Received: 28 July 2023 | Revised: 23 September 2023 | Accepted: 12 October 2023

DOI: 10.1111/mec.17177

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Ectoparasite presence and brood size manipulation interact to accelerate telomere shortening in nestling jackdaws

Elisa P. Badás[1,2] | Christina Bauch[1] | Jelle J. Boonekamp[1,3] | Ellis Mulder[1] | Simon Verhulst[1]

[1]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, Netherlands

[2]Department of Biodiversity, Ecology and Evolution, Complutense University of Madrid, Madrid, Spain

[3]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK

Correspondence
Elisa P. Badás and Simon Verhulst, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, Netherlands.
Email: eperezbadas@bio.ucm.es and s.verhulst@rug.nl

Funding information
European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie, Grant/Award Number: 8444403; DFG fellowship, Grant/Award Number: BA 5422/1-1; NWO, Grant/Award Number: 823.01.009 and 823.01.006

Handling Editor: Camille Bonneaud

Abstract

Early-life conditions impact fitness, but whether the combined effect of extrinsic stressors is additive or synergistic is not well known. This is a major knowledge gap because exposure to multiple stressors is frequent. Telomere dynamics may be instrumental when testing how stressors interact because many factors affect telomere shortening, and telomere shortening predicts survival. We evaluated the effect of manipulated brood size and natural infestation by the carnid fly *Carnus hemapterus* on nestling growth and telomere shortening of wild jackdaws (*Corvus monedula*). Telomere length, measured in blood using TRF, shortened on average by 264 bp, and on average, *Carnus* infection induced more telomere shortening. Further analyses showed that in enlarged broods, nestlings' telomeres shortened more when parasitized, while in reduced broods there was no effect of infection on telomere shortening. We conclude that there is a synergistic effect of number of siblings and *Carnus* infection on telomere shortening rate: blood-sucking parasites may negatively impact telomeres by increasing cell proliferation and/or physiological stress, and coping with infection may be less successful in enlarged broods with increased sibling competition. Larger nestlings had shorter telomeres independent of age, brood manipulation or infection. Growth was independent of infestation but in enlarged broods, nestlings were lighter at fledging. Our findings indicate that (i) evaluating consequences of early-life environmental conditions in isolation may not yield a full picture due to synergistic effects, and (ii) effects of environmental conditions may be cryptic, for example, on telomeres, with fitness consequences expressed beyond the temporal framework of the study.

KEYWORDS
body mass, brood enlargement, early-life adversity, haematophagous parasite, molecular biomarker, telomere restriction fragment

1 | INTRODUCTION

Individual variation in fitness is usually large, and at least part of that variation is caused by variation in phenotypic quality, as evidenced by positive associations between reproductive success and parental survival (Stockley & Bro-Jørgensen, 2011; Vedder & Bouwhuis, 2018). The early-life environment has been suggested to play a major role in shaping variation in phenotypic quality (Gilbert, 2001). Studies of the link between early-life conditions and fitness prospects either measure fitness components directly, by

following individuals for a significant part of their lives or use markers of morphological, physiological and/or molecular state as fitness proxy. Unfortunately, fitness prospects can be difficult to infer using such proxies because responses to environmental perturbations typically differ between markers (Boonekamp et al., 2018; Driessen et al., 2022). In this context, the length and dynamics of telomeres, complexes of proteins and repetitive DNA at the end of eukaryotic chromosomes (Blackburn, 1991) have emerged as relatively robust markers of past experiences. Telomere length and dynamics have been shown to be susceptible to early-life environmental conditions (Chatelain et al., 2019), and longer telomeres and/or lower rates of telomere shortening predict longevity and/or other fitness components in humans (Wang et al., 2018) and model (Muñoz-Lorente et al., 2019) and non-model organisms (Eastwood et al., 2023; Wilbourn et al., 2018).

Variation in telomere length is already present early in life in humans (Factor-Litvak et al., 2016) and non-model organisms (Bauch et al., 2022). While the variation present at birth is mostly maintained throughout adulthood (Aviv & Shay, 2018; Kärkkäinen et al., 2022), variation in telomere shortening in early life is associated with morbidity and mortality (Benetos et al., 2018; Boonekamp et al., 2014). However, developing individuals live in complex environments, and despite a recent surge in research efforts (Monaghan et al., 2018, 2022), little is known about the overall effects of early-life conditions on telomere length (reviewed in Monaghan and Ozanne (2018)). In particular, the outcome of the interplay between multiple stressors during growth and their effect on telomere dynamics is poorly understood. The combined effect of stressors on telomere shortening may be additive, whereby the combined result is the sum of the effect of each stressor separately, or synergistic, when multiple stressors interact to produce an effect that is greater than the sum of the individual effects in isolation. Understanding whether the combined effect of multiple stressors is additive or synergistic is of importance because developing animals will only rarely grow up in conditions that are optimal in every way; it is more likely that some of the many environmental aspects that affect development are at a sub-optimal level, and as such can be considered a stressor.

To address the question whether effects of different stressors are additive or synergistic we examined how/whether two extrinsic stressors, manipulated brood size and parasitic infections, interact to affect growth and telomere shortening of nestling jackdaws *Corvus monedula*. A stressful social environment (increased sibling competition) has proven to negatively affect growth and telomere dynamics in this (Boonekamp et al., 2014) and other bird populations (Reichert et al., 2015; Young et al., 2017). The effects of parasitic infections on telomere shortening are increasingly attracting attention, with some studies finding that parasitized individuals had shorter telomeres or higher rates of telomere loss (Asghar et al., 2015, 2016; Karell et al., 2017; Tschirren et al., 2021), while other studies did not find a clear relationship between parasitic infections and telomere dynamics (Badás et al., 2015; Slowinski, 2017; Stauffer et al., 2017; Sudyka et al., 2019). Nestling jackdaws in our population are frequently parasitized by the carnid fly *Carnus hemapterus*, a blood-sucking ectoparasite that is commonly found infecting nestlings from various medium-to-big size bird species (i.e. starlings, bee-eaters, tawny owls and raptors (Roulin et al., 2003)). Gravid females lay their eggs within the bird's nest material. The imago emerges around the time the birds' eggs hatch and the larvae then parasitizes nestlings through a large part of the development period (Veiga et al., 2019). Due to the end replication problem during cell division and/or loss of the single-strand overhang (Blackburn, 1991), telomeres shorten in length as cell division progresses. Thus, because *Carnus hemapterus* feeds on blood, the parasite could increase red blood cell replacement rate, thereby directly affecting telomere dynamics, or indirectly affecting telomere dynamics by triggering related physiological processes (i.e. oxidative stress). Nevertheless, we anticipated that parasites would accelerate telomere shortening in their hosts, as reported for great tit nestlings reared in nests experimentally infested with haematophagous hen fleas (Tschirren et al., 2021). In summary, we expect parasitized nestlings and nestlings reared in enlarged brood to show reduced growth and faster rates of telomere shortening, and we tested whether these effects were additive or synergistic.

2 | MATERIALS AND METHODS

2.1 | Study site and sampling

The study was conducted from 2012 to 2017 in a free-living population of jackdaws (*Corvus monedula*) breeding in nest boxes located south of Groningen, The Netherlands (53°14′N, 6°64′E), which has been routinely monitored since 2005. Nest boxes were routinely checked to assess egg laying and, after incubation commenced, both pair members were identified by their unique colour ring combination. Brood size manipulations are described in detail in Boonekamp et al. (2020). In brief, broods were manipulated when the oldest nestling was 4 days old (hatching date = day 0, hereafter, day 5). Dyads of broods that were manipulated were matched by clutch size and laying date (±1 day). Nestlings were cross-fostered so that the net manipulation was ±2 nestlings, creating enlarged and reduced broods with both manipulated broods containing nestlings hatched in both broods in the dyad (Bauch et al., 2022). Biometric measurements and weight (to the nearest 0.1 g) were taken on days 5 and 30, and a blood sample was taken from the brachial vein. Upon collection, all blood samples were stored in 2% EDTA buffer at 4–7°C and within 3 weeks, they underwent snap freezing in a 40% glycerol buffer for long-term storage at −80°C.

Parasite counts (hereafter *Carnus* counts) were recorded for each nestling on every visit to the nest (due to regular nest checks and experimental manipulation, data on parasite infestation were available for days 0, 1, 5, 10, 11, 20 and 30). Parasites were located by visual inspection of the nestlings' body, with particular attention to areas where either the parasite or bite marks are common (under the wings, legs and belly). From 2012 to 2014, both parasite counts and

bite marks on the belly were recorded ($n = 229$), while from 2015 to 2017, only parasite presence was recorded. Bite marks were recorded as a 'belly score' index from 0 to 4 (where 0 = no marks, 1 = under 10 marks, 2 = 10–20 marks, 3 = 20–50 marks, and 4 = 50 or more marks).

We validated the belly score index as indicator of parasite presence in the nest (for cases when no *Carnus* flies were seen but belly score was >0) using a subset of individuals for which both parasite counts and belly score data were available. Belly score and *Carnus* counts predict the same outcome (belly score = 0 *Carnus* counts = 0 vs. belly score > 0 *Carnus* counts > 0) in 94% of cases ($\chi^2_3 = 180.84$, p-value < .0001). We further validated that belly score is a good indicator of parasite presence (Table S1, Figure S1) with a generalized linear mixed model with Poisson error distribution with *Carnus* counts as dependent variable and belly score index, age, their interaction and year as fixed effects. This model included foster nest and colony as random effects to control for repeated measures and spatial variation. These successful validations allowed us to confidently unify the data collected as belly score and/or *Carnus* counts into the variable '*Carnus* presence', which was used in the main models. Because parasites often move from one sibling to another within the nest (Roulin et al., 2003), when at least one nestling was infested at any sampling point during development, we scored all nestlings from the brood as 'infested'.

We recognize that variation in parasite infestation would ideally have been experimental in origin, but in this study, we relied on natural variation. This may have biased our results when *Carnus* infection varies systematically between or within colonies, or when jackdaws could detect *Carnus* infection when selecting a nest site, but neither appears to be the case. That the flies are widespread and first appear long after (egg) incubation has led us to assume for the present study that jackdaws have little control over whether they breed in a nest box with or without *Carnus* infection.

2.2 | Telomere length analyses

Telomere length was measured in 322 individuals in blood samples using the telomere restriction fragment (TRF) analysis (Salomons et al., 2009). Samples were distributed over 34 gels, and nestlings from the same brood were partly spread over gels with repeated samples from the same individual (ages 5 and 30 days) placed always in neighbouring lanes on a gel. Briefly, DNA was extracted using the CHEF Genomic DNA Plug kit (Bio-Rad) and digested overnight with proteinase K at 50°C. About half of the digested DNA was simultaneously digested with Hind III (60 U), Hinf I (30 U) and Msp I (60 U) for 18 h at 37°C in NEB2 buffer. The digested DNA from the nucleated erythrocytes in each sample was then separated by pulsed-field gel electrophoresis at 14°C for 24 h (3 V/cm, initial switch time 0.5 s and final switch time 7.0 s). The gels were dried (Bio-Rad model 538) and hybridized overnight using a ^{32}P-end-labelled oligo $(5'\text{-CCCTAA-}3')_4$ that binds to the 3′ end-cap telomere overhang. Subsequently, a gel picture was obtained by detecting the radioactive signal (Cyclone Storage Phosphor System, PerkinElmer) with the use of a phosphor screen. Individual telomere length distributions for each sample were quantified through densitometry in the resulting gel picture using IMAGEJ v. 1.38x and following Bauch et al. (2022). For the present analyses, we used the averaged value of the individual telomere length size distributions. The intergel repeatability was measured as the coefficient of variation for a 29-day-old jackdaw control sample that was included in all 34 gels (6.01%), while the intragel repeatability is demonstrated by the high repeatability within individuals (see Results section).

2.3 | Statistical analyses

All models were fitted in R 3.6.3 (R Development Core Team, 2020). To evaluate effects of the parasitic infection and brood manipulation on the nestlings' telomere length and shortening, we built a linear mixed-effects model with telomere length as dependent variable (hereafter, telomere model). The following terms were included as fixed effects: age, *Carnus* presence, brood manipulation, sex, the four-way interaction among these variables and all lower-order terms. We also included nestling body mass and the two-way interactions between body mass and age, sex, brood manipulation and *Carnus* presence. Random effects included year of birth to control for temporal variation, colony to control for spatial variation, foster nest, gel identity to control for between-gel differences and individual identity to control for repeated measures on the same individual. Individual identity was nested in foster nest (nesting individual identity also in year of birth gave singularity problems, so this random effect was removed). This model was fitted using the R package *lme4* 1.1-27.1 (Bates et al., 2014).

Nestling body mass was standardized (mean = 0, SD = 1) by age group to control for the different scales in the mean and standard deviation of mass between day 5 (mean = 42.31 g, SD = 8.03, $n = 326$) and day 30 nestlings (mean = 229 g, SD = 20.57, $n = 326$). Standardization of mass was done separately for nestlings in reduced and enlarged broods to avoid confounding brood size manipulation with nestling mass in the telomere model.

To evaluate whether telomere shortening within individuals was (i) due to consistent parasite effects yearly (within-year effect), (ii) dependent on parasite abundance variation between years (between-year effect), or (iii) both, we followed van de Pol and Wright (2009). The model included 'average *Carnus*' (mean proportion of parasite-infested individuals in the population or between-year differences), 'delta *Carnus*' (deviation of individuals' parasite presence/absence score from the year's population mean or within-year differences), age, sex, brood manipulation, body mass scaled by age group and the two-way interaction of each with age as fixed effects, and the same random effects as in the telomere model. A model with 'average *Carnus*', *Carnus* presence, and the same fixed and random effects described in the previous model tested whether the slope of the between- and within-year effects was significantly different.

To evaluate the effects of the parasitic infection and brood manipulation on nestling growth between ages 5 and 30 days, we built a linear mixed-effects model with mass as dependent variable

TABLE 1 Parameter estimates of fixed effects from the two mixed models explaining age, brood manipulation and parasite effects on telomere length (telomere model) and body mass (mass model) of nestling jackdaws.

	Telomere model			Mass model		
Predictors	***β***	**SE**	***p-value***	***β***	**SE**	***p-value***
Intercept	7008.10	81.65	**<.001**	135.89	1.31	**<.001**
Carnus presence	−125.90	110.88	.256	0.38	1.44	.791
Age	**−263.85**	**10.16**	**<.001**	**188.08**	**0.67**	**<.001**
Sex	65.94	61.99	.287	**10.38**	**0.74**	**<.001**
Brood manipulation	84.84	69.76	.224	**−2.81**	**1.00**	**.005**
Scaled mass	**−16.50**	**6.93**	**.017**	–	–	–
AgeCarnus* presence**	**−100.07**	**32.67**	**.002**	3.10	2.05	.131
Carnus presence*Sex	57.98	212.56	.785	−0.46	2.26	.837
Age*Sex	38.03	20.99	.070	**19.41**	**1.35**	**<.001**
Carnus presence*Brood manipulation	−305.82	199.82	.126	**6.33**	**2.66**	**.017**
Age*Brood manipulation	8.00	20.61	.698	**−8.92**	**1.40**	**<.001**
Brood manipulation*Sex	−148.68	131.22	.257	−2.34	1.55	.132
Age*Scaled mass	−0.01	13.60	.999	–	–	–
Scaled mass*Sex	19.89	13.68	.146	–	–	–
Scaled mass*Brood manipulation	21.70	16.18	.180	–	–	–
Scaled mass**Carnus* presence	5.34	20.57	.795	–	–	–
Age**Carnus* presence*Sex	−26.46	72.34	.715	0.90	4.10	.827
Age*Brood manipulationCarnus* presence**	**−163.62**	**63.28**	**.010**	4.94	3.85	.200
Carnus presence*Brood manipulation*Sex	143.53	378.62	.705	−2.54	4.27	.552
Age*Brood manipulation*Sex	−35.92	47.11	.446	−3.92	2.80	.162
Age*Brood manipulation*Scaled mass	−24.56	31.85	.441	–	–	–
Age**Carnus* presence*Scaled mass	−11.58	45.54	.799	–	–	–
Age**Carnus* presence*Brood manipulation*Sex	−68.34	115.11	.553	5.15	7.71	.505
Random effects						
σ^2	11999.36			179.96		
$\tau_{00\ Chick\_ID:Fnest}$	236971.68			3.58		
$\tau_{00\ Fnest}$	21104.45			29.84		
$\tau_{00\ GelID}$	13001.77			-		
$\tau_{00\ Colony}$	26216.11			4.36		
$\tau_{00\ YEAR}$	4824.07			4.33		
ICC	0.96			0.19		
N_{YEAR}	6			6		
N_{Colony}	7			7		
N_{Chick_ID}	322			828		
N_{Fnest}	175			317		
N_{GelID}	34			–		
Observations	644			1656		
Marginal R^2/Conditional R^2	0.073/0.965			0.976/0.980		

Note: Significant parameter estimates are in bold. For variable names, in bold when significant in the telomere model and/or underlined when significant in the mass model. Nestling mass was transformed to a standard normal distribution by age and brood size manipulation group. All other parameters were centred. Data on parasite infestation and telomere length for the telomere model were available for a subset of individuals present in the mass model.

Abbreviations: CI, confidence interval; ICC, interclass correlation coefficient; SE, standard error; β, parameter estimate; σ^2, residual variance; τ, variance.

(hereafter, mass model). We specified age, *Carnus* presence, brood manipulation, sex and the interaction among them as fixed effects. The random effects were the same as in the telomere model (without gel identity). This model was fitted using the R package *blme* 1.0-4 (Chung et al., 2013), which avoids convergence and singular fit problems by using Bayes modal estimation with an inverse Wishart covariance prior for the random effects (Chung et al., 2013).

For all of the above, we present the full model, which incorporated all predictors relevant to our hypotheses. Unless stated otherwise, all predictor variables were mean centred to improve the interpretability of regression coefficients of main effects when interactions are present in the model (Schielzeth, 2010). All diagnostics plots were examined to confirm that there were no deviations from model assumptions and variance inflation factors (VIF) were calculated to check for multicollinearity (all variables had VIF < 2.5) before interpreting model estimates. To ensure that the results were not biased by the presence of extremely influential points or unbalanced sample size between groups (i.e. *Carnus* presence, see Results section for information on parasite prevalence in the models), we used parametric bootstrapping ($n = 1000$) to obtain 95% confidence intervals. Results were confirmed for all models, and thus we chose to present β-estimates, standard errors and p-values.

3 | RESULTS

3.1 | Parasite and brood manipulation effects on telomere shortening

Telomere length (TL) was on average 7008 ± 82 base pairs on day 5 (hereafter, bp ± SE) and nestlings lost on average 264 ± 10 bp over the 25-day measurement period (Table 1, age effect). TL on day 30 was highly correlated with TL on day 5 (Figure 1; $r = .99$, $n = 322$, $p < .0001$), which further confirms the high within-individual correlation of telomere length in the present population rendered by the TRF analysis (Bauch et al., 2022). TL correlated negatively with body

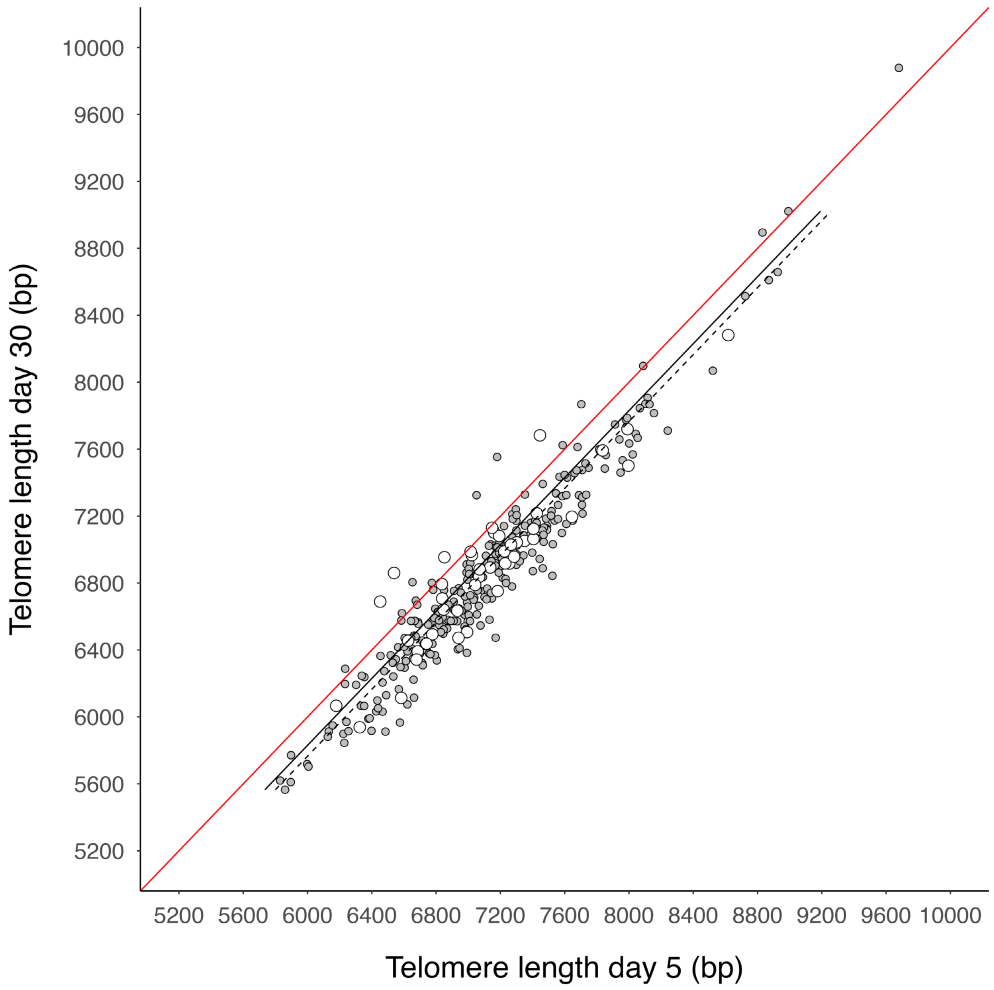

FIGURE 1 Correlation between telomere length at day 5 and day 30: the diagonal line plotted at $x = y$ (in red) represents no change in telomere length (when TL on day 5 it is equal to TL on day 30) and further points below the line indicate more telomere shortening. Raw data points and regression lines for *Carnus hemapterus* presence (dashed line, filled circles) and absence (solid line, open circles) are plotted. Note that in the model, telomere shortening was higher for parasitized nestlings reared in enlarged broods (three-way interaction, for statistics see Table 1).

mass, and this association was independent of age, as evidenced by a non-significant age×mass interaction (Table 1, telomere model; note that mass was Z-transformed by age group and brood size manipulation category). TL was on average 17±7 bp shorter per SD increase in body mass in the population (Table 1, Figure 2).

Carnid flies were recorded in 142 of the 175 nests and in 278 of the 322 nestlings. There was a significant three-way interaction between age, brood size manipulation and *Carnus* presence, indicating that the *Carnus* effect on telomere shortening with age depended on brood size (Table 1, Figure 3). The different rates of shortening between manipulated broods were further confirmed by post-hoc analyses in which we ran separate models for nestlings reared in reduced versus enlarged broods. This revealed that *Carnus* infestation accelerated telomere shortening in enlarged broods, but not in reduced broods (Table S2). Thus, while *Carnus* presence was associated with increased telomere loss over all data pooled (age×*Carnus* presence interaction, Table 1, Figure S2), this was due to enlarged broods only. The significant age×*Carnus* presence interaction in the full dataset (Table 1) shows that nestlings reared in infested nests shortened their telomeres by on average 100±33 bp more than nestlings reared in nests in which no parasite was recorded, and the subsequent post-hoc analyses show that this significance is driven by the effect in the enlarged broods.

Parasite prevalence varied between ($\chi^2_5 = 13.02$, p-value = .023) and within years, and to disentangle the effects of these different levels of variation on parasite-dependent telomere shortening, we separated within- and between-year parasite prevalence variation. We found a significant effect of parasite infestation on telomere shortening within years (age×delta *Carnus* interaction, Table S3) but not between years (no between-year effect, no age×average *Carnus* interaction, Tables S3 and S4), confirming that the parasite effect on telomere shortening is consistent within years and it is not dependent on parasite abundance variation between years.

3.2 | Parasite and brood manipulation effects on nestling growth

For the mass model, we expanded the dataset to include $N = 506$ nestlings without telomere length information but born in the same period (2012–2017) as the $N = 322$ nestlings included in the telomere

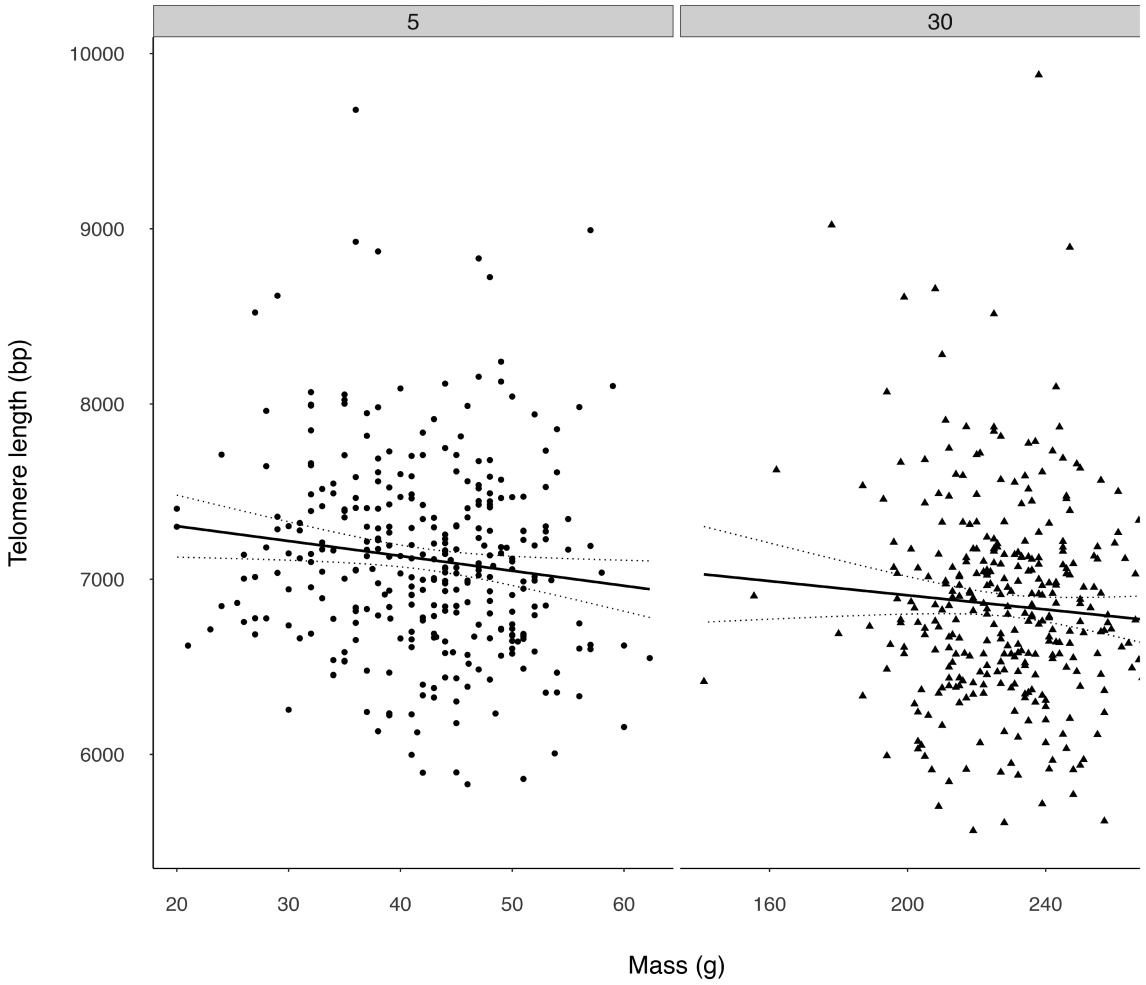

FIGURE 2 Telomere length depending on nestling body mass for nestling ages day 5 (left panel) and day 30 (right panel). Plots show raw data and regression line with 95% confidence interval, but note that mass was normalized for reduced and enlarged broods separately in the statistical analysis.

model. In this expanded data set, carnid flies were present in 247 of the 317 nests and in 708 of the 828 nestlings. Nestlings gained on average 135.9 ± 1.3 g over the 25-day measurement period (Table 1, mass model). Body mass gain was not affected by *Carnus* presence, and neither was there a significant interaction between brood size manipulation and *Carnus* presence (Table 1). However, nestlings reared in enlarged broods gained on average 8.9 ± 1.4 g less mass than nestlings reared in reduced broods (Table 1, Figure 4). Mass gain was also significantly different between sexes, with males gaining on average 19.4 ± 1.4 g more mass than females (Table 1, Figure S3).

4 | DISCUSSION

Parasitic infection by the carnid fly and sibling competition interacted to negatively affect telomere dynamics in developing individuals. Telomere shortening was accelerated in parasitized nestling jackdaws reared in enlarged broods but not in parasitized nestlings reared in reduced broods, indicating a synergistic effect of these stressors. Furthermore, ectoparasitic infections by *Carnus hemapterus* did not affect nestling growth but nestlings reared in enlarged broods gained less weight, in accordance with previous findings in the same population (Boonekamp et al., 2014).

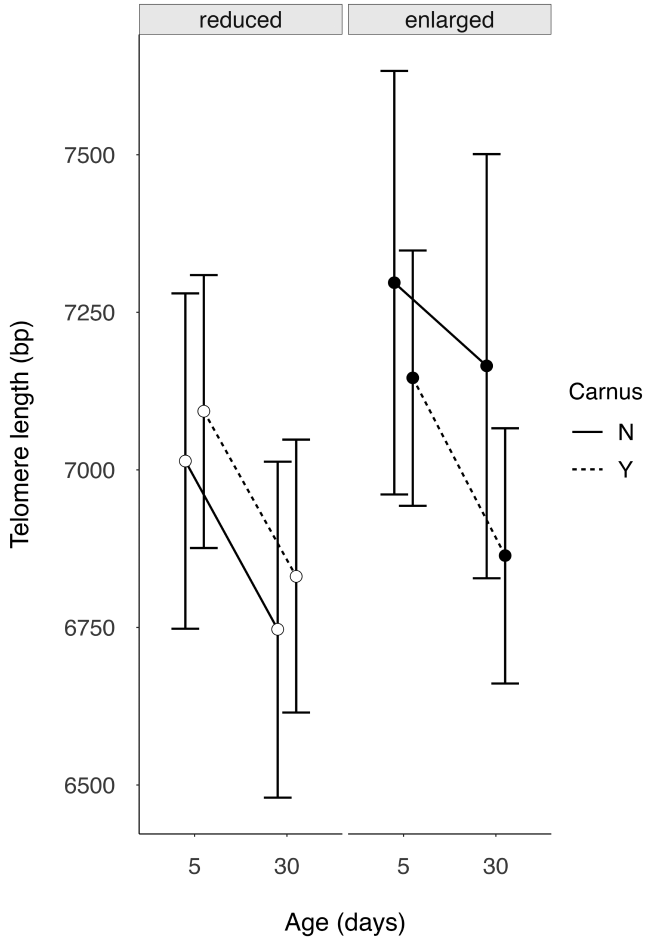

FIGURE 3 Telomere length in relation to age, brood size manipulation and *Carnus* presence in wild jackdaw nestlings. Data points (estimated marginal means) and confidence interval (bars) are shown separately for the reduced (left panel, open circles) and enlarged groups (right panel, closed circles). Telomere shortening over the development period is shown as solid line for non-parasitized individuals (N = *Carnus* absence) and dashed line for parasitized individuals (Y = *Carnus* presence).

Telomeres shortened on average 264 bp over the 25-day development period, but when nestlings in enlarged broods suffered from infections by the parasitic carnid fly, this was increased to 428 bp, a 62% increase relative to the overall mean. This increase could be a direct effect of enhanced cell proliferation to replenish the red blood cells (RBCs) depleted by the haematophagous fly, as reported for species parasitized by blowfly larvae (O'Brien et al., 2001; Sun et al., 2020). Alternatively, but not mutually exclusive, increased telomere loss could be an indirect effect of the parasitic infection, for example, through an increase in oxidative stress (Armstrong & Boonekamp, 2023). The latter possibility is supported by studies reporting increased oxidative stress in birds infected by haematophagous ectoparasites (López-Arrabé et al., 2015; Martínez-de la Puente et al., 2011; Mougeot et al., 2010). However, the effect of parasitic infections on telomere dynamics via oxidative stress remains speculative at this stage. Future studies combining brood size manipulation with anti-parasite medication would further confirm a direct effect of parasitic infections on telomere dynamics (i.e. when infected nestlings shorten their telomeres more than non-infected siblings reared in reduced broods). Additionally, measuring physiological parameters such as haemoglobin and markers of oxidative damage could shed light on the mechanism through which blood-sucking parasites increase telomere loss.

The effect of parasitic infection on telomere shortening was contingent on brood size, with *Carnus* presence accelerating telomere shortening in enlarged broods, but not in reduced broods. This result is in line with findings in other studies showing that adversity effects on telomere dynamics are more evident under ecological hardship (e.g. in the American alligator, Bae et al., 2022; McLennan et al., 2016). The finding that nestlings in enlarged broods were more susceptible to effects of *Carnus* infestation is an example of 'a reverse Matthew effect' (Zhe Jin et al., 2013). The Matthew effect is the phenomenon that individuals who are doing well benefit more from new opportunities than individuals who do less well ('to those that have shall be given'). The 'reverse Matthew effect' aggravates individual differences in a complementary way: individuals who are in a poorer state suffer more from additional adversity than individuals in a better state ('from those that have little will be taken'). This appears to be a widespread phenomenon (e.g. Briga et al., 2017). Occurrence of 'reverse Matthew effects' during early life may contribute to the fitness consequences of adverse developmental conditions, in the sense that offspring that are in a poorer state because of a particular adverse aspect of their environment may end up more disadvantaged relative to individuals in a better state due to additional challenges.

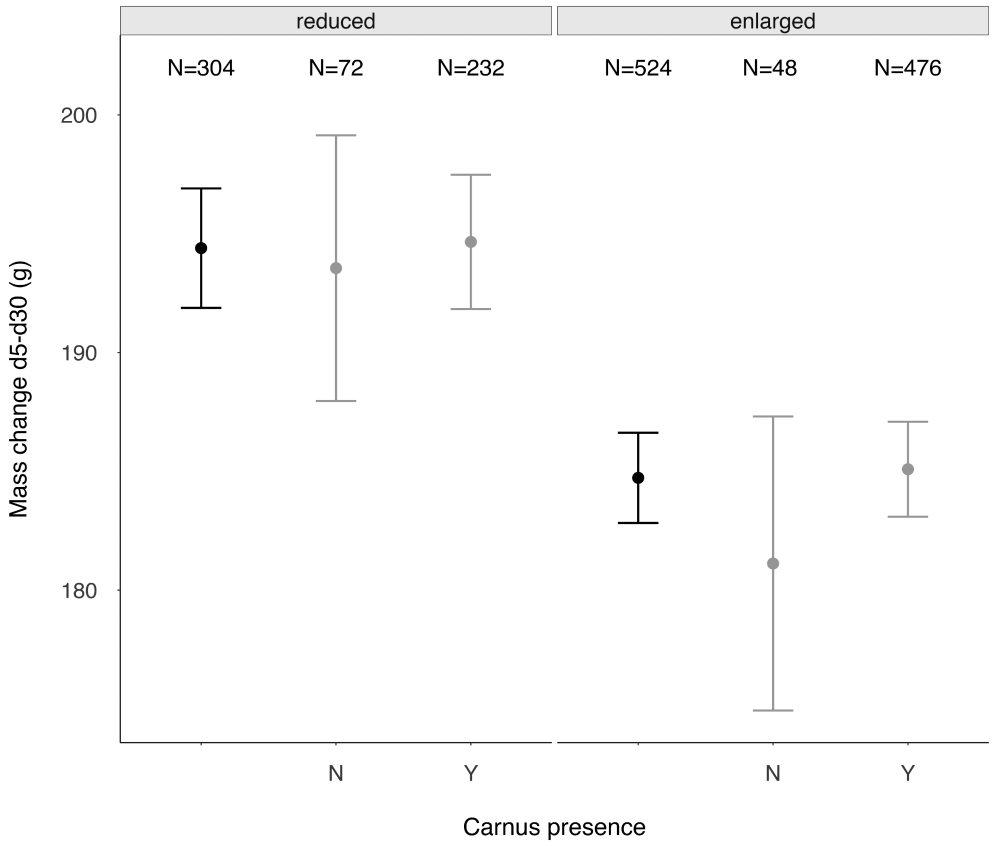

FIGURE 4 Change in jackdaw nestling body mass from day 5 to day 30 in reduced (left panel) and enlarged broods (right panel). Raw means and confidence intervals (bars) are shown for all individuals (black), or separately for unparasitized (N, grey) and parasitized (Y, grey) nestlings. The interaction between brood size manipulation and *Carnus* infestation did not significantly affect mass change.

Environmental adversity generally decreases growth rate, and adverse conditions accelerate telomere shortening. One could therefore expect fast growth to be associated with long telomeres (Monaghan & Ozanne, 2018). However, reality is more complex because studies find contrasting relationships between growth and telomere length (Vedder et al., 2017). We found TL to decrease with increasing body mass (scaled – see Methods), and this association was independent of age, brood size manipulation and *Carnus* infestation. The fact that the mass effect on TL did not change with age, despite the approximately fourfold increase in body mass, could be taken to mean that the mass effect at fledging age was in fact the day 5 mass effect that was carried over to fledging age. This underlines our earlier finding that individual variation in telomere length is largely determined very early in life (Boonekamp et al., 2014). Telomere dynamics during development is the outcome of resource allocation between growth and somatic maintenance, and depending on variation in resource allocation and availability positive and negative associations between growth and TL can arise (Vedder et al., 2017). Apparently, jackdaw nestlings canalized resources to early growth at the expense of telomere maintenance, possibly because day 5 mass has strong fitness consequences, with survival up to fledging increasing strongly with increasing day 5 mass (Boonekamp et al., 2018; Borger et al., 2023).

Had we restricted our study of the effects of *Carnus* infection to growth, we would have concluded that *Carnus* infection does not noticeably affect jackdaw nestlings, and it is only with the extension of our study to telomere dynamics that effects of *Carnus* infection were revealed. Given the earlier finding that telomere shortening predicts post-fledging survival to recruitment in this population (Boonekamp et al., 2014), we assume that *Carnus* infection thereby affects offspring fitness prospects, but we acknowledge that this remains to be established. Here, we indicate that the effects of early-life adversity depend on environmental conditions (i.e. parasite presence), complicating predictions of early-life effects on fitness. Thus, the implications of parasitic infections in combination with brood competition in the longer term remain to be explored under different scenarios. Telomere length and dynamics are proving a useful tool for this purpose (e.g. Eastwood et al. (2022)); DNA methylation is another promising candidate in this context (Tangili et al., 2023). For example, it might be that extrinsic stressors have an additive effect on survival, further confirming the impact that early-life adversity can have on fitness prospects (Cooper & Kruuk, 2018). Alternatively, when the negative effects of parasitic infections are more canalized (Boonekamp et al., 2018), one might expect no direct effect of *Carnus* parasites on survival. Furthermore, cryptic sub-lethal effects of developmental conditions that are expressed beyond the timeframe of the

study highlight the need to evaluate the effects of developmental conditions beyond morphological parameters.

AUTHOR CONTRIBUTIONS

The study was conceived by EPB and SV. Samples were collected by CB, JB and SV. Telomere analyses were performed by CB and EM. Statistical analyses were conducted by EPB with input from SV. The paper was written by EPB with extensive input from SV and additional comments and input from all authors. All authors gave final approval for publication.

ACKNOWLEDGEMENTS

We thank Martijn Salomons, the late Cor Dijkstra and many students who contributed to the fieldwork. Fieldwork was approved by the University of Groningen animal ethics committee under licence numbers (4071, 5871, 6832-A & 6832-B) before conducting research.

FUNDING INFORMATION

EPB was supported by funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 8444403. CB was supported by a DFG fellowship BA 5422/1-1. JJB was supported by NWO grant 823.01.006 awarded to Franjo Weissing and by NWO grant 823.01.009 awarded to SV, which also covered fieldwork and analyses costs.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data used for this manuscript are available from the Dryad Digital Repository (https://doi.org/10.5061/dryad.bcc2fqzjx; Badás et al., 2023) and code is available at GitHub (https://github.com/elisa-P-badas).

BENEFIT SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

ORCID

Elisa P. Badás https://orcid.org/0000-0001-9398-5440
Christina Bauch https://orcid.org/0000-0003-0218-5582
Jelle J. Boonekamp https://orcid.org/0000-0003-1900-627X
Simon Verhulst https://orcid.org/0000-0002-1143-6868

REFERENCES

Armstrong, E., & Boonekamp, J. (2023). Does oxidative stress shorten telomeres in vivo? A meta-analysis. *Ageing Research Reviews*, *85*, 101854. https://doi.org/10.1016/J.ARR.2023.101854

Asghar, M., Hasselquist, D., Zehtindjiev, P., Westerdahl, H., & Bensch, S. (2015). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, *347*(6220), 9–12.

Asghar, M., Palinauskas, V., Zaghdoudi-Allan, N., Valkiūnas, G., Mukhin, A., Platonova, E., Färnert, A., Bensch, S., Hasselquist, D., Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., Bensch, S., Blasco, M. A., Campisi, J., Hoeijmakers, J. H. J., Blackburn, E. H., … Valkiūnas, G. (2016). Parallel telomere shortening in multiple body tissues owing to malaria infection. *Proceedings. Biological Sciences/The Royal Society*, *283*(1836), 20161184. https://doi.org/10.1098/rspb.2016.1184

Aviv, A., & Shay, J. W. (2018). Reflections on telomere dynamics and ageing-related diseases in humans. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1741), 20160436. https://doi.org/10.1098/rstb.2016.0436

Badás, E. P., Bauch, C., Boonekamp, J. J., Mulder, E. & Verhulst, S. (2023). Data from: 'Ectoparasite presence and brood size manipulation interact to accelerate telomere shortening in nestling jackdaws'. Dryad Digital Repository. https://doi.org/10.5061/dryad.bcc2fqzjx

Badás, E. P., Martínez, J., Rivero-de Aguilar, J., Miranda, F., Figuerola, J., & Merino, S. (2015). Ageing and reproduction: Antioxidant supplementation alleviates telomere loss in wild birds. *Journal of Evolutionary Biology*, *28*(4), 896–905. https://doi.org/10.1111/jeb.12615

Bae, J., Bertucci, E. M., Bock, S. L., Hale, M. D., Moore, J., Wilkinson, P. M., Rainwater, T. R., Bowden, J. A., Koal, T., PhamTuan, H., & Parrott, B. B. (2022). Intrinsic and extrinsic factors interact during development to influence telomere length in a long-lived reptile. *Molecular Ecology*, *31*(23), 6114–6127. https://doi.org/10.1111/MEC.16017

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4.

Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2022). High heritability of telomere length and low heritability of telomere shortening in wild birds. *Molecular Ecology*, *31*(23), 6308–6323. https://doi.org/10.1111/mec.16183

Benetos, A., Toupance, S., Gautier, S., Labat, C., Kimura, M., Rossi, P. M., Settembre, N., Hubert, J., Frimat, L., Bertrand, B., Boufi, M., Flecher, X., Sadoul, N., Eschwege, P., Kessler, M., Tzanetakou, I. P., Doulamis, I. P., Konstantopoulos, P., Tzani, A., … Aviv, A. (2018). Short leukocyte telomere length precedes clinical expression of atherosclerosis: The blood-and-muscle model. *Circulation Research*, *122*(4), 616–623. https://doi.org/10.1161/CIRCRESAHA.117.311751

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, *350*(6319), 569–573.

Boonekamp, J. J., Bauch, C., & Verhulst, S. (2020). Experimentally increased brood size accelerates actuarial senescence and increases subsequent reproductive effort in a wild bird population. *Journal of Animal Ecology*, *89*(6), 1395–1407. https://doi.org/10.1111/1365-2656.13186

Boonekamp, J. J., Mulder, E., & Verhulst, S. (2018). Canalisation in the wild: Effects of developmental conditions on physiological traits are inversely linked to their association with fitness. *Ecology Letters*, *21*(6), 857–864. https://doi.org/10.1111/ele.12953

Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., & Verhulst, S. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1785), 20133287.

Borger, M. J., Bauch, C., Boonekamp, J. J., & Verhulst, S. (2023). Egg size effects on nestling mass in jackdaws Corvus monedula: A cross-foster experiment. *Journal of Avian Biology*, *2023*(5–6), e03099. https://doi.org/10.1111/JAV.03099

Briga, M., Koetsier, E., Boonekamp, J. J., Jimeno, B., & Verhulst, S. (2017). Food availability affects adult survival trajectories depending on early developmental conditions. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1846), 20162287. https://doi.org/10.1098/rspb.2016.2287

Chatelain, M., Drobniak, S. M., & Szulkin, M. (2019). The association between stressors and telomeres in non-human vertebrates: A meta-analysis. *Ecology Letters*, *23*, 381–398. https://doi.org/10.1111/ele.13426

Chung, Y., Rabe-Hesketh, S., Dorie, V., Gelman, A., & Liu, J. (2013). A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. *Psychometrika*, *78*(4), 685–709. https://doi.org/10.1007/s11336-013-9328-2

Cooper, E. B., & Kruuk, L. E. B. (2018). Ageing with a silver-spoon: A meta-analysis of the effect of developmental environment on senescence. *Evolution Letters*, *2*(5), 460–471. https://doi.org/10.1002/evl3.79

Driessen, M. M. G., Versteegh, M. A., Gerritsma, Y. H., Tieleman, B. I., Pen, I. R., & Verhulst, S. (2022). Effects of manipulated food availability and seasonality on innate immune function in a passerine. *Journal of Animal Ecology*, *91*(12), 2400–2411. https://doi.org/10.1111/1365-2656.13822

Eastwood, J. R., Connallon, T., Delhey, K., Hall, M. L., Teunissen, N., Kingma, S. A., La Porte, A. M., Verhulst, S., & Peters, A. (2022). Hot and dry conditions predict shorter nestling telomeres in an endangered songbird: Implications for population persistence. *Proceedings of the National Academy of Sciences*, *119*(25), e2122944119. https://doi.org/10.1073/pnas.2122944119

Eastwood, J. R., Dupoué, A., Delhey, K., Verhulst, S., Cockburn, A., & Peters, A. (2023). When does early-life telomere length predict survival? A case study and meta-analysis. *Molecular Ecology*, *32*(11), 3000–3013. https://doi.org/10.1111/MEC.16894

Factor-Litvak, P., Susser, E., Kezios, K., McKeague, I., Kark, J. D., Hoffman, M., Kimura, M., Wapner, R., & Aviv, A. (2016). Leukocyte telomere length in newborns: Implications for the role of telomeres in human disease. *Pediatrics*, *137*(4), e20153927. https://doi.org/10.1542/peds.2015-3927

Gilbert, S. F. (2001). Ecological developmental biology: Developmental biology meets the real world. *Developmental Biology*, *233*(1), 1–12. https://doi.org/10.1006/DBIO.2001.0210

Karell, P., Bensch, S., Ahola, K., & Asghar, M. (2017). Pale and dark morphs of tawny owls show different patterns of telomere dynamics in relation to disease status. *Proceedings of the Royal Society of London B: Biological Sciences*, *284*(1859), 20171127.

Kärkkäinen, T., Briga, M., Laaksonen, T., & Stier, A. (2022). Within-individual repeatability in telomere length: A meta-analysis in nonmammalian vertebrates. *Molecular Ecology*, *31*(23), 6339–6359. https://doi.org/10.1111/mec.16155

López-Arrabé, J., Cantarero, A., Pérez-Rodríguez, L., Palma, A., Alonso-Alvarez, C., González-Braojos, S., & Moreno, J. (2015). Nest-dwelling ectoparasites reduce antioxidant defences in females and nestlings of a passerine: A field experiment. *Oecologia*, *179*(1), 29–41. https://doi.org/10.1007/s00442-015-3321-7

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., & Martínez, J. (2011). Nest ectoparasites increase physiological stress in breeding birds: An experiment. *Naturwissenschaften*, *98*(2), 99–106. https://doi.org/10.1007/s00114-010-0746-z

McLennan, D., Armstrong, J. D., Stewart, D. C., Mckelvey, S., Boner, W., Monaghan, P., & Metcalfe, N. B. (2016). Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. *Molecular Ecology*, *25*(21), 5425–5438. https://doi.org/10.1111/mec.13857

Monaghan, P., Eisenberg, D. T. A. A., Harrington, L., & Nussey, D. (2018). Understanding diversity in telomere dynamics. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *373*(1741), 20160435. https://doi.org/10.1098/rstb.2016.0435

Monaghan, P., Olsson, M., Richardson, D. S., Verhulst, S., & Rogers, S. M. (2022). Integrating telomere biology into the ecology and evolution of natural populations: Progress and prospects. *Molecular Ecology*, *31*(23), 5909–5916. https://doi.org/10.1111/MEC.16768

Monaghan, P., & Ozanne, S. E. (2018). Somatic growth and telomere dynamics in vertebrates: Relationships, mechanisms and consequences. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *373*(1741), 20160446. https://doi.org/10.1098/rstb.2016.0446

Mougeot, F., Martínez-Padilla, J., Blount, J. D., Perez-Rodriguez, L., Webster, L. M. I., & Piertney, S. B. (2010). Oxidative stress and the effect of parasites on a carotenoid-based ornament. *Journal of Experimental Biology*, *213*(10), 1796. https://doi.org/10.1242/jeb.044933

Muñoz-Lorente, M. A., Cano-Martin, A. C., & Blasco, M. A. (2019). Mice with hyper-long telomeres show less metabolic aging and longer lifespans. *Nature Communications*, *10*(1), 4723. https://doi.org/10.1038/s41467-019-12664-x

O'Brien, E. L., Morrison, B. L., & Johnson, L. S. (2001). Assessing the effects of haematophagous ectoparasites on the health of nestling birds: Haematocrit vs haemoglobin levels in house wrens parasitized by blow fly larvae. *Journal of Avian Biology*, *32*(1), 73–76. https://doi.org/10.1034/j.1600-048X.2001.320110.x

R Development Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reichert, S., Criscuolo, F., Zahn, S., Arrivé, M., Bize, P., & Massemin, S. (2015). Immediate and delayed effects of growth conditions on ageing parameters in nestling zebra finches. *The Journal of Experimental Biology*, *218*(Pt 3), 491–499. https://doi.org/10.1242/jeb.109942

Roulin, A., Brinkhof, M. W. G., Bize, P., Richner, H., Jungi, T. W., Bavoux, C., Boileau, N., & Burneleau, G. (2003). Which chick is tasty to parasites? The importance of host immunology vs. parasite life history. *Journal of Animal Ecology*, *72*(1), 75–81. https://doi.org/10.1046/j.1365-2656.2003.00677.x

Salomons, H. M., Mulder, G. A., van de Zande, L., Haussmann, M. F., Linskens, M. H. K., & Verhulst, S. (2009). Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1670), 3157–3165. https://doi.org/10.1098/rspb.2009.0517

Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, *1*, 103–113. https://doi.org/10.1111/j.2041-210X.2010.00012.x

Slowinski, S. P. (2017). *How parasites affect, and are affected by, host physiology, behavior, and breeding system*. Indiana University ProQuest Dissertations Publishing.

Stauffer, J., Bruneaux, M., Panda, B., Visse, M., Vasemägi, A., & Ilmonen, P. (2017). Telomere length and antioxidant defense associate with parasite-induced retarded growth in wild brown trout. *Oecologia*, *185*(3), 365–374. https://doi.org/10.1007/s00442-017-3953-x

Stockley, P., & Bro-Jørgensen, J. (2011). Female competition and its evolutionary consequences in mammals. *Biological Reviews*, *86*(2), 341–366. https://doi.org/10.1111/J.1469-185X.2010.00149.X

Sudyka, J., Podmokła, E., Drobniak, S. M., Dubiec, A., Arct, A., Gustafsson, L., & Cichoń, M. (2019). Sex-specific effects of parasites on telomere dynamics in a short-lived passerine—The blue tit. *The Science of Nature*, *106*(1–2), 6. https://doi.org/10.1007/s00114-019-1601-5

Sun, N. W., Goodwin, S. E., Griego, M. S., Gerson, A. R., & Clotfelter, E. D. (2020). Does blood loss explain higher resting metabolic rates in nestling birds with hematophagous ectoparasites? *Journal of Avian Biology*, *51*(2), jav.02264. https://doi.org/10.1111/jav.02264

Tangili, M., Slettenhaar, A. J., Sudyka, J., Dugdale, H. L., Pen, I., Palsbøll, P. J., & Verhulst, S. (2023). DNA methylation markers of age(ing) in non-model animals. *Molecular Ecology.*, *32*, 4725–4741. https://doi.org/10.1111/MEC.17065

Tschirren, B., Romero-Haro, A. Á., Zahn, S., & Criscuolo, F. (2021). Sex-specific effects of experimental ectoparasite infestation on

telomere length in great tit nestlings. *Journal of Evolutionary Biology*, *34*(3), 584–589. https://doi.org/10.1111/jeb.13744

van de Pol, M., & Wright, J. (2009). A simple method for distinguishing within-versus between-subject effects using mixed models. *Animal Behaviour*, *77*(3), 753–758.

Vedder, O., & Bouwhuis, S. (2018). Heterogeneity in individual quality in birds: Overall patterns and insights from a study on common terns. *Oikos*, *127*(5), 719–727. https://doi.org/10.1111/OIK.04273

Vedder, O., Verhulst, S., Bauch, C., & Bouwhuis, S. (2017). Telomere attrition and growth: A life-history framework and case study in common terns. *Journal of Evolutionary Biology*, *30*(7), 1409–1419. https://doi.org/10.1111/jeb.13119

Veiga, J., Moreno, E., Benzal, J., & Valera, F. (2019). Off-host longevity of the winged dispersal stage of *Carnus hemapterus* (Insecta: Diptera) modulated by gender, body size and food provisioning. *Parasitology*, *146*(2), 241–245. https://doi.org/10.1017/S0031182018001300

Wang, Q., Zhan, Y., Pedersen, N. L., Fang, F., & Hägg, S. (2018). Telomere length and all-cause mortality: A meta-analysis. *Ageing Research Reviews*, *48*, 11–20. https://doi.org/10.1016/J.ARR.2018.09.002

Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: A meta-analysis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *373*(1741), 20160447. https://doi.org/10.1098/rstb.2016.0447

Young, R. C., Welcker, J., Barger, C. P., Hatch, S. A., Merkling, T., Kitaiskaia, E. V., Haussmann, M. F., & Kitaysky, A. S. (2017). Effects of developmental conditions on growth, stress and telomeres in black-legged kittiwake chicks. *Molecular Ecology*, *26*(13), 3572–3584. https://doi.org/10.1111/mec.14121

Zhe Jin, G., Jones, B., Feng Lu, S., & Uzzi, B. (2013). *The reverse Matthew effect: Catastrophe and consequence in scientific teams*. National Bureau of Economic Research, No. w19489. https://doi.org/10.3386/W19489

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Badás, E. P., Bauch, C., Boonekamp, J. J., Mulder, E., & Verhulst, S. (2023). Ectoparasite presence and brood size manipulation interact to accelerate telomere shortening in nestling jackdaws. *Molecular Ecology*, *32*, 6913–6923. https://doi.org/10.1111/mec.17177