Short-term elevations in glucocorticoids do not alter telomere lengths: A systematic review and meta-analysis of non-primate vertebrate studies

Lauren Zane¹*, David C. Ensminger¹,2, José Pablo Vázquez-Medina¹

¹ Department of Integrative Biology, University of California, Berkeley, CA, United States of America,
2 Department of Biological Sciences, San Jose State University, San Jose, CA, United States of America

* laurenzane@berkeley.edu

Abstract

Background

The neuroendocrine stress response allows vertebrates to cope with stressors via the activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, which ultimately results in the secretion of glucocorticoids (GCs). Glucocorticoids have pleiotropic effects on behavior and physiology, and might influence telomere length dynamics. During a stress event, GCs mobilize energy towards survival mechanisms rather than to telomere maintenance. Additionally, reactive oxygen species produced in response to increased GC levels can damage telomeres, also leading to telomere shortening. In our systematic review and meta-analysis, we tested whether GC levels impact telomere length and if this relationship differs among time frame, life history stage, or stressor type. We hypothesized that elevated GC levels are linked to a decrease in telomere length.

Methods

We conducted a literature search for studies investigating the relationship between telomere length and GCs in non-human vertebrates using four search engines: Web of Science, Google Scholar, Pubmed and Scopus, last searched on September 27th, 2020. This review identified 31 studies examining the relationship between GCs and telomere length. We pooled the data using Fisher’s Z for 15 of these studies. All quantitative studies underwent a risk of bias assessment. This systematic review study was registered in the Open Science Framework Registry (https://osf.io/rqve6).

Results

The pooled effect size from fifteen studies and 1066 study organisms shows no relationship between GCs and telomere length (Fisher’s Z = 0.1042, 95% CI = 0.0235; 0.1836). Our meta-analysis synthesizes results from 15 different taxa from the mammalian, avian, amphibian groups. While these results support some previous findings, other studies have found a direct relationship between GCs and telomere dynamics, suggesting underlying
mechanisms or concepts that were not taken into account in our analysis. The risk of bias assessment revealed an overall low risk of bias with occasional instances of bias from missing outcome data or bias in the reported result.

**Conclusion**

We highlight the need for more targeted experiments to understand how conditions, such as experimental timeframes, stressor(s), and stressor magnitudes can drive a relationship between the neuroendocrine stress response and telomere length.

**Introduction**

The vertebrate neuroendocrine stress response integrates external stimuli into a broad range of physiological adjustments through the activation of the Hypothalamic-Pituitary-Adrenal axis (HPA axis) and the concomitant secretion of glucocorticoids (GCs) [1, 2]. While the primary GC produced varies by taxa (e.g., cortisol in humans and corticosterone in birds and other mammals [3]), the impacts of GCs on organismal physiology are remarkably similar. Across species, an increase in GC secretion can typically be detected in 3–5 minutes following interaction with a stressor [4]. Additionally, GCs are relatively easy to quantify because they are present in all vertebrates and can be measured noninvasively in multiple matrices including hair and feces using a variety of assays [5, 6]. Therefore, wildlife stress physiology studies often rely on GC measurements as an indicator of the neuroendocrine stress response [7]. Following their secretion, GCs induce a myriad of acute behavioral and physiological effects to prioritize immediate survival [8, 9].

In addition to allowing animals to cope with immediate stressors, GCs can influence other cellular processes such as telomere length dynamics. Telomeres are evolutionarily conserved caps that protect chromosomes against the loss of coding nucleotides during cell replication and against chromosomal fusion [10]. Telomere shortening is associated with aging, the neuroendocrine stress response, and survival, and is thus of interest to several fields of biology [1, 11]. In humans, increased telomere loss predicts the onset of age-related diseases, cardiovascular complications, cellular senescence, and other aging phenotypes [12, 13]. Telomere attrition can be attributed to several causes including the “end replication problem” in which the terminal end of linear DNA cannot be completely replicated by the lagging strand [14]. Since the end replication problem occurs at every cell division, telomeres continuously shorten with age progression [15]. Other stressors such as inflammatory challenges erode telomeres regardless of age [16].

In non-human vertebrates including birds, mammals, fish, amphibians and reptiles, exposure to challenging environmental conditions correlates with shorter telomeres [17, 18]. Reproductive stressors such as an artificially increased brood size can also shorten telomeres in zebra finch parents compared to controls and parents with a reduced brood size [19]. Early telomere length is positively correlated with survival and lifetime breeding success in both wild purple-crowned fairy wrens and zebra finches. Thus, individuals with longer telomeres are more likely to survive and produce more offspring that survive to maturity [20, 21]. Therefore, telomere dynamics—the change in telomere length attributed to processes of elongation and shortening—is related to organismal fitness [22]. In addition to impacting telomere length, stressors that lead to energy limitation such as psychological stress, disease, accelerated growth, nutrient shortage and work load activate the HPA axis causing the release of GCs [23].
Thus, several hypothesized connections between GCs and telomere length exist. Firstly, GCs are an essential part of the vertebrate stress response, and their primary function is to mobilize energy [5]. Accordingly, the “metabolic telomere attrition hypothesis” proposes that during events that require an increased amount of energy and metabolic rates, telomeres are shortened as collateral [20]. As a result of the high energy expenditure, the energetically expensive maintenance of telomeres cannot take place as an emergency survival mechanism due to a shift in energy allocation [23]. In addition, GCs stimulate the generation of reactive oxygen species (ROS) and subsequent oxidative damage to telomeres, which are particularly susceptible to oxidation due to a high guanine content [11, 24–26]. Finally, cortisol reduces telomerase —the enzyme responsible for telomere maintenance—activity in human T lymphocytes [27]. This reduction in telomerase activity can result in excessive telomere attrition [28]. Since wildlife face an array of stressors throughout their lifetime and these stressors can erode telomeres, GCs may play a mechanistic role in telomere loss [1].

External stressors cause pleiotropic effects that can potentially influence telomere dynamics, however the evidence for a causal relationship between GCs and telomere length is sparse. Two recent literature reviews on the topic by Angelier et al. 2018 and Casagrande and Hau 2019 [11, 23] summarize the potential relationship between GCs and telomere length. However, it is essential to build a quantitative understanding of the relationships between the neuroendocrine stress response and its downstream effects. In this study, we review the existing literature for empirical evidence of the relationship between GC secretion and telomere length to better understand the underlying mechanism of telomere shortening as well as potential consequences of the neuroendocrine stress response in non-primate vertebrates. Using a meta-analytical framework, we tested whether GC levels impact telomere length and if this relationship can differ among time frame, life history stage, or stressor type. We hypothesized that elevated GC levels are linked to a decrease in telomere length.

Methods

Literature search and study selection

We conducted a literature search for studies investigating the relationship between telomere length and GC levels in non-human vertebrates using four search engines: Web of Science, Google Scholar, Pubmed and Scopus. Five subsets of the following keywords ‘reactive oxygen species,’ ‘antioxidant,’ ‘glucocorticoid,’ ‘cortisol,’ ‘corticosterone,’ ‘telomere length,’ ‘chronic stress,’ ‘oxidative stress,’ ‘acute stress,’ ‘chronic stress,’ ‘telomeres,’ and ‘HPA axis,’ were conducted in each search engine. We did not specify a time frame in our literature search. Additional records were obtained from the reference section of studies included in the meta-analysis. Our study includes a qualitative synthesis of 31 full-text, peer-reviewed studies, and we report effect sizes for 15 of these studies.

Studies were excluded if (1) GCs were administered, but physiological measurements such as feather or plasma GC levels were not taken. Such studies were excluded because it would not be possible to calculate the appropriate effect size (Fisher’s Z) for correlation data. For homogeneity in effect size calculation and statistical analysis, we did not include studies in which (2) GCs and telomere length were not specifically measured at two different time points (before or after treatment) (3) raw data was not accessible to use for the effect size calculation, or (4) telomere length measurements or GC measurements were log transformed.

Statistical data analyses

Meta-analysis. We conducted statistical analyses exclusively on studies with raw data available. When data was not publicly accessible, we contacted authors via email for consensual
access. For each study, the correlation coefficient ($R^2$) was calculated by fitting a linear mixed model using the "lme4" R package (version 3.6.1, R Development Core Team, Boston, MA). When possible, random effects such as multiple blood draws from a single individual were incorporated in the linear mixed model (LMER) to account for variability not captured by explanatory parameters. For studies where a random effect could not be determined, a linear model (LM) was fitted. From the LMs and LMERs, $R^2$ values were obtained from the model and converted into Fisher’s $Z$, then adjusted for sample size and combined into a pooled effect size (Fisher’s $Z$; $Z$) using the R package "meta". The random-effects model meta-analysis was implemented in our study as this model accounts for the assumption that studies come from different populations, rather than the same population. These pooled effect sizes were then visualized in a forest plot.

The "meta" package was also used to assess the statistical difference between observed and fixed effect model estimate of effect size (Cochrane’s Q) and the percent of variability in effect sizes that is not caused by sampling error ($I^2$). After estimating heterogeneity, we identified potential outliers. Studies were classified as outliers if the study had an effect size with a confidence interval that did not intersect with the confidence interval of the pooled effect size.

Since some studies can have a larger influence on the pooled effect size than others due to its sample size or individual effect size, we conducted an influence analysis. The analysis was conducted by omitting each study one at a time and simulating the pooled effect size, with a confidence interval had the study not been included. This influence analysis was represented in a Baujat plot, which shows the contribution of each study to heterogeneity as Cochrane’s Q, and compares this to the study’s influence on the pooled effect size.

**Subgroup analysis.** Since experimental design can affect the outcome of a study, differences in effect size may be attributed to these variables. As such, further sources of between-study heterogeneity were investigated through subgroup analysis and meta-regression. In the subgroup analysis, studies were grouped based on different categorical experimental parameters. We completed eight different subgroup analyses for the following parameters—duration of stressor, type of GC assay, telomere assay, species, taxa, study type, life history stage, and stressor type. For each subgroup analysis, a pooled effect size (Fisher’s $Z$) was calculated. We then compared pooled effect sizes and tested for between-study subgroup differences. The meta-regression was analogous to the subgroup analysis, except the parameter of investigation is continuous rather than categorical. We conducted one meta-regression for publication year and subsequently tested for between-study subgroup differences. For all analyses the significance threshold was set at $p<0.05$.

In the subgroup analysis, studies included in the meta-analysis were clustered based on categorical grouping and represented as a pooled effect size with a 95% confidence interval. The between study difference was indicated by Cochrane’s Q and the subsequent p-value for this statistical measure. The first subgroup analysis “stressor duration” organized studies based on the timeframe of the experiment—less than one week ($n=1$), one to two weeks ($n=2$), two to three weeks ($n=7$), three to four weeks ($n=1$), or longer than four weeks ($n=4$)—. The second subgroup analysis, “type of stress” compared anthropogenic ($n=5$) to naturally occurring stress ($n=7$), or if stress was simulated by GC administration ($n=3$). The subsequent subgroup analysis “life history stage,” differentiates studies based on pre-maturate study organisms ($n=12$), or post-maturate study organisms ($n=3$). Next, the subgroup “GC assay,” separates studies into those that quantified plasma GCs ($n=13$) or non-plasma GCs ($n=2$). Similarly, by performing the subgroup analysis for the variable “telomere assay” we hoped to parse out potential differences between the three methods of telomere quantification: qPCR ($n=7$), TeloTAGGG ($n=1$), and Telomerase Restriction Fragment (TRF; $n=7$). The fifth subgroup analysis contrasts avian ($n=12$) and non-avian ($n=3$) studies. To explore the
relationship between individual species, we performed an additional subgroup analysis for each species included in the study. Finally, the subgroup analysis “study type” distinguished studies based on study design: cross-sectional (n = 5), repeated measures (n = 2), or within individual (n = 8) design.

**Publication bias.** Published studies may not accurately represent the total studies investigating an area of research due to selective outcome reporting, missing studies and a higher likelihood of publication of studies reporting a significant (p < 0.05) result. While proving selective outcome reporting and other forms of publication biases is challenging, missing studies can be visually represented using a funnel plot. Commonly, studies with small effect sizes and small sample studies are likely to be missing, which can be depicted with funnel asymmetry or holes in the funnel plot. We created a funnel plot by graphing effect size against study precision, defined as the standard error of the effect size to visualize potential publication bias. We also report an Egger’s test, which is represented by the intercept, it’s confidence interval, and the associated p-value to determine if publication bias was statistically significant.

**Risk of bias in included studies.** We assessed studies for missing outcome-level data, measurement of the outcomes, and outcome reporting in each included study. For the missing outcome-level data domain, we considered studies that could not report values for telomere length or GCs in less than 10% of total study organisms as low risk. We designated studies that did not report these values for 10–50% of study organisms as moderate risk and studies that did not report values for over 50% of GCs or telomere length, as high risk. Secondly, we based risk of bias in the measurement of outcome on the type of GC and telomere measurement. Low risk studies utilized plasma GCs or salivary GCs because these quantifications capture elevations related to a short-term stress event within minutes. Studies that measured GCs in fecal matter received a ranking of some concern because fecal GCs typically encapsulate cumulative stress over the day rather than GCs related to a particular environmental stressor. Fecal GCs also received a ranking of some concern due to potential variations related to storage and collection times, which can affect the concentration of fecal GC metabolites in a sample [29]. We considered studies that measured GCs in feathers as high risk because feathers incorporate GCs in over a month. Additionally, we considered feather GC quantification as high risk because feather preparation and GC extraction can vary greatly [30]. Finally, for the risk of bias due to outcome reporting we denoted studies that based results off a subset of time points or measurements high risk. We denoted studies that report results based on all time points with low risk. We took these three domains into consideration when assessing overall risk of bias.

**Results**

**Literature search and study selection**

We electronically screened 789 records for relevance from the following databases: Google Scholar (n = 512), Web of Science (n = 105), PubMed (n = 72), and Scopus (n = 100). 2113 additional records were hand screened from the reference section of the 31 studies used in qualitative analysis. Of the total 2902 records that were screened for relevance, 78 were removed as duplicates and 2,489 did not fit criteria for our study. For example, some excluded studies include human trials, cell culture work, or studies that only assessed research questions pertaining to either telomere length or GC levels, but not both (Fig 1; S1 Table). Of the 183 assessed full-text articles, we removed 152 studies that did not fulfill our inclusion criteria. We statistically analyzed 15 of the remaining 31 studies, the ones that provided raw data for analysis either within the manuscript or after contacting the corresponding author [16, 22, 29–42]. The other 16 studies appeared to fit criteria but did not provide raw data for analysis [26, 30,
The literature and study selection process is illustrated using a PRISMA diagram (Fig 1).

**Meta-analysis**

The random-effects model meta-analysis is represented as a pooled effect size (Fisher’s $Z$) with 95% confidence intervals (Fig 2). No studies were removed as outliers. The model found no relationship between GC levels and telomere length (Fisher’s $Z = 0.1042$, CI = 0.0235; 0.1836). Both heterogeneity measures, Cochrane’s Q ($Q = 11.31$, $p = 0.6615$) and $I^2$ with 95% confidence intervals ($I^2 = 0.0%;$ CI = 0.0%; 42.6%) yielded similar results.

The influence analysis indicated that theoretically removing one study at a time did not yield pooled effect sizes (Fisher’s $Z = 0.09–0.11$) that differed from the original pooled effect size (Fisher’s $Z = 0.11$, S1 Fig). Additionally, the influence analysis demonstrated that certain studies unevenly impacted the pooled effect size and/or overall heterogeneity (S2 Fig), but no studies were removed as outliers.

**Subgroup analysis**

The subgroup analysis for “stressor duration” found no differences between any of the tested time frames (Table 1). The difference between studies was not statistically significant.

---

**Figure 1.** PRISMA diagram. PRISMA diagram showing the selection process for references included in the meta-analysis of the effects of GCs on telomere length.

https://doi.org/10.1371/journal.pone.0257370.g001

**Figure 2.** Forest plot. Distribution of effect sizes of GCs on telomere length and 95% CI of effect size. Dashed lines represent pooled effect sizes using a random and fixed effect model. Heterogeneity ($I^2$), the percent of variability in effect sizes that is not caused by sampling error indicates very little variability in effect size. Weight indicates the influence the study has on the overall pooled effect.

https://doi.org/10.1371/journal.pone.0257370.g002
### Table 1. Pooled effect sizes with 95% CI of experimental parameters investigated during the five subgroup analyses for stressor duration, stressor type, life history stage, GC assay and taxa group.

| Experimental Parameter       | Number of Studies | Effect Size (Fisher’s Z) | 95% CI            |
|------------------------------|-------------------|--------------------------|-------------------|
| **Stressor Duration**        |                   |                          |                   |
| < 1 week                     | n = 1             | 0.0135                   | -0.2717; 0.2965   |
| 1–2 weeks                    | n = 2             | 0.0959                   | -0.1663; 0.3454   |
| 2–3 weeks                    | n = 7             | 0.0741                   | -0.0157; 0.1628   |
| 3–4 weeks                    | n = 1             | 0.0957                   | -0.2950; 0.4591   |
| > 4 weeks                    | n = 4             | 0.2181                   | 0.0101; 0.4080    |
| **Type of Stress**           |                   |                          |                   |
| Anthropogenic                | n = 5             | 0.1902                   | 0.0059; 0.3621    |
| Naturally occurring          | n = 7             | 0.0451                   | -0.0388; 0.1284   |
| GC administration            | n = 3             | 0.1425                   | -0.0320; 0.3085   |
| **Life History Stage**       |                   |                          |                   |
| Pre-maturation               | n = 12            | 0.1111                   | 0.0185; 0.2019    |
| Post-maturation              | n = 3             | 0.0843                   | -0.1183; 0.2800   |
| **GC Assay**                 |                   |                          |                   |
| Plasma GCs                   | n = 12            | 0.1012                   | 0.0061; 0.1945    |
| Non-Plasma GCs               | n = 3             | 0.1161                   | -0.0437; 0.2701   |
| **Taxa Group**               |                   |                          |                   |
| Avian                        | n = 12            | 0.1012                   | 0.0088; 0.1919    |
| Non-Avian                    | n = 3             | 0.1183                   | -0.0600; 0.0936   |
| **Species**                  |                   |                          |                   |
| Capreolus capreolus          | n = 1             | 0.0993                   | [-0.2072; 0.3881] |
| Coturnix japonica            | n = 1             | 0.0596                   | [-0.1973; 0.3089] |
| Fregata magnificens          | n = 1             | 0.1306                   | [-0.1232; 0.3684] |
| Hydrobates pelagicus         | n = 1             | 0.4651                   | [0.0857; 0.7267]  |
| Parus major                  | n = 2             | 0.0707                   | [-1.322; 0.2678]  |
| Phalacrocorax aristotelis    | n = 1             | 0.1852                   | [-0.0600; 0.2893] |
| Rana temporaria              | n = 1             | 0.0657                   | [-0.1962; 0.2090] |
| Rissa tridactyla             | n = 1             | 0.0826                   | [-0.1686; 0.3238] |
| Sterna hirundo               | n = 1             | 0.0957                   | [-0.2950; 0.4591] |
| Sturnus unicolor             | n = 1             | 0.0088                   | [-1.1182; 0.1356] |
| Tachycineta bicolor          | n = 2             | 0.1134                   | [-0.2110; 0.4153] |
| Turdus merula                | n = 1             | 0.0539                   | [-0.3004; 0.3952] |
| Welsh pony                   | n = 1             | 0.2693                   | [0.0252; 0.4831]  |
| **Telomere Assay**           |                   |                          |                   |
| qPCR                         | n = 7             | 0.1186                   | [-0.0089; 0.2424] |
| TeloTAGGG                    | n = 1             | 0.1306                   | [-0.1232; 0.3684] |
| TRF                          | n = 7             | 0.0909                   | [-0.0409; 0.2197] |
| **Study Type**               |                   |                          |                   |
| Cross sectional              | n = 5             | 0.1687                   | [-0.0219; 0.3474] |
| Repeated measure             | n = 2             | 0.0271                   | [-0.1336; 0.1864] |
| Within individual            | n = 8             | 0.0984                   | [0.0040; 0.1910]  |

The meta-regression was performed for the continuous variable publication year and represented as Cochrane’s Q and the associated p-value. Publication dates ranged from 2014–2021. Publication date was not a significant predictor of effect size (Q = 1.252, p = 0.2632).

https://doi.org/10.1371/journal.pone.0257370.t001
Similarly, the subgroup analysis for “stressor type,” did not reveal a difference between types of stressors (Table 1). The between study difference was not significantly different (Q = 2.56, p = 0.2783). Likewise, our subgroup “life history stage,” did not show differences between effect sizes for pre- and post-maturation organisms (Table 1), and did not indicate a difference between groups (Q = 0.06, p = 0.8119). The fourth subgroup analysis, “GC assay” did not find a difference between plasma GCs and other GC measurements, yielding a non-significant difference between studies (Q = 0.03, p = 0.8742) (Table 1). Additionally, the between study difference for the telomere assay subgroup did not find a significant difference between the three telomere quantification methods (Q = 0.12, p = 0.9401; Table 1). Our sixth subgroup analysis examined potential differences in effect size due to taxa, which could be divided into the binary categories avian and non-avian (Table 1). There was no difference between-studies (Q = 0.03, p = 0.8666). Our analysis further explored species-specific differences and accordingly did not find a significant difference between species (Q = 9.27, p = 0.6797). Similarly, the final analysis investigated potential differences between study designs and yielded a non-significant difference between cross-sectional, repeated measures, or within individual designs (Q = 1.27, p = 0.5289).

**Publication bias**

We found publication bias against studies with small sample size and small effect size (S3 Fig; Egger’s test for small sample bias: intercept = 1.420616, CI = 0.3753223; 2.465909, p = 0.02064949).

**Risk of bias in included studies**

We represent the results of the risk of bias analysis in Table 2. Four of fifteen studies received a risk of bias ranking of moderate concern. These studies had some missing values for GCs or telomere length or selectively reported one time point in the results. The other eleven studies received a ranking of low risk and accordingly reported nearly all values for physiological parameters, measured GCs in plasma or saliva, and did not selectively report results.

### Table 2. Overall risk of bias assessed based on missing outcome data, measure of outcome and in the selection of reported results.

| Author            | Year | Bias due to missing outcome data | Bias in measure of outcome | Bias in the selection of reported result | Overall Risk of Bias   |
|-------------------|------|---------------------------------|---------------------------|------------------------------------------|-----------------------|
| Bauch et al       | 2016 | high                            | low                       | high                                     | some concern          |
| Burraco et al     | 2019 | low                             | low                       | low                                      | low risk              |
| Casagrande et al  | 2020 | high                            | low                       | low                                      | some concern          |
| Gil et al         | 2019 | low                             | low                       | low                                      | low risk              |
| Grunst et al      | 2020 | low                             | high                      | low                                      | low risk              |
| Hau et al         | 2015 | low                             | low                       | low                                      | low risk              |
| Herborn et al     | 2014 | low                             | low                       | low                                      | low risk              |
| Injaian et al     | 2019 | high                            | low                       | high                                     | high risk             |
| Lansade et al     | 2018 | low                             | low                       | low                                      | low risk              |
| Lemaître et al    | 2021 | low                             | moderate                  | low                                      | low risk              |
| Pegan et al       | 2019 | high                            | low                       | low                                      | some concern          |
| Sebastiano et al  | 2017 | low                             | low                       | low                                      | low risk              |
| Stier et al       | 2020 | low                             | low                       | low                                      | low risk              |
| Watson et al      | 2016 | low                             | low                       | low                                      | low risk              |
| Young et al       | 2017 | moderate                        | low                       | high                                     | some concern          |

https://doi.org/10.1371/journal.pone.0257370.t002
**Discussion**

External and internal stimuli can activate the neuroendocrine stress response in vertebrates, resulting in the secretion of GCs, which induces multiple downstream physiological and behavioral effects [8, 9]. GCs might directly or indirectly cause telomere erosion [1, 11, 32]. Therefore, our goal was to investigate the relationship between GCs and telomere length via meta-analysis using data from empirical studies. Though our sample size was limited (n = 15), our data do not support the hypothesis that elevated GC levels result in telomere shortening.

The empirical evidence for a relationship between GCs and telomere length is mixed, with some studies showing that telomere shortening is directly related to GC levels, and other studies finding no relationship. For example, GCs influence telomere dynamics in wild roe deer and great tits [32, 39], but not in red squirrels or magellanic penguins [46, 47]. These results suggest that the relationship between GCs and telomere length is species-specific. Alternatively, a potential relationship may be obscured by the methods used to measure GCs and telomere length or by differences in experimental design including time frame. A differential sensitivity of the HPA axis can also obscure conclusions made from GC measurements especially in free-ranging vertebrates that can potentially encounter a variety of external stimuli [1]. For example, since GC levels in plasma remain elevated for several minutes after a stressor subsides, it can be challenging to assess whether a measured GC increase results from the stressor in question, the stress involved in obtaining a sample from the experimental subject, or an unrelated event triggering HPA axis activation [6, 56]. As baseline plasma GC samples must be collected quickly in many species, it can be logistically difficult to attain a true baseline GC value in the field [57–60]. GCs can also be incorporated into other matrices such as saliva, feathers, and hair [4, 58]. The multitude of non-invasive GC sampling sources is advantageous to conservation physiology as their quantification does not require capture [6]. However, across tissues and fluids, the time required for GC incorporation varies. For example, elevations in plasma GCs can be detected within minutes of stressor exposure, whereas GCs integrate into hair a week or more after stressor exposure [4]. Hence, there are caveats in the interpretation of each measurement such as incongruencies between GC levels in plasma and other tissues, hair and saliva [60]. Therefore, GC measurements in feces may be more representative of accumulated stress, rather than the event in question [6].

GC quantification in tissues and feces can also present specific uncertainty and imprecision during sampling, storage, and extraction. In fecal samples, GC metabolites can increase up to 92% in 120 days and provide an inaccurate assessment of GC levels [61, 62]. Excrement not collected immediately or across different time scales can obscure potential differences since exposure to abiotic factors like rainfall or extreme temperature can alter the concentration of fecal glucocorticoid metabolites [63]. Moreover, diet can affect GC metabolites in fecal samples, since an increased amount of cellulose depresses fecal glucocorticoid metabolite concentrations [61]. Similarly, feather preparation and extraction can also affect GC levels [64]. Further, different parts of the feather yield different concentrations of GCs. Saliva based GC extraction and quantification hosts similar shortcomings, though salivary GCs increase on a similar timeline (5–10 minutes) to circulating plasma GCs and thus prove a close proxy for plasma GC quantification [65]. Other factors such as time since last meal and recent activity also impact salivary GC measurement [66].

Similar considerations must be taken into account when assessing telomere length. Since telomere length can be influenced by environmental, maternal, and epigenetic effects, there is a large inter-individual variability in telomere dynamics [11, 67]. Several factors may contribute to this variability including discrepancies between the repeatability of different telomere measurement assays. Seven studies included in our meta-analysis utilized the telomere...
restriction fragment (TRF) assay, which depends on the distribution of the terminal restriction fragments to average the length of telomeres in a given cell population [68]. The other eight studies used the quantitative polymerase chain reaction (qPCR, n = 7), which relies on the quantification of the highly conserved (TTAGGG)$_n$ sequence for a Southern blot variation (TelotAGGG for telomere quantification (n = 1) [69]. TRF-based studies are highly repeatable within individuals, whereas qPCR based studies are less repeatable and more variable than TRF because they are more prone to measurement errors [70]. qPCR can also bias measurements of telomere length because some species that exhibit interstitial telomeric repeats will artificially enlarge telomere length [71, 72]. In addition to methodological differences, there is large individual variability in telomere length based on tissue type [73]. In adult zebra finches, telomere length in red blood cells is correlated with telomere length in the spleen, liver and brain, but not muscle or heart [31]. While avian studies in our meta-analysis used red blood cells for telomere measurement, telomere length was measured in tail muscle and liver in mammals and amphibians, which could lead to discrepancies when comparing among studies [31, 46, 57].

A variety of biological factors also contribute to the diversity of telomere dynamics observed within a study and the large amount of observed inter-individual variability. The rate of telomere shortening can be influenced by the life histories and environmental conditions [22]. In accordance with the metabolic telomere attrition hypothesis, shortening is exacerbated by life history stages requiring more energy, such as reproduction [32]. Within an energy intensive process like reproduction, there can be a large inter-individual variability related to reproductive effort, which can be attributed to brood size and food availability [74]. Differences in reproductive roles during the breeding season account for sex-specific telomere dynamics which can contribute to differences in the variability of telomere dynamics within a study [75]. Finally, individuals respond differently to environmental challenges which can act synergistically with rapid growth or energy intensive life stages to magnify the rate of telomere shortening in non-model vertebrates [71].

Telomere dynamics can be complicated by the presence of telomerase which in some cases can elongate telomeres [22, 76]. Typically, telomerase exhibits higher activity in developing organisms as compared to adults [77]. Ectotherms such as amphibians and reptiles have telomerase that is active throughout adulthood while endotherms reduce telomerase expression almost to non-detectable levels as they reach maturity [11, 70]. However, there is conflicting evidence on these observations, as telomerase activity has been detected in adult common terns and European Storm Petrels among other species [78, 79]. Nonetheless, adult telomere shortening is observed in chickens, which have active telomerase in the adult life stage [26]. While there is an absence of empirical evidence on the long-term activity of telomerase in many avian species, even adults exhibit general shortening trends [76].

Many factors influence GC and telomere measurements. During the subgroup analysis, we attempted to disentangle the underlying causes of the variation in effect size. Ultimately, we found no impact of stressor, taxa, type of GC assay, or life history stage on the heterogeneity of the effect size. While no subgroup was identified as a predictor of heterogeneity in effect size, pooled effect sizes in certain categories with the subgroup indicate a higher pooled effect size than the overall pooled effect size. The small sample size for some parameters precluded further statistical analysis, however, we found variables of interest that may play a large role in the relationship between GCs and telomere length. For example, within “experimental timeframe,” (n = 4) the group of studies with a timeframe above four weeks had a pooled effect size of 0.2181, while all other groups’ pooled effect size was less than that of the overall pooled effect size. Since most studies took place in less than four weeks, this suggests that while almost immediate changes in GCs can be observed, the impact of GCs on telomere length cannot be
seen on short time scales. This idea is consistent with typical responses of telomere shortening observed in studies that take place for more than a year [29, 54, 79–81]. More work is needed to explore if long-term rather than short-term studies can be used to tease apart parameters that underlie the connection between GCs and telomere length such as stressor type or duration.

While GC secretion is often viewed as the endpoint of HPA axis activation in response to external stimuli, GC manipulation is an oversimplification of the stress response which involves a multitude of physiological mechanisms that can each impact energy allocation and promote telomere erosion [8]. This highlights the problematic nature of the category “GC stress” which was investigated as a category during the subgroup analysis, in which studies subjected organisms to GC manipulation via an implant or oral administration. Since previous research found that organismal stress can result in adverse physiological responses without the involvement of the HPA axis, these results underscore the issue of using only GCs as a proxy for stress [82, 83].

Overall, we found no relationship between GCs and telomere length across studies. Currently, the existing literature shows both a direct relationship and a lack of a relationship between GCs and telomere dynamics, suggesting that the underlying mechanisms driving this relationship are species-specific or altered by differences in experimental design. However, due to limited sample size, we are unable to investigate the underlying variables that play a role in this relationship. Here, we highlight the need for more studies with targeted experimental parameters to understand how conditions, such as experimental timeframes, stressor(s), and stressor magnitudes can drive a potential relationship between the neuroendocrine stress response and cellular aging. Thus, we recommend the following research priorities to groups studying similar questions.

1. Experimental timeframes and stressor magnitudes should be long enough to observe telomere erosion in relation to stressors when studying GCs.

2. When possible, studies should use a repeated measures design to measure cortisol levels and telomere lengths before and after stress exposure to account for individual variation.

3. While the avian taxa are well represented in this research topic, there is a dearth of information on other taxa. It will be important to investigate the neuroendocrine stress response in other vertebrates including mammals and reptiles to understand if similar principles hold true in these taxa or if telomere dynamics differ across taxa.

4. If possible, future research should assess the functionality of the study organisms’ HPA axis by ACTH/dexamethasone challenge prior to exposure to a stressor and completion of the study.

Certainty of evidence
We utilized the applicable Cochrane/GRADE categories “risk of bias,” “inconsistency,” and “publication bias,” for the determination of the certainty of evidence. Overall, we have a moderate confidence in the certainty of evidence. While most studies received a low risk of bias assessment, and had low heterogeneity, we report a considerable amount of publication bias as evidenced by Egger’s test and an asymmetrical funnel plot.

Supporting information
S1 Checklist. PRISMA 2020 checklist. (PDF)
S1 Fig. Influence analysis plot. The leave one out recalculation reveals a similar effect size across studies and indicates that studies evenly contribute to the pooled effect size. (TIF)

S2 Fig. Baujat plot. Studies can have an unequal influence on the pooled effect size and contribute to the heterogeneity of effect sizes. The horizontal axis represents Cochrane’s Q and influence on the pooled effect size on the vertical axis. (TIF)

S3 Fig. Funnel plot. The lack of studies in the bottom left of the “funnel” demonstrates publication bias against studies with small sample sizes and small effect sizes. (TIF)

S1 Table. Search strategy table. Details search term combinations used to search online databases and websites. (XLSX)

Acknowledgments
We would like to thank all the researchers who made their data freely available for this study. In particular, we thank Christine Bauch (University of Groningen), Stefania Casagrande (Max Planck Institute for Ornithology), Britt Heidinger (North Dakota State University), Marie-Pierre Moisan (French National Institute for Agriculture, Food, and Environment), Teresa Pegan (University of Michigan), Manrico Sebastiano (French National Centre for Scientific Research), Mathilde Tissier (Bishop’s University), and Hannah Watson (Lund University).

Author Contributions
Conceptualization: Lauren Zane, David C. Ensminger, José Pablo Vázquez-Medina.
Data curation: Lauren Zane.
Formal analysis: Lauren Zane.
Funding acquisition: José Pablo Vázquez-Medina.
Investigation: Lauren Zane.
Methodology: Lauren Zane, David C. Ensminger.
Project administration: David C. Ensminger.
Resources: David C. Ensminger.
Software: Lauren Zane.
Supervision: David C. Ensminger, José Pablo Vázquez-Medina.
Validation: David C. Ensminger, José Pablo Vázquez-Medina.
Visualization: Lauren Zane.
Writing – original draft: Lauren Zane.
Writing – review & editing: David C. Ensminger, José Pablo Vázquez-Medina.

References
1. Haussmann MF, Marchetto NM. Telomeres: Linking stress and survival, ecology and evolution. Current Zoology. 2010; 56(6):714–27.
2. Wingfield JC, Sapolsky RM. Reproduction and Resistance to Stress: When and How: Reproduction and resistance to stress. Journal of Neuroendocrinology. 2003; 15(8):711–24. https://doi.org/10.1046/j.1365-2826.2003.01033.x PMID: 12834431

3. Koren L, Whiteside D, Fahiman A, Ruckstuhl K, Kutz S, Checkley S, et al. Cortisol and corticosterone independence in cortisol-dominant wildlife. General and Comparative Endocrinology. 2012 May; 177(1):113–9. https://doi.org/10.1016/j.ygcen.2012.02.020 PMID: 22449618

4. Gormally BMG, Romero LM. What are you actually measuring? A review of techniques that integrate the stress response on distinct time-scales. Functional Ecology. 2020; 34(10):2030–44.

5. MacDougall-Shackleton SA, Bonier F, Romero LM, Moore IT. Glucocorticoids and “Stress” Are Not Synonymous. Integrative Organismal Biology. 2019; 1(1).

6. Sheriff MJ, Dantzer B, Delehanthy B, Palme R, Boonstra R. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia. 2011; 166(4):869–87. https://doi.org/10.1007/s00442-011-1943-y PMID: 21344254

7. Arnold JM, Oswald SA, Voigt CC, Palme R, Brasch A, Bauch C, et al. Taking the stress out of blood collection: comparison of field blood-sampling techniques for analysis of baseline corticosterone. Journal of Avian Biology. 2008; 39(5):588–92.

8. Angelier F, Wingfield JC. Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives. General and Comparative Endocrinology. 2013; 190:118–28. https://doi.org/10.1016/j.ygcen.2013.05.022 PMID: 23770214

9. Ricklefs RE, Wikelski M. The physiology/life-history nexus. Trends in Ecology & Evolution. 2002; 17(10):462–8.

10. Young AJ. The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. Philosophical Transactions of the Royal Society B. 2018; 373(1741):20160452. https://doi.org/10.1098/rstb.2016.0452 PMID: 29335379

11. Angelier F, Costantini D, Bévin P, Chastel O. Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. General and Comparative Endocrinology. 2018; 256:99–111. https://doi.org/10.1016/j.ygcen.2017.07.007 PMID: 28705731

12. Campisi J, Andersen JK, Kapahi P, Melov S. Cellular senescence: A link between cancer and age-related degenerative disease? Seminars in Cancer Biology. 2011; 21(1):113–9. https://doi.org/10.1016/j.semcancer.2011.09.001 PMID: 21925603

13. Donate LE, Blasco MA. Telomeres in cancer and ageing. Philosophical Transactions of the Royal Society B. 2011; 366(1561):76–84. https://doi.org/10.1098/rstb.2010.0291 PMID: 21115533

14. Levy MZ, Allsopp RC, Frazier AB, Greider CW, Harley CB. Telomere end-replication problem and cell aging. Journal of Molecular Biology. 1992; 225(4):951–60. https://doi.org/10.1016/0022-2836(92)90096-3 PMID: 1613801

15. Monaghan P. Organismal stress, telomeres and life histories. Journal of Experimental Biology. 2014; 217(1):57–66.

16. Sebastiano M, Eens M, Angelier F, Pineau K, Chastel O, Costantini D. Corticosterone, inflammation, immune status and telomere length in frigatebird nestlings facing a severe herpesvirus infection. Conservation Physiology. 2017; 5(1):cow073. https://doi.org/10.1093/conphys/cow073 PMID: 28070333

17. Chatelain M, Drobnjak SM, Szułkin M. The association between stressors and telomeres in non-human vertebrates: a meta-analysis. Ecology Letters. 2020; 23(2):381–98. https://doi.org/10.1111/ele.14326 PMID: 31773847

18. Belmaker A, Hallinger KK, Glynn RA, Winkler DW, Haussmann MF. The environmental and genetic determinants of chick telomere length in Tree Swallows (Tachycineta bicolor). Ecology and Evolution. 2019; 9(14):8175–86. https://doi.org/10.1002/eco3.5386 PMID: 31380080

19. Reichert S, Stier A, Zahn S, Arrivé M, Bize P, Masselin S, et al. Increased brood size leads to persistent eroded telomeres. Frontiers in Ecology and Evolution [Internet]. 2014; 2:9.

20. Eastwood JR, Hall ML, Teunissen N, Kingma SA, Hidalgo Aranzamendi N, Fan M, et al. Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. Molecular Ecology. 2019; 28(5):1127–37. https://doi.org/10.1111/mec.15002 PMID: 30592345

21. Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB, Monaghan P. Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences. 2012; 109(5):1743–8. https://doi.org/10.1073/pnas.1113306109 PMID: 22232671

22. Stier A, Metcalfe NB, Monaghan P. Pace and stability of embryonic development affect telomere dynamics: an experimental study in a precocial bird model. Proceedings of the Royal Society B. 2020; 287(1933):20201378. https://doi.org/10.1098/rspb.2020.1378 PMID: 32842933

23. Casagrande S, Hau M. Telomere attrition: metabolic regulation and signalling function? Biol Lett. 2019; 15(3):20180885. https://doi.org/10.1098/rsbl.2018.0885 PMID: 30890069
40. Tissier ML, Williams TD, Criscuolo F. Maternal Effects Underlie Ageing Costs of Growth in the Zebra Finch (Taeniopygia guttata). White SA, editor. PLoS ONE. 2014; 9(5):e97705. https://doi.org/10.1371/journal.pone.0097705 PMID: 24828412

41. Watson H, Bolton M, Heidinger BJ, Boner W, Monaghan P. Assessing the effects of repeated handling on the physiology and condition of semi-precocial nestlings. IBIS International Journal of Avian Science. 2016; 158(4):834–43. https://doi.org/10.1111/ibi.12402 PMID: 27708454
42. Young R, Orben R, Will A, Kitaysky A. Relationship between telomere dynamics and movement and behavior during winter in the thick-billed murre. Marine Ecological Progress Series. 2017; 578:253–61.

43. Cerchiara JA, Risques RA, Prunkard D, Smith JR, Kane OJ, Boersma PD. Magellanic penguin telomeres do not shorten with age with increased reproductive effort, investment, and basal corticosterone. Ecological Evolution. 2017; 7(15):5682–91. https://doi.org/10.1002/ece3.3128 PMID: 28811878

44. Dantzer B, van Kesteren F, Westrick SE, Boutin S, McAdam AG, Lane JE, et al. Maternal glucocorticoids promote offspring growth without inducing oxidative stress or shortening telomeres in wild red squirrels. Journal of Experimental Biology. 2020; 223(1):jeb212373. https://doi.org/10.1242/jeb.212373 PMID: 31796605

45. Drury SS, Howell BR, Jones C, Esteves K, Morin E, Schlesinger R, et al. Shaping long-term primate development: Telomere length trajectory as an indicator of early maternal maltreatment and predictor of future physiologic regulation. Developmental Psychopathology. 2017; 29(5):1539–51. https://doi.org/10.1017/S0954579417001225 PMID: 29162166

46. Gangoso L, Lambertucci SA, Cabezas S, Alarcón PAE, Wiemeyer GM, Sanchez-Zapata JA, et al. Sex-dependent spatial structure of telomere length in a wild long-lived scavenger. Ecosphere. 2016; 7(10): e01544.

47. Nogueira JC, Velando A. Reduced telomere length in embryos exposed to predator cues. Journal of Experimental Biology. 2019; 222(24):jeb216176. https://doi.org/10.1242/jeb.216176 PMID: 31796604

48. Noreikienė K, Oṣmangil Z, Moore IT, Bonier F, Haussmann MF. Do Hormones, Telomere Lengths, and Oxidative Stress form an Integrated Phenotype? A Case Study in Free-Living Tree Swallows. Integrative and Comparative Biology. 2016; 56(2):138–45. https://doi.org/10.1093/icb/icw044 PMID: 27252220

49. Stevenson JR, McManus EK, Boner W, Monaghan P, Jaatinen K. Nest cover and faecal glucocorticoid metabolites are linked to hatching success and telomere length in breeding Common Eiders (Somateria mollissima). Canadian Journal of Zoology. 2017; 95(9):695–703.

50. Quirici V, Guerrero CJ, Krause JS, Wingfield JC, Vásquez RA. The relationship of telomere length to baseline corticosterone levels in nestlings of an altricial passerine bird in natural populations. Frontiers in Zoology 2016; 13(1):1:22.

51. Schultner J, Moe B, Chastel O, Bech C, Kitaysky AS. Migration and stress during reproduction govern telomere dynamics in a seabird. Biology Letters. 2014; 10(1):20130889. https://doi.org/10.1098/rsbl.2013.0889 PMID: 24429681

52. Shen Q, Wu J, Ni Y, Xie X, Yu C, Xiao Q, et al. Exposure to jet lag aggravates depression-like behaviors and age-related phenotypes in rats subject to chronic corticosterone. Acta Biochimica et Biophysica Sinica. 2019; 51(8):834–44. https://doi.org/10.1093/abbs/gmz070 PMID: 31314053

53. Stevenson JR, McManus EK, Boner W, Haussmann MF. Oxytocin administration prevents cellular aging caused by social isolation. Psychoneuroendocrinology. 2019; 103:52–60. https://doi.org/10.1016/j.psyneuen.2019.01.006 PMID: 30640038

54. Xie X, Shen Q, Ma L, Chen Y, Zhao B, Fu Z. Chronic corticosterone-induced depression mediates premature aging in rats. Journal of Affective Disorders; 229:254–61. https://doi.org/10.1016/j.jad.2017.12.073 PMID: 29329057

55. Young R, Welcker J, Barger CP, Hatch SA, Merkling T, Kitaiskaia EV, et al. Effects of developmental conditions on growth, stress and telomeres in black-legged kittiwake chicks. Molecular Ecology. 2017; 26(13):3572–84. https://doi.org/10.1111/mec.14121 PMID: 28370751

56. Romero LM, Meister CJ, Cyr NE, Kenagy GJ, Wingfield JC. Seasonal glucocorticoid responses to capture in wild free-living mammals. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2008; 294(2):R614–22. https://doi.org/10.1152/ajpregu.00752.2007 PMID: 18094060

57. Tylan C, Camacho K, French S, Graham SP, Herr MW, Jones J, et al. Obtaining plasma to measure baseline corticosterone concentrations in reptiles: How quick is quick enough? General and Comparative Endocrinology. 2020; 287:113324. https://doi.org/10.1016/j.ygece.2019.113324 PMID: 31733208

58. Dantzer B, Fletcher QE, Boonstra R, Sheriff MJ. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conservation Biology. 2014; 28(1). https://doi.org/10.1111/comps.12023 PMID: 27293644

59. Reeder DM, Kramer KM. STRESS IN FREE-RANGING MAMMALS: INTEGRATING PHYSIOLOGY, ECOLOGY, AND NATURAL HISTORY. Journal of Mammalogy. 2005; 86(2):225–35.

60. Keckeis K, Lepschy M, Schopper H, Moser L, Trosler J, Palme R. Hair cortisol: a parameter of chronic stress? Insights from a radiomeboli sm study in guinea pigs. J Comp Physiol B. 2012; 182(7):985–96. https://doi.org/10.1007/s00360-012-0674-7 PMID: 22592900

61. Goymann W. "Noninvasive Monitoring of Hormones in Bird Droppings: Physiological Validation, Sampling, Extraction, Sex Differences, and the Influence of Diet on Hormone Metabolite Levels." Annals of the New York Academy of Sciences (2005); 1046(1): 35–53.
62. Khan M. Z., Altmann J., Isani S. S. and Yu J. "A matter of time: evaluating the storage of fecal samples for steroid analysis." General and Comparative Endocrinology (2002); 128(1): 57–64. https://doi.org/10.1016/s0016-6480(02)00063-1 PMID: 12270788

63. MILLSPAUGH J.J. & WASHBURN B.E. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General Comparative Endocrinology (2004); 138: 189–199. https://doi.org/10.1016/j.ygcen.2004.07.002 PMID: 15364201

64. Freeman N. E. and Newman A. E. M. "Quantifying corticosterone in feathers: validations for an emerging technique." Conservation Physiology (2018); 6: 9.

65. Hammond TT, Au ZA, Hartman AC, Richards-Zawacki CL. Assay validation and interspecific comparison of salivary glucocorticoids in three amphibian species. Conservation Physiology. (2018); 6(1). https://doi.org/10.1093/conphys/coy055 PMID: 30279992

66. Garde AH, Persson R, Hansen Å. M, Österberg K, Ørbaek P, Eek F, et al. Effects of lifestyle factors on concentrations of salivary cortisol in healthy individuals. Scandinavian Journal of Clinical Lab Investigations (2009); 69(2): 242–250. https://doi.org/10.1080/00365510802483708 PMID: 18985537

67. Shaley I, Entringer S, Wadhwa PD, Wolkwitz OM, Puterman E, Lin J, et al. Stress and telomere biology: A lifespan perspective. Psychoneuroendocrinology. 2013; 38(9):1835–42. https://doi.org/10.1016/j.psyneuen.2013.03.010 PMID: 23639252

68. Mender I, Shay J. Telomere Restriction Fragment (TRF) Analysis. BIO-PROTOCO L. 2015; 5(22): e1658. https://doi.org/10.2176/bioprotoc.1658 PMID: 27500189

69. Gomes NMV, Shay JW, Wright WE. Telomere biology in Metazoa. FEBS Letters. 2010; 584(17):3741–51. https://doi.org/10.1016/j.febslet.2010.07.031 PMID: 20655915

70. Kárrkäinen T, Briga M, Laaksonen T, Stier A. Within-individual repeatability in telomere length: a meta-analysis in non-mammalian vertebrates. Preprints; 2021. https://doi.org/10.1111/mec.16155 PMID: 34456645

71. Royatso S, Krateochvíl L, Altmannová M, Johnson Pokorná M. Interstitial Telomeric Motifs in Squamate Reptiles: When the Exceptions Outnumber the Rule. Stanyon R, editor. PLoS ONE. 2015; 10(8): e0142530. https://doi.org/10.1371/journal.pone.0142530 PMID: 26565632

72. Haussmann MF, Mauck RA. TECHNICAL ADVANCES: New strategies for telomere-based age estimation: TECHNICAL ADVANCES. Molecular Ecology Resources. 2008; 8(2):264–74. https://doi.org/10.1111/j.1471-8286.2007.01973.x PMID: 21585768

73. Reichert S, Criscuolo F, Verinaud E, Zahn S, Massemin S. Telomere Length Correlations among

74. Haussmann MF, Mauck RA. TECHNICAL ADVANCES: New strategies for telomere-based age estimation: TECHNICAL ADVANCES. Molecular Ecology Resources. 2008; 8(2):264–74. https://doi.org/10.1111/j.1471-8286.2007.01973.x PMID: 21585768

75. Parolini M, Romano A, Khoriauli L, Nergadze SG, Caprioli M, Rubolini D, et al. Early-Life Telomere Dynamics Differ between the Sexes and Predict Growth in the Barn Swallow (Hirundo rustica). Lustig AJ, editor. PLoS ONE. 2015; 10(11):e0142530. https://doi.org/10.1371/journal.pone.0142530 PMID: 26565632

76. Taylor HA, Delany ME. Ontogeny of telomerase in chicken: Impact of downregulation on pre- and postnatal telomere length in vivo. Development, Growth, and Differentiation. 2000; 42(6):613–21. https://doi.org/10.1046/j.1440-169x.2000.00540.x PMID: 11142683

77. Gomes NMV, Ryder OA, Houck ML, Charter SJ, Walker W, Forsyth NR, et al. Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination: The comparative biology of mammalian telomeres. Aging Cell. 2011; 10(5):761–8. https://doi.org/10.1111/j.1474-9726.2011.00718.x PMID: 21518243

78. Haussmann MF, Winkler DW, Huntington CE, Nisbet ICT, Vleck CM. Telomerase activity is maintained throughout the lifespan of long-lived birds. Experimental Gerontology. 2007; 42(7):610–8. https://doi.org/10.1016/j.exger.2007.03.004 PMID: 17470387

79. Angelier F, Vleck CM, Holberton RL, Marra PP. Telomere length, non-breeding habitat and return rate in male American redstarts. Funct Ecol.2013; 27(2):342–50.

80. Asghar M, Hasselquist D, Hansson B, Zehntlindiev P, Westerdahl H, Bensch S. Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. Science. 2015; 347 (6220):436–8. https://doi.org/10.1126/science.1261121 PMID: 25613889

81. Barrett ELB, Burke TA, Hammers M, Komdeur J, Richardson DS. Telomere length and dynamics predict mortality in a wild longitudinal study. Molecular Ecology. 2013; 22(1):249–59. https://doi.org/10.1111/mec.12110 PMID: 23167566
82. Breuner CW, Delehanty B, Boonstra R. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Functional Ecology. 2013; 27(1):24–36.

83. Gabor CR, Knutie SA, Roznik EA, Rohr JR. Are the adverse effects of stressors on amphibians mediated by their effects on stress hormones? Oecologia. 2018; 186(2):393–404. https://doi.org/10.1007/s00442-017-4020-3 PMID: 29222721