1 **Telomere length vary with sex, hatching order and year of birth in little owls,**

2 ***Athene noctua***

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15

16 **Abstract**

17 Telomeres are non-coding DNA sequences located at the end of linear chromosomes,
18 protecting genome integrity. In numerous taxa, telomeres shorten with age and telomere
19 length (TL) is positively correlated with longevity. Moreover, TL is also affected by
20 environmental stressors and/or resource-demanding situations particularly during early-life.
21 Thus, TL has been used as a physiological marker of individual quality and also as an indicator
22 of population trend in conservation physiology. In this study, we investigated the effects of
23 hatching rank, year of birth (2014 to 2017), sex and nest environment on TL of 137 little owls
24 nestlings (*Athene noctua*). Little owls' populations in Europe showed a marked declined in the
25 end of the 20th century. Nowadays, in the studied Alsatian population, the population is
26 increasing. In this study, our results indicated that telomeres are longer in females and,
27 independently of sex, in nestlings with the highest body condition. There was also a negative
28 effect of hatching rank but only for last-hatched nestlings in large clutches of 5 nestlings. We
29 did not find a marked effect of the environmental covariates on nestlings' TL. Finally, we found
30 that nestlings' TL decreased over years, while nestlings' body condition stayed unchanged
31 over the same period. This result is intriguing given the local positive population dynamics and
32 is further discussed in the context of physiological conservation. Future studies should
33 investigate the link between reduced TL and survival prospects in this species.

34

35 **Introduction**

36 Telomeres are non-coding DNA structures, located at the end of the linear chromosomes,
37 serving as a safe-keeper for preservation of coding DNA over cell duplication (Blackburn,
38 1991). Thanks to the formation of a capped structure with specific shelterin proteins,
39 telomeres help the cell to distinguish real chromosome ends from DNA breaks, thereby
40 avoiding unappropriated cell emergency responses. Still, this telomere status is degrading
41 over time, due to the progressive loss of telomere sequences at each cell division, affecting its
42 functionality and triggering cell senescence (Blackburn, 2000). In addition, telomere
43 sequences are enriched in GC bases, making them highly sensitive to a well-known ageing
44 mechanism, the oxidative stress (von Zglinicki, 2002; Reichert & Stier, 2017) (but see
45 Boonekamp *et al.*, 2017). Such a stress-related property triggered the interest of evolutionary
46 biologists to study how telomeres (length or dynamics) may explain inter-specific longevity
47 (Haussmann *et al.*, 2003; Dantzer & Fletcher, 2015; Tricola *et al.*, 2018; Criscuolo *et al.*, 2021)
48 and the link between environmental stress or life-history trade-offs and inter-individual
49 differences in lifespan and fitness (Beaulieu *et al.*, 2011; Foote *et al.*, 2011; Boonekamp *et al.*,
50 2014; Nettle *et al.*, 2017; Bichet *et al.*, 2020; Chatelain *et al.*, 2020; Fitzpatrick *et al.*, 2021;
51 Sheldon *et al.*, 2021; Salmón & Burraco, 2022).

52 The importance of how early life conditions affect inter-individual telomere length
53 quickly appears as a key question to understand how somatic growth may shape individual
54 life trajectories in the context of pleiotropy (Metcalfe & Monaghan, 2003; Monaghan &
55 Ozanne, 2018). This is based on the observation that growth is a period of high energy
56 metabolism (2-6 times basal metabolic rate, e.g. Kirkwood, 1991) to fuel intense rate of cell
57 division, both physiological traits likely to be costly in terms of telomere erosion (Vedder *et*
58 *al.*, 2017; Spurgin *et al.*, 2018). Short telomeres in fledgling may then reflect accumulated

59 stress that impaired investment in cell maintenance of the growing organism, due to
60 deleterious effects of sub-optimal nutritional, social and/or hormonal environments (Herborn
61 *et al.*, 2014; Nettle *et al.*, 2015, 2017; Reichert *et al.*, 2015; Angelier *et al.*, 2017; Quque *et al.*,
62 2021). Interestingly, telomeres may also be affected during the pre-hatching developmental
63 period. For instance, temperature instability during egg development triggers shorter
64 telomere length at hatching in Japanese quail (*Coturnix Japonica*, Stier *et al.*, 2020), and
65 decreasing incubation temperature in the common tern (*Sterna hirundo*) slows down growth
66 rate and save telomere length in matched-body sized hatchlings (Vedder *et al.*, 2018). Yet,
67 telomere dynamics are not only affected by stress effects. Producing eggs is costly for the
68 female, and depending on maternal characteristics and environmental conditions, we can
69 expect an adjustment of egg characteristics that will shape consequent embryonic traits
70 (Williams, 1994; Groothuis & Schwabl, 2008). As such, a large diversity of egg components
71 (like yolk and hormones) may vary and modulate the future offspring phenotype in a
72 synergistic or antagonistic ways, leading to the concept of multivariate egg (Postma *et al.*,
73 2014; Williams & Groothuis, 2015). In addition, because an entire clutch is produced over
74 sequential laying of consecutive eggs, intra-clutch variability in multivariate egg traits may be
75 part of a mother strategy of adaptation of the chick's phenotype, and is then expected to
76 follow the laying order (Groothuis *et al.*, 2005). In particular, according to the brood reduction
77 hypothesis, it is expected that the probability of survival of last hatched nestlings (from last
78 laid eggs) will be smaller than that of first hatched ones in case of harsh conditions (Lack, 1947;
79 Amundsen & Slagsvold, 1996). Thus, we can expect maternal investment to decrease over the
80 laying sequence. Telomere length is not an exception, and progressive shortening has been
81 observed within clutch laying order in captive zebra finches (*Taeniopygia guttata*, Noguera *et*
82 *al.*, 2016) as well as inter-individual variation within the multivariate egg concept (Criscuolo *et*

83 *al.*, 2020). In the former study, the astonishing result is that the difference in embryonic
84 telomere lengths between the 1st and the last laid eggs represents 60% of the telomere loss
85 an offspring will show over its first year of life. Given that the negative consequences of fast
86 telomere erosion during growth on future individual fitness prospects are legions, *e.g.*
87 jackdaws (*Corvus monedula*, Boonekamp *et al.*, 2018), king penguins (*Aptenodytes*
88 *patagonicus*, Geiger *et al.*, 2012) or in wild purple-crowned fairy-wrens (*Malurus coronatus*
89 *coronatus*, Eastwood *et al.*, 2019), to name a few, variability in telomere length within clutch
90 is likely not an epiphenomenon. Still, we lack data on other bird species and on how laying
91 order effect on telomere length may vary in relation to additional stress sources, like
92 environmental conditions in the wild (but see Kärkkäinen *et al.*, 2021).

93 Our study is based on 4 years of data from a wild population of Little Owl (*Athene*
94 *noctua*) reproducing in artificial nestboxes. All nestlings are ringed and measured before
95 fledging. First, we tested whether individual characteristics (sex and body mass) are
96 dependent on hatching rank and on environmental characteristics around the nest. Second,
97 using telomere length measurements made on individual feather sampling, we tested how
98 nestling telomere length varied (i) with hatching rank, controlling for nestling sex, age, body
99 condition, clutch size and year of birth, and (ii) with the local characteristics of nest
100 environment. To estimate nest environment characteristics, we calculated the proportion of
101 orchards, meadows, crops, buildings, water and forests around each nest box from land use
102 maps. In central Europe, the Little Owl is a bird species associated with traditional farmlands
103 and its optimal habitat should provide cavities, perches for hunting and short herbage with
104 invertebrates and small rodents (herbage size is linked to prey accessibility and availability,
105 van Nieuwenhuyse *et al.*, 2008). In particular, meadows and orchards are supposed to be food-
106 rich habitats (Michel *et al.*, 2017).

107 We predicted last hatched nestlings to be in worse condition (body mass, telomere
108 length) than first hatched nestlings according to the brood size reduction hypothesis. We also
109 predicted shorter telomeres in broods raised in unfavourable environments, *i.e.* more
110 proportion of buildings, water and forests around the nest box.
111
112 **Material and Methods**
113 *Model species and data collection*
114 The Little Owl is a small nocturnal raptor living in open or semi-open areas, such as farmland
115 or orchards (van Nieuwenhuyse *et al.*, 2008). The Little Owl is territorial and breeds in cavity,
116 including artificial nestboxes. In Alsace (France), numerous ringers and volunteers from the
117 French league for the protection of birds (LPO) installed and maintained more than 1,500 nest
118 boxes since 2006, thereby monitoring the yearly reproductive success of the local population.
119 Females lay 2-6 eggs in April, hatching occurs *ca.* 1 month later and nestlings are ringed
120 between 15-35 days of age. At ringing, nestlings' body mass was measured with an electronic
121 balance to the nearest 0.1 g, as well as tarsus length with a calliper to the nearest 0.1 mm, and
122 the length of the third primary feather with a ruler to the nearest mm. The measure of the
123 feather allows us to approximate the age of the nestling with the formula: age=(length of the
124 feather+36)/3.3 (Juillard, 1984; Hameau *et al.*, 2015). Using the age of each nestling in a nest,
125 the hatching order was deduced. We also collected 3-6 ventral coverts that are stored in
126 ethanol 70% at ambient temperature during fieldwork and then at 4°C in the lab.
127 For this study, we used data collected on 142 nestlings from 39 broods from 2014 to 2017. In
128 order to estimate the effect of hatching rank we used only broods with more than 1 chick
129 (n=3, n=14, n=16, n=6 for broods with respectively 2, 3, 4 and 5 chicks).
130

131 *Land use around the nestbox*

132 To determine the land use around the nest boxes, we used a land cover database for Alsace
133 (Source: BdOCS CIGAL v2 2011/2012, www.geograndest.fr) which categorizes all the habitats
134 found in our study area. We used the software QGIS version 3.4.14 (QGIS Development Team,
135 2020) to map the active nest boxes and create a circular buffer zone of a 150 m radius around
136 each one of them. This radius was established thanks to data on home range size (Exo, 1992;
137 Génot, 2005) and the field observations made during the breeding season. Due to the high
138 number of habitats, we made groupings based on the environmental characteristics of each
139 variable to calculate the area (m^2) covered by each land type within the buffer zones. Our final
140 nest environment included six categories: (1) buildings, (2) meadows, (3) crops (crop fields,
141 hedges, and vineyard), (4) orchards, (5) forest and (6) water. Because of the rarity of the last
142 two categories, forest and water were pooled together.

143 *Relative telomere length (RTL) measurement and sexing*

144 Genomic DNA was extracted from feathers using an adapted protocol of the NucleoSpin Tissue
145 kit (Macherey Nagel, Düren, Germany). RTL was measured in the 142 nestlings in one 384-
146 wells plate, using the quantitative PCR (qPCR) methodology (see Electronic Supplementary
147 Material, ESM). Intra-plate repeatability of RTL (ICC, see (Eisenberg *et al.*, 2020)) was of 0.769.
148 Molecular sexing of nestlings was determined using the same extracted DNA (following
149 Griffiths *et al.*, 1998). Briefly, the technique is based on the existence of two conserved CHD
150 (chromo-helicase-DNA-binding) genes that are located on the sex chromosomes. The CHD-W
151 gene is located on the W chromosome (only in females) and the CHD-Z gene is located on the
152 Z chromosome (both in males and females). For technical reasons, sex could not be
153 determined in 5 nestlings. All the statistical analyses were performed on the remaining 137
154 nestlings with known sex.

155 *Statistical analyses*

156 We used R version 4.2.1 (R Core Team, 2022) to compute mixed models (package lme4 version
157 1.1-30 and lmerTest version 3.1-3). In all statistical models, brood identity was included as a
158 random factor to account for the non-independence of nestlings of the same brood. We
159 checked models' assumptions (homoscedasticity, normal distribution of residuals) graphically
160 using the package DHARMa (version 0.4.6). We assessed multicollinearity among predictors
161 by calculating variance inflation factor, VIF (package car, version 3.1-0).

162 Individual phenotypic characteristics

163 We tested whether sex is dependent on hatching rank. We computed a generalized mixed
164 model with binomial family and with sex as a dependent variable and hatching rank and
165 nestling number as fixed effects. The significance of the effects was tested with type III Wald
166 chisquare tests.

167 To test for inter-individual variation in body condition, we first calculated the Scale Mass Index
168 (SMI) following Peig & Green (2009). We then computed a linear mixed model with SMI as a
169 dependent variable and hatching rank, sex, the interaction between hatching rank and sex,
170 nestling number, nestling age and cohort as fixed effects. From this global model, we fitted
171 every possible model and then selected a set of top models (AICc threshold of 2). We then
172 averaged the models from these top models set.

173 Then, we computed a linear mixed model with SMI as a dependent variable and
174 environmental covariates (proportion of buildings, meadows, crops, orchards and of water
175 and forest around the nest box) as fixed effects. The environmental covariates were scaled
176 before the analysis. Model selection was similar as described above.

177

178

179 Inter-individual variation in Relative Telomere Length

180 RTL were log-transformed before analyses. First, we computed a linear mixed model with
181 individual covariates (hatching rank, sex, the interaction between hatching rank and sex,
182 nestling number, nestling age, SMI and cohort as fixed effects). Second, we computed a linear
183 mixed model with environmental covariates (as described above). For both models, the model
184 selection procedure was the same as described above.

185

186 **Results**

187 Individual phenotypic characteristics

188 The sex of the offspring was not significantly correlated with hatching order (chi^2=4.45,
189 P=0.35) or nestling number (chi^2=0.48, P=0.49).

190 Concerning individual covariates, there were no significant variables that explained variation
191 in SMI in our models. The fixed effects retained in the top models set were nestling age,
192 nestling number and sex (see Table S1) but their effects were not significantly different from
193 0 (see Figure S1).

194 Concerning environmental covariates, the proportion of buildings, crops, meadows and
195 orchards around the nest box were kept in the best models (Table S2). The increase of
196 buildings and of crops has a marginally negative effect on the SMI of little owls (Figure S2).

197 Inter-individual variation in Relative Telomere Length (RTL)

198 Concerning individual covariates, RTL was not dependent on nestling number and there was
199 no interaction between rank and sex, the variables in the top models set were rank, sex, SMI,
200 cohort and nestling age (Table S3, Figure 1). Males have shorter telomeres than females
201 (Figures 1 and 2) and there is a small positive effect of SMI on RTL (Figure 1). In addition, last
202 hatched nestlings have shorter telomeres but only in the largest brood of 5 nestlings (Figures

203 1 and 3). The effect of the year of birth is marginally significant and is negative, meaning that
204 RTL are decreasing in recent years (Figures 1 and 3).
205 Concerning environmental covariates, the proportion of buildings, crops, orchards and forest
206 and water around the nest box were kept in the best models (Table S4). There is a marginal
207 negative effect of the proportion of forest and water around the nest box on nestlings RTL
208 (Figure S3).
209

210 **Discussion**

211 Based on the current knowledge on growth and telomeres in bird nestlings, we initially
212 predicted that RTL of little owl nestlings will be: (i) negatively related to the hatching order
213 and (ii) negatively affected by the unfavourable nature of the nest surroundings. Our results
214 indicated that RTL are longer in females and, independently of sex, in nestlings with the
215 highest body condition. They also supported a mixed negative effect of hatching order and
216 intra-brood competition on little owl nestlings' RTL, i.e. detectable only in the largest brood
217 size, suggesting that the effect of hatching rank on telomeres is dependent on a threshold
218 effect in this species. We did not find a clear effect of the environmental covariates on
219 nestlings' RTL. Finally, our longitudinal scan of nestlings' RTL over years surprisingly underlined
220 a possible progressive shortening, independent of any changes in body condition.
221 Our indication of an erosion of little owl nestlings' RTL over years need to be replaced
222 in the emerging context of conservation physiology aiming at developing physiological
223 markers of individual quality to infer consequences at the population level (Beaulieu &
224 Costantini, 2014; Lea *et al.*, 2018). Telomere length at a given age is not reflecting only the
225 negative effects of time on the cells (i.e. chronological age), it also points out the cumulative
226 effects of stressors encountered over time that may accelerate the rate of loss of telomere

227 ends (Asghar *et al.*, 2015; Louzon *et al.*, 2019; Chatelain *et al.*, 2020; Salmón & Burraco, 2022).
228 Because the rate of cell division and/or the oxidative metabolism are higher in a growing
229 organism, the period of growth is supposed to be the life stage where telomere sequences are
230 the most impacted by environmental stressors (Salomons *et al.*, 2009; Young *et al.*, 2013;
231 Monaghan & Ozanne, 2018). Thus, depending on the harshness of early life environment,
232 erosion of telomeres can be accelerated for a given age (e.g. Boonekamp *et al.*, 2014; Stier *et*
233 *al.*, 2015), leading the nestlings to be grown, prematurely, physiologically old. This has,
234 theoretically, obvious consequences for the individuals in terms of survival prospects and
235 recruitments as adult breeders in the population, as early life telomere length or rate of
236 telomere loss have been shown to predict future individuals' survival (Boonekamp *et al.*, 2014;
237 Watson *et al.*, 2015; Wood & Young, 2019). Consequently, it also has the potential to affect
238 the population dynamics. First conceptualized few years ago (Stindl, 2004), such a hypothesis
239 was recently supported by studies conducted on ectotherms' populations (Dupoué *et al.*,
240 2017, 2022). In the common lizard populations studied, analysis of telomere length in
241 yearlings of populations showing different risks of collapsing due to local global warming
242 pointed out reduced mean telomere length in the most endangered populations (Dupoué *et*
243 *al.*, 2017). Thereafter, the same group showed that short telomeres were already inherited in
244 neonates of declining populations, thereby suggesting (epi)genetic roots, i.e. progressive
245 telomere shortening being not only the result of bad early life conditions (Dupoué *et al.*, 2022).
246 We cannot draw the same conclusions in our case, particularly because we lack data on inter-
247 generational variation of telomere length. It can be noted that in vertebrates, heritability
248 estimates are moderate (Chik *et al.*, 2022), but this recent meta-analysis has no data on
249 raptors (Chik *et al.*, 2022). In addition, as low rates of recruitments of juveniles as first-
250 breeders is an important determinant of population decline in the little owl (Le Gouar *et al.*,

251 2011), the link between reduced telomere length and survival prospects of nestlings needs to
252 be established. Finally, this result is counter-intuitive in our study population of little owl since
253 the population is expanding and not decreasing (Bersuder & Wassmer, 2020), contrary to
254 other populations (Andersen *et al.*, 2017). Thus, the effect of competition or density on
255 telomere length need to be addressed in future studies.

256 Little owl female nestlings had longer telomeres than male ones. This has several
257 implications for our understanding of sex-differences in telomere dynamics and of its meaning
258 in terms of sex-biased life history. Differences in telomere length in relation to gender has
259 been previously illustrated in several taxa (reviewed in Barrett & Richardson, 2011), and
260 particularly in birds with sex-biased body size or investment in reproduction, with no
261 consensual general pattern (e.g. Caprioli *et al.*, 2013; Remot *et al.*, 2020; Saulnier *et al.*, 2022
262 for no sex differences) (e.g. Bauch *et al.*, 2020 for sex differences). In our study, sex-differences
263 in RTL were observed at the nestling stage, with longer telomeres in the females. A previous
264 study showed that females were slightly but consistently of bigger size (Tschumi *et al.*, 2019),
265 however it is not the case in our population. Yet, we did not investigate nestlings growth rates,
266 which can be different event if the final size and/or body mass is similar (e.g. Criscuolo *et al.*,
267 2008). Higher growth rates are usually associated with shorter telomeres (Geiger *et al.*, 2012;
268 Monaghan & Ozanne, 2018). However, we also found that, independently of sex, nestlings in
269 better body condition had in general longer telomeres. Thus, it is unlikely that little owl
270 nestlings had to face such a growth-body maintenance trade-off. Given that body mass is a
271 determinant of survival from hatching to fledging in little owl (Tschumi *et al.*, 2019), nestling
272 telomeres rather acts as a proxy of individual quality (Angelier *et al.*, 2019). In addition, our
273 results do not match with the idea that the heterogametic sex (*i.e.* females) would be more
274 prone to telomere erosion than the homogametic one (*i.e.* males) due to the unguarded

275 expression of deleterious alleles of sex chromosomes for telomere maintenance (see Barrett
276 & Richardson, 2011; Remot *et al.*, 2020 for a deep discussion related to telomere dynamics).
277 One alternative explanation lies on optimal parental care towards the offspring sex with the
278 highest chance of survival (Hasselquist & Kempenaers, 2002). It has been shown previously
279 that females have a higher survival probability from hatching to fledging, independent of any
280 variation in body mass (Tschumi *et al.*, 2019). However, it is not known whether this sex-
281 difference persists in older individuals. In that context, the parents would favour female
282 individuals, meaning that within little owl broods females may, on average, beneficiate from
283 better access to food resources due to specific parental investment. This may lead to an
284 attenuated body maintenance (*i.e.* telomere length) and growth rate trade-off.
285 The hypothesis that RTL is an indicator of quality is further supported by the fact that,
286 in the largest clutches, the last hatchling of little owl presented the shortest telomeres. This is
287 also in accordance with the brood size reduction hypothesis that predict a lower investment
288 with laying order. Still, our data would restrict such an effect to the last laid egg. We cannot
289 distinguish between effects of the laying order *per se* on RTL (see introduction) and postnatal
290 effects. Postnatal effects may arise from selective parental care as discussed above. Last-
291 hatched nestling may also suffer from intra-brood competition. Indeed, in a brood, larger
292 nestlings have a competitive advantage compared to smaller nestlings for feeding
293 ("Competitive advantage hypothesis", Anderson *et al.*, 1993). A previous experiment testing
294 the effect of competitive disadvantage within a brood, based on the size of the nestlings cross-
295 fostered among clutches, highlighted an interesting increased telomere attrition of less
296 competitive nestlings without affecting body mass growth (in European starlings, Nettle *et al.*,
297 2015).

Finally, our study only suggested non-significant effects of nest surroundings, with shorter telomeres in nests with higher proportion of water and forest areas, and with worse body condition in nests with higher proportion of buildings and crops. In other studies, local habitat types around nests and also the heterogeneity of habitats available have been shown to affect reproductive output in our species (Thorup *et al.*, 2010; Michel *et al.*, 2017). Moreover, it has been shown that the home range size is dependent on the environment around the nest and also is different between males and females (Michel *et al.*, 2017). Thus, it may be important to consider the habitat at a fine scale. Future studies should explore how environmental quality, food resources, parental care, chick growth, intra-brood competition and sex-specific susceptibility to stressors are intertwined factors that determine offspring telomere length and how all these factors affect population dynamics of little owls.

Ethics statement. This work is in accordance with the French legislation concerning the capture and the biological sampling of wildlife. All the ringers of the project had received ringing licenses and authorizations for feather sampling from the CRBPO (National Museum of Natural History, Paris, France) as part of a program led by Bertrand Scaar (PP N°454).

Data accessibility. Datasets used in this study are openly available on zenodo (doi: 10.5281/zenodo.7701531).

Authors' contributions. JB and FC conceived the study. BS and volunteers collected the data. SZ developed and performed the sexing and qPCR measurements. IF sorted the samples and calculated the land use around nest boxes. JB and FC ran the statistical analyses and, with SZ for the ESM, wrote the first draft of the manuscript. All authors provided comments on the manuscript and agreed on the final version of the manuscript to be submitted for publication.

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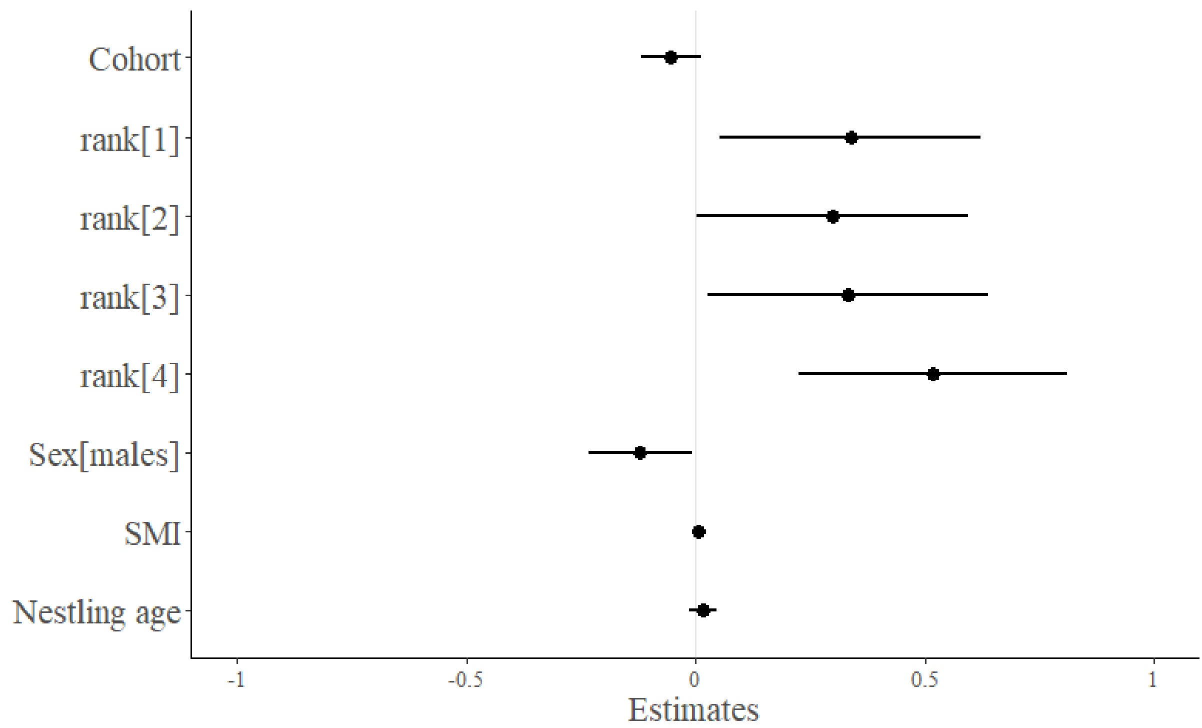
553 **Figure 1. Forest-plot of estimates for the average model of relative telomere length and**

554 **individual covariates (see Table S3).** Reference level for sex is females and for rank is 5 (last

555 hatched chicks).



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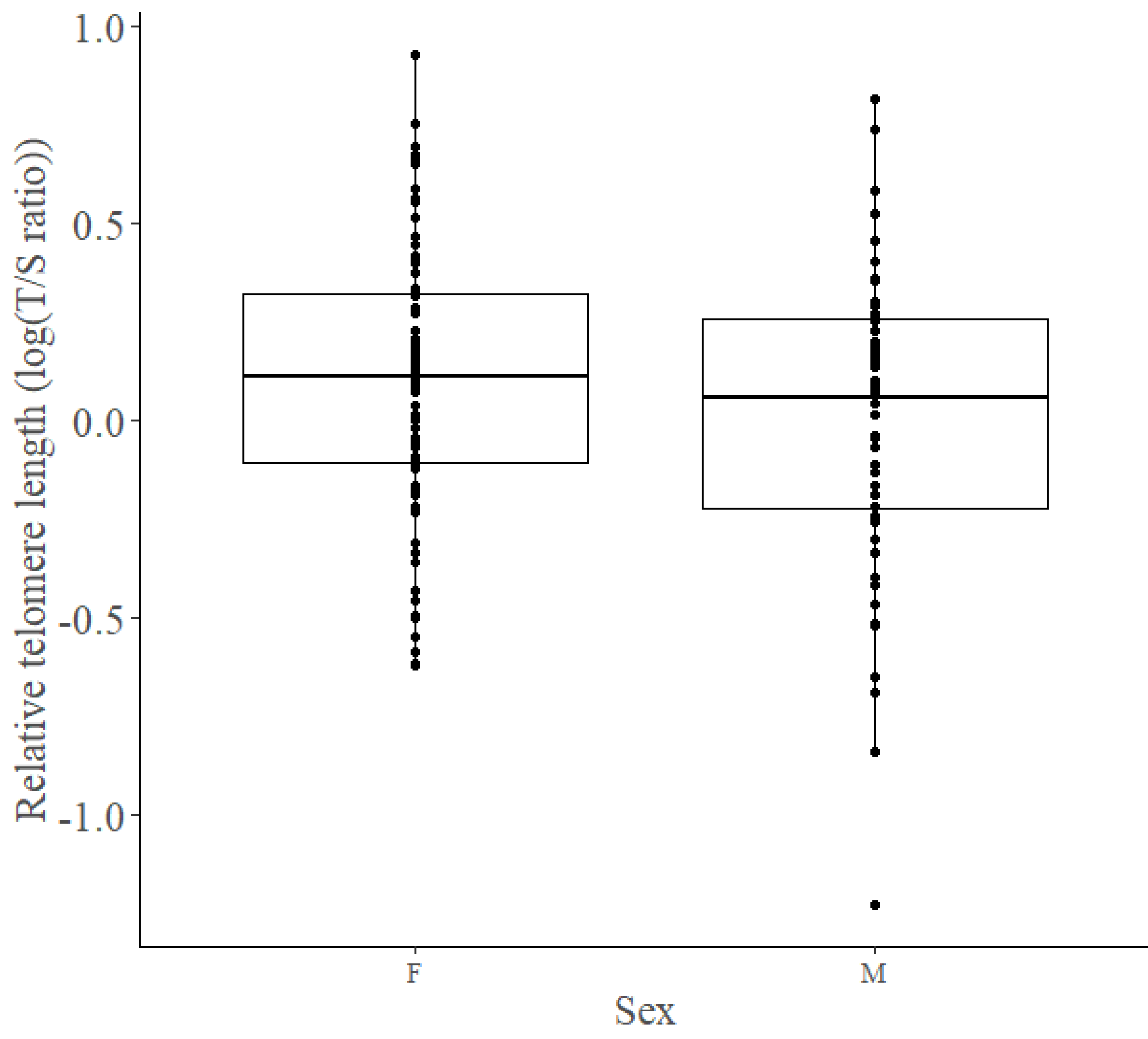
559 **Figure 2. The effect of sex on the relative telomere length before fledging.**



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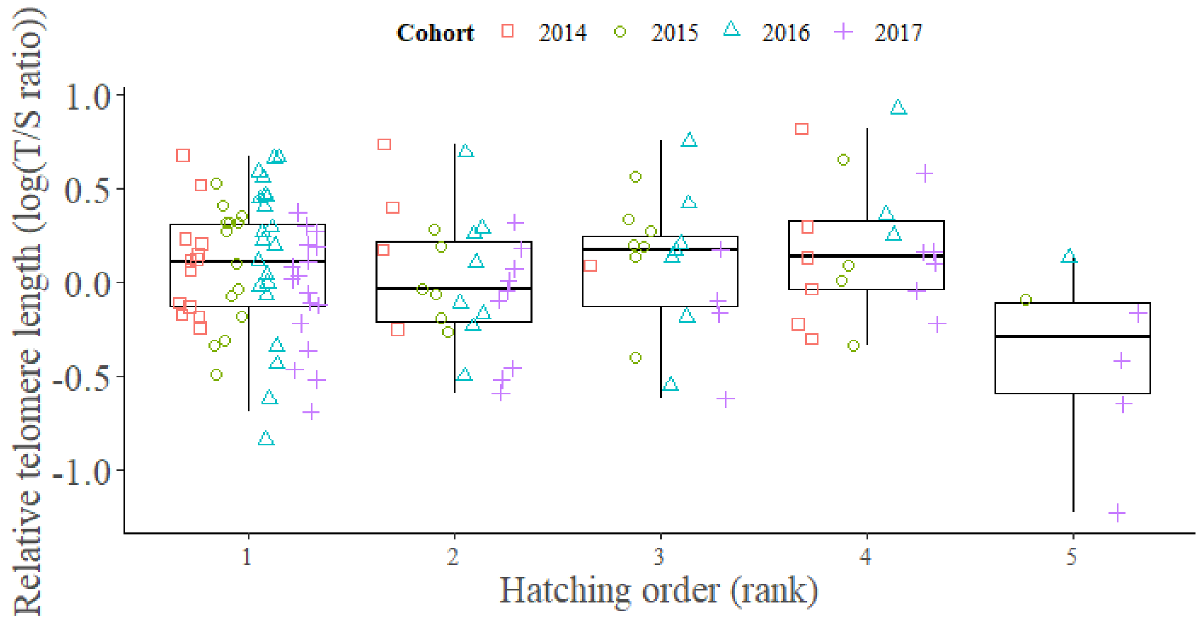
562 **Figure 3. The effect of hatching order and year of birth on the relative telomere length**

563 **before fledging.**



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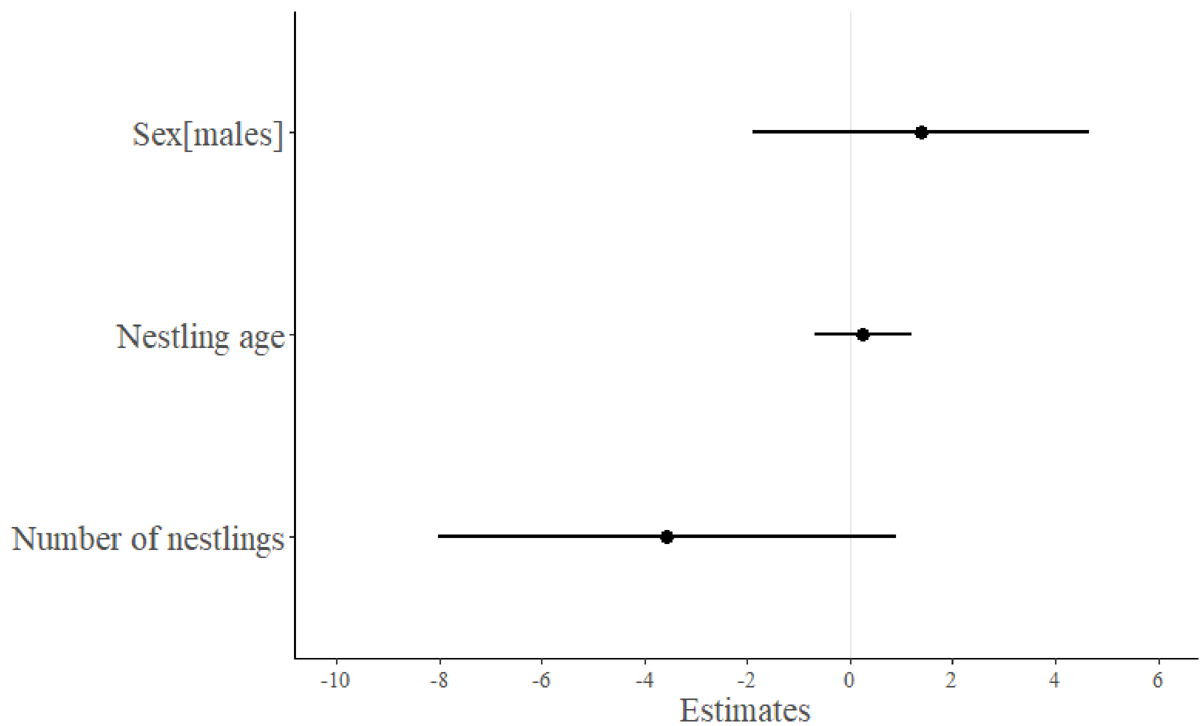
567 **Amplification of telomere repeats using q-PCR methodology**

568 The protocol for DNA extraction from feathers provided us with sufficient amount of DNA to
569 run both sexing and telomere determinations. One to three feathers per individual were
570 selected and a 0.5-1 cm piece from each feather were cut in small pieces with a sterilized
571 scissor. After digestion, feather quills will remain unlysed. For samples containing unlysed
572 quills, we centrifuge briefly and we transfer the supernatant to another tube before proceeding
573 with step 4 of the standard protocol.
574 Individual relative telomere length (RTL) were obtained following the qPCR methodology
575 previously used in several bird species by our group (*e.g.* Criscuolo et al. 2009, Bize et al. 2009,
576 Criscuolo et al. 2020, Chatelain et al. 2021). DNA quantity and quality were assessed based on
577 spectrophotometer absorbance (Nano-Drop 1000, Thermo Fisher Scientific, Waltham, MA,
578 USA, ratios A260/280 and A260/230) and gel migration. Individual DNA were all diluted to a
579 concentration of 5.0 ng/µL, and further used for RTL determination by qPCR. To control for
580 variation in DNA concentrations among diluted samples (due to potential pipetting errors),
581 which may induce a methodological bias to the final RTL values, we amplified, for each
582 individual, a genomic DNA sequence, defined so far as non-variable in copy numbers. The gene
583 used in our species was RAG-1 gene (recombination activating protein 1 gene, NCBI number
584 EU348872.1). Amplifications were conducted in two 384 wells-plates filled by a calibrated
585 automated liquid handling workstation (Epmotion, Eppendorf, Montesson, France), using one
586 distinct plate for control gene and telomere amplifications, due to the different qPCR conditions
587 due to primers sequences properties. Conditions of amplification were 2 min at 95°C followed
588 by 40 cycles of 15 s at 95°C, 30 s at 56°C and 1 min at 72°C (control gene) and of 2 min at
589 95°C followed by 30 cycles of 15 s at 95°C, 30 s at 56°C and 30 sec at 72°C, (telomere
590 sequence). Reactions were done in a master mix prepared for each primer set, with 5 µL GoTaq
591 QPCR Mix (Promega, Madison, WI, USA). We used 10 ng of DNA (in a volume of 2 µL), to
592 which we added the telomere primers at a concentration of 200 nM or the control gene primers
593 at 400 nM (for a final reaction volume of 10 µL in each well, completed with ultra-pure water).
594 In both plates (control gene and telomere sequences) we amplified individuals' DNA samples
595 plus three quality control references. A DNA golden sample (as a mix of22 individual samples
596 randomly chosen) that was used as the reference value of 1 for RTL calculations. A dilution
597 curve obtained from the amplification of a randomly chosen reference sample that was serially
598 diluted (from 10 to 0.625 ng/mL). Dilution curves enable us to assess quality of control gene
599 and telomere sequences qPCR amplifications (i.e. efficiency values (control gene 0.999;
600 telomere sequences 0.993) and r^2 (0.993 and 0.995, respectively) of the dilution curves). A
601 negative control sample (ultra-pure water) to control for putative contaminations of non-bird
602 DNA. All runs ended by a fusion curve to verify the absence of non-specific amplifications.
603 RTL values were calculated following Pfaffl (2001), shortly as the ratio between Telomere (T)
604 and Control gene (S) Cq values, controlled for their respective amplification efficiencies and
605 expressed relatively to the golden sample T/S value of 1. All samples were run in duplicates
606 and intra-individual repeatability of RTL, evaluated using the Intra Class Coefficient (Eisenberg
607 *et al.*, 2020), was of 0.769.
608

609 **Table S1. Top models set for models of SMI and individual covariates.** For continuous
610 variables, each value represents the estimate of the effect; for categorical variables, there is a
611 "+" when the variable is retained in a model.
612 df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.

Intercept	Nestling age	Nestling number	Sex	df	AICc	delta
145.30		-3.52		4	1058.0	0.00
132.30				3	1058.3	0.24
145.20		-3.66	+	5	1059.4	1.36
131.80			+	4	1059.8	1.75
139.70	0.25	-3.53		5	1059.9	1.88

613
614 **Figure S1. Forest-plot of estimates for the average model from Table S1.** Reference level
615 for sex is females.



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619 **Table S2. Top models set for models of SMI and environmental covariates.** For
620 continuous variables, each value represents the estimate of the effect.
621 df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.

Intercept	Buildings	Crops	Meadows	Orchards	df	AICc	delta
132.1	-4.98	-4.57			5	1056.8	0
132.2	-5.55	-5.96		-2.21	6	1057.7	0.9
132.0	-8.57	-8.71	-3.57	-4.06	7	1058.2	1.44
132.3					3	1058.3	1.49
132.4	-2.52				4	1058.7	1.91

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624 **Figure S2. Forest-plot of estimates for the average model from Table S2.**
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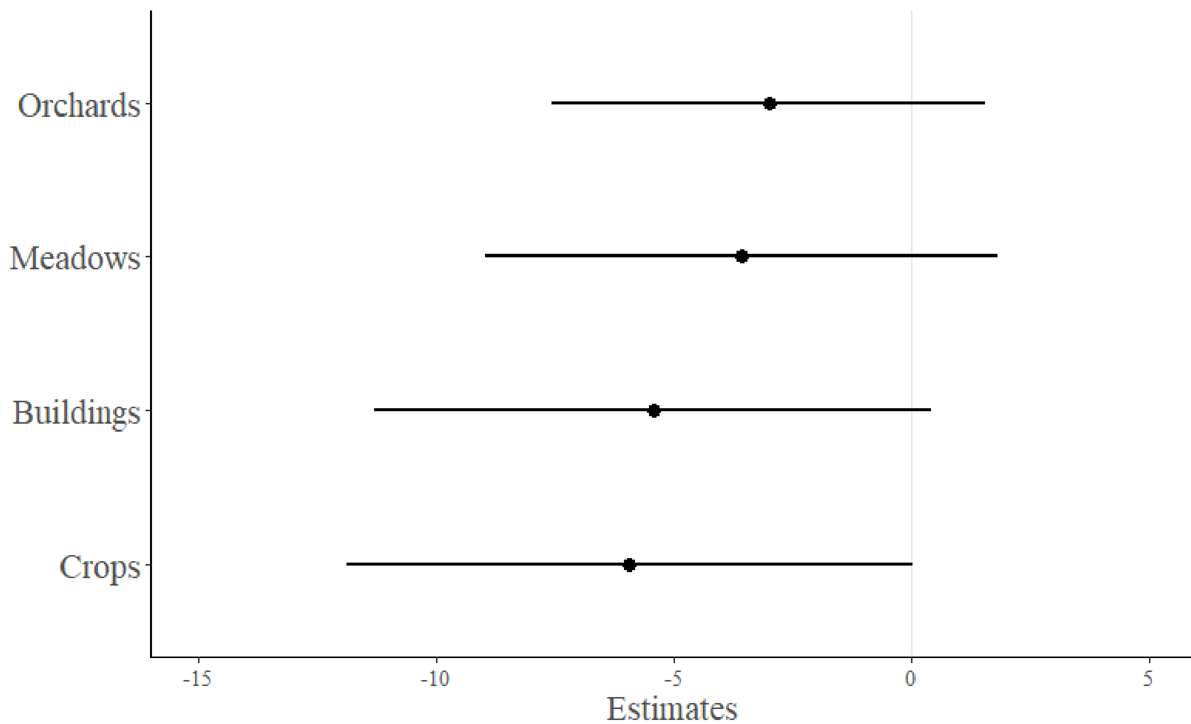

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629 **Table S3. Top models set for models of RTL and individual covariates models.** For
630 continuous variables, each value represents the estimate of the effect; for categorical
631 variables, there is a "+" when the variable is retained in a model.
632 df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.
633

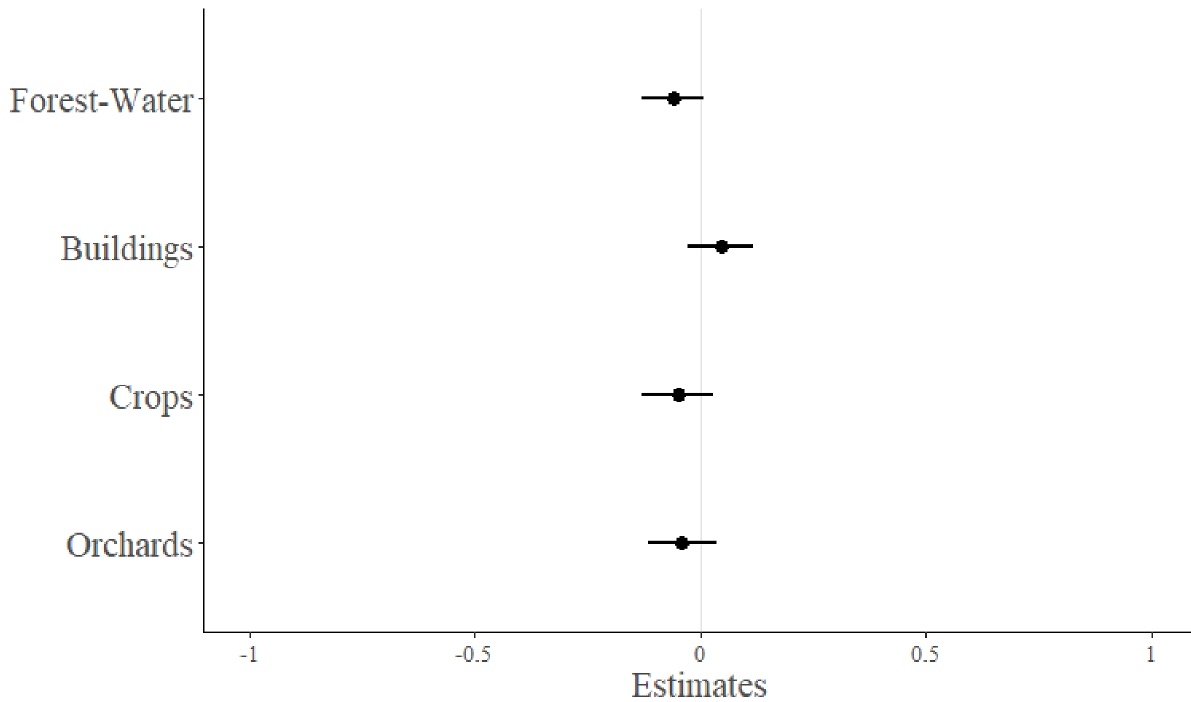
Intercept	Cohort	Nestling age	Rank	Sex	SMI	df	AICc	delta
-0.70	-0.053		+	+	0.0046	10	104.4	0.00
-0.86			+	+	0.0046	9	104.6	0.25
-0.99	-0.052	0.016	+	+	0.0045	11	105.7	1.35
-1.15		0.016	+	+	0.0045	10	105.9	1.57
-0.11	-0.053		+	+		9	106.1	1.72
-0.27			+	+		8	106.2	1.81
-0.74	-0.054		+		0.0043	9	106.3	1.97

634
635

636 **Table S4. Top models set for models of RTL and environmental covariates models.** For
637 continuous variables, each value represents the estimate of the effect.
638 df = degree of freedom. delta = difference of AICc with the model with the lowest AICc.
639

Intercept	Buildings	WaterForests	Crops	Orchards	df	AICc	delta
0.057		-0.058			4	111.8	0.00
0.057					3	112.2	0.42
0.057	0.045	-0.059			5	112.4	0.64
0.057		-0.067	-0.063	-0.059	6	112.9	1.15
0.056	0.044				4	113.0	1.24
0.056		-0.061	-0.036		5	113.1	1.26
0.059		-0.060		-0.033	5	113.1	1.31
0.058	0.048	-0.061		-0.036	6	113.6	1.78
0.058				-0.030	4	113.7	1.90

640
641 **Figure S3. Forest-plot of estimates for the average model from Table S4.**



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