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Author for correspondence:
Rebecca C. Young
e-mail: rebeccacyoung721@gmail.com

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Stressors interact across generations to influence offspring telomeres and survival

Rebecca C. Young[1], David F. Westneat[2], Jennifer Vangorder-Braid[1], Aubrey E. Sirman[1], Stefanie J. Siller[3], Jeffrey Kittilson[1], Anuj Ghimire[1] and Britt J. Heidinger[1]

[1]Department of Biological Sciences, North Dakota State University, Fargo, ND, USA
[2]Department of Biology, University of Kentucky, Lexington, KY, USA
[3]Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY, USA

RCY, 0000-0002-3227-7966; DFW, 0000-0001-5163-8096; BJH, 0000-0003-0064-209X

Parental stress often has long-term consequences for offspring. However, the mechanisms underlying these effects and how they are shaped by conditions offspring subsequently experience are poorly understood. Telomeres, which often shorten in response to stress and predict longevity, may contribute to, and/or reflect these cross-generational effects. Traditionally, parental stress is expected to have negative effects on offspring telomeres, but experimental studies in captive animals suggest that these effects may depend on the subsequent conditions that offspring experience. Yet, the degree to which parental stress influences and interacts with stress experienced by offspring to affect offspring telomeres and survival in free-living organisms is unknown. To assess this, we experimentally manipulated the stress exposure of free-living parent and offspring house sparrows (*Passer domesticus*). We found a weak, initial, negative effect of parental stress on offspring telomeres, but this effect was no longer evident at the end of post-natal development. Instead, the effects of parental stress depended on the natural sources of stress that offspring experienced during post-natal development whereby some outcomes were improved under more stressful rearing conditions. Thus, the effects of parental stress on offspring telomeres and survival are context-dependent and may involve compensatory mechanisms of potential benefit under some circumstances.

1. Background

Stress experienced by parents can have profound and lasting consequences for subsequent generations [1–4]. For example, human mothers that experienced the severe nutritional and psychological stress of the 1944–1945 Dutch Hongerwinter Famine during pregnancy produced offspring that were more susceptible to disease and had higher mortality in adulthood [4]. Cross-generational effects of stress may be common in vertebrates and often help to explain non-intuitive population dynamics [1,2]. For example, in snowshoe hares, predation causes stress-induced parental effects that can persist for multiple generations and contribute to a lag in population recovery following a decline in predator abundance [5]. Much of the detail regarding the timing and mechanism of cross-generational effects remains unclear, but this information will be critical for predicting the long-term consequences of parental stress exposure for both parents and offspring.

Telomeres may be linked to or reflect these long-term cross-generational effects [6]. Telomeres are a highly conserved nucleoprotein structure that caps and protects linear chromosomes but degrades during cellular division and in response to stress [7,8]. Individuals that experience stressful circumstances often experience greater telomere loss during development [9–13] and in adulthood [14–16,55]. Once telomeres become critically short, cells stop dividing and can secrete inflammatory compounds, and both of these processes are expected

to contribute to organismal ageing [7]. In support of this idea, individuals with longer telomeres often have longer lifespans [17–20]. In some species, this relationship is already present during early life and positively predicts lifetime reproductive success [18,21]. Accumulating evidence also suggests that stress experienced by parents can influence the telomeres of their offspring [12,22]. Thus, it seems reasonable to expect that stress experienced by parents would have negative effects on offspring telomeres regardless of the conditions that offspring subsequently experience. In support of this, several studies in humans have demonstrated that mothers that report stressful circumstances during pregnancy produce offspring with shorter telomeres at birth and in adulthood [12,13]. However, human studies are necessarily correlative, and it is difficult to separate cause and effect, as mothers that are more likely to experience circumstances as stressful may also be more likely to produce offspring with shorter telomeres.

Interestingly, experimental studies in captive rats (*Rattus norvegicus*) and zebra finches (*Taeniopygia guttata*) suggest that the effects of parental stress exposure on offspring telomeres may often be context-dependent and vary depending on the sex [22,23] and subsequent environmental conditions experienced by offspring [24,25]. However, we currently do not know how stressors experienced by parents and offspring interact to influence offspring telomeres and survival in free-living organisms [26]. Here we experimentally investigated the effects of parental and offspring stress exposures on offspring telomeres and survival in free-living house sparrows (*Passer domesticus*).

Previously, we have reported that telomere length (TL) decreases with age in this species and that females with longer telomeres at the end of post-natal development have greater longevity and higher lifetime reproductive success [21]. To test the degree to which stress experienced by parents affects and interacts with stress experienced by offspring to influence offspring telomeres and survival we experimentally manipulated parental stress exposure prior to offspring production by exposing parents to a standardized rotating series of stressors or an undisturbed control treatment. After hatching, offspring within nests of both treatments were exposed to either a standardized handling and restraint stressor or an undisturbed control treatment. We also examined the potential effects of several other post-natal nestling stressors previously shown to influence offspring TL including brood size [27,28] and relative mass within the brood [29]. This experimental design allowed us to test whether parental stress exposure negatively impacted offspring telomeres and survival and the degree to which these effects depended on the subsequent environmental conditions that offspring experienced.

2. Methods

(a) Study system and experimental manipulation of stress exposure in parents

This study was conducted between April and August 2018 and 2019 on a free-living population of house sparrows that breeds in nest-boxes on the agricultural buildings at North Dakota State University (46.9 N, −96.8 W). Nest-boxes were monitored throughout the season to determine exact reproductive timing and output including nest completion, egg laying, clutch size (the total number of eggs laid), brood size (the total number of eggs that hatched) and the total number of nestlings that survived until 10 days post-hatching. We did not check nests after 10 days post-hatching to avoid causing the nestlings to prematurely leave the nest.

During nest building, we assigned pairs randomly in a balanced design to either an experimental parental stressor (PS) or a parental control (PC) treatment. PS and PC nests were always at least 10 m apart and whenever possible on alternate sides of buildings to ensure that control parents were not inadvertently exposed to stressors directed at nearby nests; indeed, there was no orientational bias in the distribution of control and experimental nests within or among barns ($\chi^2 = 4.46$, d.f. = 3, $p = 0.21$). A small number (less than 25%) of repeated breeding attempts by unknown parents at the same nest-box received the same treatment to avoid cross-over effects between treatments. We exposed parents in the PS treatment to a standardized, unpredictable, rotating series of stressors at the nest-box between the time they began building the nest until the first egg was laid. Every other day, stressors were presented three times per day, in a series of alternating half-hour time periods of stressor presentation followed by half-hour breaks with no stressor presentation. We assigned starting times randomly during morning daylight hours. Stressors included in the rotation were relevant predator mounts (American kestrel, *Falco sparverius*; sharp-shinned hawk, *Accipiter striatus*; grey squirrel, *Sciurus carolinensis*), decoy predators (rubber coyote, artificial cat, owl decoy, hawk decoy, rubber snake), novel objects (sparkling pinwheel, plastic flower, stuffed owl toy, wooden chicken model) or a human standing under the box. Stressors were presented in a random order, and each was presented only once before repetition. Stressors were presented at experimental nests for an average of 13.4 ± 9.1 days (2 to 18 days of stressor presentation; 3 to 35 days including non-stressor days). This experimental stressor treatment has been previously shown to minimize habituation and increase glucocorticoid stress hormones, oxidative stress and telomere loss in other songbirds [30–32]. We did not present stressors at the boxes of parents in the PC treatment. Despite variation in the duration of exposure to stressors, the length of stressor exposure prior to laying did not significantly predict offspring TL ($r = 0.14$, $p = 0.14$) or day 2 mass ($r = 0.06$, $p = 0.47$).

(b) Experimental stressor manipulation in offspring and offspring sample collection

As soon as nestlings hatched, we randomly assigned them to either an experimental stressor (nestling stressor: NS) or control (nestling control: NC) treatment, with each brood containing at least one nestling from each treatment. Between days 2 and 10 after hatching, we removed nestlings in the NS treatment daily for half an hour and placed them in a small cloth bag for 30 min, which has been shown to induce telomere loss in nestling European shags (*Phalacrocorax aristotelis*) [9]. Nestlings in the NC treatment remained in the nest and did not experience this additional daily handling and restraint stressor but were disturbed briefly each day when nestlings in the NS treatment were removed from the nest. As an additional control, we included a group of PC nests where all nestlings were left unhandled between days 2 and 10 post-hatching. We term this treatment 'nestling undisturbed' (NU), and the NU treatment acts as a comparison group to offset potential stress to NC nestlings due to unavoidable proximity to NS nestlings. Thus, nestlings could belong to one of five combinations of parental and nestling treatments: PS-NS, PS-NC, PC-NS, PC-NC and PC-NU. Over two seasons we sampled a total of 46 PS nests (12 in 2018; 34 in 2019), 62 PC nests (21 in 2018 and 41 in 2019) and 46 PC nests with NU nestlings (15 in 2018;

31 in 2019) for a total of 523 nestlings (87 PS-NC; 73 PS-NS; 125 PC-NC; 107 PC-NS; 131 NU).

On days 2 and 10 post-hatching, all nestlings were blood sampled to measure TL and determine molecular sex (see details below) and their masses, tarsus lengths and wing chords were recorded. We coloured nestlings with Sharpie markers to identify them and at 10 days post-hatching they were banded with a USFWS metal band. The mass, tarsus length and wing chord of nestlings in the NS and NC groups were also recorded on days 6 and 8 post-hatching. To minimize additional disturbance, we did not mark individual eggs and monitor hatching order. However, nestling size was related to hatching order where earlier hatched nestlings were larger than their later hatched brood mates. As a proxy for rank within the brood, we calculated relative mass within the brood as day 2 mass minus the mass of the heaviest nestling within the brood. We gave the heaviest nestling a value of 0 and smaller siblings negative values. Smaller siblings at day 2 are likely those who hatched later, so they are younger as well as smaller leading to disadvantages in the competition for parental feeding.

(c) Blood sampling, telomere analysis and molecular sexing

On days 2 and 10 post-hatching small blood samples were collected from the alar vein with a 26 5/8 gauge needle and transferred to 0.5 ml Eppendorf tubes using capillary tubes. Blood was stored on ice in the field for less than 6 h before being spun down and separated into plasma and red blood cell fractions and stored at −80°C until further analysis. DNA was extracted from 4 µl of packed red blood cells using the NucleoSpin Blood kit (Machery Nagel, 740951), and extracts were frozen at −80°C until TL analysis. Extracts were measured on a Nanodrop 8000 (ThermoScientific) and only run with a 260/280 ratio of at least 1.8 and a 260/230 ratio of at least 1.9. Nestlings were genetically sexed using the P2/P8 primers [33,34].

TL was measured using quantitative real-time PCR on an Mx3000P (Agilent Technologies) [35,36]. This technique provides relative TL as a ratio of telomeric DNA to the quantity of a single-copy reference gene (here GAPDH). Primers were: telomeres - forward tel1b (5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTG GGTTTGGGTT-3′) and reverse tel2b (5′-GGCTTGCCTTACCCT TACCCTTACCCTTACCCTTACCCT-3′) and GAPDH - forward (5′-AACCAGCCAAGTACGATGACAT-3′) and reverse GAPDH (5′-CCATCAGCAGCAGCCTTCA-3′). The master mix was 6 µl water, 12.5 µl perfeCTa SYBR green supermix Low ROX (Quantabio), 0.25 µl each of the F and R primers and 6 µl of template containing 20 ng of DNA. Telomere and reference gene reactions took place on different plates. GAPDH was amplified via 40 cycles of 30 s at 95°C and 30 s at 60°C and telomeres by 27 cycles of 15 s at 95°C and 30 s at 58°C. Specificity was assessed by the presence of a single-peaked melting curve. Each pair of plates (GAPDH and telomere) was manually loaded, run on the same day, and assigned a unique assay ID. Samples were run in duplicate and rerun if the s.d. of the Ct was higher than 0.25: 16.5% of samples were re-run, but only 4 samples (0.4% of total) were unable to be brought within specifications. Samples from the same nest were run on the same assay, and treatments were balanced across assay. T/S ratios were calculated using the following formula: $2^{\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{telomere} - Ct_{GAPDH})_{reference} - (Ct_{telomere} - Ct_{GAPDH})$ [35]. For the entire dataset mean TL was 1.1 ± 0.57 units. A golden sample made of the same species was run on each plate and results were standardized to this sample. In our laboratory the $ICC_{(2,1)}$ of the reactions for house sparrows is 0.88, indicating high repeatability across plates. The standard curve was made from house sparrow DNA diluted by halves from a 40 ng reaction to a 2.5 ng reaction and run in triplicate. Slopes were linear over this range and were used to calculate primer efficiencies, which averaged 94% and 97% for telomeres and GAPDH, respectively, with a range of 85–115% and R^2 of 0.96 ± 0.3 (telo) and 0.99 ± 0.3 (GAPDH).

(d) Statistical analysis

We measured TL repeatability using the rptR program [37] in R [38]. Repeatability was assessed at the nestling and brood levels simultaneously with and without controlling for the assay in which they were analysed. We used linear mixed models to analyse TL. Although all assays met the criteria for quality, there was some among-assay variance. To control for this known source of measurement variance in both the fixed and random portions of the model, we included assay ID as a fixed effect factor. This reduced only slightly the degrees of freedom remaining for other terms in the model. For nestling survival, we employed a generalized linear mixed effect model. Nest identity within a year was included as a random factor, accounting for repeated sampling of individuals within nests and for differences among years. For all model families, collinearity assessment of predictor variables showed that variance inflation factors (VIF) were less than 1.22, and no correlation coefficient had an absolute value over 0.35 [39]. We examined residual plots to assess major violations of parametric assumptions [40]. Continuous predictors were centred on the mode to make main effects meaningful even with interactions in the model, and we used Satterthwaite's method of calculating denominator degrees of freedom. All analyses were conducted in the R package lme4 [41].

Our analyses allowed us to determine the extent to which parental stress experienced prior to offspring production affects offspring telomeres at the beginning of post-natal development and interacts with stressors experienced by offspring to influence offspring telomeres and survival at the end of post-natal development. We asked if experimental stressors in parents and offspring, and in the case of offspring, also natural stressors, influenced offspring TL and survival and if these effects interacted across generations. To determine the effect of the PS treatment on day 2 TL ($n = 499$), we used a model including parental treatment (PC/PS), clutch size and relative size within the brood at day 2 plus their interactions with parental treatment as fixed effects. These latter terms were not expected to have much influence at such an early age, but we included them due to the 48 h between hatch and sampling. Nestling sex and the date the nest hatched were included as possible contingency variables. In the entire dataset, 73% of nestlings survived from day 2 to day 10. To determine the effects of parental treatment and nestling stress treatment on day 10 TL ($n = 375$) and change in TL ($\Delta TL = TL_{Day10} - TL_{Day2}$; $n = 355$), we modelled the experimental stressor treatments of the parents (PC/PS) and nestlings (NC, NS, NU), and their interaction. We also included the likely natural stressors of brood size and relative mass within the brood and their interactions with the parental stress treatment. Sex and hatch date were also included as before. To determine the effect of experimental and natural stressors on survival between days 2 and 10 we used a binomial logit-link model ($n = 497$) with the same set of variables as above with the addition of day 2 TL. All TL response variables (day 2 TL, day 10 TL and ΔTL) were log-transformed to reduce heteroscedasticity of model residuals, and ΔTL was corrected for regression to the mean [42,43]. For ΔTL the difference between raw day 2 and day 10 TL was corrected for regression to the mean and then assessed for heteroscedasticity of model residuals. Since residuals were structured, we added a constant to make all ΔTL values positive (to avoid infinite or undefined values) and then log-transformed the values. Non-significant interactions were removed and models refitted in order to

Table 1. Model predicting log telomere length of 499 nestling house sparrows sampled at day 2. Significant estimates are bolded. Clutch size was centred on the modal size of 5 eggs. Estimates for assay ID are not shown, to save space. For the full model, including non-significant interactions, see output in electronic supplementary material, table S3.

	$\beta \pm$ SE	d.f.	*F* value	*p* value
intercept	**0.25 ± 0.11**			
parental treatment (stress)	**−0.096 ± 0.048**	**1, 127**	**4.0**	**0.047**
clutch size	**−0.054 ± 0.025**	**1, 137.6**	**4.6**	**0.035**
relative in-brood mass	0.0088 ± 0.0089	1, 398.1	0.98	0.32
sex (male)	−0.021 ± 0.026	1, 405.3	0.68	0.41
hatch date	0.00077 ± 0.0010	1, 133.6	0.60	0.44
assay ID	—	**33, 225.6**	**3.4**	**<0.0001**
random effects (nest)	var = 0.044, SE = 0.095			

Table 2. Models predicting (*a*) log telomere length in 375 house sparrow nestlings at 10 days post-hatching and (*b*) change in telomere length (days 2 to 10, also log-transformed) for 355 nestling house sparrows between days 2 and 10 post-hatching. Significant estimates are bolded. Brood size was centred on the modal size of four nestlings. Exclusion of highest TL values did not qualitatively change results (not shown). UC are unhandled controls. Estimates for assay are not shown, to save space. For the full model, including non-significant interactions, see output in electronic supplementary material, tables S4 and S6.

	(*a*) day 10 log(TL)				(*b*) ΔTL (log-transformed)			
	$\beta \pm$ SE	d.f.	*F*	*p*	$\beta \pm$ SE	d.f.	*F*	*p*
intercept	−0.15 ± 0.21				0.059 ± 0.2			
parental treatment (stress)	−0.14 ± 0.10	1, 126.1	1.9	0.17	−0.17 ± 0.086	1, 121.9	3.8	0.055
nestling treatment		2, 164.4	0.39	0.68		2, 160	1.8	0.17
(stress)	0.026 ± 0.042				0.047 ± 0.04			
(UC)	0.078 ± 0.10				−0.099 ± 0.087			
brood size	**−0.12 ± 0.036**	**1, 117.1**	**11**	**0.0012**	**−0.088 ± 0.029**	**1, 112.4**	**9.2**	**0.0030**
relative mass w/in brood	0.027 ± 0.020	1, 266	1.5	0.22	0.038 ± 0.02	1, 268.8	1.2	0.27
sex (male)	0.056 ± 0.040	1, 268.4	2.0	0.16	0.042 ± 0.039	1, 266	1.2	0.28
hatch date	0.0026 ± 0.0019	1, 110.5	2.0	0.16	0.001 ± 0.0016	1, 99.5	0.41	0.52
assay ID	—	**33, 181.5**	**2.0**	**0.0022**	—	**33, 162.6**	**1.6**	**0.036**
par. treat (stress): rel. in-brood mass	**−0.089 ± 0.029**	**1, 267.4**	**9.8**	**0.0020**	**−0.11 ± 0.028**	**1, 271.8**	**14.6**	**0.00017**
random effects (nest)	var = 0.14, SE = 0.094				var = 0.077, SE = 0.12			

provide clarity on main effects; this had little effect on the remaining terms, and all full models have been placed in the supplemental materials.

3. Results

(a) Variation in offspring telomere length

We partitioned the variance in offspring TL and assessed its repeatability. Day 2 and day 10 TL were significantly positively correlated ($r = 0.16$, $t = 3.0$, $p = 0.0032$) and offspring TL was significantly repeatable (0.14 ± 0.05, LRT = 7.4, $p = 0.003$). All of an individual's samples were run in the same assay, and controlling for assay reduced offspring level repeatability to 0.045 ± 0.052. However, repeatability at the nest level was significant even with assay included (0.20 ± 0.045, LRT = 16.8, $p < 0.0001$), indicating brood level effects on nestling TL.

(b) Main effects of parental stressor treatment

Control and stressor-exposed parents did not differ significantly in latency to lay, clutch size, hatching success, brood size, number of nestlings at day 10 or hatch date (electronic supplementary material, tables S1 and S2). Nestlings produced by PS parents had significantly shorter TL at day 2 (table 1) than nestlings produced by PC parents, but this effect did not persist to the end of post-natal development and there were no significant effects of the parental treatment on the ΔTL between days 2 and 10 or day 10 TL (table 2). There was also no significant main effect of the parental treatment on offspring survival (table 3).

(c) Main effects of experimental and natural nestling stressors

We found no significant main effect of the NS treatment on TL at Day 10 (table 2). We did find a significant main

Table 3. Logistic model predicting survival between days 2 and 10 for 497 nestling house sparrows. Significant estimates are bolded. Brood size was centred on the modal size of 4 nestlings. UC = unhandled control nestlings. For the full model, including non-significant interactions, see output in electronic supplementary material, table S5.

	estimate	*z* value	*p* value
intercept	−0.29 ± 0.72	−0.40	0.69
parental treatment (stress)	0.72 ± 0.62	1.2	0.24
day 2 telomere length	**1.4 ± 0.52**	**2.8**	**0.0055**
nestling treatment (stress)	**0.98 ± 0.36**	**2.7**	**0.0067**
nestling treatment (UC)	0.030 ± 0.63	0.047	0.96
sex (male)	−0.037 ± 0.31	−0.12	0.91
hatch date	**0.051 ± 0.012**	**4.2**	**<0.0001**
brood size	−0.38 ± 0.26	−1.4	0.15
relative mass w/in brood	**0.43 ± 0.12**	**3.5**	**0.00045**
parental treatment (stress): brood size	**1.3 ± 0.51**	**2.6**	**0.011**
random effects (nest)	var = 1.87, SE = 0.51		

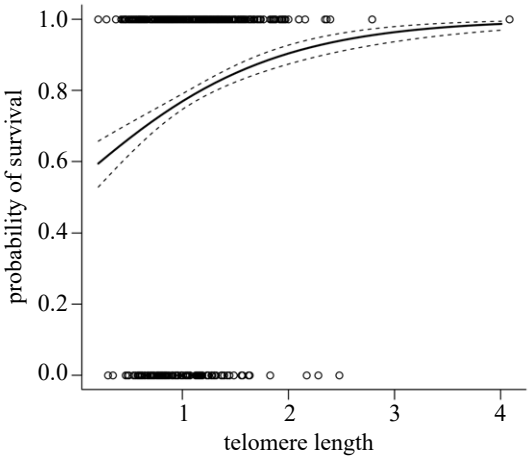

Figure 1. The relationship between telomere length (T/S ratio, measured using qPCR) at day 2 and survival from day 2 to 10 for 497 nestling house sparrows (*Passer domesticus*). Dashed lines are SE.

effect on offspring survival (table 3), where unexpectedly, experimentally stressed nestlings survived better than did control nestlings, regardless of parental treatment.

By contrast, natural stressors (like large brood size and small relative mass within the brood) had the predicted negative effects on nestlings. Nestlings in larger clutches and broods had significantly shorter TL at day 2 (table 1) and day 10 (table 2) and lost more TL between days 2 and 10 (table 2). Nestlings that were relatively smaller within the brood were significantly less likely to survive to day 10 than nestlings that were relatively larger within the brood (table 3). Nestlings with shorter day 2 TL were also significantly less likely to survive than nestlings with longer day 2 TL (table 3, figure 1). Lastly, nestlings that hatched later in the season were more likely to survive, indicating seasonal variation as a potential additional source of stress (table 3).

(d) Interactions between parental and nestling stressors

We found no significant effect of interactions between our experimental stress treatments (stressor presentation to parents and standardized nestling stress exposure) on offspring TL, ΔTL or survival (tables 2 and 3; electronic supplementary material, tables). Importantly though, we did find significant interactions between the PS treatment and the natural nestling stressors on offspring TL, ΔTL and survival. PS exposure significantly interacted with relative mass within the brood to predict day 10TL and the ΔTL (table 2). Relatively small nestlingstended to have smaller day 10 TL and more telomere loss than large nestlings in control nests, but a significantly different and opposite pattern occurred in broods of stressed parents, with smaller nestlings doing somewhat better than their larger nestmates (figure 2).

The PS treatment also significantly interacted with brood size to predict offspring survival from day 2 to day 10 post-hatching (table 3 and figure 3) with slopes of opposite signs in PS and PC nests. In PS nests, nestlings in larger broods tended to have higher survival, while in PC nests, nestlings in larger broods tended to have lower survival.

4. Discussion

Parental stress exposure often has long-term consequences for offspring [12,22,24]. Yet, the mechanisms that underlie these effects and how they are shaped by the subsequent environment that offspring experience are not well understood. We report that in free-living house sparrows the direction of the effect of the parental stress exposure on offspring telomeres and survival did indeed depend on the environmental conditions that offspring experienced during post-natal development. We found a small, but significant, initial negative effect of parental stress treatment on offspring telomeres shortly after hatching (day 2) similar to theoretical silver spoon models showing negative effects of a poor start [2], but this effect was transitory and no longer present at the end of post-natal development (day 10). Instead, the effect of parental stress exposure on offspring telomeres and survival depended on interactions with the natural, but not experimental, nestling stressors experienced during post-natal development. Relative mass within the brood predicted day 10 TL differently and with opposing slopes (figure 2)

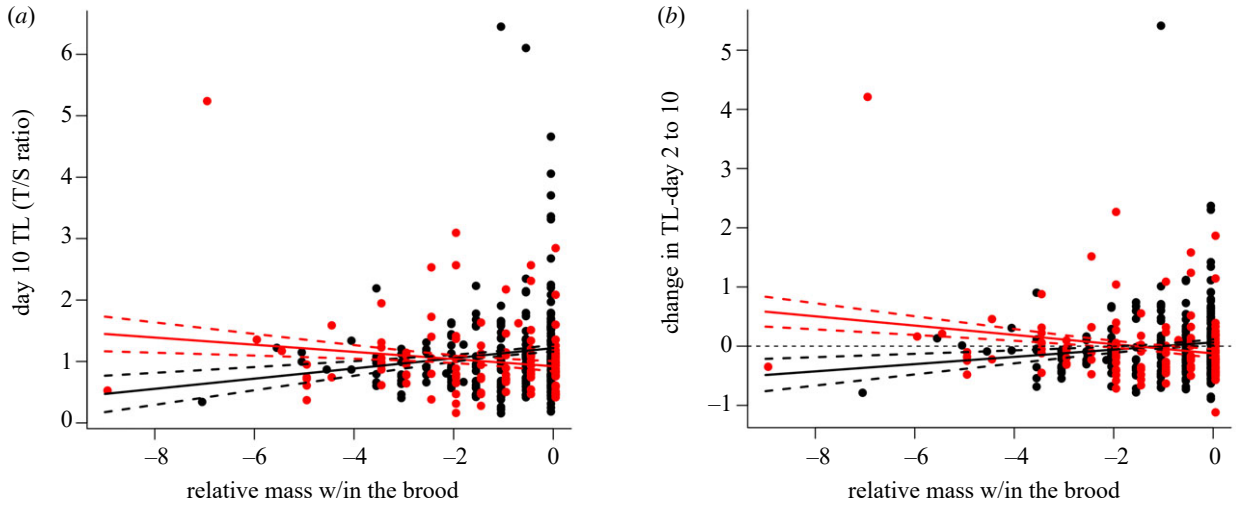

Figure 2. The relationship between relative mass within the brood and (*a*) telomere length (TL) at day 10 ($n = 375$) and (*b*) change in TL (ΔTL) ($n = 355$) in nestling house sparrows (*Passer domesticus*). Offspring from parental stress nests are in red and offspring from parental control nests are in black. The slopes of the parental treatment lines significantly differed. Data were analysed using log(TL or ΔTL) values, reducing the influence of the few high values, but are plotted as raw values to ease interpretation. Dashed lines are SE.

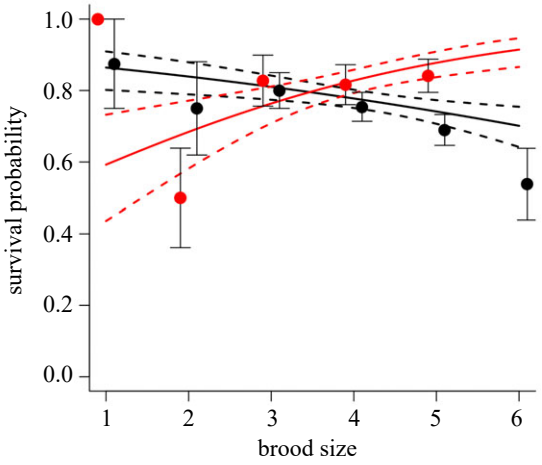

Figure 3. The relationship between brood size and nestling survival probability in 497 house sparrows (*Passer domesticus*). Offspring from parental stress nests are in red and offspring from parental control nests are in black. The direction of the slopes of the parental treatments significantly differed. Points are means ± SE for broods of that size. Dashed lines are standard errors.

when parents were experimentally stressed versus not stressed. Similarly smaller, lower ranking nestlings tended to experience less telomere attrition between days 2 and 10 than their larger siblings when their parents were exposed to stressors prior to offspring production, opposite the pattern seen for offspring of non-stressed parents. The effect of the parental stress treatment on offspring survival to the end of post-natal development also depended on a natural nestling stressor, brood size, but not on the experimental nestling stress. Brood size predicted offspring survival differently depending on parental stress treatment: in controls, survival tended to decline with larger brood size but in broods from experimentally stressed parents, the pattern was significantly different, with survival tending to increase with brood size. These results suggest that parental stress exposure may induce compensatory mechanisms in parents and/or offspring that better prepare offspring to be resilient in the face of some environmental stressors, as nestlings produced by stressed parents that experienced natural stressors during post-natal development appeared to fare better in some regards than nestlings produced by stressed parents reared under more favourable conditions. This is consistent with the hypothesis of environmental matching whereby parents are able to induce a resilient phenotype in the face of environmental challenges [2]. Importantly, these results also suggest that rather than having strictly negative effects, the influence of parental stress exposure on offspring telomeres and survival may often be context dependent.

The initially small but significant negative effect of parental stress exposure on offspring day 2 TL may have occurred through several routes including direct effects on telomeres in parental gametes or because of indirect effects on egg composition and/or incubation behaviour [6]. Future studies could distinguish between these possibilities using a cross-fostering design [44]. However, this effect did not persist to day 10, which suggests that offspring were able to overcome this initial deficit. Studies often only measure offspring telomeres at a single time point, but our longitudinal sampling design allowed us to detect this effect. Our results are consistent with recent studies in Trinidadian guppies (*Poecilia reticulata*) [45] and red squirrels (*Tamiasciurus hudsonicus*) [46] in which parental exposure to predator cues [45] or stress hormones during gestation [46] did not negatively influence offspring telomeres during post-natal development. This is in contrast with what has often been reported in humans, where exposure to stress during pregnancy shortens telomeres during early life [12,13] and has persistent negative effects on offspring telomeres into adulthood [13]. However, some results on human telomeres do fit with our results. A recent study [47] suggests that the negative effects of developmental stress on offspring telomeres are ameliorated by increased parental care behaviour. We also found the apparent negative effect of parental stress disappeared once offspring growth was completed. Several possible explanations for this exist. One is that since day 2 TL predicts offspring survival, some effects of the parental stress treatment may have disappeared due to differential mortality by day 10. Another explanation is that the many other processes influencing TL early in development may overwhelm any parental effect by day 10. Finally,

the human study [47] suggests some sort of compensation, perhaps by parents, and our other results are consistent with that as well (see below).

We also found that offspring reared in larger broods had shorter telomeres at hatching and at the end of post-natal development, which is consistent with what has been reported in other studies in birds and mammals [11,28,29,48] but see [49]. Interestingly, we did not find a significant effect of our experimental nestling stress treatment or an interaction between our parental and experimental nestling stress treatments on offspring telomeres. At day 10, handling and restraint do increase glucocorticoid stress hormone levels in nestling house sparrows [50] (B.J.H. 2020, unpublished data), and exposure to this same standardized stressor has been shown to increase telomere shortening in European shags (*Gulosus aristotelis*) [9]. Paradoxically, we also found that offspring exposed to experimental handling stress had higher, rather than lower survival to the end of post-natal development. This standardized stressor may have been relatively mild and induced hormetic mechanisms that enhanced survival. Exposure to experimentally elevated stress hormone levels does increase begging behaviour in nestling house sparrows, but does not increase parental provisioning and may even reduce it [51]. If so, it seems unlikely that our experimental treatment on nestlings led to higher survival through increased begging and food provisioning. It is also possible that despite the fact that this treatment increased survival to fledging, it may negatively influence survival at later life stages as there is evidence that exposure to experimentally elevated stress hormones during early life reduces survival over longer time scales in zebra finches [52] and house sparrows [26].

The interactive effect of parental stress and natural stressors in offspring raises a compelling question as to what mechanism(s) produce this compensatory effect. Importantly, we found no differences in reproductive measures (e.g. lay date and clutch size) between parents in the two experimental treatments, which suggests that differences in parental quality are an unlikely explanation for this effect. If parents do not innately differ, how might their responses to our experimental stressors have affected offspring responses? One possibility is that parents may have perceived the presence of stressors as lowering their own survival probabilities or their chances of breeding successfully in the future, and so shifted how they traded off future and current reproduction toward the current brood [53,54]. As in humans [47], higher parental care during development could compensate for initial poor conditions. If so, we would predict that such shifts in investment would be costly to parent residual reproductive value, but we have insufficient data to assess if that was the case in our study. Such shifts could also programme offspring stress responses and/or upregulate compensatory mechanisms (e.g. telomere regulation) in offspring that allow them to do better in the face of their own stressors [3]. Alternatively, cues provided by parents may have induced plastic responses in offspring that would have been costly under non-stressed conditions and/or may induce costs at later life stages. This array of possibilities suggests that future studies of the mechanisms that mediate the effects of parental stress exposure on offspring will be essential for understanding the long-term impacts of stressors, especially novel anthropogenic ones, on life-history strategies and population processes.

Ethics. Sampling was conducted with permissions from the NDSU IACUC committee (protocol A17035) and a USFWS banding permit (24205).

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.ncjsxksz3 [56].

The data are provided in electronic supplementary material [57].

Authors' contributions. R.C.Y.: conceptualization, data curation, formal analysis, investigation, methodology, resources, supervision, visualization, writing—original draft, writing—review and editing; D.F.W.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—review and editing; J.V.-B.: investigation, writing—review and editing; A.E.S.: conceptualization, investigation, writing—review and editing; S.J.S.: investigation, writing—review and editing; J.K.: investigation, methodology, writing—review and editing; A.G.: conceptualization, investigation, methodology, writing—review and editing; B.J.H.: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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