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Do Seasonal Glucocorticoid Changes Depend on Reproductive Investment? A Comparative Approach in Birds

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Synopsis Animals go through different life history stages such as reproduction, moult, or migration, of which some are more energy-demanding than others. Baseline concentrations of glucocorticoid hormones increase during moderate, predictable challenges and thus are expected to be higher when seasonal energy demands increase, such as during reproduction. By contrast, stress-induced glucocorticoids prioritize a survival mode that includes reproductive inhibition. Thus, many species down-regulate stress-induced glucocorticoid concentrations during the breeding season. Interspecific variation in glucocorticoid levels during reproduction has been successfully mapped onto reproductive investment, with species investing strongly in current reproduction (fast pace of life) showing higher baseline and lower stress-induced glucocorticoid concentrations than species that prioritize future reproduction over current attempts (slow pace of life). Here we test the "glucocorticoid seasonal plasticity hypothesis", in which we propose that interspecific variation in seasonal changes in glucocorticoid concentrations from the non-breeding to the breeding season will be related to the degree of reproductive investment (and thus pace of life). We extracted population means for baseline (for 54 species) and stress-induced glucocorticoids (for 32 species) for the breeding and the non-breeding seasons from the database "HormoneBase", also calculating seasonal glucocorticoid changes. We focused on birds because this group offered the largest sample size. Using phylogenetic comparative methods, we first showed that species differed consistently in both average glucocorticoid concentrations and their changes between the two seasons, while controlling for sex, latitude, and hemisphere. Second, as predicted seasonal changes in baseline glucocorticoids were explained by clutch size (our proxy for reproductive investment), with species laying larger clutches showing a greater increase during the breeding

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season—especially in passerine species. In contrast, changes in seasonal stress-induced levels were not explained by clutch size, but sample sizes were more limited. Our findings highlight that seasonal changes in baseline glucocorticoids are associated with a species' reproductive investment, representing an overlooked physiological trait that may underlie the pace of life.

Introduction

Many animals go through resource-demanding phases of parental care that are required to raise their offspring successfully (Clutton-Brock 1991). The fitness gain (in terms of reproduction) of such an investment has to be balanced against the benefits of allocating limited resources to self-maintenance processes, promoting survival (Stearns 1992; Roff 2000; Harshman and Zera 2007; but see Santos and Nakagawa 2012; Williams 2012). The trade-off between reproduction and survival is key to understanding the diversity of life-history strategies observed at species, population, and individual levels (Ricklefs and Wikelski 2002; Bókony et al. 2009; Hau et al. 2010; Reale et al. 2010; Santos and Nakagawa 2012; Zhang and Hood 2016). Life-history strategies are viewed as a continuum along a single "pace-of-life" axis, on which certain physiological and behavioral traits covary (Ricklefs and Wikelski 2002; Roff 2002; Reale et al. 2010; Pap et al. 2015; Mathot and Frankenhuis 2018). For example, species with a fast pace of life exhibit high reproductive rates, low survival rates, and high mass-specific metabolic rates, whereas species with a slow pace of life show the opposite trait values (Wikelski et al. 2003; Wiersma et al. 2007; Reale et al. 2010; Versteegh et al. 2012; Le Galliard et al. 2013; Pap et al. 2015; Auer et al. 2018). The pace of life axis has a latitudinal component, with tropical species tending to follow a slow and higher latitude species often following a fast pace of life (Wikelski et al. 2003; Wiersma et al. 2007; Hau et al. 2010; Williams et al. 2010).

Glucocorticoids are major mediators of life-history trade-offs, because they function as key metabolic and behavioral regulators of organismal energy supplies (Wingfield et al. 1998; McEwen and Wingfield 2003; Wingfield and Sapolsky 2003; Breuner et al. 2008; Romero et al. 2009; Cornelius et al. 2011; Angelier and Wingfield 2013; Romero and Wingfield 2016). At baseline levels, glucocorticoids adjust basic processes like metabolism and behavior to meet the energetic demands that an individual faces during routine activities, for example during reproductive effort (Romero 2002; Landys et al. 2006; Romero et al. 2009; Lattin et al. 2016). Stress-induced glucocorticoid levels are secreted within a few minutes after the onset of a major unpredictable challenge to support an "emergency life history stage" (Wingfield et al. 1998; Sapolsky et al. 2000; Romero 2004; Landys et al. 2006). Stress-induced glucocorticoids rapidly promote a suite of processes that serve to reallocate energy reserves to survival functions, which includes the inhibition of non-vital processes like reproduction (McEwen and Wingfield 2003; Wingfield and Sapolsky 2003; Crespi et al. 2013).

Because of their actions, stress-induced glucocorticoids have already been considered mediators of life-history trade-offs, with concentrations differing across species that diverge in life-history strategies (Breuner et al. 2003, 2008; Wingfield and Sapolsky 2003; Crespi et al. 2013). However, from a life history perspective, glucocorticoids may well play a dual role: at baseline concentrations they are expected to support energetic challenges such as investment into reproduction ("cort-adaptation hypothesis", Bonier et al. 2009; Bonier et al. 2011), whereas at stress-induced levels they should prioritize investment into self-maintenance processes and survival (McEwen and Wingfield 2003; Wingfield and Sapolsky 2003). Hence, fast pace-of-life species with a high reproductive investment should exhibit higher baseline, but lower stress-induced glucocorticoid concentrations during the breeding season compared with species following a slow pace of life (Bókony et al. 2009; Hau et al. 2010). Indeed, comparative studies have generally supported these predictions for stress-induced glucocorticoids (Goymann et al. 2006; Lancaster et al. 2008; Bókony et al. 2009; Hau et al. 2010; Palacios et al. 2012; Apfelbeck et al. 2017); although the opposite has also been reported (Breuner et al. 2003; Martin et al. 2005; Versteegh et al. 2012). Likewise, baseline glucocorticoid concentrations are higher during the breeding season in species that invest more in current versus future reproduction (i.e., in fast pace of life species; Bókony et al. 2009; Hau et al. 2010).

Until now, studies on interspecific variation in glucocorticoids relative to pace of life only included glucocorticoid traits measured during a single life history stage, usually the breeding season (Goymann et al. 2006; Bókony et al. 2009; Hau et al. 2010; Versteegh et al. 2012). However, it is known that many species change glucocorticoids seasonally and most taxa have increased baseline glucocorticoid concentrations during breeding compared

with other seasonal stages (Romero 2002). Here, we hypothesize that seasonal glucocorticoid plasticity, i.e., the magnitude of change from the non-breeding to the breeding season, is related to pace of life, and in particular to the degree of reproductive investment of a species ("glucocorticoid seasonal plasticity hypothesis"). For baseline glucocorticoids, the hypothesis builds on the "energy mobilization hypothesis" (Romero 2002), which states that glucocorticoid concentrations should be highest during energetically demanding seasons (such as the reproductive period) to mobilize energy stores. For stress-induced glucocorticoids, the hypothesis is based on findings that certain species down-regulate glucocorticoid stress responses during the parental phase, perhaps to avoid an associated reproductive disruption (O'Reilly and Wingfield 1995; Holberton and Wingfield 2003; Wingfield and Sapolsky 2003). Our hypothesis also emphasizes the fact that glucocorticoid concentrations of species are not static, and that seasonal variations in glucocorticoid levels may be as, or even more, meaningful than absolute concentrations at a single time of the year. Variations in a trait along a gradient of environmental or internal factors can be quantified through reaction norm approaches (Nussey et al. 2007). Reaction norm approaches quantify both the average trait value (i.e., the intercept) and the degree of change in the trait along a gradient (i.e., the slope of the relationship; Williams 2008; Dingemanse et al. 2010; Hau et al. 2016). In the context of our hypothesis, we would expect species with divergent degrees of reproductive investment to differ in their slope of seasonal changes between the non-breeding and the breeding season baseline glucocorticoid.

Here, we test the glucocorticoid seasonal plasticity hypothesis using data from a new and comprehensive database on hormones and life history traits of free-living vertebrates ("HormoneBase", hormonebase.org, M. N. Vitousek et al., submitted for publication). Our study aims to analyze the variation within and among bird species in both baseline and stress-induced concentrations of corticosterone measured during non-breeding and breeding. We focus on birds, firstly because they are the taxon for which the largest dataset is available in HormoneBase and secondly, because they exhibit substantial variation in clutch sizes (Jetz et al. 2008) and thus degree of parental investment from a life-history theory perspective (Saether 1988; Horrocks et al. 2015). Irrespective of life history strategy, we expect the change in (1) baseline corticosterone (the major glucocorticoid of birds) and stress-induced corticosterone from non-breeding to breeding to be species-specific. Further (2), the magnitude of the seasonal change in baseline glucocorticoids should be related to the reproductive investment, i.e., species with larger clutch sizes (and a fast pace of life) should increase baseline corticosterone from non-breeding to breeding more strongly than species with smaller clutches. With regard to stress-induced corticosterone concentrations, (3) fast pace-of-life species with larger clutches should show a larger decrease in stress-induced corticosterone from non-breeding to breeding than slow pace-of-life species with smaller clutches.

Methods

Baseline and stress-induced corticosterone concentrations were obtained from the HormoneBase dataset (M. N. Vitousek et al., submitted for publication; M. A. Johnson et al., 2018, in preparation), which has assembled steroid hormone concentrations measured in diverse life history stages for all five vertebrate classes. We assumed that parental effort represents investment into breeding (Daan et al. 1990), therefore our "breeding season" category included the phases of active parental care ranging from egg-laying to offspring independence. In our "non-breeding" category, we included the stages ranging from post-breeding (after the independence of offspring) to mating. Thus we grouped the courtship and nest building phases into the non-breeding season. Even though both stages are costly, we decided on this approach because the intensity (or degree of investment) especially of courtship should primarily depend on mating system but be independent of clutch size, our proxy for pace of life.

For each species, glucocorticoid concentrations that were extracted at a population level as multiple entries for different populations, or the same populations sampled in different seasons, were available for many species. Likewise, we kept the data separated by sex as provided by HormoneBase. All baseline glucocorticoid concentrations considered for this study were taken within 3 min from any disturbance, while stress-induced concentrations were used when taken after 30 min from the onset of a capture-restraint protocol (e.g., Hau et al. 2015). We additionally compiled life-history traits on a species level (i.e., only one entry for life-history traits per species). Life history variables such as egg mass, age at fledging, mass at fledging, life expectancy, maximal longevity, survival rate, basal metabolic rate, body mass, and clutch size have been obtained from the life history trait data compiled by the HormoneBase Consortium, and described in M. A. Johnson et al.

(2018, in preparation, for this special issue). Because we focused on degree of investment into each reproductive event, our main proxy for this trait was clutch size (with species following a fast pace-of-life laying larger clutches; Horrocks et al. 2015). We used latitude (absolute distance from equator) as a predictor, to describe large-scale differences in the environment (Jetz et al. 2008). To account for any additional variability in the environment, we also included hemisphere (North versus South) as a predictor. We did not include mating system or parental system because the majority of the species considered in this study were quite uniform with respect of mating and parental care systems as the majority was monogamous (of 54 species only 7 species were polygynous, 4 were polyandrous, and 3 showed cooperative breeding), and bi-parental (only 2 species lacked male and another 2 lacked female parental care), and we therefore lacked variance in these traits. We are confident that species with rare mating systems have not confounded our results because there is no statistical difference in baseline corticosterone levels between monogamous and non-monogamous species ($t = 0.74$, $P = 0.48$). We were not able to perform similar comparisons for stress induced traits because there were only 2 species out of 32 with non-monogamous mating systems. An exploratory analysis considered migratory habits (migratory, non-migratory, partial migratory) but since it was not related to any glucocorticoid trait, we excluded this trait in subsequent analyses.

Statistical analysis

The existence of multiple entries for different populations of the same species allowed us, as a first step, to investigate whether corticosterone concentrations in the non-breeding and breeding seasons, as well as the differences between seasons, are species-specific. If species had not systematically differed in corticosterone traits, it would not have made sense to compare seasonal changes with respect to reproductive investment. For this purpose, we built phylogenetic generalized linear mixed models (PGLMM; Hadfield and Nakagawa 2010), in which population-specific corticosterone traits were the response variables (separate models for baseline and stress-induced corticosterone, both log10-transformed). Wherever data allowed (i.e., entries for several populations of a species, for which sex and seasonal data were also available), we entered sex and season as well as absolute latitude and hemisphere (North or South) as fixed predictors. Season was treated as a centered continuous predictor as required for random-slope modeling (see below). We also considered the interaction terms between sex and season and between latitude and hemisphere to allow seasonal responses to vary between sexes, and latitude effects to be different on the two sides of the globe, respectively. When modeling stress-induced corticosterone, the predictor variables also contained baseline concentrations. The random effects were species ID and phylogeny. Information on the phylogenetic relationships of birds was taken from Jetz et al. (2012), and was always pruned to include only the species included in the model and was converted into an inverted phylogenetic covariance matrix. The null models included only random intercepts, whereas the alternative models included random slopes to allow for species-specific slopes for seasonal effects. To compare models based on relative fit we focused on the associated Deviance Information Criterion (DIC) values under the premise that a lower DIC value offers a relatively better fit to the data. We considered a given model to be significantly supported against a null-model, if the former had a considerably (deltaDIC > 10) smaller value than the latter. Significant evidence for the better fit of the alternative model to the data signifies that species differ remarkably in how they change their hormonal profiles between the two seasons.

PGLMM analyses of both hormonal traits indeed suggested that hormonal responses are species-specific traits (see the "Results" section). Therefore, in a second step to extract a proxy variable for the seasonal change in corticosterone for each species for use in further analyses, we built simple linear models with season as predictor and the focal hormonal trait as the response. Because the above repeated measure models did not show strong evidence for sex effects confounding the species-specific seasonal responses, we did not include sex among the predictors of the linear models to maximize sample size. From estimated parameters of the fitted models, we extracted a correlational "r" effect size, and the associated variance (in the form of $1/(N-3)$, where N is the number of entries in the model), to describe the species-specific seasonal responses in a standardized way (Nakagawa and Cuthill 2007). These estimates, the baseline and stress-induced reaction norm slopes (or "seasonal changes"), were brought into the next level of analyses. Higher values for these slopes indicate an increase in corticosterone concentrations from the non-breeding to the breeding season.

To investigate the interspecific determinants of seasonal corticosterone changes, we entered the species-specific effect sizes describing these slopes into a PGLMM, which also accounted for differences in the underlying sample sizes. In these phylogenetic

meta-analyses, the calculated effect sizes of the slopes were the response variable, absolute latitude, and hemisphere (including their interaction if variability in the data allowed doing so), as well as the mean corticosterone levels during the breeding season as predictors. The latter variable was included to investigate if seasonal glucocorticoid changes differed between species that inherently rely on different hormone levels (exploratory analyses indicated that including breeding season baseline levels as a predictor variable improved the model fit over the inclusion of the average baseline concentrations for the two seasons). To investigate seasonal changes in the light of reproductive investment, we introduced clutch size (log10-transformed) into the list of predictors. To examine possible allometric effects, we also considered body mass (log10-transformed). In cases where these two variables were not strongly correlated we included them simultaneously, otherwise we assessed their effects sequentially in different models. We did not include any other life history traits to avoid overfitting our models. Furthermore, we would have run into issues with collinearity as most of these traits were strongly correlated with each other (Online Appendix). We first performed models relying on data from all available species, and subsequently by focusing on passerine birds only to focus on a more homogeneous group. The latter models also allowed us to control for the fact that non-passerines differ heavily in life history strategies from passerines, thereby mediating strong body size and clutch size effects.

The PGLMMs were performed in R (R Developmental Core Team, Vienna) using the MCMCglmm package, which relies on a Markov chain Monte Carlo algorithm (Hadfield 2010). We defined priors necessary for the Bayesian modeling with inverse-Wishart distribution for the variance structure by using parameter settings for non-informative priors (expected variance, $V = 1$; degree of belief, $nu = 0.002$). The models were run for 130,000 iterations, with 30,000 samples being discarded at the beginning (burn-in), which were sampled at a thinning interval of 100. The trace and distribution of all variables were checked visually, as well as the autocorrelation between iterations. Each model was run at least four times to check for the consistency of the results (including parameter estimates and DICs). Similarly, we also checked whether longer runs, different prior settings (i.e., flat and improper priors), provided qualitatively different model outputs. Our model diagnostics also included the investigation of mixing and convergence that were tested by Gelman–Rubin statistics (Gelman and Rubin 1992).

Results

Seasonal variation in baseline levels of corticosterone

The comparison between the data fit of the random intercept (DIC = 363.84) and random slope (DIC = 321.49) models supported the latter, indicating that species differ in the slope of their baseline corticosterone concentrations across the two seasons.

Random effects: There was a strong phylogenetic signal of the variance in baseline corticosterone ($\lambda = 0.55$; Table 1), indicating that closely related species showed a similar seasonal response in baseline concentrations. Species significantly differed in both average baseline (intercept) and changes in baseline corticosterone from the non-breeding to the breeding stage (slope; Fig. 1). The interaction between intercept and slope was not significant showing that the change in baseline corticosterone was not related to average levels. The repeatability of baseline corticosterone was 0.32.

Fixed effects: Season did not explain a significant amount of the variation in baseline corticosterone.

Lower latitude birds showed higher levels of baseline corticosterone than higher latitude species (Table 1), however, this was driven by the southern hemisphere species while northern species exhibited the opposite pattern (Table 1 and Fig. 2). Further, males had higher baseline levels than females in both seasons.

Interspecific variation in seasonal changes (slopes) of baseline corticosterone

As predicted, species with larger clutches tended ($P = 0.052$) to increase baseline corticosterone from the non-breeding to the breeding season more than species with smaller clutches (Table 2 and Fig. 3). The magnitude of baseline corticosterone change was not predicted by baseline concentrations measured during the breeding season. Baseline corticosterone changes did not vary with latitude, hemisphere, or their interaction (Table 2). When running the same model only for *Passeriformes*, we could include both body mass and clutch size because they were not collinear. Among passerines, we found a strong positive association between baseline corticosterone slopes and clutch size (Table 2 and Fig. 3), indicating that in this more homogeneous group of birds the degree of reproductive investment was a strong predictor of seasonal changes in baseline corticosterone. Likewise, body mass was positively associated with the seasonal change in baseline concentrations (Table 2) showing that larger species increased baseline corticosterone more during the breeding season than smaller species. Passerines with steeper baseline

Table 1 Random and fixed effects of linear mixed models with random intercept and slopes to assess among-species variation and effect of life-history stages, sex, and absolute latitude in (a) baseline corticosterone and (b) stress-induced corticosterone variation

	Post. mean	Lower 95% CI	Upper 95% CI	*P*
a. Baseline corticosterone				
Random effects				
Intercept	0.07	0.002	0.14	*
Intercept (*) slope	−0.097	−0.057	0.047	
Slope	0.05	0.009	0.102	*
Phylogeny	0.08	0.043	0.118	*
Residuals	0.068	0.061	0.074	*
Fixed effects				
Intercept	1.26	0.98	1.55	***
Season (Breeding)	0.14	−0.11	0.34	
Sex (M)	0.05	0.015	0.089	**
Season (B) (*) Sex (M)	−0.07	−0.14	0.009	(*)
Abs. latitude	−0.09	−0.01	−0.003	***
Hemisphere (North)	−0.34	−0.53	−0.14	**
AbsLat (*) Hemisp (North)	0.008	0.002	0.013	*
b. Stress-induced corticosterone				
Random effects				
Intercept	0.026	0.003	0.055	*
Intercept (*) slope	−0.035	−0.072	−0.002	*
Slope	0.074	0.026	0.14	*
Phylogeny	0.019	0.008	0.028	*
Residuals	0.07	0.015	0.020	*
Fixed effects				
Intercept	1.18	0.93	1.44	***
Mean BL	0.30	0.25	0.34	***
Season (Breeding)	−0.04	−0.33	0.23	
Sex (M)	0.03	0.002	0.06	*
Season (B) (*) Sex (M)	0.040	−0.019	0.10	
Abs. latitude	0.003	−0.001	0.007	
Hemisphere (North)	0.21	−0.017	0.40	
AbsLat (*) Hemisp (North)	−0.04	−0.008	0.001	

Notes: M, males; B, breeding; Abs. latitude, absolute latitude. Both random and fixed effects were considered significant when their 95% credible intervals (CI) did not overlap zero. Post. means stands for posterior means of the Bayesian analysis and indicates the effect size of the predictor. We visualized significant results with asterisks in the right-most column (always $*P < 0.05$ for random effects, while for fixed effects $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; $(*)P = 0.074$).

corticosterone slopes had also higher baseline corticosterone during the breeding season. Latitude, hemisphere, and their interaction did not explain a significant proportion of the variance in the slopes of baseline corticosterone (Table 2).

Seasonal variation in stress-induced levels of corticosterone

The comparison between random intercept (DIC = −384.09) and random slope (DIC = −467.90) models supported the latter, indicating that species differed in the slopes of stress-induced corticosterone along the two seasons (Fig. 1).

Random effects: Phylogeny significantly explained the variation in stress-induced corticosterone, indicating that closely related species changed stress-induced corticosterone similarly across seasons. Species significantly differed in both average stress-induced corticosterone levels (intercept) and their changes from the non-breeding to the breeding stage (slope; Fig. 1). Species with lower average

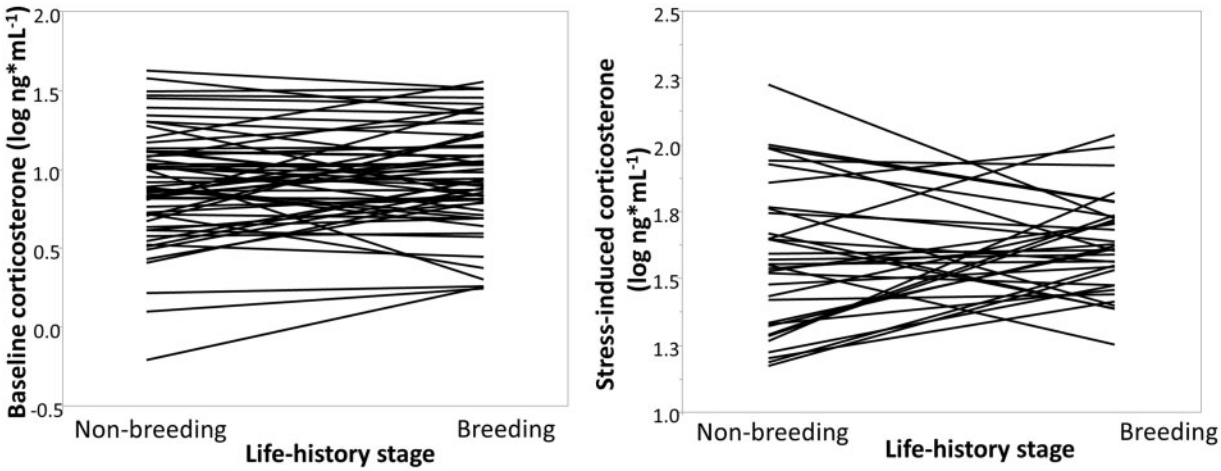

Fig. 1 Among-species variation in glucocorticoids between non-breeding and breeding stages. Left panel represents baseline and the right panel stress-induced concentrations of corticosterone.

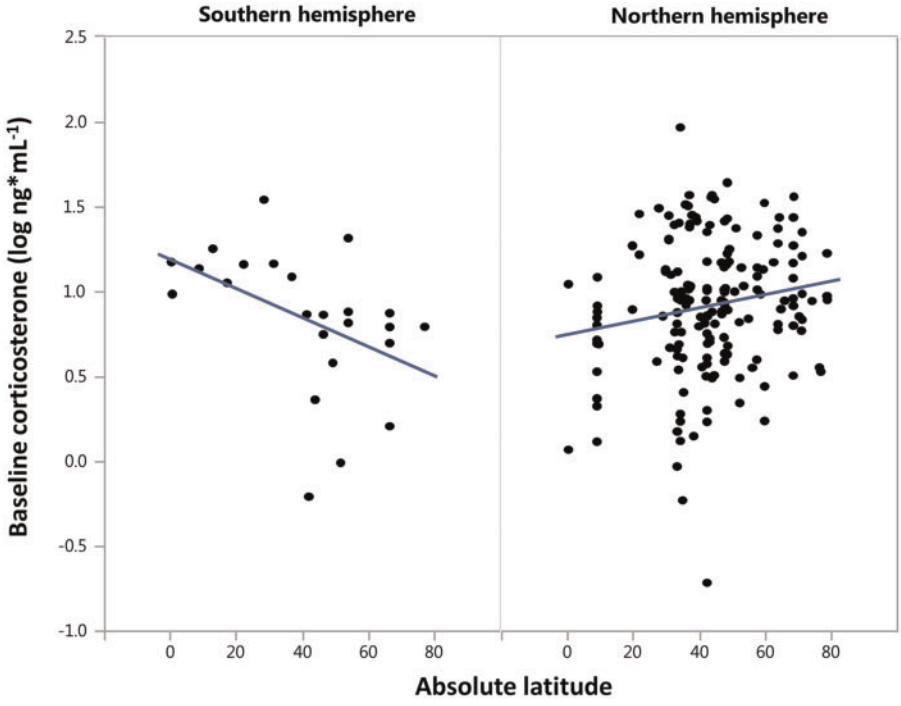

Fig. 2 Relationship between absolute latitude and average baseline corticosterone concentrations of populations of the two hemispheres.

stress-induced corticosterone concentrations showed a stronger increase during the breeding stage than species with lower average stress-induced levels (Table 1). The repeatability of stress-induced corticosterone concentrations was 0.42.

Fixed effects: Contrary to our predictions stress-induced levels did not vary significantly with season, absolute latitude, or the interaction between latitude and hemisphere (Table 1). Northern species showed higher stress-induced corticosterone, but the effect was marginally non-significant (Table 1). Males showed higher stress-induced corticosterone than females in both life history stages (Table 1).

Interspecific variation in seasonal changes (slopes) of stress-induced corticosterone

For this model we did not consider hemisphere as a predictor, because all but one species were from the

Table 2 Best models analyzing the effects of POL and environment on baseline levels (BL) of corticosterone and stress-induced levels (SL) of corticosterone slopes (a) for all species and (b) only for passeriforms

(a) Overall species	Post. mean	Lower 95% CI	Upper 95% CI	*P*
Baseline corticosterone slopes ($n = 54$)				
Intercept	−0.97	−0.88	0.78	
Breeding BLs	0.33	−0.06	0.70	
Clutch size	0.81	−0.004	1.64	(*)
Abs. latitude	−0.003	−0.02	0.01	
Hemisphere (North)	−0.30	−0.95	0.41	
AbsLat * Hemisp (North)	−0.0008	−0.017	0.015	
Stress-induced corticosterone slopes ($n = 32$)				
Intercept	−0.15	−2.53	2.32	
Breeding BLs	−0.03	−1.02	1.00	
Breeding SLs	0.58	−1.00	2.27	
Clutch size	0.80	−0.86	2.59	
Abs. latitude	−0.01	−0.04	0.09	
Hemisphere (North)	−0.32	−1.76	0.97	
AbsLat * Hemisp (North)	−0.001	−0.03	0.03	
(b) Passeriforms				
Baseline corticosterone slopes ($n = 36$)				
Intercept	−3.35	−6.06	−0.76	*
Breeding BLs	0.72	0.19	0.18	**
Clutch size	2.93	0.96	5.02	***
Body mass	0.91	0.27	1.55	**
Abs. latitude	0.02	−0.18	0.22	
Hemisphere (North)	−0.12	−1.72	1.64	
AbsLat * Hemisp (North)	−0.03	−0.22	0.17	
Stress-induced corticosterone slopes ($n = 23$)				
Intercept	−3.55	−9.99	2.04	
Breeding BLs	0.78	−0.54	2.27	
Breeding SLs	0.21	−2.10	2.00	
Clutch size	3.44	−2.51	9.47	
Body mass	1.10	−0.54	2.93	
Abs. latitude	−0.02	−0.05	0.003	

Notes: Predictors were considered significant when their 95% CI did not overlap zero. Abs. latitude, absolute latitude. We visualized significant results with asterisks in the right column (always * random effects, while for fixed effects *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, while the asterisk in brackets indicates $P = 0.052$).

northern hemisphere. None of the predictors explained a significant proportion of the variance in the data (Table 2).

Discussion

We tested the glucocorticoid seasonal plasticity hypothesis, which proposes that seasonal changes in glucocorticoid hormones from the non-breeding to the breeding season are related to the degree of reproductive investment in birds. Our first set of analyses revealed that species consistently differed in both average glucocorticoid concentrations (intercepts of reaction norms) and in their glucocorticoid changes when moving from a non-breeding to a breeding stage (slopes). This is visualized in Fig. 1, where the interspecific reaction norms across the two seasons for baseline and stress-induced corticosterone widely differ in intercept, steepness of slopes, and direction. This interspecific variability in slopes

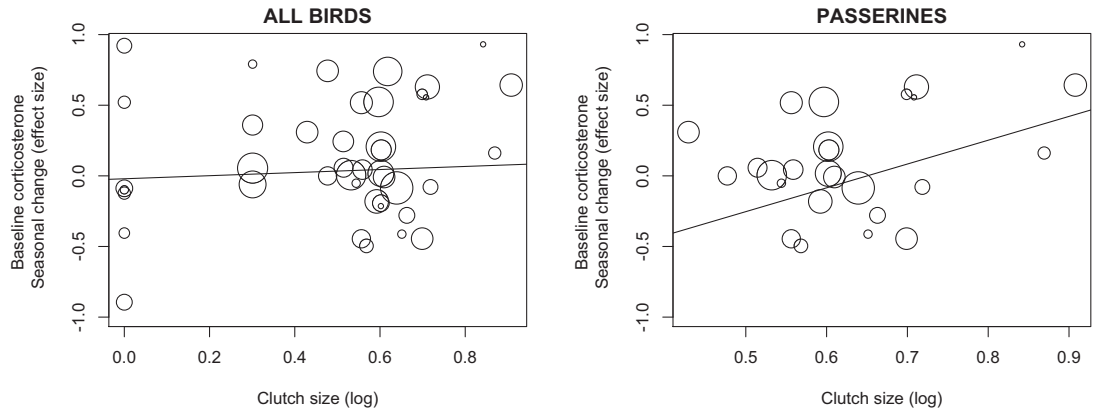

Fig. 3 Seasonal changes in baseline concentrations of corticosterone in relation to clutch size in all species (left) and only in passeriforms (right). Each circle represents a species, with the size of circles representing the variance of the hormonal trait. Black line represents regression line.

may explain the lack of an overall effect of the predictor season in these analyses. Seasonal changes in baseline corticosterone were not related to average levels nor to breeding season concentrations of a species. These results indicate that baseline corticosterone concentrations measured in a single season, or averaged across seasons, cannot predict seasonal variation. Results differed for stress-induced corticosterone, where species with lower mean values (corrected for baseline levels) showed a stronger seasonal change.

Our main prediction was that species with a high investment into each reproductive event (large clutch size) would show a stronger increase in baseline and a stronger decrease in stress-induced corticosterone when changing from the non-breeding to the breeding season than species with a lower investment. For baseline corticosterone our prediction was supported, especially when we limited our analyses to passerines. Our findings thus partially corroborate the "energy mobilization hypothesis" (Romero 2002) and are in line with the view that baseline glucocorticoid concentrations serve to support energy demanding processes (Landys et al. 2006; Patterson et al. 2011; Hau et al. 2016; Jimeno et al. 2017). The present data do not address whether these seasonal changes in baseline glucocorticoids are evolved strategies or whether they result from plastic responses to increased workload during the reproductive season.

In contrast to our expectations, seasonal changes in stress-induced corticosterone were not explained by reproductive investment (i.e., clutch size), either in all species or in passerines only. However, our analyses of stress-induced level slopes were based on a smaller sample size compared with the baseline analyses and hence have a lower statistical power. Thus, a larger sample size would be required to more conclusively test whether the degree of reproductive investment plays a role in determining species-specific concentrations of stress-induced glucocorticoids. Alternatively, one may speculate about a scenario in which baseline and stress-induced glucocorticoids may simply have divergent, non-overlapping functions, with baseline levels promoting reproductive investment and stress-induced levels supporting primarily self-maintenance functions. This view is inspired by the fact that at the two levels, glucocorticoids bind at different receptors, the mineralocorticoid and the glucocorticoid receptor, respectively (Proszkowiec-Weglarz and Porter 2010). Such a scenario would unite the (lack of) findings from the current study with those a previous study, which found that stress-induced corticosterone concentrations of male birds during the breeding season were positively related to survival rate but not to breeding season length (another proxy for investment into each reproductive effort; Hau et al. 2010).

Our findings that seasonal changes in baseline concentrations of corticosterone were positively associated with body mass in passerines (Table 2) are puzzling. We would have predicted the inverse relationship, with smaller species that have higher-mass specific metabolic rates and therefore might need to mobilize more energy reserves to support this metabolism showing stronger increases in baseline corticosterone from the non-breeding to the breeding season than larger species. During the breeding season small-bodied birds have indeed higher levels of baseline corticosterone than large-bodied species (Bókony et al. 2009; Hau et al. 2010). One possible explanation that could be tested by future studies is that smaller species, which because of their smaller size carry fewer energy stores than larger species,

might be more limited in upregulating baseline corticosterone during the breeding season. Small species may have to avoid increasing baseline corticosterone too much during the breeding season to spare crucial tissues (such as the flight muscle) from being metabolized to mobilize energy.

We found that males had overall higher levels of both baseline and stress-induced corticosterone than females. That we observed higher glucocorticoid levels in males versus females in both seasons suggest that they reflect sex differences that are unrelated to reproductive investment and pace of life. Instead, our findings suggest that males from a given species generally have a more active hypothalamo–pituitary–adrenal axis compared with females. Here it is important to note, again, that studying seasonal variations in a trait can provide better answers than studying a trait in a single season. Had we analyzed glucocorticoid concentrations only during the breeding season we would have reached an entirely different conclusion, namely that sex difference was related to reproduction (reviewed by Hau et al. 2016). Lower baseline levels in females could have indicated that they were less challenged by parental effort and lower stress-induced concentrations in females could have been taken as evidence that they decreased their endocrine stress response to avoid disrupting nest attendance (Wingfield et al. 1995).

Our first set of analyses revealed an interaction between the two extrinsic factors latitude and hemisphere, although only for baseline corticosterone concentrations (Table 1). As Fig. 2 illustrates, species from the Southern hemisphere increased baseline corticosterone concentrations toward lower latitudes, while species from the Northern hemisphere showed the opposite expected trend. This finding suggests that general extrinsic factors that vary across latitude, for example average annual temperatures, are unlikely to explain much of the interspecific variation in baseline corticosterone, as effects differed for the two hemispheres. However, these findings are not conclusive because contrary to the Northern hemisphere, southern species were represented by a limited sample size and mainly by non-passerines. Neither latitude nor hemisphere explained any variation in the seasonal slopes of both glucocorticoid traits.

Conclusions

Variation in both baseline and stress-induced corticosterone concentrations among different species of birds is substantial, but species-specific. Furthermore, interspecific variation in changes in baseline corticosterone from the non-breeding to the breeding season was explained by clutch size, a measure for the degree of investment into each breeding attempt that is related to pace of life. On the one hand, our study provides indirect support for both the "energy mobilization" (Romero 2002) and the "cort-adaptation" (Bonier et al. 2009, 2011; Ouyang et al. 2011) hypotheses, which state that glucocorticoids mediate physiological and behavioral changes to support energetically demanding phases like reproduction (Bonier et al. 2009). On the other hand, our findings suggest why some species do not show seasonal changes in baseline corticosterone (which is true for 28% of all studies, Romero 2002), as we demonstrated the slope of seasonal changes to be related to the degree of reproductive investment, especially in passerines (Bonier et al. 2009; Crespi et al. 2013; Schoenle et al. 2017). More research is needed to uncover why results sometimes differed when all species were considered versus passerines only. Likewise, it is currently unclear why baseline corticosterone decreases toward the pole in the Southern, but not the Northern hemisphere. However, our analyses have demonstrated that interspecific variation of seasonal changes in glucocorticoids, in addition to their values in a single season is related to life history strategies. Future research should address whether the observed seasonal changes in baseline glucocorticoids are the consequence of the degree of reproductive investment shown during the breeding season (e.g., of the metabolic demands) or whether they are evolved physiological strategies that underlie the pace of life of species.

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Supplementary data

Supplementary data available at *ICB* online.

References

Angelier F, Wingfield JC. 2013. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. Gen Comp Endocrinol 190:118–28.

Apfelbeck B, Helm B, Illera JC, Mortega KG, Smiddy P, Evans NP. 2017. Baseline and stress-induced levels of corticosterone in male and female Afrotropical and European temperate stonechats during breeding. BMC Evol Biol 17:114.

Auer SK, Dick CA, Metcalfe NB, Reznick DN. 2018. Metabolic rate evolves rapidly and in parallel with the pace of life history. Nat Commun 9:8–13.

Bókony V, Lendvai ÁZ, Liker A, Angelier F, Wingfield JC, Chastel O. 2009. Stress response and the value of reproduction: are birds prudent parents? Am Nat 173:589–98.

Bonier F, Martin PR, Moore IT, Wingfield JC. 2009. Do baseline glucocorticoids predict fitness? Trends Ecol Evol 24:634–42.

Bonier F, Moore IT, Robertson RJ. 2011. The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. Biol Lett 7:944–6.

Breuner CW, Orchinik M, Hahn T, Meddle S, Moore I, Owen-Ashley N, Sperry T, Wingfield J. 2003. Differential mechanisms for regulation of the stress response across latitudinal gradients. Am J Physiol Regul Integr Comp Physiol 285:R594–600.

Breuner CW, Patterson SH, Hahn TP. 2008. In search of relationships between the acute adrenocortical response and fitness. Gen Comp Endocrinol 157:288–95.

Clutton-Brock TH. 1991. The evolution of parental care. Princeton (NJ): Princeton University Press.

Cornelius JM, Perfito N, Zann R, Breuner CW, Hahn TP. 2011. Physiological trade-offs in self-maintenance: plumage molt and stress physiology in birds. J Exp Biol 214:2768–77.

Crespi EJ, Williams TD, Jessop TS, Delehanty B. 2013. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals?. Funct Ecol 27:93–106.

Daan S, Masman D, Groenewold A. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. Am J Physiol 259:R333–40.

Dingemanse NJ, Edelaar P, Kempenaers B. 2010. Why is there variation in baseline glucocorticoid levels? Trends Ecol Evol 25:261–2.

Le Galliard JF, Paquet M, Cisel M, Montes-Poloni L. 2013. Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. Funct Ecol 27:136–44.

Gelman A, Rubin D. 1992. Inference from iterative simulation using multiple sequences. Statistics 7:457–511.

Goymann W, Geue D, Schwabl I, Flinks H, Schmidl D, Schwabl H, Gwinner E. 2006. Testosterone and corticosterone during the breeding cycle of equatorial and European stonechats (*Saxicola torquata axillaris* and *S. t. rubicola*). Horm Behav 50:779–85.

Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw 33:1–22.

Hadfield JD, Nakagawa S. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. J Evol Biol 23:494–508.

Harshman LG, Zera AJ. 2007. The cost of reproduction: the devil in the details. Trends Ecol Evol 22:80–6.

Hau M, Casagrande S, Ouyang JQ, Baugh AT. 2016. Glucocorticoid-mediated phenotypes in vertebrates: multilevel variation and evolution. In: Naguib M, Mitani JC, Simmons LW, Barrett L, Healy S, Zuk M, editors. Advances in the study of behavior, vol. 48. New York (NY): Academic Press. p. 41–115.

Hau M, Haussmann MF, Greives TJ, Matlack C, Costantini D, Quetting M, Adelman JS, Miranda A, Partecke J. 2015. Repeated stressor increase the rate of biological ageing. Front Zool 12:4.

Hau M, Ricklefs RE, Wikelski M, Lee K. a, Brawn JD. 2010. Corticosterone, testosterone and life-history strategies of birds. Proc R Soc Lond B Biol Sci 277:3203–12.

Holberton RL, Wingfield JC. 2003. Modulating the corticosterone stress response: a mechanism for balancing individual risk and reproductive success in Arctic-breeding sparrows?. Auk 120:1140–50.

Horrocks NPC, Hegemann A, Ostrowski S, Ndithia H, Shobrak M, Williams JB, Matson KD, Tieleman BI. 2015. Environmental proxies of antigen exposure explain variation in immune investment better than indices of pace of life. Oecologia 177:281–90.

Jetz W, Sekercioglu CH, Bohning-Gaese K. 2008. The worldwide variation in avian clutch size across species and space. PLoS Biol 9:1–8.

Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. 2012. The global diversity of birds in space and time. Nature 491:444–8.

Jimeno B, Hau M, Verhulst S. 2017. Strong association between corticosterone and temperature dependent metabolic rate in individual zebra finches. J Exp Biol 220:4426–31.

Lancaster LT, Hazard LC, Clobert J, Sinervo BR. 2008. Corticosterone manipulation reveals differences in hierarchical organization of multidimensional reproductive trade-offs in r-strategist and K-strategist females. J Evol Biol 21:556–65.

Landys MM, Ramenofsky M, Wingfield JC. 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen Comp Endocrinol 148:132–49.

Lattin CR, Breuner CW, Michael Romero L. 2016. Does corticosterone regulate the onset of breeding in free-living birds?: The CORT-Flexibility Hypothesis and six potential mechanisms for priming corticosteroid function. Horm Behav 78:107–20.

Martin LB, Gilliam J, Han P, Lee K, Wikelski M. 2005. Corticosterone suppresses cutaneous immune function in temperate but not tropical House Sparrows, *Passer domesticus*. Gen Comp Endocrinol 140:126–35.

Mathot KJ, Frankenhuis WE. 2018. Models of pace-of-life syndromes (POLS): a systematic review. Behav Ecol Sociobiol 72:41.

McEwen BS, Wingfield JC. 2003. The concept of allostasis in biology and biomedicine. Horm Behav 43:2–15.

Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev 82:591–605.

Nussey DH, Wilson AJ, Brommer JE. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. J Evol Biol 20:831–44.

O'Reilly KM, Wingfield JC. 1995. Spring and autumn migration in Arctic shorebirds: same distance, different strategies. Am Zool 35:222–33.

Ouyang JQ, Sharp PJ, Dawson A, Quetting M, Hau M. 2011. Hormone levels predict individual differences in reproductive success in a passerine bird. Proc R Soc Lond B Biol Sci 278:2537–45.

Palacios MG, Sparkman AM, Bronikowski AM. 2012. Corticosterone and pace of life in two life-history ecotypes of the garter snake *Thamnophis elegans*. Gen Comp Endocrinol 175:443–8.

Pap LP, Vágási IC, István O, Osváth G, Veres-Szászka J, Czirják GÁ. 2015. Physiological pace of life: the link between constitutive immunity, developmental period, and metabolic rate in European birds. Oecologia 177:147–58.

Patterson SH, Winkler DW, Breuner CW. 2011. Glucocorticoids, individual quality and reproductive investment in a passerine bird. Anim Behav 81:1239–47.

Proszkowiec-Weglarz M, Porter TE. 2010. Functional characterization of chicken glucocorticoid and mineralocorticoid receptors. Am J Physiol Regul Integr Comp Physiol 298:R1257–68.

Reale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos Trans R Soc B Biol Sci 365:4051–63.

Ricklefs RE, Wikelski M. 2002. The physiology/life-history nexus. Trends Ecol Evol 17:462–8.

Roff DA. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. J Evol Biol 13:434–45.

Roff DA. 2002. Life history evolution. Sunderland (MA): Sinauer Associates.

Romero M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen Comp Endocrinol 128:1–24.

Romero LM. 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol Evol 19:249–55.

Romero LM, Dickens MJ, Cyr NE. 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. Horm Behav 55:375–89.

Romero LM, Wingfield J. 2016. Tempests, poxes, predators, and people: stress in wild animals and how they cope. New York (NY): Oxford University Press.

Saether BE. 1988. Pattern of covariation between life-history traits of European birds. Nature 331:616–7.

Santos ESA, Nakagawa S. 2012. The costs of parental care: a meta-analysis of the trade-off between parental effort and survival in birds. J Evol Biol 25:1911–7.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89.

Schoenle LA, Dudek AM, Moore IT, Bonier F. 2017. Red-winged blackbirds (*Agelaius phoeniceus*) with higher baseline glucocorticoids also invest less in incubation and clutch mass. Horm Behav 90:1–7.

Stearns SC. 1992. The evolution of life histories. Oxford: Oxford University Press.

Versteegh MA, Schwabl I, Jaquier S, Tieleman BI. 2012. Do immunological, endocrine and metabolic traits fall on a single pace-of-life axis? Covariation and constraints among physiological systems. J Evol Biol 25:1864–76.

Wiersma P, Munoz-Garcia A, Walker A, Williams JB. 2007. Tropical birds have a slow pace of life. Proc Natl Acad Sci U S A 104:9340–5.

Wikelski M, Spinney L, Schelsky W, Scheuerlein A, Gwinner E. 2003. Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. Proc R Soc Lond B Biol Sci 270:2383–8.

Williams TD. 2008. Individual variation in endocrine systems: moving beyond the "tyranny of the Golden Mean.". Philos Trans R Soc B Biol Sci 363:1687–98.

Williams TD. 2012. Hormones, life-history, and phenotypic variation: opportunities in evolutionary avian endocrinology. Gen Comp Endocrinol 176:286–95.

Williams JB, Miller RA, Harper JM, Wiersma P. 2010. Functional linkages for the pace of life, life-history, and environment in birds. Integr Comp Biol 50:855–68.

Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD. 1998. Ecological bases of hormone–behavior interactions: the "emergency life history stage." Integr Comp Biol 38:191–206.

Wingfield JC, Reilly KMO, Astheimer LB. 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. Am Zool 35:285–94.

Wingfield JC, Sapolsky RM. 2003. Reproduction and resistance to stress: when and how. J Neuroendocrinol 15:711–24.

Zhang Y, Hood WR. 2016. Current versus future reproduction and longevity: a re-evaluation of predictions and mechanisms. J Exp Biol 219:3177–89.