Parental age does not influence offspring telomeres during early life in common gulls (Larus canus)

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Abstract

Parental age can affect offspring telomere length through heritable and epigenetic-like effects, but at what stage during development these effects are established is not well known. To address this, we conducted a cross-fostering experiment in common gulls (Larus canus) that enabled us distinguish between pre- and post-natal parental age effects on offspring telomere length. Whole clutches were exchanged after clutch completion within and between parental age classes (young and old) and blood samples were collected from chicks at hatching and during the fastest growth phase (11 days later) to measure telomeres. Neither the ages of the natal nor the foster parents' predicted the telomere length or the change in telomere lengths of their chicks. Telomere length was repeatable within chicks, but increased across development (repeatability = 0.55, Intraclass Correlation Coefficient within sampling events 0.934). Telomere length and the change in telomere length were not predicted by post-natal growth rate. Taken together, these findings suggest that in common gulls, telomere length during early in life is not influenced by parental age or growth rate, which may indicate that protective mechanisms buffer telomeres from external conditions during development in this relatively long-lived species.

Keywords

Ageing, seabirds, telomere length, telomere dynamics, maternal effects, qPCR

Introduction

Parental age often has long-term effects on offspring phenotype in humans (Carlaske, Tynelius, van den Berg, & Smith, 2019) and wild animals (Bowers, Sakaluk, & Thompson, 2017). In some cases, parental age has a positive long-term effects on offspring (Bradley & Safran, 2014), possibly because older parents are more experienced breeders than younger parents and/or because reproductive investment increases with age as future reproductive opportunities decline (e.g., terminal investment) (Clutton-Brock, 1984). Alternatively, parental age may have negative long-term effects on offspring if older parents experience senescent declines in condition and/or reproductive function relative to younger parents (Bock, Jarvis, Corey, Stone, & Gribble, 2019; Maklakov & Chapman, 2019; Velarde & Menon, 2016). Regardless, the mechanisms that underlie these long-term effects of parental age on offspring phenotype are not well understood, although several candidate mechanisms have been proposed, including, for example, epigenetic factors (Bock et al. 2019).

One of the mechanism that has been proposed may mediate trans-generational effects of parental on offspring fitness could be offspring telomere length (TL). Telomeres are highly conserved, repetitive, non-coding sequences of DNA that form protective caps at the ends of chromosomes, thereby enhancing genome stability

(Blackburn, 2005). While some evidence suggests that TL is largely inherited (Blackburn, 2005), it is also known that TL and telomere dynamics are affected by a complex interaction of genetic effects and environmental factors during an organism's life (Dugdale & Richardson, 2018; Monaghan, 2010). While telomeres typically shorten with age in somatic cells, elongation of telomeres has also been described, mainly as a result of the activity of the enzyme telomerase, which can extend telomeres via the addition of terminal telomeric repeats (Cong, Wright, & Shay, 2002), and which is variably active in different cell types and at different life stages (Gomes et al. 2011). The effect of parental age on the length of offspring telomeres is currently an intensively studied (e.g. Bauch, Boonekamp, Korsten, Mulder,& Verhulst, 2019; Criscuolo, Zahn,& Bize, 2017; Heidinger et al. 2016; Froy et al. 2017; Noguera & Velando, 2020). Costs and benefits associated with maintaining the length of telomeres are particularly interesting when considering the adaptive role of telomeres in the evolution of life histories, as it is suggested that telomeres play a proximate causal role in current–future life-history trade-offs (Young, 2018). Optimal life-history strategies are both inherited and shaped by environmental effects (Stearns, 1992), and accordingly, telomere dynamics are a plausible physiological mechanisms related to life-histories (Giraudeau, Angelier, & Sepp, 2019).

While studies are accumulating that show parental age effects on offspring telomeres, it is still unknown when these effects are occuring. There are three potential routes through which parental age could impact offspring telomeres (Haussmann & Heidinger 2015; Heidinger & Young, 2020). First, age-associated changes in parental gametes may affect the telomere length of offsprings. Parental age may negatively influence offspring telomere length if older parents produce gametes with shorter telomeres (shown for example in mice, *Mus musculus* , de Frutos et al. 2015), however, studies in humans have also shown that older fathers may have offspring with longer telomeres (Broer et al. 2013; Unryn, Cook, & Riabowol, 2005), due to active telomerase in sperm cells (Kimura et al., 2008). Such inconsistences among studies, but also within species, could stem from differences in life-history strategies, likely via mechanisms related to spermatogonial stem cell telomere retention with increasing age, or selective attrition/survival of spermatogonia (Kimura et al. 2008; Eisenberg & Kuzawa 2018). Studies in humans have mostly found a link between paternal age and offspring telomere length, however, the effect of maternal age has been shown to be even stronger for some species (e.g. great reed warbler*Acrocephalus arundinaceus* , Asghar, Bensch, Tarka, Hansson, & Hasselquist, 2015). While maternal reproductive cells develop at a very early age, after which they are retained throughout life without further cell divisions, association between mother age and offspring telomere length could be explained by other mechanisms, for example age- and/or condition-dependent telomerase activity in the ovaries (Asghar et al. 2015a; Kinugawa, Murakami, Okamura, & Yajima, 2000). Second possible route is through pre-natal effects, for example age-associated changes in the amounts of glucocorticoid or androgen hormones transferred from the mother to the developing embryo with increasing age of the mother (Haussmann & Heidinger, 2015; Heidinger & Young, 2020; Stier, Metcalfe, & Monaghan 2019). This could in turn activate the production of reactive oxygen species (ROS), as well as decrease telomerase activity in the offspring (Haussmann & Heidinger, 2015), potentially leading to telomere erosion. While there are now a number of studies linking pre-natal stress to offspring telomeres (reviewed for example, in Dantzer et al. 2020; Haussmann & Heidinger; 2015, Heidinger & Young, 2020), none have included parental age in this equation. Third route are post-natal effects, as age-related variation in parental care and the characteristics of the post-birth environment could also be important mediators of offspring telomere dynamic (Tarry-Adkins et al. 2009). For example, more experienced parents may provide better care (Beamonte-Barrientos, Velando, Drummond, & Torres, 2010), but older parents may also become less capable of providing a high quality environment due to senescence effects (Torres, Drummond, & Velando, 2011). The quality of the parental care during the growth phase may hasten or reduce telomere shortening (Criscuolo et al., 2017) with long-lasting effects on the aging rate and life-history trajectories of the organisms (Young 2018).

Distinguishing between the role of genetic/pre-natal and post-natal effects on telomere length and telomere dynamics in natural populations early in life is difficult, but potentially most important from an evolutionary and ecological perspective (Dugdale & Richardson, 2018). Birds are a promising model system for this kind of study, as the embryo development takes place within a sealed system, the egg, limiting the physiological maternal effects with the moment of egg laying. Cross-fostering experiments are a useful tool, as they allow

to tease apart effects that are epigenetic and/or due to pre-natal egg effects, from effects occuring during incubation and/or chick feeding. However, to the best of our knowledge, there are only two studies that have used cross-fostering approach to separate the genetic and environmental effects of parental age on offspring telomere length. In alpine swifts (*Apus melba*), foster mother's age negatively affected offspring telomere length (Criscuolo et al., 2017). Bauch *et al* . (2019) showed that paternal age effect on offspring telomere length is independent of paternal care after conception in jackdaws (*Corvus monedula*). However, neither of these studies took repeated measures, which are necessary to assess the change in TL and to separate phenotypic variance into individual and residual variance components to calculate repeatability (Stoffel, Nakagawa, & Schielzeth, 2017). In a recent cross-fostering study of king penguins (*Aptenodytes patagonicus*), repeated measures of TL were taken, indicating that chick telomere length was positively related to foster mothers' TL at both 10 and 105 days after hatching, however, this study did not include information about parental age (Viblanc et al 2020). Our study combines both of these approaches, applying a cross-fostering manipulation between differently aged parents with repeated measures of offspring telomere length in a wild population of long-lived birds. We cross-fostered whole clutches of common gull (*Larus canus*) eggs shortly after laying within and between age classes of young and old parents, and assessed telomere length and dynamics during the chick fastest growth phase. We predicted that if there are pre-natal, epigenetic-like effects of parental age on offspring telomeres, there will be a relationship between the age of the natal parent and offspring telomeres, but if post-natal, environmental effects mediate the link between parental age and offspring telomere length, there will be a relationship between the age of the foster parent and offspring telomeres.

Materials and methods

1. Field methods.

We conducted the study between the end of May – early June 2017 on a free-living, known-age breeding population of common gulls located on Kakrarahu islet in Matsalu National Park on the west coast of Estonia (58°46' N, 23°26' E)[34]. Based on studies of offspring recruitment rate, reproductive success in common gulls increases to the 10th breeding year (12-13 years of age) and decreases thereafter (Rattiste, 2004). It is known that they undergo senescence in a number of physiological functions (Rattiste et al. 2015; Sepp et al. 2017; Urvik, Rattiste, Giraudeau, Okuliarová, Hõrak & Sepp, 2018). In addition, this species is characterized by bi-parental care and a low frequency of extra-pair mating ((Bukacinska, Bukacinski, Epplen,, Sauer, & Lubjuhn, 1998), making it possible to study the effects of age of the parent of both sexes, and the age-dependent quality of parental care. All of the birds included in the study were banded as chicks and their exact age was therefore known. Common gulls typically lay clutches of three eggs.

A total of 40 clutch nests were included in the experiment. Nests and experimental groups were chosen based on the age of the mother, but the father's age was also known. Common gulls mates assortatively with respect to age and the ages of the parents were highly positively correlated (Spearman r=0.74, p<0.0001) Half of the breeders (n = 20 females) were young, on their 1st-3rd breeding event (age exactly 5 years). Another half (n = 20 females) were middle-aged or older (15-30 years old, average age 18 ± 3.37 (SD) years). In total, 19 males were 5-7 years old (average age 5.52 ± 0.80 (SD) years) and were grouped as "young", 21 males were 10+ years (average age 16.29 ± 5.58 (SD) years) old and were grouped as "old". Distribution of the ages of parent birds are shown in figures S4-S7.

We cross-fostered whole clutches right after the clutch was completed both within and between maternal age classes (Table 1), so all of the chicks included in this experiment hatched in foster-parent's nests. Half of the clutches were cross-fostered within age classes and half were cross-fostered between age classes. The 72 chicks that were successfully caught for second sampling were included in the study. Within two days from hatching, we collected the first blood sample (10-30 µL taken, from brachial vein) for telomere measurement (average age 0.61±0.09 (SE) days) and individually marked the chicks for identification. Chick head size (the distance from the tip of the bill to the back of the head) was measured with a calliper to the nearest 0.1 mm. The day of the second blood sample varied between chicks, due to difficulties in trapping precocial chicks. We captured the chicks near their nests and collected a second blood sample between the 8th and 20th day

after hatching (with one exception of a 5-days old chick). Average age at second blood sampling was 10.62 days (±0.35 SE). Blood samples were kept on ice in an insulated box while on the islet, and stored at -20°C until the end of the field work period, when they were transferred to -80^{o}C and held there until analysed. The experimental protocol was approved by the Ministry of Rural Affairs of the Republic of Estonia (licence no. 106, issued 24.05.2017) and was performed in accordance with relevant Estonian and European guidelines and regulations.

2. Telomere measurement methods

DNA was extracted from whole blood samples using Macherey Nagel Nucleospin Blood kits following the manufacturer's protocol. Avian red blood cells are nucleated and are well suited for longitudinal telomere analysis (Nussey et al. 2014). DNA concentration and purity were assessed with a Nanodrop 8000 (Thermo Scientific). Relative telomere length was measured using qPCR (quantitative polymerase chain reaction) on an Mx3000P (Stratagene) as described in Cawthon (2002) and modified for use in common gulls (Rattiste et al., 2015). The relative telomere length (T/S) of the samples was calculated as the ratio of the telomere repeat copy number (T) to that of a single copy control gene (S), relative to the reference sample. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the single copy control gene. We used the following gull specific GAPDH forward and reverse primers (Integrated DNA Technologies): 5'-CGGAGCACCGCTTACAATTT-3' and 5'- GCATCTCCCCACTTGATGTTG-3' respectively. Amplified samples were run on a 3% agarose gel to verify that the amplification was a single product, which yielded a single band at 77 bp as expected. We used the following telomere primers (Quanta Bio, with final concentration of 200 nM): TEL 1b: 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTT-3' and TEL 2b: 5'-GGCTTGCCTTACCCTTACCCTTACCCT-3. The qPCR reactions for GAPDH and telomeres were run on separate plates. All reactions used 20 ng of DNA in a final volume of 25 µl containing 12.5 µl of SYBER green Master Mix, 0.25 µl forward and reverse primer, 6 µl water, and 6 µl of DNA sample. A negative control of water was run on each plate. All samples were run in duplicate, and average values were used to determine the T/S ratio. Treatment groups were distributed approximately equally between plates. Replicates of each sample, and the first and second sample for each bird were run on the same plate. In order to assess the efficiencies of each plate, samples were run against a standard curve of 40, 20, 10, 5, and 2.5 ng produced by serially diluting a reference sample. In all cases, plate efficiencies were in the accepted range (i.e. 100+/-15%) and all of the samples fell within the bounds of the standard curve. Average plate efficiencies and standard errors for GAPDH and telomere plates were 102.34 ± 3.00, and 98.94 ± 3.00, respectively. The average intra-plate variation of the Ct values was 0.82% for the telomere assays and 0.19% for the GAPDH assays, and the inter-plate CV-s of the Ct values for telomere and GAPDH assays were 0.64% and 0.34%, respectively. The same individual was also included on every plate and the coefficient of variation of the T/S ratio across plates was 8.12%. A subset of samples (45) was ran twice for assessing measurement accuracy. Average Intraclass Correlation Coefficient (ICC) in this subset was 0.934 (95% CI 0.879 ± 0.964), $p < 0.0001$, for single measurements ICC = 0.876 (95% CI 0.785 ± 0.930) $p < 0.0001$.

3. Statistical methods

To test for an effect of parental age on chicks' telomere erosion and telomere measurements at first and second sampling, we used repeated measures mixed models and the R package lmerTest (Kuznetsova, Brockhoff & Christensen, 2017). Chick nested in the respective nest was included as a random effect in our analysis. Models included age groups (young or old) of either mother and foster mother or father and foster father as categorical predictors, to emphasize our study design and questions. Similar models were also run with age as a continuous factor (Table S1). Separate models were run for mother's and father's age effect, since the age of parents was strongly correlated and could not be entered in the same models. Both models with and without interaction (Tables 1 and S2) with sampling occasion (first or second) were run. Interval between sampling varied between individuals, but this was not included in these models as it was not related to telomere length (Figure S8, Table S6, $p = 0.805$). Parental age effect models were run for head size as a dependent variable (Tables S4-S5). In these models, chick age was included as a predictive factor since it correlated significantly with head size. Markov Chain Monte Carlo (MCMC) multivariate generalised linear

mixed models where used for assessing the continuous effects of parental age (hatch year) on telomere length and telomere length dynamics. Repeatabilites were calculated using R package rptR (Stoffel et al., 2017). Heritabilities were obtained using one-way ANOVA from the full sib design and (for the models including parental age and treatment effects) using generalised linear mixed models for the repeated measures design (R package MCMCglmm) (Hadfield 2010, de Villemereuil, Morrissey, Nakagawa & Schielzeth, 2018; Kuznetsova & Hadfield, 2010). The models were run for both telomere measurements (first and second) as well as change as a dependent variable. The models included parent nest as random effect and the same fixed effects as the repeated measures mixed model for the effect of telomere length change. Both repeatability and heritability measures are reported together with 95% coefficient intervals (CI). The distribution of the traits was normal or close to normal distribution (Figures S1-S3), accordingly, models were ran with untransformed values. Power analyses were conducted based on Monte Carlo simulations (Green & MacLeod, 2015) (Table S3). The R code for different steps in the statistical analyses is accessible from the supplementary material.

Results

There were no significant effects of the ages of the natal or foster parents on offspring telomeres at hatching or the change in offspring telomere length from hatching to the end of post-natal development (Figure 1, see Tables 2 and S2 for results with age groups, young vs old, and Tables 3 and S1 for results with parental age as a continuous factor). When we entered the parental age squared in the models, it did not change this result (Tables S7-S9). Telomere length was individually repeatable between the first and second sampling, separated by on average 11 days (repeatability r = 0.55, CI= 0.368 to 0.69, $p < 0.0001$, Figure 2). Chick telomere length between the first and second telomere measurements increased rather than decreased (the estimated change in relative telomere length was 0.08 ± 0.05, p=0.003, Table 2; when one outlier chick with highest increase in telomere length was removed, the results stayed similar, estimated change 0.06 ± 0.04, p = 0.005). The rate of growth during post-natal development was not significantly related to the change in telomere length (measured as change of head size, $r < 0.0001$; p = 0.97, Figure 3). Chick growth rate was not related to the age of the parents and foster parents (all parental effect p-values > 0.31, Tables S4-S5). The power for detecting potential effects of parental age on telomere length and telomere shortening in our sample is reported in Table S3. The study set up also allowed us to assess wide-sense heritability of telomere length, although these results must be taken with caution, since we did not use a split nest cross-fostering design. Chick telomere length at both first (h^2=0.27 CI=0.05 to 0.50) and second (h^2=0.30 CI=0.08 to 0.54) measurement as well as the change of telomere length (calculated as telomere length 2 - telomere length 1, h^2=0.14 CI=0.03 to 0.29) were heritable.

Discussion

Parental age often has long-term consequences for offspring phenotype and fitness, but the mechanisms that mediate these effects remain insufficiently studied. Previous research suggests that telomeres may be an important mechanism mediating these effects, but the routes by which these effects occur are not well understood. The cross-fostering experiment used here allowed us to distinguish whether any effects of parental age on offspring telomeres occur as a result of epigenetic like and/or pre-natal effects vs. post-natal effects. Previous research in this colony of common gulls has demonstrated that offspring fitness decreases with increasing parental age: recruitment rate decreases in common gulls after the 10th breeding year (12-13 years of age) (Rattiste, 2004), and older gulls allocate less nutrients to their eggs (Urvik et al., 2018). However, despite these demonstrated effects of parental age on offspring fitness in this species, we did not find any evidence of the effect of parental age on offspring telomeres at hatching or the change in telomere length during post-natal development.

Although parental age has been shown to have both negative and positive effects on offspring telomere length (Table 4), this has not been found in all studies. For example, no effect of parental age on offspring telomere length was found in a study of Soay sheep (*Ovis aries*) despite a large sample size (Froy et al., 2017). Although bird studies have generally found a negative effect of parental age on offspring telomeres (see for example studies in alpine swifts, (Criscuolo et al., 2017), and European shags *Phalacrocorax aristotelis* , (Heidinger et al., 2016), this is not a universal pattern (for example, older great reed warbles *Acrocephalus*

arundinaceus produced offspring with longer telomeres, (Asghar et al., 2015b). Also in humans the effect of paternal age is positive rather than negative, as older fathers sire offspring with longer telomeres (Broer et al., 2013). A review article summarizing these results concluded that while there is no clear pattern across species, this is unlikely to be explained by statistical noise or publication bias (Eisenberg, 2014), but rather linked to the specific biology of each species. There are also several additional factors that could contribute to these mixed results: whether the study is experimental or correlative, at what age the offspring are sampled, and whether it is a longitudinal versus cross-sectional study of parental age. Accordingly, we cannot rule out that a longitudinal set-up would have revealed an effect of parental age on offspring telomere results also in our study system, as the cross-sectional design could have obscures the within individual patterns. Another possibility is that the link between parental age and offspirng telomere length in this species was too weak to be revealed from our sample size, despite relatively good test power (Table S3). In this case, and also in the case of a missing link, it is possible that telomere length in common gulls is indeed relatively buffered from environmental influence.

Telomere length was repeatable across the chick rearing period in our study, indicating a possible increase during post-natal development, and not being related to chick growth rate. Telomeres were longer at the second sampling, when chicks were approximately 11 days old. This period covered the fastest growth period of gull chicks. While telomeres shorten during growth in most studied adult vertebrates (which, as of now, mainly include mammals and some bird species), some vertebrates show increased telomerase activity during development (reviewed by Larsson, Rattiste, & Lilleleht, 1997, Monaghan & Haussmann, 2006). It has even been suggested that in free-living long-lived organisms, evolution should favour mechanisms that maintain the longest possible telomeres at the end of the most active growth period (Chan, & Blackburn, 2004). Studies in the wild have shown that telomeres elongate in at least some life stages in Seychelles warblers (*Acrocephalus sechellensis* (Spurgin et al., 2018)), edible dormouse (*Glis glis*(Hoelzl et al., 2016)), Soay sheep (Fairlie et al., 2016), Magellanic penguins (*Spheniscus magellanicus* (Cerchiara et al., 2017)), and Atlantic salmon (*Salmo salar* (McLennan et al., 2018)). However, when looking at the few studies on species more closely related to common gulls, telomere shortening during the early growth period has been reported. For example, in lesser black-backed gull (*Larus fuscus*), telomeres shortened from hatching to ten-days old (Foote et al., 2011), and in black-tailed gull chicks (*Larus crassirostris*), telomere attrition was shown for chicks growing with siblings in the nest (but not singleton chicks (Mizutani, Niizuma, & Yoda, 2016)). The latter study suggests that, in favourable growing conditions, telomere attrition might be prevented. However, more results on closely related species are needed to confirm the possibility of telomere lengthening during early growth in gulls. Growth rate was independent of telomere length or telomere dynamics (Figure 3). In general, a trade-off between rapid growth and telomere maintenance is expected, due to increased number of cell divisions required to attain larger size, and/or increased loss of telomere length during each cell division as a consequence of the conditions required for fast growth (e.g. higher metabolic rate and ROS production reviewed by Monaghan & Ozanne (2018)). The current study adds to the increasing number of studies suggesting a different pattern in long-lived seabirds (Mizutani et al., 2016; Young et al., 2017). Seabirds are distinguished from most other species by a long time period between the end of relatively fast somatic growth and the beginning of reproduction (stretching several years). More studies applying comparable methodological approaches are needed for a comparative study, including patterns of growth and life-history strategies of different species to determine if this phenomenon of delayed reproduction is causally linked with the lack of an association between fast growth and telomere shortening in seabirds.

In conclusion, our results suggest that the age of the parents at the time of offspring conception does not influence offspring telomere length or the change in telomere length in common gulls. An important area of future research is to identify other mechanisms that mediate the long-term effects of parental age on offspring and to better understand the factors that contribute to the variation in the influence of parental age on offspring telomeres across species.

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Compliance with ethical standards

The experimental protocol was approved by the Ministry of Rural Affairs of the Republic of Estonia (licence no. 106, issued 24.05.2017) and was performed in accordance with relevant Estonian and European guidelines and regulations.

Conflict of interests

The authors declare no competing interests.

Authors' contribution

TS, MG, PH and KN conceived the ideas and designed methodology; JU, KR, RM and LS collected the data; KR provided demographic data; TS, BH and MG analysed the samples; PH, RM and TS performed statistical analyses. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data available from the Dryad Digital Repository (TBA)

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Tables and figures

Table 1. Cross-fostering experiment sample sizes. A total of 72 chicks from 40 nests were included in the experiment.

Parent gender	Manipulation	Number of nests	Number of chicks
Female	Young to young	10	12
	Old to old	10	18
	Young to old	10	26
	Old to young	10	16
Male	Young to young	8	14
	Old to old	12	18
	Young to old	11	24
	Old to young	9	16

Table 2. Repeated measures mixed model of telomere length dynamics (separate models for mother and father age groups). Final models without non-significant interactions are presented in supplementary materials (Table S2).

Predictor	*Estimates*	*CI*	*p*	*Estimates*
second - first measurement	0.11	0.02 – 0.20	**0.025**	0.09
mother age group	0.04	-0.09 – 0.17	0.547	
foster mother age group	0.12	-0.02 – 0.25	0.092	
time * mother age group	-0.03	-0.14 – 0.08	0.597	
time * foster mother age group	-0.03	-0.14 – 0.08	0.552	
father age group				0.09
foster father age group				0.09
time * father age group				-0.02
time * foster father age group				0.01

Predictor	*Estimates*	*CI*	*p*	*Estimates*
Random Effects	**Random Effects**	**Random Effects**	**Random Effects**	**Random Effects**
σ^2	0.03	0.03	0.03	0.03
τ_{00}	0.02 $_{\text{chick\_ID:parent\_nest}}$	0.02 $_{\text{chick\_ID:parent\_nest}}$	0.02 $_{\text{chick\_ID:parent\_nest}}$	0.02 $_{\text{chick\_ID:parent\_n}}$
	0.02 $_{\text{parent\_nest}}$	0.02 $_{\text{parent\_nest}}$	0.02 $_{\text{parent\_nest}}$	0.01 $_{\text{parent\_nest}}$
ICC	0.58	0.58	0.58	0.57
N	72 $_{\text{chick\_ID}}$	72 $_{\text{chick\_ID}}$	72 $_{\text{chick\_ID}}$	72 $_{\text{chick\_ID}}$
	40 $_{\text{parent\_nest}}$	40 $_{\text{parent\_nest}}$	40 $_{\text{parent\_nest}}$	40 $_{\text{parent\_nest}}$
Observations	144	144	144	144

σ2 - within-group (residual) variance, τ00 - between-group-variance, ICC - intraclass-correlation (variance partition coefficient).

Table 3. Markov Chain Monte Carlo (MCMC) multivariate generalised linear mixed models of parental age (hatch year) effects on telomere length and telomere length dynamics.

	Mother's hatch year	Foster mother's hatch year
Telomere length at hatching	**Telomere length at hatching**	**Telomere length at hatching**
Mean (95% CI)	0.004 (-0.005-0.012)	0.007 (-0.001-0.015)
pMCMC	0.41	0.10
Telomere length at second trapping	**Telomere length at second trapping**	**Telomere length at second trapp**
Mean (95% CI)	0.001 (-0.010-0.014)	0.003 (-0.007-0.014)
pMCMC	0.85	0.56
Change of telomere length	**Change of telomere length**	**Change of telomere length**
Mean (95% CI)	-0.003 (-0.013-0.006)	-0.005 (-0.014-0.005)
pMCMC	0.50	0.33

CI-confidence interval.

Table 4. Overview of studies in non-human vertebrates (excluding also laboratory rodents) investigating the effect of parental age on offspring telomere length. For a review on human studies, please see Froy et al. [28]. Extended version of the table including study design and offspring developmental stage available in Supplementary Materials (Table S10).

Class	**Species**	**Sample size**	**Studied parental effects**	**Telomere method**	**Parental age effect (- / +)**
Teleost fish	Atlantic salmon (*Salmo salar*)[67]	84 HSF	M & P	qPCR (RTL)	Complex P_{fry} (+)
	Atlantic salmon (*Salmo salar*)[68]	60 HSF	M & P	qPCR (RTL)	Complex P_{fry} (-)
Reptiles	Sand lizard (*Lacerta agilis*)[12]	N_{sons}=12	P	TRF (TL in bp)	P_{sons}(-)
Birds	Great reed warbler (*Acrocephalus arundinaceus*)[18]	N_{ind}=139 N_{broods}=46	M & P	qPCR (RTL)	M (+)

Class	Species	Sample size	Studied parental effects	Telomere method	Parental age effect (- / +)
	European shag (*Phalacrocorax aristotelis*)[27]	N_{ind}=311 N_{broods}=134	M & P	qPCR (RTL) and ΔRTL	M $_{\Delta PTL ofφφσπρινγ}$ (-) P $_{\Delta PTL ofφφσπρινγ}$ (-)
	Alpine swift (*Apus melba*)[13]	N_{ind}=95 N_{broods}=54	M & P biological and foster	qPCR (RTL)	$P_{biological}$ (-) M_{foster} (-)
	Zebra finch (*Taeniopygia guttata*)[42]	N_{ind}=139 $N_{HSbroods}$=64	P	qPCR (RTL)	P (-)
	Black-browed albatross (*Thalassarche melanophrys*)[69]	N_{ind}=51	Mean M & P	TRF (TL in bp)	Par (+)
	Common tern (*Sterna hirundo*)[70]	N=142	M & P	TRF (TL in bp)	P (-)
	Jackdaw (*Corvus monedula*)[10]	N_{ind}=715 N_{nests}=298 N_{ind}=61 N_{nests}=31	M & P biological and foster	TRF (TL in bp)	P_{biol} (-)
	Common gull (*Larus canus*)[Curr. study]	N_{ind}=72 N_{nests}=40	M & P	qPCR (RTL and ΔRTL)	No effect
Mammals	Soay sheep (*Ovis aries*)[28]	N_{ind}=318	M & P	qPCR (RTL)	No effect
	Chimpanzee (*Pan troglodytes*)[71]	N_{ind}=40	P	qPCR (RTL)	P (+)

Abbreviations: TL - telomere length, RTL - relative telomere length, TRF - terminal restriction fragment, HSF - half sib families, ind - individuals, P - paternal age effect, M - maternal age effect, Par - parental age effect, (-) negative association, (+) positive association

Figure 1. Parents' and foster parents' age group did not affect offspring telomere length (A-B) and telomere dynamics (C-F). Mother's and father's age groups coincided for most, but not all chicks. Columns denote group means, whiskers are standard errors.

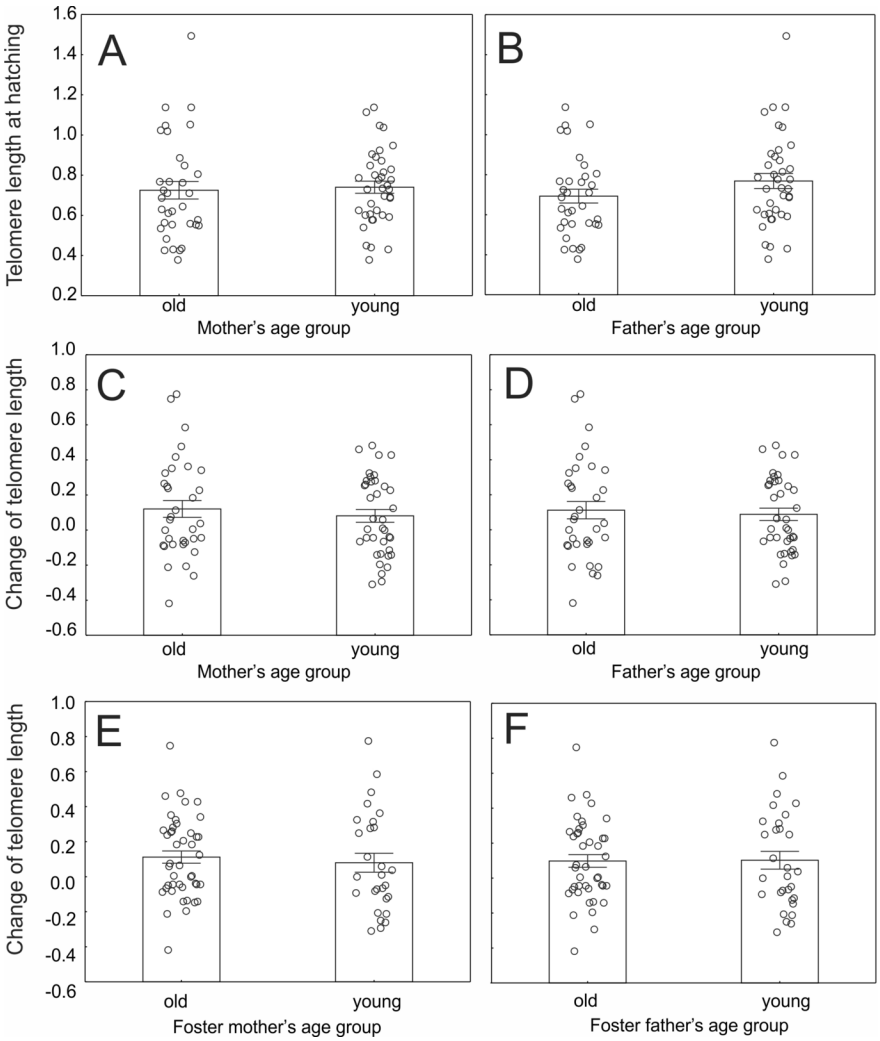


Figure 2. Telomere length was individually repeatable between first (avg. 0.61 days after hatching) and second blood sampling (avg. 10.62 days after hatching). X=Y line is depicted to illustrate the direction of individual increase of telomere length.

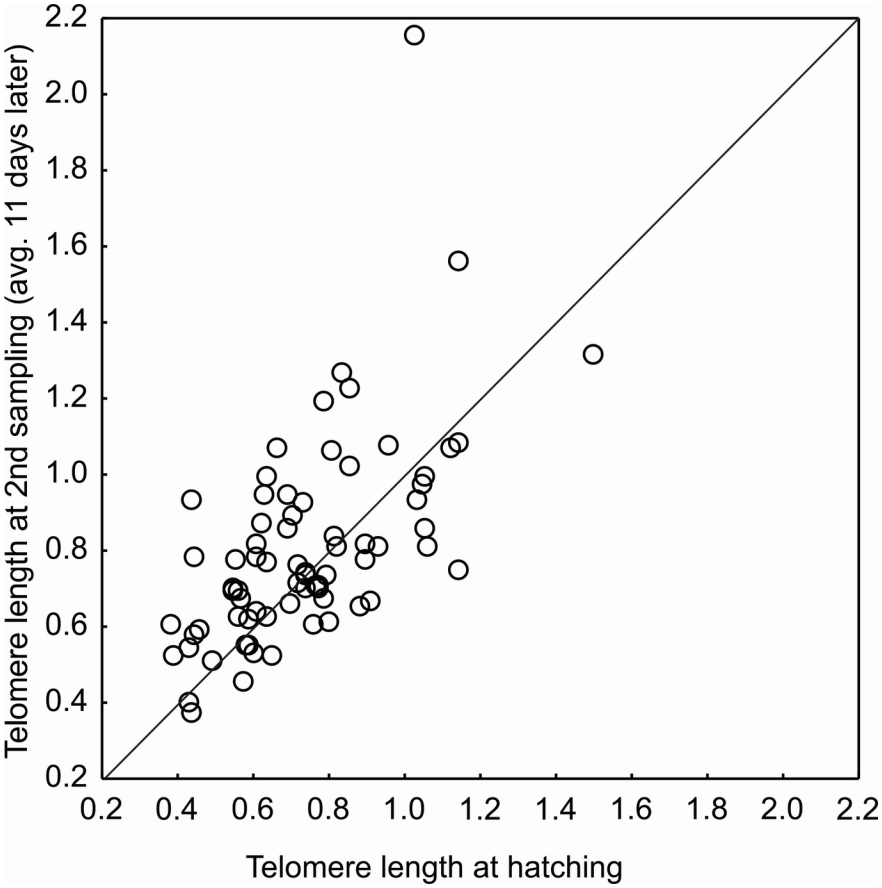
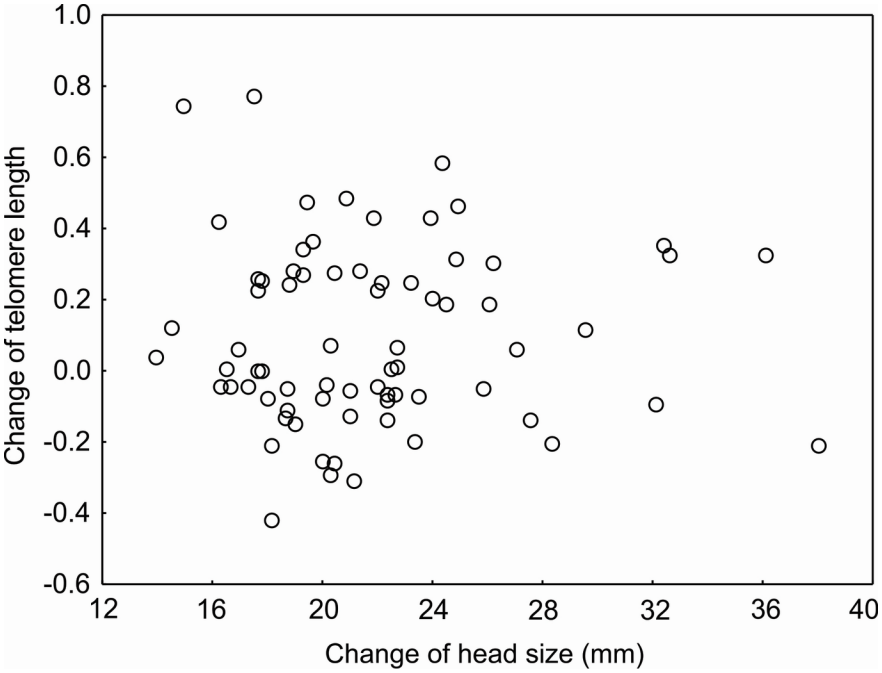


Figure 3. Relative telomere length dynamics between the first and second sampling (average time difference 10.62 days) were not related to chick growth rate (change of head size). Since head size and telomere length were measured at the same time points for individual birds, time is not included as a factor here.



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