

RESEARCH ARTICLE

Functional Ecology



Experimental brood enlargement differentially influences the magnitude of the corticosterone stress response in closely related, co-occurring songbirds

Braelei M. Hardt¹ | Daniel R. Ardia² | Meredith J. Bashaw³ | James W. Rivers⁴ 

¹Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon

²Department of Biology, Franklin & Marshall College, Lancaster, Pennsylvania

³Department of Psychology, Franklin & Marshall College, Lancaster, Pennsylvania

⁴Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon

Correspondence

James W. Rivers

Email: jim.rivers@oregonstate.edu

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Abstract

1. Rearing environments can shape offspring phenotype across taxa, yet little is known about how brood size influences hypothalamic–pituitary axis functioning, whether its expression trades off with growth, and the degree to which these relationships vary between species.
2. We evaluated how brood size influenced nestling physiological (glucocorticoids) and somatic traits (growth), and the extent to which their relationship differed between two closely related, sympatric songbirds when experiencing identical rearing environments. Specifically, we used a cross-fostering approach to alter brood size and create an experimental gradient of nestmate competition, and then tested whether experimentally manipulated brood sizes resulted in nestlings with altered concentrations of corticosterone and whether corticosterone responses traded off with growth.
3. Nestlings of both species experienced elevated concentrations of baseline and stressor-induced corticosterone when raised in enlarged broods, relative to control and reduced broods, but neither measurement was found to trade off with growth or be linked to survival to fledging.
4. In contrast, we found divergence in the magnitude of the corticosterone stress response between species across all brood treatments, with greater stressor-induced corticosterone concentrations found in the Violet-green Swallow (*Tachycineta thalassina*) relative to the closely related Tree Swallow (*T. bicolor*).
5. Our study demonstrated that brood size can lead to changes in offspring corticosterone concentrations in swallows and that nestlings of sympatric species, even those that are closely related and ecologically similar, can diverge in their corticosterone stress response when experiencing identical rearing conditions.
6. We conclude that corticosterone appears to play a key role for balancing energetic demands that arise in the face of nestmate competition in *Tachycineta* swallows and that elevated concentrations of corticosterone may enhance offspring survival during challenging environmental conditions, such as when brood competition is strong.

KEYWORDS

brood size, corticosterone, glucocorticoids, growth, *Tachycineta*, Tree Swallow, Violet-green Swallow

1 | INTRODUCTION

Glucocorticoid production is an important physiological process in vertebrates that can mediate fitness (Lendvai, Loiseau, Sorci, & Chastel, 2009; Rivers, Liebl, Owen, Martin, & Betts, 2012) and influence reproductive success (Bonier, Martin, Moore, & Wingfield, 2009; Bonier, Moore, & Robertson, 2011). Baseline glucocorticoid concentrations regulate activity patterns linked to sleep, energy balance, immune activity and reproductive functioning (Loiseau, Sorci, Dano, & Chastel, 2008; Sapolsky, Romero, & Munck, 2000); additionally, a rapid increase in glucocorticoid production can also promote fitness when organisms are confronted with unexpected stressors. Referred to as the corticosterone stress response (Wingfield et al., 1998), this process typically results in an acute increase in glucocorticoid secretion from activation of the hypothalamic–pituitary–adrenal axis that redirects energy away from non-critical processes (e.g. reproduction) and towards life-saving behaviours (e.g. flight; Sapolsky et al., 2000; Wingfield et al., 1998). Once an acute stressor is no longer perceived as a threat, glucocorticoid concentrations typically return to baseline concentrations via negative feedback (Romero, 2004). Although baseline concentrations and stressor-induced increases in glucocorticoids are physiologically distinct processes (Romero, 2004; Sapolsky et al., 2000), both should be evaluated when assessing the consequences of glucocorticoid function on fitness (Bonier et al., 2009; Breuner, Patterson, & Hahn, 2008).

Rearing conditions can affect many processes during post-natal offspring development (Monaghan, 2008; Stamps, 2003). Competition from nestmates over food is arguably one of the most important factors that influence young because competition for food often influences energy acquisition that is necessary for growth and development. Competition among nestmates is typical for species that raise offspring in broods, and the degree of competition can be a function of resource availability (Morandini & Ferrer, 2015), parent quality (Saino, Calza, & Moller, 1997; Smiseth, Ward, & Moore, 2007), and the number and characteristics of competing young (Godfray, 1995). In vertebrates, brood size can have strong consequences on some aspects of the offspring phenotype, such as body size and growth (Nilsson & Gardmark, 2001; Vitousek, Jenkins, Hubbard, Kaiser, & Safran, 2017). Brood size can also influence glucocorticoid function, although few studies have examined this topic using experimentally altered broods, and all investigations to date have been restricted to assessing baseline corticosterone. Importantly, these studies are inconsistent in their findings: although some have found increased concentrations of baseline corticosterone after experimental brood enlargement (Gil, Bulmer, Celis, & Puerta, 2008; Kozłowski & Riecklefs, 2011), others have not (Saino, Suffritti, Martinelli, Rubolini, & Moller, 2003). These mixed results are surprising given that enhanced competition and/or food shortages often result in elevated glucocorticoids (Kitaysky, Winfield, & Piatt, 1999; Love, Bird, & Shutt, 2003), and experimental increases in circulating glucocorticoids can reduce growth (Muller, Jenni-Eiermann, & Jenni, 2009; Wada & Breuner, 2008). Thus, nestlings

are expected to trade off glucocorticoids and growth, but the extent to which they do this under natural conditions is unclear. Therefore, new studies are needed that evaluate links the relationship between brood size, glucocorticoids and offspring growth to better understand internal constraints during development (Ricklefs, Starck, & Konarzewski, 1998), including investigations that address both baseline and stressor-induced hormone concentrations because of their independent consequences on fitness (Bonier et al., 2009; Breuner et al., 2008).

In this study, we tested the hypothesis that experimental changes to brood size influenced subsequent glucocorticoid functioning in developing young. We predicted that young raised in enlarged broods would have greater baseline and stressor-induced concentrations of corticosterone, the primary glucocorticoid in birds, relative to those reared in unmanipulated control broods. Likewise, nestlings in enlarged broods were also predicted to have longer nestling developmental periods and lower survival rates. In contrast, we predicted that young raised in reduced broods would show the opposite pattern relative to those in enlarged broods (i.e. lower corticosterone concentrations, shorter development periods and higher survival rates). We also tested the related hypothesis that corticosterone concentrations would trade off with growth in nestlings. Specifically, we predicted that young in enlarged broods, which were expected to have elevated corticosterone concentrations, would have reduced growth relative to those in control broods. In contrast, we predicted that nestlings in reduced broods, which were expected to have reduced corticosterone concentrations, would have increased growth rates relative to control nestlings.

We tested these hypotheses in two sympatric, closely related and ecologically similar songbirds, the Tree Swallow (*Tachycineta bicolor*) and the Violet-green Swallow (*T. thalassina*), to determine whether the links between brood size, corticosterone measures, growth and survival were similar between congeneric species experiencing identical rearing conditions. We expected both species would have similar responses to experimental variation in rearing environments because of their shared evolutionary history and because they overlap spatially and temporally during the breeding season in our study area. Thus, our investigation was undertaken not as a comparative study focused on understanding adaptation (see Garland & Adolph, 1994) but instead as an assessment of the degree of consistency between closely related species in response to the identical experimental manipulation. Given the lack of multispecies investigations on this topic, our study serves to enhance our understanding of the extent to which ecologically similar songbirds vary in their glucocorticoid functioning relative to the degree of nestmate competition they experience.

2 | MATERIALS AND METHODS

2.1 | Study species and population

Our work took place during May–August in 2015–2016 within the vicinity of Corvallis, Oregon (44.6° N, 123.3° W; elevation 70 m),

where both study species initiate nests in early May and overlap for the majority of each breeding season (J. W. Rivers, unpublished data). Both swallows exhibit female-only incubation, and have similar clutch sizes (4–7 eggs), incubation periods (13–15 days) and nestling periods (18–24 days; Brown, Knott, & Damrose, 2011; Winkler et al., 2011). Furthermore, they overlap in their foraging areas and generally feed similar food items to offspring in our study area during the period of nestling development (Garlick, Newberry, & Rivers, 2014).

2.2 | Experimental design

We monitored nestboxes every 2–3 days to determine nest initiation date, defined as the date the first egg appeared in a nest. We restricted our study to the first breeding attempt in each nestbox within each year, as in rare circumstances individuals may attempt a second nest if their initial nesting attempt fails (J. W. Rivers, unpublished data). To create an experimental gradient of nestmate competition, we used a reciprocal partial cross-fostering study design where we manipulated nest contents to create three distinct brood types. Within each species, we assigned brood treatments to nests at hatching using a blocked design, assigning every three pairs of consecutively hatching nests to a block to ensure even treatment assignment across the breeding season. Within each block, we randomly selected one pair of nests to serve as two controls, whereas each of the other two pairs was assigned as a pairing of a reduced brood and an enlarged brood. We then randomly assigned one nest in each of these pairs to be a reduced treatment and the other to be an enlarged treatment. We only used paired nests that hatched within 1 day of each other to minimize size differences among nestlings between paired nests, although most paired nests hatched on the same day for both species (82.6% of $n = 64$ nest pairs).

On nestling day 3 (where day 0 was the day of hatching), we moved nestlings between nests to create experimental broods. For control nests, we moved two nestlings from the first nest in a pair to the second nest, and vice versa. From each reduced brood nest, we moved three nestlings to the enlarged brood nest with which it was paired; at the same time, we also moved one nestling from the enlarged brood nest into the reduced brood nest. Nestling transfers took place throughout the day on nestling day 3, and no nestlings were held out of a nest >30 min during transfers. This resulted in a net increase in two nestlings for enlarged broods, a net decrease in two nestlings for reduced broods and no change in the number of nestlings for control broods. Our design also ensured that all nests, regardless of treatment, contained both unrelated young (foreign nestlings) and offspring that were related to the provisioning female (natal nestlings) in the unlikely event parents discriminated against non-kin offspring when provisioning. Experimental brood sizes (range: 1–8 nestlings) fell within range of natural broods for both species, although we note that brood sizes at the extremes of this continuum are rare under natural conditions.

2.3 | Quantification of growth, corticosterone and survival

We measured body mass when we created broods on nestling day 3 and every 3 days thereafter until day 15, resulting in five measures per nestling. Growth measurements were made between 0600 and 1300, and each nestling within a nest was marked individually using a combination of non-toxic marker and nail clipping. We sampled blood on day 15 only because neither species in our population can be handled after this age without causing premature fledging (J. W. Rivers, unpublished data). To quantify corticosterone, we selected two nestlings in the middle of the brood size continuum and collected ~75 μ l of blood from the brachial vein of each nestling. We obtained baseline blood samples within 3 min of initial contact to reflect prestressor corticosterone concentrations (Romero & Reed, 2004), and we sampled blood 30 min post-contact to quantify stressor-induced corticosterone; we placed all blood samples on ice immediately after they were obtained. Although our goal was to bleed nestlings on nestling day 15, hatching asynchrony and non-matching hatch dates forced us to bleed a minority of nestlings on either nestling day 14 (10%) or day 16 (11%). However, there was no discernible difference in corticosterone concentrations between nestlings 14 and 16 days old (B. M. Hardt & J. W. Rivers, unpublished data), so we did not include nestling age in our statistical analysis. We sampled blood from one natal and one foreign nestling within each brood but did not detect differences in corticosterone measures or growth rate between these groups for all treatment \times species combination (B. M. Hardt & J. W. Rivers, unpublished data); therefore, we used measurements from all nestlings within a given brood without considering their nest of origination. To determine fledging date, we visited nests daily prior to the expected fledging date, and every day thereafter until no nestlings were present in nest boxes. Post-fledging survival estimates are challenging to obtain in our study species because they leave the nest site shortly after fledging; therefore, we quantified survival as the proportion of nestlings that successfully fledged from nests from the brood size at the start of the experiment, as we assumed the effects of brood treatments would be strongest during this period.

2.4 | Quantification of adult provisioning rate

We quantified adult provision rates by placing a video camera >20 m from nests on nestling day 9 and filming adult feeding for a minimum of 90 min; nestling growth measurements were taken immediately thereafter. Swallows do not brood nestlings at this age (Garlick et al., 2014), so we assumed that adults entering nestboxes were feeding young. We made simultaneous recordings between 06:00 and 15:00 for pairs of nests from which offspring were swapped to eliminate variation in feeding related to time of day or weather. Swallows carry food hidden from view when arriving at their nest (Garlick et al., 2014), so we could not estimate food loads directly. Instead, we used total feeding visits as a measure of provisioning rate because this approach is an accurate index of food delivered to Tree Swallow nests

(McCarty, 2002). We extracted provisioning data from videos using MotionMeerkat (Weinstein, 2015), a programme that automates identification of motion events from a video stream and tallies the number of events recorded. We evaluated the accuracy of this approach by manually watching entire videos for a subset of nests ($n = 10$ nests/species) and found it correctly classified feeding visits with 100% accuracy. For analysis, we calculated per capita provisioning rate by taking the total number of parental feeding visits per hour and dividing it by the number of young in the nest.

2.5 | Laboratory analysis

Within 6 hr of taking blood, we returned to the laboratory where we centrifuged samples to separate blood plasma, which was then kept frozen at -80°C until analysis. To assay corticosterone in blood samples, we used an enzyme-linked immunoassay (EIA; C. J. 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 (0.05 M NaHCO_3 pH 9.6), with 50 μl /well on a 96-well Nunc-Immuno Maxisorp (Thermo-Fisher Scientific) microtitre plate, covered with a plate sealer and allowed it to sit overnight at 4°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 NaPO_4 , 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0) and then add 50 μl of diluted horseradish peroxidase-conjugated corticosterone in EIA buffer (1:70,000) to each well. We incubated plates in the dark at room temperature for 2 hr and then washed them before subjecting them to an additional 20-min incubation with 100 μl /well of ABTS [0.4 mM 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt, 1.6 mM H_2O_2 , 0.05 M citrate, pH 4.0]. For a positive control, we used a pooled plasma sample from Zebra Finches (*Taeniopygia guttata*). We read plates at 405 nm using a spectrophotometer (Gen 5, BioTek, Winooski, Vermont), and we randomized samples from different treatments and years across plates; coefficient of variation estimates for intra- and interplate variation were $<8\%$.

2.6 | Statistical analysis

Tachycineta swallows exhibit growth that is well-approximated with logistic growth curves (Ardia, 2006; McCarty, 2001), so we calculated the growth rate constant (K) of a logistic growth function (Starck & Ricklefs, 1998) for each nestling by iteratively fitting a sums of square minimization approach in MATHEMATICA (v11.0.01). We used a fixed asymptote to determine growth rate (Tree swallow: 17.95 g; Violet-green 15.27 g; J. W. Rivers, unpublished data), an approach that has the smallest error terms and least variation in parameter estimates for Violet-green Swallow nestlings (Austin, Robinson, Robinson, & Ricklefs, 2011). We then used the "nls" function in R

(v3.2.4) to fit a logistic growth curve for body mass for each species \times treatment combination.

To determine the effects of brood manipulation on baseline corticosterone, stressor-induced corticosterone, growth rate and per capita feeding rate, we constructed mixed-effects models with the "mle" function in the R statistical environment. All models were fitted using weights = varIdent for treatment and species to meet normality and variance assumptions. For corticosterone measures, our models contained brood treatment, species, and a treatment \times 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 also included the time that nestlings were blood-sampled as a covariate because baseline corticosterone exhibits a diurnal pattern in songbirds (Rich & Romero, 2001). We did not include time to bleed in our models because all samples were taken within 3 min, representing pre-stressor CORT concentrations (Romero & Reed, 2004), and because we found no relationship between time to bleed and baseline corticosterone concentration (B. M. Hardt & J. W. Rivers, unpublished data). We did not include year in our model because the design of our study resulted in modest sample sizes in each breeding season. Given that few, if any, studies have evaluated the relationship between brood size and glucocorticoid response between closely related species, we undertook pre-planned comparisons using linear contrasts within our models of corticosterone response to explore the magnitude of the glucocorticoid response to experimental treatments within and between our study species. This included an assessment of how baseline and stressor-induced corticosterone varied due to brood treatments within each species, as well as evaluating species-level differences for each brood treatment. Adopting this approach allowed us to quantify whether there were species- and treatment-level differences while also evaluating the magnitude of the response (Greenland et al., 2016; Wasserstein & Lazar, 2016), which is crucial for a broader understanding of how nestlings responded to experimental treatments in our study.

To assess how brood treatments influenced growth rate, we constructed a model that contained treatment, species and a treatment \times species interaction as fixed effects, with block and brood ID as random effects, and per capita provisioning rate as a covariate. We also tested whether brood treatments influenced adult provisioning rates, using a model that was constructed with treatment and species fixed effects and block and brood ID as random effects. For this model, nest initiation date and time of videotaping were included as covariates, as both may influence provisioning behaviour.

To ensure growth rates were not biased downward by nestlings in fatally poor condition, we removed individuals from growth rate analysis that died during days 3–15 of experiments; this resulted in 15% of nestlings being removed. With the "cor.test" function in R, we tested for association between growth rate and corticosterone measures with a Pearson's product-moment correlation, modelling each species separately. To determine the effects of brood treatments on offspring survival, we quantified the proportion of nestlings that successfully fledged from nests across treatments. We analysed this

measure 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 \times species interaction as fixed effects, block as a random effect and per capita provisioning rate as a covariate. We report marginal least squares means and their associated 95% confidence intervals (CIs), as well as effect sizes as marginal model-derived parameter estimates (B) and their associated 95% CIs; both estimates were taken at the mean value for each covariate within each model. We did not use corrections for pairwise comparisons in our models as such approaches can lead to unacceptably high type II error rates (Nakagawa, 2004).

3 | RESULTS

We created 118 experimental broods, of which 98 survived to day 15 and were available for analysis (Tree Swallow: $n = 58$, Violet-green Swallow: $n = 40$). Sample sizes ranged from $n = 18$ –21 nests/treatment for the Tree Swallow and $n = 11$ –17 nests/treatment for the Violet-green Swallow. We detected no differences in brood sizes at the start of the experiment between the two species in each of the brood treatments [reduced broods: $\hat{\beta} = 0.57$ (95% CI: $-0.06, 1.20$), $t_{37} = 1.82$, $p = .076$, control broods: $\hat{\beta} = 0.10$ (95% CI: $-0.96, 0.78$), $t_{26} = -0.23$, $p = .817$, enlarged broods: $\hat{\beta} = 0.79$ (95% CI: $-0.06, 1.65$), $t_{38} = 1.86$, $p = .070$].

3.1 | Baseline and stressor-induced corticosterone

Both species responded similarly with respect to baseline corticosterone, as we detected an effect of brood treatment ($F_{2,64} = 31.76$, $p < .001$) but not species ($F_{1,64} = 2.11$, $P = 0.15$), with no treatment \times species interaction ($F_{2,64} = 0.40$, $p = .670$; Figure 1a). For the Tree Swallow, baseline corticosterone was higher in enlarged broods relative to control broods ($\hat{\beta} = 8.16$ ng/ml [95% CI: 5.44, 10.89], $t_{64} = 5.87$, $p < .001$) and reduced broods ($\hat{\beta} = 8.020$ ng/ml [95% CI: 5.44, 10.72], $t_{64} = 5.82$, $p < .001$). In contrast, we did not detect a difference between reduced broods and control broods ($\hat{\beta} = 0.14$ ng/ml [95% CI: $-1.72, 2.02$], $t_{64} = 0.15$, $p = .88$). Similarly, we found that Violet-green Swallows exhibited higher baseline corticosterone concentrations in enlarged broods compared to control ($\hat{\beta} = 6.58$ ng/ml [95% CI: 3.83, 8.32], $t_{64} = 4.69$, $p < .001$) and reduced broods ($\hat{\beta} = 6.44$ ng/ml [95% CI: 3.89, 8.97], $t_{64} = 6.54$, $p < .001$), with no difference detected between reduced and control broods ($\hat{\beta} = 0.14$ ng/ml [95% CI: $-1.84, 2.12$], $t_{64} = 0.13$, $p = .89$). We found that Violet-green Swallows did not have baseline corticosterone concentrations that differed from Tree Swallows in either reduced ($\hat{\beta} = -1.33$ ng/ml [95% CI: $-3.12, 0.45$], $t_{64} = -1.46$, $p = .15$), control ($\hat{\beta} = -1.33$ ng/ml [95% CI: $-3.63, 0.96$], $t_{64} = -1.13$, $p = .26$) or enlarged broods ($\hat{\beta} = -2.91$ ng/ml [95% CI: $-6.15, 0.30$], $t_{64} = -1.77$, $p < .08$).

For stressor-induced corticosterone, both brood treatment ($F_{2,64} = 48.04$, $p < .001$) and species ($F_{1,64} = 20.91$, $p < .001$) influenced the response of nestlings, with no treatment \times species interaction ($F_{2,64} = 2.48$, $p = .091$; Figure 1b). For Tree Swallows, we

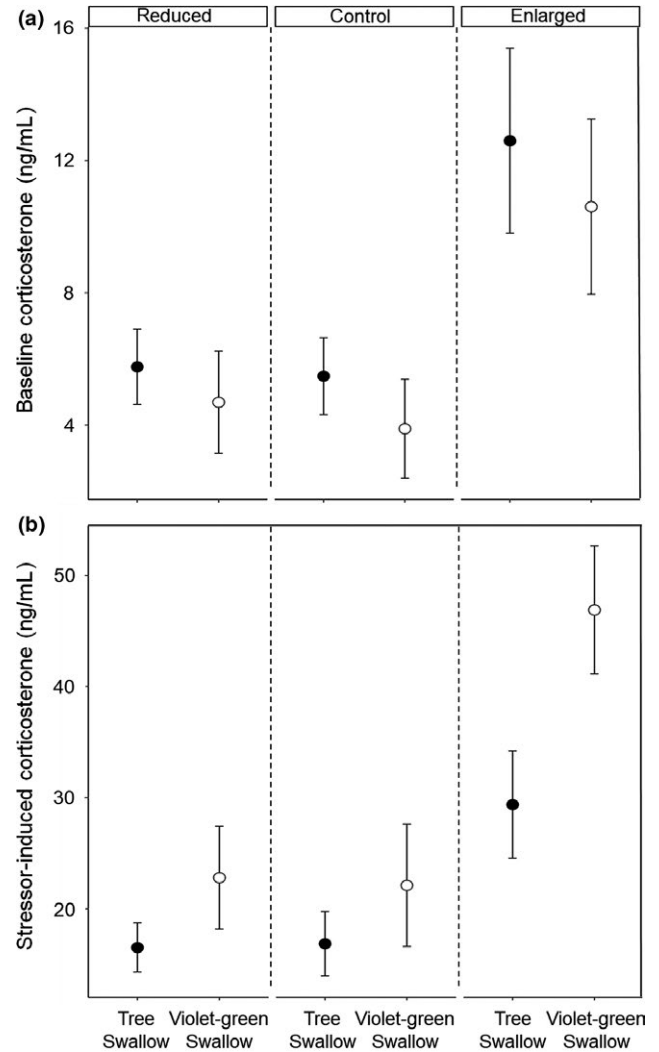


FIGURE 1 Mean (\pm 95% CI) estimates for (a) baseline corticosterone and (b) stressor-induced corticosterone relative to experimental brood treatments for Tree Swallows (filled circles) and Violet-green Swallows (open circles). In both species, baseline and stressor-induced concentrations were greater in enlarged broods, whereas reduced broods showed no difference from control. Note that the scales in the two panels are different

found that stressor-induced corticosterone was higher in enlarged broods relative to control broods ($\hat{\beta} = 15.6$ ng/ml [95% CI: 10.9, 20.3], $t_{64} = 6.54$, $p < .001$) and reduced broods ($\hat{\beta} = 13.8$ ng/ml [95% CI: 9.0, 18.6], $t_{64} = 5.64$, $p < .001$), whereas we did not detect a difference between reduced and control broods ($\hat{\beta} = 1.8$ ng/ml [95% CI: $-1.6, 5.2$], $t_{64} = -1.06$, $p = .294$). The same was true for Violet-green Swallows as stressor-induced corticosterone was greater in enlarged broods relative to control broods ($\hat{\beta} = 23.9$ ng/ml [95% CI: 17.1, 30.6], $t_{64} = 6.94$, $p < .001$) and to reduced broods ($\hat{\beta} = 22.4$ ng/ml [95% CI: 15.9, 28.9], $t_{64} = 6.76$, $p < .001$), but not between reduced and control broods ($\hat{\beta} = 1.5$ ng/ml [95% CI: $-3.5, 6.5$], $t_{64} = 0.56$, $p = .574$). With respect to species differences, Violet-green Swallows had greater stressor-induced corticosterone concentrations than Tree Swallows for all brood treatments: reduced broods ($\hat{\beta} = 5.3$ ng/ml [95% CI: 1.0,

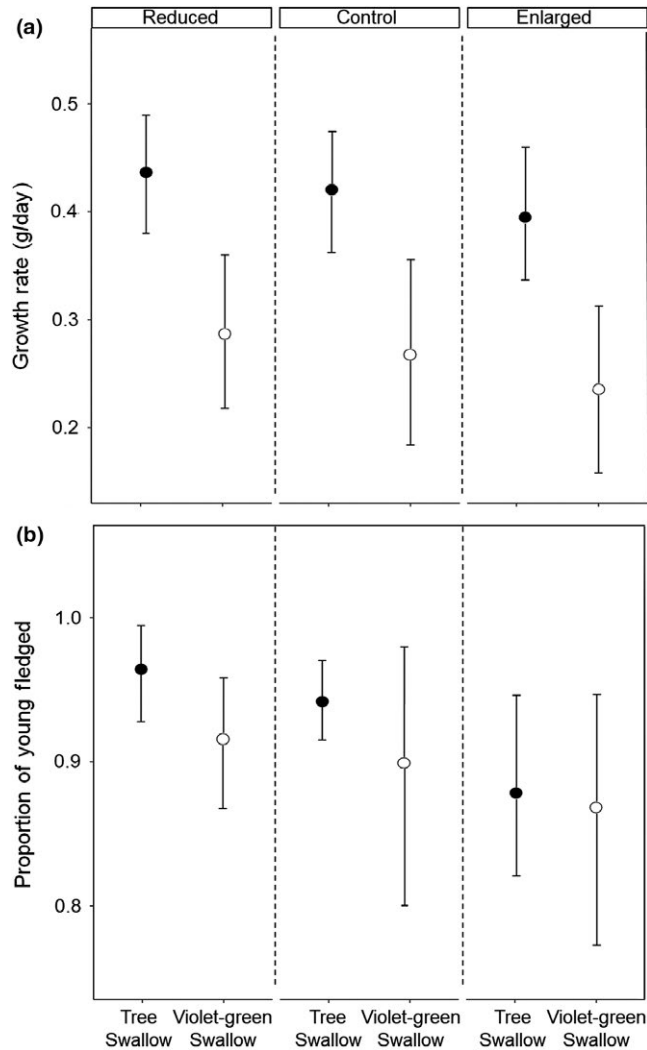


FIGURE 2 Mean (\pm 95% CI) estimates for (a) growth rate and (b) fledging success relative to experimental brood treatments for Tree Swallows (filled circles) and Violet-green Swallows (open circles)

9.6], $t_{64} = 2.44$, $p = .018$), control broods ($\hat{\beta} = 5.7$ ng/ml [95% CI: 0.9, 10.6], $t_{64} = 2.31$, $p = .024$) and enlarged broods ($\hat{\beta} = 13.6$ ng/ml [95% CI: 16.6, 20.6], $t_{64} = 1.32$, $p < .001$; Figure 1b).

3.2 | Growth, development and corticosterone-growth trade-offs

We did not detect an effect of brood treatment on nestling growth rate ($F_{2,72} = 3.46$, $p = .178$) although we did detect an effect of species ($F_{1,72} = 21.40$, $p < .001$), with no treatment \times species interaction ($F_{2,72} = 0.041$, $p = .960$; Figure 2a). Tree Swallow growth rates were similar between enlarged broods and control broods ($\hat{\beta} = -0.02$ g/day [95% CI: -0.08, 0.04], $t_{72} = -0.72$, $p = .470$), between enlarged and reduced broods ($\hat{\beta} = -0.04$ g/day [95% CI: -1.00, 0.01], $t_{72} = -1.51$, $p = .135$), and between reduced broods and control broods ($\hat{\beta} = 0.02$ g/day [95% CI: -0.04, 0.08], $t_{72} = 0.69$, $p = .512$). Similarly, we did not detect a difference for Violet-green Swallows between enlarged and control broods

($\hat{\beta} = -0.03$ g/day [95% CI: -0.11, 0.04], $t_{72} = -0.84$, $p = .401$), between enlarged and reduced broods ($\hat{\beta} = -0.04$ g/day [95% CI: -0.11, 0.03], $t_{72} = -1.16$, $p = .251$), or between reduced broods and control broods ($\hat{\beta} = 0.01$ g/day [95% CI: -0.05, 0.08], $t_{72} = -0.19$, $p = .853$). Overall, Violet-green Swallows grew slower than Tree Swallows across all treatments ($\hat{\beta} = -0.13$ g/day [95% CI: -0.22, -0.05], $t_{72} = -3.12$, $p = .002$; Figure 3).

Although fledging success did appear to decrease slightly in enlarged broods (Figure 2b; Table S1), we detected no differences in the proportion of nestlings that successfully fledged relative to experimental treatment ($F_{2,79} = 0.55$, $p = .759$) or species ($F_{1,79} = 0.40$, $p = .976$), with no species \times treatment interaction ($F_{2,79} = 0.00$, $p = .988$). When assessing the length of the nestling developmental period, we did not detect an effect of brood treatment ($F_{2,79} = 0.08$, $p = .91$), species ($F_{1,79} = 1.44$, $p = .160$) or a treatment \times species interaction ($F_{2,79} = 1.09$, $p = .341$).

For both species, baseline corticosterone did not appear to trade off with offspring growth rate across all treatments, as very little variance in growth was attributed to baseline corticosterone (Tree Swallow: $\hat{\beta} = -0.31$ [95% CI: -0.54, -0.04], $r^2 = .10$, $t_{49} = -2.29$, $p = .03$; Violet-green Swallow: $\hat{\beta} = -0.26$ [95% CI: -0.59, 0.09], $r^2 = .07$, $t_{32} = -1.52$, $p = .14$; Figure 4a). A similar relationship was observed between stressor-induced corticosterone and growth rate (Tree Swallow: $\hat{\beta} = -0.27$ [95% CI: -0.50, 0.01], $r^2 = .07$, $t_{49} = -1.93$, $p = .05$; Violet-green Swallow: $\hat{\beta} = -0.32$ [95% CI: -0.60, 0.01], $r^2 = .11$, $t_{32} = -1.97$, $p = .05$; Figure 4b).

3.3 | Adult provisioning rate

We did not detect an effect of treatment ($F_{2,37} = 0.47$, $p = .626$) or species ($F_{2,37} = 1.52$, $p = .224$) on per capita provisioning rate by adults, with no species \times treatment interaction ($F_{2,37} = 0.30$, $p = .740$). Provisioning rates were similar between enlarged broods and control broods ($\hat{\beta} = -0.5$ visits per nestling per hour [95% CI: -1.9, 0.7], $t_{37} = -0.85$, $p = .40$), between enlarged and reduced broods ($\hat{\beta} = -1.7$ visits per nestling per hour [95% CI: -3.7, 0.3], $t_{37} = 1.95$, $p = .059$), and between reduced broods and control broods ($\hat{\beta} = 1.18$ visits per nestling per hour [95% CI: -0.5, 2.8], $t_{37} = 1.44$, $p = .16$). Tree Swallows provisioned offspring at similar rates as Violet-green Swallows ($\hat{\beta} = 0.84$ visits per nestling per hour [95% CI: -3.4, 5.1], $t_{37} = 0.39$, $p = .70$).

4 | DISCUSSION

Our study provides three main findings that advance our understanding of how post-natal rearing environments influence glucocorticoids in developing young. First, we found that swallow nestlings in experimentally enlarged broods exhibited greater concentrations of baseline and stressor-induced corticosterone concentrations relative to young in smaller broods, a pattern that was found across both of our study species. Second, we found no evidence of a trade-off between either glucocorticoid measure and growth in either species

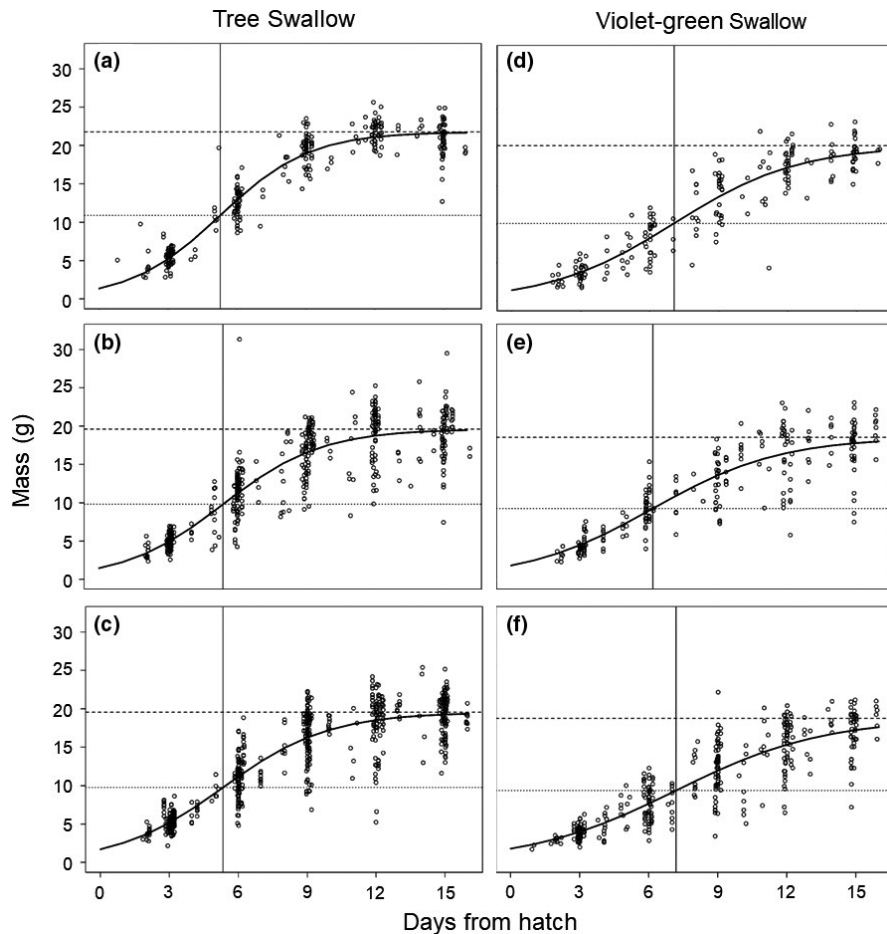


FIGURE 3 Mean logistic growth curves for each brood treatment × species combination. Tree Swallows grew faster than Violet-green Swallows in reduced broods (a, d), control broods (b, e), and enlarged broods (c, f). Dashed horizontal lines indicate asymptotic value, dotted horizontal lines indicate inflection point, and solid vertical lines indicate predicted age when nestlings reach half of total growth

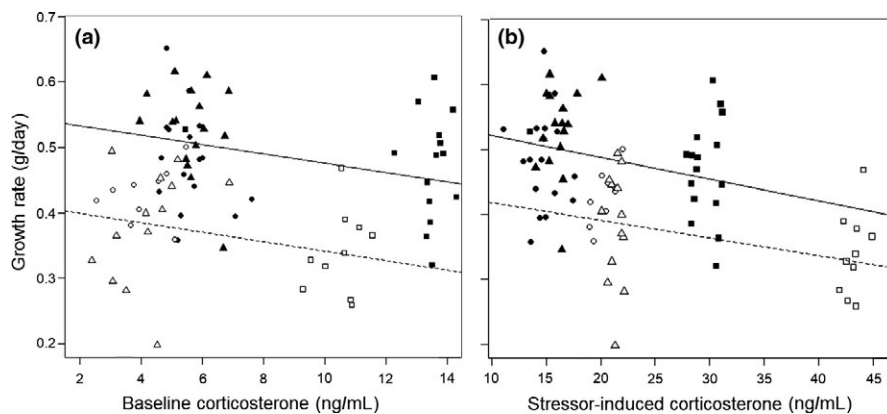


FIGURE 4 Relationship between measures of corticosterone and nestling growth rate for the Tree Swallow (filled symbols, solid line) and Violet-green Swallow (open symbols, dashed line). Circles indicate reduced broods, triangles indicate control broods, and squares indicate enlarged broods

or in any experimental brood treatment. Finally, the magnitude of the corticosterone stress response was greater for Violet-green Swallows relative to Tree Swallows, and this was consistent across experimental broods of different sizes.

4.1 | Corticosterone responses to experimental brood size manipulation

We found support for the hypothesis that brood size was linked to corticosterone function in developing nestlings, as both species

exhibited greater concentrations with an increasing number of nest-mate competitors. One explanation for this pattern is that baseline concentrations may have enhanced food intake, as elevated baseline corticosterone can facilitate begging and food consumption in birds (Loiseau et al., 2008; Sapolsky et al., 2000). Greater concentrations of baseline corticosterone of young in enlarged broods may have therefore helped maintain energetic homeostasis by augmenting nestling begging and food consumption in the presence of heightened competition. Thus, corticosterone may have allowed offspring to match their behavioural (e.g. begging) and/or physiological

processes (e.g. metabolism) to the rearing environments in which they were found (Schwabl & Lipar, 2002). Of note, brood enlargement did not lead to detectable differences in growth rate or survival among treatments, indicating that nestlings and/or adults responded in ways that maintained offspring condition. We were unable to address potential mechanisms behind this results directly in our study; however, so additional investigation of this topic is needed to parse out the relative contributions of parents and their offspring that promote offspring growth and survival.

Previous investigations evaluating the effects of brood size have reported mixed effects of brood enlargement on baseline corticosterone, so the effect of competition on glucocorticoid function may depend on the species under study. For example, corticosterone concentrations increased in experimentally enlarged broods of the Barn Swallow (*Hirundo rustica*; Saino et al., 2003; Vitousek et al., 2017), whereas no change was observed in other altricial species (Gil et al., 2008; Kozłowski & Ricklefs, 2011). It is worth noting that both studies finding an effect have been conducted on swallows (family Hirundinidae), a group that is particularly susceptible to cold weather that grounds their flying insect prey (Winkler, Luo, & Rakhimberdiev, 2013). Thus, it may be that glucocorticoids play an especially important role in mediating environmental challenges as they relate to food in members of this group, relative to other species that are not obligate aerial insectivores.

In addition to increases in baseline corticosterone, we also found that the response to an acute noxious stressor was enhanced when offspring experienced increased nestmate competition during development. Thus, elevated concentrations of stressor-induced corticosterone of nestlings in enlarged broods may have been a response to suboptimal food during post-natal development (Kitaysky et al., 1999; Love et al., 2003) that enhanced adaptive behavioural and physiological responses (Breuner et al., 2008; Sapolsky et al., 2000; Schwabl & Lipar, 2002). We suspect that this may have occurred in our study because adult provisioning did not vary across brood sizes. Nevertheless, these remain important questions to address, and additional work is needed to understand the consequences of altered glucocorticoid responses that arise from early rearing conditions characterized by large broods and heightened competition for food resources.

4.2 | Trade-offs between corticosterone and growth

We did not find support for our prediction that nestlings in larger broods would have reduced growth. This suggests that growth may be less variable and has a higher threshold for being negatively impacted by brood treatments, relative to glucocorticoids. Our results provide an interesting contrast with previous studies that have found decreasing growth rates in individuals with high concentrations of baseline corticosterone (Muller et al., 2009; Wada, Hahn, & Breuner, 2007; Wayne & Mason, 2008; Williams, Kitaysky, Kettle, & Buck, 2008). Unlike many other passerines, swallows must be able to fly upon leaving the nest, so there is likely strong selection pressure for them to allocate sufficient energy towards stable growth during their development. Furthermore, our results showed that although

growth rates remained constant despite elevated brood competition, corticosterone did appear to shift towards higher concentrations. Thus, from a fitness-based perspective, the hierarchy of how energy is used by nestlings seems to place optimizing growth above corticosterone function. Although we expected increased competition to have some negative effects on fledging success, we did not detect differences in the proportion of young surviving to fledge between treatments. Taken together with the lack of an effect of brood enlargement on growth, this indicates that nestlings of both species tolerated enlarged brood sizes that increased stress hormone concentrations without reducing growth or survival to fledgling. It also suggests that corticosterone may be an adaptive strategy for maintaining growth rate and surviving to fledge in swallows in response to competition within the nest.

Despite the many similarities shared by our two study species, Violet-green Swallows exhibited a slower growth rate than Tree Swallows across brood treatments. Growth is likely to be influenced, in part, by the thermal properties of broods, and it can trade off with the development of endothermy because of constraints on energy allocation (Andreasson, Nord, & Nilsson, 2016; Olson, 1992; Węgrzyn, 2013). Thus, the differences we observed between species could have been an endothermic consequence of size differences. Indeed, Violet-green Swallow nestlings were slightly smaller than Tree Swallow nestlings at the time we measured corticosterone (B. M. Hardt & J. W. Rivers, unpublished data), so they may have allocated more energy away from growth and towards endothermic development. Moreover, recent experiments suggest that *Tachycineta* swallow broods of reduced size undergo more rapid cooling than larger broods, particularly for small species (D. R. Ardia, unpublished data), so more work in this area will be valuable for a more complete understanding of how thermoregulatory needs influence nestling growth.

4.3 | Species-level differences in the corticosterone stress response

An unexpected outcome of our study was that the magnitude of the corticosterone stress response was consistently greater in Violet-green Swallows relative to Tree Swallows, a result that was consistent across brood treatments. This finding was surprising because both species are very similar in their life history and general ecology (Brown et al., 2011; Winkler et al., 2011). In addition, both species nest alongside one another, overlap in their foraging ranges and feed similar prey to offspring in our study population (Garlick et al., 2014; J. W. Rivers, unpublished data). One possible explanation for these differences relates to phylogenetic variation that has developed over evolutionary time. For example, the Violet-green Swallow is more closely related to the Caribbean clade of *Tachycineta* swallows than it is to the Tree Swallow (Cerasale, Dor, Winkler, & Lovette, 2012), so it may be that contemporary corticosterone responses have been shaped by evolutionary change that took place in a common ancestor living in a tropical location. Other potential explanations for the observed differences may

be related to species differences in wintering ranges, migratory movements, or annual survival. Unfortunately, very little is known about the ecology of the Violet-green Swallow, even on its breeding range, making it difficult to know with certainty which factor(s) may contribute to the differences we observed in the corticosterone stress response. What is clear, however, is that sympatric and ecologically similar congeneric songbirds can diverge in their glucocorticoid response as nestlings even when faced with identical rearing conditions. Therefore, researchers should use caution and not simply assume that offspring of closely related species will have concordant glucocorticoid responses, even when they share numerous similarities and nest within the same habitat.

Our study found that baseline and stressor-induced corticosterone responses during the nestling period were influenced by brood size, although the mechanism(s) responsible for this pattern remains unknown. Therefore, additional investigations should focus on testing how corticosterone varies within a setting that allows for controlling key factors, such as food intake and thermal conditions. We also found that neither baseline nor stressor-induced corticosterone traded off with growth when young encountered heightened sibling competition, and fledging success was uninfluenced by experimental changes to brood size. Thus, increased corticosterone concentrations in response to poor conditions may not always be an adverse outcome with negative consequences for fitness, as elevated glucocorticoid concentrations in recently fledged young have been linked to a positive effect on survival (Rivers et al., 2012), and some critical behavioural responses during the post-fledging period, such as begging, are facilitated by glucocorticoid actions (Schwabl & Lipar, 2002). Finally, given that corticosterone concentrations, rather than growth, were predictors of rearing environments in our study, corticosterone samples collected prior to fledging may serve as useful indicators of the relative rearing conditions that nestlings have faced during development, at least in aerial insectivores.

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CONFLICT OF INTEREST

The authors declare no conflict of interest as it pertains to this work.

AUTHORS' CONTRIBUTIONS

B.M.H. and J.W.R. conceived and designed the study. B.H.M. collected data and analysed samples with input from D.R.A. and M.J.B. B.M.H. performed statistical analysis with input from J.W.R. and D.R.A. B.M.H. and J.W.R. wrote the manuscript with input from D.R.A. and M.J.B.

DATA ACCESSIBILITY

Data associated with this paper have been deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.ms38c8k> (Hardt, Ardia, Bashaw, & Rivers, 2018).

ORCID

James W. Rivers  <http://orcid.org/0000-0001-5041-6002>

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SUPPORTING INFORMATION

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