MOLECULAR ECOLOGY

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

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Manuscripts

1   **Foster rather than biological parental telomere length predicts offspring**

2   **survival and telomere length in king penguins**

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21 **ABSTRACT**

22 Because telomere length and dynamics relate to individual growth, reproductive investment

23 and survival, telomeres have emerged as possible markers of individual quality. Here, we

24 tested the hypothesis that, in species with parental care, parental telomere length can be a

25 marker of parental quality that predicts offspring phenotype and survival. In king penguins,

26 we experimentally swapped the single egg of 66 breeding pairs just after egg laying to

27 disentangle the contribution of pre-laying parental quality (*e.g.* genetics, investment in the

28 egg) and/or post-laying parental quality (*e.g.* incubation, postnatal feeding rate) on offspring

29 growth, telomere length and survival. Parental quality was estimated through the joint effects

30 of biological and foster parent telomere length on offspring traits, both soon after hatching

31 (day 10) and at the end of the pre-winter growth period (day 105). We expected that offspring

32 traits would be mostly related to the telomere lengths (i.e. quality) of biological parents at day

33 10 and to the telomere lengths of foster parents at day 105. Results show that chick survival

34 up to 10 days was negatively related to biological fathers' telomere length whereas survival

35 up to 105 days was positively related to foster fathers' telomere lengths. Chick growth was

36 neither related to biological nor to foster parents' telomere length. Chick telomere length was

37 positively related to foster mothers' telomere length at both 10 and 105 days. Overall, our

38 study shows that, in a species with bi-parental care, parents' telomere length is foremost a

39 proxy of post-laying parental care quality, supporting the "telomere – parental quality

40 hypothesis".

41

1 | INTRODUCTION

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

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

67 estimates of telomere length heritability appear to be largely variable across species (Asghar

68 et al. 2014; Atema et al., 2015; Becker et al., 2015; Stier et al., 2015; Belmaker et al., 2019),

69 suggesting that both genetics and environmental factors (including parental care) may

70 influence offspring telomere length. Assessing the effects of parental care quality on telomere

71 length is however complex, since it requires disentangling the contribution of additive genetic

72 effects (i.e. heritability) from parental care *per se* on offspring telomeres. In fact, individual

73 telomere length within its cohort appears not to be fully established at the embryonic stage but

74 changes rapidly in early-life (Fairlie et al., 2016), mostly during growth when cell division

75 rates are high (Monaghan & Ozanne, 2018). A large number of non-exclusive mechanisms

76 can account for inter-individual variability in telomere length early in life. In birds for

77 instance, telomere length may vary according to embryo exposure to maternal corticosterone

78 in the egg (Haussman et al. 2012), incubation temperature (Stier et al., 2019), and/or variation

79 in post-hatching environmental conditions (Nettle et al., 2015; Reichert et al., 2015; Soler et

80 al., 2017). Those post-hatching factors include the quality of parental care and/or parental

81 effort (as suggested by positive links between parental telomere length and breeding

82 performance; Le Vaillant et al., 2015; Angelier et al., 2019, but see Bauch et al., 2013; Young

83 et al., 2016). In this context, the use of cross-fostering designs combined with longitudinal

84 measurements of offspring growth trajectories, telomere length dynamics, and survival

85 (Boonekamp et al. 2014; Bauch, et al. 2019; Criscuolo et al., 2017; Dugdale & Richardson,

86 2018; McCarty, 2017) may prove particularly powerful in gaining new insights on the

87 proximate genetic and post-laying environmental determinants of telomere length variability

88 in the next generation.

89   We applied such an approach to study the growth trajectories and telomere length

90 dynamics of king penguin chicks (*Aptenodytes patagonicus*) during the first 3 months of their

91 development. King penguins are slow-breeding seabirds where bi-parental care is required to

92   successfully rear a single chick over a 14-month period. Parental quality is therefore of critical

93   importance in this species (Stonehouse 1960). In this study, we exchanged eggs between

94   breeding pairs soon after egg laying, and we measured both adult telomere length shortly after

95   mating and their chick phenotype at 10 and 105 days after hatching (shortly after hatching and

96   towards the end of their pre-winter growth period, respectively). During the winter period,

97   chicks gather into "crèches" with almost no parental care (Stonehouse 1960; Geiger et al.,

98   2012; Saraux et al., 2012). This experimental cross-fostering design allowed us to disentangle

99   the contribution of biological (mostly investment in eggs and genetics) *vs*. foster (mostly

100   incubation and chick rearing) parental quality assessed as parental telomere length (i.e.

101   telomere length is positively associated with breeding success in adults; Le Vaillant et al.,

102   2015) on chick structural size, body condition, telomere length and survival in early life (i.e.

103   at 10 and 105 days). In the king penguin, chick body condition and telomere length soon after

104   hatching (day 10) are good predictors of survival (Geiger et al., 2012; Stier et al., 2014).

105   Telomere length also shortens with age during chick growth (Geiger et al., 2012; Stier et al.,

106   2014), but does not appear to be related with age in adults (aged 5 to 9 years old; Le Vaillant

107   et al., 2015). If chick phenotype and telomere length soon after hatching are mostly

108   determined through genetic and/or early maternal effects (i.e. investment in eggs), we

109   expected chick phenotypes, including chick telomere length, to be positively related to the

110   telomere lengths of their biological parents. However, because post-laying parental quality,

111   measured through telomere length of foster parents, is likely to become apparent as chicks

112   grow and receive increasing amounts of parental care, we predicted foster parental telomere

113   lengths to be positively related to chick structural size, body condition and survival at 105

114   days. Telomere inheritance was previously found to be moderate ($h^2 \sim 0.2$), being stronger

115   early in development (day 10 after hatching) and fading during development (up to day 300

116   after hatching) in this species (Reichert et al., 2015). Hence, in this study we also tested

117   whether the resemblance between biological parent and offspring telomere length (i.e. genetic

118   effects) diminished during offspring development and was replaced by post-hatching

119   environmental influences measured through a positive resemblance between foster parent-

120   offspring telomeres, as offspring aged.

121   When investigating the effects of parental quality on offspring phenotype and survival,

122   it is essential to keep in mind that parental quality typically increases with age as individuals

123   gain experience over successive breeding seasons (Forslund & Pärt, 1995; Lecomte et al.,

124   2010). Interestingly, the 'age – parental quality' and 'telomere – parental quality' hypotheses

125   lead to opposite predictions. On one hand the 'age – parental quality hypothesis' predicts that

126   older parents should be of higher quality. On the other hand, older parents are expected to

127   have shorter telomeres and therefore to be of lower quality according to the 'telomere –

128   parental quality hypothesis'.

129

130   2 | MATERIAL AND METHODS

131   **2.1 | Study species and breeding pair monitoring**

132   This study was conducted in the king penguin colony of "La Baie du Marin" (Possession

133   Island, Crozet Archipelago, 46°26' S – 51°52'E), home to some 24,000 pairs of breeding

134   birds. In 2012-2013, we monitored 66 breeding pairs of unknown age from courtship (early

135   November) up to the onset of the Austral winter (early April). In king penguins, the breeding

136   cycle is long and complex, starting by a courtship period of ~15 days during which pairs will

137   form, select a breeding territory, and females lay their single egg (Stonehouse, 1960).

138   Following egg-laying, males and females alternate between periods on land, incubating the

139   egg or caring for the chick, and periods foraging at sea for the rest of the summer

140   (Weimerskirch et al., 1992). The female is the first to leave for sea, the male taking charge of

141   the first incubation shift (Weimerskirch et al., 1992). Incubation lasts for ~53 days

142   (Stonehouse, 1960), the egg typically hatching during incubation shift 4 (the female's second

143   incubation shift). The chick's growth period extends over 10-11 months, including an energy-

144   constraining winter period (April to September) during which it is seldom fed and loses

145   substantial body weight (Cherel et al., 1985; Weimerskirch et al., 1992). Chick feeding and

146   growth resume the following summer (Weimerskirch et al., 1992). Following chick fledging,

147   parents have to moult and replenish their energy stores before they are ready for a subsequent

148   breeding season (Weimerskirch et al., 1992). Divorce rates between breeding seasons are high

149   (*ca.* 80%; Olsson, 1998), however, within a season cooperation between partners is critical to

150   successfully raise the chick, *i.e.* a single parent can not succeed. Parental quality is key and

151   mutual mate choice for high quality partners is high in this species (Jouventin & Dobson

152   2017).

153   We first marked both male and female pair members on the chest from a 1-m distance

154   using animal spray dye (Porcimark®, Kruuse, Lageskov; Denmark) when they were settling

155   on their final breeding territory. The pair was monitored daily at a distance, using binoculars,

156   until a single bird was observed incubating the egg. This bird was identified as the male at day

157   1 of incubation and, 3 days after egg-laying (to minimize disturbance until the bird was

158   motivated to incubate), was flipper-banded with semi-rigid PVC Darvic bands (25.8mm wide,

159   1.9mm thick, 7.4g), allowing its identification and subsequent monitoring during the study.

160   The female was caught and flipper-banded when she returned from her first foraging trip at

161   sea. All flipper-bands were removed from birds at the end of the study.

162

163   **2.2 | Cross-fostering design, blood sampling and bird monitoring**

164   Three days after the egg was laid (first incubation shift of the male), we cross-fostered (*i.e.*

165   swapped) eggs between penguin pairs that had laid their egg on the same day. In total, we

166   swapped eggs between 66 breeding pairs grouped in 33 dyads. This required 3 persons. First,

167   two males were immobilized while incubating in the colony and rapidly hooded to minimize

168   stress. Their respective egg was carefully removed from the brood pouch and replaced by a

169   warm dummy plaster egg during the exchange. Eggs were weighed to the nearest 1-g using a

170   Pesola® spring-slide scale. One person then proceeded to exchange the eggs while the 2 other

171   persons remained by the birds in the breeding colony at all times to ensure the procedure went

172   smoothly. Once the eggs were swapped and individuals released, we monitored bird

173   behaviour to ensure they settled down once again on their breeding territory. We never

174   witnessed breeding abandonment by the birds at this stage.

175

176   *Adult monitoring*

177   For males and females, blood samples (2 mL) were collected from the marginal flipper vein

178   using a G22-1½ needle fitted to a 2.5 mL heparinized syringe. Males were sampled at the

179   time cross-fostering occurred (day 3, incubation shift 1). Females were sampled during their

180   first incubation shift (day 2). The bird's head was covered with a hood to minimize stress and

181   agitation during blood sampling, and samples were kept on crushed ice in the field until

182   further processing, usually within 15 min. After centrifugation (3000g for 10 min), plasma

183   and blood cells were separated and kept frozen dried at -20°C until the end of the day, before

184   being moved to -80°C until assayed. Penguin pairs were monitored twice daily until hatching

185   (confirmed by the presence of a newly hatched chick and the presence of broken egg shells).

186   That day was marked as hatching day. Ten days later, we caught the adults as described

187   above, and temporarily replaced the chick with a warm dummy plaster egg.

188

189   *Chick monitoring*

190   On day 10 post-hatching (*i.e.* early during development), chicks were measured for flipper

191   length, beak length and tarsus length to closest 1-mm using a solid metal ruler. They were

192   weighed (closest 5g) using a spring-slide Pesola® scale, and a small blood sample (~100 μL)

193   was obtained from the marginal flipper vein using a G27-1½ needle and 75 μL heparinized

194   capillary tubes. Chicks were then individually identified using color-coded fish tags (Floy Tag

195   and MFG, Inc. Seattle, WA, USA) attached subcutaneously to their upper-back (Stier et al.,

196   2014). On day 105 post-hatching, the same procedure was repeated, when chicks had been

197   emancipated for approximately two months and had gathered in crèches in anticipation of the

198   austral winter period. We then collected 1 mL of blood from the marginal flipper vein, and

199   measured flipper length, beak length and tarsus length as described above.

200   From these data, we calculated chick structural size as the first principal component of

201   separate PCA analyses on flipper length, beak length and tarsus length both at 10 and 105

202   days (SSz_{10} = -28.44 + 0.29 beak + 0.12 flipper + 0.11 tarsus; SSz_{105} = -33.80 + 0.12 beak +

203   0.03 flipper + 0.08 tarsus; ΔSSz = -21.16 + 0.12 beak + 0.04 flipper + 0.08 tarsus). Because

204   chick body mass and structural size indices were highly correlated (at day 10: Pearson's r =

205   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

206   calculated chick body condition at day 10 and day 105 by regressing body mass on structural

207   size at those different time points (Schulte-Hostedde et al. 2005). Chick structural size and

208   body condition were then used as uncorrelated dependent variables in subsequent analyses

209   (see below).

210   Chicks were monitored up until the subsequent summer (November-December), when

211   they departed from the colony for their first trip at sea. Of the 66 eggs produced by the

212   monitored breeding pairs 54 chicks survived up to 10 days and 44 chicks survived up to 105

213   days.

214

215   **2.3 | Measurement of telomere length in adult and chick king penguins**

216   King penguin relative telomere length (RTL) was measured using a protocol specifically

217   developed and routinely used on king penguins (Geiger et al., 2012; Reichert et al., 2015; Le

218   Vaillant et al., 2015; Stier et al., 2014; Schull et al., 2018). DNA was extracted from

219   nucleated red blood cells (Nucleospin Blood QuickPure, Macherey-Nagel, Düren, Germany)

220   and checked for quality using gel-migration and a NanoDrop 1000 (Thermo Scientific)

221   spectrophotometer (absorbance ratio A260/280; A260/230.). Extracted DNA was then used to

222   amplify both the telomere and a control gene (non-variable in copy numbers within our

223   population, Smith, Turbill & Penn, 2011) by quantitative real-time amplification (qPCR)

224   based on Cawthon's original development (Cawthon, 2002). Control gene (*Aptenodytes*

225   *patagonicus* zinc finger) and primer sequences were identical to those used in previous

226   penguin telomere studies, as well as the conditions of qPCR amplifications (see Stier *et al.,*

227   2014 for details). We used 2.5 ng DNA per reaction and the BRYT Green fluorescent probe

228   (GoTaq_qPCR Master Mix; Promega, Charbonniere, France). The samples were amplified on

229   a 384 wells thermocycler (CFX-384, Biorad Hercules), in duplicates over three runs, the

230   telomere sequence and the control gene sequence being amplified using the same conditions.

231   Samples were distributed over 3 plates and individual birds randomly distributed on each

232   plate. Intra-plate repeatability based on duplicate runs was of 0.785 for the final calculated

233   relative telomere length value (T/S ratio based on Cq values). Inter-plate repeatability based

234   on 13 samples (*i.e.* 13 different individuals) repeated over all plates was of 0.894 for final

235   calculated relative telomere length value (T/S ratio). Mean amplification efficiencies of

236   telomere sequence and control gene were of 100% and 99.9% (plate 1), of 100.2% and 99.8%

237   (plate 2) and of 100% and 100.3% (plate 3), respectively. Relative telomere lengths were

238   calculated following (Pfaffl, 2001) and using the plate efficiencies amplification values

239   corresponding specifically to each sample. No apparent well-position bias was observed

240   (Eisenberg, Kuzawa, & Hayes, 2015) (see Online Supporting Information). We obtained

241   telomere data for 61 adult breeding pairs and 42 chicks throughout growth.

242

243   **2.4 | Statistical analyses**

244   All analyses were run using R v.3.5.1. Forest plots and marginal effects plot with 95% CI

245   were obtained using the 'sjPlot' package in R (Lüdecke, 2017). In all models presented

246   below, Relative Telomere Length (RTL) was systematically log-transformed and standardized

247   (z scores) prior to analyses (see Verhulst et al. 2019). Other continuous variables were

248   standardized so that model coefficients could be directly comparable in their magnitude.

249   Where appropriate, we ensured residuals were normally distributed by visual inspection of

250   density distributions, Q-Q plots, cumulative distribution functions and P-P plots using the

251   'fitdistrplus' package in R (Delignette-Muller & Dutang, 2015). We also ensured that no

252   substantial collinearity occurred between independent variables (Variance Inflation Factors

253   ranged $1.05 < VIF < 2.05$; suggested cut-off at 3; Zuur, Ieno, & Elphick, 2010). For each

254   model, sample sizes are reported in the tables. Sample sizes can vary across models due to

255   variation in egg and chick mortality and/or due to difficulties at sampling blood from some

256   chicks or amplifying DNA (telomeres) from some blood samples.

257

258   *Chick telomere dynamics during growth*

259   We investigated chick RTL dynamics in early life using linear mixed models (LMMs) with

260   RTL as the dependent variable, chick age (categorical: 10 or 105 days after hatching) as an

261   independent variable, and chick ID as a random factor. Hence, the model was specified as:

262   *z-RTL~ Chick age$_{10\ or\ 105}$ + (1|chick ID)*

263   From this model, we computed repeatability in chick RTL during early life as the ratio

264   of among-individual variance (V_G) over the total phenotypic variance (V_P) equal to $V_G + V_R$

265   (the within-individual or residual variance in RTL) (see Nakagawa & Schielzeth 2010; Stoffel

266   et al. 2017). Hence, repeatability $= R = \frac{V_G}{V_P} = \frac{V_G}{V_G + V_R}$. This LMM-based repeatability estimate

267   allowed to control for the confounding effects of age (see Nakagawa & Schielzeth 2010) since

268   chick RTL shortened with time. Repeatability was calculated using the 'rptR' R cran package;

269   Stoffel *et al.,* 2017). Confidence intervals around the repeatability estimate were computed by

270   parametric bootstrapping (10,000 iterations). This repeatability allowed us to assess whether

271   chicks starting their post-hatching growth period with long telomeres also entered the winter

272   period with long telomeres, which informs on the importance of 'starting' telomere length in

273   determining later life telomere length and potentially life histories.

274

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

276   *Chick survival:* We tested for influences of parental (both biological and foster) RTL on chick

277   survival up to day 10, or up to day 105, using separate Generalized Linear Mixed Models

278   (GLMMs: binomial, logit-link). These were specified as:

279   *Survival (0 =failure, 1 = success)$_{10 (or 105)}$ ~ z-RTL$_{biological♂}$ + z-RTL$_{biological♀}$ + z-*

280   *RTL$_{foster♂}$ + z-RTL$_{foster♀}$ + z-egg mass + (1|dyad)*

281   Here, we included cross-fostering dyad identity as a random factor in the model and

282   accounted for egg mass as a covariate to test for potential effects occurring from early

283   maternal investments in the egg (Bize et al. 2002; Krist 2011). From these models, odd ratios

284   were calculated to illustrate the relative influence of the different fixed factors (mainly in our

285   case biological and foster parental telomere lengths) on offspring survival. The odd-ratio can

286   be interpreted for a given predictor in terms of increasing (>1) or decreasing (<1) the

287   likelihood to survive for a one unit increase in that predictor, holding all other variables

288   constant. For instance, holding all other variables constant, an odds ratio of 2 for a given

289   predictor would imply that the odds of surviving increase by a factor 2 for each unit increase

290   in the considered predictor.

291

292   *Chick phenotype:* The influence of biological and foster parent RTL on chick phenotypic

293   traits (structural size, body condition and RTL) both early (10 days post-hatching) and later

294   (105 days post-hatching) in life were tested using separate LMMs. Here also, we accounted

295   for egg mass as a covariate in the models, and controlled for cross-fostering dyad identity as a

296   random factor. These were thus specified as:

297        $z\text{-}Phenotypic\ trait \sim z\text{-}RTL_{biological\male} + z\text{-}RTL_{biological\female} + z\text{-}RTL_{foster\male} + z\text{-}RTL_{foster\female} + z\text{-}$

298        $egg\ mass + (1|dyad)$

299

300   Finally, we tested the influences of both biological and foster parent RTL on the

301   change in chick RTL between days 10 and 105 ($RTL_{105} - RTL_{10}$) using a Linear Mixed

302   Model (LMM). We specifically chose not to control for chick initial telomere length in this

303   model (RTL_{10}), since this may lead to biased estimated of rate of attrition even when

304   correcting for regression to the mean (Bateson et al. 2019). We included cross-fostering dyad

305   identity as a random factor in the model to account for potential temporal effects associated

306   with the cross-fostering design (eggs being swapped on the same date between dyads of

307   penguin pairs). The model was thus specified as:

308        $z\text{-}(RTL_{105} - RTL_{10}) \sim z\text{-}RTL_{biological\male} + z\text{-}RTL_{biological\female} + z\text{-}RTL_{foster\male} + z\text{-}RTL_{foster\female} +$

309        $(1|dyad)$

310

311   3 | RESULTS

312   **3.1 | Chick telomere dynamics in early life**

313   On average, chick telomere length decreased over time (LMM; $z\ RTL_{105vs10}$ = -0.40 ± 0.14, t =

314   -2.78, CI = [-0.68; -0.12], P = 0.008; Fig. 1). Using the variance explained by chick ID in this

315   model (var = 0.55), we found that chick telomere length was repeatable (LMM; r = 0.56 ±

316   0.11, CI = [0.33; 0.74], P < 0.001): chicks starting their post-hatching growth period with

317   longer telomeres also entered the winter period with longer telomeres (see Figs. 1a and 1b).

318

319   **3.2 | Chick survival and phenotype at 10 days**

320   Chick survival up to 10 days was weakly and negatively related to the RTL of the biological

321   male, but not to the RTL of the biological mother, the RTL of foster parents, or egg mass

322   (Table 1, Fig. 2a and 3a). At 10 days, neither chick structural size or body condition were

323   significantly related to biological or foster parental RTL telomere length, or egg mass (Table

324   1, Figs. 2b and 2c). In contrast, chick RTL was positively associated with the RTL of foster

325   mothers (Table 1, Figs. 2d and 3b), and positively (though not significantly, P = 0.071) with

326   the RTL of foster fathers, but not with the RTL of genetic parents or egg mass (Table 1).

327

328   **3.3 | Chick survival and phenotype at 105 days**

329   At 105 days, chick survival was significantly and positively related to foster male RTL, but

330   not to the RTL of the foster mother, the RTL of biological parents, or egg mass (Table 2, Fig.

331   4a and 5a). At 105 days, neither was chick's structural size or body condition significantly

332   related to biological or foster parental RTL telomere length, or egg mass (Table 2, Figs. 4b

333   and 4c). In contrast, chick RTL was significantly and positively associated with the RTL of

334   foster mothers (Table 2, Fig. 4d and 5b). The change in chick telomere length between days

335   10 and 105 was not significantly associated with parental RTL when both biological and

336   foster parents were included in the same model (Table 3).

337

338

4 | DISCUSSION

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

351

4.1 | Parental telomere length effects on chick survival

Because of their susceptibility to environmental stress, telomeres have been proposed as integrative markers that can be used to reflect an individual's life stress and by extension stress coping mechanisms, thus perhaps allowing to gauge individual quality (Angelier *et al.,* 2019). From an evolutionary perspective, high quality individuals are expected to perform well in a suite of correlated phenotypic traits, including investment in parental care (Wilson & Nussey, 2010). Hence, one of the aims of this study was to test the 'telomere – parental quality hypothesis' hypothesizing that parents with long telomeres were of higher quality, and therefore predicting that they should produce heavier and larger chicks more likely to survive early in life. Accordingly, previous studies have reported positive links between telomere length and reproductive success in seabirds, including king penguin (Le Vaillant et al. 2015,

363   Angelier et al. 2019; but see Bauch et al. 2013 for a negative association, and Olsson et al.

364   2011a for a quadratic association in a reptile).

365   Surprisingly, after controlling for egg mass (*i.e.* maternal effects; Krist 2011), we

366   found a negative effect of biological father telomere length on chick survival at 10 days, but

367   no significant effect of foster parent telomere length (*i.e.* early post-hatching environmental

368   effects). Contrary to our expectation based on the 'telomere – parental quality hypothesis',

369   this result suggests that fathers with longer telomeres (expected to be of good quality)

370   somehow reduced the chances of survival of their chicks in the first days after hatching. This

371   negative effect was rather marginal (Table 3) and the mechanism explaining such an

372   association remains unclear. It seems unlikely this result was explained by the 'age – parental

373   quality hypothesis' (Forslund & Pärt, 1995; Lecomte et al., 2010), given a lack of association

374   between telomere length and chronological age in king penguins (Le Vaillant et al., 2015;

375   note however that birds in this study were aged 5 to 9 and king penguins have been reported

376   to live up to 26 year old in captivity, Flower 1938). Furthermore, if father's age and

377   experience were important determinants of chick survival in penguins, we would have

378   expected to detect a negative impact of foster father telomere length on chick survival at 105

379   days.

380   In contrast, chick survival at 105 days increased with foster male telomere length,

381   even when controlling for egg mass. This is predicted by the 'telomere – parental quality

382   hypothesis' if indeed telomere length acts as a proxy of individual quality and positively

383   correlates with post-hatching paternal care. Interestingly, this effect was apparently

384   independent of any effect of paternal telomere length on chick body mass or growth,

385   suggesting other benefits than those purely related to energy investments in the offspring.

386   Telomere length has been positively associated to foraging efficiency, but not to parental

387   investment, in other seabird species (Young et al., 2015, 2016). In king penguins, if parental

388 foraging efficiency was also related to telomere length, we might expect parents with longer

389 telomeres to be better at provisioning their chicks during development, ultimately affecting

390 chick body mass or structural size. We found however no support for such mechanism.

391 Remarkably, in king penguins on-land predation of brooded chicks is high (i.e. 51 % of

392 crèching chicks in a given reproductive season; Descamps et al., 2005), and an important

393 source of extrinsic mortality. Hence, an alternative mechanism could be that foster males with

394 long telomeres are more territorial and aggressive birds and therefore better at coping with

395 predators during their brooding shifts. This alternative mechanism remains to be tested.

396

397 **4.2 | Parental telomere length effects on chick telomere length**

398 Individual variation in telomere length in early life may come from (i) how zygote telomere

399 length is determined and (ii) what inherited and environmental factors are going to change the

400 way offspring lose and repair their telomeres. Disentangling those genetic and pre/post-laying

401 influences is far from being an easy task because telomere length is a complex structure

402 underpinned by the expression of multiple genes, by epigenetic modulation (Bauch et al.,

403 2019), as well as by a wide number of environmental factors (Dugdale & Richardson, 2018).

404 In addition, any modulation of development, of genetic (*i.e.* parental age, Bauch et al. 2019)

405 or environmental origins (Metcalfe & Monaghan, 2003), may have pervasive impact on the

406 future phenotype of offspring, including telomere length (Metcalfe & Monaghan, 2003;

407 Tarry-Adkins et al., 2009). In this study, we swapped eggs soon after laying to investigate

408 whether offspring telomere length were more alike the telomere length of their biological

409 (genetic effect) or foster parents (pre/post-hatching parental effect).

410   Our results show that chick telomere lengths at 10 and 105 days were both related to

411 foster maternal telomere length. At day 105, offspring telomere length was also positively

412 related to biological mother and foster father telomere length, though not significantly.

413   Previous data based on biological mother-offspring regressions have reported significant

414   maternal heritability for telomere length in king penguin (around $h^2 = 0.2$), which weakened

415   over the period of chick growth (Reichert et al., 2015). Thus, although telomere length in king

416   penguin chicks may be determined in part before egg-laying (e.g. Olsson et al. 2011a; Bauch

417   et al., 2019), our data suggest a stronger effect of the post-laying environment on chick

418   telomere length (see Becker et al., 2015 for similar findings in another bird species). King

419   penguin chicks are raised in an unpredictable environment (high predation risk, socially

420   aggressive adults, inclement weather conditions), and are subject to periods of intermittent to

421   prolonged fasting early in life (Cherel & Le Maho, 1985). Thus, variation in parental care and

422   ability to efficiently provision and defend their offspring will have critical consequences on

423   offspring phenotype. Our results in king penguins suggests that selection on telomere length

424   might be sex-specific (see also Olsson et al. 2011b for similar finding in a lizard species).

425   However, why this should occur is unclear. We do know that feeding strategies differ

426   somewhat between male and female adult king penguins during chick rearing (Le Vaillant et

427   al. 2013; see also Saraux et al., 2012 for sex-related differences over winter). Females for

428   instance, appear to perform more prey pursuits than males during chick care (Le Vaillant et al.

429   2013), which might result in subtle sex-related differences in offspring feeding strategies,

430   leading mothers to display a larger effect on chick telomere length during early growth.

431   Focusing on food elements known to buffer deleterious effect on telomeres (*e.g.* dietary

432   antioxidants, Reichert & Stier, 2017), and the quality of the diet provided by mothers and

433   fathers, may provide new insights into this question. In king penguin chicks, telomeres seem

434   to erode faster in rapidly growing individuals (Geiger et al., 2012). This suggests that

435   variation in maternal provisioning patterns early in life is likely another important factor

436   affecting chick telomere length. Adequate or more regular rates of food provisioning by high

437   quality adults may allow chicks to better balance out the allocation of energy towards growth

438 and other somatic compartments, without affecting body mass *per se*, allowing higher

439 telomere maintenance. Additionally, development does not only concern cell multiplication

440 and an increase in body mass but also physiological maturation. A recent study in birds

441 suggested that maturation may be done at a cost of telomere loss (Criscuolo et al., 2019).

442 Whether early maternal care may enable chicks to mature in a way that allows to better

443 preserve telomere ends afterwards is intriguing and a call for further research. Finally, it is

444 worth keeping in mind that, in this study, parental age was unknown. Because parental age

445 can explain substantial variation in offspring telomere length (*e.g.* Criscuolo et al. 2017;

446 Bauch et al. 2019; but see Le Vaillant et al., 2015 for a lack of relationship in adult king

447 penguins aged 5 to 9 years of age), the reported association between foster and biological

448 parental might be an underestimation of any true association between parental and offspring

449 telomere length.

450   Overall, our study provides experimental evidence that the quality of environmental

451 rearing conditions mediated by the parents partly influence variation in offspring telomere

452 length and survival in a long-lived seabird, and adds to the growing evidence that telomeres

453 may be a useful proxy of individual (parental) quality in wild animals. Such an approach

454 opens perspectives as to the finer characterization of the nature and timing of environmental

455 effects conditioning individual survival chances in the wild.

456

464 **TABLES**

465 **Table 1**. Standardized model estimates for the relationship between parental relative telomere

466 length (RTL) and chick survival (binary 0 = failure / 1 = success) and phenotype (structural

467 size, body condition and telomere length) early in the development (day 10 post-hatching).

468 Significant effects have CI95 not overlapping 1 for the binomial model, and not overlapping 0

469 for linear models. All parents were included in the same model. Variance inflation factors

470 (VIFs) are provided. The number of chicks (n) and dyads (N) are given. Sample sizes vary

471 across models due to variation in chick mortality and/or difficulties at sampling blood from

472 some chicks or amplifying DNA (telomeres) from some blood samples.

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Chick survival and phenotype early during development (day 10)							
(A) Survival (binary 1/0)	*Odds ratio ± SE*	*CI*	*z*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.15 ± 0.87	$0.03 - 0.82$	-2.18	0.029*	2.05	0.549	56 (28)
z RTL$_{biological\female}$	1.68 ± 0.65	$0.47 - 6.00$	0.80	0.422	1.88		
z RTL$_{foster\male}$	3.21 ± 0.73	$0.77 - 13.37$	1.61	0.108	1.96		
z RTL$_{foster\female}$	3.25 ± 0.89	$0.57 - 18.61$	1.33	0.185	1.93		
(B) z Structural size	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.21 ± 0.29	$-0.36 - 0.77$	0.71	0.481	1.46	0.084	49 (28)
z RTL$_{biological\female}$	-0.03 ± 0.26	$-0.53 - 0.48$	-0.10	0.921	1.33		
z RTL$_{foster\male}$	0.32 ± 0.29	$-0.25 - 0.88$	1.10	0.271	1.32		
z RTL$_{foster\female}$	0.02 ± 0.28	$-0.53 - 0.56$	0.06	0.956	1.40		
(C) z Body condition	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.30 ± 0.20	$-0.09 - 0.68$	1.51	0.139	1.47	0.078	49 (28)
z RTL$_{biological\female}$	-0.20 ± 0.18	$-0.54 - 0.15$	-1.13	0.267	1.33		
z RTL$_{foster\male}$	-0.03 ± 0.20	$-0.41 - 0.36$	-0.15	0.885	1.32		
z RTL$_{foster\female}$	-0.20 ± 0.19	$-0.57 - 0.17$	-1.05	0.301	1.40		
(D) z RTL	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.14 ± 0.18	$-0.20 - 0.49$	0.81	0.423	1.54	0.335	40 (26)
z RTL$_{biological\female}$	0.22 ± 0.17	$-0.12 - 0.56$	1.26	0.216	1.12		
z RTL$_{foster\male}$	0.34 ± 0.18	$-0.02 - 0.70$	1.86	0.071	1.38		
z RTL$_{foster\female}$	0.42 ± 0.17	$0.08 - 0.76$	2.42	0.021*	1.16		

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476

477   **Table 2**. Standardized model estimates for the relationship between parental relative telomere

478   length (RTL) and chick survival (binary 0 = failure / 1 = success) and phenotype (structural

479   size, body condition and telomere length) late in the development (day 105 post-hatching; the

480   end of the pre-winter growth phase). Significant effects have CI95 not overlapping 1 for the

481   binomial model, and not overlapping 0 for linear models. All parents were included in the

482   same model. Variance inflation factors (VIFs) are provided. The number of chicks (n) and

483   dyads (N) are given. Sample sizes vary across models due to variation in chick mortality

484   and/or difficulties at sampling blood from some chicks or amplifying DNA (telomeres) from

485   some blood samples.

486

Chick survival and phenotype late in development (day 105)							
(A) Survival (binary 1/0)	*Odds ratio ± SE*	*CI*	*z*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.57 ± 0.45	0.24 – 1.37	-1.25	0.210	1.32	0.238	56 (28)
z RTL$_{biological\female}$	1.00 ± 0.41	0.45 – 2.21	-0.00	0.999	1.25		
z RTL$_{foster\male}$	2.99 ± 0.44	1.26 – 7.08	2.49	0.013*	1.30		
z RTL$_{foster\female}$	1.15 ± 0.42	0.50 – 2.64	0.33	0.745	1.29		
(B) z Structural size	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.15 ± 0.18	-0.20 – 0.50	0.91	0.412	1.30	0.050	41 (27)
z RTL$_{biological\female}$	0.10 ± 0.19	-0.27 – 0.47	0.60	0.592	1.32		
z RTL$_{foster\male}$	-0.15 ± 0.20	-0.54 – 0.25	-0.63	0.474	1.19		
z RTL$_{foster\female}$	-0.21 ± 0.20	-0.60 – 0.19	-1.09	0.308	1.38		
(C) z Body condition	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	*R^2*	*n (N)*
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$_{biological\male}$	0.06 ± 0.20	-0.32 – 0.45	0.31	0.757	1.26	0.078	41 (27)
z RTL$_{biological\female}$	-0.08 ± 0.21	-0.50 – 0.34	-0.37	0.716	1.46		
z RTL$_{foster\male}$	0.25 ± 0.22	-0.19 – 0.68	1.11	0.274	1.15		
z RTL$_{foster\female}$	-0.32 ± 0.23	-0.76 – 0.13	-1.39	0.174	1.53		
(D) z RTL	*Estimate ± SE*	*CI*	*t*	*P*		*R^2*	*n (N)*
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$_{biological\male}$	0.08 ± 0.18	-0.28 – 0.43	0.44	0.664	1.40	0.330	40 (26)
z RTL$_{biological\female}$	0.31 ± 0.19	-0.05 – 0.67	1.67	0.104	1.23		
z RTL$_{foster\male}$	0.28 ± 0.19	-0.09 – 0.65	1.48	0.148	1.25		
z RTL$_{foster\female}$	0.54 ± 0.19	0.18 – 0.91	2.90	0.007*	1.28		

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489   **Table 3.** Standardized linear mixed model estimates for the relationship between parental

490   relative telomere lengths (RTL) and chick change in relative telomere length over growth (i.e.

491   between days 10 and 105 post-hatching). All parents were included in the same model.

492   Variance inflation factors (VIFs) are provided. The number of chicks (n) and dyads (N) are

493   given.

494

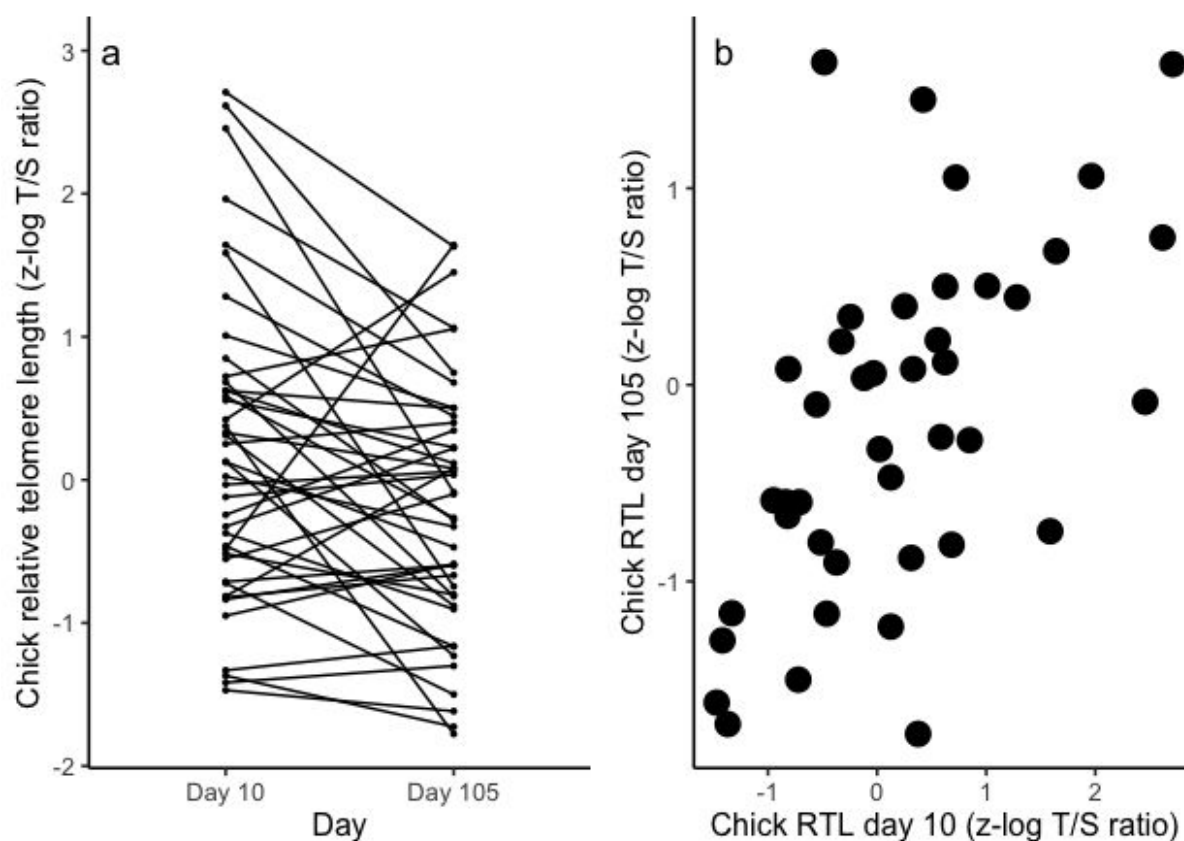| Chick RTL change over growth ($RTL_{chick105} - RTL_{chick10}$)							
z RTL change	*Estimate ± SE*	*CI*	*t*	*P*	*VIF*	*R^2*	*n (N)*
Intercept	0.02 ± 0.17	-0.31 – 0.34	0.10	0.924			
$z\ RTL_{biological\male}$	-0.11 ± 0.21	-0.53 – 0.30	-0.53	0.597	1.41		
$z\ RTL_{biological\female}$	0.04 ± 0.21	-0.38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

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

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

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

505   Relationship between RTL values at day 10 and 105. Different colours indicate different
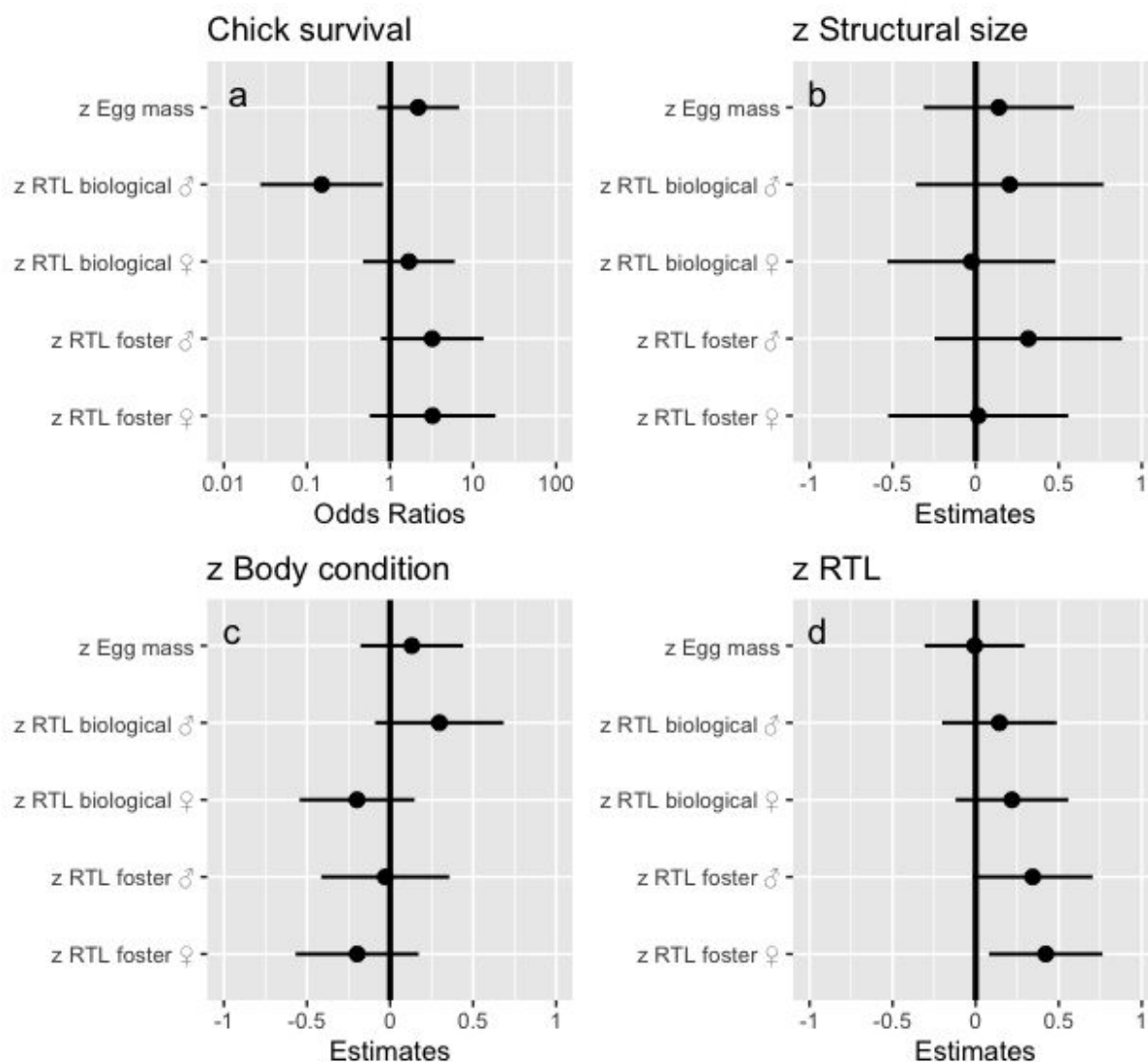
506   birds.
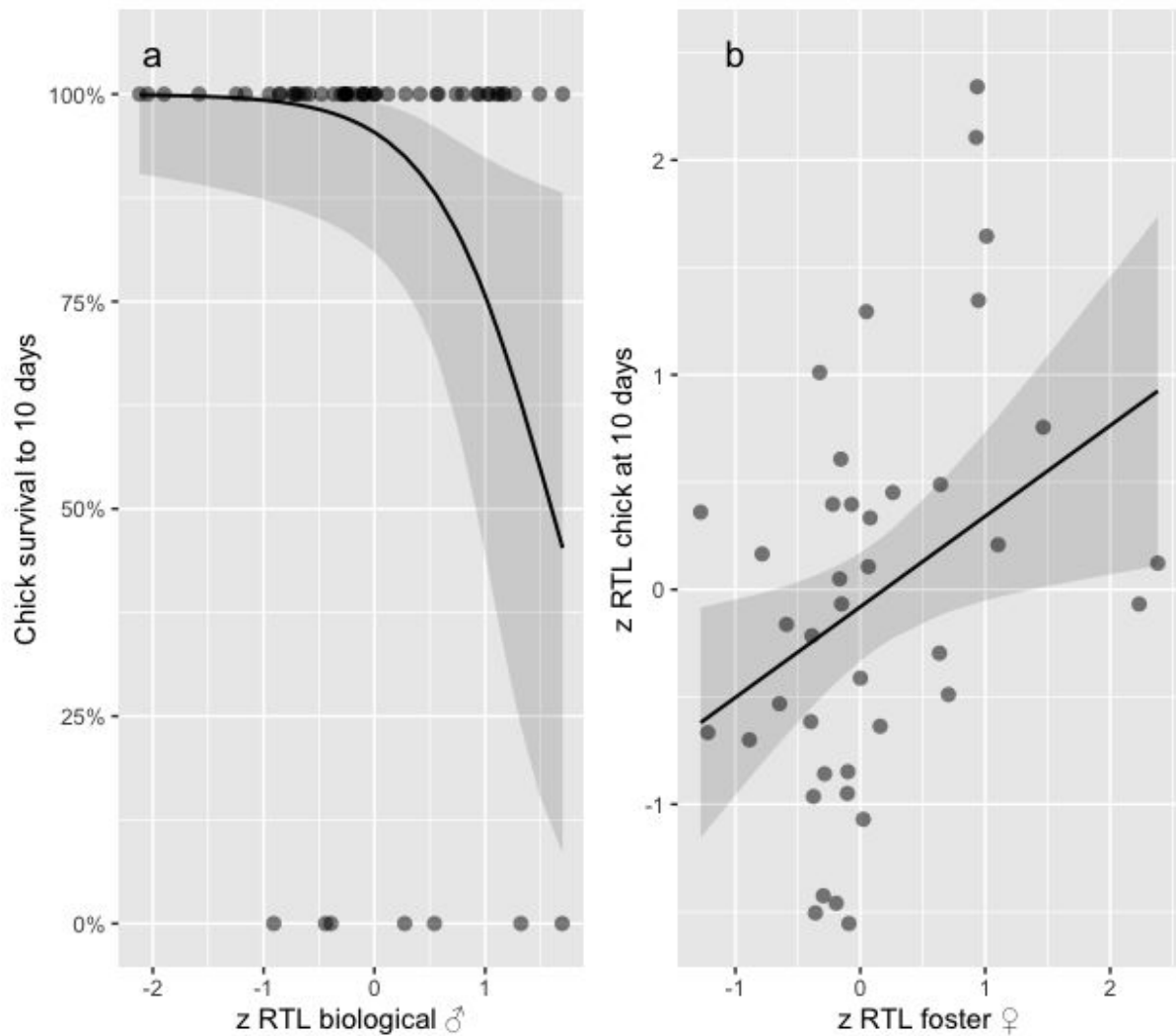
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Fig. 2. Relationships between king penguin parental telomere length (RTL) and chick survival

and phenotype early in development (day 10 post-hatching). All parents were included in the

same model, and different mixed models were run for (a) chick survival (binary 0/1); (b)

chick structural size (principal components axis, see Methods); (c) chick body condition (see

Methods); and (d) chick RTL. Standardized mixed model estimates are given with 95% CI.

Significant effects have CI_{95} not overlapping 1 for the binomial model, and not overlapping 0

for linear models. Positive and negative effects fall to the right and left of the vertical line,

respectively. RTL is expressed as log (T/S ratio), and all variables were standardized (z-

scores) priori to analyses.

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525

526   **Fig. 3.** (a) Predicted probability and 95% CI of chick survival at 10 days as a function of

527   biological male relative telomere length (RTL). (b) Relationship between foster female RTL

528   and chick RTL at 10 days. RTL is expressed as log (T/S ratio), and was standardized (*z*-
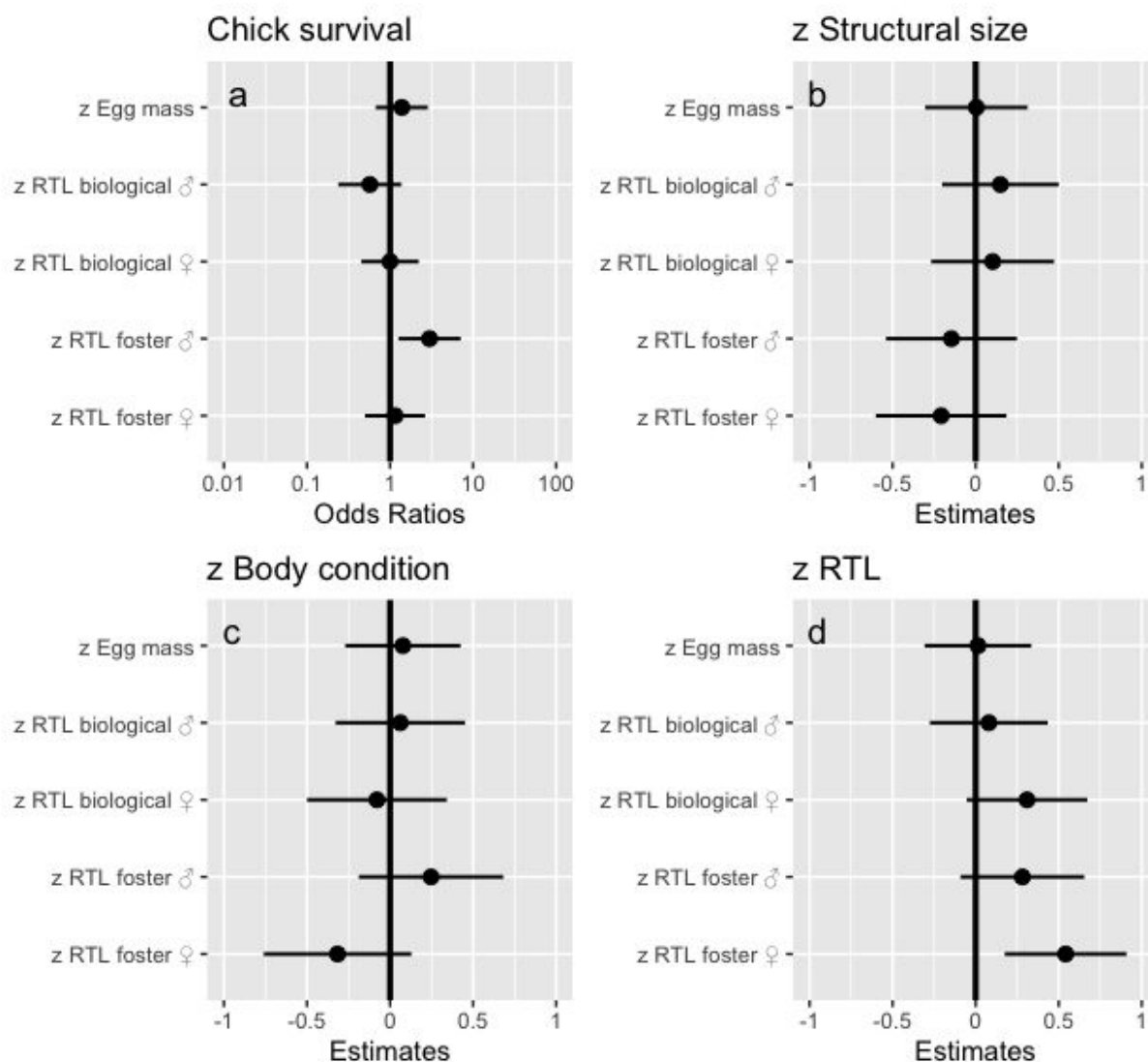
529   scores) priori to analyses.

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

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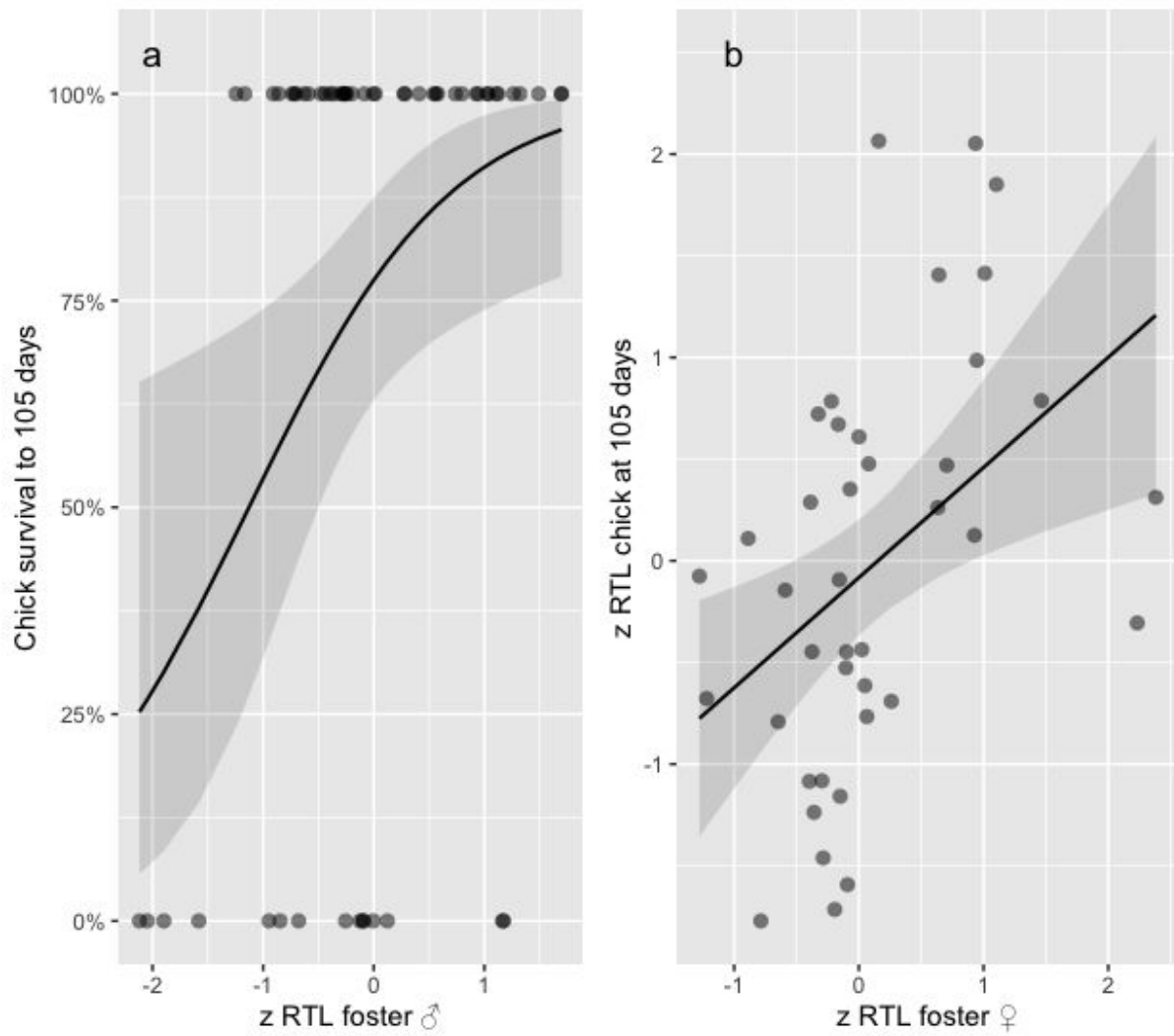
546

547   **Fig. 5.** (a) Predicted effect and 95% CI of chick survival at 105 days as a function of foster

548   male relative telomere length (RTL). (b) Relationship between foster female RTL and chick

549   RTL at 105 days. RTL is expressed as log (T/S ratio), and was standardized (*z*-scores) priori

550   to analyses.

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555 **CONFLICT OF INTEREST**

556 None declared

557

558 **AUTHOR CONTRIBUTION**

559 J.-P.R. is the PI of the polar research program 119. V.A.V. and P.B. conceived the

560 experiment; Q.S., A.S., L.D, E.L. conducted the experiment, Q.S., S.Z. and F.C. extracted the

561 DNA and performed the qPCR measurements and RTL analyses, F.C. and V.A.V. ran the

562 statistical analyses and wrote a first version of the manuscript. All authors drafted the final

563 manuscript and gave their approval for publication.

564

565 **DATA ACCESSIBILITY**

566 The data associated with this manuscript are available online at figshare doi:

567 10.6084/m9.figshare.12249902 (Viblanc et al. 2020).

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