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1 **Foster rather than biological parental telomere length predicts offspring**
2 **survival and telomere length in king penguins**

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14
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17
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19 **ABSTRACT**

20 Because telomere length and dynamic relate to individual growth, reproductive investment
21 and survival, telomeres have emerged as possible markers of individual quality. Here, we
22 tested the hypothesis that, in species with parental care, parental telomere length can be a
23 marker of parental quality that predicts offspring phenotype and survival. In king penguins,
24 we experimentally swapped the single egg of 66 breeding pairs just after egg laying to
25 disentangle the contribution of pre-laying parental quality (*e.g.* genetics, investment in the
26 egg) and/or post-laying parental quality (*e.g.* incubation, postnatal feeding rate) on offspring
27 growth, telomere length and survival. Parental quality was estimated through the joint effects
28 of biological and foster parent telomere length on offspring traits, both soon after hatching
29 (day 10) and at the end of the pre-winter growth period (day 105). We expected that offspring
30 traits were related mostly to telomere length (*i.e.* quality) of biological parents at day 10 and
31 to telomere length of foster parents at day 105. Results show that chick survival up to 10 days
32 was negatively related to biological fathers' telomere length whereas survival up to 105 days
33 was positively related to foster fathers' telomere length. Chick growth was neither related to
34 biological nor to foster parents' telomere length. Chick telomere length was positively related
35 to foster mothers' telomere length at both 10 and 105 days. Overall, our study shows that, in a
36 species with bi-parental care, parents' telomere length is foremost a proxy of post-laying
37 parental care quality, supporting the "telomere – parental quality hypothesis".

38

39 1 | INTRODUCTION

40 Telomeres are repeated DNA sequences at the end of chromosomes that play a key role in
41 maintaining genome integrity (Gomes, Shay, & Wright, 2010). Telomere length can shorten
42 over time in response both to cell division and stressors (including environmental stressors,
43 psychosocial stressors, or poor early life conditions) (Levy, Allsopp, Futcher, Greider, &
44 Harley, 1992; Tomiyama et al. 2012; Boonekamp et al. 2014; Hanssen et al. 2017; Chatelin et
45 al. 2019; Noguera et al. 2019; Saulnier et al. 2020; but see Cerchiara et al. 2017). As a
46 consequence, telomere lengths and their dynamics have been related to individual health and
47 stress at a proximate level (Verhulst et al., 2016) and to fitness-outcomes at various life
48 history stages (Bauch et al. 2013; Bize et al. 2009; Heidinger et al., 2012; Salomons et al.,
49 2009). Therefore, telomeres are increasingly considered as a cellular proxy of multiple
50 correlated phenotypic traits that define individual quality (Angelier et al., 2019). This
51 ‘telomere - individual quality hypothesis’ predicts that individuals with longer telomeres may
52 benefit from both higher survival and reproductive rates (Angelier et al., 2019). For species
53 with parental care, an extrapolation of this ‘telomere - individual quality hypothesis’ is that
54 parental telomere length may reflect parental quality, parents with longer telomeres being
55 better at raising a large number of high quality offspring with high survival rates (i.e.
56 ‘telomere - parental quality hypothesis’). Remarkably, because telomeres are genetic material
57 passed on from parents to offspring, one topical question is also to which extent parent-
58 offspring resemblance in telomere length is explained by genetic additive variance
59 (heritability) and/or by other environmental effects caused by variation in the quality of pre-
60 and post-hatching parental care (Belmaker et al. 2019).

61 Early studies suggested that telomere length is fixed in the zygote (*i.e.* inherited from
62 the gametes in a sex- and age-dependent way; Eisenberg, 2019), remaining unchanged for life
63 relative to others individuals from the same cohort (Graakjaer et al., 2004). However,

64 estimates of telomere length heritability appear to be largely variable across species (Asghar
65 et al. 2014; Atema et al., 2015; Becker et al., 2015; Stier et al., 2015; Belmaker et al., 2019),
66 suggesting that both genetics and environmental factors (including parental care) may
67 influence offspring telomere length. Assessing the effects of parental care quality on telomere
68 length is however complex, since it requires disentangling the contribution of additive genetic
69 effects (i.e. heritability) from parental care *per se* on offspring telomeres. In fact, individual
70 telomere length within its cohort appears not to be fully established at the embryonic stage but
71 changes rapidly in early-life (Fairlie et al., 2016), mostly during growth when cell division
72 rates are high (Monaghan & Ozanne, 2018). A large number of non-exclusive mechanisms
73 can account for inter-individual variability in telomere length early in life. In birds for
74 instance, telomere length may vary according to embryo exposure to maternal corticosterone
75 in the egg (Hausman et al. 2012), incubation temperature (Stier et al., 2019), and/or variation
76 in post-hatching environmental conditions (Nettle et al., 2015; Reichert et al., 2015; Soler et
77 al., 2017). Those post-hatching factors include the quality of parental care and/or parental
78 effort (as suggested by positive links between parental telomere length and breeding
79 performance; Le Vaillant et al., 2015; Angelier et al., 2019, but see Bauch et al., 2013; Young
80 et al., 2016). In this context, the use of cross-fostering designs combined with longitudinal
81 measurements of offspring growth trajectories, telomere length dynamics, and survival
82 (Boonekamp et al. 2014; Bauch, et al. 2019; Criscuolo et al., 2017; Dugdale & Richardson,
83 2018; McCarty, 2017) may prove particularly powerful in gaining new insights on the
84 proximate genetic and post-laying environmental determinants of telomere length variability
85 in the next generation.

86 We applied such an approach to study the growth trajectories and telomere length
87 dynamics of king penguin chicks (*Aptenodytes patagonicus*) during the first 3 months of their
88 development. King penguins are slow-breeding seabirds where bi-parental care is required to

89 successfully rear a single chick over a 14-month period. Parental quality is therefore of critical
90 importance in this species (Stonehouse 1960). In this study, we exchanged eggs between
91 breeding pairs soon after egg laying, and we measured both adult telomere length shortly after
92 mating and their chick phenotype at 10 and 105 days after hatching (shortly after hatching and
93 towards the end of their pre-winter growth period, respectively). During the winter period,
94 chicks gather into “crèches” with almost no parental care (Stonehouse 1960; Geiger et al.,
95 2012; Saraux et al., 2012). Hence, this experimental cross-fostering design allowed us to
96 disentangle the contribution of biological (mostly investment in eggs and genetics) vs. foster
97 (mostly incubation and chick rearing) parental quality assessed as parental telomere length
98 (i.e. telomere length is positively associated with breeding success in adults; Le Vaillant et al.,
99 2015) on chick structural size, body condition, telomere length and survival in early life (i.e.
100 at 10 and 105 days). In the king penguin, chick body condition and telomere length soon after
101 hatching (day 10) are good predictors of survival (Geiger et al., 2012; Stier et al., 2014).
102 Telomere length also shortens with age during chick growth (Geiger et al., 2012; Stier et al.,
103 2014), but does not appear to be related with age in adults (aged 5 to 9 years old; Le Vaillant
104 et al., 2015). If chick phenotype and telomere length soon after hatching are mostly
105 determined through genetic and/or early maternal effects (i.e. investment in eggs), we
106 expected chick phenotypes, including chick telomere length, to be positively related to the
107 telomere lengths of their biological parents. However, because post-laying parental quality,
108 measured through telomere length of foster parents, is likely to become apparent as chicks
109 grow and receive increasing amounts of parental care, we predicted foster parental telomere
110 lengths to be positively related to chick structural size, body condition and survival at 105
111 days. Telomere inheritance was previously found to be moderate ($h^2 \sim 0.2$), being stronger
112 early in development (day 10 after hatching) and fading during development (up to day 300
113 after hatching) in this species (Reichert et al., 2015). Hence, in this study we also tested

114 whether the resemblance between biological parent and offspring telomere length (i.e. genetic
115 effects) diminished during offspring development and was replaced by post-hatching
116 environmental influences measured through a positive resemblance between foster parent-
117 offspring telomeres, as offspring aged.

118 When investigating the effects of parental quality on offspring phenotype and survival,
119 it is essential to keep in mind that parental quality typically increases with age as individuals
120 gain experience over successive breeding seasons (Forslund & Pärt, 1995; Lecomte et al.,
121 2010). Interestingly, the ‘age – parental quality’ and ‘telomere – parental quality’ hypotheses
122 lead to opposite predictions. On one hand the ‘age – parental quality hypothesis’ predicts that
123 older parents should be of higher quality. On the other hand, older parents are expected to
124 have shorter telomeres and therefore to be of lower quality according to the ‘telomere –
125 parental quality hypothesis’.

126

127 2 | MATERIAL AND METHODS

128 2.1 | Study species and breeding pair monitoring

129 This study was conducted in the king penguin colony of “La Baie du Marin” (Possession
130 Island, Crozet Archipelago, 46°26’ S – 51°52’E), home to some 24,000 pairs of breeding
131 birds. In 2012-2013, we monitored 66 breeding pairs of unknown age from courtship (early
132 November) up to the onset of the Austral winter (early April). In king penguins, the breeding
133 cycle is long and complex, starting by a courtship period of ~15 days during which pairs will
134 form, select a breeding territory, and females lay their single egg (Stonehouse, 1960).
135 Following egg-laying, males and females alternate between periods on land, incubating the
136 egg or caring for the chick, and periods foraging at sea for the rest of the summer
137 (Weimerskirch et al., 1992). The female is the first to leave for sea, the male taking charge of
138 the first incubation shift (Weimerskirch et al., 1992). Incubation lasts for ~53 days

139 (Stonehouse, 1960), the egg typically hatching during incubation shift 4 (the female's second
140 incubation shift). The chick's growth period extends over 10-11 months, including an energy-
141 constraining winter period (April to September) during which it is seldom fed and loses
142 substantial body weight (Cherel et al., 1985; Weimerskirch et al., 1992). Chick feeding and
143 growth resume the following summer (Weimerskirch et al., 1992). Following chick fledging,
144 parents have to moult and replenish their energy stores before they are ready for a subsequent
145 breeding season (Weimerskirch et al., 1992). Divorce rates between breeding seasons are high
146 (*ca.* 80%; Olsson, 1998), however, within a season cooperation between partners is critical to
147 successfully raise the chick, *i.e.* a single parent can not succeed. Parental quality is key and
148 mutual mate choice for high quality partners is high in this species (Jouventin & Dobson
149 2017).

150 We first marked both male and female pair members on the chest from a 1-m distance
151 using animal spray dye (Porcimark®, Kruuse, Lageskov; Denmark) when they were settling
152 on their final breeding territory. The pair was monitored daily at a distance, using binoculars,
153 until a single bird was observed incubating the egg. This bird was identified as the male at day
154 1 of incubation and, 3 days after egg-laying (to minimize disturbance until the bird was
155 motivated to incubate), was flipper-banded with semi-rigid PVC Darvic bands (25.8mm wide,
156 1.9mm thick, 7.4g), allowing its identification and subsequent monitoring during the study.
157 The female was caught and flipper-banded when she returned from her first foraging trip at
158 sea. All flipper-bands were removed from birds at the end of the study.

159

160 **2.2 | Cross-fostering design, blood sampling and bird monitoring**

161 Three days after the egg was laid (first incubation shift of the male), we cross-fostered (*i.e.*
162 swapped) eggs between penguin pairs that had laid their egg on the same day. In total, we
163 swapped eggs between 66 breeding pairs grouped in 33 dyads. This required 3 persons. First,

164 two males were immobilized while incubating in the colony and rapidly hooded to minimize
165 stress. Their respective egg was carefully removed from the brood pouch and replaced by a
166 warm dummy plaster egg during the exchange. Eggs were weighed to the nearest 1-g using a
167 Pesola® spring-slide scale. One person then proceeded to exchange the eggs while the 2 other
168 persons remained by the birds in the breeding colony at all times to ensure the procedure went
169 smoothly. Once the eggs were swapped and individuals released, we monitored bird
170 behaviour to ensure they settled down once again on their breeding territory. We never
171 witnessed breeding abandonment by the birds at this stage.

172

173 *Adult monitoring*

174 For males and females, blood samples (2 mL) were collected from the marginal flipper vein
175 using a G22-1½ needle fitted to a 2.5 mL heparinized syringe. Males were sampled at the
176 time cross-fostering occurred (day 3, incubation shift 1). Females were sampled during their
177 first incubation shift (day 2). The bird's head was covered with a hood to minimize stress and
178 agitation during blood sampling, and samples were kept on crushed ice in the field until
179 further processing, usually within 15 min. After centrifugation (3000g for 10 min), plasma
180 and blood cells were separated and kept frozen dried at -20°C until the end of the day, before
181 being moved to -80°C until assayed. Penguin pairs were monitored twice daily until hatching
182 (confirmed by the presence of a newly hatched chick and the presence of broken egg shells).
183 That day was marked as hatching day. Ten days later, we caught the adults as described
184 above, and temporarily replaced the chick with a warm dummy plaster egg.

185

186 *Chick monitoring*

187 On day 10 post-hatching (*i.e.* early during development), chicks were measured for flipper
188 length, beak length and tarsus length to closest 1-mm using a solid metal ruler. They were

189 weighed (closest 5g) using a spring-slide Pesola® scale, and a small blood sample (~100 µL)
190 was obtained from the marginal flipper vein using a G27-1½ needle and 75 µL heparinized
191 capillary tubes. Chicks were then individually identified using color-coded fish tags (Floy Tag
192 and MFG, Inc. Seattle, WA, USA) attached subcutaneously to their upper-back (Stier et al.,
193 2014). On day 105 post-hatching, the same procedure was repeated, when chicks had been
194 emancipated for approximately two months and had gathered in crèches in anticipation of the
195 austral winter period. We then collected 1 mL of blood from the marginal flipper vein, and
196 measured flipper length, beak length and tarsus length as described above.

197 From these data, we calculated chick structural size as the first principal component of
198 separate PCA analyses on flipper length, beak length and tarsus length both at 10 and 105
199 days ($SSz_{10} = -28.44 + 0.29 \text{ beak} + 0.12 \text{ flipper} + 0.11 \text{ tarsus}$; $SSz_{105} = -33.80 + 0.12 \text{ beak} +$
200 $0.03 \text{ flipper} + 0.08 \text{ tarsus}$; $\Delta SSz = -21.16 + 0.12 \text{ beak} + 0.04 \text{ flipper} + 0.08 \text{ tarsus}$). Because
201 chick body mass and structural size indices were highly correlated (at day 10: Pearson's $r =$
202 0.87 , $t = 12.72$, $df = 52$, $P < 0.0001$; at day 105: $r = 0.76$, $t = 7.42$, $df = 41$, $P < 0.0001$), we
203 calculated chick body condition at day 10 and day 105 by regressing body mass on structural
204 size at those different time points (Schulte-Hostedde et al. 2005). Chick structural size and
205 body condition were then used as uncorrelated dependent variables in subsequent analyses
206 (see below).

207 Chicks were monitored up until the subsequent summer (November-December), when
208 they departed from the colony for their first trip at sea. Of the 66 eggs produced by the
209 monitored breeding pairs 54 chicks survived up to 10 days and 44 chicks survived up to 105
210 days.

211

212 **2.3 | Measurement of telomere length in adult and chick king penguins**

213 King penguin relative telomere length (RTL) was measured using a protocol specifically
214 developed and routinely used on king penguins (Geiger et al., 2012; Reichert et al., 2015; Le
215 Vaillant et al., 2015; Stier et al., 2014; Schull et al., 2018). DNA was extracted from
216 nucleated red blood cells (Nucleospin Blood QuickPure, Macherey-Nagel, Düren, Germany)
217 and checked for quality using gel-migration and a NanoDrop 1000 (Thermo Scientific)
218 spectrophotometer (absorbance ratio A260/280; A260/230.). Extracted DNA was then used to
219 amplify both the telomere and a control gene (non-variable in copy numbers within our
220 population, Smith, Turbill & Penn, 2011) by quantitative real-time amplification (qPCR)
221 based on Cawthon's original development (Cawthon, 2002). Control gene (*Aptenodytes*
222 *patagonicus* zinc finger) and primer sequences were identical to those used in previous
223 penguin telomere studies, as well as the conditions of qPCR amplifications (see Stier *et al.*,
224 2014 for details). We used 2.5 ng DNA per reaction and the BRYT Green fluorescent probe
225 (GoTaq_qPCR Master Mix; Promega, Charbonniere, France). The samples were amplified on
226 a 384 wells thermocycler (CFX-384, Biorad Hercules), in duplicates over three runs, the
227 telomere sequence and the control gene sequence being amplified using the same conditions.
228 Samples were distributed over 3 plates and individual birds randomly distributed on each
229 plate. Intra-plate repeatability based on duplicate runs was of 0.785 for the final calculated
230 relative telomere length value (T/S ratio based on Cq values). Inter-plate repeatability based
231 on 13 samples (*i.e.* 13 different individuals) repeated over all plates was of 0.894 for final
232 calculated relative telomere length value (T/S ratio). Mean amplification efficiencies of
233 telomere sequence and control gene were of 100% and 99.9% (plate 1), of 100.2% and 99.8%
234 (plate 2) and of 100% and 100.3% (plate 3), respectively. Relative telomere lengths were
235 calculated following (Pfaffl, 2001) and using the plate efficiencies amplification values
236 corresponding specifically to each sample. No apparent well-position bias was observed

237 (Eisenberg, Kuzawa, & Hayes, 2015) (see Online Supporting Information). We obtained
238 telomere data for 61 adult breeding pairs and 42 chicks throughout growth.

239

240 **2.4 | Statistical analyses**

241 All analyses were run using R v.3.5.1. Forest plots and marginal effects plot with 95% CI
242 were obtained using the ‘sjPlot’ package in R (Lüdtke, 2017). In all models presented
243 below, Relative Telomere Length (RTL) was systematically log-transformed and standardized
244 (z scores) prior to analyses (see Verhulst et al. 2019). Other continuous variables were
245 standardized so that model coefficients could be directly comparable in their magnitude.
246 Where appropriate, we ensured residuals were normally distributed by visual inspection of
247 density distributions, Q-Q plots, cumulative distribution functions and P-P plots using the
248 ‘fitdistrplus’ package in R (Delignette-Muller & Dutang, 2015). We also ensured that no
249 substantial collinearity occurred between independent variables (Variance Inflation Factors
250 ranged $1.05 < \text{VIF} < 2.05$; suggested cut-off at 3; Zuur, Ieno, & Elphick, 2010). For each
251 model, sample sizes are reported in the tables. Sample sizes can vary across models due to
252 variation in egg and chick mortality and/or due to difficulties at sampling blood from some
253 chicks or amplifying DNA (telomeres) from some blood samples.

254

255 ***Chick telomere dynamics during growth***

256 We investigated chick RTL dynamics in early life using linear mixed models (LMMs) with
257 RTL as the dependent variable, chick age (categorical: 10 or 105 days after hatching) as an
258 independent variable, and chick ID as a random factor. Hence, the model was specified as:

$$259 \quad z\text{-RTL} \sim \text{Chick age}_{10 \text{ or } 105} + (1|\text{chick ID})$$

260 From this model, we computed repeatability in chick RTL during early life as the ratio
261 of among-individual variance (V_G) over the total phenotypic variance (V_P) equal to $V_G + V_R$

262 (the within-individual or residual variance in RTL) (see Nakagawa & Schielzeth 2010; Stoffel
 263 et al. 2017). Hence, repeatability = $R = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_R}$. This LMM-based repeatability estimate
 264 allowed to control for the confounding effects of age (see Nakagawa & Schielzeth 2010) since
 265 chick RTL shortened with time. Repeatability was calculated using the ‘rptR’ R cran package;
 266 Stoffel *et al.*, 2017). Confidence intervals around the repeatability estimate were computed by
 267 parametric bootstrapping (10,000 iterations). This repeatability allowed us to assess whether
 268 chicks starting their post-hatching growth period with long telomeres also entered the winter
 269 period with long telomeres, which informs on the importance of ‘starting’ telomere length in
 270 determining later life telomere length and potentially life histories.

271

272 ***Chick survival and phenotype in relation to parental RTL***

273 *Chick survival:* We tested for influences of parental (both biological and foster) RTL on chick
 274 survival up to day 10, or up to day 105, using separate Generalized Linear Mixed Models
 275 (GLMMs: binomial, logit-link). These were specified as:

$$276 \quad \text{Survival } (0 = \text{failure}, 1 = \text{success})_{10 \text{ (or 105)}} \sim z\text{-RTL}_{\text{biological}\text{♂}} + z\text{-RTL}_{\text{biological}\text{♀}} + z\text{-}$$

$$277 \quad \text{RTL}_{\text{foster}\text{♂}} + z\text{-RTL}_{\text{foster}\text{♀}} + z\text{-egg mass} + (1|\text{dyad})$$

278 Here, we included cross-fostering dyad identity as a random factor in the model and
 279 accounted for egg mass as a covariate to test for potential effects occurring from early
 280 maternal investments in the egg (Bize et al. 2002; Krist 2011). From these models, odd ratios
 281 were calculated to illustrate the relative influence of the different fixed factors (mainly in our
 282 case biological and foster parental telomere lengths) on offspring survival. The odd-ratio can
 283 be interpreted for a given predictor in terms of increasing (>1) or decreasing (<1) the
 284 likelihood to survive for a one unit increase in that predictor, holding all other variables
 285 constant. For instance, holding all other variables constant, an odds ratio of 2 for a given

310 On average, chick telomere length decreased over time (LMM; z $RTL_{105vs10} = -0.40 \pm 0.14$, $t =$
311 -2.78 , $CI = [-0.68; -0.12]$, $P = 0.008$; Fig. 1). Using the variance explained by chick ID in this
312 model ($0.55 \pm$ s.d. 0.74), we found that chick telomere length was repeatable (LMM; $r = 0.56$
313 ± 0.11 , $CI = [0.33; 0.74]$, $P < 0.001$): chicks starting their post-hatching growth period with
314 longer telomeres also entered the winter period with longer telomeres (see Figs. 1a and 1b).

315

316 **3.2 | Chick survival and phenotype at 10 days**

317 Chick survival up to 10 days was weakly and negatively related to the RTL of the biological
318 male, but not to the RTL of the biological mother, the RTL of foster parents, or egg mass
319 (Table 1, Fig. 2a and 3a). At 10 days, neither chick structural size or body condition were
320 significantly related to biological or foster parental RTL telomere length, or egg mass (Table
321 1, Figs. 2b and 2c). In contrast, chick RTL was positively associated with the RTL of foster
322 mothers (Table 1, Figs. 2d and 3b), and positively (though not significantly, $P = 0.071$) with
323 the RTL of foster fathers, but not with the RTL of genetic parents or egg mass (Table 1).

324

325 **3.3 | Chick survival and phenotype at 105 days**

326 At 105 days, chick survival was significantly and positively related to foster male RTL, but
327 not to the RTL of the foster mother, the RTL of biological parents, or egg mass (Table 2, Fig.
328 4a and 5a). At 105 days, neither was chick's structural size or body condition significantly
329 related to biological or foster parental RTL telomere length, or egg mass (Table 2, Figs. 4b
330 and 4c). In contrast, chick RTL was significantly and positively associated with the RTL of
331 foster mothers (Table 2, Fig. 4d and 5b). The change in chick telomere length between days
332 10 and 105 was not significantly associated with parental RTL when both biological and
333 foster parents were included in the same model (Table 3).

334

335

336 4 | DISCUSSION

337 Using an experimental cross-fostering approach in the king penguin, our study aimed at
338 identifying the contributions of pre-laying (genetics and egg mass) and post-laying
339 (incubation, brooding and feeding) parental quality on offspring phenotype and survival. We
340 hypothesised that parents with longer telomeres were of higher quality. We tested whether
341 offspring phenotype either soon after hatching (day 10) or at the end of the pre-winter growth
342 period (day 105) were best explained by pre-laying and post-laying parental quality measured
343 via, respectively, the measures of telomere length of their biological and foster parents. Our
344 results highlight an overall larger impact of foster parental RTL on chick survival over the
345 growth period, as well as concomitant impact on chick RTL. This supports the idea that
346 telomere length is a measure of parental quality that can (i) predict post-laying parental
347 investment into their offspring and (ii) modulate next generation telomere length.

348

349 **4.1 | Parental telomere length effects on chick survival**

350 Because of their susceptibility to environmental stress, telomeres have been proposed as
351 integrative markers that can be used to reflect an individual's life stress and by extension
352 stress coping mechanisms, thus perhaps allowing to gauge individual quality (Angelier *et al.*,
353 2019). From an evolutionary perspective, high quality individuals are expected to perform
354 well in a suite of correlated phenotypic traits, including investment in parental care (Wilson &
355 Nussey, 2010). Hence, one of the aims of this study was to test the 'telomere – parental
356 quality hypothesis' hypothesizing that parents with long telomeres were of higher quality, and
357 therefore predicting that they should produce heavier and larger chicks more likely to survive
358 early in life. Accordingly, previous studies have reported positive links between telomere
359 length and reproductive success in seabirds, including king penguin (Angelier *et al.* 2019, Le

360 Vaillant et al. 2015; but see Olsson et al. 2011a for a quadratic association, and Bauch et al.
361 2013 for a negative association).

362 Surprisingly, after controlling for egg mass (*i.e.* maternal effects; Krist 2011), we
363 found a negative effect of biological father telomere length on chick survival at 10 days, but
364 no significant effect of foster parent telomere length (*i.e.* early post-hatching environmental
365 effects). Contrary to our expectation based on the ‘telomere – parental quality hypothesis’,
366 this result suggests that fathers with longer telomeres (expected to be of good quality)
367 somehow reduced the chances of survival of their chicks in the first days after hatching. This
368 negative effect was rather marginal (Table 3) and the mechanism explaining such an
369 association remains unclear. It seems unlikely this result was explained by the ‘age – parental
370 quality hypothesis’ (Forslund & Pärt, 1995; Lecomte et al., 2010), given a lack of association
371 between telomere length and chronological age in king penguins (Le Vaillant et al., 2015).
372 Furthermore, if father’s age and experience were important determinants of chick survival in
373 penguins, we would have expected to detect a negative impact of foster father telomere length
374 on chick survival at 105 days.

375 In contrast, chick survival at 105 days increased with foster male telomere length,
376 even when controlling for egg mass. This is predicted by the ‘telomere – parental quality
377 hypothesis’ if indeed telomere length acts as a proxy of individual quality and positively
378 correlates with post-hatching paternal care. Interestingly, this effect was apparently
379 independent of any effect of paternal telomere length on chick body mass or growth,
380 suggesting other benefits than those purely related to energy investments in the offspring.
381 Telomere length has been positively associated to foraging efficiency, but not to parental
382 investment, in other seabird species (Young et al., 2015, 2016). In king penguins, if parental
383 foraging efficiency was also related to telomere length, we might expect parents with longer
384 telomeres to be better at provisioning their chicks during development, ultimately affecting

385 chick body mass or structural size. We found however no support for such mechanism.
386 Remarkably, in king penguins on-land predation of brooded chicks is high (i.e. 51 % of
387 crèching chicks in a given reproductive season; Descamps et al., 2005), and an important
388 source of extrinsic mortality. Hence, an alternative mechanism could be that foster males with
389 long telomeres are more territorial and aggressive birds and therefore better at coping with
390 predators during their brooding shifts. This alternative mechanism remains to be tested.

391

392 **4.2 | Parental telomere length effects on chick telomere length**

393 Individual variation in telomere length in early life may come from (i) how zygote telomere
394 length is determined and (ii) what inherited and environmental factors are going to change the
395 way offspring lose and repair their telomeres. Disentangling those genetic and pre/post-laying
396 influences is far from being an easy task because telomere length is a complex structure
397 underpinned by the expression of multiple genes, by epigenetic modulation (Bauch et al.,
398 2019), as well as by a wide number of environmental factors (Dugdale & Richardson, 2018).
399 In addition, any modulation of development, of genetic (*i.e.* parental age, Bauch et al. 2019)
400 or environmental origins (Metcalf & Monaghan, 2003), may have pervasive impact on the
401 future phenotype of offspring, including telomere length (Metcalf & Monaghan, 2003;
402 Tarry-Adkins et al., 2009). In this study, we swapped eggs soon after laying to investigate
403 whether offspring telomere length were more alike the telomere length of their biological
404 (genetic effect) or foster parents (pre/post-hatching parental effect).

405 Our results show that chick telomere lengths at 10 and 105 days were both related to
406 foster maternal telomere length. At day 105, offspring telomere length was also positively
407 related to biological mother and foster father telomere length, though not significantly.
408 Previous data based on biological mother-offspring regressions have reported significant
409 maternal heritability for telomere length in king penguin (around $h^2 = 0.2$), which weakened

410 over the period of chick growth (Reichert et al., 2015). Thus, although telomere length in king
411 penguin chicks may be determined in part before egg-laying (e.g. Olsson et al. 2011a; Bauch
412 et al., 2019), our data suggest a stronger effect of the post-laying environment on chick
413 telomere length (see Becker et al., 2015 for similar findings in another bird species). King
414 penguin chicks are raised in an unpredictable environment (high predation risk, socially
415 aggressive adults, inclement weather conditions), and are subject to periods of intermittent to
416 prolonged fasting early in life (Cherel & Le Maho, 1985). Thus, variation in parental care and
417 ability to efficiently provision and defend their offspring will have critical consequences on
418 offspring phenotype. Our results in king penguins suggests that selection on telomere length
419 might be sex-specific (see also Olsson et al. 2011b for similar finding in a lizard species).
420 However, why maternal and paternal contributions should differ is unclear. We do know that
421 feeding strategies differ between sexes in adult king penguins, but mostly during the winter
422 period (Saraux et al., 2012), which is outside of the experimental window of the present
423 study. However, subtle differences in incubation and feeding strategies between males and
424 females may lead mothers to display a larger effect on chick telomere length during
425 incubation and the first three months of growth. Whether this effect stems from the quality of
426 incubation and/or of the food provided specifically by the mother remains to be determined,
427 and focusing on food elements known to buffer deleterious effect on telomeres (e.g. dietary
428 antioxidants, Reichert & Stier, 2017) may provide new insights. In king penguin chicks,
429 telomeres seem to erode faster in rapidly growing individuals (Geiger et al., 2012). This
430 suggests that variation in maternal provisioning patterns early in life is likely another
431 important factor affecting chick telomere length. Adequate or more regular rates of food
432 provisioning by high quality adults may allow chicks to better balance out the allocation of
433 energy towards growth and other somatic compartments, without affecting body mass *per se*,
434 allowing higher telomere maintenance. Additionally, development does not only concern cell

435 multiplication and an increase in body mass but also physiological maturation. A recent study
436 in birds suggested that maturation may be done at a cost of telomere loss (Criscuolo et al.,
437 2019). Whether early maternal care may enable chicks to mature in a way that allows to better
438 preserve telomere ends afterwards is intriguing and a call for further research. Finally, it is
439 worth keeping in mind that, in this study, parental age was unknown. Because parental age
440 can explain substantial variation in offspring telomere length (*e.g.* Criscuolo et al. 2017;
441 Bauch et al. 2019), the reported association between foster and biological parental age
442 probably an underestimation of any true association between parental and offspring telomere
443 length.

444 Overall, our study provides experimental evidence that the quality of environmental
445 rearing conditions mediated by the parents partly influence variation in offspring telomere
446 length and survival in a long-lived seabird. Such an approach opens perspectives as to the
447 finer characterization of the nature and timing of environmental effects conditioning
448 individual survival chances in the wild.

449

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453 ECONERGY). We are grateful to 5 anonymous reviewers for constructive and useful
454 comments on previous drafts of the paper.

455 **TABLES**

456 **Table 1.** Standardized model estimates for the relationship between parental relative telomere
 457 length (RTL) and chick survival (binary 0 = failure / 1 = success) and phenotype (structural
 458 size, body condition and telomere length) early in the development (day 10 post-hatching).
 459 Significant effects have CI95 not overlapping 1 for the binomial model, and not overlapping 0
 460 for linear models. All parents were included in the same model. Variance inflation factors
 461 (VIFs) are provided. The number of chicks (n) and dyads (N) are given. Sample sizes vary
 462 across models due to variation in chick mortality and/or difficulties at sampling blood from
 463 some chicks or amplifying DNA (telomeres) from some blood samples.

464
 465

Chick survival and phenotype early during development (day 10)							
(A) Survival (binary 1/0)	<i>Odds ratio ± SE</i>	<i>CI</i>	<i>z</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	19.99 ± 0.80	4.16 – 96.00	3.74	<0.001*			
z egg mass	2.18 ± 0.58	0.70 – 6.78	1.35	0.178	1.30		
z RTL_{biological}♂	0.15 ± 0.87	0.03 – 0.82	-2.18	0.029*	2.05	0.549	56 (28)
z RTL_{biological}♀	1.68 ± 0.65	0.47 – 6.00	0.80	0.422	1.88		
z RTL_{foster}♂	3.21 ± 0.73	0.77 – 13.37	1.61	0.108	1.96		
z RTL_{foster}♀	3.25 ± 0.89	0.57 – 18.61	1.33	0.185	1.93		
(B) z Structural size	<i>Estimate ± SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	-0.17 ± 0.22	-0.39 – 0.17	-0.77	0.444			
z egg mass	0.14 ± 0.23	-0.31 – 0.59	0.61	0.548	1.12		
z RTL_{biological}♂	0.21 ± 0.29	-0.36 – 0.77	0.71	0.481	1.46	0.084	49 (28)
z RTL_{biological}♀	-0.03 ± 0.26	-0.53 – 0.48	-0.10	0.921	1.33		
z RTL_{foster}♂	0.32 ± 0.29	-0.25 – 0.88	1.10	0.271	1.32		
z RTL_{foster}♀	0.02 ± 0.28	-0.53 – 0.56	0.06	0.956	1.40		
(C) z Body condition	<i>Estimate ± SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	0.06 ± 0.15	-0.25 – 0.36	0.39	0.696			
z egg mass	0.13 ± 0.16	-0.18 – 0.44	0.83	0.409	1.12		
z RTL_{biological}♂	0.30 ± 0.20	-0.09 – 0.68	1.51	0.139	1.47	0.078	49 (28)
z RTL_{biological}♀	-0.20 ± 0.18	-0.54 – 0.15	-1.13	0.267	1.33		
z RTL_{foster}♂	-0.03 ± 0.20	-0.41 – 0.36	-0.15	0.885	1.32		
z RTL_{foster}♀	-0.20 ± 0.19	-0.57 – 0.17	-1.05	0.301	1.40		
(D) z RTL	<i>Estimate ± SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	-0.17 ± 0.13	-0.43 – 0.10	-1.24	0.222			
z egg mass	-0.01 ± 0.15	-0.31 – 0.29	-0.04	0.968	1.21		
z RTL_{biological}♂	0.14 ± 0.18	-0.20 – 0.49	0.81	0.423	1.54	0.335	40 (26)
z RTL_{biological}♀	0.22 ± 0.17	-0.12 – 0.56	1.26	0.216	1.12		
z RTL_{foster}♂	0.34 ± 0.18	-0.02 – 0.70	1.86	0.071	1.38		
z RTL_{foster}♀	0.42 ± 0.17	0.08 – 0.76	2.42	0.021*	1.16		

466

467

468 **Table 2.** Standardized model estimates for the relationship between parental relative telomere
469 length (RTL) and chick survival (binary 0 = failure / 1 = success) and phenotype (structural
470 size, body condition and telomere length) late in the development (day 105 post-hatching; the
471 end of the pre-winter growth phase). Significant effects have CI95 not overlapping 1 for the
472 binomial model, and not overlapping 0 for linear models. All parents were included in the
473 same model. Variance inflation factors (VIFs) are provided. The number of chicks (n) and
474 dyads (N) are given. Sample sizes vary across models due to variation in chick mortality
475 and/or difficulties at sampling blood from some chicks or amplifying DNA (telomeres) from
476 some blood samples.

477

Chick survival and phenotype late in development (day 105)							
(A) Survival (binary 1/0)	<i>Odds ratio ± SE</i>	<i>CI</i>	<i>z</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	3.35 ± 0.36	1.66 – 6.74	3.38	0.001*			
z egg mass	1.38 ± 0.37	0.67 – 2.86	0.88	0.378	1.13		
z RTL^{biological}♂	0.57 ± 0.45	0.24 – 1.37	-1.25	0.210	1.32	0.238	56 (28)
z RTL^{biological}♀	1.00 ± 0.41	0.45 – 2.21	-0.00	0.999	1.25		
z RTL^{foster}♂	2.99 ± 0.44	1.26 – 7.08	2.49	0.013*	1.30		
z RTL^{foster}♀	1.15 ± 0.42	0.50 – 2.64	0.33	0.745	1.29		
(B) z Structural size	<i>Estimate ± SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	0.01 ± 0.17	-0.32 – 0.34	0.07	0.944			
z egg mass	0.00 ± 0.16	-0.30 – 0.31	0.08	0.978	1.05		
z RTL^{biological}♂	0.15 ± 0.18	-0.20 – 0.50	0.91	0.412	1.30	0.050	41 (27)
z RTL^{biological}♀	0.10 ± 0.19	-0.27 – 0.47	0.60	0.592	1.32		
z RTL^{foster}♂	-0.15 ± 0.20	-0.54 – 0.25	-0.63	0.474	1.19		
z RTL^{foster}♀	-0.21 ± 0.20	-0.60 – 0.19	-1.09	0.308	1.38		
(C) z Body condition	<i>Estimate ± SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>	<i>VIF</i>	<i>R²</i>	<i>n (N)</i>
Intercept	-0.04 ± 0.19	-0.42 – 0.34	-0.22	0.825			
z egg mass	0.08 ± 0.18	-0.27 – 0.42	0.44	0.665	1.05		
z RTL^{biological}♂	0.06 ± 0.20	-0.32 – 0.45	0.31	0.757	1.26	0.078	41 (27)
z RTL^{biological}♀	-0.08 ± 0.21	-0.50 – 0.34	-0.37	0.716	1.46		
z RTL^{foster}♂	0.25 ± 0.22	-0.19 – 0.68	1.11	0.274	1.15		
z RTL^{foster}♀	-0.32 ± 0.23	-0.76 – 0.13	-1.39	0.174	1.53		
(D) z RTL	<i>Estimate ± SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>		<i>R²</i>	<i>n (N)</i>
Intercept	-0.16 ± 0.15	-0.45 – 0.13	-1.09	0.288			
z egg mass	0.01 ± 0.16	-0.31 – 0.33	0.09	0.932	1.19		
z RTL^{biological}♂	0.08 ± 0.18	-0.28 – 0.43	0.44	0.664	1.40	0.330	40 (26)
z RTL^{biological}♀	0.31 ± 0.19	-0.05 – 0.67	1.67	0.104	1.23		
z RTL^{foster}♂	0.28 ± 0.19	-0.09 – 0.65	1.48	0.148	1.25		
z RTL^{foster}♀	0.54 ± 0.19	0.18 – 0.91	2.90	0.007*	1.28		

478

479

480 **Table 3.** Standardized linear mixed model estimates for the relationship between parental
 481 relative telomere lengths (RTL) and chick change in relative telomere length over growth (i.e.
 482 between days 10 and 105 post-hatching). All parents were included in the same model.
 483 Variance inflation factors (VIFs) are provided. The number of chicks (n) and dyads (N) are
 484 given.

485

Chick RTL change over growth ($RTL_{chick105} - RTL_{chick10}$)							
<i>z</i> RTL change	<i>Estimate</i> ± <i>SE</i>	<i>CI</i>	<i>t</i>	<i>P</i>	<i>VIF</i>	<i>R</i> ²	<i>n</i> (<i>N</i>)
Intercept	0.02 ± 0.17	-0.31 – 0.34	0.10	0.924			
<i>z</i> RTL ^{biological♂}	-0.11 ± 0.21	-0.53 – 0.30	-0.53	0.597	1.41		
<i>z</i> RTL ^{biological♀}	0.04 ± 0.21	-0.38 – 0.46	0.20	0.844	1.09	0.019	40 (26)
<i>z</i> RTL ^{foster♂}	-0.08 ± 0.23	-0.54 – 0.37	-0.37	0.717	1.38		
<i>z</i> RTL ^{foster♀}	0.05 ± 0.22	-0.38 – 0.47	0.21	0.831	1.14		

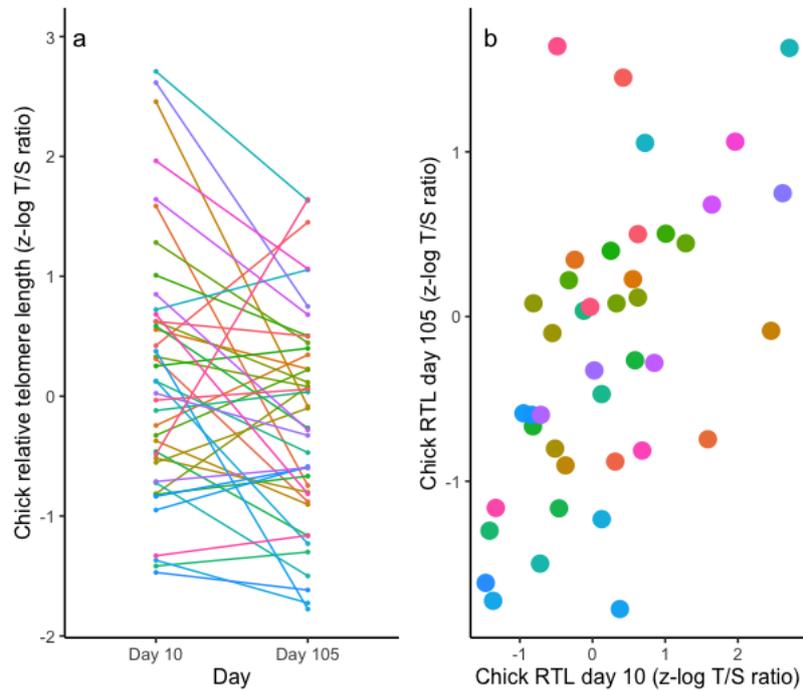
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493 **Fig. 1.** King penguin chick relative telomere length (RTL, T/S ratio) dynamics in early life.

494 RTL was log transformed, and all values were standardized (z-scores). (a) Individual

495 trajectories in RTL between days 10 and 105, i.e. the pre-winter growth period. (b)

496 Relationship between RTL values at day 10 and 105. Different colours indicate different

497 birds.

498

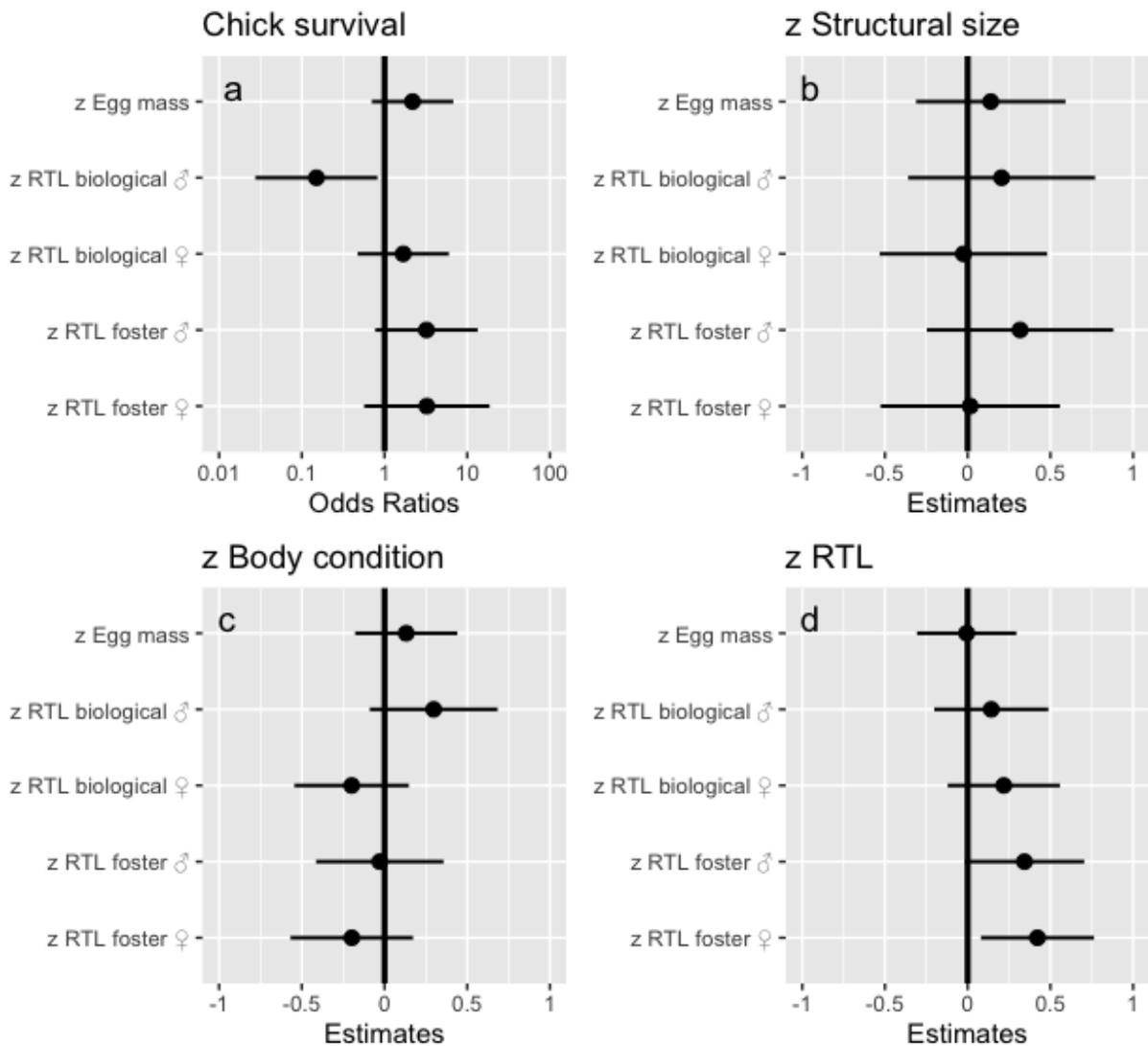
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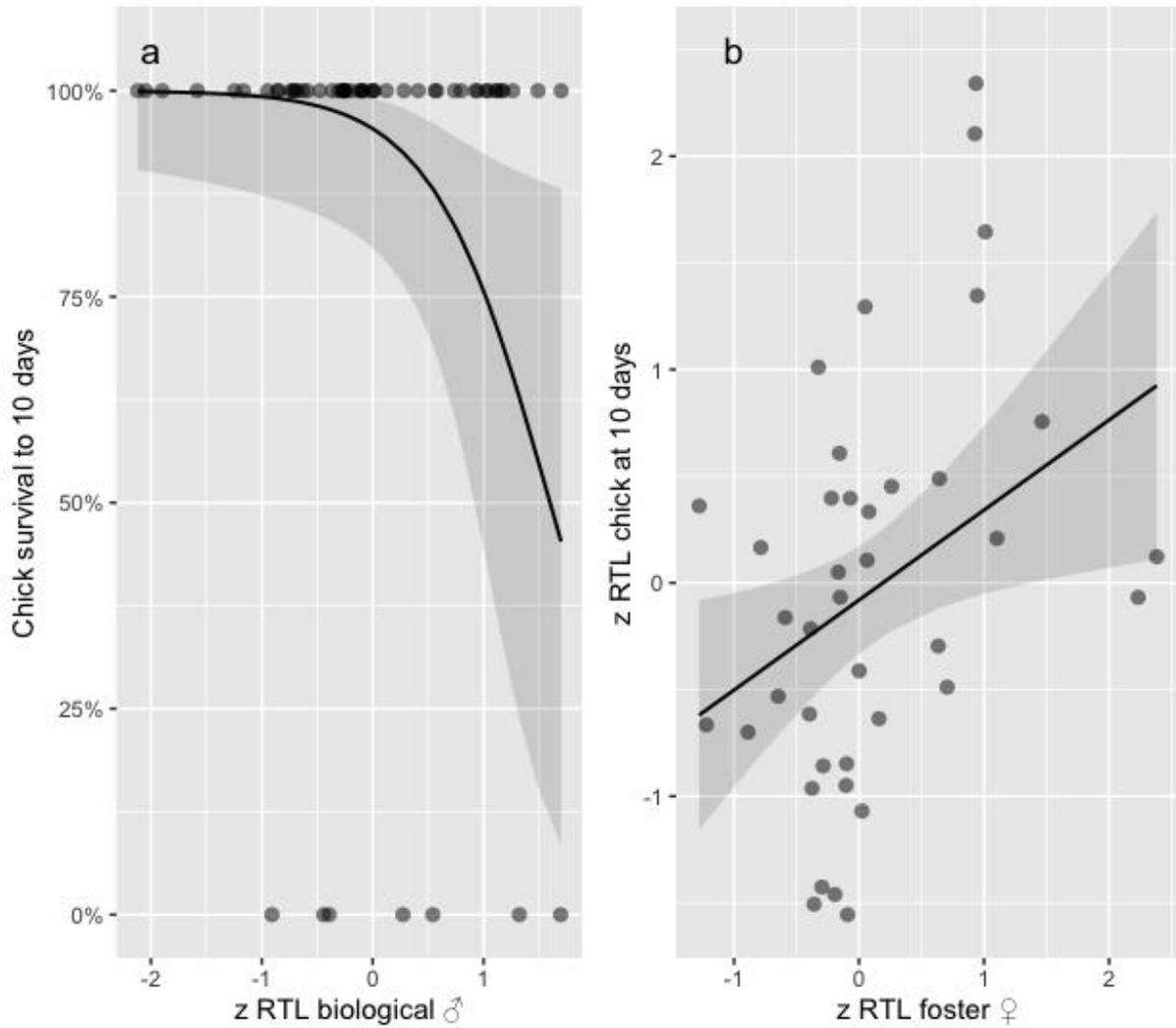
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506 **Fig. 2.** Relationships between king penguin parental telomere length (RTL) and chick survival
 507 and phenotype early in development (day 10 post-hatching). All parents were included in the
 508 same model, and different mixed models were run for (a) chick survival (binary 0/1); (b)
 509 chick structural size (principal components axis, see Methods); (c) chick body condition (see
 510 Methods); and (d) chick RTL. Standardized mixed model estimates are given with 95% CI.
 511 Significant effects have CI_{95} not overlapping 1 for the binomial model, and not overlapping 0
 512 for linear models. Positive and negative effects fall to the right and left of the vertical line,
 513 respectively. RTL is expressed as $\log(T/S)$ ratio, and all variables were standardized (z -
 514 scores) priori to analyses.



516

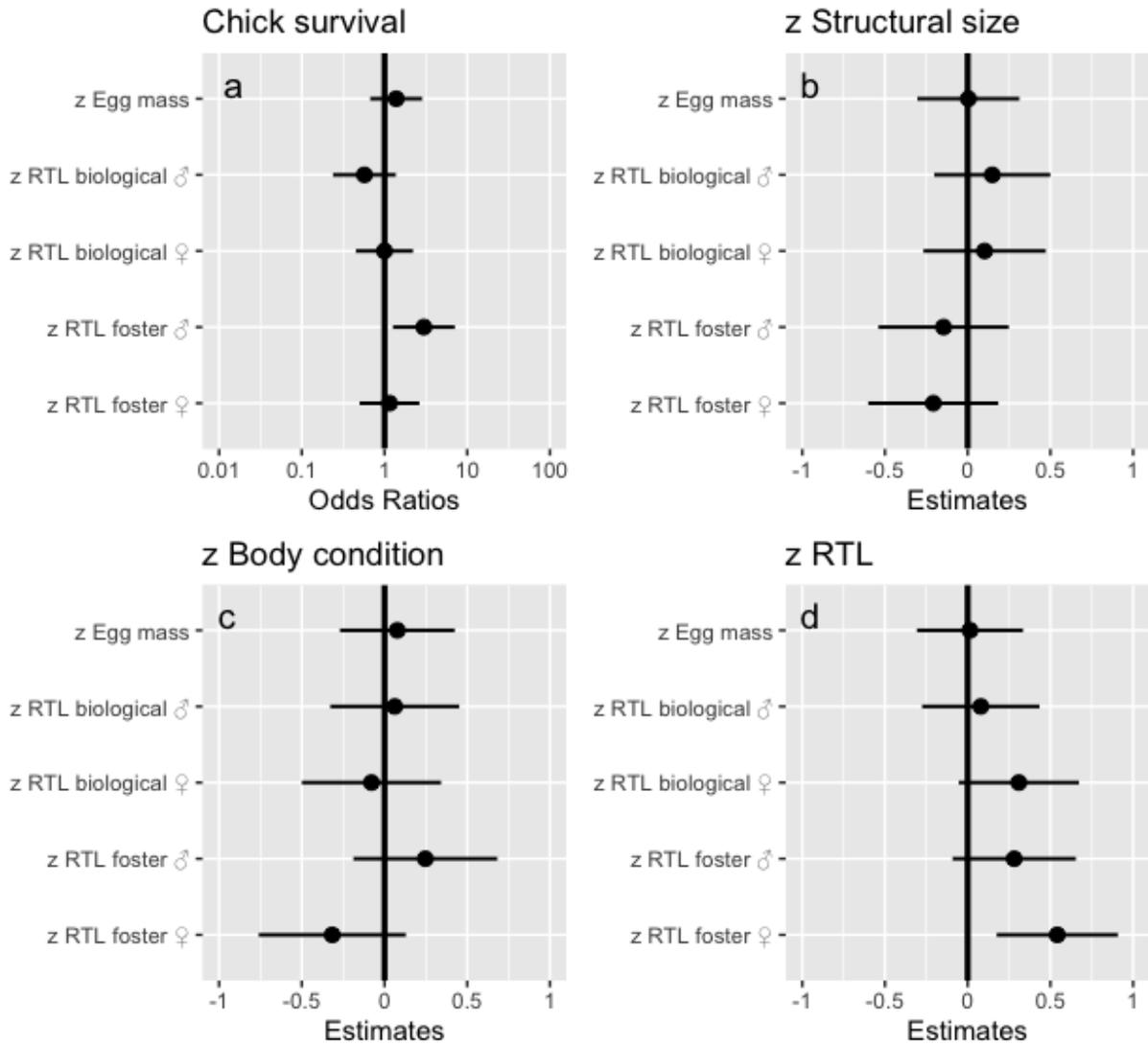
517 **Fig. 3.** (a) Predicted probability and 95% CI of chick survival at 10 days as a function of
 518 biological male relative telomere length (RTL). (b) Relationship between foster female RTL
 519 and chick RTL at 10 days. RTL is expressed as $\log(T/S)$ ratio, and was standardized (z -
 520 scores) priori to analyses.

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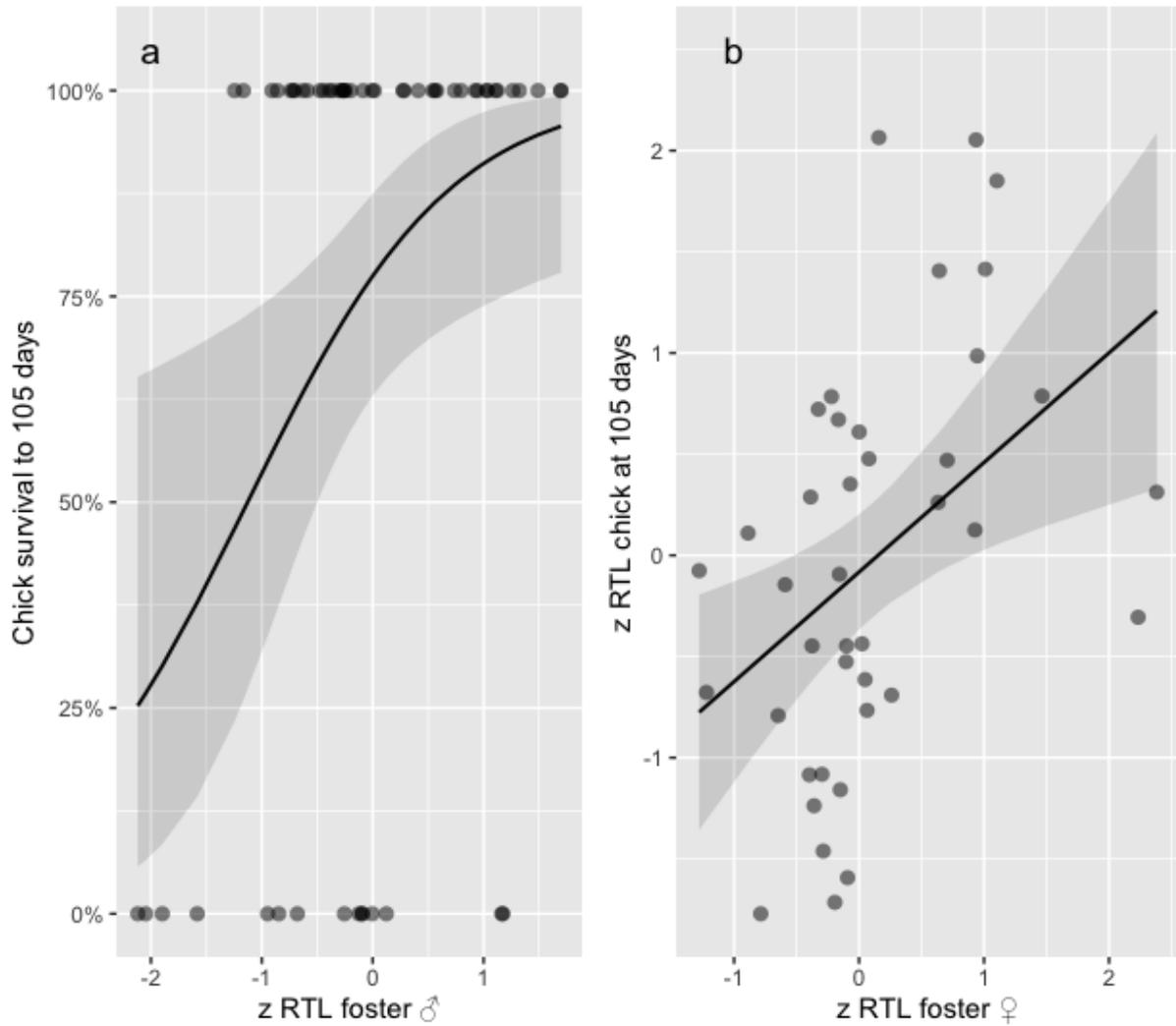
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527 **Fig. 4.** Relationships between king penguin parental telomere length (RTL) and chick survival
 528 and phenotype late in the development (day 105 post-hatching, the end of the pre-winter
 529 growth phase). All parents were included in the same model, and different mixed models were
 530 run (a) chick survival (binary 0/1); (b) chick structural size (principal components axis, see
 531 Methods); (c) chick body condition (see Methods); and (d) chick RTL. Standardized mixed
 532 model estimates are given with 95% CI. Significant effects have CI₉₅ not overlapping 1 for the
 533 binomial model, and not overlapping 0 for linear models. Positive and negative effects fall to
 534 the right and left of the vertical line, respectively. RTL is expressed as log (T/S ratio), and all
 535 variables were standardized (z -scores) priori to analyses.



537

538 **Fig. 5.** (a) Predicted effect and 95% CI of chick survival at 105 days as a function of foster
 539 male relative telomere length (RTL). (b) Relationship between foster female RTL and chick
 540 RTL at 105 days. RTL is expressed as log (T/S ratio), and was standardized (z-scores) priori
 541 to analyses.

542

543

544

545

546 **CONFLICT OF INTEREST**

547 None declared

548

549 **AUTHOR CONTRIBUTION**

550 J.-P.R. is the PI of the polar research program 119. V.A.V. and P.B. conceived the
551 experiment; Q.S., A.S., L.D, E.L. conducted the experiment, Q.S., S.Z. and F.C. extracted the
552 DNA and performed the qPCR measurements and RTL analyses, F.C. and V.A.V. ran the
553 statistical analyses and wrote a first version of the manuscript. All authors drafted the final
554 manuscript and gave their approval for publication.

555

556 **DATA ACCESSIBILITY**

557 The data associated with this manuscript are available online at figshare xxxxxxxx.

558

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