Early competitive environments differentially influence stress axis responses in sympatric, ecologically similar songbirds

By

Braelei Hardt

A THESIS

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Abstract approved:_____

James Rivers

1. Early competitive environments can shape the development of offspring across taxa. For example, competition for food can have downstream effects on offspring phenotypes and fitness; however, little is known about how early competition influences the development of the hypothalamic-pituitary axis and its potential tradeoff with growth. Because baseline and stressor-induced concentrations of glucocorticoids serve different purposes yet are central to survival, both should be evaluated both when assessing these potential tradeoffs.

2. We used a reciprocal partial cross-fostering study design in a free-living population of two species of sympatric, closely related bird species to test the hypothesis that increased competition during post-natal development leads to tradeoffs in the production of corticosterone (CORT), the primary glucocorticoid in birds, and growth. In addition, we tested the extent to which these responses differed between closely related species experiencing the same experimental conditions.

3. We found that nestlings of both species had elevated concentrations of both baseline and stressor-induced CORT in enlarged broods relative to control and reduced broods. In addition, we detected higher stressor-induced CORT in Violet-green Swallows (*Tachycineta thalassina*) when compared to Tree Swallows (*T. bicolor*) across treatments.

4. Violet-green Swallows grew slower than Tree Swallows in all treatments, and growth rate tended to decrease as brood size increased in both species. Further, we did not detect a treatment effect on the offspring survivorship to fledge or length of nesting period. Thus, elevated levels of CORT appear to be adaptive via changes that maintain nest survival rates under challenging conditions, such as when brood competition is high.

5. Our study showed that nestlings of closely related species that experience the same environmental stressors can diverge in their CORT response, indicating that species-level variation in CORT expression may be more important than environmental drivers. Further, we found that variation in early competition can lead to changes in CORT expression in swallows, and that this expression trades off with growth when brood competition is high. CORT may play a key role for balancing energetic demands that arise in the face of nestmate competition.

Key Words: corticosterone, glucocorticoids, growth, *Tachycineta*, tradeoffs

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Introduction

Early rearing environments often play an important role in the development of offspring across taxa. The post-natal developmental conditions that organisms experience can have lasting effects on a variety of behaviors and physiological processes, such as the propensity for learning and spatial memory (Nowicki, Peters, and Podos 1998; Roy and Bhat 2016), stimuli discrimination (Farrell and MacDougall-Shackleton 2016), and growth and cellular metabolism (DiRienzo et al. 2012; Garcia-Torres et al. 2015). In turn, these can lead to variation in fitness by priming organisms for the environmental conditions they might face as adults (Monaghan 2008; Crino and Breuner 2015). For example, male Zebra Finches (*Taeniopygia guttata*) who were experimentally stressed during post-natal development sired more and higher quality young (Crino et al. 2014c), possibly as a frontloading tactic to compensate for stressor-induced decreases in longevity (Tissier, Williams, and Criscuolo 2014).

Of the developmental conditions that organisms face, the degree of sibling competition may be one of the most important in determining juvenile and adult traits, as it often mediates energy acquisition necessary for adequate growth and development. Competition among siblings for food resources is typical among species that raise young in broods (Mock and Parker 1997), with the degree of competition being a function of both resource availability (Divya et al. 2012; Morandini et al. 2013) and factors that relate to offspring characteristics such as relative size (Harper 1986; Kacelnik et al. 1995; Ostreiher and Heifetz 2016), as well as parental quality (Saino et al. 1997; Smiseth et al.

2007). In birds, environmentally mediated competition can have long-lasting and sometimes permanent downstream effects on offspring phenotypes of fitness-related traits such as body mass at time of fledging, immune function, and glucocorticoid (GC) production (Saino et al. 1997; Eraud et al. 2008; Gil et al. 2008; Kozlowski et al. 2011; Nettle et al. 2016). While it is generally accepted that competition negatively affects body mass and immune functioning, studies testing the effects of within-brood competition on the development of the GC response have seen conflicting results (Saino et al. 2003; Eraud et al. 2008; Gil et al. 2008; Kozlowski et al. 2011). For example, although Saino et al. (2003) saw increased GC concentrations in response to brood enlargement, other studies (Eraud et al. 2008; Gil et al. 2008; Kozlowski et al. 2011) did not detect any effect.

The secretion of GCs is a typical endocrine response to the stressors experienced by individuals (Sapolsky, Romero, and Munck 2001). GCs are the end product of the cascade of the hypothalamic-pituitary-adrenal (HPA) axis for the purpose of maintaining or altering physiology and behavior during exposure to stressors; this is achieved though permissive, suppressive, or stimulating action (Sapolsky et al. 2001; Romero 2004). In vertebrates, GC production is an important physiological process which may mediate fitness through influencing juvenile survival (Lendvai et al. 2009; Rivers et al. 2012) and reproductive success (Bonier et al. 2009a, 2011; Angelier et al. 2007). Furthermore, baseline concentrations of circulating GCs are strongly linked to metabolism and energy balance. For example, baseline concentrations of corticosterone (CORT), the primary GC in birds and reptiles, is essential for stimulating appetite, promoting glucogenesis, and bolstering feeding behaviors (Sapolsky and Romero 2000)—processes which are critical

for maintaining energetic homeostasis. For post-natal individuals, elevated baseline concentrations can increase begging behavior (Kitaysky et al. 2001b; Quillfeldt et al. 2006; Loiseau et al. 2008, Wada et al. 2008), although they have also been linked to reduced growth rate and body condition (Morici, Elsey, and Lance 1997; Muller, Jenni-Eiermann, and Jenni 2009, Wada et al. 2008). Similarly, the amount of additional hormone released into the blood in response to an acute stressor, known as acute stress response reactivity (Wada and Breuner 2007), also acts as an important contributor to survival by priming an organism to escape from life-threatening situations (Wingfield et al. 1998; Sapolsky et al. 2000). This is achieved by facilitation of locomotor activity and exploration (Thaker, Lima, and Hews 2009), problem solving ability (Oitzl and Kloet 1992), and learning and memory acquisition (Thaker et al. 2010; Beylin and Shors 2003), which are necessary traits for the successful assessment and survival of acute stressors. These temporary, stressor-induced spikes in CORT concentration can therefore enhance survival by redirecting energy away from non-critical processes such as feeding and reproductive functioning (Sapolsky et al. 2000) and towards life-saving behaviors in times of need (Patterson et al. 2014).

In altricial nestlings, which hatch naked, blind, and unable to thermoregulate, the development of the stress axis likely begins within a few days of hatch but in a hyporesponsive state where concentrations cannot be detected; it isn't until the latter stages of the nestling phase that the response becomes identifiable and continues to mature until fledge (Schwabl 1999). Once detectable, the baseline and stressor-induced CORT concentrations found in nestlings can predict CORT expression in adulthood. Several studies have shown that post-natal CORT concentrations alter HPA axis

responsiveness when individuals are tested as adults (reviewed by Schoech et al. 2011). Thus, study of the baseline and stressor-induced CORT systems can provide information about an individual's current state as well as its potential adult phenotype. It is important to note that baseline and stressor-induced CORT bind to separate sets of receptors (baseline CORT binds to Type I receptors whereas stressor-induced CORT binds to Type II receptors) and can therefore be considered separate physiological processes (Romero 2004).

Possible tradeoffs between CORT and nestling growth have been studied in just a few instances. Previous studies investigating baseline CORT have found it does trade off with nestling growth in White-crowned Sparrows (*Zonotrichia leucophrys*) (Wada and Breuner 2008), though relationships between stressor-induced CORT and growth have not yet been investigated. Because baseline and stressor-induced CORT have different binding receptors (Sapolsky et al. 2000) and contribute to different aspects of avian phenotypes (e.g. baseline concentrations are linked to fitness and metabolic homeostasis whereas induced CORT mediates the response to acute stressors), it is important to assess both when exploring developmental tradeoffs. Moreover, previous studies of this topic have been generally restricted to an array of phylogenetically distinct species or related species living in contrasted environments (e.g. allopatric subspecies of Swamp Sparrow [*Melospiza georgiana*]; Angelier et al. 2011), and none have examined whether closely-related, sympatric species have divergent physiological responses when confronted with identical challenges during their development.

In this study, we used an experimental reciprocal partial cross-fostering experimental design to evaluate potential tradeoffs in the development of the stress axis

and growth when nestmate competition was experimentally manipulated. Specifically, our goals were to (1) quantify variation in the expression of baseline and stressor-induced CORT due to experimental changes in rearing environment, (2) assess whether experimentally-induced changes in CORT trade off with growth and if those tradeoffs might influence fitness, and (3) evaluate whether ecologically similar, sympatric, closely related species responded similarly when confronted with the same range of rearing environments. We did this by altering brood size of two sympatric species of *Tachycineta* swallows, the Tree Swallow (*T. bicolor*) and the Violet-green Swallow (*T. thalassina*), shortly after hatching to create broods that were within the natural range of variation of these species (Brown et al. 2011; Winkler et al. 2011), and then measuring potential tradeoffs between nestling CORT and growth rate during development. These two species are ecologically similar close relatives (Brown et al. 2011; Winkler et al. 2011; Cerasale et al. 2012) and at our study site in western Oregon they breed concurrently in a nest-box population and feed their offspring similar diets (Garlick, Newberry, and Rivers 2014); further, both species typically raise a single brood each season, exhibit female-only incubation and biparental provisioning, and are similar in their clutch size, incubation period, and nestling developmental period (Winkler et al. 2011, Brown et al. 2011).

We hypothesized that, relative to offspring in control broods, nestlings raised in experimentally enlarged broods would have higher baseline and stressor-induced CORT concentrations and lower growth rates, with longer nestling phases and lower nest survival rates as consequences of these tradeoffs. In contrast, we hypothesized that individuals raised in reduced broods would show the opposite pattern. Furthermore, we

hypothesized that close relatives would have consistent responses to variation in rearing environments because of similar environmental pressures experienced during the breeding season. We focus on production of endogenous CORT as a function of rearing environments across a natural range of environmental conditions; our study stands as the first investigation to quantify the rearing conditions → post-natal physiological development → survival pathway in free-living populations of two sympatric species thought to experience very similar selection pressures during breeding. This study will provide context on how hormonal physiology and survival are linked during post-natal development in similar species.

Materials and methods

Study Species and Population

Our work took place during May-August in 2015 and 2016 within the vicinity of Corvallis, Oregon where we have established a nestbox-breeding population of Tree Swallows and Violet-green Swallows that used approximately 45% of the 225 nest boxes available to them in each year. In our study sites, these species use the same breeding locations and prey resources (Garlick et al. 2014), though Tree Swallows generally begin nesting activity two weeks earlier than Violet-green Swallows. We monitored nest boxes every 2-3 days from the start of nesting to determine the nest initiation date, defined as the date the first egg appeared in the clutch. The first nest initiation date for 2015 was 2 May for Tree Swallows and 20 May for Violet-green Swallows, and the first initiation date for 2016 was 22 April for Tree Swallows and 16 May for Violet-green Swallows.

We restricted our study to the first breeding attempt in each nest box in each year to maintain independence between broods.

Experimental Design

We manipulated the contents of nests on nestling day 3 (where nestling day 0 is the day the first egg in a clutch hatched) to create three brood types across a gradient of competition. Reduced broods were created via a net reduction of two nestlings relative to starting brood size, whereas enlarged broods were created by a net increase of two nestlings relative to starting brood size; this created broods that were between 40% and 66% smaller or larger than initial brood sizes. Control broods experienced no net change in number of nestlings, although they too consisted of a mix of offspring from different nests (see below). Mean starting brood sizes were similar among treatments for both Tree Swallows (reduced broods = 3.1 nestlings ± 1.1 SD, control broods = 4.5 nestlings ± 1.2 SD, enlarged broods = 6.9 nestlings ± 1.3 SD) and Violet-green Swallows (reduced broods = 2.9 nestlings ± 0.7 SD, control broods = 4.5 nestlings ± 1.3 SD, enlarged broods = 6.7 nestlings ± 1.1 SD).

To create broods, we first paired nests that hatched within 24 h of one another, although the great majority of pairs hatched on the same day (82.6%, n = 64 pairs for both species combined). To ensure even treatment assignment across each breeding season, we assigned every three consecutively-hatching nest pairs for each species into a study block. Within each block, we randomly assigned one pair to serve as controls, and each of the two remaining pairs became reduced and enlarged brood. For each of these non-control brood pairs, we randomly assigned one nest each to an enlarged and reduced

treatment. On nestling day 3, we moved three nestlings from each reduced brood into the enlarged brood with which it was paired while simultaneously removing one nestling from the enlarged brood and moving it into the reduced brood. For control nest pairs, we moved two nestlings from the first nest to the second nest, and vice versa. This approach resulted in a net increase of two nestlings in enlarged broods, a net decrease of two nestlings in reduced broods, and no net change in the number of nestlings in control broods. Moreover, it ensured that all nests in our study experienced a combination of nestlings that were related to the provisioning female (hereafter, natal nestlings), and nestlings that were unrelated to the provisioning female (hereafter, foreign nestlings) in the unlikely event that parents discriminated against non-kin young during provisioning bouts. Nestling transfers took place between the hours of 6:30am and 6:00pm, and no nestlings were held out of the nest longer than 30 min. We detected no differences in CORT measures or growth rate between natal and foreign nestlings within each of our six treatment × species combinations (Hardt et al., unpublished data) so we took combined measures for all nestlings in a brood, regardless of their nest origination. One treatment group did not receive older or heavier nestlings by chance (One-way ANOVA, age: $F_{2,473} = 0.983$, $P = 0.38$; mass: $F_{2,473} = 0.76$).

Quantification of Nestling Growth and CORT Production

At the time broods were created, we measured each nestling for body mass and clipped the tip of one or more of its claws in a unique pattern relative to its nestmates so that we were able to quantify variation in growth rate between individuals in each brood. We returned to nests to re-measure each nestling every three days until day 15 when we

collected CORT samples, resulting in 5 growth measures for each individual (i.e., days 3, 6, 9, 12, and 15). To quantify CORT, we selected two nestlings in the middle of the size continuum and collected ~75 μL of blood from each by pricking the brachial vein of the wing with a sterile 26-gauge needle, collecting blood with a capillary tube, and then transferring it to a 3 mL sterile centrifuge tube. We took baseline blood samples within 3 min of initial contact to ensure pre-stressor hormone concentrations (Romero and Romero 2002), and handling-induced CORT was measured from blood taken 30 min after initial contact. All blood samples were placed on ice immediately after being obtained. Because of slight differences in broods that resulted from hatching asynchrony and non-matching hatch dates, the majority of nestlings were bled for CORT on day 15 (79%), with a minority bled on day 14 (10%) or day 16 (11%). Between baseline and stress-induced sample collections, we kept all nestlings from a nest together in a small cloth bag. To control for differences in CORT between natal and foreign nestlings, we sampled from at least one natal and one foreign nestling within each brood, except for in the rare cases in which nestlings in each group had died prior to sampling (n = 2 out of 98 broods).

Quantification of Adult Provisioning Rates

The amount of food a nestling obtains during development is directly linked to its growth and development, so we quantified parental provisioning rates for nests in our study. We did this by hiding a digital video camera >20 m from each nest box for a minimum of 90 min on nestling day 9, and measured chicks immediately after filming to avoid influencing delivery rates. We made simultaneous recordings for each set of paired

nests within each study block to control for time of day and weather effects. Swallows carry food within their mouth when arriving at the nest box, so we were unable to quantify food loads and were restricted to quantifying provisioning rates. Nevertheless, prior work with Tree Swallows has shown that nest visitation rate provides an accurate index for the amount of food delivered to the nest (McCarty 2002), so we assumed that was the case in our study. To obtain data on parental provisioning from videos we used an automated program, MotionMeerkat (Weinstein 2015), that used foreground detection followed by contour analysis to determine when adults entered nest boxes to feed young. In our population, female swallows significantly reduce brooding by day 9 and enter nest boxes with food as soon as they return to the nest (Garlick et al. 2014); therefore, we assumed that parents visiting the nests were doing so to feed their offspring. We ground-truthed a random subset of approximately 10% of feeding videos from each species (n = 10) to assess precision and found that this program had 100% detection accuracy. For analysis, we calculated per capita provisioning rate as the number of parental feeding visits divided by the number of nestlings present in the nest on day 9.

Laboratory Analysis

Within 6 hours of taking blood samples we returned to a laboratory where we centrifuged samples to separate blood plasma, which was then kept frozen at -80°C for 2-17 months until analysis. To assay corticosterone in blood samples we used an enzyme linked-immunoassay (EIA) supplied by CJ Munro (University of California-Davis). First, we extracted samples in methylene chloride followed by drying under a gentle stream of N, and then reconstituted samples in assay buffer. We then diluted polyclonal

corticosterone antiserum (CJM006) at 1:15,000 in coating buffer (0.05M NaHCO3 pH 9.6), with 50 µl/well on a 96-well Nunc-Immuno Maxisorp (Thermo-Fisher Scientific) microtiter plate, covered with a plate sealer and allowed it to sit overnight at °C. The next day, we washed plates five times (0.15 M NaCl, 0.05% Tween 20) before adding standards or samples in duplicate; standards ranged from 3.9 to 200,000 pg/well. Next, we diluted samples (50 µl/well) in a 1:20 ratio in EIA Buffer (0.1 M NaPO4, 0.149 M NaCl, 0.1% bovine serumalbumin, pH 7.0) and then add 50 µl of diluted horseradish peroxidase conjugate in EIA buffer (1:70,000) to each well. We incubated plates in the dark at room temperature for two hours and then washed them before subjecting them to an additional 20-minute incubation with 100 µl/well of ABTS [0.4 mM 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, 1.6 mM H2O2, 0.05 M citrate, pH 4.0]. Finally, we read plates at 405 nm using a spectrophotometer (Gen 5, BioTek, Winooski, Vermont). To avoid plate-level variation, we randomized samples from different treatments across plates. Coefficient of variation estimates for intra- and inter-plate variation were < 8%. For a positive control we used a pooled blood plasma samples from zebra finches.

Statistical Methods

To quantify the rate of growth, r, across the period during which we evaluated nestlings (day 3-15), we used the program Mathematica (version 11.0.1) to create individual body mass curves using the ‘Growth Fit’ and ‘Logistic Growth’ programs. We then used the ‘nls’ function in R (version 3.2.4) to fit a logistic growth curve for body mass for each of our species × treatment combinations.

To determine the effects of brood manipulation on response variables (i.e., baseline CORT, stressor-induced CORT, and growth rate), we used multiple linear mixed effects models with the 'mle' function in R (version 3.2.4). All models were fitted using weights = varIdent for treatment and species to meet assumptions of residual variance and normality. To evaluate the effect of our experimental brood treatments on baseline and stressor-induced CORT, we constructed similar models that contained brood treatment, species, and a treatment × species interaction as fixed effects, block and brood ID as random effects, and the time of sample collection and per capita provisioning rate as covariates. We included the time when each brood was sampled as a covariate because baseline CORT exhibits diurnal patterns of variation in other songbird species (Romero and Remage-Healey 2000; Rich and Romero 2001). We did not include the time from first contact to the time blood samples were obtained because all samples were taken within 3 min, which is thought to represent pre-stressor levels in birds (Romero and Romero 2002). To assess how growth rate was influenced by brood treatments, we constructed a model that contained treatment, species, and a treatment × species interaction as fixed effects, with block and brood ID as random effects, and per capita provisioning rate as a covariate. We used a model with the same parameters to assess whether treatments influenced the length of the nestling phase. For our results, we report least-squares marginal means and their 95% confidence intervals (CIs), which take into account the mean of each covariate within the model, unless otherwise noted. To ensure that growth was not biased downward by nestlings that were in fatally poor condition, we removed individuals from analysis that did not survive during the time of our experiments (day 3-15); this resulted in a minority of individuals being removed (15 %).

Finally, to quantify the effects of brood treatments on brood survival, we quantified the proportion of nestlings that successfully fledged from the nest after broods were created. We analyzed this proportion in a binomial distributed model with a logit link using the 'glmer' function in R package 'lme4; this model contained species, treatment and a treatment × species interaction as fixed effects, block as a random effect, and per capita provisioning rate as a covariate.

Results

We created 118 experimental broods over two years, of which 98 survived to day 15 and could be used for analysis (n = 58 Tree Swallow, n = 40 Violet-green Swallow); the majority of nest failures (approximately 60%) were due to predation. Sample sizes ranged from 18-21 nests/treatment for the Tree Swallow, where we had 11-17 nests/treatment for Violet-green Swallows. We detected no differences in brood sizes between the two species when comparing within each treatment [reduced: β = 0.57 (95% CI: -0.06, 1.20), t_{37} = 1.82, P = 0.076, control: β = 0.10 (95% CI: -0.96, 0.78), t_{26} = -0.23, P = 0.817, enlarged: β = 0.79 (95% CI: -0.06, 1.65), t_{38} = 1.86, P = 0.070] (see Fig. 1).

Baseline Corticosterone

We found that baseline CORT was influenced by treatment ($F_{1,2}$ = 31.76, P < 0.001) (see Fig. 2a). We found no evidence that chicks in reduced broods had baseline concentrations [4.27 ng mL^{-1} (95% CI: 0.91, 7.863)] that differed from those in control broods [4.13 ng mL^{-1} (95% CI: 0.45, 7.80), β = 0.15 ng mL^{-1} (95% CI: -1.72, 2.02), $t_{105,64}$ = 0.15, P = 0.871], although mean baseline CORT was greater in enlarged broods [12.29

ng mL^{-1} (95% CI: 8.52, 16.07)] than in control broods [4.13 ng mL^{-1} (95% CI: 0.45, 7.80), β = 8.16 ng mL^{-1} (95% CI: 5.44, 10.89), $t_{105,64}$ = 5.88, P < 0.001)] and this relationship was consistent across both species [β = -1.33 ng mL^{-1} (95% CI: -3.62, 0.95), $t_{105,64}$ = -1.16, P = 0.260]; treatment × species interaction: β = -0.004 ng mL^{-1} (95% CI: -2.78, 2.78), $F_{1,2}$ = 0.40, P = 0.671]. Baseline CORT concentrations of nestlings in enlarged broods were about 3.0 × (± 1.7 SD) greater than those of nestlings in enlarged broods.

Stressor-induced Corticosterone

Stressor-induced CORT generally showed a similar pattern across treatments as baseline CORT. Although we did not detect a difference between reduced [11.75 ng mL^{-1} (95% CI: 5.24, 18.25)] and control broods [9.91 ng mL^{-1} (95% CI: 3.06, 16.79), β = 1.84 ng mL^{-1} (95% CI: -1.57, 5.24), $t_{103,64}$ = -1.06, P = 0.294)], induced concentrations of CORT were higher in enlarged broods than in control broods [β = 15.62 ng mL^{-1} (95% CI: 10.94, 20.31), $F_{103,64}$ = -6.54, P < 0.001] (see Fig. 2b). However, unlike baseline CORT, the stressor-induced CORT varied by species [β = 5.75 ng mL^{-1} (95% CI: 0.88, 10.60), $t_{105,64}$ = 2.31, P = 0.024], and although this pattern was similar across treatments, the magnitude of the effect was much greater for enlarged broods [treatment × species interaction: $F_{1,2}$ = 2.48, P = 0.092]: induced CORT in Violet-green Swallows in enlarged broods was 23.87 ng mL^{-1} (95% CI: 17.13, 30.60) higher than in control ($t_{103,64}$ = -6.94, P < 0.001), whereas induced CORT in Tree Swallows in enlarged broods was only 15.63 ng mL^{-1} (95% CI: 10.94, 20.32) higher than in control ($t_{103,64}$ = -6.54, P < 0.001).

Growth Rate

We found evidence for a relationship between brood treatment and growth rate ($F_{1,2}$ = 5.07, P = 0.008) (see Fig. 2c). In general, as brood size increased, growth rate decreased, although this difference was only evident when we compared reduced broods to enlarged broods (β = -0.04 per day, $t_{270,79}$ = -2.25, P = 0.027) Both species showed a slight decrease in growth rate from reduced [TRES: 0.51 per day (95% CI: 0.49, 0.60); VGSW: 0.37 per day (95% CI: 0.31, 0.43)] to control broods [TRES: 0.46 per day (95% CI: 0.40, 0.52); VGSW: 0.36 per day (95% CI: 0.30, 0.42)] and from control broods to enlarged broods [TRES: 0.44 per day (95% CI: 0.40, 0.48); VGSW: 0.31 per day (95% CI: 0.25, 0.37)]. Further, we detected a relatively slower growth rate in Violet-green Swallows when compared to Tree Swallows, and this reduction was similar across treatments [species: β = -0.14 per day (95% CI: -0.22, -0.05), $t_{270,79}$ = -2.25, P = 0.003; species × treatment interaction: $F_{1,2}$ = 0.04, P = 0.96] (see Fig. 3).

Length of Nestling Phase

We did not detect a difference in the length of the nestling phase, measured as days between hatch and fledge, between treatments [25 days (95% CI: 23, 28), $F_{1,2}$ = 0.084, P = 0.91] or between species [β = 2 days (95% CI: -1, 5), $F_{1,2}$ = 1.44, P = 0.16], and we saw no species × treatment interaction ($F_{1,2}$ = 1.09, P = 0.34), although nestling phases did tend to be slightly longer in Violet-green Swallows. Estimated mean Tree Swallow nestling phase was 24 days (95% CI: 22, 26) in reduced broods, 25 days (95% CI: 23, 28) in control broods, and 24 days (95% CI: 23, 26) in enlarged broods. Estimated

mean nestling phase for Violet-green Swallows was 28 days for all treatments (reduced 95% CI: 27, 30; control 95% CI: 25, 30; enlarged 95% CI: 26, 30).

Nest Survival

We detected no difference in the proportion of nestlings that successfully fledged from nests in different treatments [88.5% fledged (95% CI: 86.3%, 90.7%), $F_{1,2} = 0.08$, $P = 0.759$], or species (species: $F_{1,2} = 0.393$, $P = 0.976$), with no species × treatment interaction ($F_{1,2} = 0.000$, $P = 0.988$). Mean survival rates did appear to decrease slightly in enlarged broods, but not significantly so. Estimated Tree Swallow survival rate was 98.9% (95% CI: 92.9%, 99.8%) in reduced broods, 92.2% (95% CI: 84.2%, 96.9%) in control broods, and 85.7% (95% CI: 79.2%, 93.2%) in enlarged broods. For Violet-green Swallows, estimated survival rate was 92.8% (95% CI: 78.9%, 97.8%) in reduced broods, 92.6% (95% CI: 80.7%, 97.5%) in control broods, and 84.8% (95% CI: 71.6%, 92.5%) in enlarged broods.

Discussion

Measures of CORT

As predicted, both Tree and Violet-green Swallow nestlings exhibited increased baseline CORT in response to experimentally increased competition, which may have the potential to cause changes in nestling survival. Although some studies have achieved similar results, baseline CORT responses to brood manipulation seem to be inconsistent across species. For example, when Saino et al. (2003) experimentally enlarged the broods of the Barn Swallow (*Hirundo rustica*), CORT concentrations increased; however,

studies of other altricial species detected no effect of brood alteration on baseline concentrations (Gil et al. 2008; Kozlowski et al. 2011). It may be that these discrepancies can be explained by differences in the feeding compensation ability of parents; in our study populations, at least, parents were unable to fully compensate for increased provisioning demand (unpublished data). Alternatively, increased CORT concentrations may be a mediator of food intake, not just a consequence of it. Because persistently elevated baseline CORT facilitates begging behavior, appetite, and food intake in birds (Sapolsky et al. 2001; Kitaysky et al. 2001a, 2003; Loiseau et al. 2008), it may be adaptive by ensuring that nestlings get enough food when confronted with an especially challenging developmental conditions. Importantly, in our study, brood enlargement did not lead to treatment specific differences in survival. Thus, greater concentrations of baseline CORT in enlarged broods could be a mechanism that helps maintain energetic homeostasis by ramping up food consumption in response to reduced provisioning, acting as part of a negative feedback loop which ultimately preserves nest survival rates in the short term.

Although other studies have found relationships between sibling competition and baseline CORT expression, ours is the first to test the effects of brood size on baseline and stressor-induced CORT, each of which may have consequences for survival (Breuner et al. 2008; Bonier et al. 2009). We found that stressor-induced CORT was significantly higher in experimentally enlarged broods. Because induced CORT concentrations are necessary for an adequate physiological and behavioral response to acute stressors (Wingfield et al. 1998; Sapolsky et al. 2000), high concentrations may be an adaptive reaction to unexpected stressors that arise during nesting. For example, inclement

weather has been shown to increase stressor-induced CORT in Lapland Longspurs (*Calcarius lapponicus*), which could in part be due to short-term fasting during bad weather (Aestheimer, Buttemer, and Wingfield 1995); indeed, fasting experiments with White-crowned Sparrows elicit elevated stressor-induced CORT responses (Lynn et al. 2003). During storms, nestlings raised in large broods may be even further food-stressed due to heightened competition for an extremely-limited resource, in which case an elevated stressor-induced CORT response may be beneficial for navigating these low-feeding periods. Additionally, competition-altered induced CORT responses could be programmed during the nestling stage for use in later life history stages. In House Sparrows (*Passer domesticus*), for example, recently fledged juveniles from experimentally enlarged broods maintained higher stressor-induced CORT responses to handling protocol, showing that nest environment can have lasting effects on HPA axis reactivity (Lendvai et al. 2009). Our study suggests that this heightened stress reactivity develops during the nestling stage, possibly so that it is fully functional and available for use from the start of the fledging period.

Perhaps the most noteworthy result of our study was the strong difference between the stressor-induced CORT responses of our two study species such that Violet-green Swallows had higher stressor-induced CORT reactivity than Tree Swallows did across treatments. Because both species appear to use similar nest (Brown et al. 2011; Winkler et al. 2011) and food (Garlick et al. 2014) resources in our study, the differences we observed are difficult to elucidate. One explanation arises from phylogenic differences that have developed over evolutionary time. Violet-green Swallows are more closely related to the Caribbean clade of *Tachycineta* swallows (i.e. the Bahama Swallow

[*T. cyaneoviridis*] and the Golden Swallow [*T. euchrysea*]) than Tree Swallows (Cerasale et al. 2012); therefore, current-day differences in CORT response may linked to having an ancestor that evolved in a tropical location. Indeed, several studies have shown that closely related temperate and tropical species have differing CORT responses (Wingfield et al. 2008b), and glucocorticoid reactivity is relatively higher in lower latitude populations (Silverin 1997, 1998; O'Reilly and Wingfield 2001). Although it is interesting to speculate that evolutionary ancestry could contribute more to variation in the stressor-induced CORT response than current environmental factors, data that allow for conclusively addressing this is lacking. A broader study investigating several descendants of the same evolutionary line, using both sympatric and allopatric species, would be informative for understanding the relative contributions of environmental versus genetic factors influencing post-natal CORT development.

Tradeoffs Between CORT and Growth Rate

The nestlings in our study trended toward exhibiting an inverse linear relationship between brood size and growth rate (see Fig. 4). Because endogenous CORT production increased while growth decreased in response to brood enlargement in our study, within-brood competition seems to be a strong enough stressor to elicit tradeoffs between growth trajectories and HPA axis functioning in nestling swallows. This aligns with previous studies that have found negative relationships between baseline CORT and growth (Morici et al. 1997; Wada et al. 2008; Muller et al. 2009) while showing that a relationship exists for stressor-induced CORT as well.

When environmental conditions are challenging, especially when food intake is low, there may be a strategic shift from energy allocated to growth to energy allocated to physiological maintenance mediated by CORT. If this shift is temporary, no permanent effects may result; however, if CORT is maintained over long timescales and becomes chronic, nestlings may experience reduced growth which could negatively affect survival rates (Wada et al. 2008). Although we expected increased early competition to have some negative effects on survival, we did not observe any differences in the proportion of nestlings successfully fledged between treatments, indicating the tradeoffs we detected were not linked to fitness consequences for offspring. Nevertheless, because fledging mass is strongly correlated with juvenile survival in many species of migratory songbird (Perlut and Strong 2016; Naef-Daenzer and Grueebler 2016), our study may have had consequences for the quality of those chicks that do survive. Indeed, in other brood manipulation experiments, nestlings raised in experimentally enlarged broods commonly weigh less than those raised in smaller broods during the pre-fledge period (Saino et al. 2003; Gil et al. 2008; Wada et al. 2008; Kozlowski et al. 2011). Further, though we expected that individuals from enlarged broods would stay nest-bound longer to compensate for reduced growth rates, we saw no evidence that the length of the nestling period was influenced by brood size. This suggests that, given the same amount of time to grow, nestlings in enlarged broods will fledge in poorer condition for these species. Thus, competition-induced tradeoffs between CORT and growth may be an adaptive strategy for surviving to fledge, although we cannot be certain of its downstream effects on juvenile and adult fitness.

Interestingly, despite ecological and physiological similarities between our two study species, Violet-green swallows showed a relatively slower growth rate than Tree Swallows across treatments. Growth rate is likely influenced by the thermal properties of broods, as growth has been shown to trade off with the development of endothermy in other songbirds because of constraints on energy allocation (Olson 1992; Wegrzyn et al. 2013; Andreasson et al. 2016). The differences seen between our species could be an endothermic consequence of size differences; because Violet-green Swallow chicks were slightly smaller than Tree Swallow chicks at the time we measured CORT (Tree Swallow 19.8 (95% CI: 19.11, 20.4), Violet-green Swallow 18.3 g (95% CI: 17.5, 19.2); Hardt et al. unpublished data), they may need to allocate more energy away from growth and toward endothermic development; Indeed, initial brood cooling work suggests very rapid cooling in reduced broods for other *Tachycineta* swallows, especially for the smaller species (Ardia et al., unpublished data).

Species differences notwithstanding, our study found that for two sympatric, ecologically similar species, both baseline and stressor-induced CORT responses during the nestling period are influenced by early competitive environment, although the reason why remains untested. To determine underlying mechanisms responsible for what drives the CORT response to brood enlargement, further study should test how CORT varies within a lab setting that can control for differences in food intake and thermal properties of nests. We also showed that both baseline and stressor-induced CORT trades off with growth when nestlings of these species are faced with heightened sibling competition. However, we should take care in considering increased CORT levels in response to poor conditions as an adverse outcome that may negative influence fitness, as it facilitates

behavioral responses such as begging and predator avoidance that promote survival in these less-than-ideal conditions. Regardless of the environmental circumstances in which a nestling develops, stress axis functioning and growth must be carefully balanced so that survival chances and future reproductive output are optimized.

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References

Andreasson, F., Nord, A. & Nilsson, J.-Å. (2016) Brood size constrains the development of endothermy in blue tits. *The Journal of Experimental Biology*, 219, 2212–2219.

Angelier, F., Ballentine, B., Holberton, R.L., Marra, P.P. & Greenberg, R. (2011) What drives variation in the corticosterone stress response between subspecies? A

common garden experiment of swamp sparrows (*Melospiza georgiana*). *Journal of Evolutionary Biology*, 24, 1274–1283.

Astheimer, L.B., Buttemer, W.A. & Wingfield, J.C. (1995) Seasonal and Acute Changes in Adrenocortical Responsiveness in an Arctic-Breeding Bird. *Hormones and Behavior*, 29, 442–457.

Beylin, A. V. & Shors, T.J. (2003) Glucocorticoids are necessary for enhancing the acquisition of associative memories after acute stressful experience. *Hormones and Behavior*, 43, 124–131.

Bonier, F., Martin, P.R., Moore, I.T. & Wingfield, J.C. (2009a) Do baseline glucocorticoids predict fitness? *Trends in Ecology and Evolution*, 24, 634–642.

Bonier, F., Moore, I.T. & Robertson, R.J. (2011) The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. *Biology Letters*, 7, 944–946.

Brown, Charles R., A. M. Knott and E. J. Damrose. (2011). Violet-green Swallow (Tachycineta thalassina), The Birds of North America (P. G. Rodewald, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America: https://birdsna.org/Species-Account/bna/species/vigswa DOI: 10.2173/bna.14

Cerasale, D.J., Dor, R., Winkler, D.W. & Lovette, I.J. (2012) Phylogeny of the Tachycineta genus of New World swallows: Insights from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution*, 63, 64–71.

Crino, O.L., Prather, C.T., Driscoll, S.C., Good, J.M. & Breuner, C.W. (2014) Developmental stress increases reproductive success in male zebra finches. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20141266–20141266.

Crino, O.L. & Breuner, C.W. (2015c) Developmental stress: evidence for positive phenotypic and fitness effects in birds. *Journal of Ornithology*, 156, 389–398.

DiRienzo, N., Pruitt, J.N. & Hedrick, A. V. (2012) Juvenile exposure to acoustic sexual signals from conspecifics alters growth trajectory and an adult personality trait. *Animal Behaviour*, 84, 861–868.

Divya, S.P., Kathiresan, K., Asha, P., Sekar, V. & Rajasekaran, R. (2012) Experimental study of the interspecific competition between two sibling marine herbivorous rotifers in relation to food availability and initial population density. *Acta Oceanologica Sinica*, 31, 113–126.

Eraud, C., Trouvé, C., Dano, S., Chastel, O. & Faivre, B. (2008) Competition for resources modulates cell-mediated immunity and stress hormone level in nestling collared doves (*Streptopelia decaocto*). *General and Comparative Endocrinology*, 155, 542–551.

Farrell, T.M., Morgan, A. & MacDougall-Shackleton, S.A. (2016) Developmental stress impairs performance on an association task in male and female songbirds, but impairs auditory learning in females only. *Animal Cognition*, 19, 1–14.

García-Torres, E., Hudson, R., Castelán, F., Martínez-Gómez, M. & Bautista, A. (2015) Differential metabolism of brown adipose tissue in newborn rabbits in relation to position in the litter huddle. *Journal of Thermal Biology*, 51, 33–41.

Garlick, N.W., Newberry, G.N. & Rivers, J.W. (2014) An Assessment of Nestling Diet Composition in the Violet-Green Swallow (*Tachycineta thalassina*). *Northwest Science*, 88, 49–54.

Gil, D., Bulmer, E., Celis, P. & Puerta, M. (2008) Increased sibling competition does not increase testosterone or corticosterone levels in nestlings of the spotless starling (*Sturnus unicolor*). *Hormones and Behavior*, 54, 238–243.

Harper, A. (1986) The Evolution of Begging: Sibling Competition and Parent-Offspring Conflict on JSTOR. *The American Naturalist*, 128, 99–114.

Heath, J., Flynn, L. & Kilpatrick, K. (2015) Corticosterone Levels during Nest Departure of Juvenile American Kestrels. The Condor, 99, 806–811.

Holberton, R.L. & Able, K.P. (2000) Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). *Proceedings of the Royal Society B: Biological Sciences*, 267, 1889–1896.

Horton, B.M. & Holberton, R.L. (2010) Morph-Specific Variation in Baseline Corticosterone and the Adrenocortical Response in Breeding White-Throated Sparrows (*Zonotrichia albicollis*). *The Auk*, 127, 540–548.

Kacelnik, A., Cotton, P.A., Stirling, L. & Wright, J. (1995) Food Allocation among Nestling Starlings: Sibling Competition and the Scope of Parental Choice. *Proceedings of the Royal Society of London B: Biological Sciences*, 259.

Kitaysky, A., Kitaiskaia, E., Wingfield, J. & Piatt, J. (2001a) Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 171, 701–709.

Kitaysky, A.S., Wingfield, J.C. & Piatt, J. (2001b) Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behavioral Ecology*, 12, 619–625.

Kitaysky, A.., Kitaiskaia, E.., Piatt, J. & Wingfield, J. (2003) Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior*, 43, 140–149.

Kozlowski, C.P. & Ricklefs, R.E. (2011) The effects of brood size on growth and steroid hormone concentrations in nestling eastern bluebirds (*Sialia sialis*). *General and Comparative Endocrinology*, 173, 447–453.

Lendvai, A.Z., Loiseau, C., Sorci, G. & Chastel, O. (2009) Early developmental conditions affect stress response in juvenile but not in adult house sparrows (Passer domesticus). *General and Comparative Endocrinology*, 160, 30–35.

Lindström, K.M., Hawley, D.M., Davis, A.K. & Wikelski, M. (2005) Stress responses and disease in three wintering house finch (*Carpodacus mexicanus*) populations along a latitudinal gradient. *General and Comparative Endocrinology*, 143, 231–239.

Loiseau, C., Sorci, G., Dano, S. & Chastel, O. (2008) Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *General and Comparative Endocrinology*, 155, 101–108.

Lynn, S.E., Breuner, C.W. & Wingfield, J.C. (2003) Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Hormones and Behavior*, 43, 150–157.

Mock D.W., Parker, G.A. (1997) The evolution of sibling rivalry. Oxford: Oxford University Press.

Monaghan, P. (2008) Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363, 1635–1645.

Morandini, V. & Ferrer, M. (2015) Sibling aggression and brood reduction: a review. *Ethology Ecology & Evolution*, 27, 2–16.

Morici, L.A., Elsey, R.M. & Lance, V.A. (1997) Effects of long -term corticosteror implants on growth and immune function in juvenile alligators, *Alligator mississippiensis*. *The Journal of Experimental Zoology*, 279, 156–162.

Muller, C., Jenni-Eiermann, S. & Jenni, L. (2009) Effects of a short period of elevated circulating corticosterone on postnatal growth in free-living Eurasian kestrels *Falco tinnunculus*. *Journal of Experimental Biology*, 212, 1405–1412.

Naef-Daenzer, B. & Grüebler, M.U. (2016) Post-fledging survival of altricial birds: ecological determinants and adaptation. *Journal of Field Ornithology*, 87, 227–250.

Nowicki, S., Peters, S. & Podos, J. (1998) Song learning, early nutrition and sexual selection in songbirds. *American Zoologist*, 38, 179–190.

Nettle, D., Andrews, C., Reichert, S., Bedford, T., Gott, A., Parker, C., Kolenda, C., Martin-Ruiz, C., Monaghan, P. & Bateson, M. (2016) Brood size moderates associations between relative size, telomere length, and immune development in European starling nestlings. *Ecology and Evolution*, 6, 8138–8148.

Oitzl, M.S. & Kloet, E.R. De. (1992) Selective Corticosteroid Antagonists Modulate Specific Aspects of Spatial Orientation Learning. *Behavioral Neuroscience*, 106, 62–71.

Olson, J.M. (1992) Growth, the Development of Endothermy, and the Allocation of Energy in Red-Winged Blackbirds (*Agelaius phoeniceus*) during the Nestling Period. *Physiological Zoology*, 65, 124–152.

O'Reilly, K.M. & Wingfield, J.C. (2001) Ecological Factors Underlying the Adrenocortical Response to Capture Stress in Arctic-Breeding Shorebirds. *General and Comparative Endocrinology*, 124, 1–11.

Ostreiher, R. & Heifetz, A. (2016) The blessing of having younger nestmates: the case of the Arabian babbler. *Behavioral Ecology*, 27, 393–400.

Patterson, S.H., Hahn, T.P., Cornelius, J.M. & Breuner, C.W. (2014) Natural selection and glucocorticoid physiology. *Journal of Evolutionary Biology*, 27, 259–274.

Perlut, N.G. & Strong, A.M. (2016) Comparative analysis of factors associated with first-year survival in two species of migratory songbirds. *Journal of Avian Biology*, 47, 858–864.

Quillfeldt, P., Masello, J.F., Strange, I.J. & Buchanan, K.L. (2006) Begging and provisioning of thin-billed prions, Pachyptila belcheri, are related to testosterone and corticosterone. *Animal Behaviour*, 71, 1359–1369.

Rich, E. & Romero, L.M. (2001) Daily and photoperiod variations of basal and stress-induced corticosterone concentrations in house sparrows (*Passer domesticus*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 171, 543–547.

Rivers, J.W., Liebl, A.L., Owen, J.C., Martin, L.B. & Betts, M.G. (2012) Baseline corticosterone is positively related to juvenile survival in a migrant passerine bird. *Functional Ecology*, 26, 1127–1134.

Romero, L.M. & Remage-Healey, L. (2000) Daily and Seasonal Variation in Response to Stress in Captive Starlings (Sturnus vulgaris): Corticosterone. *General and Comparative Endocrinology*, 119, 52–59.

Romero, L.M. & Romero, R.C. (2002) Corticosterone Responses in Wild Birds: The Importance of Rapid Initial Sampling. *The Condor*, 104, 129–135.

Romero, L.M. (2004) Physiological stress in ecology: Lessons from biomedical research. *Trends in Ecology and Evolution*, 19, 249–255.

Roy, T. & Bhat, A. (2016) Learning and Memory in Juvenile Zebrafish: What makes the Difference - Population or Rearing Environment? *Ethology*, 122, 308–318.

Saino, N., Calza, S., Moller, A., Saino, N., Calza, S. & Mollert, A.P. (1997) Immunocompetence of Nestling Barn Swallows in Relation to Brood Size and Parental Effort. *British Ecological Society Journalof Animal Immunocompetence*. 66, 827-836.

Saino, N., Suffritti, C., Martinelli, R., Rubolini, D. & Moller, A.P. (2003) Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hirundo rustica*). *Behavioral Ecology*, 14, 318–325.

Sapolsky, R.M., Romero, M.L. & Munck, A.U. (2000) How Do Glucocorticoids Influence Stress Responses? Preparative Actions. *Endocrine Reviews*, 21, 55–89.

Schoech, S.J., Rensel, M. a & Heiss, R.S. (2011) Short- and long-term effects of developmental corticosterone exposure on avian physiology,behavioral phenotype,cognition,and fitness: A review. *Current Zoology*, 57, 514–530.

Schwabl, H. (116AD) Developmental Changes and Among-Sibling Variation of Corticosterone Levels in an Altricial Avian Species. *General and Comparative Endocrinology*, 408, 403–408.

Silverin, B., Arvidsson, B. & Wingfield, j. (1997) The adrenocortical responses to stress in breeding Willow Warblers *Phylloscopus trochilus* in Sweden: effects of latitude and gender. *Functional Ecology*, 11, 376–384.

Smiseth, P.T., Ward, R.J.S. & Moore, A.J. (2007) Parents influence asymmetric sibling competition: experimental evidence with partially dependent young. *Ecology*, 88, 3174–3182.

Thaker, M., Lima, S.L. & Hews, D.K. (2009) Acute corticosterone elevation enhances antipredator behaviors in male tree lizard morphs. *Hormones and Behavior*, 56, 51–57.

Thaker, M., Vanak, A.T., Lima, S.L. & Hews, D.K. (2010) Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. *The American naturalist*, 175, 50–60.

Tissier, M.L., Williams, T.D. & Criscuolo, F. (2014) Maternal Effects Underlie Ageing Costs of Growth in the Zebra Finch (Taeniopygia guttata). *PLoS ONE*, 9, e97705.

Wada, H. & Breuner, C.W. (2007) Development of stress reactivity in white-crowned sparrow nestlings: total corticosterone response increases with age, while free corticosterone response remains low. *General Comparative Endocrinology*, 150, 405–413.

Wada, H. & Breuner, C.W. (2008) Transient elevation of corticosterone alters begging behavior and growth of white-crowned sparrow nestlings. *The Journal of experimental biology*, 211, 1696–1703.

Weinstein, B.G. (2015) MotionMeerkat: integrating motion video detection and ecological monitoring. *Methods in Ecology and Evolution*, 6, 357–362.

Wegrzyn, E. (2013) Resource allocation between growth and endothermy allows rapid nestling development at low feeding rates in a species under high nest predation. *Journal of Avian Biology*, 44, 383–389.

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M. & Richardson, R.D. (1998) Ecological bases of hormone-behavior interactions: The "emergency life history stage". *American Zoologist*, 38, 191–206.

Wingfield, J.C., Moore, I.T., Vasquez, R. a., Sabat, P., Clark, A., Addis, E., Prado, F. & Wada, H. (2008b) Modulation of the Adrenocortical Responses To Acute Stress in Northern and Southern Populations of Zonotrichia. *Ornitologia Neotropical*, 19, 241–251.

Winkler, David W., Kelly K. Hallinger, Daniel R. Ardia, R. J. Robertson, B. J. Stutchbury and R. R. Cohen. (2011). Tree Swallow (Tachycineta bicolor), The Birds of North America (P. G. Rodewald, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America: https://birdsna.org/Species-Account/bna/species/treswa DOI: 10.2173/bna.11

Figure Legends

Fig. 1. Mean brood size across treatments for both Tree Swallows (TRES) and Violet-green Swallows (VGSW) showing that our experiment was effective at creating a stepwise increase in mean brood size. We used the average number of nestlings present over the day 3-15 period, as it more accurately represents the competitive conditions nestlings experience during development than brood size on any single day.

Fig. 2. Predicted differences between control broods and manipulated broods for baseline CORT (a), stressor-induced CORT (b), and growth rate (c). Both baseline and stressor-induced CORT concentrations were higher in enlarged broods while reduced broods showed no difference from control. Growth rates in both species trended toward an increase in reduced broods and a reduction in enlarged broods. Dashed lines indicate the reference levels in control broods.

Fig. 3. Growth curves for each species/treatment combination. Tree Swallows (TRES; a-c) grew faster than Violet-green Swallows (VGSW; d-f) across all treatments (p-value = 0.014). Solid lines represent mean logistic growth curves whereas dashed lines indicate asymptotic value (horizontal, across the top of the curve) and inflection point (horizontal, halving the curve), and age at half of day 15 mass (vertical).

Fig. 4. Correlation between measures of CORT (ng/mL) and nestling growth rate (r). Both baseline CORT (a-b) (correlation coefficient = -0.23) and stressor-induced CORT (c-d) (coefficient = -0.26) were slightly negatively correlated with growth rate.

Figure 1

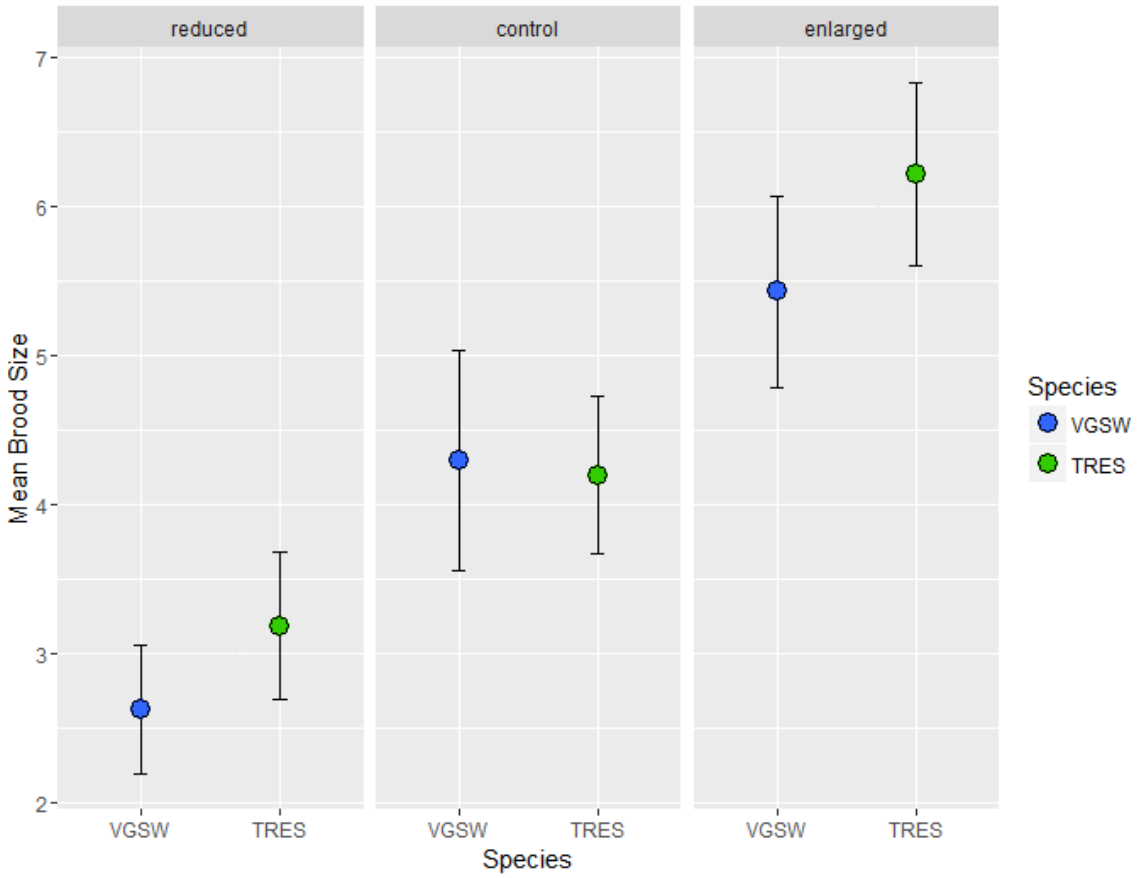
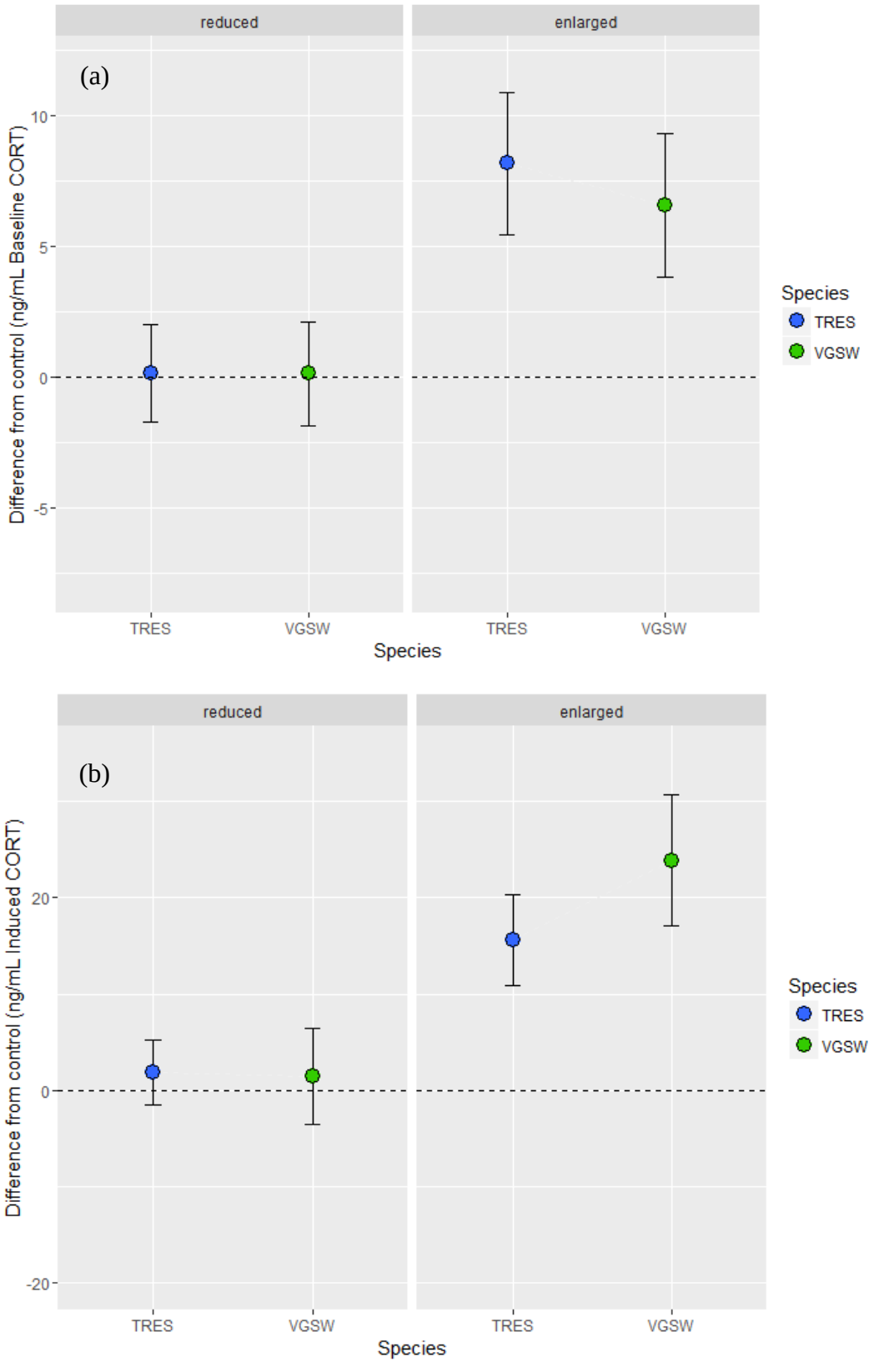

reduced
control
enlarged
Mean Brood Size
7
6
5
4
3
2
VGSW
TRES
VGSW
TRES
VGSW
TRES
Species
Species
VGSW
TRES

Figure 2


reduced
enlarged
(a)
Difference from control (ng/mL Baseline CORT)
10
5
0
-5
TRES
VGSW
TRES
VGSW
Species
Species
TRES
VGSW
(b)
Difference from control (ng/mL Induced CORT)
20
0
-20
TRES
VGSW
TRES
VGSW
Species
Species
TRES
VGSW

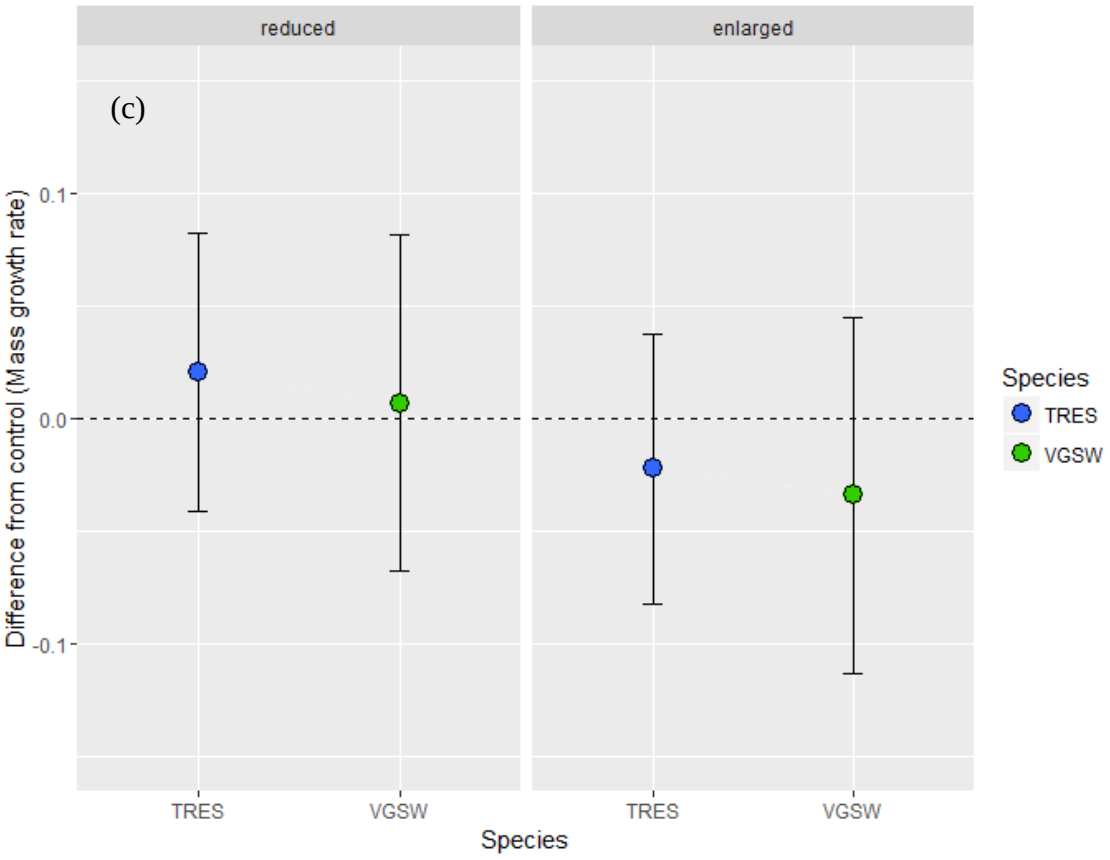

reduced
enlarged
(c)
0.1
0.0
-0.1
Difference from control (Mass growth rate)
TRES
VGSW
TRES
VGSW
Species
Species
TRES
VGSW

Figure 3

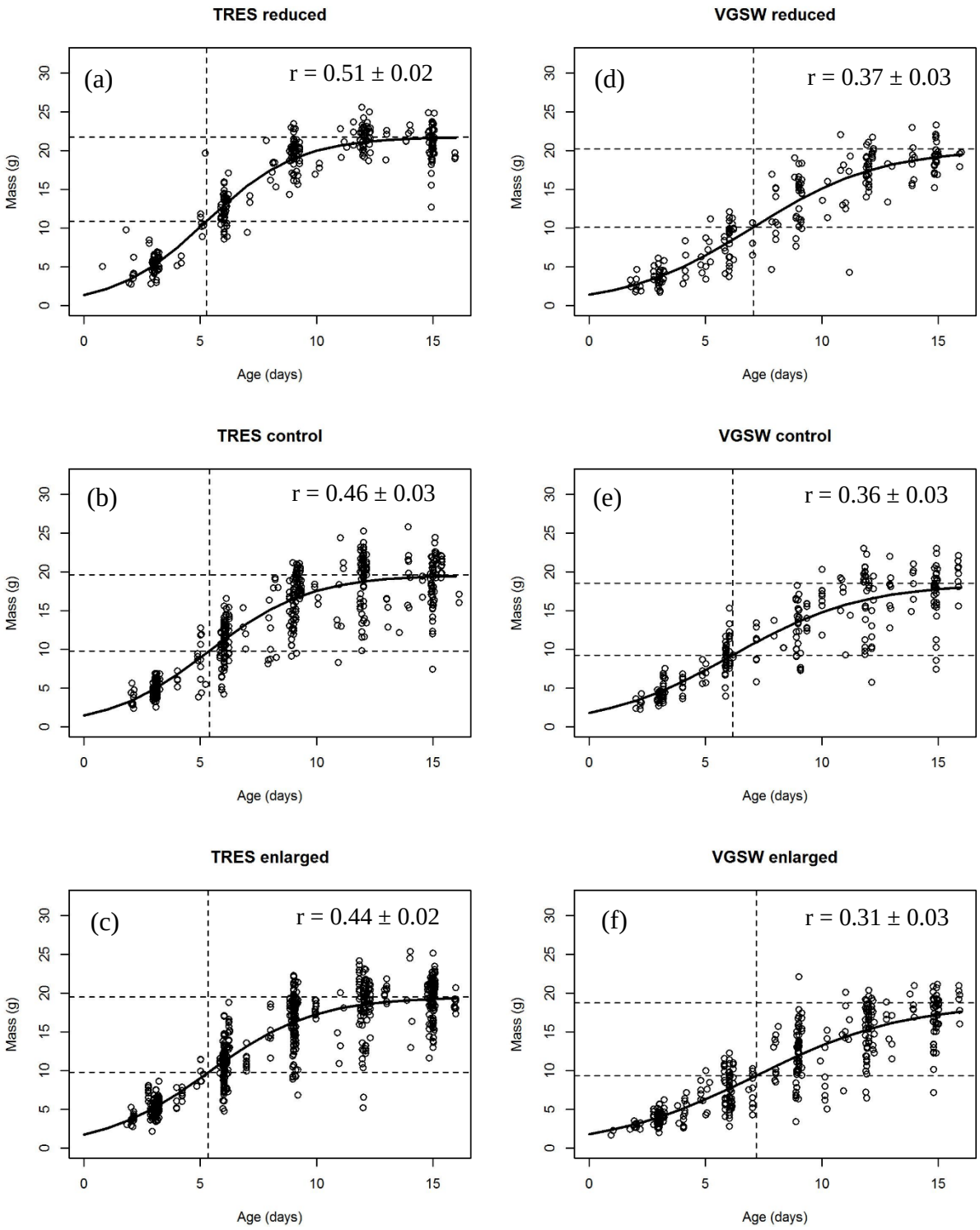
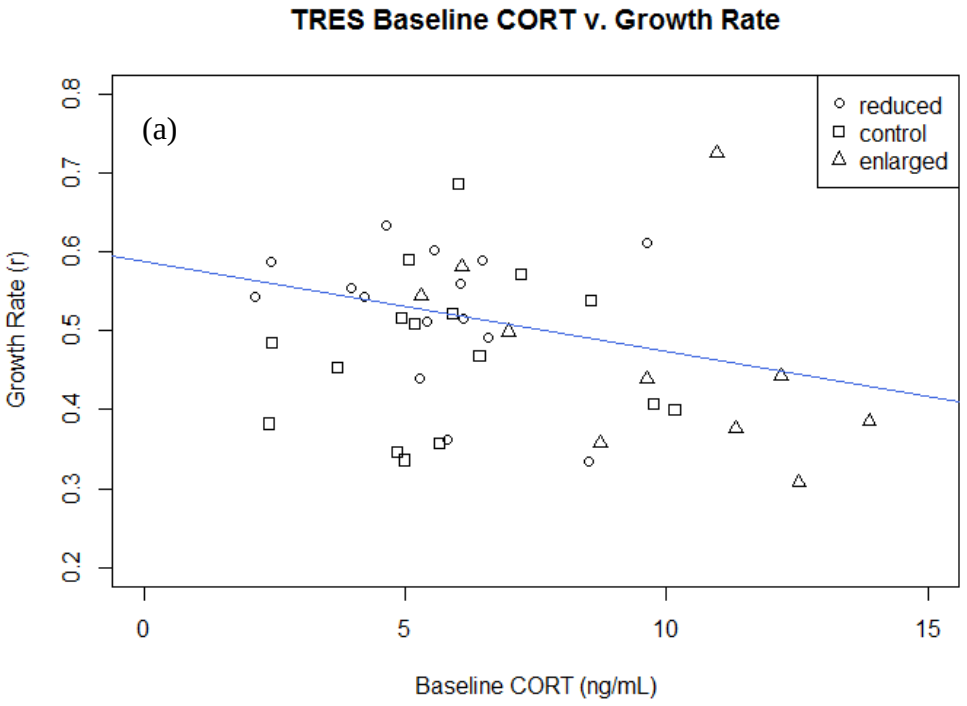
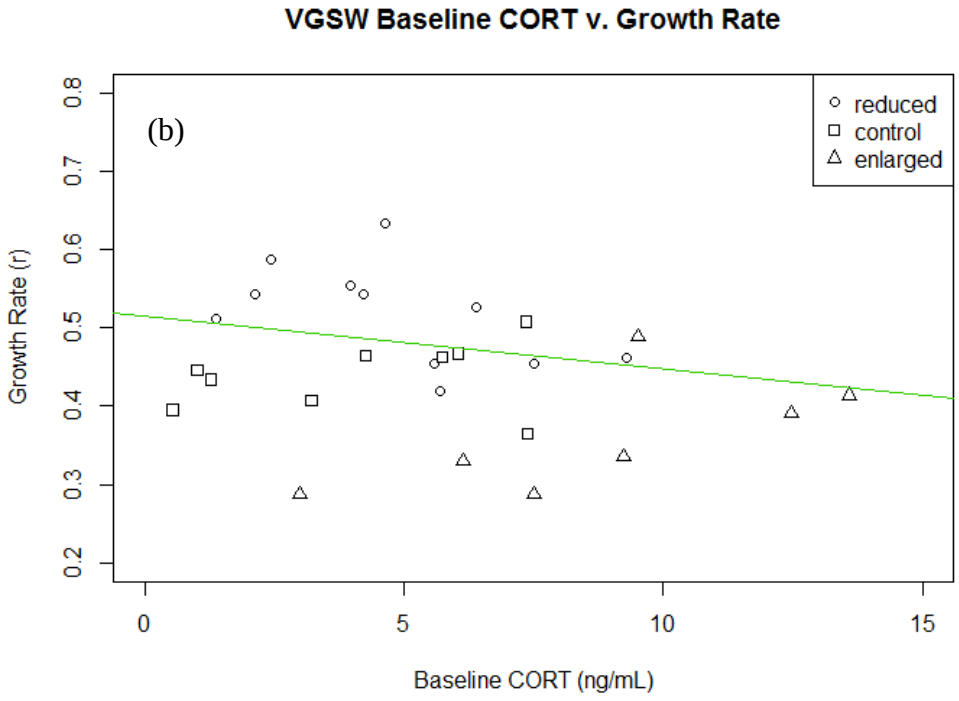
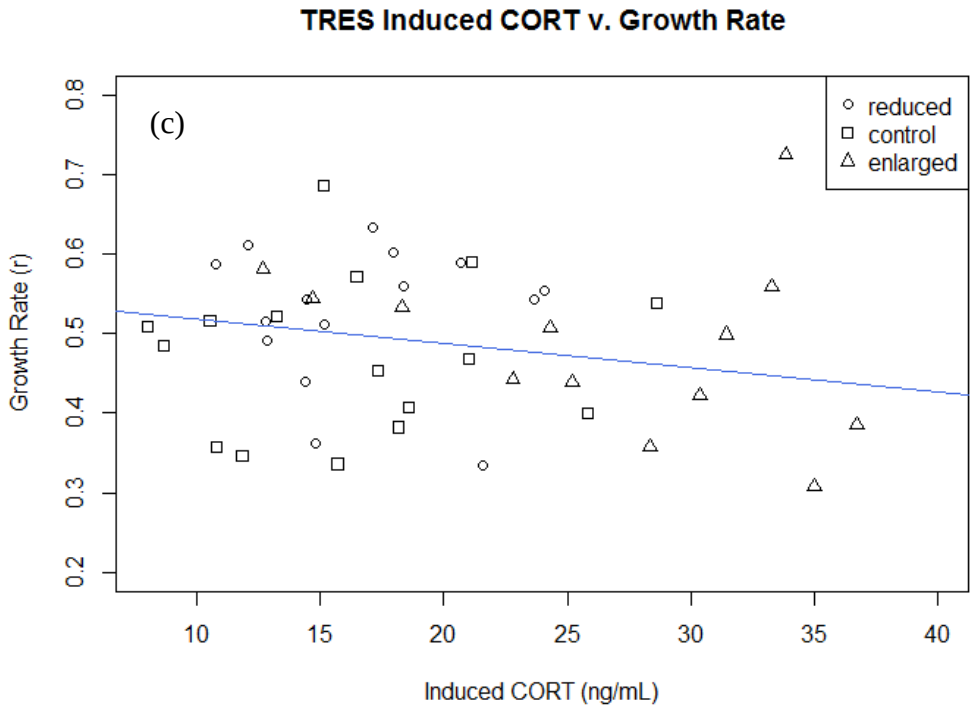
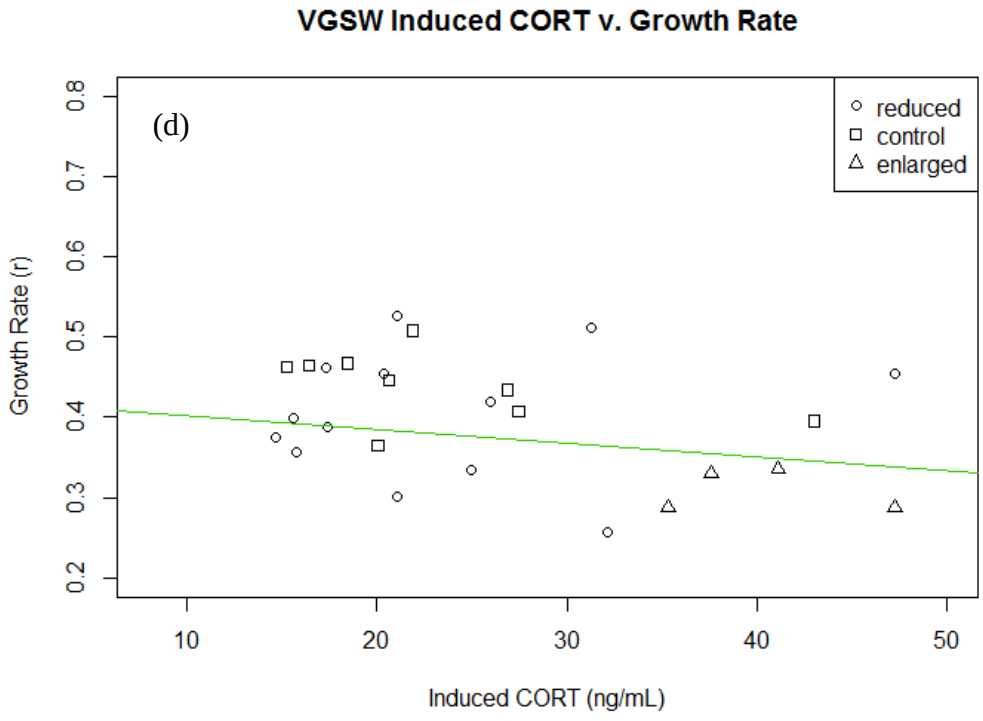

TRES reduced
(a)
r = 0.51 ± 0.02
VGSW reduced
(d)
r = 0.37 ± 0.03
TRES control
(b)
r = 0.46 ± 0.03
VGSW control
(e)
r = 0.36 ± 0.03
TRES enlarged
(c)
r = 0.44 ± 0.02
VGSW enlarged
(f)
r = 0.31 ± 0.03
Mass (g)
Age (days)
0
5
10
15
20
25
30

Figure 4


TRES Baseline CORT v. Growth Rate
(a)
reduced
control
enlarged
Growth Rate (r)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
5
10
15
Baseline CORT (ng/mL)


VGSW Baseline CORT v. Growth Rate
(b)
reduced
control
enlarged
Growth Rate (r)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
5
10
15
Baseline CORT (ng/mL)


TRES Induced CORT v. Growth Rate
(c)
reduced
control
enlarged
Growth Rate (r)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
10
15
20
25
30
35
40
Induced CORT (ng/mL)


VGSW Induced CORT v. Growth Rate
(d)
reduced
control
enlarged
Growth Rate (r)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
10
20
30
40
50
Induced CORT (ng/mL)