Telomere length vary with sex, hatching rank and year of birth in little owls, *Athene noctua*

François Criscuolo[1], Inès Fache[1,2], Bertrand Scaar[3], Sandrine Zahn[1] and Josefa Bleu[1]

[1] Université de Strasbourg, CNRS, IPHC UMR 7178, 67000 Strasbourg, France

[2] Université du Québec à Rimouski (UQAR), Département de Biologie, Chimie et Géographie, Rimouski, QC, G5L 3A1, Canada.

[3] Ligue pour la Protection des Oiseaux (LPO) Alsace, 1 rue du Wisch, 67560 Rosenwiller, France

Running title: telomere length in little owl

Key words: telomere, little owl, hatching rank, early-life effects, sex differences

Correspondance: josefa.bleu@iphc.cnrs.fr

16 **Abstract**

17 Telomeres are non-coding DNA sequences located at the end of linear chromosomes,
18 protecting genome integrity. In numerous taxa, telomeres shorten with age and telomere
19 length (TL) is positively correlated with longevity. Moreover, TL is also affected by
20 environmental stressors and/or resource-demanding situations particularly during early-life.
21 Thus, TL has been used as a physiological marker of individual quality and also as an indicator
22 of population trend in conservation physiology. In this study, we investigated the effects of
23 hatching rank, year of birth (2014 to 2017), sex and nest environment on TL of 137 little owls
24 nestlings (*Athene noctua*). Little owls' populations in Europe showed a marked declined in the
25 end of the 20th century. Nowadays, in the studied Alsatian population, the population is
26 increasing. In this study, our results indicated that telomeres are longer in females and,
27 independently of sex, in nestlings with the highest body condition. There was also a negative
28 effect of hatching rank but only for last-hatched nestlings in large clutches of 5 nestlings. We
29 did not find any effect of the environmental covariates on nestlings' TL. Finally, we found that
30 nestlings' TL were shorter the last year of the study, while nestlings' body condition stayed
31 unchanged over the same period. This result is intriguing given the local positive population
32 dynamics and is further discussed in the context of physiological conservation. Future studies
33 should investigate the link between reduced TL and survival prospects in this species.

34

35 **Introduction**

36 Telomeres are non-coding DNA structures, located at the end of the linear chromosomes,
37 serving as a safe-keeper for preservation of coding DNA over cell duplication (Blackburn,
38 1991). Thanks to the formation of a capped structure with specific shelterin proteins,
39 telomeres help the cell to distinguish real chromosome ends from DNA breaks, thereby
40 avoiding unappropriated cell emergency responses. Still, this telomere status is degrading
41 over time, due to the progressive loss of telomere sequences at each cell division, affecting its
42 functionality and triggering cell senescence (Blackburn, 2000). In addition, telomere
43 sequences are enriched in GC bases, making them highly sensitive to a well-known ageing
44 mechanism, the oxidative stress (von Zglinicki, 2002; Reichert & Stier, 2017) (but see
45 Boonekamp *et al.*, 2017). Such a stress-related property triggered the interest of evolutionary
46 biologists to study how telomeres (length or dynamics) may vary with age and thus be used
47 as a proxy to address the question of the existing variance in inter-specific longevity
48 (Haussmann *et al.*, 2003; Dantzer & Fletcher, 2015; Tricola *et al.*, 2018; Criscuolo *et al.*, 2021)
49 or inter-individual differences in lifespan and fitness (Beaulieu *et al.*, 2011; Foote *et al.*, 2011;
50 Boonekamp *et al.*, 2014; Nettle *et al.*, 2017; Bichet *et al.*, 2020; Chatelain *et al.*, 2020;
51 Fitzpatrick *et al.*, 2021; Sheldon *et al.*, 2021; Salmón & Burraco, 2022).

52 The importance of how early life conditions affect inter-individual telomere length
53 quickly appears as a key question to understand how somatic growth may shape individual
54 life trajectories in the context of life history trade-offs (Metcalfe & Monaghan, 2003;
55 Monaghan & Ozanne, 2018). This is based on the observation that growth is a period of high
56 energy metabolism (2-6 times basal metabolic rate, e.g. Kirkwood, 1991) to fuel intense rate
57 of cell division, which is likely to be costly in terms of telomere erosion (Vedder *et al.*, 2017;
58 Spurgin *et al.*, 2018). Studies have shown juveniles exposed to challenging conditions in early

59 life to have shorter telomeres. This could be due to reduced investment in somatic
60 maintenance as a consequence of low resource availability when conditions are harsh
61 (Herborn *et al.*, 2014; Nettle *et al.*, 2015, 2017; Reichert *et al.*, 2015; Angelier *et al.*, 2017;
62 Quque *et al.*, 2021). Interestingly, telomeres may also be affected during the pre-hatching
63 developmental period. For instance, temperature instability during egg development triggers
64 shorter telomere length at hatching in Japanese quail (*Coturnix Japonica*, Stier *et al.*, 2020),
65 and decreasing incubation temperature in the common tern (*Sterna hirundo*) slows down
66 growth rate and preserve telomere length in matched-body sized hatchlings (Vedder *et al.*,
67 2018). Yet, telomere dynamics are not only affected by stress effects. Producing eggs is costly
68 for the female, and depending on maternal characteristics and environmental conditions, we
69 can expect an adjustment of egg characteristics that will shape consequent embryonic traits
70 (Williams, 1994; Groothuis & Schwabl, 2008). As such, a large diversity of egg components
71 (like yolk and hormones), that may be positively or negatively correlated with each other, may
72 vary and modulate future offspring phenotype (Postma *et al.*, 2014; Williams & Groothuis,
73 2015). In addition, because an entire clutch is produced over sequential laying of consecutive
74 eggs, intra-clutch variability in egg traits may be part of a mother strategy of adaptation of the
75 chick's phenotype, and is then expected to follow the laying order (Groothuis *et al.*, 2005). In
76 particular, according to the brood reduction hypothesis, it is expected that the probability of
77 survival of last hatched nestlings (from last laid eggs) will be smaller than that of first hatched
78 ones in case of harsh conditions (Lack, 1947; Amundsen & Slagsvold, 1996). Thus, we can
79 expect maternal investment to decrease over the laying sequence. Telomere length is not an
80 exception, and progressive shortening has been observed within clutch laying order in captive
81 zebra finches (*Taeniopygia guttata*, Noguera *et al.*, 2016) . In this study, the astonishing result
82 is that the difference in embryonic telomere lengths between the 1st and the last laid eggs

83 represents 60% of the telomere loss an offspring will show over its first year of life. This source
84 of variation in telomere length may be important to consider since many studies have shown
85 negative consequences of telomere erosion on future individual fitness, *e.g.* jackdaws (*Corvus*
86 *monedula*, Boonekamp *et al.*, 2018), king penguins (*Aptenodytes patagonicus*, Geiger *et al.*,
87 2012) or in wild purple-crowned fairy-wrens (*Malurus coronatus coronatus*, Eastwood *et al.*,
88 2019), to name a few. Still, we lack data on the effect of laying order in many bird species and
89 on how laying order effect on telomere length may vary in relation to additional stress sources,
90 like environmental conditions in the wild (but see Kärkkäinen *et al.*, 2021).

91 Our study is based on 4 years of data from a wild population of Little Owl (*Athene*
92 *noctua*) reproducing in artificial nestboxes. All nestlings are ringed and measured before
93 fledging. After checking for hatching rank and environmental effects on chick phenotype, we
94 used telomere length measurements made on individual feather sampling to test how nestling
95 telomere length varied with hatching rank and with the local characteristics of nest
96 environment. To do so, we controlled for nestling sex, age, body condition, clutch size and
97 year of birth. To estimate nest environment characteristics, we calculated the proportion of
98 orchards, meadows, crops, buildings, water and forests around each nest box from land use
99 maps. In central Europe, the Little Owl is a bird species associated with traditional farmlands
100 and its optimal habitat should provide cavities, perches for hunting and short herbage with
101 invertebrates and small rodents (herbage size is linked to prey accessibility and availability,
102 van Nieuwenhuyse *et al.*, 2008). In particular, meadows and orchards are supposed to be food-
103 rich habitats (Michel *et al.*, 2017).

104 We predicted last hatched nestlings to be in worse condition (body mass, telomere
105 length) than first hatched nestlings according to the brood size reduction hypothesis. We also

106 predicted shorter telomeres in broods raised in unfavourable environments, *i.e.* more
107 proportion of buildings, water and forests around the nest box.
108
109 **Material and Methods**
110 *Model species and data collection*
111 The Little Owl is a small nocturnal raptor living in open or semi-open areas, such as farmland
112 or orchards (van Nieuwenhuyse *et al.*, 2008). The Little Owl is territorial and breeds in cavity,
113 including artificial nestboxes. In Alsace (France), numerous ringers and volunteers from the
114 French league for the protection of birds (LPO) installed and maintained more than 1,500 nest
115 boxes since 2006, thereby monitoring the yearly reproductive success of the local population.
116 Females lay 2-6 eggs in April, hatching occurs *ca.* 1 month later and nestlings are ringed
117 between 15-35 days of age. At ringing, nestlings' body mass was measured with an electronic
118 balance to the nearest 0.1 g, as well as tarsus length with a calliper to the nearest 0.1 mm, and
119 the length of the third primary feather with a ruler to the nearest mm. The measure of the
120 feather allows us to approximate the age of the nestling with the formula: age=(length of the
121 feather+36)/3.3, where the age is in days and the length of the feather is in mm (Juillard, 1984;
122 Hameau *et al.*, 2015). This formula is valid between age 15 and 35 when there is a linear
123 growth of the feather. Using the age of each nestling in a nest, the hatching rank was deduced.
124 When two nestling had the same estimated age, we assigned them the same hatching rank.
125 We also collected 3-6 ventral feathers that are stored in ethanol 70% at ambient temperature
126 during fieldwork and then at 4°C in the lab.
127 For this study, we used data collected on 142 nestlings from 39 broods from 2014 to 2017. All
128 those broods had more than 1 chick. We included in our study only broods with more than 1

129 chick in order to estimate the effect of hatching rank (n=3, n=14, n=16, n=6 for broods with
130 respectively 2, 3, 4 and 5 chicks).

131 *Land use around the nestbox*

132 To determine the land use around the nest boxes, we used a land cover database for Alsace
133 (Source: BdOCS CIGAL v2 2011/2012, www.geograndest.fr) which categorizes all the habitats
134 found in our study area. We used the software QGIS version 3.4.14 (QGIS Development Team,
135 2020) to map the active nest boxes and create a circular buffer zone of a 150 m radius around
136 each one of them. This radius was established thanks to data on home range size (Exo, 1992;
137 Génot, 2005) and the field observations made during the breeding season. Due to the high
138 number of habitats, we made groupings based on the environmental characteristics of each
139 variable to calculate the area (m^2) covered by each land type within the buffer zones. Our final
140 nest environment included six categories: (1) buildings, (2) meadows, (3) crops (crop fields,
141 hedges, and vineyard), (4) orchards, (5) forest and (6) water. Because of the rarity of the last
142 two categories, forest and water were pooled together. The surface of habitat of the different
143 categories were correlated with each other and thus we used in the model only the proportion
144 of surface of favorable habitat defined as the proportion of meadows and orchards in the
145 buffer.

146 *Relative telomere length (RTL) measurement and sexing*

147 Genomic DNA was extracted from feathers using an adapted protocol of the NucleoSpin Tissue
148 kit (Macherey Nagel, Düren, Germany). RTL was measured in the 142 nestlings in one 384-
149 wells plate, using the quantitative PCR (qPCR) methodology (see Electronic Supplementary
150 Material, ESM). Intra-plate repeatability of RTL (ICC, see (Eisenberg *et al.*, 2020)) was of 0.769.
151 Molecular sexing of nestlings was determined using the same extracted DNA (following
152 Griffiths *et al.*, 1998). Briefly, the technique is based on the existence of two conserved CHD

153 (chromo-helicase-DNA-binding) genes that are located on the sex chromosomes. The CHD-W
154 gene is located on the W chromosome (only in females) and the CHD-Z gene is located on the
155 Z chromosome (both in males and females). For technical reasons, sex could not be
156 determined in 5 nestlings. All the statistical analyses were performed on the remaining 137
157 nestlings with known sex.

158 *Statistical analyses*

159 We used R version 4.3.1 (R Core Team, 2023) to compute mixed models (package lme4 version
160 1.1-33 and lmerTest version 3.1-3). In all statistical models, brood identity was included as a
161 random factor to account for the non-independence of nestlings of the same brood. We
162 checked models' assumptions (homoscedasticity, normal distribution of residuals) graphically
163 using the package DHARMa (version 0.4.6). We assessed multicollinearity among predictors
164 by calculating variance inflation factor, VIF (package car, version 3.1-2).

165 Individual phenotypic characteristics

166 To test for inter-individual variation in body condition, we first calculated the Scale Mass Index
167 (SMI) following the formula of Peig & Green (2009): SMI = M_i $[L_0/L_i]^b$ where M_i and L_i are the
168 body mass and size measurements of individual i, b is the slope of the standardised major axis
169 (SMA) regression of log-transformed M on log-transformed L and L_0 is the arithmetic mean of
170 L for the study population. We then computed a linear mixed model with SMI as a dependent
171 variable and hatching rank, sex, nestling number, nestling age, cohort, the proportion of
172 meadows and orchards, the interaction between hatching rank and sex, and the interaction
173 between hatching rank and the proportion of meadows and orchards as fixed effects. From
174 this global model, we fitted every possible model and then selected a set of top models (AICc
175 threshold of 2). Then, if the null model was not the best model, we averaged the models from
176 these top models set (conditional average, package MuMIn, version 1.47.5).

177 Inter-individual variation in Relative Telomere Length

178 RTL were log-transformed before analyses. We computed a linear mixed model with individual
179 covariates (hatching rank, sex, the interaction between hatching rank and sex, nestling
180 number, nestling age, SMI and cohort) and environmental covariates (the proportion of
181 meadows and orchards, the interaction between hatching rank and this proportion) as fixed
182 effects. The model selection procedure was the same as described above.

183

184 **Results**

185 Individual phenotypic characteristics

186 Concerning individual covariates, there were no significant variables that explained variation
187 in SMI in our models. The fixed effects retained in the top models set (5 models) were the
188 proportion of meadows and orchards, nestling number and sex (see Table S1) but their effects
189 were not significantly different from 0 (see Figure S1). This is consistent with the fact that the
190 null model was in the top models set (see Table S1).

191

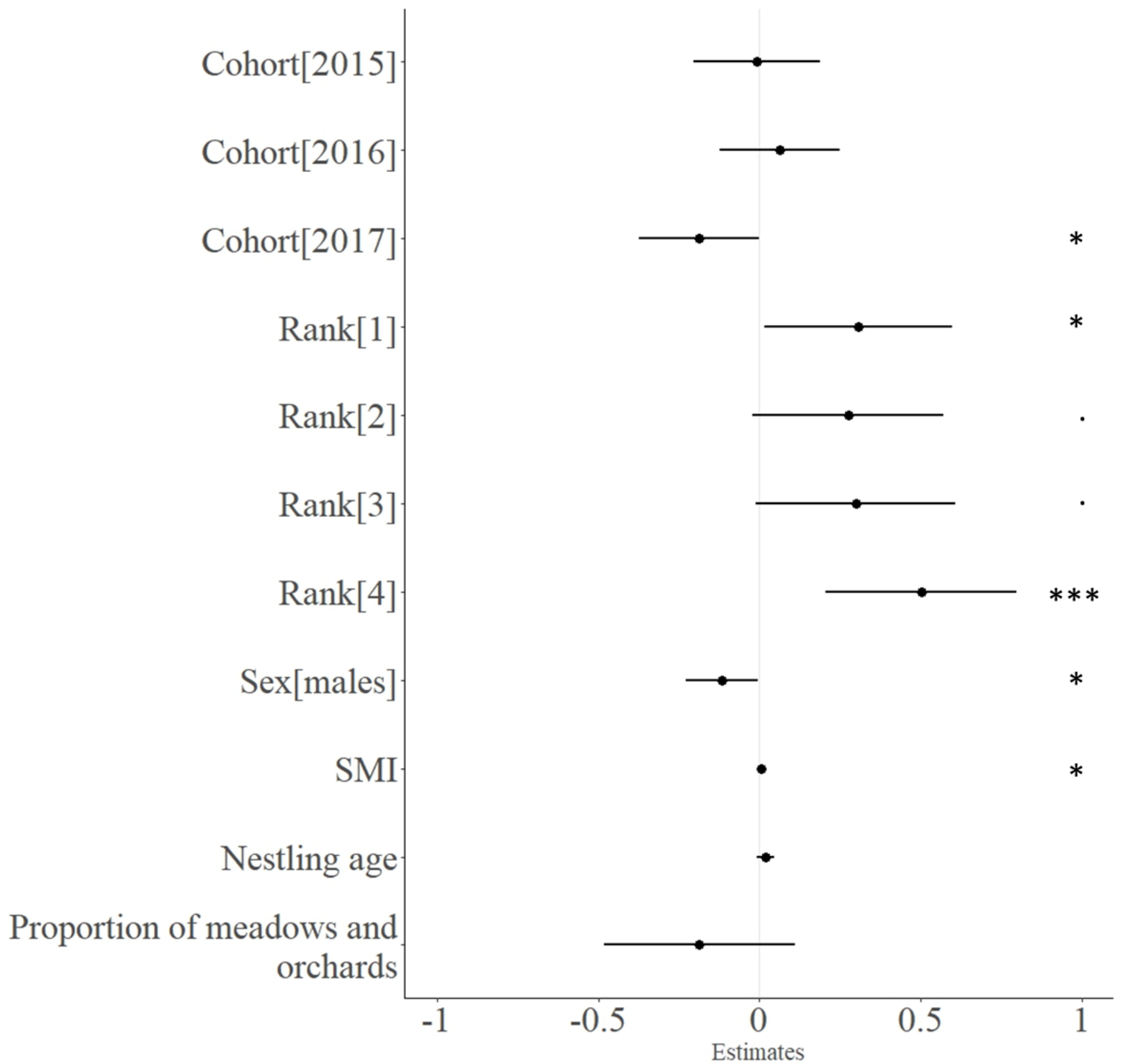
192

193 **Figure 1. Forest-plot of estimates for the average model of relative telomere length and**

194 **individual covariates (see Table S3).** Reference level for sex is females, for cohort is 2014

195 (the first year of the study) and for rank is 5 (last hatched chicks). Significance levels are

196 annotated with asterisks: *** p<00.1,**p<0.01,*p<0.05, . p<0.10



197

198

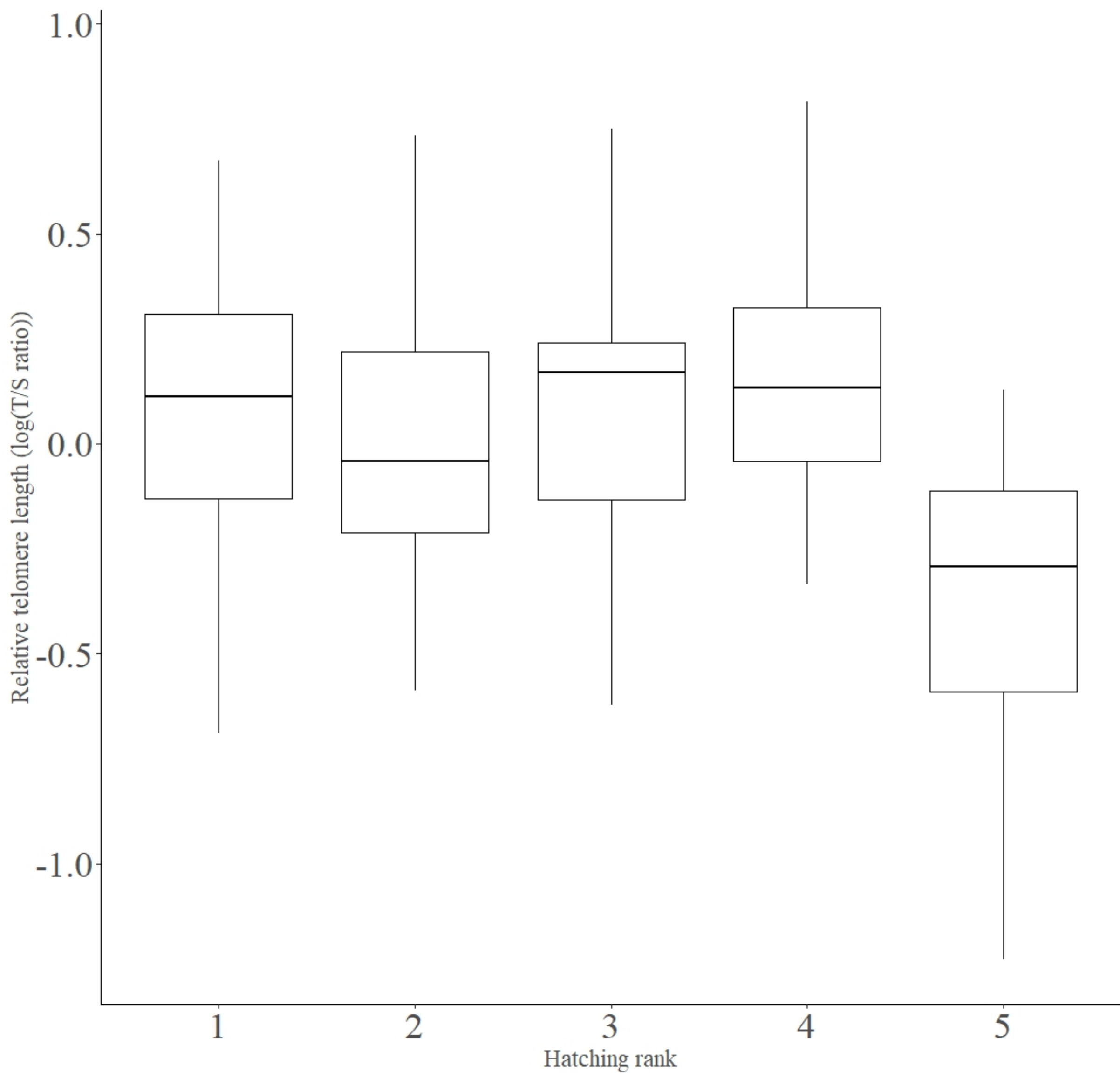
199

200

201 Inter-individual variation in Relative Telomere Length (RTL)

202 Concerning individual covariates, RTL was not dependent on nestling number and there was
203 no interaction between rank and sex, or between rank and the proportion of meadows and
204 orchards. The variables in the top models set (6 models) were rank, sex, SMI, cohort, nestling
205 age and the proportion of meadows and orchards (Table S3, Figure 1). Males have significantly
206 shorter telomeres than females and there is a small significant positive effect of SMI on RTL
207 (Figure 1). In addition, last hatched nestlings have shorter telomeres but only in the largest
208 brood of 5 nestlings (Figures 1 and 2). The effect of the year of birth is significant for the last
209 year of study, meaning that individuals born in 2017 have shorter telomeres than individuals
210 born earlier (Figures 1 and 3).Concerning environmental covariates, the proportion of
211 meadows and orchards was kept In the best model but has no significant effect on RTL (Figure
212 1).
213

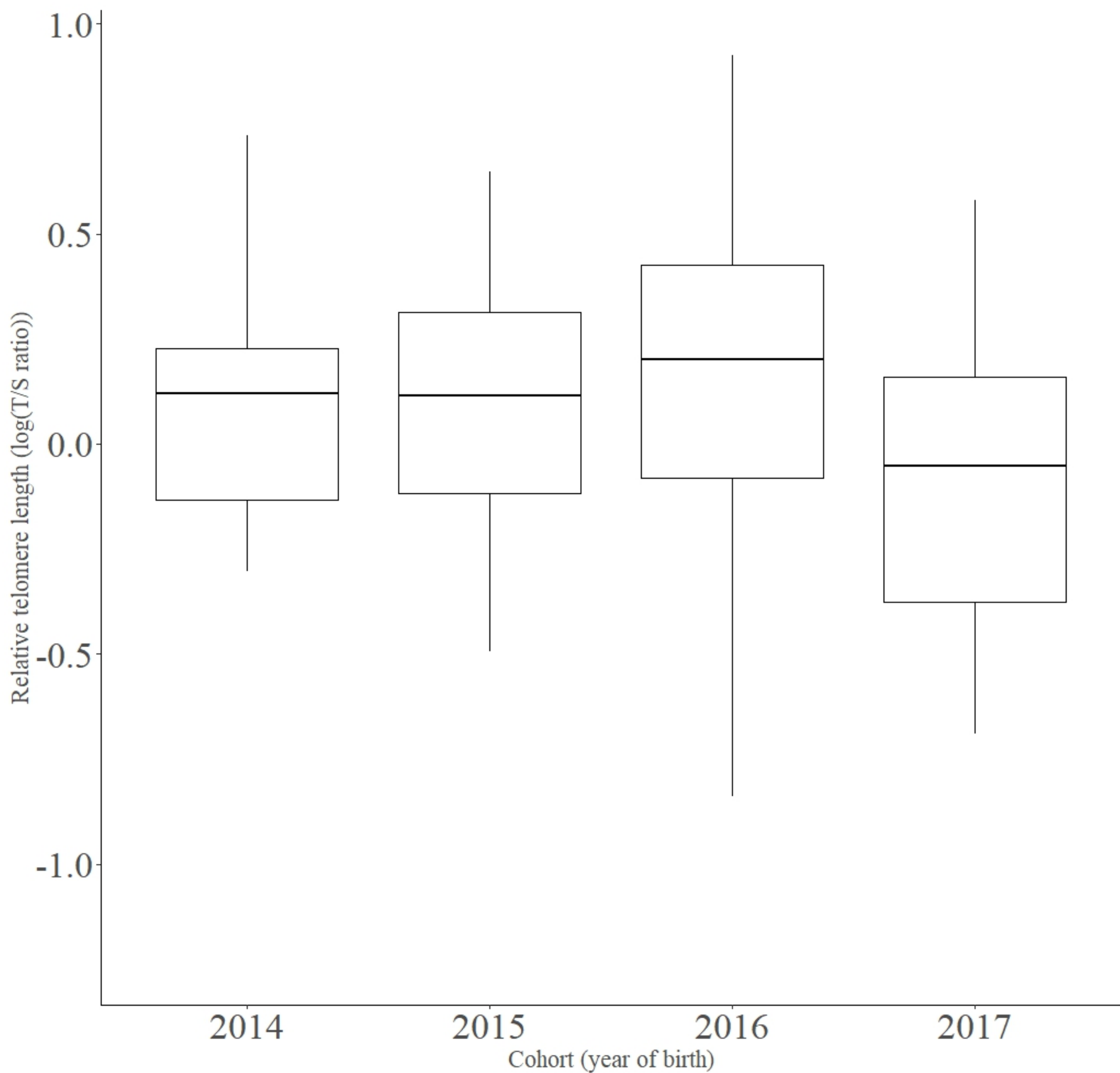
214 **Figure 2. The effect of hatching rank on the relative telomere length before fledging.**



215

216

217 **Figure 3. The effect of the cohort on the relative telomere length before fledging.**



218

219 **Discussion**

220 Based on the current knowledge on growth and telomeres in bird nestlings, we initially
221 predicted that RTL of little owl nestlings will be: (i) negatively related to the hatching rank and
222 (ii) negatively affected by the unfavourable nature of the nest surroundings. Our results
223 indicated that RTL are longer in females and, independently of sex, in nestlings with the
224 highest body condition. They also supported a mixed negative effect of hatching rank and
225 intra-brood competition on little owl nestlings' RTL, i.e. detectable only in the largest brood
226 size, suggesting that the effect of hatching rank on telomeres is dependent on a threshold

227 effect in this species. We did not find an effect of the environmental covariates on nestlings'
228 RTL. Finally, our scan of nestlings' RTL over years surprisingly underlined a possible progressive
229 shortening, independent of any changes in body condition.

230 Little owl nestlings' RTL were shorter in the last year of the study (2017) in comparison
231 to previous years (2014 onwards). Both telomere data and such year effect are of great
232 interest in the context of conservation physiology aiming at developing physiological markers
233 of individual quality to infer consequences at the population level (Beaulieu & Costantini,
234 2014; Lea *et al.*, 2018). Telomeres are good candidate to be such marker because telomere
235 length at a given age is not reflecting only the negative effects of time on the cells (i.e.
236 chronological age), it also points out the cumulative effects of stressors encountered over time
237 that may accelerate the rate of loss of telomere ends over the expected rate at a given age for
238 a given species (Asghar *et al.*, 2015; Louzon *et al.*, 2019; Chatelain *et al.*, 2020; Salmón &
239 Burraco, 2022). Thus, the use of telomere assay is potentially providing data that are useful to
240 establish survival rates at specific age stages, like the nestling period. Since deleterious
241 environmental conditions can affect negatively telomere length, the period of growth is
242 supposed to be the life stage where telomere sequences can be the most impacted (Salomons
243 *et al.*, 2009; Young *et al.*, 2013; Monaghan & Ozanne, 2018). Beside the classical explanation
244 that the growing period is particularly sensitive to environmental stressors because the rate
245 of cell division and/or the oxidative metabolism are higher in a growing organism, it is likely
246 that chicks can just hardly escape the trade-off between growth and survival. As such,
247 sustaining a fast (but not too fast, see below) rate of growth to shorten as much as possible
248 the nestling period may be done at a cost for telomere length. Thus, depending on the
249 harshness of early life environment, erosion of telomeres can be accelerated for a given age
250 (e.g. Boonekamp *et al.*, 2014; Stier *et al.*, 2015), leading the fledglings to be grown

251 physiologically old. In addition, variation in growth rate, due to changes in food availability,
252 may affect telomere length and not body mass or body condition. As an example, growth rate
253 may accelerate after a stunt when optimal feeding conditions are re-established, which are
254 known to trigger transient over-optimal compensatory growth rate and faster telomere
255 erosion (Metcalfe & Monaghan, 2001; Geiger *et al.*, 2012). This has, theoretically, obvious
256 consequences for the individuals in terms of survival prospects and recruitments as adult
257 breeders in the population, as early life telomere length or rate of telomere loss have been
258 shown to predict future individuals' survival (Boonekamp *et al.*, 2014; Watson *et al.*, 2015;
259 Wood & Young, 2019). Consequently, it also has the potential to affect the population
260 dynamics. First conceptualized few years ago (Stindl, 2004), such a hypothesis was recently
261 supported by studies conducted on ectotherms' populations (Dupoué *et al.*, 2017, 2022). In
262 the common lizard populations studied, analysis of telomere length in yearlings of populations
263 showing different risks of collapsing due to local global warming pointed out reduced mean
264 telomere length in the most endangered populations (Dupoué *et al.*, 2017). Thereafter, the
265 same group showed that short telomeres were already inherited in neonates of declining
266 populations, thereby suggesting (epi)genetic roots, i.e. progressive telomere shortening being
267 not only the result of bad early life conditions (Dupoué *et al.*, 2022). We cannot draw the same
268 conclusions in our case, particularly because (i) our data indicate that 2017 was the only year
269 with shorter telomeres and (ii) we lack data on inter-generational variation of telomere length.
270 It can be noted that in vertebrates, heritability estimates are moderate (Chik *et al.*, 2022), but
271 this recent meta-analysis has no data on raptors (Chik *et al.*, 2022). In addition, as low rates of
272 recruitments of juveniles as first-breeders is an important determinant of population decline
273 in the little owl (Le Gouar *et al.*, 2011), the link between reduced telomere length and survival
274 prospects of nestlings needs to be established. Finally, this result is counter-intuitive in our

275 study population of little owl since the population is expanding and not decreasing (Bersuder
276 & Wassmer, 2020), contrary to other populations (Andersen *et al.*, 2017). Whether 2017 is a
277 transient year with unknown bad conditions for chicks or is actually the start of a longer
278 adverse period for our population is currently unknown. Thus, the effects of yearly variations
279 in food availability, intra-nest competition or density on telomere length need to be addressed
280 in future studies.

281 Little owl female nestlings had longer telomeres than male ones. This has several
282 implications for our understanding of sex-differences in telomere dynamics and of its meaning
283 in terms of sex-biased life history. Differences in telomere length in relation to sex has been
284 previously illustrated in several taxa (reviewed in Barrett & Richardson, 2011), and particularly
285 in birds with sex-biased body size or investment in reproduction, producing no consistent
286 male-female differences (e.g. Caprioli *et al.*, 2013; Remot *et al.*, 2020; Saulnier *et al.*, 2022 for
287 no sex differences) (e.g. Bauch *et al.*, 2020 for sex differences). In our study, sex-differences
288 in RTL were observed at the nestling stage, with longer telomeres in the females. A previous
289 study showed that females were slightly but consistently of bigger size (Tschumi *et al.*, 2019),
290 however it is not the case in our population. Yet, we did not investigate nestlings growth rates,
291 which can be different even if the final size and/or body mass is similar (e.g. Criscuolo *et al.*,
292 2008). Higher growth rates are usually associated with shorter telomeres (Geiger *et al.*, 2012;
293 Monaghan & Ozanne, 2018) and generally the larger sex is growing at a slower rate in sexually
294 dimorphic bird species (e.g. Teather & Weatherhead, 1994).This may potentially account for
295 our sex-difference in telomere length as females may dilute the growth-body maintenance
296 trade-off over a longer period. However, we also found that, independently of sex, nestlings
297 in better body condition had in general longer telomeres. Thus, it is either unlikely that little
298 owl nestlings had to face such a growth-body maintenance trade-off, or that our result is

299 driven by high quality individuals that can sustain growth without showing any associated cost
300 in terms of telomere loss. Given that body mass is a determinant of survival from hatching to
301 fledging in little owl (Tschumi *et al.*, 2019), nestling telomeres rather acts as a proxy of
302 individual quality (Angelier *et al.*, 2019). In addition, our results do not match with the idea
303 that the heterogametic sex (*i.e.* females) would be more prone to telomere erosion than the
304 homogametic one (*i.e.* males) due to the unguarded expression of deleterious alleles of sex
305 chromosomes for telomere maintenance (see Barrett & Richardson, 2011; Remot *et al.*, 2020
306 for a deep discussion related to telomere dynamics). One alternative explanation lies on
307 optimal parental care towards the offspring sex with the highest chance of survival
308 (Hasselquist & Kempenaers, 2002). It has been shown previously that females have a higher
309 survival probability from hatching to fledging, independent of any variation in body mass
310 (Tschumi *et al.*, 2019). However, it is not known whether this sex-difference persists in older
311 individuals. In that context, the parents would favour female individuals, meaning that within
312 little owl broods females may, on average, benefit from better access to food resources due
313 to specific parental investment. This may lead to an attenuated body maintenance (*i.e.*
314 telomere length) and growth rate trade-off. Still, further study in our case is needed to
315 determine whether adaptive brood sex ratio actually occurs, since it may result from non-
316 adaptive additional effects (e.g. sex specific mortality, see Bortolotti, 1986; Hasselquist &
317 Kempenaers, 2002).

318 The hypothesis that RTL is an indicator of quality is further supported by the fact that,
319 in the largest clutches, the last hatchling of little owl presented the shortest telomeres. Even
320 if our sample size is small (i.e., 6 clutches with 5 eggs), our data are in accordance with the
321 brood size reduction hypothesis that predicts a lower investment with laying order. Still, our
322 data would restrict such an effect to the last laid egg. We cannot distinguish between effects

323 of the laying order *per se* on RTL (see introduction) and postnatal effects. Postnatal effects
324 may arise from selective parental care as discussed above. Last-hatched nestling may also
325 suffer from intra-brood competition. Indeed, in a brood, larger nestlings have a competitive
326 advantage compared to smaller nestlings for feeding ("Competitive advantage hypothesis",
327 Anderson *et al.*, 1993). A previous experiment testing the effect of competitive disadvantage
328 within a brood, based on the size of the nestlings cross-fostered among clutches, highlighted
329 an interesting increased telomere attrition of less competitive nestlings without affecting
330 body mass growth (in European starlings, Nettle *et al.*, 2015).

331 Finally, our study only suggested non-significant effects of nest surroundings. In other
332 studies, local habitat types around nests and also the heterogeneity of habitats available have
333 been shown to affect reproductive output in our species (Thorup *et al.*, 2010; Michel *et al.*,
334 2017). Moreover, it has been shown that the home range size is dependent on the
335 environment around the nest and also is different between males and females (Michel *et al.*,
336 2017). Thus, it may be important to consider the habitat at a fine scale. Future studies should
337 explore how environmental quality, food resources, parental care, chick growth, intra-brood
338 competition and sex-specific susceptibility to stressors are intertwined factors that determine
339 offspring telomere length and how all these factors affect population dynamics of little owls.

340

341 **Ethics statement.** This work is in accordance with the French legislation concerning the
342 capture and the biological sampling of wildlife. All the ringers of the project had received
343 ringing licenses and authorizations for feather sampling from the CRBPO (National Museum
344 of Natural History, Paris, France) as part of a program led by Bertrand Scaar (PP N°454).

345 **Data accessibility.** Datasets used in this study are openly available on zenodo (doi:
346 10.5281/zenodo.7701530).

347 **Authors' contributions.** JB and FC conceived the study. BS and volunteers collected the data.
348 SZ developed and performed the sexing and qPCR measurements. IF sorted the samples and
349 calculated the land use around nest boxes. JB and FC ran the statistical analyses and, with SZ
350 for the ESM, wrote the first draft of the manuscript. All authors provided comments on the
351 manuscript and agreed on the final version of the manuscript to be submitted for publication.

352 **Conflict of interest disclosure.** The authors declare that they comply with the PCI rule of
353 having no financial conflicts of interest in relation to the content of the article.

354 **Acknowledgements.** This study would not have been possible without the continuous
355 investment of local bird watchers and the league for the protection of birds (LPO), heavily
356 concerned by the preservation of the Little Owl in Alsace. We wish to thank warmly all of
357 them, and particularly Aurélie Barboiron, Marc Baumann, Jean Baysang, Dominique Bersuder,
358 Jean-Marc Bronner, Jérôme Isambert, Bernard Meurer, Nicolas Minéry, Anne Reszka, Pierre
359 Robellet and Freddy Sturm from the LPO. We also thank Mégane Jeannelle and Emma Jamann
360 for the help with the laboratory analyses. We are also grateful for all the persons that
361 financially supported our study though their donation to the Foundation of the University of
362 Strasbourg.

363 **Funding statement.** This work was supported by the CNRS and the Foundation of the
364 University of Strasbourg (https://fondation.unistra.fr/tag/iphc/).

365 **References**

366 Amundsen, T. & Slagsvold, T. 1996. Lack's brood reduction hypothesis and avian hatching
367 asynchrony: what's next? *Oikos* **76**: 613–620.

368 Andersen, L.H., Sunde, P., Pellegrino, I., Loeschcke, V. & Pertoldi, C. 2017. Using
369 population viability analysis, genomics, and habitat suitability to forecast future
370 population patterns of Little Owl *Athene noctua* across Europe. *Ecol. Evol.* **7**: 10987–
371 11001.

372 Anderson, D.J., Budde, C., Apanius, V., Gomez, J.E.M., Bird, D.M. & Weathers, W.W. 1993.
373 Prey size influences female competitive dominance in nestling american kestrels
374 (*Falco sparverius*). *Ecology* **74**: 367–376.

375 Angelier, F., Costantini, D., Blévin, P. & Chastel, O. 2017. Do glucocorticoids mediate the
376 link between environmental conditions and telomere dynamics in wild vertebrates? A
377 review. *Gen. Comp. Endocrinol.* **256**: 99–111.

378 Angelier, F., Weimerskirch, H., Barbraud, C. & Chastel, O. 2019. Is telomere length a
379 molecular marker of individual quality? Insights from a long-lived bird. *Funct. Ecol.*
380 **33**: 1076–1087.

381 Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H. & Bensch, S. 2015.
382 Hidden costs of infection: Chronic malaria accelerates telomere degradation and
383 senescence in wild birds. *Science* **347**: 436–438. American Association for the
384 Advancement of Science.

385 Barrett, E.L.B. & Richardson, D.S. 2011. Sex differences in telomeres and lifespan. *Aging*
386 *Cell* **10**: 913–921.

387 Bauch, C., Gatt, M.C., Granadeiro, J.P., Verhulst, S. & Catry, P. 2020. Sex-specific telomere
388 length and dynamics in relation to age and reproductive success in Cory's shearwaters.
389 *Mol. Ecol.* **29**: 1344–1357.

390 Beaulieu, M. & Costantini, D. 2014. Biomarkers of oxidative status: missing tools in
391 conservation physiology. *Conserv. Physiol.* **2**: cou014.

392 Beaulieu, M., Reichert, S., Le Maho, Y., Ancel, A. & Criscuolo, F. 2011. Oxidative status and
393 telomere length in a long-lived bird facing a costly reproductive event. *Funct. Ecol.*
394 **25**: 577–585.

395 Bersuder, D. & Wassmer, B. 2020. La chevêche d'Athéna *Athene noctua* dans l'Arrière-
396 Kochersberg (Alsace) : statut, habitat, reproduction et perspectives. *Ciconia* **44**: 89–
397 136.

398 Bichet, C., Bouwhuis, S., Bauch, C., Verhulst, S., Becker, P.H. & Vedder, O. 2020. Telomere
399 length is repeatable, shortens with age and reproductive success, and predicts
400 remaining lifespan in a long-lived seabird. *Mol. Ecol.* **29**: 429–441.

401 Blackburn, E.H. 1991. Structure and function of telomeres. *Nature* **350**: 569–573. Nature
402 Publishing Group.

403 Blackburn, E.H. 2000. Telomere states and cell fates. *Nature* **408**: 53–56. Nature Publishing
404 Group.

405 Boonekamp, J.J., Bauch, C., Mulder, E. & Verhulst, S. 2017. Does oxidative stress shorten
406 telomeres? *Biol. Lett.* **13**: 20170164.

407 Boonekamp, J.J., Mulder, E. & Verhulst, S. 2018. Canalisation in the wild: effects of
408 developmental conditions on physiological traits are inversely linked to their
409 association with fitness. *Ecol. Lett.* **21**: 857–864.

410 Boonekamp, J.J., Mulder, G.A., Salomons, H.M., Dijkstra, C. & Verhulst, S. 2014. Nestling
411 telomere shortening, but not telomere length, reflects developmental stress and
412 predicts survival in wild birds. *Proc. R. Soc. Lond. B Biol. Sci.* **281**: 20133287.

413 Bortolotti, G.R. 1986. Influence of sibling competition on nestling sex ratios of sexually
414 dimorphic birds. *Am. Nat.* **127**: 495–507. The University of Chicago Press.

415 Caprioli, M., Romano, M., Romano, A., Rubolini, D., Motta, R., Folini, M., *et al.* 2013.
416 Nestling telomere length does not predict longevity, but covaries with adult body size
417 in wild barn swallows. *Biol. Lett.* **9**: 20130340. Royal Society.

418 Chatelain, M., Drobniak, S.M. & Szulkin, M. 2020. The association between stressors and
419 telomeres in non-human vertebrates: a meta-analysis. *Ecol. Lett.* **23**: 381–398.

420 Chik, H.Y.J., Sparks, A.M., Schroeder, J. & Dugdale, H.L. 2022. A meta-analysis on the
421 heritability of vertebrate telomere length. *J. Evol. Biol.* **35**: 1283–1295.

422 Criscuolo, F., Dobson, F.S. & Schull, Q. 2021. The influence of phylogeny and life history on
423 telomere lengths and telomere rate of change among bird species: A meta-analysis.
424 *Ecol. Evol.* **11**: 12908–12922.

425 Criscuolo, F., Monaghan, P., Nasir, L. & Metcalfe, N.B. 2008. Early nutrition and phenotypic
426 development: 'catch-up' growth leads to elevated metabolic rate in adulthood. *Proc.*
427 *R. Soc. B Biol. Sci.* **275**: 1565–1570.

428 Dantzer, B. & Fletcher, Q.E. 2015. Telomeres shorten more slowly in slow-aging wild
429 animals than in fast-aging ones. *Exp. Gerontol.* **71**: 38–47.

430 Dupoué, A., Blaimont, P., Angelier, F., Ribout, C., Rozen-Rechels, D., Richard, M., *et al.*
431 2022. Lizards from warm and declining populations are born with extremely short
432 telomeres. *Proc. Natl. Acad. Sci.* **119**: e2201371119. Proceedings of the National
433 Academy of Sciences.

434 Dupoué, A., Rutschmann, A., Le Galliard, J.F., Clobert, J., Angelier, F., Marciau, C., *et al.*
435 2017. Shorter telomeres precede population extinction in wild lizards. *Sci. Rep.* **7**:
436 16976. Nature Publishing Group.

437 Eastwood, J.R., Hall, M.L., Teunissen, N., Kingma, S.A., Hidalgo Aranzamendi, N., Fan, M.,
438 *et al.* 2019. Early-life telomere length predicts lifespan and lifetime reproductive
439 success in a wild bird. *Mol. Ecol.* **28**: 1127–1137.

440 Eisenberg, D., Nettle, D. & Verhulst, S. 2020. How to calculate the repeatability (ICC) of
441 telomere length measures.

442 Exo, K.M. 1992. Population ecology of little owls *Athene noctua* in Central Europe: a review.
443 *Ecol. Conserv. Eur. Owls* 64–75. Joint Nature Conservation Committee.

444 Fitzpatrick, L.J., Olsson, M., Pauliny, A., While, G.M. & Wapstra, E. 2021. Individual
445 telomere dynamics and their links to life history in a viviparous lizard. *Proc. R. Soc. B*
446 *Biol. Sci.* **288**: 20210271. Royal Society.

447 Foote, C.G., Gault, E.A., Nasir, L. & Monaghan, P. 2011. Telomere dynamics in relation to
448 early growth conditions in the wild in the lesser black-backed gull. *J. Zool.* **283**: 203–
449 209.

450 Geiger, S., Le Vaillant, M., Lebard, T., Reichert, S., Stier, A., Le Maho, Y., *et al.* 2012.
451 Catching-up but telomere loss: half-opening the black box of growth and ageing trade-
452 off in wild king penguin chicks. *Mol. Ecol.* **21**: 1500–1510.

453 Génot, J.-C. 2005. *La chevêche d'athéna,* Athene noctua*, dans la Réserve de la biosphère des*
454 *Vosges du Nord: de 1984 à 2004*.

455 Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.G. 1998. A DNA test to sex most birds.
456 *Mol. Ecol.* **7**: 1071–1075.

457 Groothuis, T.G.G., Müller, W., von Engelhardt, N., Carere, C. & Eising, C. 2005. Maternal
458 hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav.*
459 *Rev.* **29**: 329–352.

460 Groothuis, Ton.G.G. & Schwabl, H. 2008. Hormone-mediated maternal effects in birds:
461 mechanisms matter but what do we know of them? *Philos. Trans. R. Soc. B Biol. Sci.*
462 **363**: 1647–1661.

463 Hameau, P.O., Hardouin, L., Lecomte, P., Penpeny-Lecomte, M., Scaar, B., Sève, D., *et al.*
464 2015. Protocole minimal commun pour le suivi de la Chevêche d'Athéna (*Athene*
465 *noctua*) par capture-recapture en nichoirs dans le cadre d'un programme personnel de
466 baguage en France. Muséum National d'Histoire Naturelle, Paris, France.

467 Hasselquist, D. & Kempenaers, B. 2002. Parental care and adaptive brood sex ratio
468 manipulation in birds. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **357**: 363–372. Royal
469 Society.

470 Haussmann, M.F., Winkler, D.W., O'Reilly, K.M., Huntington, C.E., Nisbet, I.C.T. & Vleck,
471 C.M. 2003. Telomeres shorten more slowly in long-lived birds and mammals than in
472 short–lived ones. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 1387–1392.

473 Herborn, K.A., Heidinger, B.J., Boner, W., Noguera, J.C., Adam, A., Daunt, F., *et al.* 2014.
474 Stress exposure in early post-natal life reduces telomere length: an experimental
475 demonstration in a long-lived seabird. *Proc. R. Soc. B Biol. Sci.* **281**: 20133151. Royal
476 Society.

477 Juillard, M. 1984. *La chouette chevêche*. "Nos oiseaux" Société romande pour l'étude et la
478 protection des oiseaux.

479 Kärkkäinen, T., Teerikorpi, P., Schuett, W., Stier, A. & Laaksonen, T. 2021. Interplays
480 between pre- and post-natal environments affect early-life mortality, body mass and
481 telomere dynamics in the wild. *J. Exp. Biol.* **224**: jeb231290.

482 Kirkwood, J.K. 1991. Energy requirements for maintenance and growth of wild mammals,
483 birds and reptiles in captivity. *J. Nutr.* **121**: S29–S34.

484 Lack, D. 1947. The significance of clutch-size. *Ibis* **89**: 302–352.

485 Le Gouar, P.J., Schekkerman, H., van der Jeugd, H.P., Boele, A., van Harxen, R., Fuchs, P., *et*
486 *al.* 2011. Long-term trends in survival of a declining population: the case of the little
487 owl (*Athene noctua*) in the Netherlands. *Oecologia* **166**: 369–379.

488 Lea, J.M.D., Walker, S.L., Kerley, G.I.H., Jackson, J., Matevich, S.C. & Shultz, S. 2018.
489 Non-invasive physiological markers demonstrate link between habitat quality, adult
490 sex ratio and poor population growth rate in a vulnerable species, the Cape mountain
491 zebra. *Funct. Ecol.* **32**: 300–312.

492 Louzon, M., Coeurdassier, M., Gimbert, F., Pauget, B. & de Vaufleury, A. 2019. Telomere
493 dynamic in humans and animals: Review and perspectives in environmental
494 toxicology. *Environ. Int.* **131**: 105025.

495 Metcalfe, N.B. & Monaghan, P. 2001. Compensation for a bad start: grow now, pay later?
496 *Trends Ecol. Evol.* **16**: 254–260.

497 Metcalfe, N.B. & Monaghan, P. 2003. Growth versus lifespan: perspectives from
498 evolutionary ecology. *Exp. Gerontol.* **38**: 935–940.

499 Michel, V.T., Naef-Daenzer, B., Keil, H. & Grüebler, M.U. 2017. Reproductive consequences
500 of farmland heterogeneity in little owls (*Athene noctua*). *Oecologia* **183**: 1019–1029.

501 Monaghan, P. & Ozanne, S.E. 2018. Somatic growth and telomere dynamics in vertebrates:
502 relationships, mechanisms and consequences. *Philos. Trans. R. Soc. B Biol. Sci.* **373**:
503 20160446. Royal Society.

504 Nettle, D., Andrews, C., Reichert, S., Bedford, T., Kolenda, C., Parker, C., *et al.* 2017. Early-
505 life adversity accelerates cellular ageing and affects adult inflammation: Experimental
506 evidence from the European starling. *Sci. Rep.* **7**: 1–10.

507 Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T. & Bateson, M. 2015. An
508 experimental demonstration that early-life competitive disadvantage accelerates
509 telomere loss. *Proc. R. Soc. B Biol. Sci.* **282**: 20141610. Royal Society.

510 Noguera, J.C., Metcalfe, N.B., Reichert, S. & Monaghan, P. 2016. Embryonic and postnatal
511 telomere length decrease with ovulation order within clutches. *Sci. Rep.* **6**: 25915.
512 Nature Publishing Group.

513 Peig, J. & Green, A.J. 2009. New perspectives for estimating body condition from
514 mass/length data: the scaled mass index as an alternative method. *Oikos* **118**: 1883–
515 1891.

516 Postma, E., Siitari, H., Schwabl, H., Richner, H. & Tschirren, B. 2014. The multivariate egg:
517 quantifying within- and among-clutch correlations between maternally derived yolk
518 immunoglobulins and yolk androgens using multivariate mixed models. *Oecologia*
519 **174**: 631–638.

520 QGIS Development Team. 2020. QGIS Geographic Information System. QGIS Association.

521 Quque, M., Paquet, M., Zahn, S., Théron, F., Faivre, B., Sueur, C., *et al.* 2021. Contrasting
522 associations between nestling telomere length and pre and postnatal helpers' presence
523 in a cooperatively breeding bird. *Oecologia* **196**: 37–51.

524 R Core Team. 2023. R: a language and environment for statistical computing.

525 Reichert, S., Criscuolo, F., Zahn, S., Arrivé, M., Bize, P. & Massemin, S. 2015. Immediate
526 and delayed effects of growth conditions on ageing parameters in nestling zebra
527 finches. *J. Exp. Biol.* **218**: 491–499.

528 Reichert, S. & Stier, A. 2017. Does oxidative stress shorten telomeres in vivo? A review. *Biol.*
529 *Lett.* **13**: 20170463.

530 Remot, F., Ronget, V., Froy, H., Rey, B., Gaillard, J.-M., Nussey, D.H., *et al.* 2020. No sex
531 differences in adult telomere length across vertebrates: a meta-analysis. *R. Soc. Open*
532 *Sci.* **7**: 200548. Royal Society.

533 Salmón, P. & Burraco, P. 2022. Telomeres and anthropogenic disturbances in wildlife: A
534 systematic review and meta-analysis. *Mol. Ecol.* **in press**.

535 Salomons, H.M., Mulder, G.A., Zande, L. van de, Haussmann, M.F., Linskens, M.H.K. &
536 Verhulst, S. 2009. Telomere shortening and survival in free-living corvids. *Proc. R.*
537 *Soc. Lond. B Biol. Sci.* **276**: 3157–3165.

538 Saulnier, A., Bleu, J., Lemonnier, G., Uhlrich, P., Zahn, S. & Massemin, S. 2022. Does the
539 urban environment act as a filter on the individual quality of birds? *Birds* **3**: 84–98.
540 Multidisciplinary Digital Publishing Institute.

541 Sheldon, E.L., Eastwood, J.R., Teunissen, N., Roast, M.J., Aranzamendi, N.H., Fan, M., *et al.*
542 2021. Telomere dynamics in the first year of life, but not later in life, predict lifespan
543 in a wild bird. *Mol. Ecol.* **in press**.

544 Spurgin, L.G., Bebbington, K., Fairfield, E.A., Hammers, M., Komdeur, J., Burke, T., *et al.*
545 2018. Spatio-temporal variation in lifelong telomere dynamics in a long-term
546 ecological study. *J. Anim. Ecol.* **87**: 187–198.

547 Stier, A., Massemin, S., Zahn, S., Tissier, M.L. & Criscuolo, F. 2015. Starting with a
548 handicap: effects of asynchronous hatching on growth rate, oxidative stress and
549 telomere dynamics in free-living great tits. *Oecologia* **179**: 999–1010.

550 Stier, A., Metcalfe, N.B. & Monaghan, P. 2020. Pace and stability of embryonic development
551 affect telomere dynamics: an experimental study in a precocial bird model. *Proc. R.*
552 *Soc. B Biol. Sci.* **287**: 20201378. Royal Society.

553 Stindl, R. 2004. Is telomere erosion a mechanism of species extinction? *J. Exp. Zool.* **302B**:
554 111–120.

555 Teather, K.L. & Weatherhead, P.J. 1994. Allometry, Adaptation, and the Growth and
556 Development of Sexually Dimorphic Birds. *Oikos* **71**: 515–525. [Nordic Society
557 Oikos, Wiley].

558 Thorup, K., Sunde, P., Jacobsen, L.B. & Rahbek, C. 2010. Breeding season food limitation
559 drives population decline of the Little Owl Athene noctua in Denmark. *Ibis* **152**: 803–
560 814.

561 Tricola, G.M., Simons, M.J.P., Atema, E., Boughton, R.K., Brown, J.L., Dearborn, D.C., *et*
562 *al.* 2018. The rate of telomere loss is related to maximum lifespan in birds. *Phil Trans*
563 *R Soc B* **373**: 20160445.

564 Tschumi, M., Humbel, J., Erbes, J., Fattebert, J., Fischer, J., Fritz, G., *et al.* 2019. Parental sex
565 allocation and sex-specific survival drive offspring sex ratio bias in little owls. *Behav.*
566 *Ecol. Sociobiol.* **73**: 85.

567 van Nieuwenhuyse, D.V., Génot, J.-C. & Johnson, D.H. 2008. *The Little Owl: Conservation,*
568 *Ecology and Behavior of* Athene Noctua. Cambridge University Press.

569 Vedder, O., Verhulst, S., Bauch, C. & Bouwhuis, S. 2017. Telomere attrition and growth: a
570 life-history framework and case study in common terns. *J. Evol. Biol.* **30**: 1409–1419.

571 Vedder, O., Verhulst, S., Zuidersma, E. & Bouwhuis, S. 2018. Embryonic growth rate affects
572 telomere attrition: an experiment in a wild bird. *J. Exp. Biol.* **221**: jeb181586.

573 von Zglinicki, T. 2002. Oxidative stress shortens telomeres. *Trends Biochem. Sci.* **27**: 339–
574 344.

575 Watson, H., Bolton, M. & Monaghan, P. 2015. Variation in early-life telomere dynamics in a
576 long-lived bird: links to environmental conditions and survival. *J. Exp. Biol.* **218**: 668–
577 674.

578 Williams, T.D. 1994. Intraspecific variation in egg size and egg composition in birds: effects
579 on offspring fitness. *Biol. Rev.* **69**: 35–59.

580 Williams, T.D. & Groothuis, T.G.G. 2015. Egg quality, embryonic development, and post-
581 hatching phenotype: an integrated perspective. In: *Nests, eggs, and incubation: new*
582 *ideas about avian reproduction*, pp. 113–126. Oxford University Press Oxford.

583 Wood, E.M. & Young, A.J. 2019. Telomere attrition predicts reduced survival in a wild social
584 bird, but short telomeres do not. *Mol. Ecol.* **28**: 3669–3680.

585 Young, R.C., Kitaysky, A.S., Haussmann, M.F., Descamps, S., Orben, R.A., Elliott, K.H., *et*
586 *al.* 2013. Age, Sex, and Telomere Dynamics in a Long-Lived Seabird with Male-
587 Biased Parental Care. *PLOS ONE* **8**: e74931. Public Library of Science.

588

589

ESM

Amplification of telomere repeats using q-PCR methodology

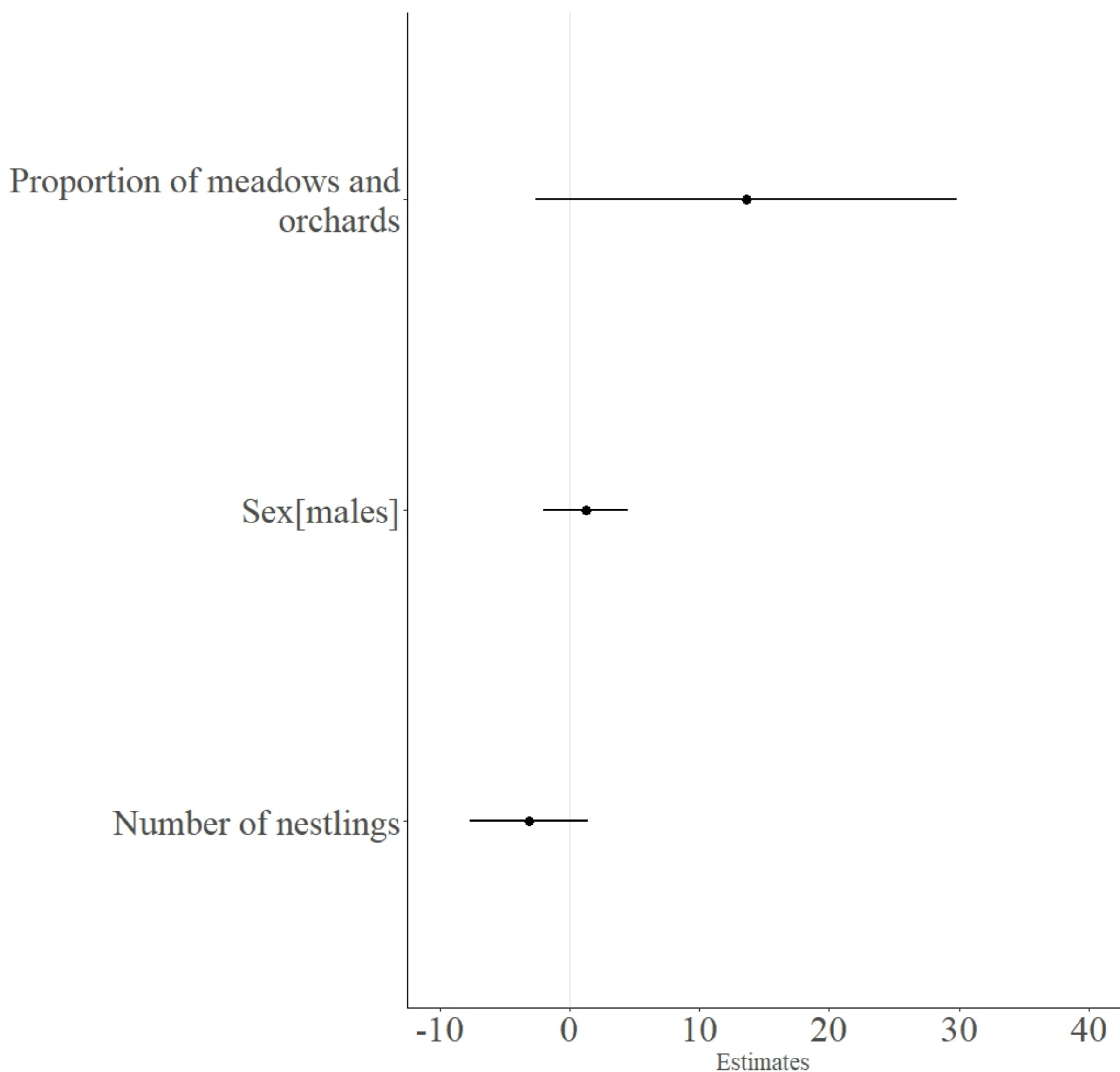
The protocol for DNA extraction from feathers provided us with sufficient amount of DNA to run both sexing and telomere determinations. One to three feathers per individual were selected and a 0.5-1 cm piece from each feather were cut in small pieces with a sterilized scissor. After digestion, feather quills will remain unlysed. For samples containing unlysed quills, we centrifuge briefly and we transfer the supernatant to another tube before proceeding with step 4 of the standard protocol.

Individual relative telomere length (RTL) were obtained following the qPCR methodology previously used in several bird species by our group (*e.g.* Criscuolo et al. 2009, Bize et al. 2009, Criscuolo et al. 2020, Chatelain et al. 2021). DNA quantity and quality were assessed based on spectrophotometer absorbance (Nano-Drop 1000, Thermo Fisher Scientific, Waltham, MA, USA, ratios A260/280 and A260/230) and gel migration. Individual DNA were all diluted to a concentration of 5.0 ng/µL, and further used for RTL determination by qPCR. To control for variation in DNA concentrations among diluted samples (due to potential pipetting errors), which may induce a methodological bias to the final RTL values, we amplified, for each individual, a genomic DNA sequence, defined so far as non-variable in copy numbers. The gene used in our species was RAG-1 gene (recombination activating protein 1 gene, NCBI number EU348872.1). Amplifications were conducted in two 384 wells-plates filled by a calibrated automated liquid handling workstation (Epmotion, Eppendorf, Montesson, France), using one distinct plate for control gene and telomere amplifications, due to the different qPCR conditions due to primers sequences properties. Conditions of amplification were 2 min at 95°C followed by 40 cycles of 15 s at 95°C, 30 s at 56°C and 1 min at 72°C (control gene) and of 2 min at 95°C followed by 30 cycles of 15 s at 95°C, 30 s at 56°C and 30 sec at 72°C, (telomere sequence). Reactions were done in a master mix prepared for each primer set, with 5 µL GoTaq QPCR Mix (Promega, Madison, WI, USA). We used 10 ng of DNA (in a volume of 2 µL), to which we added the telomere primers at a concentration of 200 nM or the control gene primers at 400 nM (for a final reaction volume of 10 µL in each well, completed with ultra-pure water). In both plates (control gene and telomere sequences) we amplified individuals' DNA samples plus three quality control references. A DNA golden sample (as a mix of22 individual samples randomly chosen) that was used as the reference value of 1 for RTL calculations. A dilution curve obtained from the amplification of a randomly chosen reference sample that was serially diluted (from 10 to 0.625 ng/mL). Dilution curves enable us to assess quality of control gene and telomere sequences qPCR amplifications (i.e. efficiency values (control gene 0.999; telomere sequences 0.993) and r^2 (0.993 and 0.995, respectively) of the dilution curves). A negative control sample (ultra-pure water) to control for putative contaminations of non-bird DNA. All runs ended by a fusion curve to verify the absence of non-specific amplifications. RTL values were calculated following Pfaffl (2001), shortly as the ratio between Telomere (T) and Control gene (S) Cq values, controlled for their respective amplification efficiencies and expressed relatively to the golden sample T/S value of 1. All samples were run in duplicates and intra-individual repeatability of RTL, evaluated using the Intra Class Coefficient (Eisenberg *et al.*, 2020), was of 0.769.

634 **Table S1. Top models set for models of SMI.** For continuous variables, each value
635 represents the estimate of the effect; for categorical variables, there is a "+" when the variable
636 is retained in a model.
637 df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.
638

Intercept	Nestling number	Proportion of meadows and orchards	Sex	df	AICc	delta
125.8		14.44		4	1057.3	0.00
145.3	-3.52			4	1058.0	0.70
136.7	-2.66	11.93		5	1058.1	0.82
132.3				3	1058.3	0.93
125.3		14.32	+	5	1058.9	1.59

639
640 **Figure S1. Forest-plot of estimates for the average model from Table S1.** Reference level
641 for sex is females.



642
643
644

645 **Table S3. Top models set for models of RTL.** For continuous variables, each value
646 represents the estimate of the effect; for categorical variables, there is a "+" when the variable
647 is retained in a model.
648 df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.
649

Intercept	Proportion of meadows and orchards	Nestling age	Cohort	Rank	Sex	SMI	df	AICc	delta
-0.82			+	+	+	0.0049	12	103.8	0.00
-0.86				+	+	0.0046	9	104.6	0.81
-1.16		0.019	+	+	+	0.0047	13	104.6	0.83
-0.84	-0.17		+	+	+	0.0055	13	104.9	1.12
-0.86			+	+		0.0046	11	105.3	1.46
-1.23	-0.20	0.021	+	+	+	0.0054	14	105.3	1.48

650
651

652 **Supplementary references**
653

654 Bize, P., Criscuolo, F., Metcalfe, N.B., Nasir, L. & Monaghan, P. 2009. Telomere dynamics rather than
655 age predict life expectancy in the wild. *Proc. R. Soc. Lond. B* **276**: 1679–1683.

656 Chatelain, M., Massemin, S., Zahn, S., Kurek, E., Bulska, E. & Szulkin, M. 2021. Urban metal pollution
657 explains variation in reproductive outputs in great tits and blue tits. *Science of The Total*
658 *Environment* **776**: 145966.

659 Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N.B., Foote, C.G., Griffiths, K., *et al.* 2009. Real-time
660 quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology* **40**:
661 342–347.

662 Criscuolo, F., Torres, R., Zahn, S. & Williams, T.D. 2020. Telomere dynamics from hatching to sexual
663 maturity and maternal effects in the 'multivariate egg.' *Journal of Experimental Biology* **223**:
664 jeb232496.

665 Eisenberg, D., Nettle, D. & Verhulst, S. 2020. How to calculate the repeatability (ICC) of telomere
666 length measures.

667 Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic*
668 *Acids Research* **29**: e45.

669
670