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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

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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)³⁴. 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, Hõrak & 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 ± 3.37 (SD) years). In total, 19 males were 5-7
176 years old (average age 5.52 ± 0.80 (SD) years) and were grouped as “young”, 21 males were
177 10+ years (average age 16.29 ± 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 μ L taken, from brachial vein) for
197 telomere measurement (average age 0.61 ± 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 (± 0.35 SE). Blood samples were kept on ice in an insulated box while on the
204 islet, and stored at -20°C until the end of the field work period, when they were transferred to -
205 80°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 μ l
232 containing 12.5 μ l of SYBR green Master Mix, 0.25 μ l forward and reverse primer, 6 μ l
233 water, and 6 μ 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 ± 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 ± 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

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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

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

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

685 σ^2 - within-group (residual) variance, τ_{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)
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% 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
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 _{sons} =12	P	TRF (TL in bp)	P _{sons} (-)	Olsson et al., 2011
Birds	Great reed warbler (<i>Acrocephalus arundinaceus</i>)	N _{ind} =139 N _{broods} =46	M & P	qPCR (RTL)	M (+)	Ashgar et al., 2015a
	European shag (<i>Phalacrocorax aristotelis</i>)	N _{ind} =311 N _{broods} =134	M & P	qPCR (RTL) and Δ RTL	M Δ RTLoffspring (-) P Δ RTLoffspring (-)	Noguera et al., 2018
	Alpine swift (<i>Apus melba</i>)	N _{ind} =95 N _{broods} =54	M & P biological and foster	qPCR (RTL)	P _{biological} (-) M _{foster} (-)	Heidinger et al. 2016
	Zebra finch (<i>Taeniopygia guttata</i>)	N _{ind} =139 N _{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

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