

# On again, off again: Acute stress response and negative feedback together predict resilience to experimental challenges

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## Abstract

- Individuals often vary markedly in their ability to cope with stressors, but the drivers of this variation remain poorly understood. Many studies have tested relationships among individual variation in glucocorticoid levels and the response to challenges—often finding inconsistent patterns; however, few have addressed whether variation in the capacity to terminate the stress response through negative feedback is associated with stress resilience.
- While conceptual models predict that interactions among different components of hypothalamic–pituitary–adrenal (HPA) axis regulation may be important predictors of the phenotypic and fitness effects of stress, we are aware of no previous experimental tests of this hypothesis.
- We investigate whether individual variation in HPA axis regulation is related to resilience to experimental challenges in free-living tree swallows (*Tachycineta bicolor*). We mimicked salient natural challenges by temporarily reducing flight efficiency or increasing perceived predation risk during incubation, and determined whether HPA axis responsiveness prior to treatments predicted resilience.
- Females that exhibited both a robust HPA axis activation and strong negative feedback were less likely to abandon nests during incubation.
- Our results suggest that exhibiting a strong HPA axis activation coupled with effective negative feedback may predict stress resilience. Therefore, the ability to turn on and then off the HPA axis efficiently may be important for fitness.
- Our results also suggest that the interactions between different components of the HPA axis may provide greater insight into differences in stress coping capacity.

## KEY WORDS

dexamethasone, feather restraint, predation, stress-induced corticosterone, tree swallow

## 1 | INTRODUCTION

Adaptive behavioural flexibility in dynamic environments is well supported (Taff & Vitousek, 2016). Although behavioural responses are often assumed to be mediated by physiological processes, our knowledge about the underlying physiological mechanisms is

limited (Crossin, Love, Cooke, & Williams, 2016; Taff & Vitousek, 2016). Hormonal systems, which respond to environmental, internal and social conditions, are important mediators of phenotypic flexibility and life-history trade-offs (Taff & Vitousek, 2016; Zera & Harshman, 2001). Glucocorticoid hormones may be particularly central mediators of life-history trade-offs due to their role in

regulating homeostasis and metabolism, and their involvement in the response to stressful events (Romero, 2004; Sapolsky, Romero, & Munck, 2000; Wingfield et al., 1998). At low levels, glucocorticoids are mainly involved in regulating energy balance (Romero, 2002; Sapolsky et al., 2000). Circulating glucocorticoids increase dramatically in organisms facing challenges, mediating the response to and recovery from these challenges (Angelier & Wingfield, 2013; Sapolsky et al., 2000; Wingfield et al., 1998). Mounting a physiological stress response is crucial to coping with challenges; individuals that are unable to elevate glucocorticoids after exposure to a stressor often fail to mount an appropriate phenotypic response (Darlington, Chew, Ha, Keil, & Dallman, 1990; Thaker, Vanak, Lima, & Hews, 2010). Thus, individuals that show stronger HPA axis activity (e.g., higher circulating glucocorticoid levels) when facing major challenges are often predicted to have higher survival compared to those that mount a weaker response (Angelier & Wingfield, 2013; Wingfield et al., 2015). However, exposure to glucocorticoids can also be costly. Prolonged exposure can inflict phenotypic damage (Wingfield & Sapolsky, 2003), and even relatively short-term exposure can impair processes central to fitness, including reproductive behaviour (Wingfield & Sapolsky, 2003). Because elevated glucocorticoids suppress reproduction, it has been suggested to be adaptive for organisms engaged in high value reproductive attempts to reduce the acute glucocorticoid stress response (e.g., Bókony et al., 2009).

Although the known actions of glucocorticoids predict clear links with fitness, studies often find inconsistent relationships between natural variation in glucocorticoid levels and fitness (e.g., Breuner, Patterson, & Hahn, 2008; Bonier, Martin, Moore, & Wingfield, 2009). Some support has been found for a negative relationship between baseline glucocorticoids and fitness, but this pattern is highly inconsistent (Bonier et al., 2009), and likely context dependent (Love, Madliger, Bourgeon, Semeniuk, & Williams, 2014).

Previous studies looking at the consequences of variation in the acute glucocorticoid stress response on fitness have focused almost exclusively on its magnitude (e.g., Breuner et al., 2008; Bókony et al., 2009; Ouyang, Quetting, & Hau, 2012; Vitousek, Jenkins, & Safran, 2014). Individual differences in the ability to rapidly and effectively terminate the glucocorticoid stress response have received much less attention (but see, e.g., Baugh, Senft et al., 2017; MacDougall-Shackleton, Schmidt, Furlonger, & MacDougall-Shackleton, 2013; Romero & Wikelski, 2010). When the HPA axis is activated, negative feedback is coordinated by glucocorticoids binding to glucocorticoid (GR) and mineralocorticoid (MR) receptors particularly in the hippocampus, hypothalamus and pituitary gland resulting in a decrease in circulating glucocorticoids (Breuner & Orchinik, 2001; Romero, 2004). Thus, negative feedback, which has been largely neglected in free-living organisms, may be a critical aspect of the HPA axis regulation, and measuring its efficacy may reveal different patterns than studies measuring only the immediate glucocorticoid response to acute stress (Baugh, Davidson, Hau, & van Oers, 2017; Lattin, Breuner, & Michael Romero, 2016; Schmidt, Furlonger, Lapierre, MacDougall-Shackleton, & MacDougall-Shackleton, 2012). Some of the best evidence to date for causal effects of negative feedback

efficacy on fitness comes from work in Galapagos marine iguanas (*Amblyrhynchus cristatus*). Adult male marine iguanas with stronger negative feedback are less likely to die during El Niño-induced food shortages (Romero & Wikelski, 2010).

Here, we investigate whether individual variation in HPA axis regulation is related to stress resilience—defined as the ability to continue breeding efforts despite challenges—in a free-living population of tree swallows (*Tachycineta bicolor*) during breeding. During mid-incubation (day 6 or 7), we assessed individual variation in response to a standardized restraint stressor by measuring baseline and stress-induced corticosterone (CORT) levels, and negative feedback after injection with a synthetic glucocorticoid. Incubating females were then exposed to one of two treatments chosen to simulate particularly salient challenges for breeding tree swallows: increased foraging workload (as occurs during periods of low food availability: Winkler, Luo, & Rakhamberdiev, 2013) and elevated risk of predation. To temporarily increase foraging workload, we restrained feathers to reduce flight efficiency. Previous reductions in flight efficiency (via feather clipping) have been shown to reduce body mass and/or reproductive investment in this species (Ardia & Clotfelter, 2007; Madliger & Love, 2016; Patterson, Winkler, & Breuner, 2011; Winkler & Allen, 1995) to a similar extent to that which occurs during cold weather events (Winkler et al., 2013). To increase perceived predation risk, a predator mount was presented to incubating females inside the nest box. This treatment was intended to mimic a short predator encounter that could induce transient changes in glucocorticoid levels or other physiological mediators, or directly influence behaviour (Harris & Carr, 2016; Kavaliers & Choleris, 2001; Lima, 1998). Our measure of resilience was nest abandonment during incubation (the life-history stage during which stressors were manipulated). Because tree swallows in this population do not typically re-nest, nest abandonment is likely to have significant fitness consequences.

The overall goal of our study was to determine whether aspects of HPA axis function predict stress resilience. We predicted that individual variation in response to the standardized restraint stressor would be associated with the response to experimental challenges because HPA axis activity is relatively consistent within individuals over time (e.g., Baugh, Davidson et al., 2017; Taff, Schoenle, & Vitousek, 2018), including in this population (Vitousek, Taff, Hallinger, Zimmer, & Winkler, 2018) and can predict the behavioural response to natural challenges (e.g., Vitousek et al., 2014). We predicted that (a) females that respond to a standardized restraint stressor with a greater elevation in CORT would be more strongly impacted by experimental challenges, and thus have a higher probability of abandoning nests during incubation. Alternatively, if acute elevations in glucocorticoids enable more effective coping, a greater increase in CORT following exposure to a standardized restraint stressor could be associated with greater stress resilience. Because the ability to rapidly terminate the stress response may be important when facing ongoing challenges (Romero & Wikelski, 2010; Zimmer, Larriva, Boogert, & Spencer, 2017), we predicted that (b) females with stronger negative feedback (lower CORT levels following a dexamethasone challenge) would be more resilient to experimental

challenges and show a lower probability of abandonment. We also predicted that (c) individual variation in baseline CORT levels, when considered alone, would be poorer predictors of nest abandonment than measures of the glucocorticoid stress response (stress-induced CORT and/or negative feedback efficacy).

Although conceptual models suggest that interactions among components of the glucocorticoid stress response may be important determinants of the effects of stress, such interactions have rarely been examined. Based on both previous predictions and correlational work (e.g., Breuner et al., 2008), we predicted that (d) stress resilience would be positively predicted by having either (a) low baseline CORT levels and high stress-induced CORT levels, or (b) a robust stress response and strong negative feedback.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling and stress manipulation

The experiment was conducted in female tree swallows breeding in nest boxes at two field sites in Ithaca, New York (~42°29'N, 76°27'W) between April and July 2016. Nest boxes were checked at least every other day from the initiation of activity at the site until nestling day 12 to record nest building, clutch initiation and nest abandonment or failure. We were unable to test treatment effects on nestlings because a bout of unusually cold weather caused widespread nestling mortality (only 11 of 58 focal nests successfully fledged any young).

Females were caught in their nest boxes on incubation day 6 or 7 by hand, or using a manually activated trap door installed in the box. Captures occurred between 07:00 and 10:00 to minimize variation in circulating glucocorticoid hormones due to circadian rhythms. A baseline blood sample was taken (~70 µl) within 3 min of disturbance to measure baseline circulating CORT levels. Body mass, head-bill length, and flattened wing length were measured, and females received USGS leg bands. Birds were placed in a cloth bag until the stress-induced CORT sample (~30 µl) was taken 30 min after initial disturbance (see Figure S1 in Supporting Information for a comparison of CORT levels 30 vs. 60 min post-capture). Females were then injected intramuscularly with the synthetic glucocorticoid dexamethasone (0.5 µl/g; Dexamethasone Sodium Phosphate, 4 µg/µl; equivalent to 3 µg/µl dexamethasone; Mylan Institutional LLC, Rockford, IL, USA; Taff, Zimmer, & Vitousek, 2018). Dexamethasone binds to GRs, and with a lower affinity to MRs, reducing the endogenous release of CORT by stimulating negative feedback within the HPA axis. Then, a third blood sample (~30 µl) was taken 30 min after the injection to measure the individual's ability to suppress CORT output via negative feedback (post-dex CORT level). On incubation day 12 or 13, all females were recaptured (except those that had abandoned) to collect a baseline blood sample and measure body mass. At all sampling events blood samples were taken by brachial venipuncture; blood was collected in heparinized microhematocrit capillary tubes, and then transferred to

microcentrifuge tubes and kept on ice until centrifugation (within 4 hr). After separation, the plasma was stored at -30°C until analysis.

Females were randomly assigned to treatments prior to capture. Two treatments manipulated locomotion costs in order to mimic the physiological effects of a cold weather event—when few flying insects, the primary food source of tree swallows, are available. In the Low Feather Restraint group (Low FR:  $n = 13$ ), primary feathers 4, 5 and 6 were attached using miniature zip ties. In the High Feather Restraint group (High FR:  $n = 12$ ), two bundles of primary feathers were attached: 2, 3 and 4, and 6, 7 and 8 (Figure 1). After 5 to 6 full days of treatment, birds were recaptured and the zip ties were removed allowing females to regain their original flight efficiency. In a separate group, we manipulated predation risk ( $n = 12$ ) by exposing females to a mink (*Neovison vison*; a common predator of juvenile and adult tree swallows) mount once per day for 5 days, starting the day after initial capture. At a random time during the day when the female was known to be incubating, we simulated a predation attempt by inserting the mink mount into the nest box through the entrance hole for 10 s. Control females ( $n = 14$ ) were subjected to the same capture and handling procedures (blood samples, weighing, body measures, banding) but were not exposed to experimental stressors.

### 2.2 | Dexamethasone injection validation

Between May and July 2018, 25 birds were used to validate the effectiveness of dexamethasone injection in female tree swallows. Blood samples (~40 µl) were collected between 15:30 and 18:30 on incubation day 6 or 7. Following the stress-induced bleed (30 min post-capture), birds were either injected with dexamethasone as described above ( $n = 13$ ) or with an equivalent volume of saline solution ( $n = 12$ ); post-injection blood samples were collected 30 min later. All methods were approved by Cornell IACUC and conducted with appropriate state and federal permits.



**FIGURE 1** Female tree swallow from the High Feather Restraint group. Primary feathers 2–4 and 6–8 are restrained with miniature zip ties

## 2.3 | Corticosterone assay

Plasma samples were extracted using a triple ethyl acetate extraction and assayed using a commercial corticosterone enzyme immunoassay (EIA) kit (DetectX Corticosterone, Arbor Assays: K014-H5, Ann Arbor, MI, USA). This kit was chosen because of its low level of cross-reactivity of its antibody with dexamethasone (0.12%), and has been validated for measuring plasma corticosterone in tree swallows (Taff, Zimmer, & Vitousek, 2019). All samples were run in duplicate alongside a 9-point standard curve. In total, we ran 18 assays with all samples of an individual in the same assay and with samples of the different treatments randomized across plates. The average extraction efficiency was 93.3% and the detection limit 0.47 ng/ml. The inter-assay variation was 5.7% and the intra-assay variation 10.6%.

## 2.4 | Data analysis

We first examined the main effects of our treatments on incubation abandonment, and initial adult phenotype using generalized linear models (GLMs). We also looked at how corticosterone levels changed over time after capture using a generalized linear mixed model (GLMM) fitted with a gamma distribution. Treatment, sample type, and their interaction were fixed factors, and individual was a random factor. We next asked which aspects of the glucocorticoid stress response predicted stress resilience. With incubation abandonment as the response variable, we fitted a set of binomial GLMs. Best-fit models were identified by comparing the corrected Akaike information criterion ( $AIC_c$ ) scores of the candidate models. The models included combinations of the variables clutch initiation day, clutch size, body mass at first capture, treatment, baseline CORT concentration at first capture, stress-induced CORT concentration and post-dex CORT concentration. All CORT levels included in models were raw, untransformed concentrations. Pre-treatment baseline CORT levels were not correlated with pre-treatment stress-induced CORT ( $R^2 = 0.001, p = 0.79$ ) or with pre-treatment post-dex CORT ( $R^2 = 0.014, p = 0.41$ ). Stress-induced and post-dex CORT were moderately positively correlated ( $R^2 = 0.29, p = 0.0002$ ). To test whether stressor type influenced the predictors of stress resilience we constructed models that included interactions between treatment and each of the three components of HPA axis activity we measured (Table 1). Finally, to take into account the possibility that the response to stress is influenced by a combination of aspects of HPA axis activity, we constructed models that included two-way interactions between the measured components of HPA axis activity (Table 1).

In birds that completed incubation, and thus were captured a second time, we tested whether the treatments affected body mass and baseline CORT at the second capture. We used GLMMs to model body mass and baseline CORT changes between the two captures. Both models were fitted with a gamma distribution as the residuals of the normal models were not normally distributed.

Data from the dexamethasone validation experiment were analyzed using a GLMM of the difference between stress-induced and post-injection CORT. Treatment, sample type and their interaction

were fixed factors and individual identity a random effect. Post-injection CORT was further examined using a GLM fitted with a gamma distribution, with treatment, stress-induced CORT, and their interaction as fixed effects.

Generalized linear models were run using the GENMOD procedure and GLMMs were run using the GLIMMIX procedure in SAS University Edition (SAS Institute Inc., Cary, NC, USA). Post-hoc comparisons for treatment effect were performed using Tukey-Kramer multiple comparison adjustment to obtain corrected  $p$ -values. Probability levels  $<0.05$  were considered significant.

## 3 | RESULTS

Treatment groups did not differ in clutch initiation day ( $\chi^2_{3,51} = 1.69, p = 0.64$ ), clutch size ( $\chi^2_{3,51} = 0.35, p = 0.95$ ) or HPA axis function at initial capture (baseline:  $\chi^2_{3,51} = 3.37, p = 0.34$ , stress-induced:  $\chi^2_{3,51} = 6.11, p = 0.11$  and post-dex CORT levels:  $\chi^2_{3,51} = 2.95, p = 0.40$ ). Although females were randomly allocated to treatments at first capture, body mass at initial capture differed by treatment ( $\chi^2_{3,51} = 13.00, p = 0.005$ ). None of the treatment groups differed significantly in body mass from controls (Control:  $21.35 \pm 0.47$  g; Predator:  $21.39 \pm 0.38$  g), but the body mass of High Feather Restraint (High FR) females ( $22.55 \pm 0.29$  g) was significantly higher than that of Low Feather Restraint (Low FR) females at initial capture ( $20.62 \pm 0.25$  g;  $z = 3.81, p = 0.0008$ ). Corticosterone level significantly changed over time after capture ( $F_{2,102} = 322.13, p < 0.0001$ ). Corticosterone levels increased between baseline and stress-induced samples ( $t = 25.18, df = 102, p < 0.0001$ ; Figure 2). Dexamethasone injection effectively decreased CORT levels (stress-induced CORT vs. post-dex CORT:  $t = 9.85, df = 102, p < 0.0001$ , Figure 2), but CORT levels post-dex remained higher than baseline CORT levels ( $t = 15.33, df = 102, p < 0.0001$ , Figure 2). Baseline ( $\chi^2_{1,51} = 0.32, p = 0.57$ ), stress-induced ( $\chi^2_{1,51} = 2.40, p = 0.12$ ) and post-dex ( $\chi^2_{1,51} = 0.02, p = 0.89$ ) CORT levels were not related to the time of day.

Experimental stressor treatment influenced the likelihood of nest abandonment during incubation ( $\chi^2_{3,51} = 11.78, p = 0.008$ ). Females from the High FR group (66.7%, 8 of 12) abandoned nests at higher rates than Control females (14.3%, 2 of 14) ( $z = 2.59, p = 0.047$ ). Females from the Low FR (30.8%, 4 of 13) and Predator treatments (16.7%, 2 of 12) showed similar rates of abandonment to the Control group (14.3%, 2 of 14) ( $z \leq 2.38, p \geq 0.08$ ).

We fitted a different model set to explain nest abandonment as a function of treatment and HPA axis function (Table 1). When comparing these models using  $AIC_c$  scores, there was strong support for the model that included treatment, clutch initiation day, the interaction between stress-induced and post-dex CORT levels and their main effects (model relative weight = 0.60, Table 1). No other model in the set received substantial support (relative weight of second-ranked model: 0.11, Table 1). In the best-fit model, the probability of abandoning the nest was predicted by the interaction between stress-induced and post-dex CORT concentrations (Table 2); females

**TABLE 1** Candidate model set for incubation abandonment

Candidate models	N, df	LL	K	$\Delta AIC_c$	Weight
Treatment + Stress-induced CORT + Post-dex CORT + Stress-induced CORT × Post-dex CORT + Clutch initiation	51, 7	-17.4	9	0	0.60
Treatment + Stress-induced CORT + Clutch initiation	51, 5	-20.1	7	3.4	0.11
Treatment + Stress-induced CORT + Post-dex CORT + Clutch initiation	51, 6	-18.9	8	3.5	0.11
Stress-induced CORT + Clutch initiation	51, 2	-24.5	3	4.6	0.06
Treatment + Baseline 1 + Stress-induced CORT + Clutch initiation	51, 6	-19.8	8	5.3	0.04
Treatment + Clutch initiation	51, 4	-23.2	6	6.9	0.02
Treatment + Baseline 1 + Clutch initiation	51, 5	-22.7	7	7.4	0.01
Treatment + Stress-induced CORT + Treatment × Stress-induced CORT + Clutch initiation	51, 6	-17.9	11	7.4	0.01
Treatment + Baseline 1 + Stress-induced CORT + Baseline 1 × Stress-induced CORT + Clutch initiation	51, 7	-19.8	9	8.1	0.01
Stress-induced CORT	51, 1	-27.7	2	8.8	0.01
Treatment + Post-dex CORT + Clutch initiation	51, 5	-23.2	7	9.4	0.01
Treatment + Baseline 1 + Post-dex CORT + Clutch initiation	51, 6	-22.7	9	11.1	0.00
Clutch initiation	51, 1	-29.1	2	11.6	0.00
Treatment	51, 3	-26.8	5	11.6	0.00
Treatment + Post-dex CORT + Treatment × Post-dex CORT + Clutch initiation	51, 6	-20.2	11	11.9	0.00
Treatment + Baseline 1 + Treatment × Baseline 1 + Clutch initiation	51, 6	-20.7	11	12.9	0.00
Post-dex CORT + Clutch initiation	51, 2	-29.0	3	13.6	0.00
Treatment + Baseline 1 + Post-dex CORT + Baseline 1 × Post-dex CORT + Clutch initiation	51, 7	-22.5	9	13.6	0.00
Baseline 1 + Clutch initiation	51, 2	-29.0	3	13.7	0.00
Null model	51, 1	-31.7	1	14.7	0.00
Body mass	51, 1	-30.8	2	14.9	0.00
Baseline 1	51, 1	-31.5	2	16.4	0.00
Post-dex CORT	51, 1	-31.6	2	16.5	0.00
Clutch size	51, 1	-31.7	2	16.8	0.00

Note. CORT: corticosterone.

that exhibited both higher stress-induced and lower post-dex CORT levels were less likely to abandon their nest (Figure 3).

In females that did not abandon, the interaction between treatment and capture number significantly predicted body mass ( $F_{3,34.5} = 3.30, p = 0.032$ ). Body mass at first capture (pre-treatment) did not differ by treatment among females that completed incubation ( $t \leq 0.87, df = 59, p \geq 0.98$ , Figure 3). Body mass significantly decreased between the first and the second captures in females in the High FR group ( $t = 3.05, df = 34, p = 0.021$ , Figure 4), but did not significantly change in the other groups ( $t \leq 1.18, df = 34, p \geq 0.25$ , Figure 3). At the second capture, body mass of females in the High FR groups was lower than females in the Control group ( $t = 2.53, df = 59, p = 0.041$ ) but did not differ from females in the Low FR and Predator groups ( $t \leq 1.81, df = 59, p \geq 0.15$ , Figure 4). Body mass of females in the Low FR and Predator groups did not differ from Control females at second capture ( $t \leq 1.34, df = 59, p \geq 0.19$ , Figure 4). Baseline CORT was not significantly predicted by treatment ( $F_{3,70} = 1.80, p = 0.16$ ), capture number ( $F_{1,70} = 0.72, p = 0.40$ ) or their interaction ( $F_{3,70} = 0.36, p = 0.78$ ).

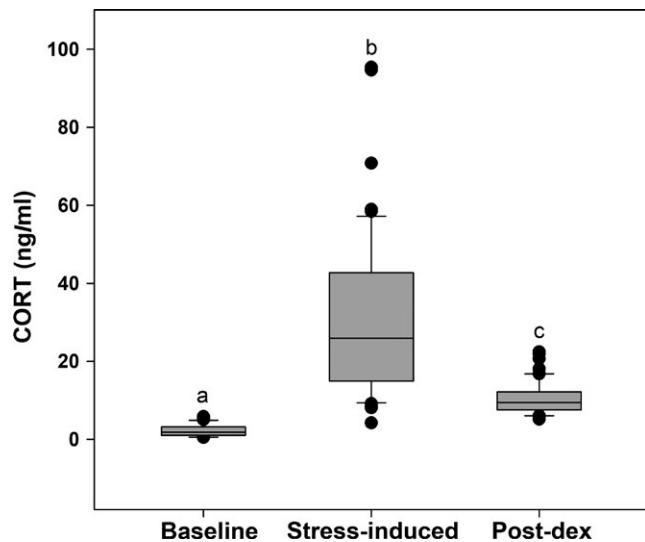
In the dexamethasone validation experiment, dexamethasone-treated but not saline-injected birds significantly reduced their

CORT in the 30 min following injection (GLMM: treatment × sample type:  $F_{1,25} = 5.81, p = 0.024$ ; multiple comparisons: dexamethasone:  $t = 6.20, df = 25, p < 0.0001$ , saline:  $t = 2.62, df = 25, p = 0.067$ ; Supporting Information Figure S1). Multiple comparisons indicated that mean post-injection CORT levels did not differ significantly between treatments ( $t = 1.32, df = 41, p = 0.56$ ). However, GLMs revealed that post-injection CORT was significantly affected by the interaction between treatment and stress-induced CORT ( $\chi^2_{121} = 13.25, p = 0.0003$ ; Figure 5). In saline-injected birds, post-injection CORT was positively predicted by stress-induced CORT, but in dexamethasone-treated birds, post-dex CORT was independent of stress-induced CORT levels.

## 4 | DISCUSSION

### 4.1 | Endocrine predictors of stress resilience

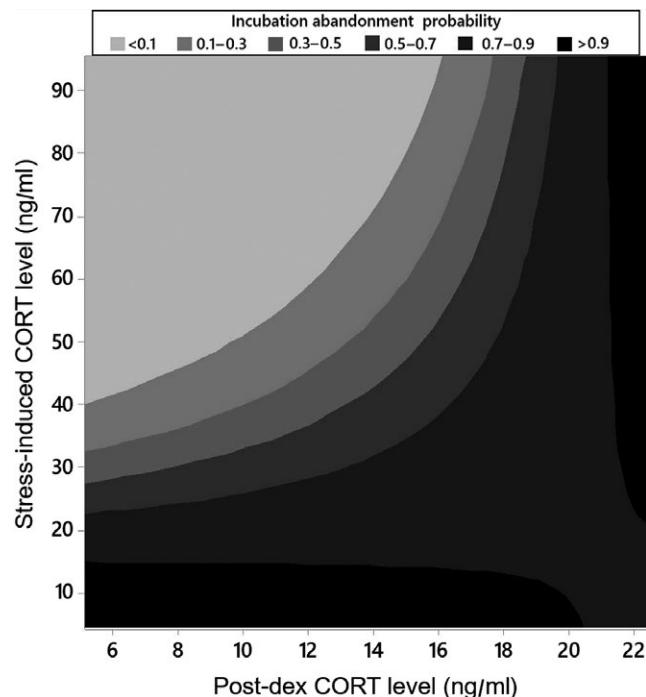
Stress resilience, measured here as the ability to continue reproductive effort despite experimental challenges, was predicted by individual variation in stress physiology in tree swallows. The likelihood that females would abandon nests during incubation was best predicted



**FIGURE 2** Mean ( $\pm$ SE) pre-treatment corticosterone levels in baseline, stress-induced and post-dexamethasone samples. Different letters indicate significant differences

by the interaction between stress-induced and post-dexamethasone corticosterone concentrations. Female tree swallows that showed both higher corticosterone levels following a standardized capture and restraint stressor, and lower corticosterone concentrations following dexamethasone injections (stronger negative feedback), were less likely to abandon. Nest abandonment was higher in females with significantly reduced flight efficiency (High Feather Restraint group), suggesting that individual differences in stress physiology likely have greater fitness consequences under challenging conditions.

Abandoning the nest is a major reproductive decision. There is evidence that glucocorticoids are involved in this reproductive decision: studies in other bird species have found that individuals with naturally higher baseline corticosterone concentrations (Lotherry, Thompson, Lawler, & Sakaluk, 2014; Love et al., 2014) or experimentally elevated corticosterone (Silverin, 1986; Spée et al., 2011) are more likely to abandon. Higher stress-induced corticosterone levels during capture and restraint have also been associated with an increased likelihood of abandoning the nest immediately after release



**FIGURE 3** Contour plot for nest abandonment probability during incubation relative to stress-induced and post-dex corticosterone (CORT) levels. Darker shades denote increasing probability of nest abandonment

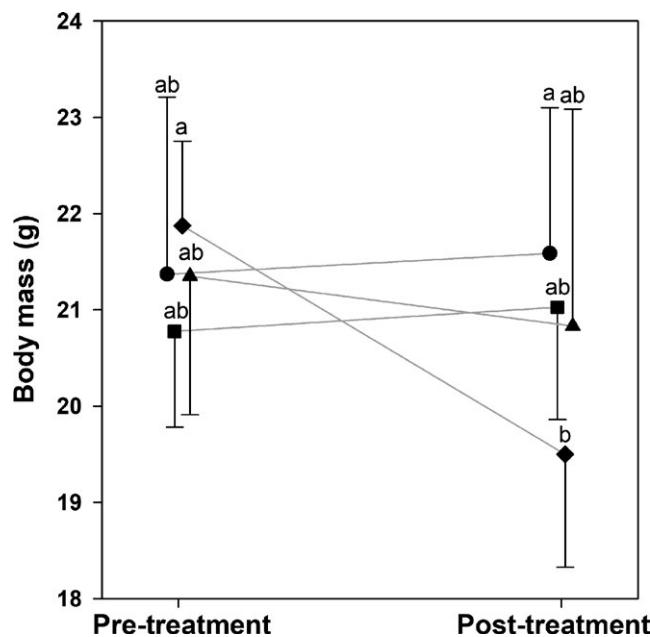
(Ouyang et al., 2012). Here, in accordance with our initial prediction, we found that individuals with high stress-induced corticosterone levels were less likely to abandon—as long as they also had strong negative feedback. This suggests that a robust corticosterone increase followed by a quick induction of negative feedback—which may facilitate an effective response to the challenge while avoiding negative effects of sustained corticosterone elevation—may be adaptive, at least under these conditions (sensu Breuner et al., 2008). To our knowledge, our study is the first to report a link between negative feedback efficacy and a proxy of reproductive fitness.

The observed relationships between HPA activity and fitness could have at least two functional explanations. First, abandonment

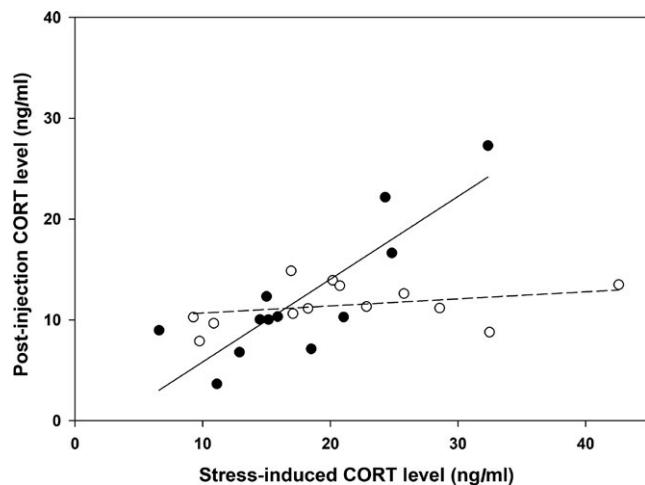
**TABLE 2** Best-fit model for incubation abandonment

Parameter	Estimate	SE	Confidence interval	N, df	$\chi^2$	P
Intercept	25.76	10.56	5.07 to 46.45	51, 1	5.95	0.015
Treatment: High FR	-2.76	1.34	-5.38 to -0.14	51, 1	4.28	0.039
Treatment: Low FR	-0.55	1.33	-3.16 to 2.06	51, 1	0.17	0.678
Treatment: Predator	1.48	1.58	-1.62 to 4.59	51, 1	0.88	0.349
Stress-induced CORT (Str)	-0.23	0.10	0.03 to 0.43	51, 1	7.01	0.008
Post-dex CORT (Dex)	-0.17	0.22	-0.27 to 0.001	51, 1	0.55	0.457
Str × Dex	0.01	0.01	-0.023 to -0.002	51, 1	3.36	0.062
Clutch initiation date	0.21	0.08	-0.37 to -0.06	51, 1	7.12	0.008

Note. GLM with binomial distribution and logit link function. The Control treatment is coded as the reference value.  
CORT: corticosterone; FR: feather restraint.



**FIGURE 4** Mean ( $\pm$ SE) body mass before and after treatment in females in the Control group (circles), the Low Feather Restraint group (squares), the High Feather Restraint group (diamonds) and the Predator group (triangles). Different letters indicate significant differences



**FIGURE 5** The relationship between stress-induced and post-injection corticosterone levels in incubating females injected with saline solution (closed circles) or dexamethasone (open circles)

may be primarily driven by the duration of corticosterone elevation; females that had stronger negative feedback would have had a shorter stress response, and may thus have recovered faster, and been less likely to abandon nests. In this case, the significant interaction with stress-induced corticosterone could be simply an artifact of this measure being positively correlated with the strength of negative feedback. The acute stress response and negative feedback may be functionally linked, as negative feedback is induced by corticosterone binding to GR, and to a lesser extent to MR (de Kloet, Vreugdenhil, Oitzl, & Joëls, 1998; Smulders, 2017). Consequently,

higher corticosterone release in response to a stressor activates more receptors (Breuner & Orchinik, 2001), which can result in a faster induction of negative feedback and lower post-dex corticosterone levels (de Kloet et al., 1998; Liebl, Shimizu, & Martin, 2013; Romero, 2004). We do see this correlation between stress-induced and post-dex corticosterone levels in our population but it is moderate, suggesting that this mechanistic relationship could be broken downstream by differential receptor or transporter expression. A second (non-mutually exclusive) possibility is that low stress-induced CORT levels promote abandonment, as low levels do not enable birds to cope effectively with immediate challenges imposed by the stressor. Although higher stress responses are more commonly predicted to promote investment in survival over reproductive success (Wingfield et al., 1998; Patterson, Hahn, Cornelius, & Breuner, 2014), mounting a more effective immediate stress response—particularly when coupled with robust negative feedback—could improve stress resilience in ways that promote reproductive success (Krause et al., 2016). Therefore, our results suggest that (a) mounting a strong stress response may in some cases be positively associated with reproductive success, and (b) the ability to efficiently turn on and then turn off the HPA axis is important for coping with challenges.

These results suggest that measurements of negative feedback efficacy may be important for resolving several remaining questions. If individual variation in stress-induced corticosterone is positively correlated with the strength of negative feedback (as suggested by previous investigations that used similar measures of HPA activity (e.g., Schmidt et al., 2012; Baugh, Senft et al., 2017))—then correlations between stress-induced glucocorticoids and survival or stress coping capacity could be explained at least in part by stronger stress responders also having stronger negative feedback. Individual variation in measures of HPA axis activity could also reflect downstream variation in receptor densities within brain areas involved in negative feedback regulation. Our results also highlight that interactions among different aspects of the HPA axis (i.e., stress response and negative feedback) may influence stress resilience and life-history investment. Similarly, recent analyses of non-manipulated individuals in this population indicate that reproductive success is best predicted by interactions among different components of HPA axis activity (Vitousek et al., 2018). Taken together, these results imply that exploring only the main effects of baseline and/or stress-induced corticosterone levels may not be sufficient to characterize HPA axis reactivity. It also suggests that negative feedback may be critical for coping with challenges and regulating reproductive investment under challenging conditions.

Here, we characterized the stress response using circulating corticosterone levels. However, the hormonal stress response is an intricate system that involves different levels of regulation. Thus, HPA axis reactivity could be adjusted via modification of downstream components such as the levels of the transporter protein corticosteroid-binding globulin, 11 $\beta$ -hydroxysteroid dehydrogenase 1 and 2 (an enzyme that mediates the interconversion of corticosterone to a less active metabolite), and receptor expression

(Breuner, Delehanty, & Boonstra, 2013; Breuner & Orchinik, 2001). Negative feedback is coordinated by GR and MR in the hypothalamus, hippocampus and pituitary gland (de Kloet et al., 1998; Smulders, 2017), and differential expression of these receptors has been associated with changes in negative feedback efficacy (de Kloet et al., 1998; Zimmer & Spencer, 2014). As it seems likely that the differences in hormonal profiles covary with differences in receptor numbers, measuring receptor expression is an important future direction.

## 4.2 | The impact of experimental stressors

Our treatments were designed to mimic salient challenges for breeding tree swallows: cold weather and predation risk. During cold weather events, the availability of flying insects decreases drastically (Winkler et al., 2013), forcing tree swallows to spend more effort to obtain sufficient food. The high FR treatment appeared to increase the cost of foraging to a level similar to that seen during naturally occurring cold snaps (or from feather clipping experiments: Winkler & Allen, 1995; Ardia & Clotfelter, 2007; Madliger & Love, 2016). The reduction in body mass seen over the treatment interval is similar to that seen in tree swallows facing naturally occurring periods of cold weather (M. N. Vitousek, unpublished data). This treatment also increased abandonment rates, as is seen during naturally occurring cold snaps (Winkler et al., 2013). The Low FR and Predator treatments did not differ significantly from Controls in abandonment rate, and showed no change in body mass.

Experimental treatments did not significantly predict baseline corticosterone levels at recapture. Previous work in this population has found that baseline corticosterone levels are higher under poor conditions; thus, we predicted elevated baseline corticosterone in the High FR group. However, previous feather clipping experiments in tree swallows showed inconsistent effects on baseline corticosterone (decrease: Patterson et al., 2011, increased: Madliger & Love, 2016). It is possible that in this study, low sample sizes at recapture, particularly in the High FR group, may have prevented detection of a change in baseline corticosterone. We also cannot rule out that the females that abandoned had higher baseline corticosterone levels. Experimentally, elevating the perceived risk of predation in breeding birds does in some cases result in an increase in circulating corticosterone (Clinchy, Zanette, Boonstra, Wingfield, & Smith, 2004; Sheriff, Krebs, & Boonstra, 2009); however, both the short- and long-term effects of predators on glucocorticoids appear to be highly variable (Harris & Carr, 2016). Despite the high level of risk simulated by each encounter, it is possible that our protocol did not elevate perceived predation risk over longer time-scales because of the low frequency and duration of these encounters. Alternatively, our protocol may have increased perceived predation risk without affecting HPA axis activity and reproductive investment, as the costs associated with the response to predation risk do not increase proportionally with increasing risk (LaManna & Martin, 2016).

## 4.3 | Dexamethasone injections

The dexamethasone validation experiments revealed an interesting context-dependency between treatment and stress-induced CORT. Among saline-injected birds, post-injection CORT levels were strongly and positively predicted by stress-induced CORT levels. In contrast, among dexamethasone-treated birds, post-injection CORT levels were unrelated to stress-induced CORT levels. Thus, dexamethasone treatment may only be necessary for maximally stimulating negative feedback in naturally strong stress responders. Overall, these results suggest that dexamethasone treatment enables measuring negative feedback in a standardized way across individuals with varying degrees of CORT stress responsiveness.

## 5 | CONCLUSIONS

Our results suggest that the ability to both mount a strong acute stress response and shut it down effectively is crucial for appropriately dealing with challenges and for regulating investment in reproduction. Although most studies consider different aspects of HPA axis function independently, interactions among them may be important determinants of individual differences in stress coping capacity. As relationships between glucocorticoids and fitness are complex and context-dependent, optimal HPA axis regulation will likely differ across environmental contexts and life-history stages, as well as between individuals. Future research on the relationships among different components of HPA axis regulation and stress resilience across a diversity of contexts and environmental gradients could help to reveal how phenotypic variation influences resilience in the face of stress.

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## AUTHORS' CONTRIBUTION

M.N.V. conceived of the study. M.N.V., C.Z., C.C.T., D.R.A. and D.W.W. designed the study. C.C.T., C.Z. and M.N.V. collected data. C.Z. and C.C.T. assayed hormones. C.Z. analyzed data and wrote the manuscript with feedback from the other authors.

## DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.5mr10r5> (Zimmer et al., 2019)

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