.26	0.186	0.667
Model III. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=32)	Intercept	0.1909	0.4369				
Nest of origin (n=21)	Intercept	0.2422	0.4921				
Residual		0.5170	0.7191				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		0.173	1.483	0.117			
Heating (No-heat)		-0.786	0.245	-3.207			
Sex (male)		0.143	0.197	0.728			
Day-14 body mass		0.009	0.081	0.114			
Nest temperature (mean-centered within treatment)		0.051	0.113	0.452			
Heating × temperature		0.036	0.136	0.266	1, 18.85	0.065	0.802

Table S13: Results of linear mixed models for juvenile blood cell mtDNA copy number

Juvenile mtDNA copy number (n=23)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9976	0.9988			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-7.514	4.240	-1.772			
Heating (No-heat)	0.404	0.515	0.784	1, 7.97	0.382	0.554
Sex (male)	-0.237	0.441	-0.538	1, 17.13	0.212	0.651
Juvenile body mass	0.404	0.224	1.803	1, 15.60	1.979	0.179
Heating × sex	0.833	0.936	0.891	1, 15.37	0.493	0.493
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9743	0.9871			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.209	3.641	-1.705			
Sex (male)	-0.139	0.438	-0.318			
Juvenile body mass	0.325	0.200	1.627			
Nest temperature	-0.057	0.096	-0.589	1, 8.02	0.123	0.735
(Nest temperature) ²	0.070	0.052	1.359	1, 6.91	1.435	0.270
Sex × (nest temperature) ²	-0.107	0.109	-0.982	1, 12.42	0.663	0.431
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	0.0158	0.1258			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		1.049	1.0244			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.805	4.475	-1.521			
Heating (No-heat)	0.494	0.553	0.894			
Sex (male)	-0.200	0.490	-0.409			
Juvenile body mass	0.359	0.238	1.505			
Nest temperature (mean-centered within treatment)	0.281	0.332	0.847			
Heating × temperature	-0.327	0.361	-0.906	1, 10.73	0.508	0.491

Table S14: Results of linear mixed models for nestling blood cell relative telomere length

Day-14 nestling telomere length (n=90)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.3542	0.5951			
Nest of origin (n=21)	Intercept	0.0614	0.2477			
	Residual	0.6041	0.7773			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.977	1.534	-1.288			
Heating (No-heat)	0.161	0.280	0.574	1, 21.12	0.315	0.580
Sex (male)	-0.212	0.210	-1.011	1, 81.19	0.959	0.331
Day-14 body mass	0.110	0.084	1.317	1, 84.06	1.589	0.211
Heating × sex	-0.264	0.399	-0.663	1, 77.89	0.406	0.526
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.2174	0.4662			
Nest of origin (n=21)	Intercept	0.2136	0.4621			
	Residual	0.6089	0.7803			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.999	1.604	-1.246			
Sex (male)	-0.211	0.208	-1.014			
Day-14 body mass	0.108	0.086	1.251			
Nest temperature	-0.043	0.077	-0.557	1, 29.11	0.512	0.480
(Nest temperature) ²	0.030	0.035	0.857	1, 27.41	0.662	0.423
Sex × (nest temperature) ²	-0.008	0.055	-0.154	1, 82.35	0.021	0.884
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.1579	0.3973			
Nest of origin (n=21)	Intercept	0.2808	0.5299			
	Residual	0.6093	0.7805			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-2.149	1.575	-1.364			
Heating (No-heat)	0.040	0.247	0.162			
Sex (male)	-0.197	0.209	-0.941			
Day-14 body mass	0.121	0.086	1.397			
Nest temperature (mean-centered within treatment)	0.069	0.116	0.596			
Heating × temperature	-0.229	0.138	-1.663	1, 18.36	2.516	0.130

Table S15: Results of linear mixed models for juvenile blood cell relative telomere length

Juvenile telomere length (n=24)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	<0.0001	0.0011			
Nest of origin (n=13)	Intercept	0.1108	0.3329			
	Residual	0.4093	0.6398			
Fixed effects:	Estimate	Std. Error	t	df	F	p
	Intercept	1.648	2.887			
	Heating (No-heat)	1.475	0.373	3.952	1, 5.90	0.033
	Sex (male)	0.260	0.313	0.833	1, 17.38	0.473
	Juvenile body mass	-0.153	0.153	-0.999	1, 13.25	0.575
	Heating × sex	0.088	0.683	0.129	1, 16.87	0.011
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	0.9899	0.9949			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
	Residual	0.3033	0.5507			
Fixed effects:	Estimate	Std. Error	t	df	F	p
	Intercept	4.777	2.939	1.625		
	Sex (male)	-0.037	0.365	-0.101		
	Juvenile body mass	-0.250	0.158	-1.582		
	Nest temperature	-0.072	0.134	-0.536	1, 10.61	0.295
	(Nest temperature) ²	-0.033	0.073	-0.457	1, 8.93	0.159
	Sex × (nest temperature) ²	0.113	0.123	0.913	1, 14.34	0.604
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	0.2534	0.5034			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
	Residual	0.2857	0.5345			
Fixed effects:	Estimate	Std. Error	t	df	F	p
	Intercept	1.164	2.714	0.429		
	Heating (No-heat)	1.530	0.412	3.712		
	Sex (male)	-0.045	0.314	-0.143		
	Juvenile body mass	-0.118	0.144	-0.823		
	Nest temperature					
	(mean-centered within treatment)	-0.087	0.251	-0.349		
	Heating × temperature	0.296	0.275	1.075	1, 9.18	0.709

Table S16: Results of linear mixed models for nr3c1 expression levels

Day-14 nestling nr3c1 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6435	0.8022			
Nest of origin (n=20)	Intercept	<0.0001	0.0004			
Residual		0.2912	0.5396			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	2.460	2.414	1.019			
Heating (No-heat)	-0.756	0.340	-2.226	1, 17.16	4.667	0.045
Sex (male)	0.032	0.274	0.117	1, 25.80	0.011	0.917
Day-14 body mass	-0.103	0.129	-0.799	1, 34.87	0.534	0.470
Plate	-0.444	0.260	-1.705	1, 22.84	2.380	0.137
Heating × sex	0.651	0.563	1.157	1, 28.28	1.027	0.320
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6502	0.8063			
Nest of origin (n=20)	Intercept	<0.0001	0.0009			
Residual		0.3231	0.5684			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	0.888	2.689	0.330			
Sex (male)	0.033	0.282	0.117			
Day-14 body mass	-0.036	0.141	-0.252			
Plate	-0.418	0.272	-1.536			
Nest temperature	0.163	0.087	1.868	1, 23.59	3.506	0.074
(Nest temperature) ²	-0.016	0.043	-0.379	1, 25.80	0.125	0.727
Sex × (nest temperature) ²	0.023	0.072	0.315	1, 27.42	0.079	0.780
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6244	0.7902			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3194	0.5652			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.402	2.626	0.534			
Heating (No-heat)	-0.780	0.342	-2.279			
Sex (male)	0.051	0.291	0.176			
Day-14 body mass	-0.046	0.141	-0.329			
Plate	-0.429	0.281	-1.530			
Nest temperature (mean-centered within treatment)	0.110	0.155	0.711			
Heating × temperature	-0.011	0.202	-0.056	1, 21.87	0.003	0.958

Table S17: Results of linear mixed models for TERF2 expression levels

Day-14 nestling TERF2 expression (n=40)							
Model I. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=31)	Intercept	0.6949	0.8336				
Nest of origin (n=20)	Intercept	<0.0001	<0.0001				
Residual		0.2067	0.4546				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-2.937	2.292	-1.281			
Heating (No-heat)		0.625	0.336	1.860	1, 17.27	3.264	0.088
Sex (male)		-0.149	0.250	-0.595	1, 21.63	0.288	0.597
Day-14 body mass		0.138	0.122	1.126	1, 33.70	1.046	0.314
Plate		0.184	0.236	0.781	1, 19.22	0.501	0.488
Heating × sex		0.340	0.517	0.657	1, 22.66	0.329	0.572
Model II. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=31)	Intercept	0.5250	0.7246				
Nest of origin (n=20)	Intercept	0.0857	0.2927				
Residual		0.1931	0.4395				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-0.397	2.334	-0.170			
Sex (male)		-0.163	0.238	-0.685			
Day-14 body mass		0.025	0.123	0.201			
Plate		0.178	0.226	0.786			
Nest temperature		-0.245	0.081	-3.031	1, 25.35	7.765	0.010
(Nest temperature) ²		-0.027	0.039	-0.673	1, 26.67	0.395	0.535
Sex × nest temperature		0.061	0.120	0.508	1, 24.87	0.208	0.652
Sex × (nest temperature) ²		-0.015	0.060	-0.248	1, 21.81	0.049	0.826
Model III. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=31)	Intercept	0.4607	0.6787				
Nest of origin (n=20)	Intercept	0.1587	0.3983				
Residual		0.1880	0.4335				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-1.891	2.297	-0.823			
Heating (No-heat)		0.619	0.298	2.077			
Sex (male)		-0.059	0.245	-0.242			
Day-14 body mass		0.081	0.123	0.661			
Plate		0.065	0.231	0.283			
Nest temperature (mean-centered within treatment)		-0.073	0.143	-0.513			
Heating × temperature		-0.225	0.178	-1.260	1, 20.77	1.394	0.251

Table S18: Results of linear mixed models for TERT expression levels

Day-14 nestling TERT expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	<0.0001	<0.0001			
Nest of origin (n=20)	Intercept	0.4313	0.6567			
Residual		0.5662	0.7524			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.172	2.426	-0.483			
Heating (No-heat)	0.537	0.274	1.959	1, 13.88	3.452	0.085
Sex (male)	-0.116	0.293	-0.397	1, 29.48	0.126	0.726
Day-14 body mass	0.051	0.131	0.389	1, 32.00	0.126	0.725
Plate	0.039	0.278	0.141	1, 26.65	0.017	0.899
Heating × sex	0.070	0.621	0.113	1, 32.50	0.010	0.921
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	<0.0001	<0.0001			
Nest of origin (n=20)	Intercept	0.1894	0.4352			
Residual		0.5335	0.7304			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	0.309	2.324	0.133			
Sex (male)	-0.107	0.266	-0.403			
Day-14 body mass	-0.019	0.123	-0.153			
Plate	-0.081	0.263	-0.308			
Nest temperature	-0.268	0.073	-3.672	1, 23.37	12.876	0.002
(Nest temperature) ²	0.035	0.035	0.991	1, 24.90	0.819	0.374
Sex × nest temperature	0.120	0.128	0.938	1, 29.54	0.681	0.416
Sex × (nest temperature) ²	-0.099	0.065	-1.523	1, 32.70	1.803	0.189
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	<0.0001	<0.0001			
Nest of origin (n=20)	Intercept	0.2481	0.4981			
Residual		0.4797	0.6926			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.235	2.273	-0.103			
Heating (No-heat)	0.612	0.249	2.457			
Sex (male)	0.004	0.271	0.015			
Day-14 body mass	0.002	0.123	0.015			
Plate	-0.190	0.266	-0.716			
Nest temperature (mean-centered within treatment)	-0.103	0.128	-0.805			
Heating × temperature	-0.265	0.154	-1.714	1, 15.78	2.448	0.138

Table S19: Results of linear mixed models for HSP70 expression levels

Day-14 nestling HSP70 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.7109	0.8431			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.2268	0.4762			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-2.537	2.353	-1.078			
Heating (No-heat)	0.378	0.342	1.104	1, 17.25	1.150	0.298
Sex (male)	-0.008	0.259	-0.033	1, 22.34	0.001	0.977
Day-14 body mass	0.119	0.126	0.950	1, 34.02	0.746	0.394
Plate	0.183	0.244	0.141	1, 19.82	0.461	0.505
Heating × sex	0.022	0.539	0.040	1, 23.71	0.001	0.972
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.5995	0.7743			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.2111	0.4959			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.419	2.388	-0.175			
Sex (male)	-0.035	0.245	-0.143			
Day-14 body mass	0.022	0.125	0.176			
Plate	0.172	0.234	0.736			
Nest temperature	-0.216	0.080	-2.710	1, 23.95	6.164	0.020
(Nest temperature) ²	-0.023	0.040	-0.577	1, 26.15	0.290	0.595
Sex × nest temperature	0.045	0.123	0.366	1, 25.68	0.108	0.745
Sex × (nest temperature) ²	0.007	0.062	0.113	1, 22.86	0.010	0.920
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6003	0.7748			
Nest of origin (n=20)	Intercept	<0.0001	0.0005			
Residual		0.2077	0.4558			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.613	2.359	-0.684			
Heating (No-heat)	0.394	0.319	1.236			
Sex (male)	0.058	0.253	0.229			
Day-14 body mass	0.070	0.126	0.555			
Plate	0.068	0.242	0.280			
Nest temperature (mean-centered within treatment)	-0.073	0.143	-0.507			
Heating × temperature	-0.231	0.187	-1.235	1, 22.41	1.356	0.256

Table S20: Results of linear mixed models for HSP90 expression levels

Day-14 nestling HSP90 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.4735	0.6881			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3562	0.5968			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-7.956	2.331	-3.414			
Heating (No-heat)	-0.006	0.317	-0.019	1, 17.07	<0.001	0.985
Sex (male)	-0.153	0.275	-0.558	1, 30.07	0.254	0.618
Day-14 body mass	0.419	0.125	3.360	1, 34.57	9.541	0.004
Plate	0.308	0.263	1.170	1, 26.80	1.122	0.299
Heating × sex	0.189	0.557	0.338	1, 28.92	0.088	0.769
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.4259	0.6526			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3544	0.5953			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.470	2.469	-2.621			
Sex (male)	-0.177	0.269	-0.659			
Day-14 body mass	0.346	0.130	2.661			
Plate	0.287	0.261	1.098			
Nest temperature	-0.130	0.077	-1.677	1, 23.17	2.268	0.146
(Nest temperature) ²	-0.020	0.038	-0.530	1, 25.24	0.243	0.627
Sex × (nest temperature) ²	0.030	0.067	0.453	1, 30.00	0.164	0.689
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.3964	0.6296			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3644	0.6037			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.564	2.364	-2.777			
Heating (No-heat)	0.026	0.302	0.085			
Sex (male)	-0.185	0.268	-0.691			
Day-14 body mass	0.345	0.127	2.724			
Plate	0.278	0.258	1.076			
Nest temperature (mean-centered within treatment)	-0.160	0.085	-1.874			
Heating × temperature	-0.372	0.173	-2.151	1, 20.54	4.054	0.057

Table S21: Results of linear mixed models for NRF2 expression levels

Day-14 nestling NRF2 expression (n=40)							
Model I. Heating treatment							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=31)	Intercept	0.4850	0.6964				
Nest of origin (n=20)	Intercept	<0.0001	<0.0001				
Residual		0.4215	0.6492				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-6.242	2.446	-2.552			
Heating (No-heat)		0.233	0.330	0.705	1, 17.05	0.465	0.504
Sex (male)		0.294	0.291	1.011	1, 31.01	0.834	0.368
Day-14 body mass		0.308	0.131	2.354	1, 34.32	4.694	0.037
Plate		0.316	0.279	1.131	1, 27.74	1.048	0.315
Heating × sex		-0.047	0.591	-0.080	1, 29.52	0.005	0.945
Model II. Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=31)	Intercept	0.3795	0.6160				
Nest of origin (n=20)	Intercept	<0.0001	<0.0001				
Residual		0.4109	0.6410				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-4.624	2.501	-1.848			
Sex (male)		0.266	0.276	0.961			
Day-14 body mass		0.228	0.132	1.730			
Plate		0.256	0.269	0.951			
Nest temperature		-0.182	0.077	-2.355	1, 22.89	5.194	0.032
(Nest temperature) ²		0.011	0.038	0.285	1, 24.93	0.070	0.794
Sex × nest temperature		0.164	0.132	1.241	1, 32.20	1.226	0.277
Sex × (nest temperature) ²		0.007	0.069	0.097	1, 31.17	0.008	0.932
Model III. Heating treatment × Nest box temperature							
Random effects:		Variance	Std. Dev.				
Nest of rearing (n=31)	Intercept	0.2992	0.5470				
Nest of origin (n=20)	Intercept	0.0394	0.1985				
Residual		0.3334	0.5774				
Fixed effects:		Estimate	Std. Error	t	df	F	p
Intercept		-5.598	2.266	-2.470			
Heating (No-heat)		0.234	0.279	0.840			
Sex (male)		0.486	0.263	1.848			
Day-14 body mass		0.276	0.122	2.268			
Plate		0.045	0.256	0.174			
Nest temperature (mean-centered within treatment)		0.070	0.129	0.543			
Heating × temperature		-0.461	0.167	-2.758	1, 20.35	6.649	0.018

References

1. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* 1997;25: 4692–4693. doi:10.1093/nar/25.22.4692
2. Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, et al. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *The Journal of Physiology.* 2012;590: 3349–3360. doi:10.1113/jphysiol.2012.230185
3. Stier A, Bize P, Hsu B-Y, Ruuskanen S. Plastic but repeatable: rapid adjustments of mitochondrial function and density during reproduction in a wild bird species. *Biology Letters.* 2019;15: 20190536. doi:10.1098/rsbl.2019.0536
4. Atema E, Mulder E, Noordwijk AJV, Verhulst S. Ultralong telomeres shorten with age in nestling great tits but are static in adults and mask attrition of short telomeres. *Molecular Ecology Resources.* 2019;19: 648–658. doi:10.1111/1755-0998.12996
5. Stier A, Massemin S, Zahn S, Tissier ML, Criscuolo F. Starting with a handicap: effects of asynchronous hatching on growth rate, oxidative stress and telomere dynamics in free-living great tits. *Oecologia.* 2015;179: 999–1010. doi:10.1007/s00442-015-3429-9
6. Stier A, Delestrade A, Bize P, Zahn S, Criscuolo F, Massemin S. Investigating how telomere dynamics, growth and life history covary along an elevation gradient in two passerine species. *Journal of Avian Biology.* 2016;47: 134–140. doi:10.1111/jav.00714
7. Salmon P, Nilsson JF, Nord A, Isaksson C. Urban environment shortens telomere length in nestling great tits, *Parus major*. *Biology Letters.* 2016; 1–4. doi:10.1098/rsbl.2016.0155&domain=pdf&date_stamp=2016-06-14
8. Salmón P, Nilsson JF, Watson H, Bensch S, Isaksson C. Selective disappearance of great tits with short telomeres in urban areas. *Proceedings of the Royal Society of London Series B: Biological Sciences.* 2017;284: 20171349–8. doi:10.1098/rspb.2017.1349
9. Kärkkäinen T, Briga M, Laaksonen T, Stier A. Within-individual repeatability in telomere length: A meta-analysis in nonmammalian vertebrates. *Mol Ecol.* 2021. doi:10.1111/mec.16155
10. Ellegren H, Fridolfsson AK. Male-driven evolution of DNA sequences in birds. *Nature genetics.* 1997;17: 182–184. doi:10.1038/ng1097-182
11. Chang H-W, Cheng C-A, Gu D-L, Chang C-C, Su S-H, Wen C-H, et al. High-throughput avian molecular sexing by SYBR green-based real-time PCR combined with melting curve analysis. *BMC Biotechnology.* 2008;8: 12–8. doi:10.1186/1472-6750-8-12
12. Casagrande S, Stier A, Monaghan P, Loveland JL, Boner W, Lupi S, et al. Increased glucocorticoid concentrations in early life cause mitochondrial inefficiency and short telomeres. *Journal Of Experimental Biology.* 2020;223: jeb222513-14. doi:10.1242/jeb.222513
13. Verhagen I, Laine VN, Mateman AC, Pijl A, Wit R de, Lith B van, et al. Fine-tuning of seasonal timing of breeding is regulated downstream in the underlying neuro-endocrine system in a small songbird. *Journal Of Experimental Biology.* 2019;222: jeb202481-22. doi:10.1242/jeb.202481
14. Criscuolo F, Bize P, Nasir L, Metcalfe NB, Foote CG, Griffiths K, et al. Real-time quantitative PCR assay for measurement of avian telomeres. *J Avian Biol.* 2009;40: 342–347. doi:10.1111/j.1600-048x.2008.04623.x
15. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw.* 2015;67. doi:10.18637/jss.v067.i01
16. Halekoh U, Højsgaard S. A Kenward-Roger Approximation and Parametric Bootstrap Methods for Tests in Linear Mixed Models - The R Package pbkrtest. *J Stat Softw.* 2014;59. doi:10.18637/jss.v059.i09