

## RESEARCH PAPER

# Developmental stress and telomere dynamics in a genetically polymorphic species

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National Institutes of Health, Grant/Award Number: 1R01GM084229; The School of Graduate and Professional Studies at Indiana State University; The National Science Foundation, Grant/Award Number: DUE-0934648

**Abstract**

A central objective of evolutionary biology is understanding variation in life-history trajectories and the rate of aging, or senescence. Senescence can be affected by trade-offs and behavioural strategies in adults but may also be affected by developmental stress. Developmental stress can accelerate telomere degradation, with long-term longevity and fitness consequences. Little is known regarding whether variation in developmental stress and telomere dynamics contributes to patterns of senescence during adulthood. We investigated this question in the dimorphic white-throated sparrow (*Zonotrichia albicollis*), a species in which adults of the two morphs exhibit established differences in behavioural strategy and patterns of senescence, and also evaluated the relationship between oxidative stress and telomere length. Tan morph females, which exhibit high levels of unassisted parental care, display faster reproductive senescence than white females, and faster actuarial senescence than all of the other morph-sex classes. We hypothesized that high oxidative stress and telomere attrition in tan female nestlings could contribute to this pattern, since tan females are small and potentially at a competitive disadvantage even as nestlings. Nestlings that were smaller than nest mates had higher oxidative stress, and nestlings with high oxidative stress and fast growth rates displayed shorter telomeres. However, we found no consistent morph-sex differences in oxidative stress or telomere length. Results suggest that oxidative stress and fast growth contribute to developmental telomere attrition, with potential ramifications for adults, but that developmental oxidative stress and telomere dynamics do not account for morph-sex differences in senescence during adulthood.

**KEYWORDS**

developmental stress, disassortative mating, genetic polymorphism, telomeres, white-throated sparrow

## 1 | INTRODUCTION

Understanding factors that affect individual-level differences in life-history trajectories is a central objective of evolutionary biology. Of particular interest are factors affecting rates of senescence,

or the decline in physical and reproductive performance with age (Bouwhuis, Choquet, Sheldon, & Verhulst, 2012; Monaghan, 2008; Ricklefs, 2010). Much of life-history theory focuses on trade-offs affecting adult organisms, most notably the trade-off between self-maintenance and reproductive effort (Fisher, 1930; Nussey et al.,

2009; Roff, 1992). How different forms of reproductive effort affect life-history trajectories and senescence has also generated debate. For instance, does intense competition over mates, as observed under sexual selection, lead to earlier or faster senescence than does investment in parental care (Bonduriansky, Maklakov, Zajitschek, & Brooks, 2008)?

In addition to dynamics in adults, developmental trade-offs and environments can also play a role in determining individual differences in life-history trajectories and patterns of senescence. Exposure to developmental stress can have wide-ranging phenotypic effects that persist into adulthood (Metcalf & Monaghan, 2001; Monaghan, 2008; Spencer & MacDougall-Shackleton, 2011). For instance, developmental stress can reduce growth rates and body size (Searcy, Peters, & Nowicki, 2004; Verhulst, Holveck, & Riebel, 2006), affect cognitive ability (Bateson, Emmerson, Ergün, Monaghan, & Nettle, 2015; Peters, Searcy, & Nowicki, 2014) and metabolic rate (Criscuolo, Monaghan, Nasir, & Metcalfe, 2008) and alter stress physiology and expression of sexually selected ornaments in adults (Banerjee, Arterbery, Fergus, & Adkins-Regan, 2012; Marasco, Robinson, Herzyk, & Spencer, 2012; Spencer, Buchanan, Goldsmith, & Catchpole, 2003; Spencer, Evans, & Monaghan, 2009; Spencer & MacDougall-Shackleton, 2011).

Stress-related changes in telomere dynamics during development may have particularly strong effects on life-history trajectories (Boonekamp, Mulder, Salmons, Dijkstra, & Verhulst, 2014; Haussmann, Longenecker, Marchetto, Juliano, & Bowden, 2012; Monaghan, 2014; Monaghan & Haussmann, 2006). Telomeres are conserved terminal repeats of the sequence TTAGGG, in vertebrates, which protect DNA from degradation and distinguish healthy from damaged chromosomes (Blackburn, 1991). Telomere attrition regulates senescence by triggering apoptosis and can lead to genomic instability, disease and decreases in reproductive performance and survivorship (Haussmann, Winkler, & Vleck, 2005; Monaghan & Haussmann, 2006). Fast growth and cellular division during development can result in rapid telomere attrition (Heidinger et al., 2012), and developing organisms may be sensitive to stress-related increases in telomere degradation due to poorly developed physiological coping mechanisms (Boonekamp et al., 2014; Herborn et al., 2014; Monaghan, 2014). A longitudinal study in zebra finches (*Taeniopygia guttata*) found that post-fledging telomere length more strongly predicted longevity than telomere length later in life, suggesting that developmental telomere dynamics can fundamentally alter life-history trajectories and lifetime fitness (Heidinger et al., 2012).

The mechanisms that influence telomere degradation rates during development remain contentious. Research suggests that stressful environmental conditions during development, such as intense sibling competition (Nettle, Monaghan, Boner, Gillespie, & Bateson, 2013; Nettle et al., 2015), can accelerate telomere degradation, with fitness and longevity consequences (Heidinger et al., 2012). Elevated stress hormone (corticosterone) levels (Haussmann et al., 2012; Quirici, Guerrero, Krause, Wingfield, & Vasquez, 2016) and rapid growth (Geiger et al., 2012; Stier, Massemin,

Zahn, Tissier, & Criscuolo, 2015) during development have been linked to increased telomere loss and might both influence telomere dynamics by increasing oxidative stress (OS) (Geiger et al., 2012). Understanding how OS influences rates of developmental telomere attrition has received particular attention. Telomeres are sensitive to OS in cell culture (von Zglinicki, 2002). However, studies that assess the relationship between OS and telomere length in vivo have yielded mixed results. Some report a positive relationship between OS and telomere degradation rate (Geiger et al., 2012; Stier et al., 2015). For instance, king penguin (*Aptenodytes patagonicus*) chicks that engaged in catch-up growth did so at the expense of oxidative status and telomere length (Geiger et al., 2012). On other hand, other studies find no relationship between OS and telomere degradation rate (Boonekamp, Bauch, Mulder, & Verhulst, 2017), calling the relationship between OS and telomere dynamics into question.

Despite growing interest in the mechanisms and implications of developmental telomere dynamics, little is known regarding whether variation in developmental telomere dynamics is related to differences in life-history strategies and senescence during adulthood. We employed a unique model species, the dimorphic white-throated sparrow (*Zonotrichia albicollis*) (Figure 1), to address this question and evaluate the contentious relationship between OS, growth rate and telomere length. White-throated sparrows display a genetically determined polymorphism in both sexes (Figure 1). White morph birds are heterozygous for an inversion-based supergene on chromosome 2 (ZAL2<sup>m</sup>/2), whereas tan morph birds are homozygous, lacking the rearrangement (ZAL2/2) (Thornycroft, 1966; Tuttle et al., 2016). White birds are more aggressive, less parental and sing more than tan counterparts, and white males (WMs) are promiscuous (Knapton & Falls, 1983; Kopachena & Falls, 1993; Tuttle, 2003; Zinzow-Kramer et al., 2015). Furthermore, white-throated sparrows pair disassortatively by morph, producing a pair type with biparental care (tan males [TMs] × white females [WFs]; T × W), and a pair type with female-biased care (WMs × tan females [TFs]; W × T) (Knapton



**FIGURE 1** The white morph of the white-throated sparrow (left) is visually distinguished from the tan morph (right) by brighter crown plumage coloration, and genetically distinguished by an inversion-based supergene on chromosome 2

& Falls, 1983; Tuttle, 2003). We previously found that TFs, which engage in relatively unassisted parental care, display faster reproductive senescence than WFs (Grunst et al., 2018), and faster actuarial senescence (decreased survivorship with age) than all other morph-sex classes (Grunst et al., accepted). These results suggest that parental care, rather than intense competition for mates as observed in WMs, is particularly likely to promote senescence. Here, we address the contingency that variation in developmental stress and telomere attrition also contributes to morph-sex differences in senescence.

Most published work on morph differences in the white-throated sparrow focuses on adults. Thus, whether nestlings differ in physiology is largely unknown. However, white birds are larger than tan birds even as nestlings (Tuttle et al., 2017). We predicted that the genetic and behavioural divergence between the morphs could also affect OS and telomere dynamics in nestlings. Compared to white counterparts, tan nestlings might suffer more developmental stress via sibling competition, since tan birds are generally smaller and less aggressive (Kopachena & Falls, 1993; Tuttle, 2003; Tuttle et al., 2016). On the other hand, since they attain higher body mass, WM nestlings might suffer accelerated telomere degradation rates during development (Tuttle et al., 2017). Indeed, some past studies report that male nestlings display faster telomere degradation than females (Stier et al., 2015). Our study is the first to investigate morph differences in developmental OS and telomere dynamics in a species with genetically determined morphs that display differences in reproductive strategy and life history. Moreover, the unique disassortative pairing and parental system of the white-throated sparrow provided an opportunity to explore whether parental strategies exert distinct physiological effects on offspring of different morphs.

## 2 | MATERIALS AND METHODS

### 2.1 | Field methods

We studied white-throated sparrows breeding at Cranberry Lake Biological Station (44°15'N, 74°48'W; Adirondack Mountains, New York). The study site consists of ~32 ha, with white-throated sparrows favouring forest edges, bogs and glades. White-throated sparrows at Cranberry Lake have been monitored for over 27 years, and territory boundaries are relatively stable and well delineated. We banded adults with Fish and Wildlife aluminium bands and a combination of three colour bands. We morphed adults using visual criteria (Lowther, 1961; Piper & Wiley, 1989; Tuttle, 1993, 2003), and sexed adults based on the presence of a brood patch (females) or cloacal protuberance (males).

From early May to early August, 2014 and 2015, we located nests through behavioural observations and systematic search. White-throated sparrows sometimes rear two broods per season and repeatedly re-nest after clutch loss. Thus, for some pairs, we collected data from multiple nests per season. We located most nests during the building or laying stages and were thus able to predict hatching dates. We checked nests found during incubation at least every 2 days, so that hatching

date was known. We marked the tarsi of nestlings using non-toxic markers, to enable individual identification. From day 0 (hatch day) to ~day 6, we measured mass ( $\pm 0.25$  g) daily. We calculated growth rates using the slope of a linear regression of mass versus nestling age (in days; no evidence of nonlinearity). We determined nestling size rank by ordering nestlings within broods from largest to smallest. The largest nestling received a rank of 1 and the smallest a rank equal to the brood size. Size rank may influence the competitive stress experienced by nestlings. To minimize disruption and nestling stress levels, we never removed all nestlings from the nest simultaneously, took measurements ~10 m from the nest, and returned nestlings to the nest as quickly as possible. Daily handling could nonetheless have elevated nestling stress levels. However, this is unlikely to have biased our results, since nestlings of both morphs and sexes were treated equivalently.

We aimed to band and bleed nestlings on day 6. Some nestlings were banded on day 5, because some nestlings hatched 1 day later than nest mates. Only data from 5- and 6-day-old nestlings were included in models involving telomere length, which is known to erode during development (Bateson, Brilot, Gillespie, Monaghan, & Nettle, 2015; Bateson, Emmerson, et al., 2015; Boonekamp et al., 2014; Heidinger et al., 2012). We occasionally located nests when nestlings were older than 6 days, in which case nestlings were immediately banded and bled, and also occasionally banded and bled 4-day-old nestlings due to time constraints. We included these nestlings in models involving OS, because age had no effect on OS in statistical models (age range 4–10 days, mean  $\pm$  SE =  $5.9 \pm 0.04$ ). We collected ~100  $\mu$ l blood samples immediately before banding using 26-gage needles and two heparinized microcapillary tubes. Blood samples were stored on ice in the field and processed within 5 hr.

### 2.2 | Processing samples

Following collection of blood samples, we separated cell fraction from plasma via centrifugation. We expelled erythrocytes from the first microcapillary into Longmire's buffer (Longmire, Gee, Handenkipf, & Mark, 1992), and stored these samples at 4°C for use in genetic sexing and morphing. We expelled erythrocytes from the second microcapillary into glycerol buffer (50 mM Tris-Cl, 5 mM MgCl, 0.1 mM EDTA, 40% glycerol) for telomere length determination, and plasma from both microcapillaries into a microvial. We stored erythrocytes for telomere length determination and plasma samples in liquid nitrogen pending transport to Indiana State University (ISU). At ISU, we stored samples at -20°C until performing telomere length and OS assays, 6–8 months after sample collection.

### 2.3 | Genetic sexing and morphing

We extracted DNA from samples in Longmire's buffer using the DNA IQ<sup>®</sup> magnetic extraction system (Promega Corp, Madison,

For both assays, the standard curve and all samples were run in duplicate. Intra-assay coefficients of variation averaged 11.7% for the OXY-adsorbent assay and 13.1% for the d-ROMs assay. We calculated OS as: mM ROMs/mM HOCl neutralized (TAC)  $\times$  1,000 (Costantini et al., 2007).

## 2.6 | Statistical analyses

We performed statistics in R 3.1.2 (R Core Team, 2014). We used linear mixed effect models (R packages lme4; Bates, Maechler, & Bolker, 2012) to analyse differences in OS and RTL, with nest, father and mother identity as random effects. As fixed effects, we entered nestling morph–sex (e.g., TM vs. WF), the morph composition of the parental pair, brood size, clutch initiation date, time of blood sampling (excluded in the telomere model), year, growth rate, size rank and age. OS level was additionally included as a fixed effect in the model predicting RTL.

To assess whether rearing conditions affected the morph–sex classes differently, we tested two-way interactions, with nestling type interacting with parental pair composition, brood size, clutch initiation date, year and size rank. Testing overly complex global models can lead to problems with overfitting, inaccurate parameter estimates, model convergence and interpretability (Harrison et al., 2018). Moreover, robustly testing interactions involving four-level (nestling type) or two-level (sex, morph) factors is especially difficult and requires a relatively large sample size, even for one such interaction (Harrison et al., 2018). Thus, to avoid the problems discussed above, we tested each interaction separately, by including all main effects in the global model but only one interaction at a time.

We performed an additional model to assess the effect of brood composition on both OS and RTL. In this model, we used the morph and sex composition of the brood (proportion male and proportion white morph). We included an interaction with nestling morph–sex class in the initial model and year and time of sampling as covariates.

We sequentially reduced models by eliminating the predictor variable with the highest  $p$ -value first. When morph–sex type was nonsignificant, this factor was simplified to morph and sex, entered separately, to further assess the potential effects of these variables. In final models, all predictor variables were significant at  $\alpha = 0.05$ .  $p$ -values were derived by using Satterthwaite approximations to estimate degrees of freedom (R package lmerTest; Kuznetsova, Brockhoff, & Christensen, 2016). To normalize residuals for the model involving OS, we natural log-transformed OS and eliminated four outlying data points.

## 3 | RESULTS

### 3.1 | Oxidative stress

Nestling OS (mM ROMs/mM TAC  $\times 1,000$ ) averaged  $3.91 \pm 0.13$  (range: 0.57–15.77;  $N = 244$ ). TAC averaged  $288.47 \pm 4.44$  mM (range: 111.80–499.3 mM;  $N = 248$ ), and ROMs averaged  $1.09 \pm 0.03$  mM (range: 0.18–3.20 mM;  $N = 248$ ).

Nestlings had lower OS in 2015 than in 2014, and higher OS later in the day (Table 1). In addition, OS was higher in nestlings that were smaller than others relative to nest mates (in nestlings with higher size ranks; Table 1, Figure 2). Nestling size rank

tends to vary with morph–sex class, with WMs less likely to place low in the hierarchy than TFs (Poisson GLMM:  $\beta = -0.31 \pm 0.13$ ,  $z = -2.32$ ,  $p = 0.020$ ). Thus, nestling morph–sex class could affect OS via an effect on size rank. However, we found no consistent evidence that nestling morph–sex affects OS. Rather, the relationship between nestling morph and OS differed between years (significant morph  $\times$  year interaction; Table 1). In 2014, white nestlings tended to have lower OS than tan nestlings (LMM:  $\beta = -0.12 \pm 0.07$ ,  $t_{114} = -1.76$ ,  $p = 0.087$ ). In 2015, this relationship reversed, with white nestlings tending to display higher OS ( $\beta = 0.16 \pm 0.09$ ,  $t_{117} = 1.76$ ,  $p = 0.080$ ).

Across years, OS did not vary with nestling morph–sex type ( $F_{3, 211} = 0.07$ ,  $p = 0.973$ ), being  $3.94 \pm 0.25$  in WMs,  $4.04 \pm 0.27$  in TMs,  $3.89 \pm 0.27$  in WFs, and  $4.00 \pm 0.27$  in TFs (Figure 3a). Moreover, when data were combined across years, the morphs had virtually identical OS ( $\beta = 0.01 \pm 0.05$ ,  $t_{230} = 0.246$ ,  $p = 0.805$ ), with white nestlings having levels of  $3.91 \pm 0.18$ , and tan nestlings displaying levels of  $3.91 \pm 0.17$ . The two sexes also displayed similar OS ( $\beta = 0.03 \pm 0.05$ ,  $t_{213} = 0.51$ ,  $p = 0.608$ ), with males displaying levels of  $3.98 \pm 0.18$ , and females displaying levels of  $3.92 \pm 0.19$ .

The other predictors examined were also unrelated to nestling OS. Nestling OS was unrelated to the morph composition of the parental pair ( $\beta = -0.04 \pm 0.06$ ,  $t_{76} = -0.69$ ,  $p = 0.487$ ), growth rate ( $\beta = -0.11 \pm 0.15$ ,  $t_{161} = -0.73$ ,  $p = 0.461$ ), brood size (LMM:  $\beta = -0.02 \pm 0.03$ ,  $t_{131} = -0.57$ ,  $p = 0.566$ ), nest date ( $\beta = 0.002 \pm 0.001$ ,  $t_{85} = 1.42$ ,  $p = 0.157$ ) or nestling age ( $\beta = -0.02 \pm 0.03$ ,  $t_{86} = -0.76$ ,  $p = 0.452$ ). Furthermore, these variables did not interact with nestling sex or morph to predict OS ( $p > 0.06$  for interaction terms). Brood composition, quantified by the proportions of male and white nestlings, was also unrelated to OS ( $p > 0.10$  for main effects and interactions).  $p$ -values for nonsignificant predictors are reported from models containing only variables that were retained in the final model and the nonsignificant predictor of interest.

### 3.2 | Telomere length

Relative telomere length in nestlings ranged from 0.334 to 2.37 with a mean  $\pm$  SE of  $1.137 \pm 0.028$ . Nestlings had shorter telomeres in 2015 (mean  $\pm$  SE =  $0.974 \pm 0.034$ ,  $N = 113$ ) than in 2014 (mean  $\pm$  SE =  $1.311 \pm 0.039$ ,  $N = 105$ ). When accounting for this year effect on RTL, nestlings with higher growth rates and higher OS had shorter telomeres (Table 2; Figure 4a,b). Removing one outlying data point (OS = 15.77; Figure 4a) did not qualitatively change results of this model ( $\beta = -0.02 \pm 0.01$ ,  $t_{156} = -2.44$ ,  $p = 0.036$ ; effect of OS with this data point removed).

We found no evidence for an effect of nestling ( $F_{3, 160} = 0.11$ ,  $p = 0.951$ ) or parental ( $\beta = -0.12 \pm 0.08$ ,  $t_{56} = -1.46$ ,  $p = 0.150$ ) morph–sex type on nestling RTL. RTL of nestlings averaged  $1.154 \pm 0.058$  for WMs,  $1.108 \pm 0.049$  for TMs,  $1.169 \pm 0.058$  for WFs, and  $1.169 \pm 0.070$  for TFs (Figure 3b). RTL was similar between nestlings from broods produced by the two pair types, being  $1.153 \pm 0.040$  for nestlings of W  $\times$  T pairs, and  $1.124 \pm 0.040$  for nestlings of T  $\times$  W pairs.



**TABLE 1** LMM predicting nestling OS from size rank, morph and year

	$\beta \pm SE$	df	t	$p >  t $
Intercept	1.09 $\pm$ 0.16	111.76	6.81	<0.001
Size rank	0.05 $\pm$ 0.02	217.52	2.08	0.038
Time	0.02 $\pm$ 0.01	73.52	2.02	0.047
Nestling morph <sup>a</sup>	-0.11 $\pm$ 0.08	229.49	-1.42	0.156
Year (factor) <sup>b</sup>	-0.54 $\pm$ 0.08	128.09	-6.73	<0.001
Morph $\times$ year	0.26 $\pm$ 0.11	233.97	2.26	0.024
<b>Random effects</b>	<b>Variance</b>	<b>SD</b>	<b>N</b>	
Nest ID	0.0006	0.02	84	
Father ID	<0.0001	<0.0001	51	
Mother ID	<0.0001	<0.0001	53	
Residual	0.201	0.449	240	

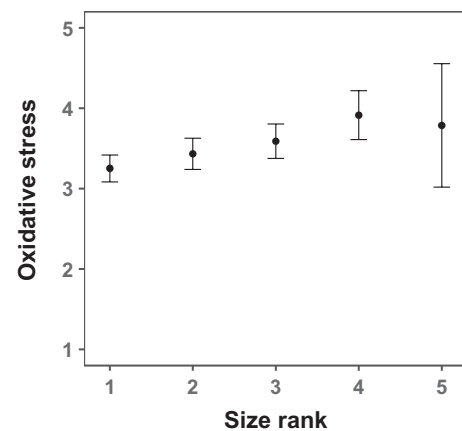
Note. <sup>a</sup>White contrasted to tan morph. <sup>b</sup>2015 contrasted to 2014.

Relative telomere length was unrelated to brood size ( $\beta = -0.03 \pm 0.03$ ,  $t_{66} = -0.978$ ,  $p = 0.331$ ) and nest date ( $\beta = 0.001 \pm 0.001$ ,  $t_{43} = 0.546$ ,  $p = 0.587$ ), which could both affect environmental stress. Size rank was not related to RTL ( $\beta = 0.03 \pm 0.02$ ,  $t_{178} = 1.27$ ,  $p = 0.204$ ), despite the association between size rank and OS. However, nestlings that were smaller than brood mates also exhibited slow growth rates ( $\beta = -0.10 \pm 0.01$ ,  $t_{244} = -10.76$ ,  $p < 0.001$ ), potentially explaining the absence of high telomere attrition in these individuals. Finally, the morph-sex composition of broods was also unrelated to RTL ( $p > 0.05$  for all main effects and interaction terms).

## 4 | DISCUSSION

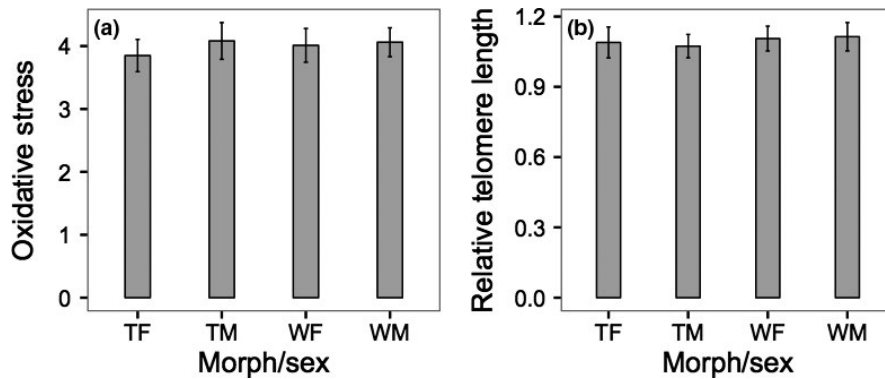
A primary objective of our study was to assess whether morph-sex differences in OS or telomere length occur in nestling white-throated sparrows and have the potential to explain different patterns of senescence during adulthood. Apart from our investigation of morph differences, our study also contributes to the current debate regarding the relationship between OS and telomere dynamics (Boonekamp et al., 2017; Monaghan, 2014). Results provide no evidence that nestlings of different morphs or sexes differ in developmental stress levels or telomere length. Lack of morph-sex differences in nestling OS and telomere lengths suggests that the morph-sex differences in reproductive and actuarial senescence observed in white-throated sparrows are not linked to developmental telomere dynamics.

On the other hand, results support a role for both OS and growth rate in determining rates of developmental telomere degradation. Individuals with higher OS and faster growth rates had shorter telomeres at day 5–6 of the nestling stage. OS clearly leads to telomere attrition in cell cultures, but whether OS leads to telomere degradation in vivo is the subject of more debate (Boonekamp et al., 2017). A few past studies report a relationship between accelerated growth, OS and telomere length, in



**FIGURE 2** Relationship between size rank and oxidative stress (OS) in nestling white-throated sparrows. Nestlings with larger size ranks were smaller than nest mates and had higher OS levels. Points are back-transformed least square mean values extracted using the model in Table 1, and error bars represent standard error

accordance with our results (Bádas et al., 2015; Geiger et al., 2012; Stier et al., 2015). However, others find no relationship between OS and telomere length or attrition (Boonekamp et al., 2017; Nettle et al., 2015, 2017; Reichert et al., 2014; Young et al., 2017). Boonekamp et al. (2017) failed to find a relationship between multiple markers of OS and telomere attrition in jackdaw (*Corvus monedula*) nestlings and suggested that cell proliferation rates during development may be more important to determining rates of telomere loss than OS. However, our results suggest a role for both variables in determining nestling telomere lengths. Studies that do not concurrently measure both growth rate and OS could potentially fail to find an effect of either variable. However, in our dataset, the relationship between OS and telomere length remained significant even when excluding growth rate from the model ( $p = 0.025$ ). Of course, our results are correlational and cannot prove a causal relationship between OS and telomere length, which would require experimental manipulation.

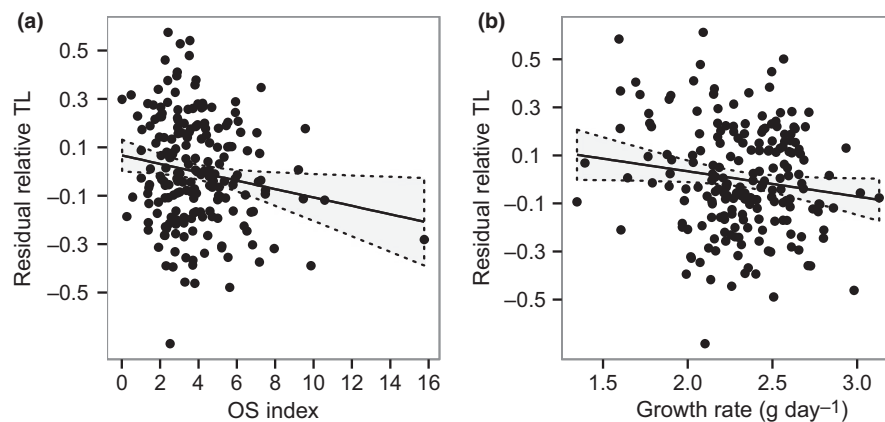


**FIGURE 3** Bar graphs showing no relationship between OS (a) or telomere length (b) and morph-sex class in nestling white-throated sparrows. Bars represent means and error bars represent standard error

	$\beta \pm SE$	<i>df</i>	<i>T</i>	$p >  t $
Intercept	$1.96 \pm 0.20$	170.20	9.63	<0.001
OS	$-0.02 \pm 0.01$	156.50	-2.44	0.015
Growth rate (g/day)	$-0.20 \pm 0.08$	177.17	-2.50	0.013
Year (factor) <sup>a</sup>	$-0.35 \pm 0.06$	60.69	-5.27	<0.001
Random effects	Variance	SD	N	
Nest ID	0.02	0.14	76	
Father ID	0.05	0.23	46	
Mother ID	<0.001	<0.001	46	
Residual	0.06	0.25	196	

**TABLE 2** LMM predicting RTL from OS and growth rate

Note. <sup>a</sup>2015 contrasted to 2014.



**FIGURE 4** The relationship between OS (a), growth rate (b) and RTL. RTL is represented as residuals from the mixed model shown in Table 2, excluding the focal variable of interest (i.e., OS for plot a. and growth rate for plot b.). Shaded regions represent 95% confidence intervals

Results also suggest that the competitive environment within broods can contribute to levels of developmental stress, and particularly to OS. Specifically, we found that nestlings that were smaller than nest mates had higher OS. The finding that relatively small nestlings had higher OS is consistent with past research reporting higher stress levels in nestlings at the bottom of brood hierarchies (Martínez-Padilla et al., 2004; Merklings et al., 2014; Stier et al., 2014, 2015). On the other hand, unlike some studies (Nettle et al., 2013, 2015; Stier et al., 2015), we did not find that nestlings that were smaller than brood mates suffered higher telomere attrition. Given our finding that fast growth was associated with

shorter telomeres, one explanation for this null result is that higher OS in smaller nestlings was accompanied by slower growth, depressing overall rates of telomere loss. Indeed, nestlings low in the competitive hierarchy displayed slow growth rates. The sex and morph composition of broods did not affect OS, suggesting that the morph-sex identity of nestlings does not strongly influence competitive dynamics.

Although TF nestlings tend to be smaller than nest mates, whereas WM nestlings tend to be larger, we also found no evidence for morph-sex differences in nestling OS or telomere length. Some studies have found sex differences in telomere dynamics during

development. For instance, male great tit (*Parus major*) nestlings suffer faster telomere degradation than females, potentially because males attain higher mass and are under more OS (Stier et al., 2015). Higher testosterone in males could contribute to faster telomere degradation, although the above study found no sex difference in testosterone levels (Stier et al., 2015). However, consistent with our results, many studies also fail to find sex differences in telomere dynamics (Barrett & Richardson, 2011; Herborn et al., 2014; Reichert et al., 2014, 2015).

These results suggest that neither developmental telomere dynamics nor OS during development can explain morph–sex differences in patterns of reproductive and actuarial senescence in adult white-throated sparrows. Rather, as we propose elsewhere, differences in reproductive strategies and pairing dynamics may explain morph–sex differences in senescence (Grunst et al., 2018). Specifically, unassisted parental care might elevate stress and induce faster reproductive and actuarial senescence in TFs. As a caveat, we only measured telomere length near the end of the developmental period, not telomere degradation rate. Some research suggests that the rate of telomere attrition is more reflective of developmental stress and more predictive of longevity than nestling telomere length alone (Boonekamp et al., 2014). However, others have found that the rate of telomere attrition and telomere length in nestlings are strongly correlated and can both predict fitness and behaviour in adults (Bateson, Brilot, et al., 2015; Heidinger et al., 2012).

We also found no evidence that the morph–sex composition of the parental pair affects nestling OS or telomere length. The pair types tend to exhibit unique parental systems, with T × W pairs exhibiting biparental care and W × T pairs exhibiting female-biased care (Tuttle, 2003; Tuttle et al., 2016). However, results suggest that nestlings from the two different pair types do not experience different developmental stress levels, perhaps because overall provisioning rates do not consistently differ between pair types.

Finally, we found year effects on both OS and telomere length, suggesting cohort effects. Other studies have also found significant cohort effects on these variables (Watson, Bolton, & Monaghan, 2015). Thus, cohort effects could play a substantial role in determining levels of developmental stress and telomere length, and in shaping future life-history trajectories.

In conclusion, our study supports the hypothesis that OS and growth rates (levels of cell proliferation) concurrently affect telomere attrition during development. On the other hand, results do not support our prediction that genetically determined morphs differ in developmental OS levels and telomere dynamics. Thus, results suggest no role for developmental telomere dynamics in explaining morph–sex differences in life histories, and particularly ageing rates. However, the absence of morph–sex effects does not preclude a role for developmental telomere dynamics in influencing individual life histories, independent of morph–sex identity. OS and telomere loss in developing nestlings may contribute to the fitness and longevity of adults within each morph–sex class. Establishing a link between telomere attrition during development and adult fitness and longevity will require longitudinal studies that track individuals into adulthood.

## ACKNOWLEDGMENT

We thank the White-throated Sparrow field crews, Cranberry Lake Biological Station, The Center for Genomic Advocacy at Indiana State University, the Tuttle and Gonser labs, C.A.T. Gonser, and Zonotrichia Zeke. Funding sources included the School of Graduate and Professional Studies at Indiana State University, the National Science Foundation (grant DUE-0934648) and the National Institutes of Health (grant 1R01GM084229 to E.M.T. and R.A.G.). Procedures were approved by Indiana State University's Institutional Animal Care and Use Committee (protocols 562158-1 and 562192-1).

## DATA ACCESSIBILITY

Data deposited at Dryad: <https://doi.org/10.5061/dryad.6j4n154>.

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**How to cite this article:** Grunst AS, Grunst ML, Gonser RA, Tuttle EM. Developmental stress and telomere dynamics in a genetically polymorphic species. *J Evol Biol*. 2019;32:134–143. <https://doi.org/10.1111/jeb.13400>