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Parental age does not influence offspring telomeres during early life in common gulls

(*Larus canus*)

Running title: Parental age and telomeres in common gulls

Tuul Sepp^{a#*}, Richard Meitern^{a#}, Britt Heidinger^b, Kristina Noreikiene^c, Kalev Rattiste^d,
Peeter Hõrak^a, Lauri Saks^e, Jeffrey Kittilson^b, Janek Urvik^{af#}, Mathieu Giraudeau^{g#}

^a*Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia*

^b*Biological Sciences Department, North Dakota State University, Stevens Hall, Fargo, ND 58108, USA*

^c*Institute of Veterinary Medicine, Estonian University of Life Sciences, Kreutzwaldi 62, 51006 Tartu, Estonia*

^d*Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51006 Tartu, Estonia*

^e*Estonian Marine Institute, University of Tartu, Vanemuise 46a, 51014, Tartu, Estonia*

^f*Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 19, 50411, Tartu, Estonia*

^g*CREEC, 911 Avenue Agropolis, BP 6450134394 Montpellier Cedex 5, France and MIVEGEC,*

UMR IRD/CNRS/UM 5290, 911 Avenue Agropolis, BP 6450134394 Montpellier Cedex 5, France

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26 * Corresponding author, tuul.sepp@gmail.com

27 # these authors contributed equally to the study

28

29 **Abstract**

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

45

46 **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, increased 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 mechanisms that could mediate trans-generational effects of parental on offspring fitness could be offspring and parental 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, 1991). While some evidence suggests that TL is largely inherited (Nordfjäll, Larefalk, Lindgren, Holmberg & Roos, 2005; Njajou et al., 2007; Olsson et al., 2011, Horn et al., 2011; Reichert et al. 2014), 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 (but sometimes also elongate) 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, and in different taxonomic groups (Gomes et al. 2011). The effect of parental age on the length of offspring telomeres is currently 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 an increasing number of studies indicate parental age effects on offspring telomeres, the timing of these effects in terms of offspring development is still unknown. 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 between 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 (Broer et al. 2013; Unryn, Cook, & Riabowol, 2005), however, the effect of maternal age has been shown to be even stronger than paternal age 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 (Hausmann & 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 (Hausmann & 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; Hausmann & 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 dynamics (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 offsprings (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 occurring 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 during chick growth. Measuring chicks repeatedly allows to assess the change in TL during growth 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 (Viblanç 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 (from hatching to approximately two weeks old), based on blood samples. This time period was based on the feasibility of repeated trapping of precocial bird chicks. The red blood cells (a major component of the blood) of birds have a maximum lifespan of about 30-40 days (Rodnan et al., 1957), and accordingly, it is reasonable to expect to see an effect on telomere shortening in this time period. 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 biparental 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, independent of maternal age.

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 mate 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 between age classes (to test for the effects of parental age – young vs old - on offspring telomere length) and half of the clutches within age classes (to exclude the possibility that we are measuring the effect of cross-fostering). We opted for cross-fostering whole clutches instead of a split-nest design

for the following reasons. (1.) Swapping half clutches would have required very careful and logistically complicated monitoring of hatching order within nests, to know which chick hatched from which egg. (2.) Swapping part of the clutch would have required very careful timing with each two nests exactly on the same breeding schedule, to avoid age differences between „own“ and „foster“ chicks. (3.) In case of split-nest cross-fostering with a 3-egg clutch, if one of the chicks dies, the nest is lost for the analysis. (4) A split-nest design with a 3-egg clutch would reduce the robustness and power with the relatively few clutches available. (5) Since the main approach was to swap between parental age categories, cross-fostering whole clutches was a feasible study design. The 72 chicks that were successfully caught for second sampling were included in the study. From the nests of “old” parents, we recaptured on average 1.6 chicks, from “young” parents, 2.0 chicks (t-test $t=-1.7$, $p=0.1$). Within two days from hatching, we collected the first blood sample (10-30 μ L 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°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 and molecular sexing

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), all our samples had high purity. 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 (concentration in reaction mixture 200 nM). 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'-CGGTTTGTGGTTTGGGTTTGGGTT-3' and TEL 2b: 5'-GGCTTGCCTTACCCTTACCCTTACCCT-3. The qPCR reactions for GAPDH and telomeres were run on separate plates. The cycling parameters for telomere plate were following: 1 cycle – 10 min at 95°C, 27 cycles -15 sec at 95°C, 30 sec at 58°C, 30 sec at 72°C, 1 cycle of dissociation curve (melt curve). The cycling parameters for the GAPDH plate were following: 1 cycle -10 min at 95°C, 40 cycles – 30 sec at 95°C, 30 sec at 60°C, 1 cycle of

dissociation curve (melt curve). All reactions used 20 ng of DNA in a final volume of 25 μ l containing 12.5 μ l of SYBR green Master Mix, 0.25 μ l forward and reverse primer, 6 μ l water, and 6 μ l 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. Technical replicates of each sample, and the first and second sample for each bird were run on the same plate. Duplicates that had a SD higher of 0.25 were rerun again as duplicates. 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 $\pm$ 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 $\pm$ 3.00, and 98.94 $\pm$ 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%. Standards and Golden (reference sample) were from birds not included in the experiment. 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 $\pm$ 0.964), $p < 0.0001$, for single measurements ICC = 0.876 (95% CI 0.785 $\pm$ 0.930) $p < 0.0001$.

The CHD gene (Chromo Helicase DNA-binding gene) was use as a molecular marker for sexing the birds. PCR was used to amplify the CHD genes in DNA extracted from the red blood cells. The primers used in the PCR were as follows: 2550F (5'-

GTTACTGATTCGTCTACGAGA-3') and 2718R (5'- ATTGAAATGATCCAGTGCTTG-3') described by Fridolfsson and Ellergen (1999). The PCR products were visualized by gel electrophoresis and a FluorChem FC2 imaging system. One band on the gel indicates a male and two bands indicates a female.

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). Brood ID and the sex of the chick were included as a random effect in our analysis to account for the common origin of the chicks originating from the same brood. 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 2 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 were used for assessing the continuous effects of parental age (hatch year) on telomere length and telomere length dynamics. Repeatabilities 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 data repository (Sepp et al. 2021).

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, neither for male nor for female chicks (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). Parental age effect did not become significant after removal of the non-significant interactions and chick sex from the model (Table S2). 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 suggest 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 obscured the within individual patterns. Another possibility is that the link between parental age and offspring 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 no effect of parental age, it is possible that telomere length in common gulls is indeed relatively buffered from environmental influence.

Another possibility is that our experimental time scale (approximately two weeks) was too short to detect effects on telomeres. However, as the maximum lifespan of red blood cells of birds is estimated to be 30-45 days (Rodnan, Ebaugh & Fox 1957), we should have been able to detect telomere loss if it took place. Indeed, several studies have detected telomere loss within 30 days or less (e.g. Foote et al., 2011, Boonekamp et al., 2014; Heidinger et al., 2016).

We also considered the possibility that selective disappearance played a role in our finding of a lack of parental age effect on offspring telomere length and shortening, but consider it unlikely. The probability of trapping chicks from old and young parents did not differ statistically significantly. During the early age, recapture rate mainly depends on nest location – it is more difficult to trap chicks from nests that are close to water or surrounded by dense vegetation. In our colony, about half of the chicks die before fledging, mainly due to common hazards of the environment. Parental care quality might play a small role in chick mortality, but it is very likely that this role is eclipsed by the effect of random mortality. Accordingly, we consider the role of selective disappearance to be negligible in our results.

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). In several bird species, telomerase activity appears to be highest in the hatchling age-class, when the proliferative demands of most organs are the highest (Haussmann, Winkler, Huntington, Nisbet & Vleck, 2007). 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. The physiological mechanism mediating the link between parental age and recruitment rate in common gulls remains therefore open and deserves further attention. 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. Future studies applying this design should also consider measuring parental telomere length, to understand if telomere length is inherited to the offsprings in a parental-age-independent manner. Additionally, studies testing the same hypothesis over years with different breeding conditions could indicate how much the links between parental age and offspring quality are dependent on environmental conditions.

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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, JK 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)

References

- Asghar, M., Bensch, S., Tarka, M., Hansson, B., & Hasselquist, B. (2015a). Maternal and genetic factors determine early life telomere length. *Proceedings of the Royal Society B: Biological Sciences*, 282 20142263. doi: 10.1098/rspb.2014.2263
- Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., & Bensch, S. (2015 b). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* 347(6220), 436-438.
- Bateson, M. & Nettle, D. (2017). The telomere lengthening conundrum – it could be biology. *Aging Cell* 16, 312-319 (2017).
- Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2019). Epigenetic inheritance of telomere length in wild birds. *PLoS Genetics*. 15, e1007827 (2019).
- Beamonte-Barrientos, R., Velando, A., Drummond, H. & Torres, R. Senescence of maternal effects: aging influences egg quality and rearing capacities of a long-lived bird. *American Naturalist* 175, 469–480 (2010).
- Blackburn, E. H. (1991). Structure and function of telomeres. *Nature* 350, 569–573.
- Bock, M. J., Jarvis, G. C., Corey, E. L. Stone, E. E. & Gribble, K.E. (2019). Maternal age alters offspring lifespan, fitness, and lifespan extension under caloric restriction. *Scientific Reports* 9, 3138.
- Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., Verhulst, S. (2014) Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B: Biological Sciences*, 281: 20133287. <https://doi.org/10.1098/rspb.2013.3287>.
- Bouwhuis, S., Verhulst, S., Bauch, C. & Vedder, O. (2018) Reduced telomere length in offspring of old fathers in a longlived seabird. *Biology Letters*, 14, 20180213.
- Bowers, E. K., Sakaluk, S. K., & Thompson, C. F. (2017). Interactive effects of parental age on offspring fitness and age-assortative mating in a wild bird. *Journal of experimental zoology. Part A, Ecological and integrative physiology*, 327, 302–310. <https://doi.org/10.1002/jez.2090>
- Bradley R. J., & Safran, R. J. (2014). Conceptual revision and synthesis of proximate factors associated with age-related improvement in reproduction. *Ethology*, 120, 1–16.
- Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G. ... Boomsma, D.I. (2013). Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics* 21, 1163–1168.
- Bukacinska, M., Bukacinski, D., Epplen, J. T., Sauer, K. P., & Lubjuhn, T. (1998). Low frequency of extra-pair paternity in common gulls (*Larus canus*) as revealed by DNA fingerprinting. *Journal of Ornithology* 139, 413-420 (1998).
- Carslake, D., Tynelius, P., van den Berg, G. J., & Smith, G. D. (2019). Associations of parental age with offspring all-cause and cause-specific adult mortality. *Scientific Reports*, 9, 17097. <https://doi.org/10.1038/s41598-019-52853-8>

- Cerchiara, J. A., Risques, R. A., Prunkard, D., Smith, J. R., Kane, O. J., 1 & Boersma, P. D. (2017). Telomeres shorten and then lengthen before fledging in Magellanic penguins (*Spheniscus magellanicus*). *Aging* 9, 487-493.
- Chan, S. R. & Blackburn, E. H. (2004). Telomeres and telomerase. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359, 109-121.
- Clutton-Brock, T. H. (1984). Reproductive Effort and Terminal Investment in Iteroparous Animals *The American Naturalist*, 123, 212-229.
- Cong, Y. S., Wright, W. E. & Shay, J. W. Human telomerase and its regulation. *Microbiol. Mol. Biol. Rev.* 66, 407 (2002).
- Cawthon, R. M. Telomere measurment by quantitative PCR. *Nucleic Acids Res.* 129, 550-557 (2002).
- Criscuolo, F., Zahn, S., & Bize, P. (2017) Offspring telomere length in the long lived Alpine swift is negatively related to the age of their biological father and foster mother. *Biology Letters*, 13, 20170188.
- Dagnall, C. L., Hicks, B., Teshome, K., Hutchinson, A. A., Gadalla, S. M., Khincha, P. P. ... Savage, S. A. (2017). Effect of pre-analytic variables on the reproducibility of qPCR relative telomere length measurement. *PloS One*, 12, e0184098.
- Dantzer, B., van Kesteren, F., Westrick, S. E., Boutin, S., McAdam, A. G., Lane, J. E., ... Pat Monaghan. (2020). Maternal glucocorticoids promote offspring growth without inducing oxidative stress or shortening telomeres in wild red squirrels. *Journal of Experimental Biology* 2020 223: jeb212373 doi: 10.1242/jeb.212373 Published 6 January 2020
- de Frutos, C., López-Cardona A.P., Fonseca Balvís, N., Laguna-Barraza, R., Rizos, D., Gutierrez-Adán, A., & Bermejo-Álvarez, P. (2016) Spermatozoa telomeres determine telomere length in early embryos and offspring. *Reproduction*, 151, 1–7.
- de Villemereuil, P., Morrissey, M. B., Nakagawa, S., & Schielzeth, H. (2018). Fixed-effect variance and the estimation of repeatabilities and heritabilities: issues and solutions. *Journal of Evolutionary Biology*, 31, 621-632.
- Dugdale, H. L., & Richardson, D. S. (2018). Heritability of telomere variation: it is all about the environment! *Philosophical Transactions of the Royal Society B: Biological Sciences* 373, 20160450.
- Dupont, S. M., Barbraud, C., Chastel, O., Delord, K., Ruault, S., Weimerskirch, H. & Angelier, F. (2018). Young parents produce offspring with short telomeres: A study in a long-lived bird, the Black-browed Albatross (*Thalassarche melanophrys*). *PLoS ONE*, 13, e0193526.
- Eisenberg, D. T. A., & Kuzawa, C. W. (2018). The paternal age at conception effect on offspring telomere length: mechanistic, comparative and adaptive perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences* 373, 20160442.
- Eisenberg, D.T.A. Tackney, J., Cawthon, R.M., Cloutier, C.T. & Hawkes, K. (2016). Paternal and grandpaternal ages at conception and descendant telomere lengths in chimpanzees and humans. *American Journal of Physical Anthropology*, 162, 201–207.
- Eisenberg, D.T.A. (2014). Inconsistent inheritance of telomere length (TL): is offspring TL more strongly correlated with maternal or paternal TL? *European Journal of Human Genetics* 22, 8-9.
- Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, 15, 140–148.

- 527 Foote, C. G. Gault, E. A., Nasir, L., & Monaghan, P. (2011). Telomere dynamics in relation to
528 early growth conditions in the wild in the lesser black-backed gull. *Journal of Zoology*,
529 283, 203–209.
- 530 Fridolfsson, A.K., Ellegren, E. 1999. A simple and universal method for molecular sexing of
531 non-ratite birds. *Journal of Avian Biology* 30, 116-121.
- 532 Froy, H., Bird, E. J., Wilbourn, R. V., Fairlie, J., Underwood, S. L., Salvo-Chirnside, E., ...
533 Nussey, D. H. (2017). No evidence for parental age effects on offspring leukocyte
534 telomere length in free-living Soay sheep. *Scientific Reports* 7, 9991.
- 535 Giraudeau, M., Angelier, F., & Sepp, T. (2019). Do telomeres influence
536 pace- of- life- strategies in response to environmental conditions over a lifetime and
537 between generations? *BioEssays* 41, 1800162.
- 538 Gomes, N. M. V, Ryder, O.A., Houck, M.L., Charter, S.J., Walker, W., Forsyth N.R., ...
539 Wright W.E. (2011). Comparative biology of mammalian telomeres: hypotheses on
540 ancestral states and the roles of telomeres in longevity determination. *Aging Cell* 10,
541 761–768.
- 542 Green, P., MacLeod, C. (2015). SIMR: an R package for power analysis of generalized linear
543 mixed models by simulation. *Methods in Ecology and Evolution*, 7, 493-498.
- 544 Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed
545 Models: The MCMCglmm R Package. *Journal of Statistical Software*, 33, 1–22.
- 546 Haussmann, M.F., Winkler, D.W., Huntington, C.E., Nisbet, I.C. & Vleck, C.M. (2007)
547 Telomerase activity is maintained throughout the lifespan of long-lived birds.
548 *Experimental Gerontology*, 42, 610-618.
- 549 Haussmann, M. F., & Heidinger, B. J. (2015). Telomere dynamics may link stress exposure
550 and ageing across generations. *Biology Letters* 11, 20150396.
- 551 Heidinger, B.J., Herborn K. A., Granroth- Wilding H. M.V., Boner, W., Burthe, S., Newell,
552 M., ... Monaghan, P. (2016) Parental age influences offspring telomere loss. *Functional*
553 *Ecology*, 30, 1531-1538.
- 554 Hoelzl, F., Smith, S., Cornilsv, J.S., Aydinonat, D., Bieber, D., & Ruf, T. (2016) Telomeres
555 are elongated in older individuals in a hibernating rodent, the edible dormouse (*Glis*
556 *glis*). *Scientific Reports*, 6, 36856.
- 557 Horn, T., Robertson, B.C., Will, M., Eason, D.K., Elliott, G.P. & Gemmell, N.J. (2011).
558 Inheritance of telomere length in a bird. *PLoS ONE* 6: e17199.
- 559 Kimura, M., Cherkas, L. F., Kato, B. S., Demissie, S., Hjelmberg, J. B., Brimacombe, M., ...
560 Aviv, A. (2008). Offspring's leukocyte telomere length, paternal age, and telomere
561 elongation in sperm. *PLoS Genetics* 4, e37.
- 562 Kinugawa, C., Murakami, T., Okamura K. & Yajima. A. (2000). Telomerase activity in
563 normal ovaries and premature ovarian failure. *Tohoku Journal of Experimental*
564 *Medicine*, 190, 231-238.
- 565 Kuznetsova, A., Brockhoff, P.B. & Christensen, R. B. H. (2017). LmerTest Package: Tests in
566 Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1–26.
- 567 Larsson, K., Rattiste, K. & Lilleleht, V. (1997). Heritability of head size in the common gull
568 *Larus canus* in relation to environmental conditions during offspring growth. *Heredity*,
569 79, 201-20
- 570 Maklakov, A.A., & Chapman, T. (2019). Evolution of ageing as a tangle of trade-offs: energy
571 versus function. *Proceedings of the Royal Society B: Biological Sciences*, 286,
572 20191604.

- McLennan, D. Armstrong, J.D., Stewart, D.C., Mckelvey, S., Boner, W., Monaghan, P., Metcalfe, N.B. (2016). Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. *Molecular Ecology*, 25, 5425-5438.
- McLennan, D., Armstrong, J. D., Stewart, D. C., Mckelvey, S., Boner, W., Monaghan, P., & Metcalfe, N. B. (2018). Telomere elongation during early development is independent of environmental temperatures in Atlantic salmon. *Journal of Experimental Biology*, 221, 178616.
- Mizutani, Y., Niizuma, Y., & Yoda, K. (2016). How do growth and sibling competition affect telomere dynamics in the first month of life of long-lived seabird? *PLoS ONE*, 11, e0167261.
- Monaghan, P., & Ozanne, S. E. (2018). Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373, 20160446.
- Monaghan, P. (2010). Telomeres and life histories: the long and the short of it. *Annals of the New York Academy of Sciences*, 1206, 130–142.
- Monaghan, P. & Haussmann, M.F. (2006). Do telomere dynamics link lifestyle and lifespan? *Trends in Ecology and Evolution*, 21, 47-53.
- Nawrot, T.S., Staessen, J.A., Gardner, J.P. & Aviv, A. (2004). Telomere length and possible link to X chromosome. *Lancet*, 363, 507-510.
- Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T., & Bateson, M. (2015). An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20141610.
- Njajou, O.T., Cawthon, R.M., Damcott, C.M., Wu, S.H., Ott, S., Garant, M.J., Blackburn, E.H., Mitchell, B.D., Shuldiner, A.R. & Hsueh, W.-C. (2007). Telomere length is paternally inherited and is associated with parental lifespan. *Proceedings of the National Academy of Sciences of USA* 104, 12135–12139.
- Noguera, J. C., & Velando, A. (2020). Gull chicks grow faster but lose telomeres when prenatal cues mismatch the real presence of sibling competitors. *Proceedings of the Royal Society B: Biological Sciences*, 287, 20200242
- Noguera, J. C., Metcalfe, N. B. & Monaghan, P. (2018). Experimental demonstration that offspring fathered by old males have shorter telomeres and reduced lifespans. *Proceedings of the Royal Society B: Biological Sciences*, 285, 1874.
- Nordfjäll, K., Larefalk, Å., Lindgren, P., Holmberg, D. & Roos, G . (2005). Telomere length and heredity: Indications of paternal inheritance. *Proceedings of the National Academy of Sciences of USA*, 102, 16374–16378.
- Nussey, D. H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N. ... Monaghan, P. (2014). Measuring telomere length and telomere dynamics in evolutionary biology and ecology. *Methods in Ecology and Evolution*, 5, 299-310.
- Nussey, D. H., Froy, H., Lemaitre, J.-F., Gaillard, J. M., & Austad, S. N. (2013). Senescence in natural populations of animals: widespread evidence and its implications for biogerontology. *Ageing Research Reviews*, 12, 214-225.
- Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T. & Blomqvist, D . (2011). Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. *PLoS ONE* 6: e17473.

- Rattiste, K., Klandorf, K., Urvik, J., Sepp, T., Asghar, M. Hasselquist, D., Cooey, C., & Hřrak, P. (2015). Skin pentosidine and telomere length do not covary with age in a long-lived seabird. *Biogerontology*, *16*, 435-441.
- Rattiste, K. (2004). Reproductive success in presenescent common gulls (*Larus canus*): the importance of the last year of life. *Proceedings of the Royal Society B: Biological Sciences*, *271*, 2059-2064.
- Reichert, S., Rojas, E., Zahn, S., Robin, J.-P., Criscuolo, F. & Massemin, S. (2015) Maternal telomere length inheritance in the king penguin. *Heredity*, *114*, 10-16.
- Ricklefs, R. E. Insights from comparative analyses of aging in birds and mammals. *Aging cell*, *9*, 273-284 (2009).
- Rodnan, G. P., Ebaugh, Jr. F.G., Spivey Fox, M. R. & Chambers, D.M. (1957). the life span of the red blood cell and the red blood cell volume in the chicken, pigeon and duck as estimated by the use of Na²Cr⁵¹O₄: with observations on red cell turnover rate in the mammal, bird and reptile. *Blood*, *124*, 355-366.
- Sepp, T., Meitern, R., Heidinger, B., Noreikiene, K., Rattiste, K., Hřrak, P. saks, L., Urvik, J., Giraudeau, M. (2021). Supplementary data for the article: Parental age does not influence offspring telomeres during early life in common gulls (*Larus canus*) (Version 0.1) [Data set]. Molecular Ecology. *Zenodo*. <http://doi.org/10.5281/zenodo.4485871>
- Sepp, T., Rattiste K., Saks, L., Meitern, R., Urvik, J., Kaasik, A., & Hřrak, P. (2017). A small badge of longevity: opposing survival selection on the size of white and black wing markings. *Journal of Avian Biology*, *48*, 570-580.
- Simons, M. J., Stulp, G. & Nakagawa, S. (2014). A statistical approach to distinguish telomere elongation from error in longitudinal datasets. *Biogerontology*, *15*, 99-103.
- Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T. ... Richardson, D. S. (2018). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. *Journal of Animal Ecology*, *87*, 187-198.
- Stearns, S. C. (1992). The evolution of life-histories, Oxford University Press.
- Stier, A., Metcalfe, N. B., & Monaghan, P. (2019). Ageing before birth: pace and stability of prenatal growth affect telomere dynamics. *bioRxiv*, 809087. doi: <https://doi.org/10.1101/809087>
- Stoffel, M. A., Nakagawa, S., & Schielzeth H. (2017). RptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*, 1639-1644.
- Tarry-Adkins, J. L., Chen, J. H., Smith, N. S., Jones, R. H., Cherif, H. & Ozanne, S.E. (2009). Poor maternal nutrition followed by accelerated postnatal growth leads to telomere shortening and increased markers of cell senescence in rat islets. *The FASEB Journal*, *23*, 152-1528.
- Torres, R., Drummond, H., & Velando, A. (2011). Parental age and lifespan influence offspring recruitment: a long-term study in a seabird. *PloS ONE*, *6*, e2724.
- Unryn, B. M., Cook, L. S. & Riabowol, K. T. (2005). Paternal age is positively linked to telomere length of children. *Aging Cell*, *4*, 97-101.
- Urvik, J., Rattiste, K., Giraudeau, M., Okuliarová, M., Hřrak, P., & Sepp, T. (2018) Age-specific patterns of maternal investment in common gull egg yolk. *Biology Letters*, *14*, 20180346.
- Velarde, M., & Menon, R. (2016). Positive and negative effects of cellular senescence during female reproductive aging and pregnancy, *Journal of Endocrinology*, *230*, R59-R76.

- Viblanç, V. A., Schull, Q., Stier, A., Durand, L., Lefol, E., Robin, J-P. ... Criscuolo, F.(2020). Foster rather than biological parental telomere length predicts offspring survival and telomere length in king penguins. *Molecular Ecology*, Wiley, 2020, [ff10.1111/mec.15485](https://doi.org/10.1111/mec.15485)[ff](https://doi.org/10.1111/mec.15485). [ffhal-02868474f](https://doi.org/10.1111/mec.15485).
- Young, A. J. (2018). The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373, 20160452.
- Young, R. C., Welcker, J., Barger, C. P., Hatch, S. A., Merklings T., Kitaiskaia, E. V. ... Kitaysky, A. S. (2017). Effects of developmental conditions on growth, stress and telomeres in black-legged kittiwake chicks. *Molecular Ecology*, 26, 3572-3584.

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	CI	p
second - first measurement	0.11	0.02 – 0.20	0.022	0.09	-0.01 – 0.18	0.066
chick sex	-0.02	-0.12 – 0.09	0.749	-0.01	-0.11 – 0.09	0.796
mother age group	0.04	-0.10 – 0.17	0.592			
foster mother age group	0.11	-0.02 – 0.25	0.099			
time * mother age group	-0.03	-0.14 – 0.08	0.550			

time * foster mother age group	-0.03	-0.14 – 0.08	0.552
father age group	0.09	-0.04 – 0.22	0.179
foster father age group	0.09	-0.04 – 0.21	0.199
time * father age group	-0.02	-0.12 – 0.09	0.737
time * foster father age group	0.01	-0.10 – 0.11	0.899

Random Effects

σ^2	0.03	0.03
τ_{00}	0.02 chick_ID:parent_nest	0.02 chick_ID:parent_nest
	0.02 parent_nest	0.01 parent_nest
ICC	0.59	0.58
N	72 chick_ID	72 chick_ID
	40 parent_nest	40 parent_nest
Observations	144	144

σ^2 - within-group (residual) variance, τ_{00} - 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	Random effect (parent nest)	Father's hatch year	Foster father's hatch year	Random effect (parent nest)
Telomere length at hatching						
Mean (95% CI)	0.004 (- 0.005- 0.012)	0.007 (- 0.001- 0.015)	0.016 (0.003- 0.032)	0.006 (- 0.003- 0.015)	0.006 (- 0.004- 0.016)	0.016 (0.003- 0.032)
pMCMC	0.41	0.10		0.23	0.25	
Telomere length at second trapping						
Mean (95% CI)	0.001 (- 0.010-	0.003 (- 0.007-	0.021 (0.004-	0.009 (- 0.002-	0.009 (- 0.003-	0.019 (0.004-

	CI)	0.014)	0.014)	0.043)	0.020)	0.020)	0.039)
	pMCMC	0.85	0.56		0.11	0.16	
Change of telomere length							
Mean (95%	-0.003 (-	-0.005 (-	0.011	-0.031 (-	0.001 (-	0.011	
CI)	0.013-	0.014-	(0.002-	0.174-	0.01-	(0.002-	
	0.006)	0.005)	0.023)	0.103)	0.012)	0.023)	
	pMCMC	0.50	0.33		0.65	0.093	

CI-confidence interval.

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693 Table 4. Overview of studies in non-human vertebrates (excluding also laboratory rodents) investigating the effect of parental age
 694 on offspring telomere length. For a review on human studies, please see Froy et al (2017). Extended version of the table including
 695 study design and offspring developmental stage available in Supplementary Materials (Table S10).

Class	Species	Sample size	Studied parental effects	Telomere method	Parental effect (- / +)	age	Reference
Teleost fish	Atlantic salmon (<i>Salmo salar</i>)	84 HSF	M & P	qPCR (RTL)	Complex P_{fry} (+)		McLennan et al. 2016
	Atlantic salmon (<i>Salmo salar</i>)	60 HSF	M & P	qPCR (RTL)	Complex P_{fry} (-)		McLennan et al. 2018
Reptiles	Sand lizard (<i>Lacerta agilis</i>)	$N_{\text{sons}}=12$	P	TRF (TL in bp)	$P_{\text{sons}}(-)$		Olsson et al., 2011
Birds	Great reed warbler (<i>Acrocephalus arundinaceus</i>)	$N_{\text{ind}}=139$ $N_{\text{broods}}=46$	M & P	qPCR (RTL)	M (+)		Ashgar et al., 2015a
	European shag (<i>Phalacrocorax aristotelis</i>)	$N_{\text{ind}}=311$ $N_{\text{broods}}=134$	M & P	qPCR (RTL) and Δ RTL	$M_{\Delta\text{RTLoffspring}}(-)$ $P_{\Delta\text{RTLoffspring}}(-)$		Noguera et al., 2018
	Alpine swift (<i>Apus melba</i>)	$N_{\text{ind}}=95$ $N_{\text{broods}}=54$	M & P biological and foster	qPCR (RTL)	$P_{\text{biological}}(-)$ $M_{\text{foster}}(-)$		Heidinger et al. 2016
	Zebra finch (<i>Taeniopygia guttata</i>)	$N_{\text{ind}}=139$ $N_{\text{HSbroods}}=64$	P	qPCR (RTL)	P (-)		Criscuolo et al., 2017

	Black-browed albatross (<i>Thalassarche melanophrys</i>)	N _{ind} =51	Mean M & P	TRF (TL in bp)	Par (+)	Dupont et al. 2018
	Common tern (<i>Sterna hirundo</i>)	N=142	M & P	TRF (TL in bp)	P (-)	Bouwhuis et al. 2018
	Jackdaw (<i>Corvus monedula</i>)	N _{ind} =715 N _{nests} =298 N _{ind} =61 N _{nests} =31	M & P biological and foster	TRF (TL in bp)	P _{biol} (-)	Bauch et al., 2019
	Common gull (<i>Larus canus</i>) ^{Curr. study}	N _{ind} =72 N _{nests} =40	M & P	qPCR (RTL and ΔRTL)	No effect	Sepp et al. (current study)
Mammals	Soay sheep (<i>Ovis aries</i>)	N _{ind} =318	M & P	qPCR (RTL)	No effect	Froy et al. 2017
	Chimpanzee (<i>Pan troglodytes</i>)	N _{ind} =40	P	qPCR (RTL)	P (+)	Eisenberg et al. 2016

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.