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

2 **(*Larus canus*)**

3  
4 Running title: Parental age and telomeres in common gulls

5  
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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**

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

48

## 49 **Introduction**

50 Parental age often has long-term effects on offspring phenotype in humans (Carlaske,  
51 Tynelius, van den Berg, & Smith, 2019) and wild animals (Bowers, Sakaluk, & Thompson,  
52 2017). In some cases, increased parental age has a positive long-term effects on offspring  
53 (Bradley & Safran, 2014), possibly because older parents are more experienced breeders than  
54 younger parents and/or because reproductive investment increases with age as future  
55 reproductive opportunities decline (e.g., terminal investment) (Clutton-Brock, 1984).  
56 Alternatively, parental age may have negative long-term effects on offspring if older parents  
57 experience senescent declines in condition and/or reproductive function relative to younger  
58 parents (Bock, Jarvis, Corey, Stone, & Gribble, 2019; Maklakov & Chapman, 2019; Velarde  
59 & Menon, 2016). Regardless, the mechanisms that underlie these long-term effects of parental  
60 age on offspring phenotype are not well understood, although several candidate mechanisms  
61 have been proposed, including, for example, epigenetic factors (Bock et al. 2019).

62 One of the mechanisms that could mediate trans-generational effects of parental on offspring  
63 fitness could be offspring and parental telomere length (TL). Telomeres are highly conserved,  
64 repetitive, non-coding sequences of DNA that form protective caps at the ends of  
65 chromosomes, thereby enhancing genome stability (Blackburn, 1991). While some evidence  
66 suggests that TL is largely inherited (Nordfjäll, Larefalk, Lindgren, Holmberg & Roos, 2005;  
67 Njajou et al., 2007; Olsson et al., 2011, Horn et al., 2011; Reichert et al. 2014), it is also  
68 known that TL and telomere dynamics are affected by a complex interaction of genetic effects  
69 and environmental factors during an organism's life (Dugdale & Richardson, 2018;

70 Monaghan, 2010). While telomeres typically shorten (but sometimes also elongate) with age  
71 in somatic cells, elongation of telomeres has also been described, mainly as a result of the  
72 activity of the enzyme telomerase, which can extend telomeres via the addition of terminal  
73 telomeric repeats (Cong, Wright, & Shay, 2002), and which is variably active in different cell  
74 types and at different life stages, and in different taxonomic groups (Gomes et al. 2011). The  
75 effect of parental age on the length of offspring telomeres is currently intensively studied (e.g.  
76 Bauch, Boonekamp, Korsten, Mulder, & Verhulst, 2019; Criscuolo, Zahn, & Bize, 2017;  
77 Heidinger et al. 2016; Froy et al. 2017; Noguera & Velando, 2020). Costs and benefits  
78 associated with maintaining the length of telomeres are particularly interesting when  
79 considering the adaptive role of telomeres in the evolution of life histories, as it is suggested  
80 that telomeres play a proximate causal role in current–future life-history trade-offs (Young,  
81 2018). Optimal life-history strategies are both inherited and shaped by environmental effects  
82 (Stearns, 1992), and accordingly, telomere dynamics are a plausible physiological mechanisms  
83 related to life-histories (Giraudeau, Angelier, & Sepp, 2019).

84 While an increasing number of studies indicate parental age effects on offspring telomeres, the  
85 timing of these effects in terms of offspring development is still unknown. There are three  
86 potential routes through which parental age could impact offspring telomeres (Hausmann &  
87 Heidinger 2015; Heidinger & Young, 2020). First, age-associated changes in parental gametes  
88 may affect the telomere length of offsprings. Parental age may negatively influence offspring  
89 telomere length if older parents produce gametes with shorter telomeres (shown for example  
90 in mice, *Mus musculus*, de Frutos et al. 2015), however, studies in humans have also shown  
91 that older fathers may have offspring with longer telomeres (Broer et al. 2013; Unryn, Cook, &  
92 Riabowol, 2005), due to active telomerase in sperm cells (Kimura et al., 2008). Such

93 inconsistencies among studies, but also between species, could stem from differences in life-  
94 history strategies, likely via mechanisms related to spermatogonial stem cell telomere  
95 retention with increasing age, or selective attrition/survival of spermatogonia (Kimura et al.  
96 2008; Eisenberg & Kuzawa 2018). Studies in humans have mostly found a link between  
97 paternal age and offspring telomere length (Broer et al. 2013; Unryn, Cook, & Riabowol,  
98 2005), however, the effect of maternal age has been shown to be even stronger than paternal  
99 age for some species (e.g. great reed warbler *Acrocephalus arundinaceus*, Asghar, Bensch,  
100 Tarka, Hansson, & Hasselquist, 2015). While maternal reproductive cells develop at a very  
101 early age, after which they are retained throughout life without further cell divisions,  
102 association between mother age and offspring telomere length could be explained by other  
103 mechanisms, for example age- and/or condition-dependent telomerase activity in the ovaries  
104 (Asghar et al. 2015a; Kinugawa, Murakami, Okamura, & Yajima, 2000). Second possible  
105 route is through pre-natal effects, for example age-associated changes in the amounts of  
106 glucocorticoid or androgen hormones transferred from the mother to the developing embryo  
107 with increasing age of the mother (Hausmann & Heidinger, 2015; Heidinger & Young, 2020;  
108 Stier, Metcalfe, & Monaghan 2019). This could in turn activate the production of reactive  
109 oxygen species (ROS), as well as decrease telomerase activity in the offspring (Hausmann &  
110 Heidinger, 2015), potentially leading to telomere erosion. While there are now a number of  
111 studies linking pre-natal stress to offspring telomeres (reviewed for example, in Dantzer et al.  
112 2020; Hausmann & Heidinger; 2015, Heidinger & Young, 2020), none have included  
113 parental age in this equation. Third route are post-natal effects, as age-related variation in  
114 parental care and the characteristics of the post-birth environment could also be important  
115 mediators of offspring telomere dynamics (Tarry-Adkins et al. 2009). For example, more

116 experienced parents may provide better care (Beamonte-Barrientos, Velando, Drummond, &  
117 Torres, 2010), but older parents may also become less capable of providing a high quality  
118 environment due to senescence effects (Torres, Drummond, & Velando, 2011). The quality of  
119 the parental care during the growth phase may hasten or reduce telomere shortening  
120 (Criscuolo et al., 2017) with long-lasting effects on the aging rate and life-history trajectories  
121 of the offsprings (Young 2018).

122 Distinguishing between the role of genetic/pre-natal and post-natal effects on telomere length  
123 and telomere dynamics in natural populations early in life is difficult, but potentially most  
124 important from an evolutionary and ecological perspective (Dugdale & Richardson, 2018).

125 Birds are a promising model system for this kind of study, as the embryo development takes  
126 place within a sealed system, the egg, limiting the physiological maternal effects with the  
127 moment of egg laying. Cross-fostering experiments are a useful tool, as they allow to tease  
128 apart effects that are epigenetic and/or due to pre-natal egg effects, from effects occurring  
129 during incubation and/or chick feeding. However, to the best of our knowledge, there are only  
130 two studies that have used cross-fostering approach to separate the genetic and environmental  
131 effects of parental age on offspring telomere length. In alpine swifts (*Apus melba*), foster  
132 mother's age negatively affected offspring telomere length (Criscuolo et al., 2017). Bauch *et*  
133 *al.* (2019) showed that paternal age effect on offspring telomere length is independent of  
134 paternal care after conception in jackdaws (*Corvus monedula*). However, neither of these  
135 studies took repeated measures during chick growth. Measuring chicks repeatedly allows to  
136 assess the change in TL during growth and to separate phenotypic variance into individual and  
137 residual variance components to calculate repeatability (Stoffel, Nakagawa, & Schielzeth,  
138 2017). In a recent cross-fostering study of king penguins (*Aptenodytes patagonicus*), repeated

139 measures of TL were taken, indicating that chick telomere length was positively related to  
140 foster mothers' TL at both 10 and 105 days after hatching, however, this study did not include  
141 information about parental age (Viblanç et al 2020). Our study combines both of these  
142 approaches, applying a cross-fostering manipulation between differently aged parents with  
143 repeated measures of offspring telomere length in a wild population of long-lived birds. We  
144 cross-fostered whole clutches of common gull (*Larus canus*) eggs shortly after laying within  
145 and between age classes of young and old parents, and assessed telomere length and dynamics  
146 during the chick fastest growth phase (from hatching to approximately two weeks old), based  
147 on blood samples. This time period was based on the feasibility of repeated trapping of  
148 precocial bird chicks. The red blood cells (a major component of the blood) of birds have a  
149 maximum lifespan of about 30-40 days (Rodnan et al., 1957), and accordingly, it is reasonable  
150 to expect to see an effect on telomere shortening in this time period. We predicted that if there  
151 are pre-natal, epigenetic-like effects of parental age on offspring telomeres, there will be a  
152 relationship between the age of the natal parent and offspring telomeres, but if post-natal,  
153 environmental effects mediate the link between parental age and offspring telomere length,  
154 there will be a relationship between the age of the foster parent and offspring telomeres.

155

## 156 **Materials and methods**

### 157 **1. Field methods.**

158 We conducted the study between the end of May – early June 2017 on a free-living, known-  
159 age breeding population of common gulls located on Kakrarahu islet in Matsalu National Park  
160 on the west coast of Estonia (58°46' N, 23°26' E)<sup>34</sup>. Based on studies of offspring recruitment  
161 rate, reproductive success in common gulls increases to the 10th breeding year (12-13 years of

162 age) and decreases thereafter (Rattiste, 2004). It is known that they undergo senescence in a  
163 number of physiological functions (Rattiste et al. 2015; Sepp et al. 2017; Urvik, Rattiste,  
164 Giraudeau, Okuliarová, Hōrak & Sepp, 2018). In addition, this species is characterized by bi-  
165 parental care and a low frequency of extra-pair mating (Bukacinska, Bukacinski, Epplen.,  
166 Sauer, & Lubjuhn, 1998), making it possible to study the effects of age of the parent of both  
167 sexes, and the age-dependent quality of parental care. All of the birds included in the study  
168 were banded as chicks and their exact age was therefore known. Common gulls typically lay  
169 clutches of three eggs, independent of maternal age.

170 A total of 40 clutch nests were included in the experiment. Nests and experimental groups  
171 were chosen based on the age of the mother, but the father's age was also known. Common  
172 gulls mates assortatively with respect to age and the ages of the parents were highly positively  
173 correlated (Spearman  $r=0.74$ ,  $p<0.0001$ ) Half of the breeders ( $n = 20$  females) were young, on  
174 their 1st-3rd breeding event (age exactly 5 years). Another half ( $n = 20$  females) were middle-  
175 aged or older (15-30 years old, average age  $18 \pm 3.37$  (SD) years). In total, 19 males were 5-7  
176 years old (average age  $5.52 \pm 0.80$  (SD) years) and were grouped as "young", 21 males were  
177 10+ years (average age  $16.29 \pm 5.58$  (SD) years) old and were grouped as "old". Distribution  
178 of the ages of parent birds are shown in figures S4-S7.

179 We cross-fostered whole clutches right after the clutch was completed both within and  
180 between maternal age classes (Table 1), so all of the chicks included in this experiment  
181 hatched in foster-parent's nests. Half of the clutches were cross-fostered between age classes  
182 (to test for the effects of parental age – young vs old - on offspring telomere length) and half  
183 of the clutches within age classes (to exclude the possibility that we are measuring the effect  
184 of cross-fostering). We opted for cross-fostering whole clutches instead of a split-nest design

185 for the following reasons. (1.) Swapping half clutches would have required very careful and  
186 logistically complicated monitoring of hatching order within nests, to know which chick  
187 hatched from which egg. (2.) Swapping part of the clutch would have required very careful  
188 timing with each two nests exactly on the same breeding schedule, to avoid age differences  
189 between „own“ and „foster“ chicks. (3.) In case of split-nest cross-fostering with a 3-egg  
190 clutch, if one of the chicks dies, the nest is lost for the analysis. (4) A split-nest design with a  
191 3-egg clutch would reduce the robustness and power with the relatively few clutches available.  
192 (5) Since the main approach was to swap between parental age categories, cross-fostering  
193 whole clutches was a feasible study design. The 72 chicks that were successfully caught for  
194 second sampling were included in the study. From the nests of “old” parents, we recaptured on  
195 average 1.6 chicks, from “young” parents, 2.0 chicks (t-test  $t=-1.7$ ,  $p=0.1$ ). Within two days  
196 from hatching, we collected the first blood sample (10-30  $\mu$ L taken, from brachial vein) for  
197 telomere measurement (average age  $0.61 \pm 0.09$  (SE) days) and individually marked the chicks  
198 for identification. Chick head size (the distance from the tip of the bill to the back of the head)  
199 was measured with a calliper to the nearest 0.1 mm. The day of the second blood sample  
200 varied between chicks, due to difficulties in trapping precocial chicks. We captured the chicks  
201 near their nests and collected a second blood sample between the 8th and 20th day after  
202 hatching (with one exception of a 5-days old chick). Average age at second blood sampling  
203 was 10.62 days ( $\pm 0.35$  SE). Blood samples were kept on ice in an insulated box while on the  
204 islet, and stored at  $-20^{\circ}\text{C}$  until the end of the field work period, when they were transferred to -  
205  $80^{\circ}\text{C}$  and held there until analysed. The experimental protocol was approved by the Ministry  
206 of Rural Affairs of the Republic of Estonia (licence no. 106, issued 24.05.2017) and was  
207 performed in accordance with relevant Estonian and European guidelines and regulations.

208

## 209 **2. Telomere measurement methods and molecular sexing**

210 DNA was extracted from whole blood samples using Macherey Nagel Nucleospin Blood kits  
211 following the manufacturer's protocol. Avian red blood cells are nucleated and are well suited  
212 for longitudinal telomere analysis (Nussey et al. 2014). DNA concentration and purity were  
213 assessed with a Nanodrop 8000 (Thermo Scientific), all our samples had high purity. Relative  
214 telomere length was measured using qPCR (quantitative polymerase chain reaction) on an  
215 Mx3000P (Stratagene) as described in Cawthon (2002) and modified for use in common gulls  
216 (Rattiste et al., 2015). The relative telomere length (T/S) of the samples was calculated as the  
217 ratio of the telomere repeat copy number (T) to that of a single copy control gene (S), relative  
218 to the reference sample. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as  
219 the single copy control gene. We used the following gull specific GAPDH forward and reverse  
220 primers (Integrated DNA Technologies): 5'-CGGAGCACCGCTTACAATTT-3' and 5'-  
221 GCATCTCCCCACTTGATGTTG-3' respectively (concentration in reaction mixture 200  
222 nM). Amplified samples were run on a 3% agarose gel to verify that the amplification was a  
223 single product, which yielded a single band at 77 bp as expected. We used the following  
224 telomere primers (Quanta Bio, with final concentration of 200 nM): TEL 1b: 5'-  
225 CGGTTTGTGGTTGGTTGGTTGGTT-3' and TEL 2b: 5'-  
226 GGCTTGCCTTACCCTTACCCTTACCCT-3. The qPCR reactions for GAPDH and  
227 telomeres were run on separate plates. The cycling parameters for telomere plate were  
228 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,  
229 1 cycle of dissociation curve (melt curve). The cycling parameters for the GAPDH plate were  
230 following: 1 cycle -10 min at 95°C, 40 cycles – 30 sec at 95°C, 30 sec at 60°C, 1 cycle of

231 dissociation curve (melt curve). All reactions used 20 ng of DNA in a final volume of 25  $\mu$ l  
232 containing 12.5  $\mu$ l of SYBR green Master Mix, 0.25  $\mu$ l forward and reverse primer, 6  $\mu$ l  
233 water, and 6  $\mu$ l of DNA sample. A negative control of water was run on each plate. All  
234 samples were run in duplicate, and average values were used to determine the T/S ratio.  
235 Treatment groups were distributed approximately equally between plates. Technical replicates  
236 of each sample, and the first and second sample for each bird were run on the same plate.  
237 Duplicates that had a SD higher of 0.25 were rerun again as duplicates. In order to assess the  
238 efficiencies of each plate, samples were run against a standard curve of 40, 20, 10, 5, and 2.5  
239 ng produced by serially diluting a reference sample. In all cases, plate efficiencies were in the  
240 accepted range (i.e. 100 $\pm$ 15%) and all of the samples fell within the bounds of the standard  
241 curve. Average plate efficiencies and standard errors for GAPDH and telomere plates were  
242 102.34  $\pm$  3.00, and 98.94  $\pm$  3.00, respectively. The average intra-plate variation of the Ct  
243 values was 0.82% for the telomere assays and 0.19% for the GAPDH assays, and the inter-  
244 plate CV-s of the Ct values for telomere and GAPDH assays were 0.64% and 0.34%,  
245 respectively. The same individual was also included on every plate and the coefficient of  
246 variation of the T/S ratio across plates was 8.12%. Standards and Golden (reference sample)  
247 were from birds not included in the experiment. A subset of samples (45) was ran twice for  
248 assessing measurement accuracy. Average Intraclass Correlation Coefficient (ICC) in this  
249 subset was 0.934 (95% CI 0.879  $\pm$  0.964),  $p < 0.0001$ , for single measurements ICC = 0.876  
250 (95% CI 0.785  $\pm$  0.930)  $p < 0.0001$ .

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

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

258

### 259 **3. Statistical methods**

260 To test for an effect of parental age on chicks' telomere erosion and telomere measurements at  
261 first and second sampling, we used repeated measures mixed models and the R package  
262 lmerTest (Kuznetsova, Brockhoff & Christensen, 2017). Brood ID and the sex of the chick  
263 were included as a random effect in our analysis to account for the common origin of the  
264 chicks originating from the same brood. Models included age groups (young or old) of either  
265 mother and foster mother or father and foster father as categorical predictors, to emphasize our  
266 study design and questions. Similar models were also run with age as a continuous factor  
267 (Table S1). Separate models were run for mother's and father's age effect, since the age of  
268 parents was strongly correlated and could not be entered in the same models. Both models  
269 with and without interaction (Tables 2 and S2) with sampling occasion (first or second) were  
270 run. Interval between sampling varied between individuals, but this was not included in these  
271 models as it was not related to telomere length (Figure S8, Table S6,  $p = 0.805$ ). Parental age  
272 effect models were run for head size as a dependent variable (Tables S4-S5). In these models,  
273 chick age was included as a predictive factor since it correlated significantly with head size.  
274 Markov Chain Monte Carlo (MCMC) multivariate generalised linear mixed models were  
275 used for assessing the continuous effects of parental age (hatch year) on telomere length and  
276 telomere length dynamics. Repeatabilities were calculated using R package rptR (Stoffel et al.,

277 2017). Heritabilities were obtained using one-way ANOVA from the full sib design and (for  
278 the models including parental age and treatment effects) using generalised linear mixed  
279 models for the repeated measures design (R package MCMCglmm) (Hadfield 2010, de  
280 Villemereuil, Morrissey, Nakagawa & Schielzeth, 2018; Kuznetsova & Hadfield, 2010). The  
281 models were run for both telomere measurements (first and second) as well as change as a  
282 dependent variable. The models included parent nest as random effect and the same fixed  
283 effects as the repeated measures mixed model for the effect of telomere length change. Both  
284 repeatability and heritability measures are reported together with 95% coefficient intervals  
285 (CI). The distribution of the traits was normal or close to normal distribution (Figures S1-S3),  
286 accordingly, models were ran with untransformed values. Power analyses were conducted  
287 based on Monte Carlo simulations (Green & MacLeod, 2015) (Table S3). The R code for  
288 different steps in the statistical analyses is accessible from the data repository (Sepp et al.  
289 2021).

290

## 291 **Results**

292 There were no significant effects of the ages of the natal or foster parents on offspring  
293 telomeres at hatching or the change in offspring telomere length from hatching to the end of  
294 post-natal development, neither for male nor for female chicks (Figure 1, see Tables 2 and S2  
295 for results with age groups, young vs old, and Tables 3 and S1 for results with parental age as  
296 a continuous factor). Parental age effect did not become significant after removal of the non-  
297 significant interactions and chick sex from the model (Table S2). When we entered the  
298 parental age squared in the models, it did not change this result (Tables S7-S9). Telomere  
299 length was individually repeatable between the first and second sampling, separated by on

300 average 11 days (repeatability  $r = 0.55$ , CI= 0.368 to 0.69,  $p < 0.0001$ , Figure 2). Chick  
301 telomere length between the first and second telomere measurements increased rather than  
302 decreased (the estimated change in relative telomere length was  $0.08 \pm 0.05$ ,  $p=0.003$ , Table 2;  
303 when one outlier chick with highest increase in telomere length was removed, the results  
304 stayed similar, estimated change  $0.06 \pm 0.04$ ,  $p = 0.005$ ). The rate of growth during post-natal  
305 development was not significantly related to the change in telomere length (measured as  
306 change of head size,  $r < 0.0001$ ;  $p = 0.97$ , Figure 3). Chick growth rate was not related to the  
307 age of the parents and foster parents (all parental effect  $p$ -values  $> 0.31$ , Tables S4-S5). The  
308 power for detecting potential effects of parental age on telomere length and telomere  
309 shortening in our sample is reported in Table S3. The study set up also allowed us to assess  
310 wide-sense heritability of telomere length, although these results must be taken with caution,  
311 since we did not use a split nest cross-fostering design. Chick telomere length at both first  
312 ( $h^2=0.27$  CI=0.05 to 0.50) and second ( $h^2=0.30$  CI=0.08 to 0.54) measurement as well as the  
313 change of telomere length (calculated as telomere length 2 - telomere length 1,  $h^2=0.14$   
314 CI=0.03 to 0.29) were heritable.

315

## 316 **Discussion**

317 Parental age often has long-term consequences for offspring phenotype and fitness, but the  
318 mechanisms that mediate these effects remain insufficiently studied. Previous research suggest  
319 that telomeres may be an important mechanism mediating these effects, but the routes by  
320 which these effects occur are not well understood. The cross-fostering experiment used here  
321 allowed us to distinguish whether any effects of parental age on offspring telomeres occur as a  
322 result of epigenetic like and/or pre-natal effects vs. post-natal effects. Previous research in this

323 colony of common gulls has demonstrated that offspring fitness decreases with increasing  
324 parental age: recruitment rate decreases in common gulls after the 10th breeding year (12-13  
325 years of age) (Rattiste, 2004), and older gulls allocate less nutrients to their eggs (Urvik et al.,  
326 2018). However, despite these demonstrated effects of parental age on offspring fitness in this  
327 species, we did not find any evidence of the effect of parental age on offspring telomeres at  
328 hatching or the change in telomere length during post-natal development.

329 Although parental age has been shown to have both negative and positive effects on offspring  
330 telomere length (Table 4), this has not been found in all studies. For example, no effect of  
331 parental age on offspring telomere length was found in a study of Soay sheep (*Ovis aries*)  
332 despite a large sample size (Froy et al., 2017). Although bird studies have generally found a  
333 negative effect of parental age on offspring telomeres (see for example studies in alpine swifts,  
334 (Criscuolo et al., 2017), and European shags *Phalacrocorax aristotelis*, (Heidinger et al.,  
335 2016), this is not a universal pattern (for example, older great reed warblers *Acrocephalus*  
336 *arundinaceus* produced offspring with longer telomeres, (Asghar et al., 2015b). Also in  
337 humans the effect of paternal age is positive rather than negative, as older fathers sire  
338 offspring with longer telomeres (Broer et al., 2013). A review article summarizing these  
339 results concluded that while there is no clear pattern across species, this is unlikely to be  
340 explained by statistical noise or publication bias (Eisenberg, 2014), but rather linked to the  
341 specific biology of each species. There are also several additional factors that could contribute  
342 to these mixed results: whether the study is experimental or correlative, at what age the  
343 offspring are sampled, and whether it is a longitudinal versus cross-sectional study of parental  
344 age. Accordingly, we cannot rule out that a longitudinal set-up would have revealed an effect  
345 of parental age on offspring telomere results also in our study system, as the cross-sectional

346 design could have obscures the within individual patterns. Another possibility is that the link  
347 between parental age and offspring telomere length in this species was too weak to be revealed  
348 from our sample size, despite relatively good test power (Table S3). In this case, and also in  
349 the case of no effect of parental age, it is possible that telomere length in common gulls is  
350 indeed relatively buffered from environmental influence.

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

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

365 Telomere length was repeatable across the chick rearing period in our study, indicating a  
366 possible increase during post-natal development, and not being related to chick growth rate.

367 Telomeres were longer at the second sampling, when chicks were approximately 11 days old.

368 This period covered the fastest growth period of gull chicks. While telomeres shorten during

369 growth in most studied adult vertebrates (which, as of now, mainly include mammals and  
370 some bird species), some vertebrates show increased telomerase activity during development  
371 (reviewed by Larsson, Rattiste, & Lilleleht, 1997, Monaghan & Haussmann, 2006). In several  
372 bird species, telomerase activity appears to be highest in the hatchling age-class, when the  
373 proliferative demands of most organs are the highest (Haussmann, Winkler, Huntington,  
374 Nisbet & Vleck, 2007). It has even been suggested that in free-living long-lived organisms,  
375 evolution should favour mechanisms that maintain the longest possible telomeres at the end of  
376 the most active growth period (Chan, & Blackburn, 2004). Studies in the wild have shown that  
377 telomeres elongate in at least some life stages in Seychelles warblers (*Acrocephalus*  
378 *sechellensis* (Spurgin et al., 2018)), edible dormouse (*Glis glis* (Hoelzl et al., 2016)), Soay  
379 sheep (Fairlie et al., 2016), Magellanic penguins (*Spheniscus magellanicus* (Cerchiara et al.,  
380 2017)), and Atlantic salmon (*Salmo salar* (McLennan et al., 2018)). However, when looking at  
381 the few studies on species more closely related to common gulls, telomere shortening during  
382 the early growth period has been reported. For example, in lesser black-backed gull (*Larus*  
383 *fuscus*), telomeres shortened from hatching to ten-days old (Foote et al., 2011), and in black-  
384 tailed gull chicks (*Larus crassirostris*), telomere attrition was shown for chicks growing with  
385 siblings in the nest (but not singleton chicks (Mizutani, Niizuma, & Yoda, 2016)). The latter  
386 study suggests that, in favourable growing conditions, telomere attrition might be prevented.  
387 However, more results on closely related species are needed to confirm the possibility of  
388 telomere lengthening during early growth in gulls. Growth rate was independent of telomere  
389 length or telomere dynamics (Figure 3). In general, a trade-off between rapid growth and  
390 telomere maintenance is expected, due to increased number of cell divisions required to attain  
391 larger size, and/or increased loss of telomere length during each cell division as a consequence

392 of the conditions required for fast growth (e.g. higher metabolic rate and ROS production  
393 reviewed by Monaghan & Ozanne (2018)). The current study adds to the increasing number of  
394 studies suggesting a different pattern in long-lived seabirds (Mizutani et al., 2016; Young et  
395 al., 2017). Seabirds are distinguished from most other species by a long time period between  
396 the end of relatively fast somatic growth and the beginning of reproduction (stretching several  
397 years). More studies applying comparable methodological approaches are needed for a  
398 comparative study, including patterns of growth and life-history strategies of different species  
399 to determine if this phenomenon of delayed reproduction is causally linked with the lack of an  
400 association between fast growth and telomere shortening in seabirds.

401 In conclusion, our results suggest that the age of the parents at the time of offspring  
402 conception does not influence offspring telomere length or the change in telomere length in  
403 common gulls. The physiological mechanism mediating the link between parental age and  
404 recruitment rate in common gulls remains therefore open and deserves further attention. An  
405 important area of future research is to identify other mechanisms that mediate the long-term  
406 effects of parental age on offspring and to better understand the factors that contribute to the  
407 variation in the influence of parental age on offspring telomeres across species. Future studies  
408 applying this design should also consider measuring parental telomere length, to understand if  
409 telomere length is inherited to the offsprings in a parental-age-independent manner.  
410 Additionally, studies testing the same hypothesis over years with different breeding conditions  
411 could indicate how much the links between parental age and offspring quality are dependent  
412 on environmental conditions.

413

414

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423

424 **Compliance with ethical standards**

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

428

429 **Conflict of interests**

430 The authors declare no competing interests.

431

432 **Authors' contribution**

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

437

438 **Data accessibility**

439 Data available from the Dryad Digital Repository (TBA)

440

441 **References**

- 442 Asghar, M., Bensch, S., Tarka, M., Hansson, B., & Hasselquist, B. (2015a). Maternal and  
443 genetic factors determine early life telomere length. *Proceedings of the Royal Society B:  
444 Biological Sciences*, 282 20142263. doi: 10.1098/rspb.2014.2263
- 445 Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., & Bensch, S. (2015  
446 b). Hidden costs of infection: Chronic malaria accelerates telomere degradation and  
447 senescence in wild birds. *Science* 347(6220), 436-438.
- 448 Bateson, M. & Nettle, D. (2017). The telomere lengthening conundrum – it could be biology.  
449 *Aging Cell* 16, 312-319 (2017).
- 450 Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2019). Epigenetic  
451 inheritance of telomere length in wild birds. *PLoS Genetics*. 15, e1007827 (2019).
- 452 Beamonte-Barrientos, R., Velando, A., Drummond, H. & Torres, R. Senescence of maternal  
453 effects: aging influences egg quality and rearing capacities of a long-lived bird.  
454 *American Naturalist* 175, 469–480 (2010).
- 455 Blackburn, E. H. (1991). Structure and function of telomeres. *Nature* 350, 569–573.
- 456 Bock, M. J., Jarvis, G. C., Corey, E. L. Stone, E. E. & Gribble, K.E. (2019). Maternal age  
457 alters offspring lifespan, fitness, and lifespan extension under caloric restriction.  
458 *Scientific Reports* 9, 3138.
- 459 Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., Verhulst, S. (2014) Nestling  
460 telomere shortening, but not telomere length, reflects developmental stress and predicts  
461 survival in wild birds. *Proceedings of the Royal Society B: Biological Sciences*, 281:  
462 20133287. <https://doi.org/10.1098/rspb.2013.3287>.
- 463 Bouwhuis, S., Verhulst, S., Bauch, C. & Vedder, O. (2018) Reduced telomere length in  
464 offspring of old fathers in a longlived seabird. *Biology Letters*, 14, 20180213.
- 465 Bowers, E. K., Sakaluk, S. K., & Thompson, C. F. (2017). Interactive effects of parental age  
466 on offspring fitness and age-assortative mating in a wild bird. *Journal of experimental  
467 zoology. Part A, Ecological and integrative physiology*, 327, 302–310.  
468 <https://doi.org/10.1002/jez.2090>
- 469 Bradley R. J., & Safran, R. J. (2014). Conceptual revision and synthesis of proximate factors  
470 associated with age-related improvement in reproduction. *Ethology*, 120, 1–16.
- 471 Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G. ... Boomsma, D.I.  
472 (2013). Meta-analysis of telomere length in 19,713 subjects reveals high heritability,  
473 stronger maternal inheritance and a paternal age effect. *European Journal of Human  
474 Genetics* 21, 1163–1168.
- 475 Bukacinska, M., Bukacinski, D., Epplen, J. T., Sauer, K. P., & Lubjuhn, T. (1998). Low  
476 frequency of extra-pair paternity in common gulls (*Larus canus*) as revealed by DNA  
477 fingerprinting. *Journal of Ornithology* 139, 413-420 (1998).
- 478 Carslake, D., Tynelius, P., van den Berg, G. J., & Smith, G. D. (2019). Associations of  
479 parental age with offspring all-cause and cause-specific adult mortality. *Scientific  
480 Reports*, 9, 17097. <https://doi.org/10.1038/s41598-019-52853-8>

- 481 Cerchiara, J. A., Risques, R. A., Prunkard, D., Smith, J. R., Kane, O. J., 1 & Boersma, P. D.  
482 (2017). Telomeres shorten and then lengthen before fledging in Magellanic penguins  
483 (*Spheniscus magellanicus*). *Aging* 9, 487-493.
- 484 Chan, S. R. & Blackburn, E. H. (2004). Telomeres and telomerase. *Philosophical*  
485 *Transactions of the Royal Society B: Biological Sciences* 359, 109-121.
- 486 Clutton-Brock, T. H. (1984). Reproductive Effort and Terminal Investment in Iteroparous  
487 Animals *The American Naturalist*, 123, 212-229.
- 488 Cong, Y. S., Wright, W. E. & Shay, J. W. Human telomerase and its regulation. *Microbiol.*  
489 *Mol. Biol. Rev.* 66, 407 (2002).
- 490 Cawthon, R. M. Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 129, 550-557  
491 (2002).
- 492 Criscuolo, F., Zahn, S., & Bize, P. (2017) Offspring telomere length in the long lived Alpine  
493 swift is negatively related to the age of their biological father and foster mother. *Biology*  
494 *Letters*, 13, 20170188.
- 495 Dagnall, C. L., Hicks, B., Teshome, K., Hutchinson, A. A., Gadalla, S. M., Khincha, P. P. ...  
496 Savage, S. A. (2017). Effect of pre-analytic variables on the reproducibility of qPCR  
497 relative telomere length measurement. *PLoS One*, 12, e0184098.
- 498 Dantzer, B., van Kesteren, F., Westrick, S. E., Boutin, S., McAdam, A. G., Lane, J. E., ... Pat  
499 Monaghan. (2020). Maternal glucocorticoids promote offspring growth without inducing  
500 oxidative stress or shortening telomeres in wild red squirrels. *Journal of Experimental*  
501 *Biology* 2020 223: jeb212373 doi: 10.1242/jeb.212373 Published 6 January 2020
- 502 de Frutos, C., López-Cardona A.P., Fonseca Balvís, N., Laguna-Barraza, R., Rizos, D.,  
503 Gutierrez-Adán, A., & Bermejo-Álvarez, P. (2016) Spermatozoa telomeres determine  
504 telomere length in early embryos and offspring. *Reproduction*, 151, 1–7.
- 505 de Villemereuil, P., Morrissey, M. B., Nakagawa, S., & Schielzeth, H. (2018). Fixed-effect  
506 variance and the estimation of repeatabilities and heritabilities: issues and solutions.  
507 *Journal of Evolutionary Biology*, 31, 621-632.
- 508 Dugdale, H. L., & Richardson, D. S. (2018). Heritability of telomere variation: it is all about  
509 the environment! *Philosophical Transactions of the Royal Society B: Biological*  
510 *Sciences* 373, 20160450.
- 511 Dupont, S. M., Barbraud, C., Chastel, O., Delord, K., Ruault, S., Weimerskirch, H. & Angelier,  
512 F. (2018). Young parents produce offspring with short telomeres: A study in a long-lived  
513 bird, the Black-browed Albatross (*Thalassarche melanophrys*). *PLoS ONE*, 13,  
514 e0193526.
- 515 Eisenberg, D. T. A., & Kuzawa, C. W. (2018). The paternal age at conception effect on  
516 offspring telomere length: mechanistic, comparative and adaptive perspectives.  
517 *Philosophical Transactions of the Royal Society B: Biological Sciences* 373, 20160442.
- 518 Eisenberg, D.T.A. Tackney, J., Cawthon, R.M., Cloutier, C.T. & Hawkes, K. (2016). Paternal  
519 and grandpaternal ages at conception and descendant telomere lengths in chimpanzees  
520 and humans. *American Journal of Physical Anthropology*, 162, 201–207.
- 521 Eisenberg, D.T.A. (2014). Inconsistent inheritance of telomere length (TL): is offspring TL  
522 more strongly correlated with maternal or paternal TL? *European Journal of Human*  
523 *Genetics* 22, 8-9.
- 524 Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H.  
525 (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal.  
526 *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.

- 573 McLennan, D. Armstrong, J.D., Stewart, D.C., Mckelvey, S., Boner, W., Monaghan, P.,  
574 Metcalfe, N.B. (2016). Interactions between parental traits, environmental harshness  
575 and growth rate in determining telomere length in wild juvenile salmon. *Molecular*  
576 *Ecology*, 25, 5425-5438.
- 577 McLennan, D., Armstrong, J. D., Stewart, D. C., Mckelvey, S., Boner, W., Monaghan, P., &  
578 Metcalfe, N. B. (2018). Telomere elongation during early development is independent of  
579 environmental temperatures in Atlantic salmon. *Journal of Experimental Biology*, 221,  
580 178616.
- 581 Mizutani, Y., Niizuma, Y., & Yoda, K. (2016). How do growth and sibling competition affect  
582 telomere dynamics in the first month of life of long-lived seabird? *PLoS ONE*, 11,  
583 e0167261.
- 584 Monaghan, P., & Ozanne, S. E. (2018). Somatic growth and telomere dynamics in vertebrates:  
585 relationships, mechanisms and consequences. *Philosophical Transactions of the Royal*  
586 *Society B: Biological Sciences*, 373, 20160446.
- 587 Monaghan, P. (2010). Telomeres and life histories: the long and the short of it. *Annals of the*  
588 *New York Academy of Sciences*, 1206, 130–142.
- 589 Monaghan, P. & Haussmann, M.F. (2006). Do telomere dynamics link lifestyle and lifespan?  
590 *Trends in Ecology and Evolution*, 21, 47-53.
- 591 Nawrot, T.S., Staessen, J.A., Gardner, J.P. & Aviv, A. (2004). Telomere length and possible  
592 link to X chromosome. *Lancet*, 363, 507-510.
- 593 Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T., & Bateson, M. (2015). An  
594 experimental demonstration that early-life competitive disadvantage accelerates  
595 telomere loss. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20141610.
- 596 Njajou, O.T., Cawthon, R.M., Damcott, C.M., Wu, S.H., Ott, S., Garant, M.J., Blackburn,  
597 E.H., Mitchell, B.D., Shuldiner, A.R. & Hsueh, W.-C. (2007). Telomere length is  
598 paternally inherited and is associated with parental lifespan. *Proceedings of the National*  
599 *Academy of Sciences of USA* 104, 12135–12139.
- 600 Noguera, J. C., & Velando, A. (2020). Gull chicks grow faster but lose telomeres when  
601 prenatal cues mismatch the real presence of sibling competitors. *Proceedings of the*  
602 *Royal Society B: Biological Sciences*, 287, 20200242
- 603 Noguera, J. C., Metcalfe, N. B. & Monaghan, P. (2018). Experimental demonstration that  
604 offspring fathered by old males have shorter telomeres and reduced lifespans.  
605 *Proceedings of the Royal Society B: Biological Sciences*, 285, 1874.
- 606 Nordfjäll, K., Larefalk, Å., Lindgren, P., Holmberg, D. & Roos, G . (2005). Telomere length  
607 and heredity: Indications of paternal inheritance. *Proceedings of the National Academy*  
608 *of Sciences of USA*, 102, 16374–16378.
- 609 Nussey, D. H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N. ... Monaghan, P.  
610 (2014). Measuring telomere length and telomere dynamics in evolutionary biology and  
611 ecology. *Methods in Ecology and Evolution*, 5, 299-310.
- 612 Nussey, D. H., Froy, H., Lemaitre, J.-F., Gaillard, J. M., & Austad, S. N. (2013). Senescence  
613 in natural populations of animals: widespread evidence and its implications for  
614 biogerontology. *Ageing Research Reviews*, 12, 214-225.
- 615 Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T. & Blomqvist, D . (2011). Sex  
616 differences in sand lizard telomere inheritance: paternal epigenetic effects increases  
617 telomere heritability and offspring survival. *PLoS ONE* 6: e17473.

618 Rattiste, K., Klandorf, K., Urvik, J., Sepp, T., Asghar, M. Hasselquist, D., Cooney, C., &  
619 Hrõak, P. (2015). Skin pentosidine and telomere length do not covary with age in a long-  
620 lived seabird. *Biogerontology*, *16*, 435-441.

621 Rattiste, K. (2004). Reproductive success in presenescent common gulls (*Larus canus*): the  
622 importance of the last year of life. *Proceedings of the Royal Society B: Biological*  
623 *Sciences*, *271*, 2059-2064.

624 Reichert, S., Rojas, E., Zahn, S., Robin, J.-P., Criscuolo, F. & Masseurin, S. (2015) Maternal  
625 telomere length inheritance in the king penguin. *Heredity*, *114*, 10-16.

626 Ricklefs, R. E. Insights from comparative analyses of aging in birds and mammals. *Aging cell*,  
627 *9*, 273-284 (2009).

628 Rodnan, G. P., Ebaugh, Jr. F.G., Spivey Fox, M. R. & Chambers, D.M. (1957). the life span of  
629 the red blood cell and the red blood cell volume in the chicken, pigeon and duck as  
630 estimated by the use of Na<sup>2</sup>Cr<sup>51</sup>O<sub>4</sub>: with observations on red cell turnover rate in the  
631 mammal, bird and reptile. *Blood*, *124*, 355-366.

632 Sepp, T., Meitern, R., Heidinger, B., Noreikiene, K., Rattiste, K., Hõrak, P. saks, L., Urvik, J.,  
633 Giraudeau, M. (2021). Supplementary data for the article: Parental age does not  
634 influence offspring telomeres during early life in common gulls (*Larus canus*) (Version  
635 0.1) [Data set]. *Molecular Ecology*. *Zenodo*. <http://doi.org/10.5281/zenodo.4485871>

636 Sepp, T., Rattiste K., Saks, L., Meitern, R., Urvik, J., Kaasik, A., & Hõrak, P. (2017). A small  
637 badge of longevity: opposing survival selection on the size of white and black wing  
638 markings. *Journal of Avian Biology*, *48*, 570-580.

639 Simons, M. J., Stulp, G. & Nakagawa, S. (2014). A statistical approach to distinguish telomere  
640 elongation from error in longitudinal datasets. *Biogerontology*, *15*, 99-103.

641 Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T. ...  
642 Richardson, D. S. (2018). Spatio-temporal variation in lifelong telomere dynamics in a  
643 long-term ecological study. *Journal of Animal Ecology*, *87*, 187-198.

644 Stearns, S. C. (1992). *The evolution of life-histories*, Oxford University Press.

645 Stier, A., Metcalfe, N. B., & Monaghan, P. (2019). Ageing before birth: pace and stability of  
646 prenatal growth affect telomere dynamics. *bioRxiv*, 809087. doi:  
647 <https://doi.org/10.1101/809087>

648 Stoffel, M. A., Nakagawa, S., & Schielzeth H. (2017). rptR: Repeatability estimation and  
649 variance decomposition by generalized linear mixed-effects models. *Methods in Ecology*  
650 *and Evolution*, *8*, 1639-1644.

651 Tarry-Adkins, J. L., Chen, J. H., Smith, N. S., Jones, R. H., Cherif, H. & Ozanne, S.E. (2009).  
652 Poor maternal nutrition followed by accelerated postnatal growth leads to telomere  
653 shortening and increased markers of cell senescence in rat islets. *The FASEB Journal*,  
654 *23*, 152-1528.

655 Torres, R., Drummond, H., & Velando, A. (2011). Parental age and lifespan influence  
656 offspring recruitment: a long-term study in a seabird. *PloS ONE*, *6*, e2724.

657 Unryn, B. M., Cook, L. S. & Riabowol, K. T. (2005). Paternal age is positively linked to  
658 telomere length of children. *Aging Cell*, *4*, 97-101.

659 Urvik, J., Rattiste, K., Giraudeau, M., Okuliarová, M., Hõrak, P., & Sepp, T. (2018) Age-  
660 specific patterns of maternal investment in common gull egg yolk. *Biology Letters*, *14*,  
661 20180346.

662 Velarde, M., & Menon, R. (2016). Positive and negative effects of cellular senescence during  
663 female reproductive aging and pregnancy, *Journal of Endocrinology*, *230*, R59-R76.

664 Viblanc, V. A., Schull, Q., Stier, A., Durand, L., Lefol, E., Robin, J-P. ... Criscuolo, F.(2020).  
665 Foster rather than biological parental telomere length predicts offspring survival and  
666 telomere length in king penguins. *Molecular Ecology*, Wiley, 2020,  
667 [ff10.1111/mec.15485](https://doi.org/10.1111/mec.15485)[ff. fffhal-02868474f](https://doi.org/10.1111/mec.15485).  
668 Young, A. J. (2018). The role of telomeres in the mechanisms and evolution of life-history  
669 trade-offs and ageing. *Philosophical Transactions of the Royal Society B: Biological*  
670 *Sciences*, 373, 20160452.  
671 Young, R. C., Welcker, J., Barger, C. P., Hatch, S. A., Merkling T., Kitaiskaia, E. V. ...  
672 Kitaysky, A. S. (2017). Effects of developmental conditions on growth, stress and  
673 telomeres in black-legged kittiwake chicks. *Molecular Ecology*, 26, 3572-3584.  
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675

676 **Tables and figures**

677  
678 Table 1. Cross-fostering experiment sample sizes. A total of 72 chicks from 40 nests were  
679 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

680

681

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

<i>Predictor</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
second - first measurement	0.11	0.02 – 0.20	<b>0.022</b>	0.09	-0.01 – 0.18	0.066
chick sex	-0.02	-0.12 – 0.09	<b>0.749</b>	-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**

$\sigma^2$	0.03	0.03
$\tau_{00}$	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

685  $\sigma^2$  - within-group (residual) variance,  $\tau_{00}$  - between-group-variance, ICC - intraclass-  
686 correlation (variance partition coefficient).

687

688 Table 3. Markov Chain Monte Carlo (MCMC) multivariate generalised linear mixed models  
689 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)
<b>Telomere length at hatching</b>						
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	
<b>Telomere length at second trapping</b>						
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	
<b>Change of telomere length</b>						
Mean (95% CI)	-0.003 (- 0.013- 0.006)	-0.005 (- 0.014- 0.005)	0.011 (0.002- 0.023)	-0.031 (- 0.174- 0.103)	0.001 (- 0.01- 0.012)	0.011 (0.002- 0.023)
pMCMC	0.50	0.33		0.65	0.093	

690 CI-confidence interval.  
691

692

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 age effect (- / +)	Reference
<b>Teleost fish</b>	Atlantic salmon ( <i>Salmo salar</i> )	84 HSF	M & P	qPCR (RTL)	Complex P <sub>fry</sub> (+)	McLennan et al. 2016
	Atlantic salmon ( <i>Salmo salar</i> )	60 HSF	M & P	qPCR (RTL)	Complex P <sub>fry</sub> (-)	McLennan et al. 2018
<b>Reptiles</b>	Sand lizard ( <i>Lacerta agilis</i> )	N <sub>sons</sub> =12	P	TRF (TL in bp)	P <sub>sons</sub> (-)	Olsson et al., 2011
<b>Birds</b>	Great reed warbler ( <i>Acrocephalus arundinaceus</i> )	N <sub>ind</sub> =139 N <sub>broods</sub> =46	M & P	qPCR (RTL)	M (+)	Ashgar et al., 2015a
	European shag ( <i>Phalacrocorax aristotelis</i> )	N <sub>ind</sub> =311 N <sub>broods</sub> =134	M & P	qPCR (RTL) and $\Delta$ RTL	M $\Delta$ RTLoffspring (-) P $\Delta$ RTLoffspring (-)	Noguera et al., 2018
	Alpine swift ( <i>Apus melba</i> )	N <sub>ind</sub> =95 N <sub>broods</sub> =54	M & P biological and foster	qPCR (RTL)	P <sub>biological</sub> (-) M <sub>foster</sub> (-)	Heidinger et al. 2016
	Zebra finch ( <i>Taeniopygia guttata</i> )	N <sub>ind</sub> =139 N <sub>HSbroods</sub> =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> ) <sup>Curr. study</sup>	$N_{ind}=72$ $N_{nests}=40$	M & P	qPCR (RTL and $\Delta RTL$ )	No effect	Sepp et al. (current study)
<b>Mammals</b>	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

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696 Abbreviations: TL - telomere length, RTL - relative telomere length, TRF - terminal  
697 restriction fragment, HSF - half sib families, ind - individuals, P - paternal age effect, M -  
698 maternal age effect, Par - parental age effect, (-) negative association, (+) positive association  
699

700 Figure 1. Parents' and foster parents' age group did not affect offspring telomere length (A-B)  
701 and telomere dynamics (C-F). Mother's and father's age groups coincided for most, but not all  
702 chicks. Columns denote group means, whiskers are standard errors.  
703

704 Figure 2. Telomere length was individually repeatable between first (avg. 0.61 days after  
705 hatching) and second blood sampling (avg. 10.62 days after hatching). X=Y line is depicted to  
706 illustrate the direction of individual increase of telomere length.  
707

708 Figure 3. Relative telomere length dynamics between the first and second sampling (average  
709 time difference 10.62 days) were not related to chick growth rate (change of head size). Since  
710 head size and telomere length were measured at the same time points for individual birds, time  
711 is not included as a factor here.  
712

713