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

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Running title: telomere length in little owl

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

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Abstract

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

Introduction

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

The importance of how early life conditions affect inter-individual telomere length quickly appears as a key question to understand how somatic growth may shape individual life trajectories in the context of pleiotropy (Metcalfe & Monaghan, 2003; Monaghan & Ozanne, 2018). This is based on the observation that growth is a period of high energy metabolism (2-6 times basal metabolic rate, e.g. Kirkwood, 1991) to fuel intense rate of cell division, both physiological traits likely to be costly in terms of telomere erosion (Vedder *et al.*, 2017; Spurgin *et al.*, 2018). Short telomeres in fledgling may then reflect accumulated stress that impaired investment in cell maintenance of the growing organism, due to deleterious effects of sub-optimal nutritional, social and/or hormonal environments (Herborn *et al.*, 2014; Nettle *et al.*, 2015, 2017; Reichert *et al.*, 2015; Angelier *et al.*, 2017; Quque *et al.*, 2021). Interestingly, telomeres may also be affected during the pre-hatching developmental period. For instance, temperature instability during egg development triggers shorter telomere length at hatching in Japanese quail (*Coturnix Japonica*, Stier *et al.*, 2020), and decreasing incubation temperature in the common tern (*Sterna hirundo*) slows down growth rate and save telomere length in matched-body sized hatchlings (Vedder *et al.*, 2018). Yet, telomere dynamics are not only affected by stress effects. Producing eggs is costly for the female, and depending on maternal characteristics and environmental conditions, we can expect an adjustment of egg characteristics that will shape consequent embryonic traits (Williams, 1994; Groothuis & Schwabl, 2008). As such, a large diversity of egg components (like yolk and hormones) may vary and modulate the future offspring phenotype in a synergistic or antagonistic ways, leading to the concept of multivariate egg (Postma *et al.*, 2014; Williams & Groothuis, 2015). In addition, because an entire clutch is produced over sequential laying of consecutive eggs, intra-clutch variability in multivariate egg traits may be part of a mother strategy of adaptation of the chick’s phenotype, and is then expected to follow the laying order (Groothuis *et al.*, 2005). In particular, according to the brood reduction hypothesis, it is expected that the probability of survival of last hatched nestlings (from last laid eggs) will be smaller than that of first hatched ones in case of harsh conditions (Lack, 1947; Amundsen & Slagsvold, 1996). Thus, we can expect maternal investment to decrease over the laying sequence. Telomere length is not an exception, and progressive shortening has been observed within clutch laying order in captive zebra finches (*Taeniopygia guttata*, Noguera *et al.*, 2016) as well as inter-individual variation within the multivariate egg concept (Criscuolo *et* *al.*, 2020). In the former study, the astonishing result is that the difference in embryonic telomere lengths between the 1st and the last laid eggs represents 60% of the telomere loss an offspring will show over its first year of life. Given that the negative consequences of fast telomere erosion during growth on future individual fitness prospects are legions, *e.g.* jackdaws (*Corvus monedula*, Boonekamp *et al.*, 2018), king penguins (*Aptenodytes patagonicus*, Geiger *et al.*, 2012) or in wild purple-crowned fairy-wrens (*Malurus coronatus coronatus*, Eastwood *et al.*, 2019), to name a few, variability in telomere length within clutch is likely not an epiphenomenon. Still, we lack data on other bird species and on how laying order effect on telomere length may vary in relation to additional stress sources, like environmental conditions in the wild (but see Kärkkäinen *et al.*, 2021).

Our study is based on 4 years of data from a wild population of Little Owl (*Athene noctua*) reproducing in artificial nestboxes. All nestlings are ringed and measured before fledging. First, we tested whether individual characteristics (sex and body mass) are dependent on hatching rank and on environmental characteristics around the nest. Second, using telomere length measurements made on individual feather sampling, we tested how nestling telomere length varied (i) with hatching rank, controlling for nestling sex, age, body condition, clutch size and year of birth, and (ii) with the local characteristics of nest environment. To estimate nest environment characteristics, we calculated the proportion of orchards, meadows, crops, buildings, water and forests around each nest box from land use maps. In central Europe, the Little Owl is a bird species associated with traditional farmlands and its optimal habitat should provide cavities, perches for hunting and short herbage with invertebrates and small rodents (herbage size is linked to prey accessibility and availability, van Nieuwenhuyse *et al.*, 2008). In particular, meadows and orchards are supposed to be food-rich habitats (Michel *et al.*, 2017).

We predicted last hatched nestlings to be in worse condition (body mass, telomere length) than first hatched nestlings according to the brood size reduction hypothesis. We also predicted shorter telomeres in broods raised in unfavourable environments, *i.e.* more proportion of buildings, water and forests around the nest box.

Material and Methods

Model species and data collection

The Little Owl is a small nocturnal raptor living in open or semi-open areas, such as farmland or orchards (van Nieuwenhuyse *et al.*, 2008). The Little Owl is territorial and breeds in cavity, including artificial nestboxes. In Alsace (France), numerous ringers and volunteers from the French league for the protection of birds (LPO) installed and maintained more than 1,500 nest boxes since 2006, thereby monitoring the yearly reproductive success of the local population. Females lay 2-6 eggs in April, hatching occurs *ca.* 1 month later and nestlings are ringed between 15-35 days of age. At ringing, nestlings' body mass was measured with an electronic balance to the nearest 0.1 g, as well as tarsus length with a calliper to the nearest 0.1 mm, and the length of the third primary feather with a ruler to the nearest mm. The measure of the feather allows us to approximate the age of the nestling with the formula: age=(length of the feather+36)/3.3 (Juillard, 1984; Hameau *et al.*, 2015). Using the age of each nestling in a nest, the hatching order was deduced. We also collected 3-6 ventral coverts that are stored in ethanol 70% at ambient temperature during fieldwork and then at 4°C in the lab.

For this study, we used data collected on 142 nestlings from 39 broods from 2014 to 2017. In order to estimate the effect of hatching rank we used only broods with more than 1 chick (n=3, n=14, n=16, n=6 for broods with respectively 2, 3, 4 and 5 chicks).

Land use around the nestbox

To determine the land use around the nest boxes, we used a land cover database for Alsace (Source: BdOCS CIGAL v2 2011/2012, www.geograndest.fr) which categorizes all the habitats found in our study area. We used the software QGIS version 3.4.14 (QGIS Development Team, 2020) to map the active nest boxes and create a circular buffer zone of a 150 m radius around each one of them. This radius was established thanks to data on home range size (Exo, 1992; Génot, 2005) and the field observations made during the breeding season. Due to the high number of habitats, we made groupings based on the environmental characteristics of each variable to calculate the area (m²) covered by each land type within the buffer zones. Our final nest environment included six categories: (1) buildings, (2) meadows, (3) crops (crop fields, hedges, and vineyard), (4) orchards, (5) forest and (6) water. Because of the rarity of the last two categories, forest and water were pooled together.

Relative telomere length (RTL) measurement and sexing

Genomic DNA was extracted from feathers using an adapted protocol of the NucleoSpin Tissue kit (Macherey Nagel, Düren, Germany). RTL was measured in the 142 nestlings in one 384-wells plate, using the quantitative PCR (qPCR) methodology (see Electronic Supplementary Material, ESM). Intra-plate repeatability of RTL (ICC, see (Eisenberg *et al.*, 2020)) was of 0.769. Molecular sexing of nestlings was determined using the same extracted DNA (following Griffiths *et al.*, 1998). Briefly, the technique is based on the existence of two conserved CHD (chromo-helicase-DNA-binding) genes that are located on the sex chromosomes. The CHD-W gene is located on the W chromosome (only in females) and the CHD-Z gene is located on the Z chromosome (both in males and females). For technical reasons, sex could not be determined in 5 nestlings. All the statistical analyses were performed on the remaining 137 nestlings with known sex.

Statistical analyses

We used R version 4.2.1 (R Core Team, 2022) to compute mixed models (package lme4 version 1.1-30 and lmerTest version 3.1-3). In all statistical models, brood identity was included as a random factor to account for the non-independence of nestlings of the same brood. We checked models' assumptions (homoscedasticity, normal distribution of residuals) graphically using the package DHARMa (version 0.4.6). We assessed multicollinearity among predictors by calculating variance inflation factor, VIF (package car, version 3.1-0).

Individual phenotypic characteristics

We tested whether sex is dependent on hatching rank. We computed a generalized mixed model with binomial family and with sex as a dependent variable and hatching rank and nestling number as fixed effects. The significance of the effects was tested with type III Wald chisquare tests.

To test for inter-individual variation in body condition, we first calculated the Scale Mass Index (SMI) following Peig & Green (2009). We then computed a linear mixed model with SMI as a dependent variable and hatching rank, sex, the interaction between hatching rank and sex, nestling number, nestling age and cohort as fixed effects. From this global model, we fitted every possible model and then selected a set of top models (AICc threshold of 2). We then averaged the models from these top models set.

Then, we computed a linear mixed model with SMI as a dependent variable and environmental covariates (proportion of buildings, meadows, crops, orchards and of water and forest around the nest box) as fixed effects. The environmental covariates were scaled before the analysis. Model selection was similar as described above.

Inter-individual variation in Relative Telomere Length

RTL were log-transformed before analyses. First, we computed a linear mixed model with individual covariates (hatching rank, sex, the interaction between hatching rank and sex, nestling number, nestling age, SMI and cohort as fixed effects). Second, we computed a linear mixed model with environmental covariates (as described above). For both models, the model selection procedure was the same as described above.

Results

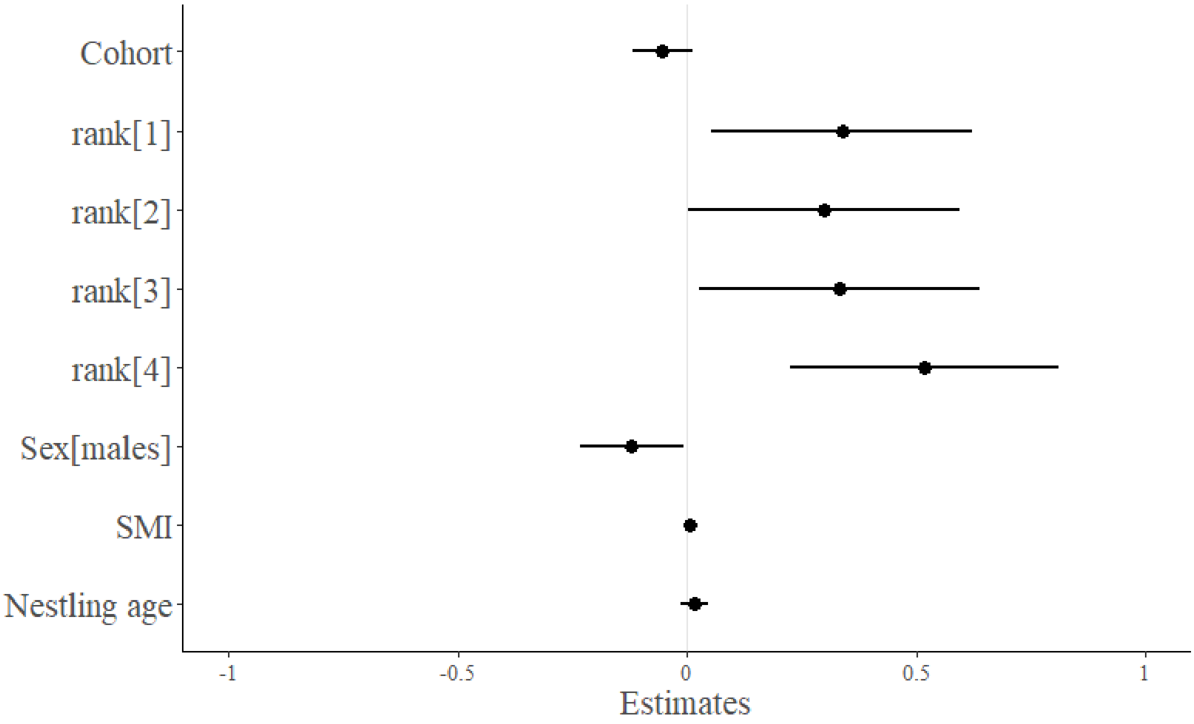
Individual phenotypic characteristics

The sex of the offspring was not significantly correlated with hatching order (chi^2=4.45, P=0.35) or nestling number (chi^2=0.48, P=0.49).

Concerning individual covariates, there were no significant variables that explained variation in SMI in our models. The fixed effects retained in the top models set were nestling age, nestling number and sex (see Table S1) but their effects were not significantly different from 0 (see Figure S1).

Concerning environmental covariates, the proportion of buildings, crops, meadows and orchards around the nest box were kept in the best models (Table S2). The increase of buildings and of crops has a marginally negative effect on the SMI of little owls (Figure S2).

Figure 1. Forest-plot of estimates for the average model of relative telomere length and individual covariates (see Table S3). Reference level for sex is females and for rank is 5 (last hatched chicks).



<u>Inter-individual variation in Relative Telomere Length (RTL)</u>

Concerning individual covariates, RTL was not dependent on nestling number and there was no interaction between rank and sex, the variables in the top models set were rank, sex, SMI, cohort and nestling age (Table S3, Figure 1). Males have shorter telomeres than females (Figures 1 and 2) and there is a small positive effect of SMI on RTL (Figure 1). In addition, last hatched nestlings have shorter telomeres but only in the largest brood of 5 nestlings (Figures 1 and 3). The effect of the year of birth is marginally significant and is negative, meaning that RTL are decreasing in recent years (Figures 1 and 3).

Concerning environmental covariates, the proportion of buildings, crops, orchards and forest and water around the nest box were kept in the best models (Table S4). There is a marginal negative effect of the proportion of forest and water around the nest box on nestlings RTL (Figure S3).

Figure 2. The effect of sex on the relative telomere length before fledging.

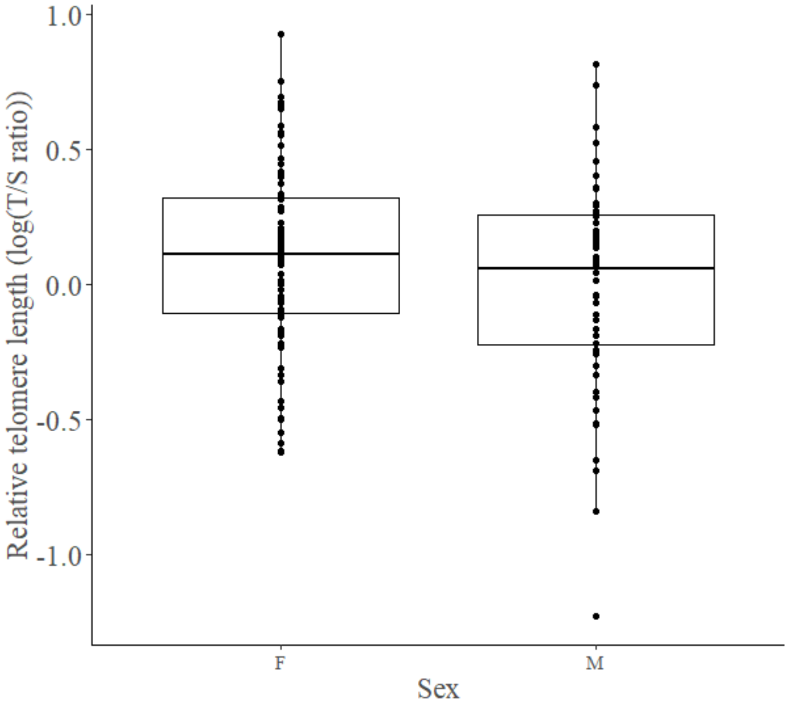
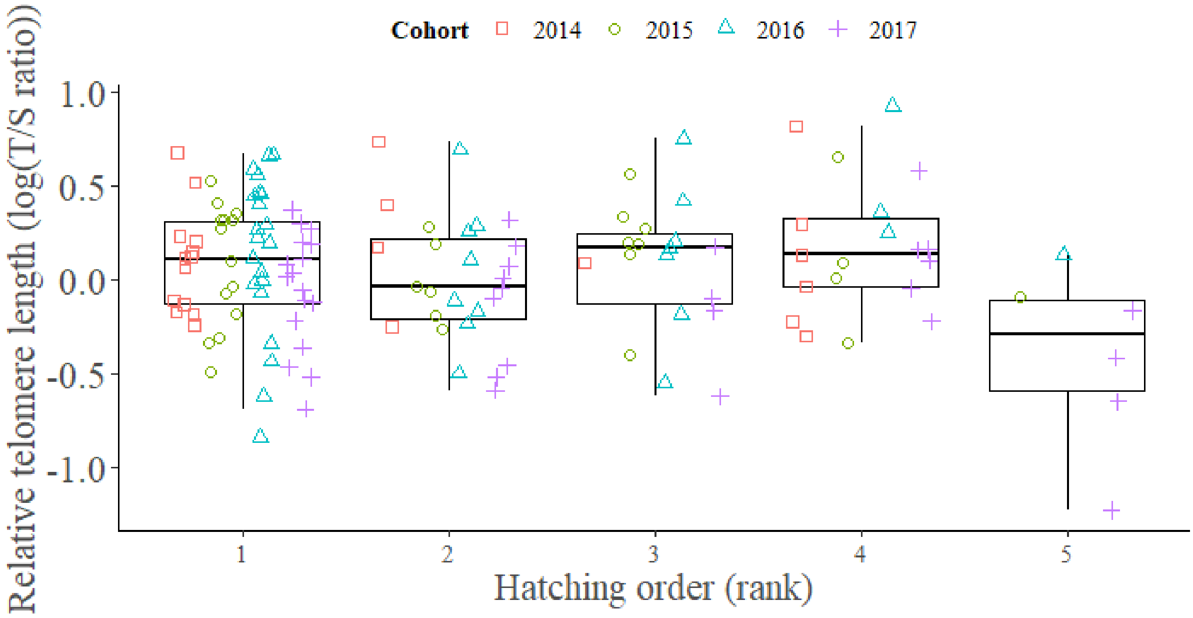


Figure 3. The effect of hatching order and year of birth on the relative telomere length before fledging.



Discussion

Based on the current knowledge on growth and telomeres in bird nestlings, we initially predicted that RTL of little owl nestlings will be: (i) negatively related to the hatching order and (ii) negatively affected by the unfavourable nature of the nest surroundings. Our results indicated that RTL are longer in females and, independently of sex, in nestlings with the highest body condition. They also supported a mixed negative effect of hatching order and intra-brood competition on little owl nestlings' RTL, i.e. detectable only in the largest brood size, suggesting that the effect of hatching rank on telomeres is dependent on a threshold effect in this species. We did not find a clear effect of the environmental covariates on nestlings' RTL. Finally, our longitudinal scan of nestlings' RTL over years surprisingly underlined a possible progressive shortening, independent of any changes in body condition.

Our indication of an erosion of little owl nestlings' RTL over years need to be replaced in the emerging context of conservation physiology aiming at developing physiological markers of individual quality to infer consequences at the population level (Beaulieu & Costantini, 2014; Lea *et al.*, 2018). Telomere length at a given age is not reflecting only the negative effects of time on the cells (i.e. chronological age), it also points out the cumulative effects of stressors encountered over time that may accelerate the rate of loss of telomere ends (Asghar *et al.*, 2015; Louzon *et al.*, 2019; Chatelain *et al.*, 2020; Salmón & Burraco, 2022). Because the rate of cell division and/or the oxidative metabolism are higher in a growing organism, the period of growth is supposed to be the life stage where telomere sequences are the most impacted by environmental stressors (Salomons *et al.*, 2009; Young *et al.*, 2013; Monaghan & Ozanne, 2018). Thus, depending on the harshness of early life environment, erosion of telomeres can be accelerated for a given age (e.g. Boonekamp *et al.*, 2014; Stier *et al.*, 2015), leading the nestlings to be grown, prematurely, physiologically old. This has, theoretically, obvious consequences for the individuals in terms of survival prospects and recruitments as adult breeders in the population, as early life telomere length or rate of telomere loss have been shown to predict future individuals' survival (Boonekamp *et al.*, 2014; Watson *et al.*, 2015; Wood & Young, 2019). Consequently, it also has the potential to affect the population dynamics. First conceptualized few years ago (Stindl, 2004), such a hypothesis was recently supported by studies conducted on ectotherms' populations (Dupoué *et al.*, 2017, 2022). In the common lizard populations studied, analysis of telomere length in yearlings of populations showing different risks of collapsing due to local global warming pointed out reduced mean telomere length in the most endangered populations (Dupoué *et al.*, 2017). Thereafter, the same group showed that short telomeres were already inherited in neonates of declining populations, thereby suggesting (epi)genetic roots, i.e. progressive telomere shortening being not only the result of bad early life conditions (Dupoué *et al.*, 2022). We cannot draw the same conclusions in our case, particularly because we lack data on inter-generational variation of telomere length. It can be noted that in vertebrates, heritability estimates are moderate (Chik *et al.*, 2022), but this recent meta-analysis has no data on raptors (Chik *et al.*, 2022). In addition, as low rates of recruitments of juveniles as first-breeders is an important determinant of population decline in the little owl (Le Gouar *et al.*, 2011), the link between reduced telomere length and survival prospects of nestlings needs to be established. Finally, this result is counter-intuitive in our study population of little owl since the population is expanding and not decreasing (Bersuder & Wassmer, 2020), contrary to other populations (Andersen *et al.*, 2017). Thus, the effect of competition or density on telomere length need to be addressed in future studies.

Little owl female nestlings had longer telomeres than male ones. This has several implications for our understanding of sex-differences in telomere dynamics and of its meaning in terms of sex-biased life history. Differences in telomere length in relation to gender has been previously illustrated in several taxa (reviewed in Barrett & Richardson, 2011), and particularly in birds with sex-biased body size or investment in reproduction, with no consensual general pattern (e.g. Caprioli *et al.*, 2013; Remot *et al.*, 2020; Saulnier *et al.*, 2022 for no sex differences) (e.g. Bauch *et al.*, 2020 for sex differences). In our study, sex-differences in RTL were observed at the nestling stage, with longer telomeres in the females. A previous study showed that females were slightly but consistently of bigger size (Tschumi *et al.*, 2019), however it is not the case in our population. Yet, we did not investigate nestlings growth rates, which can be different event if the final size and/or body mass is similar (e.g. Criscuolo *et al.*, 2008). Higher growth rates are usually associated with shorter telomeres (Geiger *et al.*, 2012; Monaghan & Ozanne, 2018). However, we also found that, independently of sex, nestlings in better body condition had in general longer telomeres. Thus, it is unlikely that little owl nestlings had to face such a growth-body maintenance trade-off. Given that body mass is a determinant of survival from hatching to fledging in little owl (Tschumi *et al.*, 2019), nestling telomeres rather acts as a proxy of individual quality (Angelier *et al.*, 2019). In addition, our results do not match with the idea that the heterogametic sex (*i.e.* females) would be more prone to telomere erosion than the homogametic one (*i.e.* males) due to the unguarded expression of deleterious alleles of sex chromosomes for telomere maintenance (see Barrett & Richardson, 2011; Remot *et al.*, 2020 for a deep discussion related to telomere dynamics). One alternative explanation lies on optimal parental care towards the offspring sex with the highest chance of survival (Hasselquist & Kempenaers, 2002). It has been shown previously that females have a higher survival probability from hatching to fledging, independent of any variation in body mass (Tschumi *et al.*, 2019). However, it is not known whether this sex-difference persists in older individuals. In that context, the parents would favour female individuals, meaning that within little owl broods females may, on average, beneficiate from better access to food resources due to specific parental investment. This may lead to an attenuated body maintenance (*i.e.* telomere length) and growth rate trade-off.

The hypothesis that RTL is an indicator of quality is further supported by the fact that, in the largest clutches, the last hatchling of little owl presented the shortest telomeres. This is also in accordance with the brood size reduction hypothesis that predict a lower investment with laying order. Still, our data would restrict such an effect to the last laid egg. We cannot distinguish between effects of the laying order *per se* on RTL (see introduction) and postnatal effects. Postnatal effects may arise from selective parental care as discussed above. Last-hatched nestling may also suffer from intra-brood competition. Indeed, in a brood, larger nestlings have a competitive advantage compared to smaller nestlings for feeding ("Competitive advantage hypothesis", Anderson *et al.*, 1993). A previous experiment testing the effect of competitive disadvantage within a brood, based on the size of the nestlings cross-fostered among clutches, highlighted an interesting increased telomere attrition of less competitive nestlings without affecting body mass growth (in European starlings, Nettle *et al.*, 2015).

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

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

Data accessibility. Datasets used in this study are openly available on zenodo (doi: 10.5281/zenodo.7701531).

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

Competing interests. We declare we have no competing interests.

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

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

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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.

Table S1. Top models set for models of SMI and individual covariates. For continuous variables, each value represents the estimate of the effect; for categorical variables, there is a "+" when the variable is retained in a model.

df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.

Intercept	Nestling age	Nestling number	Sex	df	AICc	delta
145.30		-3.52		4	1058.0	0.00
132.30				3	1058.3	0.24
145.20		-3.66	+	5	1059.4	1.36
131.80			+	4	1059.8	1.75
139.70	0.25	-3.53		5	1059.9	1.88

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

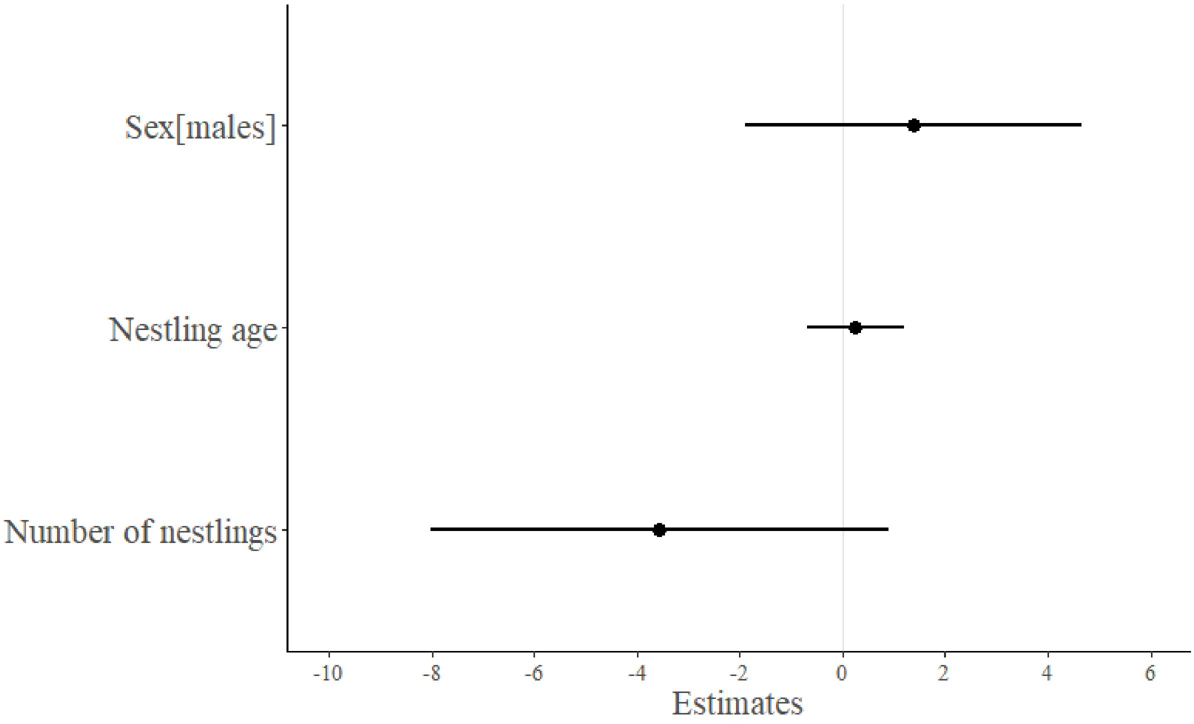


Table S2. Top models set for models of SMI and environmental covariates. For continuous variables, each value represents the estimate of the effect.
df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.

Intercept	Buildings	Crops	Meadows	Orchards	df	AICc	delta
132.1	-4.98	-4.57			5	1056.8	0
132.2	-5.55	-5.96		-2.21	6	1057.7	0.9
132.0	-8.57	-8.71	-3.57	-4.06	7	1058.2	1.44
132.3					3	1058.3	1.49
132.4	-2.52				4	1058.7	1.91

Figure S2. Forest-plot of estimates for the average model from Table S2.

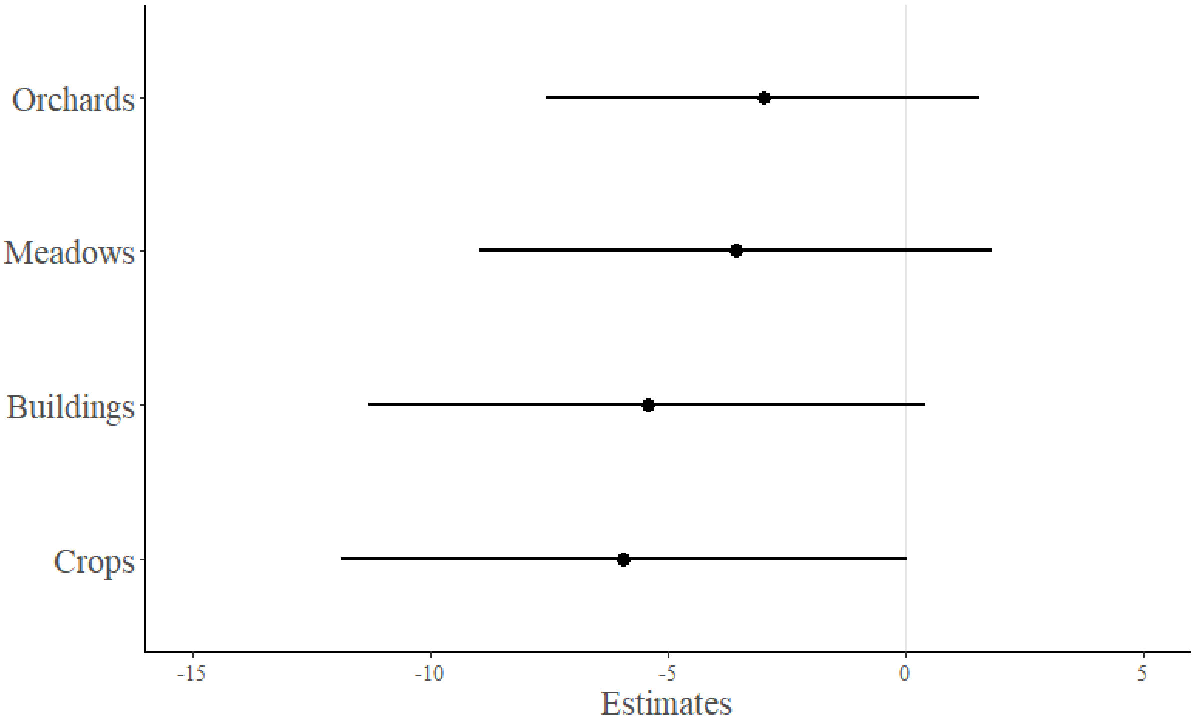


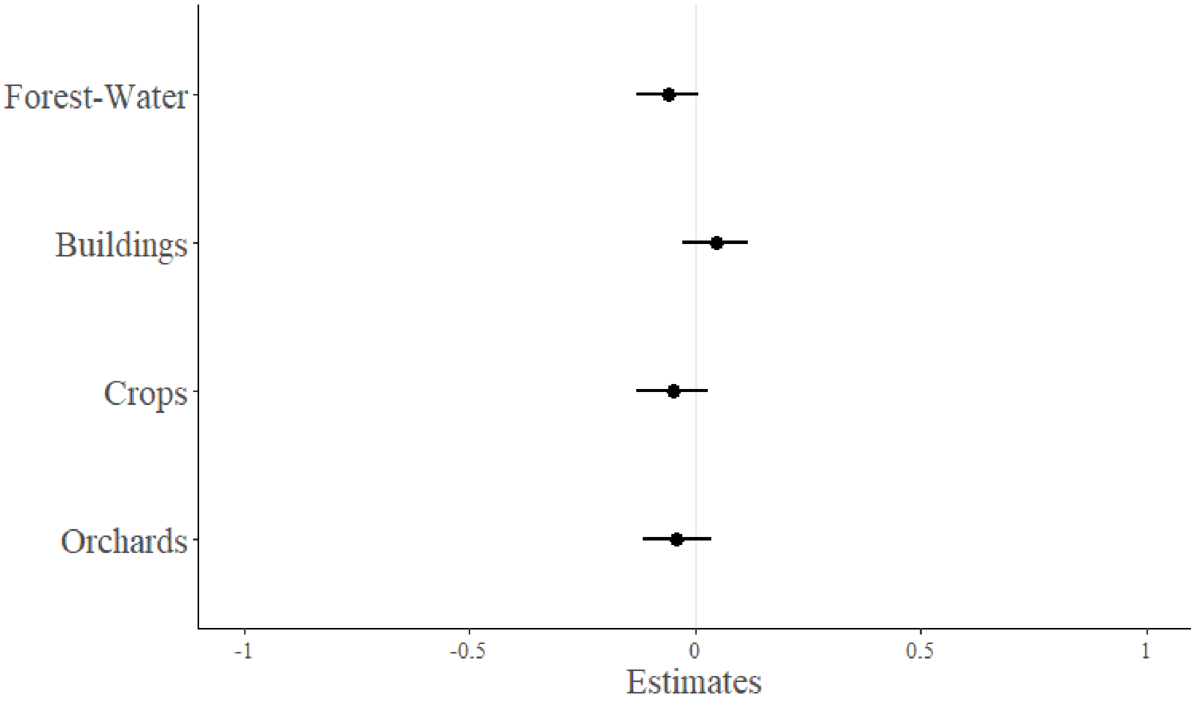
Table S3. Top models set for models of RTL and individual covariates models. For continuous variables, each value represents the estimate of the effect; for categorical variables, there is a "+" when the variable is retained in a model.
df = degree of freedom. delta = difference of AICc with the model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

Intercept	Cohort	Nestling age	Rank	Sex	SMI	df	AICc	delta
-0.70	-0.053		+	+	0.0046	10	104.4	0.00
-0.86			+	+	0.0046	9	104.6	0.25
-0.99	-0.052	0.016	+	+	0.0045	11	105.7	1.35
-1.15		0.016	+	+	0.0045	10	105.9	1.57
-0.11	-0.053		+	+		9	106.1	1.72
-0.27			+	+		8	106.2	1.81
-0.74	-0.054		+		0.0043	9	106.3	1.97

Table S4. Top models set for models of RTL and environmental covariates models. For continuous variables, each value represents the estimate of the effect.
df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.

Intercept	Buildings	WaterForests	Crops	Orchards	df	AICc	delta
0.057		-0.058			4	111.8	0.00
0.057					3	112.2	0.42
0.057	0.045	-0.059			5	112.4	0.64
0.057		-0.067	-0.063	-0.059	6	112.9	1.15
0.056	0.044				4	113.0	1.24
0.056		-0.061	-0.036		5	113.1	1.26
0.059		-0.060		-0.033	5	113.1	1.31
0.058	0.048	-0.061		-0.036	6	113.6	1.78
0.058				-0.030	4	113.7	1.90

Figure S3. Forest-plot of estimates for the average model from Table S4.



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