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Key words: growth, body mass, physiological traits, telomere, bird

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Author Contributions: T.D.W. and A. C. collected the data and A.C. run all the physiological analyses, V.F. and S.Z. extracted the DNA and ran the qPCR measurements of telomere length, V.F., S.Z. and F.C. analysed the qPCR data, F.C. and T.D.W. did the statistical analyses, and T.D.W. and F.C. drafted the final manuscript on which A.C. made comments.

ABSTRACT

Telomere length may be considered as an indicator of an organism's somatic state, long telomeres reflecting effective self-maintenance. Early-life is a period of intense investment in somatic growth and in physiological maturation but how this is traded-off with telomere length remains unclear. Using European starling chicks we tested: (i) how telomere length is predicted by somatic and physiological maturity at pre-fledging (17 days-old); (ii) how telomere length at 17 days then predicts the changes in somatic and physiological maturity occurring just prior to fledging (17-21 days); (iii) how growth and telomere length covary when chicks are under experimentally good (fed) or bad (stressed mothers) growth conditions. Depending on environmental conditions or sexes, we found the predicted relationships between somatic growth and body maintenance parameters (positive with oxidative stress and negative with telomere length), but also that higher levels of physiological maturation were associated with shorter telomeres in pre-fledging chicks. Telomere length at day 17 predicted subsequent change in one variable of physiological maturation observed during the fledging stage, but only in second-brood chicks: chicks with shorter telomeres had a higher pre-fledging rate of increase in reticulocytes numbers. Finally, fed chicks grew faster in size at no physiological cost, while chicks raised by stressed mothers invested more in somatic growth/maturity but at a cost of higher oxidative damage and shorter telomeres at pre-fledging. Our results confirm that physiological maturation prior to fledging is a hardwired process, which may occur at the expense of telomere length when environmental conditions are sub-optimal.

INTRODUCTION

Early life development is a critical period for new-born organisms because it is a time during which the future functioning of the organism is set-up in a way to sustain maximized fitness at adulthood (Monaghan 2008; West-Eberhard 2003). Regulation of somatic growth is believed to be shaped by life-history trade-offs through the optimized allocation of available resources to growth and self-maintenance (Stearns 1992). Thus, growth rate is subjected to among and within species plasticity in relation to context-specific environmental conditions (Dantzer et al. 2013; Dmitriew 2011) and consequences for the future organism's fitness are substantial: *e.g.* faster growth trades off with individual lifespan (Dmitriew 2011; Metcalfe and Monaghan 2003), even when controlling for any confounding effects of resource availability during growth (Lee et al. 2013). In this context, evaluating the output of growth trade-offs at the levels of the soma has been an important objective for evolutionary biologists (Monaghan and Ozanne 2018). Rapid growth in mammals and birds has been associated with pleiotropic effects of signalling or hormonal pathways involved in both growth and ageing (Flatt and Heyland 2011), or to an imbalance in the oxidative status (Monaghan et al. 2009; Speakman et al. 2015). Growth could trigger either a rise in oxidative damage or reactive oxygen species production (Christensen et al. 2016; Geiger et al. 2012; Rollo et al. 1996), or a decrease in antioxidant capacities (Alonso-Alvarez et al. 2007; Blount et al. 2003).

Another marker of ageing that is part of the mechanisms underlying the cost of growth is telomere erosion; telomere length maintenance is impaired in fast growing individuals (Geiger et al. 2012) or when growth conditions are sub-optimal, energetically or socially (Nettle et al. 2017; Nettle et al. 2015; Reichert et al. 2015). Telomeres are repeats of T_2AG_3 sequences (in vertebrates) protecting the linear ends of telomeres, and may be considered as a proxy of cell maintenance that is experienced over the long-term. In fact, telomere length and rate of telomere erosion have been linked to fitness-related traits such as reproductive success or lifespan in multiple vertebrate species (Bize et al. 2009; Fairlie et al.

2016; Heidinger et al. 2012; Olsson et al. 2011; Rollings et al. 2017; Seeker et al. 2018). Since telomeres shorten at each cell division or due to oxidative stress (Blackburn 1991; Reichert and Stier 2017), this predicts a causal relationship between the rate of growth and the rate of telomere erosion during development, and thus with life-history trade-offs at adulthood (Monaghan and Ozanne 2018). In fact, telomeres are lost more rapidly during early-life than later-on (Daniali et al. 2013; Frenck et al. 1998; Hall et al. 2004). However, the growth-telomere link remains debated (Monaghan and Ozanne 2018) and needs further study notably when growth takes place under variable environmental conditions (Vedder et al. 2017).

Growth is not restricted to the gain of body mass or body size but also involves a gain of functionality, *via* maturation processes. Functional, or physiological, maturation involves more than simple somatic changes and interpreting growth-telomere relationships based on mass alone may lead to false conclusions (Durant et al. 2008). For example, maturation of tissues includes the development of physiological traits encompassing aerobic capacity, muscular performances or metabolic pathways that are key components of adaptations to adult life (Ricklefs and Starck 1998). Physiological maturation at fledging thus represents a key life-history transition as chicks shift from a mostly non-active nestling to an active adult life. If this is right, in most altricial species (with a non-active nestling period of development), maturation processes should invariably take place before fledging independent of the prevailing environmental conditions. Such a hardwired pattern of physiological maturation has recently been described in European starlings, *Sturnus vulgaris* (Cornell and Williams 2017). This study showed that poor environmental conditions do not affect the ultimate fledging physiological maturation processes but rather induce a cost of maintaining the programmed maturation trajectory, *i.e.* increased oxidative stress (Cornell and Williams 2017). Interestingly, such a canalization phenomenon (*i.e.* resilience of traits to environmental variations due to robust relationships with fitness (Waddington 1942)) has been recently proposed for telomere length in another bird species, the common tern (*Sterna*

hirundo) (Vedder et al. 2017). In those chicks, between-individual variance in telomere loss during growth has been found to be low, and not correlated to variance in somatic growth (body mass). This suggests that, at least when looking at somatic growth, telomere length is a cellular trait that needs to be preserved perhaps due to its potential importance in defining the adult organism's fitness (Heidinger et al. 2012; Le Vaillant et al. 2015; Salomons et al. 2009). However, there is to date no study looking at how telomere length and physiological maturation process are interrelated over the entire growth period (*i.e.* at the pre-fledging and fledging stages).

In the present study, we aimed to fill this gap by recording telomere length, somatic growth and physiological maturation at the pre-fledging (day 17) and/or fledging (day 21) stages. In European starling chicks, we assessed somatic growth through body condition measurement (mass / size residuals) and physiological maturity as oxygen transport traits of the chicks (hemoglobin blood concentration, hematocrit and reticulocyte blood count). We then measured the dynamics of change of somatic growth and physiological maturity during the last days before the chicks leave the nest, and tested if pre-fledging telomere length predicts growth changes just prior to fledging. If telomere length reflects the cost of growth, we expected to see negative relationships with pre-fledging somatic growth and physiological maturation. Similarly, as a proxy of past-investment, telomere length at the pre-fledging stage should negatively influence the ultimate growth patterns before fledging. In contrast, following the hypothesis that fledging maturation and telomere length will show low variance among individuals, both should stay unaffected by sub-optimal environmental conditions. This canalization should then be achieved at a higher cost for the chicks, here evaluated by the measurement of chicks' oxidative status. We tested this hypothesis further by comparing somatic and physiological maturation, telomere length and oxidative status in chicks provided with supplemental food or raised by mothers slightly handicapped when fitted with a radio transmitter, between day 4-17 post-hatching.

MATERIAL AND METHODS

Species and area of study

Field work was conducted on a natural population of European starlings at Davistead Farm, Langley, British Columbia, Canada (49°08' N, 122°37' W), which includes ca 150 nest boxes used by c.75 breeding pairs each year (see Cornell and Williams 2017). In this study we measured telomere length for a sub-sample of chicks used in studies previously reported in Cornell & Williams (2017) and Cornell et al. (2017). Specifically, we analysed telomere length in relation to growth, somatic and physiological maturity in: a) control chicks from 1st and 2nd broods in two years of differing productivity (see below); and b) chicks from a supplemental feeding experiment (Cornell and Williams 2017) and a maternal stress manipulation (attachment of radio-transmitters (Serota and Williams 2019)) both conducted in 2015. Breeding productivity of control pairs (brood size at fledging, BSF) was 2.5 chicks in 2013 and 2.9 chicks in 2015 including birds fledging zero chicks; both values were lower than the long-term average for our population (3.1 chicks). Breeding productivity of successful birds (BSF ≥ 1 chick) was 3.5 chicks in 2013 and 4.5 chicks in 2015 (long-term average, 4.1 chicks). So, based on these data we categorised 2013 as a "poor" year and 2015 as a "good" year.

Measurement of somatic and physiological maturity and cost of growth

We measured somatic maturation using body mass and wing lengths, physiological maturation using hematocrit, hemoglobin and reticulocytes (measures of aerobic capacity), and potential costs of growth as oxidative damage (d-ROMs) relative to antioxidant capacity (OXY).

Chicks were sampled at 17 days and 21 days post-hatching, body mass (± 0.01 g) and wing length (± 0.01 mm) recorded, and blood samples (> 200 µL) were taken from the brachial vein using a 26 ½-gauge needle. All blood samples were obtained within 3 min of chicks being handled. Fresh blood was used for

97 hematocrit and hemoglobin measurements and two blood smears were prepared for reticulocytes
98 counting (following Cornell and Williams 2017). Remaining blood was transferred to heparinized tubes
99 and kept at 4°C until centrifugation in the laboratory (3000 g for 10 min). Separated plasma and red
100 blood cells were immediately frozen (- 20°C) until further assaying.
101 Hematocrit was measured as packed cell volume (PCV) divided by total volume with digital calipers (±
102 0.01 mm) following centrifugation of whole blood for 3 min at 13,000 g (Microspin 24; Vulcon
103 Technologies, Grandview, MO, USA).
104 Hemoglobin concentration ($g.dl^{-1}$ whole blood) was measured using the cyanomethemoglobin method
105 (Drabkin and Austin, 1932) modified for use with a microplate spectrophotometer using 5 µl whole blood
106 diluted in 1.25 ml Drabkin's reagent (D5941; SigmaAldrich Canada, Oakville, Ontario, Canada) with
107 absorbance measured at 540 nm. Intra-assay CV% was 0.7%, based on duplicate measurements and
108 inter-assay CV% was 1.6%. Reticulocytes (% immature red blood cells) were calculated as number of
109 immature red blood cells/total red blood cells counted from whole blood smears after supravital staining
110 with new Methylene Blue (R4132, Sigma Aldrich Canada, Canada). A total of 1000 red blood cells were
111 counted per slide, and reticulocytes were identified following Fowler and Williams (2017).
112 We assessed chicks' oxidative status based on plasma levels of oxidative damage (d-ROMs) and of
113 antioxidant capacity (OXY) tests (following Tissier et al. 2014; Cornell and Williams 2017). All samples
114 were measured in duplicate to calculate coefficient of variation (OXY: 5.1%; d-ROMs: 6.4%) as a measure
115 of intra-assay variation. To determine inter-assay variation we used a single pooled sample each year
116 across all plates to calculate average inter-assay coefficient of variation (OXY: 5.9%; d-ROMs: 11.6%).
117
118 **Experimental manipulation: supplemental feeding and maternal stress**
119 In 2015, we measured telomere length in a sub-sample of chicks from a supplemental feeding
120 experiment described in Cornell and Williams (2017). Briefly, two chicks per nest were provided with

supplemental food between day 4-17 post-hatching; two chicks in each nest were handled as controls but did not receive food. Chicks were weighed at the end of each day (after supplementation) to monitor body mass and fed chicks received supplemental food twice a day with the daily total amount of food per day (summing both meals) equivalent to 10% of predicted daily mass gain in European starling chicks as reported in Westerterp et al. (1982) (see Cornell and Williams for further details). In addition, we measured telomeres in a sample of non-manipulated chicks where mothers were fitted with radio-transmitters during mid-incubation (as part of a different study,(Serota and Williams 2019)). Females were fitted with a digitally-coded Nanotag radio transmitter (Lotek Wireless Inc.) using a leg loop harness (Rappole and Tipton, 1991). The weight of the transmitters (≤ 2.1g) or about 2.5% of mean body mass of females at incubation (82.9 g).

Telomere length assay

Telomere length was measured in chicks at 17-days post-hatching on DNA extracted from frozen red blood cells using a real-time quantitative PCR technique (qPCR) initially developed by Cawthon and collaborators (Cawthon 2002) (see also (Criscuolo et al. 2009) for a full description of the principle and general methodology applied to two bird species). Specific amplification of a reference - non-variable in copy number among individuals – gene (see (Smith et al. 2011) in European starlings was obtained using recombination activating 1 (RAG 1) sequences (accession number XM_014873522). This gene is used to control for small DNA quantity variation among samples (thereafter referred as the S value) used during the qPCR amplification.

Chicks' telomere length were measured on 12 separate 96-wells plates (6 for RAG1 amplification and 6 for telomere sequences amplification due to different amplification conditions, see below). Total reaction volume in each well was of 10 µL, *i.e.* 5 µL of SYBR green mix, 10 ng of DNA and 500 nmol/L or 200 nmol/L of primers of telomere and RAG1, respectively. Reverse and forward primers' sequences for

both telomeric and RAG1 genes were, respectively: Tel1b: 5' – CGGTTTGTTT GGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'; Tel2b: 5' –GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACC CT-3'; RAG1-F: 5' – TGCAAAGAGATTTCGATATGATG-3'; RAG1-R: 5' – TCACTGACATCTCCCATTCC-3'. Amplification of telomere sequences were done using the following temperatures: 2 min at 95°C, followed by 30 cycles of: 15 seconds at 95°C, 30 seconds at 58°C and 30 seconds at 72°C. RAG1 amplification thermal profile was 2 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 30 sec at 56°C and 1 min at 72°C. All qPCR runs encompasses a dilution curve (40 ng to 1.25 ng) to calculate each plates' qPCR efficiencies, two negative control wells (to check for non-specific amplification due to water contamination) and were ended by a melting curve (to check for single peak amplification and primer-dimer artefact). qPCR amplifying efficiencies varied from 94 to 109 % for RAG1 and from 99 to 104 % for telomeres (an efficiency of 100 % corresponded to a doubling of DNA at each cycle). Samples were randomly assigned over the plates and assayed in duplicates and each plate contained 4 different samples chosen randomly and repeated over all runs were used to calculate repeatability in amplification values. Intra-plate and inter-plate coefficients of variation (based on Cq values) were 0.34 ± 0.03 % and 0.64 ± 0.07 % for the RAG1 gene assay and 1.92 ± 0.22 % and 2.10 ± 0.71 % for the telomere assay, respectively. Telomere length was finally calculated as a T/S ratio as stated in (Pfaffl 2001). qPCR telomere length, as a ratio (T/S), is a relative and not absolute evaluation of a mean telomere length of chromosomes and red-blood cells populations. Intra-plate and inter-plate coefficients of variation (based on T/S ratio) were 10.64 ± 0.84 % and 11.77 ± 4.59 %, respectively.

Statistical analysis

The dataset was split in two for the statistical analysis. First, only control chicks from 2013 and 2015 were considered (n=70, but one chick of unknown gender was excluded) to test how natural growth pattern are related to telomere length, 28 from 1st brood and 11 from 2nd brood in 2013, 15 from 1st

169 brood and 15 from 2nd brood in 2015, (see below). In a second step, only chicks of 2015 were selected
170 (n=84, 43 from 1st brood and 41 from 2nd brood), to assess how our experimental design affected growth
171 patterns and telomere length. We checked for brood effects since, in our population: (i) the incidence of
172 second clutches is relatively high (~40% of the females) but (ii) chicks from 2nd clutches are generally of
173 lower quality. While being rather independent of timing of breeding and of female individual quality,
174 second broods suffer nevertheless of lower rates of success (Cornell and Williams 2016) and the
175 physiological mechanisms underlying the lower survival of 2nd brood chicks remains to be better
176 understood. Differences among experimental groups were assessed using Bonferroni posthoc tests.
177 Statistics were done on SPSS 18.0. Normality of the data was double-checked with Shapiro-Wilk's test on
178 residuals of the models, and using QQ plots distribution. Only telomere length data were beforehand
179 log10 transformed (T/S ratio) to reach normality. $P < 0.05$ was considered as significant, and models
180 presented in the tables were selected on the basis of the lowest Akaike information criterion (AIC) value
181 (with a step threshold of 2).
182 *Natural growth pattern and telomeres*
183 Analyses of natural variation in growth was done based on a sub-sample (i.e. individuals for which
184 telomere lengths were measured) of a data file previously analysed (Cornell et al. 2016). Reanalysis of
185 our sample of telomere chicks produced the same key results: overall being born as 1st brood chicks and
186 in 2015 seemed to favour growth and maturation processes.
187 We first sought to explain chicks' variation in telomere length at the age of 17 days, using a mixed model
188 approach. We thereby developed several models with telomere length as the response variable, and
189 either chick body condition (mass / wing length residuals), oxidative status [residuals of plasma oxidative
190 damage (d-ROMs) and antioxidant capacity (OXY) levels], and physiological maturation as fixed
191 explanatory variables. Regarding physiological maturation, we used a variable representing the individual
192 values obtained from a principal component analysis merging the hematocrit, hemoglobin content and

193 reticulocytes count of each individual (see Electronic Supplementary Material, ESM). Using the 3
194 variables independently did not change the output of the analysis (data not shown). For the analysis at
195 day 17, we further detailed the results using independently the three physiological variables. In each
196 mixed model, the year (2013-2015), the brood (1^{st} or 2^{nd}), the brood size at day 17 and the interactions
197 with body condition, oxidative status and PCA values were included as covariates to explore the context-
198 dependency of the respective relationship evaluated. Finally, the nest identity was included as a random
199 factor in each of our model to control for the non-independency of the chicks (*i.e.* nestlings within the
200 same brood or from 1^{st} and 2^{nd} broods that have been raised by the same parents). In addition, given the
201 existing links between oxidative damage and telomere dynamics, we tested the significance of an
202 oxidative cost of growth using another mixed model, with the chick's oxidative status as a response
203 variable and body condition at day 17 as explanatory factor.
204 The second objective of our statistical analysis was to test whether telomere length at 17 days indeed
205 predicts the pre-fledging changes in somatic / physiological maturation, *i.e.* changes recorded between
206 day 17 and day 21 (fledging day). To do so, we used different mixed models with the following response
207 variables: (i) body condition at day 21, (ii) body mass loss between day 17 and day 21, (iii) wing length
208 gain between day 17 and day 21, (iv) change in hematocrit between day 17 and day 21, (v) change in
209 hemoglobin content between day 17 and day 21 and (vi) change in reticulocytes count between day 17
210 and day 21. For each model, year, brood, brood size and chick gender were included as explanatory
211 variables in addition to telomere length, and nest identity was added as random factor.
212 *Experimental manipulation of growth and telomeres*
213 The third objective was to test how an experimental manipulation of food availability and maternal
214 stress influences growth and maturation patterns, and what is the outcome for chick's oxidative status
215 and telomere length. To do so, we used the same mixed model approach with explanatory factors as
216 above (except the Year effect since only 2015 chicks were considered), and with experimental treatment

as an additional factor (Food, n=29; Maternal Stress, n=20; Control, n=30). In all models nest identity was considered as random factor.

220

221 **RESULTS**

222 We first repeated the analysis presented in Cornell et al. (2017) to confirm patterns of growth and
223 maturation for the subset of chicks for which we obtained telomere data. Mixed model analysis (using
224 year, brood and sex as fixed factors, and nest ID as random factor) of body mass, wing length,
225 hemoglobin concentration, and hematocrit yielded results that were mostly consistent with the
226 description of changes of somatic and physiological traits previously presented for starling chicks (Cornell
227 et al. 2016; Cornell and Williams 2017). The description of the dynamics of reticulocytes' count is given in
228 the Electronic Supplementary Material. In short, reticulocytes' counts were higher in 2015 than in 2013
229 and in 1st brood chicks than in 2nd brood chicks (only in 2013). However, in 2015, 2nd brood chicks had
230 higher reticulocytes' count at day 17 and 21. Overall, chicks reared in 1st broods and in 2015 had faster
231 growth and physiological maturation processes, but 2nd brood chicks in 2015 had an overall higher
232 reticulocyte count.

233

234 **1. Natural growth patterns and telomeres**

235 *Somatic maturity and telomere length in pre-fledging chicks*

236 Body condition was calculated as the residuals of the regression between body mass and wing length at
237 day 17 ($r = 0.548$, $D_{1, 69} = 29.258$, $P < 0.001$, body mass = 0.547 wing length + 26.651). Results of the
238 mixed models are presented in Table 1A. Chick body condition was significantly related to telomere
239 length at day 17, but in relation to brood and sex (Figure 1A and 1B): in first brood chicks or in males,
240 individuals that reached a better body condition (*i.e.* heavier for their body size) had shorter telomeres.
241 Telomere length of chicks did not vary with brood size or year, despite a tendency for telomeres to be
242 longer in 2013 ($P = 0.081$).

243

244 *Oxidative cost of growth and link with telomere length in pre-fledging chicks*

245 d-ROMs and OXY levels were significantly correlated (r = 0.421, $D_{1, 63}$ = 13.344, P=0.001) and residuals
246 were then used as a proxy of oxidative status: individuals with positive residuals had higher levels of
247 oxidative damage when controlling for their plasma antioxidant capacity. Chick's oxidative status was
248 significantly related to chick's body condition at day 17 (Table 1B). As shown in Figure 2A, the
249 relationship between oxidative status and body condition were positive in both years, highlighting the
250 fact that chicks that were in better body condition at day 17 paid a cost in terms of their oxidative status.
251 However, this relationship was different in 2013 and 2015 (significant body condition x year effect, Table
252 1B), indicating that for the same body condition reached at day 17 the cost in terms of oxidative
253 damages was higher in 2013 than in 2015 (Figure 2A). Oxidative status significantly predicted telomere
254 length of chicks at the age of 17 days (Table 2D), but this effect was again year-dependent. Only in 2013
255 did the chicks with higher levels of d-ROMs, controlling for antioxidant capacity, also have shorter
256 telomeres (Figure 2B).

257

258 *Current physiological maturity and telomere length in pre-fledging chicks*

259 PCA1 scores of physiological maturation were positively associated with hematocrit and plasma
260 hemoglobin content and negatively with reticulocytes count recorded in 17 days-old chicks (ESM). There
261 was a significant effect of physiological maturation (PCA1) on telomere length in 17 days-old chicks
262 (Table 1E). More precisely, the significant interactions PCA1 x Sex and PCA1 x Year indicated that male
263 (and not female, Figure 3A) chicks with higher scores of physiological maturation were also characterized
264 by shorter telomeres. Furthermore, this PCA1 – telomere length relationship was in opposite directions
265 in 2013 (negative) and in 2015 (positive; Figure 3B). Looking at the variables independently (see ESM)
266 showed that the reverse relationship observed by sexes is driven by males with short telomeres having
267 higher hematocrit levels while females with shorter telomeres having higher reticulocytes' counts (which

drives lower PCA values). In 2015, chicks with higher hemoglobin levels were also those with longer telomeres (see ESM).

Pre-fledging telomere length as a predictor of fledging somatic maturity

Body condition of fledging chicks was evaluated using the residuals of the regression between body mass and wing length measured at day 21 ($r = 0.545$, $F_{1, 68} = 28.320$, $P < 0.001$, body mass = 0.531 wing length + 17.892). Telomere length of pre-fledging chicks (at 17 days old) did not predict their body condition at the end of the nesting period (i.e. at 21 days old; Table 2A). We only found a year effect on chicks' body condition at day 21, fledging being in worse body condition in 2013 than in 2015. We also found a year and brood effect on body mass loss from day 17 to day 21 (Table 2B), with chicks that were raised in 2013 or in a first brood losing significantly more body mass before fledging (-4.0 ± 0.3 g and -3.2 ± 0.3 g, respectively) than those raised in 2015 (0.4 ± 0.4 g) or in a second brood (-0.4 ± 0.4 g). However, the body mass loss between days 17 and 21 was not predicted by telomere length at 17 days old (Table 2B). Similarly, the gain in wing length between day 17 and 21 was not predicted by telomere length at day 17 (Table 2B) but was influenced by brood and brood size: chicks from 1st brood had a higher rate of wing growth between day 17 and day 21 (15.4 ± 0.4 mm *vs.* 13.0 ± 0.5 mm), while brood size has an overall negative effect on pre-fledging wing length gain.

Pre-fledging telomere length as a predictor of fledging physiological maturity

The change (increase) in blood hemoglobin concentration between days 17 and 21 was predicted by pre-fledging telomere length (Table 2C), both variables being negatively associated: chicks that increased their hemoglobin content more also had shorter telomeres at day 17 (Figure 4), independently of year, brood or sex effects. Telomere length at day 17 did not significantly predict any of the changes in hematocrit and reticulocytes count between day 17 and day 21. Being a 1st brood chick was significantly

292 associated to a larger increase in hematocrit, and the final decrease in reticulocytes' number between
293 day 17 and day 21 was lower in 2013 (Table 2C).
294
295 **2. Experimental growth manipulation and telomeres**
296 At day 4 (before the beginning of the feeding / mother stress experiments), all chicks were of similar
297 body mass. Food provisioned chicks and chicks raised by stressed mothers had comparable body mass at
298 day 17 to control chicks, despite a tendency to be slightly lighter (fed group) or heavier (stressed group,
299 Table 3A, $P = 0.058$). At day 21, no significant differences were found among groups (Table 3A), due to an
300 overall higher body mass loss observed in the stress group compared to control chicks (Bonferroni
301 posthoc, $P = 0.037$) during the pre-fledging period (day 17-21, Table 3C). At day 17, fed chicks had longer
302 wings than controls (Bonferroni posthoc, $P=0.047$), while this difference was not significant between
303 fed/stress and control/stress chicks (Bonferroni posthoc, $P > 0.820$). At day 21, no differences in wing
304 length remained (Table 3B), essentially due to the fact that fed chicks were characterized by a lower wing
305 growth rate than control chicks between day 17 and day 21 (Bonferroni posthoc, $P=0.041$). First brood
306 chicks had longer wings at that stage than 2nd brood chicks (Table 3B).
307 The oxidative status of chicks at day 17 was also influenced by our experimental treatment:
308 levels of oxidative damages were higher in stressed females' chicks than control chicks (Bonferroni
309 posthoc, $P=0.014$). Brood and sex had a significant effect on chicks' oxidative status, 1st brood chicks and
310 females having lower levels of oxidative damages at day 17 (Table 3D). Our experimental treatment had
311 no impact on physiological maturity of chicks at day 17, the only notable effect being the brood effect:
312 chicks raised in a 1st brood had higher physiological maturity proxies than 2nd brood chicks (Table 3E). A
313 similar brood effect was noticed on telomere length, and the experimental treatment affected telomere
314 length at day 17 (Table 3F): chicks raised by stressed females had shorter telomeres than control chicks
315 (Bonferroni posthoc, $P=0.017$), other comparisons being non-significant (Bonferroni posthoc, $P > 0.219$).

316 The fledging maturation processes we measured (the changes in hematocrit, hemoglobin concentration,
317 and reticulocytes count between day 17-day 21) were not found to be affected by our experimental
318 design (Table 3G). However, 1st brood chicks had a greater increase in their blood hemoglobin
319 concentration and in their hematocrit, and a larger decrease in their reticulocytes count during the last
320 days before fledging.
321

DISCUSSION

Our paper focused on the potential pro-ageing costs, *i.e.* telomere shortening, of natural variation (over two years and between 1st and 2nd broods) in developmental trajectories of chicks prior to fledging, both in somatic and physiological traits. In accordance with the literature, we observed that somatic growth was reflected in a deteriorated oxidative status (but only in a 'bad' reproductive year, *i.e.* 2013) and shortened telomeres (but only in 1st brood and male chicks) at the pre-fledging stage (day 17). One of our main results is that physiological maturity is also balanced with telomere erosion at that stage (but again in relation to environmental context – only in 2013 and in males), since higher hematocrit, hemoglobin concentration, and lower levels of reticulocyte counts (PCA 1 axis) are negatively related to telomere length. Our experimental approach confirmed that a marginally increased rate of somatic body mass growth (in chicks raised by stressed mothers) is reflected in terms of higher oxidative stress and telomere erosion at pre-fledging, while no cost was found for an enhanced wing growth in fed chicks. When looking at the final developmental changes at the fledging stage (days 17-21), we found that telomere length measured at day 17 significantly predicts one of the physiological maturation processes: chicks having short telomeres at day 17 had larger increases in hemoglobin concentration whole blood content. While our overall results confirm that early developmental trajectories are mostly resilient to both positive and negative environmental factors, we showed that maintaining them when conditions are sub-optimal seems to be paid in terms of shorter telomere length at the end of growth.

Somatic growth and ageing costs

Life-history trade-offs are most frequently based on the idea of allocation of limited resources among competitive traits, and natural growth trajectories are generally expected to be derived from such trade-offs (Monaghan and Ozanne 2018). In support of the idea of telomere length as a cellular indicator of an individual's biological state (Monaghan 2014), numerous previous studies have reported that telomere

346 length or telomere loss is negatively correlated with body mass at the end of the growth period
347 (Boonekamp et al. 2014; Herborn et al. 2014; Noguera et al. 2015). Our data confirmed this negative
348 correlation but only in first brood chicks or in males. In both cases shorter telomeres may reflect the cost
349 of selection for higher rates of sustained growth. First brood chicks generally benefit from better
350 seasonal food availability, supporting higher growth rates, and fledge at higher mass, both probably
351 favouring higher survival prospects (Cornell et al. 2016; Naef-Daenzer et al. 2001). Similarly, a stronger
352 selection for larger size/mass may exist in males if future reproductive success of males is condition/size
353 dependent, *e.g.* due to male-male competition. Previous data on starlings suggested that growth rate is
354 more resilient to the quality of the rearing environment in male chicks than in female chicks (Rowland et
355 al. 2007). Consequently, any cost of sustained growth is more likely to be detected in male chicks. In our
356 study, the oxidative cost of growth was found to be stronger and negatively related to telomere length
357 only in 2013, when rearing conditions were less favourable. This suggests that the causes of shorter
358 telomeres at the end of growth implicated in the brood/sex or in the year effects might be different. The
359 year effect matches well with an increased energy allocation to cell proliferation and away from
360 telomere maintenance because of food shortage. The brood/sex effects may be rather related to indirect
361 consequences of a hormone-driven trade-off favouring large mass/size in males (potentially involving a
362 testosterone-induced stress for telomeres, (Drury et al. 2014)) and/or to higher chick competition within
363 larger broods (Nettle et al. 2017; Reichert et al. 2015). Interestingly, a recent study conducted on
364 spotless starlings (*Sturnus unicolor*) showed that experimental manipulation of growth before day 14
365 affected telomere length of female chicks especially *via* an increased oxidative stress, while this
366 modulation was indirect in males (Gil et al. 2019). A better understanding of the concurrent modification
367 of cellular pathways interacting with telomere dynamics when growth patterns are perturbed is deeply
368 needed, as it has been explored for other life-history trade-offs (Criscuolo et al. 2018). Brood sizes at 6
369 and 17 days were larger in 1st broods in our study, and competition among chicks has been previously

shown to impact telomere variance in starlings' chicks (Nettle et al. 2016). However, brood size did not impact directly ageing parameters in our study, and the social modulation of the cost of growth remains to be properly tested in our population.

Telomere loss as a cost of physiological maturation at day 17

Short telomeres at the end of growth may result from the cost of growing under sub-optimal conditions due to resource dependent trade-offs. However, as suggested before (Arendt 1997), growth trajectories may have evolved also in relation to intrinsic developmental constraints, *e.g.* the need to reach functional maturity of tissues and organs early in life. Here we focused on two components of developmental maturation, somatic and physiological, in relation to aerobic capacity and metabolism. Interestingly, we found the same patterns of association between our proxies of physiological maturity and telomere length as we observed with somatic growth. First, pre-fledging chicks that faced sub-optimal rearing conditions (in 2013, the 'poor' year) had shorter telomeres associated with more rapid maturation at day 17. Second, independently of conditions, in male chicks' maturation levels and telomere length were also negatively associated. Our results confirmed that (i) developmental (growth and maturation) trajectories have evolved in such a way that they should be maintained even when environmental conditions declines (Cornell and Williams 2017), probably because the ultimate cost of reduced development is high (*i.e.* decreased survival, (Bowers et al. 2014)); (ii) sustaining developmental trajectories (and long-term fitness) under sub-optimal conditions occur with a potential ageing cost (shorter telomere length at the end of growth). While oxidative stress has been previously suggested to be a conserved mechanism mediating the somatic growth / lifespan trade-off (Carney Almroth et al. 2012; Kim et al. 2010), the ageing cost of somatic / physiological maturation processes has been less studied. In amphibian larvae, growth but not development (here transition in life stages which is also related to maturation) has been found to be balanced with oxidative stress (Burraco et al. 2017). In our

study system, while oxidative stress at fledging has been previously characterized as an overall cost of growth, none of the physiological parameters of maturation were found to be correlated with oxidative damages (Cornell and Williams 2017). Accordingly, we did not find any direct oxidative cost of maturity levels at day 17, questioning the nature of the mechanisms leading to shortened telomeres. This indicates that there is a still strong selection for rapid maturation of physiological mechanisms allowing the functioning of the future active fledging outside the nest (*i.e.* flying metabolism (Riera et al. 1983)), which relies on red blood cells for the transport of oxygen to organs. This process is likely to be sustained by an increase in the rate of division of the stem cells from the haematopoietic tissue (Orkin and Zon 2008), and then to translate into a parallel reduction in red blood cells' telomere length. However, the significance of this relationship between maturation and telomere length is again related to sex, only males presenting a negative relationship (*i.e.* potentially reflecting a cost) while females presented a positive relationship (*i.e.* potentially reflecting a permissive relationship). This supports the idea that males and females may have distinct optimal developmental trajectories which may be reflected by different short-term (Costantini et al. 2006), but also long-term costs. Our data suggest that those maturation priorities and their physiological impact may be differently scheduled depending on sexes: males with higher hematocrit (*i.e.* had matured red blood cells) had shortened telomeres at day 17, while this is the case for females having higher reticulocytes' counts (*i.e.* with still numerous immature red blood cells). Whether this reflects a common consequence of recent or on-going cell division leading to red blood cell maturation remains to be defined.

Along with these differences in telomere shortening related to sex-specific maturation trajectories, chicks born in 2013 (bad year) had lower body condition, and also showed a negative relationship between physiological maturation levels and telomere length. In 2015 (good year), physiological maturation levels and telomere length were positively related. This means that either a part of the telomere erosion remained under the influence of an energy-related driver, or that the dynamics of the

maturation process is changed, in a way that is more costly to the organism, for instance if it took place later-on in 2013 than in 2015. When looking more specifically at each variable separately, it seems that the negative relationship in 2013 is mainly driven by hematocrit and reticulocyte count variables, while hemoglobin levels and telomere length were positively related in 2015. This suggests that cell division rate on the one hand and the process of hemoglobin production per se (which in birds may take place both in reticulocytes and in mature nucleated red blood cells) on the other hand do not have the same impact on telomere maintenance. When conditions are optimal, sufficient energy is available to sustain both hemoglobin production and telomere maintenance, while reticulocyte's precursors division and reticulocyte maturation in erythrocytes is traded off with telomere shortening. In fact, as reduced hemoglobin levels or lower hematocrit reflects reproduction costs in adult starlings (Fowler and Williams 2017), it is understandable that those variables may also reflect, under some conditions, the cost of maturation.

Pre-fledging (day 17) telomere length and subsequent somatic and physiological maturation

Hematocrit and hemoglobin concentration increase (while reticulocytes count decrease) during physiological maturation in the last days before fledging (after day 17) in starling chicks (see ESM), whereas most somatic traits (tarsus, mass) reach close to adult values at this point (Cornell et al. 2016). We evaluated whether telomere length measured at day 17 predicted subsequent somatic and physiological changes that are observed in chicks immediately prior to fledging (day 21): mass loss (recession) and increased wing length, hemoglobin. Somatic changes were not found to be related to telomere length, which may be due to the fact that the final body mass loss and wing lengthening are either uncoupled from trade-offs with other traits (and then from ageing costs) or do not require any substantial additional energy investment (Cornell et al. 2016). The last possibility is supported by our experimental approach, showing that stress or food provisioning both altered the somatic growth and

442 ultimate changes before fledging (higher body mass gain before day 17 and higher body mass loss before
443 fledging in stressed-mothers' chicks; lower body mass gain but higher wing growth before day 17 and
444 lower wing gain in fed chicks) but not with the same consequences for ageing (higher oxidative stress
445 and telomere loss in chicks of stressed females). Therefore, two explanations may be proposed: (i) It is
446 not energy *per se* that modulate the ageing cost of somatic maturation at fledging, but rather the
447 investment of energy over the entire growth process that had consequences for the final somatic
448 maturation. We suggest that the way energy is modulating fledging maturation is rather related to the
449 intrinsic control of how growth and maturation are traded off; (ii) the ageing cost experienced by chicks
450 raised by stressed mothers are mostly deriving from parent-offspring or nestling conflicts.
451 Telomere length at day 17 did predict the subsequent change in hemoglobin concentration, but not
452 hematocrit and reticulocytes blood count: chicks having short telomeres underwent the larger increases
453 in hemoglobin just before fledging. Given that hemoglobin content is a predictor of fledging and post-
454 fledging survival in others passerines (Bowers et al. 2014; Nadolski et al. 2006), our results support the
455 hypothesis that chicks adopting a slow developmental trajectory may have to catch-up to fulfil the
456 maturation requirements associated to fledging. Such a catch-up response has been previously shown to
457 come from both an energy and/or time-constrained window of optimal growth (Mangel and Munch
458 2005; Metcalfe and Monaghan 2001). Evolution of faster-than-normal growth has attracted extended
459 interest for several years, and several studies both correlative and experimental suggested that such
460 catch-up growth incurs oxidative stress and/or shortened telomeres (Smith et al. 2016; Tarry-Adkins et
461 al. 2009). Our experimental data, by showing that physiological maturation at fledging (day 17-21) is not
462 responding to food provisioning or mother's stress, confirmed that physiological maturation has been
463 under strong selection probably to promote immediate survival. The most remarkable effect was
464 obtained when the mothers were equipped with transmitters: in that case, chicks' somatic growth
465 trajectories was slightly modified, oxidative stress was increased and telomere length decreased, while

maturation processes remained unaffected. This supports the idea that, either (i) growth and ageing parameters are intimately related while ageing costs of maturation seem to results from the way energy is invested over the whole developmental process, or (ii) ageing costs of maturation may take place later-on, since in many altricial birds chicks are rather somatically mature (close to adult size/mass) before fledging, but are still physiologically immature (Cornell et al. 2016).

Conclusion

In our study, growth rate and developmental maturity appeared as finely-tuned processes in relation to ecological context, ensuring a competitive phenotype of chicks at fledging. As such, they remained, within certain limits, mostly resilient to environmental variations. Our paper highlights correlative evidences that reaching maturity early in life when resources are limited is done at a cost of larger telomere loss. We also experimentally underlined that somatic maturation just prior to fledging may also lead to additional telomere attrition. Our results are in accordance with the idea that development (growth and maturation) has been canalized because of large effects on fitness (Boonekamp et al. 2018). Previous studies conducted in European starlings have stressed that adverse early-life conditions of growth induced both precocious telomere shortening and have delayed impact on physiological condition at adulthood (*i.e.* inflammation status (Nettle et al. 2017)). If telomere length at fledging is of key importance in defining the fitness prospects of fledging, we may expect that canalization has also take place in a way for telomere maintenance (Vedder et al. 2017). Therefore, how the ultimate maturation process is actually reflected in telomere length during the last days before fledging and when chicks entered their active life urgently needs to be evaluated.

ACKNOWLEDGEMENTS

We would like to thank David Davis and the whole Davis Family for their continued support for our European starling work on the Davistead Farm in Langley, BC. Melinda A. Fowler provided invaluable assistance with fieldwork. We are grateful to Dr Mathilde Tissier for helpful comments on the paper.

FUNDING INFORMATION

The present study was supported by a CNRS grant for international collaborations (*TALISMAN,* PICS n° 231662) to F.C. and T.D.W., and Natural Sciences and Engineering Research Council Discovery (155395-2012) and Accelerator (429387-2012) grants to T.D.W.

AUTHORS' CONTRIBUTIONS

T.D.W. and A. C. collected the data and A.C. run all the physiological analyses, V.F. and S.Z. extracted the DNA and ran the qPCR measurements of telomere length, V.F., S.Z. and F.C. analysed the qPCR data, F.C. and T.D.W. did the statistical analyses, and T.D.W. and F.C. drafted the final manuscript on which A.C. made comments.

DATA ACCESSIBILITY

Data will be deposited on Dryad once the paper is accepted

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671 **FIGURE LEGENDS**

672 Figure 1:

673 Linear relationships between starling chick body condition after 17 days of post-hatching development
674 (residuals of wing/mass regression) and telomere length (Log10-transformed T/S ratio), in relation to
675 brood number (**A**: filled circles = 1st brood; open circles = 2nd brood) and chick sex (**B**: filled circles =
676 males; open circles = females). The lines represent the significant negative linear regressions for 1st
677 brood chicks (A) and in males (B): chicks being heavier for a given wing size did it at the expense of
678 shorter telomere lengths at day 17, this effect being significant in males. See Table 1a for statistics.

679

680 Figure 2:

681 Mean slopes illustrating the linear regressions between growth and ageing-related parameters measured
682 in 17 days-old starling chicks: (A) body condition index (residuals body mass / wing length) and oxidative
683 status (residuals of d-ROMs and OXY), and (B) oxidative status (residuals of d-ROMs and OXY) and red-
684 blood cells' telomere length (Log10-transformed T/S ratio). In both figures, filled and open circles
685 correspond to measurements of both variables in 2013 and 2015, respectively. Regression lines show
686 that: (i) while in both years chicks in better condition at the end of growth presented higher levels of
687 plasmatic oxidative damage when controlled for antioxidant capacity of the plasma, the chicks born in
688 2013 had a worse oxidative status for a same body condition than in 2015; (ii) in 2013, chicks with higher
689 oxidative damages presented shorter telomere lengths at day 17, while the relationships was reversed in
690 2015. See Table 1B-D for statistics.

691

692 Figure 3:

693 Red-blood cells' telomere length (Log10-transformed T/S ratio) in response to change in physiological
694 maturation proxies measured at day 17 (PCA 1 individual values based on hematocrit, hemoglobin
695 content reticulocytes blood count), in relation to year (A) and chick sex (B). Filled and open points
696 correspond to (A) 2013 and 2015 and (B) measurements made in males and females, respectively. In
697 2013 and in males, chicks which had higher maturation values also presented shorter telomeres. These
698 relationships were positive in 2015 and in females. See Table 2C for statistics.
699
700 Figure 4:
701 Increase in hemoglobin content of whole blood of fledging chicks (day 21) in relation to red blood cells'
702 telomere length measured at day 17. Independently of sex, brood or year effects, chicks with the shorter
703 telomeres at pre-fledging were those which increased the most their hemoglobin blood concentrations
704 in the last days before leaving the nest. See Table 2C for statistics.
705

706 **FIGURES**
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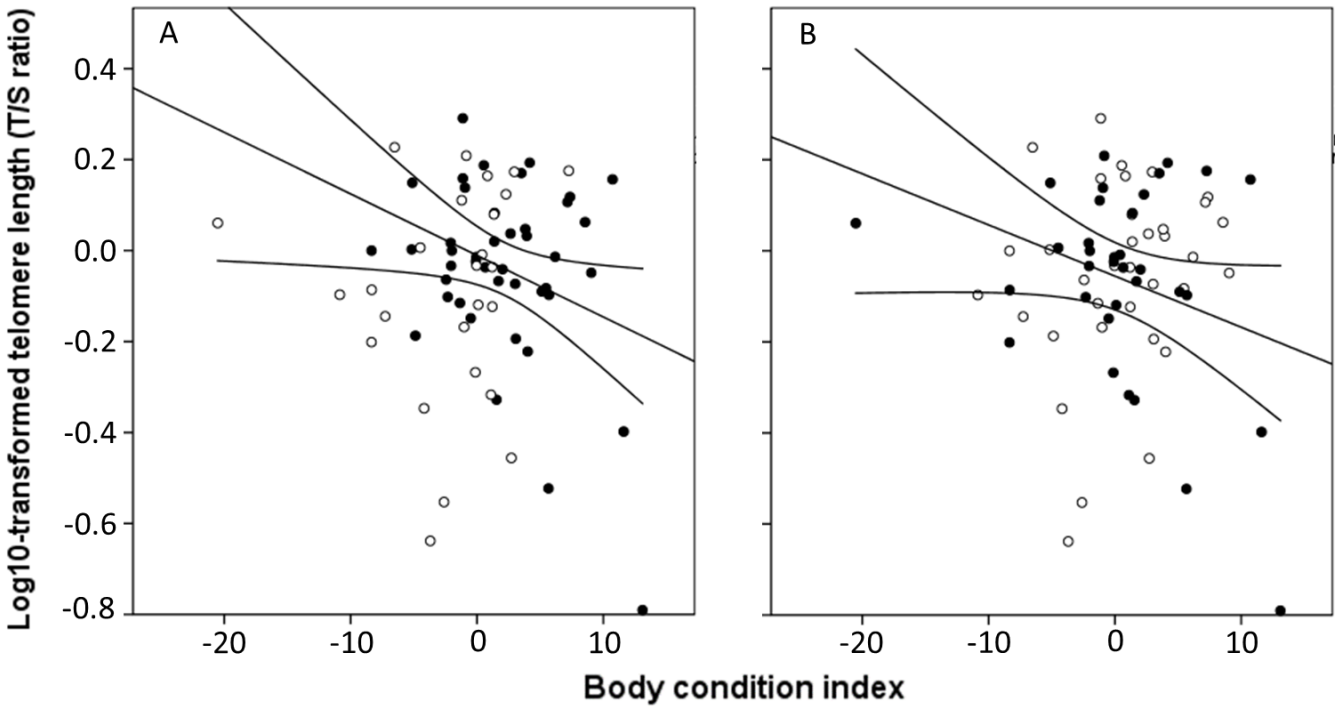
708 Figure 1



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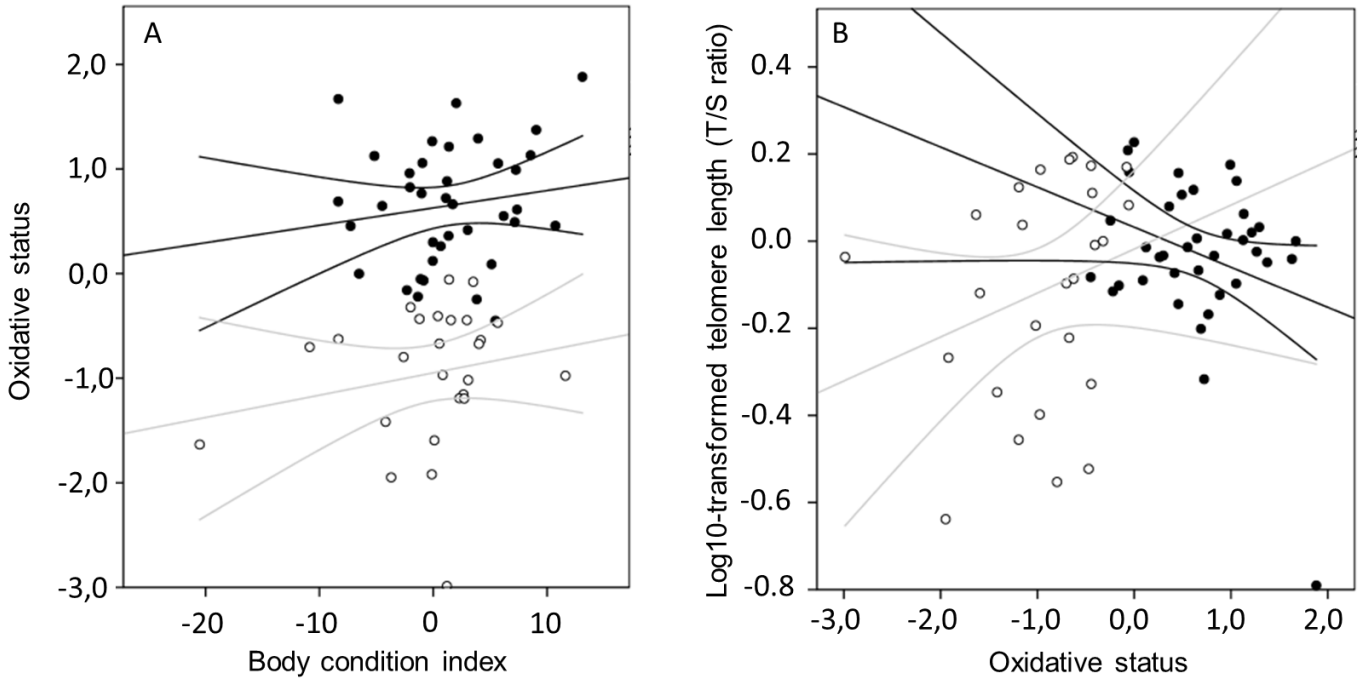

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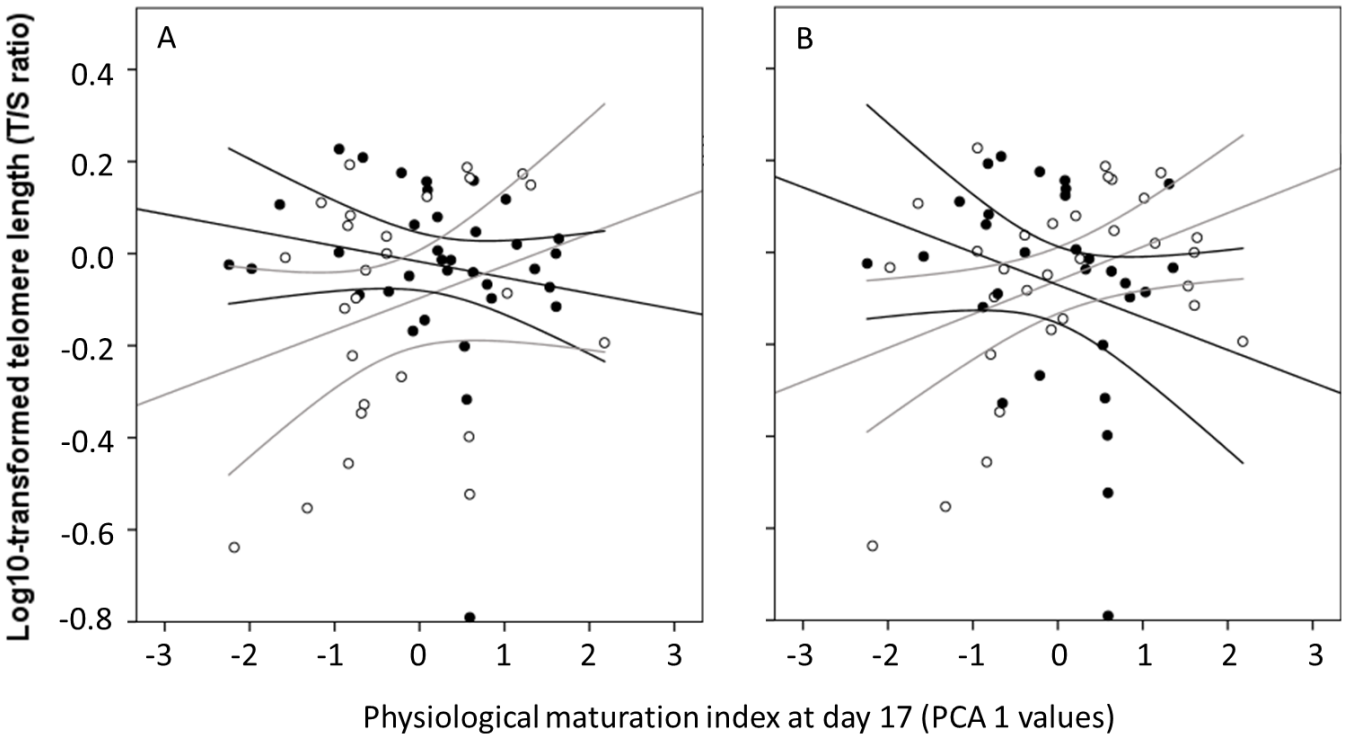

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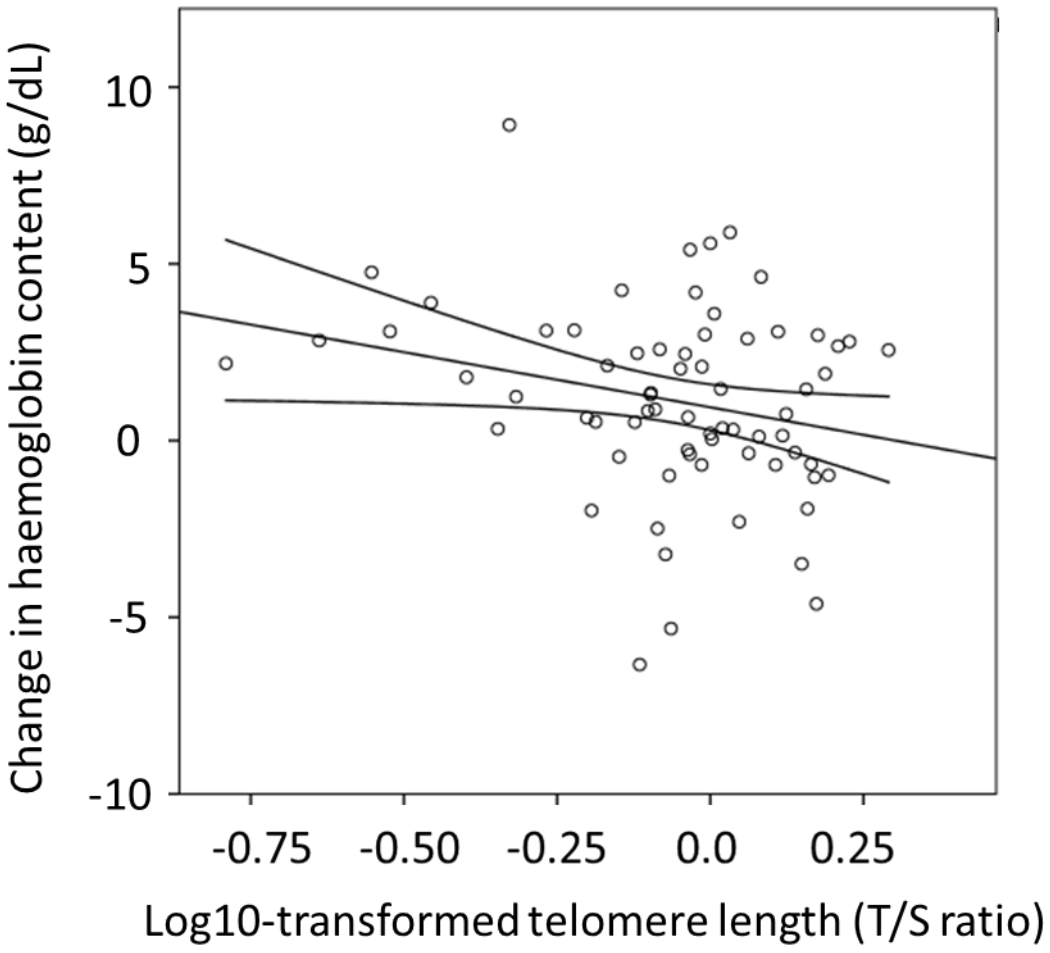

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TABLES

Table 1. Summary of Mixed Models testing for relationships between Log10-transformed telomere length (TS ratio) of starling chicks (17 days-old) and variables all measured at the end of the growth period in non-manipulated chicks in 2013 and 2015: (A) body condition (residuals of body mass/wing length linear regression), (C) plasma level of oxidative damage (d-ROMs), (D) total antioxidant plasma capacity (OXY), and (E) physiological maturation (PCA of hematocrit, hemoglobin content and reticulocytes blood count). Mixed model was also used to check for oxidative cost of growth (B). Nest identity (ID) was used as a random factor to control for the fact that some chicks were raised in the same nest. Significant results are indicated in bold (P<0.05), and results which P<0.1 are indicated in italics.

A. Body condition at the end of growth				
Response variable: ***Log10 (T/S ratio)***	Estimates	D.F	F	P
Intercept	-0.260 ± 0.115	1, 61	4.038	0.049
Year (2013 vs. 2015)	0.096 ± 0.054	1, 61	3.156	*0.081*
Brood (1^{st} vs. 2^{nd})	0.023 ± 0.057	1, 61	0.167	0.684
Brood size at day 17	0.042 ± 0.026	1, 61	2.718	0.104
Body condition at day 17	-0.004 ± 0.008	1, 61	0.094	0.760
Sex (F *vs.* M)	-0.006 ± 0.049	1, 61	0.015	0.904
Brood x Body condition at day 17 (1^{st} vs. 2^{nd})	**-0.020 ± 0.010**	**1, 61**	**4.252**	**0.043**
Sex x Body condition at day 17 (F *vs.* M)	**0.026 ± 0.009**	**1, 61**	**7.561**	**0.008**
B. Oxidative cost of growth				
Response variable: ***Residuals d-ROM/OXY***	Estimates	D.F	F	P
Intercept	-0.152 ± 0.202	1, 61	1.344	0.387
Year (2013 vs. 2015)	**0.551 ± 0.093**	**1, 61**	**35.420**	**<0.001**
Brood (1^{st} vs. 2^{nd})	**0.311 ± 0.099**	**1, 61**	**9.809**	**0.004**
Brood size at day 17	-0.063 ± 0.045	1, 61	1.968	0.167
Sex (F *vs.* M)	-0.144 ± 0.082	1, 61	3.033	*0.088*
Body condition at day 17	-0.039 ± 0.012	1, 61	2.686	0.107
Year x Body condition at day 17 (in 2013)	**0.050 ± 0.015**	**1, 61**	**10.66**	**0.002**
C. Oxidative balance				
Response variable: ***Log10 (T/S ratio)***	Estimates	D.F	F	P
Intercept	-0.092 ± 0.126	1, 57	0.128	0.722
Year (2013 vs. 2015)	0.019 ± 0.074	1, 57	0.067	0.797
Brood (1^{st} vs. 2^{nd})	0.049 ± 0.059	1, 57	0.679	0.413
Brood size at day 17	0.013 ± 0.028	1, 57	0.209	0.649

Sex (F *vs.* M)	0.035 ± 0.053	1, 57	0.432	0.514
Oxidative balance (d-ROMs – OXY residuals)	0.329 ± 0.123	1, 57	1.301	0.259
Year x Oxidative balance (in 2013)	**-0.483 ± 0.151**	**1, 57**	**10.22**	**0.002**
E. Physiological maturity at the end of growth	Estimates	D.F	F	P
Response variable: ***Log10 (T/S ratio)***				
Intercept	-0.227 ± 0.127	1, 61	2.675	0.108
Year (2013 vs. 2015)	0.117 ± 0.060	1, 61	3.751	*0.058*
Brood (1^{st} vs. 2^{nd})	-0.025 ± 0.059	1, 61	0.184	0.670
Brood size at day 17	0.031 ± 0.027	1, 61	1.267	0.265
Sex (F *vs.* M)	0.012 ± 0.052	1, 61	0.053	0.819
PCA 1 – Physiological condition proxies	-0.037 ± 0.054	1, 61	0.119	0.731
Year x PCA 1 (in 2013)	**-0.111 ± 0.054**	**1, 61**	**4.187**	**0.046**
Sex x PCA 1 (F *vs.* M)	**0.164 ± 0.055**	**1, 61**	**8.893**	**0.004**

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739 Table 2. Results of Mixed Models testing for the reliability of Log10-transformed telomere length (TS
740 ratio) of starling chicks (17 days-old) to predict: (A) body condition (residuals of body mass/wing length
741 linear regression) at fledging (day 21), (B) individual pre-fledging somatic maturation (change in body
742 mass and wing length between day 17 and day 21) and (C) individual physiological maturation (changes
743 in hemoglobin content, reticulocytes blood count and hematocrit between day 17 and day 21). Nest
744 identity (ID) was used as a random factor to control for the fact that some chicks were raised in the same
745 nest. The models that are presented are those corresponding to the best AIC value. Significant results are
746 indicated in bold (P<0.05), and results which P<0.1 are indicated in italics.

A. Body condition at fledging (21 days old)				
Response variable: ***Residuals mass/wing***	Estimates	D.F	F	P
Intercept	3.071 ± 1.806	1, 51.24	1.790	0.187
Year (2013 vs. 2015)	-1.838 ± 0.838	1, 51.32	4.815	**0.033**
Brood (1^{st} vs. 2^{nd})	1.116 ± 0.828	1, 52.72	1.817	0.183
Brood size at day 17	-0.489 ± 0.393	1, 50.90	1.548	0.219
Sex (F *vs.* M)	-1.203 ± 0.741	1, 51.12	2.641	0.110
Log10 (T/S ratio)	2.399 ± 1.838	1, 47.76	1.704	0.198
B. Pre-fledging somatic maturation				
Response variable: ***Body mass loss (days 17-21)***	Estimates	D.F	F	P
Intercept	0.913 ± 0.293	1, 15.39	0.293	0.596
Year (2013 vs. 2015)	-3.212 ± 1.001	1, 48.90	10.307	**0.002**
Brood (1^{st} vs. 2^{nd})	-1.531 ± 0.983	1, 43.45	2.428	0.126
Brood size at day 17	-0.014 ± 0.437	1, 56.28	0.001	0.976
Sex (F *vs.* M)	-0.425 ± 0.686	1, 34.24	0.383	0.540
Log10 (T/S ratio)	1.141 ± 1.782	1, 55.35	0.410	0.525
Response variable: ***wing length gain (days 17-21)***	Estimates	D.F	F	P
Intercept	18.734 ± 2.700	1, 869.74	54.90	**<0.001**
Year (2013 vs. 2015)	-1.305 ± 0.701	1, 50.66	3.460	*0.069*
Brood (1^{st} vs. 2^{nd})	2.322 ± 0.707	1, 53.17	10.774	**0.002**
Brood size at day 17	-1.327 ± 0.335	1, 52.79	15.514	**<0.001**
Sex (F *vs.* M)	0.408 ± 0.590	1, 50.04	0.477	0.493
Log10 (T/S ratio)	1.696 ± 1.603	1, 27.92	1.118	0.299
C. Pre-fledging physiological maturation				
Response variable: ***Hemoglobin content change***	Estimates	D.F	F	P
Intercept	8.920 ± 1.405	1, 12.76	40.323	<0.001
Year (2013 vs. 2015)	-0.640 ± 0.437	1, 52.33	2.144	0.149

Brood (1st vs. 2nd)	0.306 ± 0.483	1, 45.70	0.401	0.530
Brood size at day 17	0.592 ± 0.189	1, 59.18	9.813	**0.003**
Sex (F *vs.* M)	0.393 ± 0.357	1, 48.17	1.215	0.276
Log10 (T/S ratio)	-1.082 ± 0.502	1, 63.04	4.640	**0.035**
Hemoglobin at day 17	-0.720 ± 0.067	1, 62.21	112.962	**<0.001**
Response variable: ***Reticulocytes count change***	Estimates	D.F	F	P
Intercept	20.159 ± 7.608	1, 7.032	4.569	0.070
Year (2013 vs. 2015)	-10.125 ± 2.662	1, 39.64	14.467	**<0.001**
Brood (1st vs. 2nd)	-1.250 ± 2.393	1, 30.73	0.273	0.605
Brood size at day 17	-0.274 ± 1.116	1, 45.60	0.060	0.808
Sex (F *vs.* M)	1.278 ± 2.000	1, 39.63	0.408	0.527
Log10 (T/S ratio)	-2.317 ± 2.805	1, 52.06	0.682	0.413
Reticulocytes count change at day 17	-0.916 ± 0.100	1, 47.63	85.62	**<0.001**
Response variable: ***hematocrit change***	Estimates	D.F	F	P
Intercept	**32.299 ± 6.191**	**1, 19,64**	**28.302**	**<0.001**
Year (2013 vs. 2015)	-0.392 ± 1.679	1, 46,83	0.055	0.816
Brood (1st vs. 2nd)	3.537 ± 1.694	1, 38.45	4.361	**0.043**
Brood size at day 17	0.546 ± 0.759	1, 56,63	0.517	0.475
Sex (F *vs.* M)	-0.856 ± 1.357	1, 50.15	0.398	0.531
Log10 (T/S ratio)	-1.158 ± 1.877	1, 61,28	0.381	0.539
Hematocrit at day 17	-0.757 ± 0.128	1, 58.58	35.285	**<0.001**

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Table 3. Results of Mixed Models testing for the effect of the experimental treatment conducted on chicks (additional feeding) and on adult females (stress) on growth (A and B), pre-fledging maturation, oxidative status and telomere length (C, D, E and F) and physiological maturation at fledging (G). Nest identity (ID) was used as a random factor to control for the fact that some chicks were raised in the same nest. The models that are presented are those corresponding to the best AIC value. Significant results are indicated in bold (P<0.05), and results which P<0.1 are indicated in italics.

A. Body mass growth					
Response variable: ***Body mass day 4***		Estimates	D.F	F	P
Intercept		19.584 ± 3.597	1, 7.03	23.314	**0.002**
Experimental treatment (*vs.* control)			2, 18.76	2.306	0.127
	Food	2.344 ± 1.475			
	Stress	-5.227 ± 4.462			
Response variable: ***Body mass day 17***		Estimates	D.F	F	P
Intercept		72.994 ± 3.496	1, 6.97	473.07	**<0.001**
Experimental treatment (*vs.* control)			2, 71.79	2.967	*0.058*
	Food	-0.819 ± 1.378			
	Stress	3.737 ± 1.882			
Response variable: ***Body mass day 21***		Estimates	D.F	F	P
Intercept		72.724 ± 2.808	1, 5.60	693.075	**<0.001**
Experimental treatment (*vs.* control)			2, 54.33	1.796	0.176
	Food	-1.721 ± 1.141			
	Stress	1.299 ± 1.960			
B. Body size growth					
Response variable: ***Wing length day 17***		Estimates	D.F	F	P
Intercept		86.940 ± 3.540	1, 13.20	642.064	**<0.001**
Experimental treatment (*vs.* control)			2, 57.45	3.230	**0.047**
	Food	2.473 ± 0.983			
	Stress	1.784 ± 1.613			
Response variable: ***Wing length day 21***		Estimates	D.F	F	P
Intercept		100.054 ± 2.031	1, 3.39	2890.304	**<0.001**
Brood (1^{st} vs. 2^{nd})		3.628 ± 1.018	1, 32.27	12.705	**0.001**
Experimental treatment (*vs.* control)			2, 54.13	1.580	0.215
	Food	0.695 ± 0.905			
	Stress	2.641 ± 1.501			
C. Pre-fledging somatic maturation			D.F	F	P

Response variable: ***Body mass loss (day17-21)***		Estimates			
Intercept		-0.205 ± 2.963	1, 23.08	0.392	0.537
Experimental treatment (*vs.* control)			2, 41.44	4.786	**0.013**
	Food	-1.205 ± 0.561			
	Stress	-3.728 ± 1.429			
Response variable: ***Wing gain (day17-21)***		Estimates	D.F	F	P
Intercept		14.650 ± 1.852	1, 8.50	63.186	**<0.001**
Experimental treatment (*vs.* control)			2, 53.55	3.773	**0.029**
	Food	-1.777 ± 0.693			
	Stress	0.207 ± 1.153			
Brood (1^{st} vs. 2^{nd})		1.452 ± 0.888	1, 32.41	2.674	0.112
Brood size at day 17		-0.174 ± 0.381	1, 40.45	0.209	0.650
Sex (F *vs.* M)		0.778 ± 0.718	1, 60.61	1.173	0.283
D. Oxidative balance at day 17					
Response variable: ***Residuals d-ROMs/OXY***		Estimates	D.F	F	P
Intercept		-0.250 ± 0.199	1, 46	2.084	0.156
Experimental treatment (*vs.* control)			2, 46	5.034	**0.011**
	Food	0.164 ± 0.094			
	Stress	0.548 ± 0.184			
Brood (1^{st} vs. 2^{nd})		-0.306 ± 0.112	1, 46	7.509	**0.009**
Brood size at day 17		0.041 ± 0.051	1, 46	0.652	0.424
Sex (F *vs.* M)		-0.270 ± 0.093	1, 46	8.416	**0.006**
E. Physiological maturity at the end of growth					
Response variable: ***PCA 1 – Physiological condition proxies***		Estimates	D.F	F	P
Intercept		-1.276 ± 0.781	1, 12.25	0.604	0.452
Experimental treatment (*vs.* control)			2, 39.31	0.561	0.575
	Food	0.019 ± 0.201			
	Stress	0.368 ± 0.354			
Brood (1^{st} vs. 2^{nd})		**0.965 ± 0.321**	**1, 27.82**	**9.062**	**0.005**
Brood size at day 17		0.180 ± 0.136	1, 30.71	1.750	0.196
Sex (F *vs.* M)		0.072 ± 0.224	1, 52.17	0.104	0.749
F. Pre-fledging telomere length					
Response variable: ***Log10 (T/S ratio)***		Estimates	D.F	F	P
Intercept		-0.145 ± 0.087	1, 1.327	5.113	0.213
Experimental treatment (*vs.* control)			2, 71.23	4.232	**0.018**
	Food	-0.066 ± 0.051			
	Stress	-0.182 ± 0.063			
Brood (1^{st} vs. 2^{nd})		0.103 ± 0.050	1, 30.49	4.179	**0.050**
G. Fledging physiological maturation					
Response variable: ***Hemoglobin content change***		Estimates	D.F	F	P

Intercept		10.788 ± 1.380	1, 15.75	65.591	**<0.001**
Experimental treatment (*vs.* control)			2, 51.09	2.267	0.114
	Food	0.319 ± 0.382			
	Stress	1.252 ± 0.591			
Brood (1st vs. 2nd)		**1.143 ± 0.526**	**1, 30.25**	**4.730**	**0.038**
Brood size at day 17		-0.023 ± 0.212	1, 34.38	0.012	0.914
Sex (F *vs.* M)		0.442 ± 0.396	1, 62.59	1.246	0.269
Hemoglobin content at day 17		**-0.784 ± 0.077**	**1, 62.61**	**102.785**	**<0.001**
Response variable: ***reticulocytes count change***		Estimates	D.F	F	P
Intercept		23.487 ± 8.896	1, 9.37	6.731	**0.028**
Experimental treatment (*vs.* control)			2, 37.95	1.215	0.308
	Food	-2.409 ± 2.552			
	Stress	5.776 ± 3.706			
Brood (1st vs. 2nd)		-3.435 ± 3.580	1, 26.13	0.921	0.346
Brood size at day 17		-1.135 ± 1.459	1, 24.59	0.605	0.444
Sex (F *vs.* M)		-2.409 ± 2.552	1, 50.90	0.891	0.350
Reticulocytes count at day 17		**-0.887 ± 0.116**	**1, 48.12**	**58.839**	**<0.001**
Response variable: ***hematocrit change***		Estimates	D.F	F	P
Intercept		**40.404 ± 6.800**	**1, 34.34**	**41.455**	**<0.001**
Experimental treatment (vs. control)			2, 47.17	1.114	0.337
Food		1.858 ± 1.255			
Stress		1.364 ± 2.442			
Brood (1st vs. 2nd)		**7.433 ± 2.201**	**1, 39.68**	**11.408**	**0.002**
Brood size at day 17		-0.977 ± 0.918	1, 41.52	1.133	0.293
Sex (F vs. M)		0.273 ± 1.444	1, 59.16	0.036	0.851
Hematocrit at day 17		**-0.911 ± 0.114**	**1, 61.78**	**63.971**	**<0.001**

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