Received: 3 April 2022 | Accepted: 29 June 2022

DOI: 10.1111/1365-2435.14144

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Natural and experimental cold exposure in adulthood increase the sensitivity to future stressors in a free-living songbird

Maren N. Vitousek[1,2] | Jennifer L. Houtz[1,2] | Monique A. Pipkin[1,2] | David A. Chang van Oordt[1,2] | Kelly K. Hallinger[1,2,3] | Jennifer J. Uehling[1,2] | Cedric Zimmer[1,4] | Conor C. Taff[1,2]

[1]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA

[2]Cornell Lab of Ornithology, Ithaca, NY, USA

[3]Department of Biology, Albion College, Albion, MI, USA

[4]Laboratoire d'Ethologie Expérimentale et Comparée, LEEC, Université Sorbonne Paris Nord, Villetaneuse, France

Correspondence
Maren N. Vitousek
Email: mnv6@cornell.edu

Funding information
Defense Advanced Research Projects Agency, Grant/Award Number: D17AP00033; National Science Foundation, Grant/Award Number: IOS-1457251 and IOS-2128337; U.S. Department of Agriculture, Grant/Award Number: NIFA Hatch 1017321

Handling Editor: Frédéric Angelier

Abstract

1. As the global climate shifts, many species are imperilled by changing thermal regimes. Despite rising global temperatures, some populations must contend with more frequent or extreme cold. In these populations, the ability to cope with cold may be an important determinant of fitness.
2. Experiments in captive animals have shown that extreme cold or rapid temperature declines typically elicit an increase in glucocorticoid hormones (mediators of the stress response); however, it is not known whether free-living adults, which may be better at buffering the effects of cold, show a similar response to ecologically relevant thermal challenges. The effects of cold on the sensitivity to future challenges are also poorly understood.
3. Using targeted manipulations of nest temperature in free-living tree swallows *Tachycineta bicolor* and a long-term dataset (2,888 samples; 8 years), we tested the relationship between cold exposure and glucocorticoid levels in adults.
4. Both natural and experimental cold exposure altered glucocorticoid regulation. This manifested primarily in terms of an upregulation in the sensitivity to future challenges (stress-induced levels). Experimental cold exposure also increased the initial speed and duration of the acute stress response. Analyses of the long-term dataset found that baseline glucocorticoids were higher in cold conditions; however, the magnitude of this effect was weak. A sliding window analysis revealed differences in the timeline over which temperature best predicted baseline and stress-induced glucocorticoids.
5. Although unpredictability is often regarded as a defining factor of stressors, we found that recent ambient temperatures better predicted circulating corticosterone than measures of thermal unpredictability, including the degree of recent temperature change and negative prediction error (the difference between experienced temperatures and long-term averages).
6. Together, these results suggest that thermally induced increases in the sensitivity to future challenges may prime individuals to respond more strongly or rapidly to worsening conditions, or to subsequent challenges of a different type,

without incurring the costs that can result from substantial elevations in baseline glucocorticoids. This kind of preparatory response could be adaptive; however, an increased sensitivity to other challenges could also represent an overlooked cost of changing thermal regimes.

KEYWORDS
climate change, cold exposure, corticosterone, glucocorticoids, stress, thermal physiology, tree swallow

1 | INTRODUCTION

As our global climate shifts, many species are imperilled by changing thermal regimes (e.g. Andreasson et al., 2020; Conradie et al., 2019; Riddell et al., 2019). Despite the rise in global temperatures, some populations must also contend with more frequent or extreme cold; this can result from increased environmental variability or from climate-induced shifts in distribution or the timing of breeding (e.g. Burggren, 2018; La Sorte et al., 2021; Shipley et al., 2020). In these populations, the ability to cope effectively with low temperatures is likely to be an important determinant of fitness.

Vertebrates respond to a diversity of challenges by initiating a glucocorticoid stress response. This response triggers a suite of phenotypic changes, some of which are particularly important for coping with thermal challenges, including modifications to metabolism, thermoregulatory capacity and foraging behaviour (Astheimer et al., 1992; Breuner & Wingfield, 2000; Jimeno et al., 2018; Ruuskanen et al., 2021; Sapolsky et al., 2000; Wingfield & Ramenofsky, 2011). Experiments in captive animals, including birds, have shown that extreme cold or rapid declines in temperature typically elicit an immediate glucocorticoid stress response (de Bruijn & Romero, 2018; Jessop et al., 2016). To our knowledge, similar experiments have not been done in free-living vertebrates, which may be better at buffering the effects of cold through other means (e.g. behavioural thermoregulation). But observational studies suggest that the glucocorticoid response to low temperatures varies: some free-living birds and mammals elevate baseline or faecal concentrations in cold weather, but this response is not seen in others (reviewed in de Bruijn & Romero, 2018).

Initiating a glucocorticoid stress response is likely to be an important tool for coping with life-threatening thermal challenges; however, increasing glucocorticoid levels during more moderate thermal challenges can be costly because of the diversity of traits affected by these hormones (Wingfield et al., 2017). An alternative though not mutually exclusive response to cold is to alter the sensitivity to future challenges (Astheimer et al., 1995). Through this route, moderately challenging conditions could prime individuals to respond more strongly or rapidly to deteriorating conditions, or to additional challenges of a different type, without incurring the costs of an altered baseline physiological state. Work in Lapland longspurs *Calcarius lapponicus*, birds that breed in extreme Arctic environments, supports this idea. When confronted with sudden snowstorms during breeding, longspurs often show no change in baseline corticosterone; however, they release more corticosterone in response to an additional stressor (capture and handling) than birds sampled during milder conditions (Astheimer et al., 1995; Krause et al., 2018; Romero et al., 2000). It is not clear, however, whether responding to cold primarily by altering the sensitivity to subsequent challenges is an adaptation to life in an extreme environment, or whether species in more temperate regions show a similar response to smaller fluctuations in temperature. If thermal stressors commonly increase the sensitivity to other challenges (of the same type or a different type), this could represent an overlooked cost of changing thermal regimes.

Another fundamental but unresolved question relates to how organisms perceive and evaluate changing temperatures and other stressors. Many of the classic conceptual models of stress in ecology, psychology and biomedical science consider unpredictability to be a defining characteristic of stressors (Del Giudice et al., 2011, 2018; Koolhaas et al., 2011; Romero et al., 2009; Ursin & Eriksen, 2004; Wingfield, 2003). Some of these models posit that the generalized stress response is triggered by a negative prediction error—when current conditions are more challenging than predicted (Del Giudice et al., 2018; Ursin & Eriksen, 2004). If so, then it stands to reason that the glucocorticoid response to a given thermal challenge should be more strongly associated with the degree to which it represents a prediction error than by its absolute value. As far as we are aware, this prediction has not been tested in natural environments. Comparisons across species and life-history stages have supported the basic idea that the perception of weather as 'extreme' varies across populations and life-history stages (Wingfield et al., 2011, 2017). However, to date, empirical studies have focused almost exclusively on assessing how ambient temperatures affect glucocorticoids, rather than on quantifying the degree to which those conditions represent a departure from expected or previous conditions.

Here we test whether recent temperatures, temperature change and the degree to which temperatures represent prediction errors, affect circulating corticosterone and the response to future challenges in a free-living population of tree swallows *Tachycineta bicolor*. As early spring temperate zone breeders, tree swallows sometimes face periods of cold weather during reproduction (Cox et al., 2019;

McCarty, 1995; Winkler et al., 2013). Recent analyses in our long-term study population found that the likelihood of experiencing a cold snap during reproduction (defined as days in which ambient temperature does not exceed 18.5°C: Winkler et al., 2013) is increasing, as tree swallows are advancing the onset of breeding to match warming springs (Shipley et al., 2020). These unpredictable events pose major challenges: they increase the thermoregulatory costs of parents, the demand for incubating or brooding and the energetic demands of nestlings (Ardia et al., 2010; McCarty, 1995). Food availability also declines when temperatures fall below a threshold that impairs insect flight behaviour (Winkler et al., 2013). As a result, cold snaps reduce reproductive success—and if severe, cause widespread nest failure (Cox et al., 2020; Griebel & Dawson, 2019; Shipley et al., 2020). Responding effectively to cold snaps is thus likely to represent a strong selective pressure in this species.

First, we test the causal effects of temperature on circulating corticosterone and the acute corticosterone stress response in adults by experimentally reducing the temperature of nest boxes at our long-term study site. Second, we use data collected over an 8-year period ($n = 2{,}888$ samples) to test how corticosterone levels, and the response to future challenges, vary with ambient temperature. Using a sliding window analysis, we identify the time-scale over which ambient temperature best predicts variation in baseline corticosterone, stress-induced corticosterone and the strength of negative feedback (which affects the duration of the corticosterone response). We also test the prediction that corticosterone levels will be more strongly associated with temperature during incubation than after hatching. This prediction stems from the hypothesis that parents will be less sensitive to stressors during particularly high-value reproductive attempts or stages of reproduction because elevated glucocorticoids can impair reproduction (Bókony et al., 2009; Wingfield & Sapolsky, 2003). Tree swallows in this population commonly lay a second clutch if their first fails during incubation; however, they almost never initiate a second reproductive attempt if their first nest fails after hatching. Thus, we predict that tree swallows will show a reduced sensitivity to thermal challenges during provisioning, when the fitness costs of elevated corticosterone are likely to be higher. Finally, we test whether corticosterone regulation is best predicted by ambient temperature, recent temperature change or negative prediction error (the difference between current temperature and long-term average conditions).

2 | MATERIALS AND METHODS

Blood samples were collected in 2013–2021 from female tree swallows breeding in nest boxes at our long-term study site in Tompkins County, New York (~42°29′N, 76°27′W, 340–530 m elevation). Nests were monitored daily during the laying period so that the reproductive stage of each female was known. Birds were captured while inside their nest boxes by covering the entrance hole by hand or with a manually triggered trap and sampled immediately upon capture. All captures took place between 06:00 and 10:00 to reduce the likelihood of circadian rhythms in corticosterone affecting measured concentrations. All methods were approved by Cornell IACUC and conducted with appropriate federal and state permits.

2.1 | Experimental manipulation of nest temperature

In 2021, we tested whether experimental nest cooling affects glucocorticoid regulation in adult female tree swallows. Nest boxes ($n = 19$) were artificially cooled for 3 days during the early nestling period (from 4 to 6 days after hatching) using ice packs placed below nests and in an 'attic' installed above the nest box and lined with steel hardware cloth. Four ice packs (~11 cm × 11 cm × 4 cm) were placed inside; an additional ice pack was placed under each nest and separated from nesting material via a cardboard nest liner a and film vapour barrier underlayment (Moisturbloc). Foam insulation (0.5 in) was placed under the lower ice pack. Ice packs were swapped every 3 hr from 06:00 to 18:00. Control boxes ($n = 17$) were visited on the same schedule and had a similar volume of room temperature cooling packs added. Nest temperatures were recorded every 1 min using HOBO data loggers (MX2201 waterproof Bluetooth pendants; Onset Computer Corporation) installed on the inner wall of each nest box, just above nest cups. Females were captured and sampled on the morning of the third day of experimental cooling (day 6 post-hatch; ~48–52 hr after the onset of cooling).

To test whether experimental cold exposure affected the glucocorticoid stress response of adult females, we collected blood samples at different times relative to capture from each individual. Each individual was sampled at three randomly chosen times between 0 and 60 min post-capture ($n = 98$ samples in total from 36 females) from among a set of possible target times that included: 0 (as soon as possible after capture), 5, 10, 15, 20, 30 and 60 min post-capture. The exact latency from capture to sampling was recorded once each sample had been obtained and was used as a covariate in analyses. We used this sampling approach because recent simulations have shown that when the number of blood samples that can be safely collected from each individual during a single capture is limited (in this case, no more than three), it will often not be possible to detect *true* differences between groups in the speed of the corticosterone response if all samples are collected at the same three time points (Taff, 2021). Instead, these simulations showed that a random sampling scheme, as we used here, or a weighted sampling approach (in which each individual is sampled at two times chosen from a normal distribution with a mean around expected peak levels) are better able to correctly identify differences in the speed of the response between groups, when such differences exist.

2.2 | Long-term dataset: Plasma sample collection

Blood samples ($n = 2{,}888$) collected from 2013 to 2020, as a part of our long-term study, were used to assess the relationship between

ambient temperature and glucocorticoid regulation during both incubation and the nestling provisioning period. Upon capture, a first blood sample was collected within 3 min of initial disturbance to measure baseline corticosterone levels (n = 1,365 samples from 689 individuals). Birds were then restrained in a bag until a second blood sample was collected 30 min after the initial disturbance to measure stress-induced corticosterone (n = 1,011 samples from 589 individuals). This measure enabled us to test how temperature affected the response to an additional stressor of a different type (capture and restraint). Birds were then injected in the pectoral muscle with the synthetic glucocorticoid dexamethasone (0.5 μl/g Dexamethasone Sodium Phosphate; Mylan Institutional LLC), which initiates negative feedback by binding to glucocorticoid receptors (validated in tree swallows in Zimmer et al., 2019). A third blood sample was collected 30 min post-dexamethasone injection (n = 512 samples from 346 individuals). This measure enabled us to test whether birds respond to cold by increasing (or decreasing) the strength of negative feedback, and thereby the duration of the corticosterone stress response. Birds were held for the duration of collection of all three blood samples and then released at the site of capture.

2.3 | Glucocorticoid assays

From 2015 to 2020, corticosterone levels were measured using an enzyme immunoassay (EIA) (DetectX Corticosterone, Arbor Assays: K014-H5), following a triple ethyl acetate extraction (validated in Taff et al., 2019). Extraction efficiency averaged 91.5%. Intra-assay variation was 7.6% and inter-assay variation was 9.4% (detection limits: 0.8 ng/ml). Corticosterone levels in all samples collected in 2013 and 2014 and approximately half of the samples collected in 2015 were measured using a miniaturized double antibody radioimmunoassay (RIA) kit (ImmuChem Corticosterone I-RIA, MP Biomedicals: 07-120103). Intra-assay variation was 4.3% and inter-assay variation was 8.5% (detection limit: 0.6 ng/ml). A previous analysis comparing samples analysed using both methods found that measured results were highly correlated, but absolute levels differed; concentrations measured using RIA were therefore adjusted to EIA-equivalent concentrations using the inverse of the formula described in Stedman et al. (2017).

2.4 | Temperature and prediction errors

Hourly weather data were obtained from the Northeast Regional Climate Center's monitoring station on Game Farm Road (https://www.nrcc.cornell.edu/). We selected this station because it is located between our sub-sites and has data on hourly mean temperatures since 1983. We calculated average daily temperature (06:00–20:00) for each day. As a standardized measure of ambient temperature on the morning of sampling, we also calculated mean temperature for the first 2 hr of the activity period (from 06:00 to 08:00) on the morning of capture. We only included the first 2 hr of the day in this measure because after 08:00 many of the target birds had been captured, and thus were no longer experiencing normal ambient temperatures. To determine the degree to which the conditions each individual experienced represented a departure from predicted conditions (prediction error), we first created a distribution of temperatures for the same time intervals and dates using the long term (38 years) weather records. The mean of this distribution represents the expected temperature at a given date and time. Using the distribution, we determined the difference between temperature in the year of capture and the long-term average in standard deviation units. For these calculations, we used the interval determined by the sliding window analysis to be most predictive of each measure of corticosterone (baseline, stress-induced and post-dexamethasone). The most predictive time window for each measure was considered to be the window with the greatest absolute value of the sum of coefficient estimates derived from models of corticosterone during incubation and provisioning.

2.5 | Data analyses

Analyses were conducted using R v. 4.1.1 (R Core Team, 2021). Prior to analyses involving the long-term dataset, we excluded samples collected after the onset of manipulations in which experimental stressors were introduced or hormone levels were manipulated.

2.6 | Experimental data analysis

We first examined the effect of temperature manipulations using data collected from the HOBO temperature loggers placed in each nest. We fit a single GLMM with nest temperature as the response and treatment as a fixed effect. To account for repeated observations at each nest and temporal autocorrelation in measures, we included random effects for nest and day of year.

We then modelled the effect of experimental nest cooling on the shape of the response curve in corticosterone levels using a generalized additive mixed model (GAMM) fit with the 'MGCV' package version 1.8-31 in R (Wood, 2011). In this model, corticosterone was the response variable with treatment as a fixed effect, and individual ID as a random effect. Latency from capture to sampling was fit as a smoothed predictor using the default package settings. We allowed for a different smoothed response between treatments using the 'by' argument. Overall differences in corticosterone levels between the two groups were determined by inspecting the fixed effect of treatment in the fit GAM. To examine differences in the time course of corticosterone change between the two groups, we evaluated the shape of response curves following the method outlined in Rose et al. (2012). We interpret periods of the time course in which the confidence intervals of response curves do not overlap as having support for a difference in corticosterone at that time between treatment groups. The timing of maximum corticosterone secretion

in each group was estimated by comparing GAMM predicted values for each minute.

2.7 | Identifying the time-scale of the corticosterone response to temperature

Using the long-term dataset, we identified the interval over which recent temperatures best predict corticosterone levels using a sliding window analysis. For baseline and stress-induced corticosterone, we compared the degree to which ambient temperatures the morning of capture, and over an average of 1–8 days prior to capture, predicted variation. We expected that any effects of the thermal environment on negative feedback might take longer to manifest, because of its dependence on glucocorticoid receptor expression (de Kloet et al., 1998; Jacobson & Sapolsky, 1991) which can take days to weeks after stressor onset to change (Lattin & Romero, 2014; Paskitti et al., 2000). Thus, we tested whether ambient temperatures the morning of capture, or over an average of 1–30 days prior to capture, predicted variation in post-dexamethasone corticosterone.

To implement this sliding window approach, we first calculated the average daytime temperature for each possible predictor interval. We next fit a series of generalized linear mixed models in the 'LME4' package version 1.1-27.1 (Bates et al., 2015) in R with log-transformed corticosterone as the response variable and temperature, breeding stage and their two-way interaction as predictor variables. Each model also included random effects for female identity and year. Models for stress-induced corticosterone also included baseline corticosterone as a predictor; models for post-dexamethasone corticosterone included stress-induced corticosterone as a predictor. All continuous measures were standardized to a mean of 0 and a standard deviation of 1 so that effect sizes are directly comparable. Using this basic model construction, we proceeded through each of the selected time intervals and substituted temperature from that interval into the model as the key predictor. After fitting each model, we saved the standardized parameter estimate for the relationship between temperature and corticosterone along with the confidence interval for this effect separately for the incubation and provisioning periods. We plotted and examined the strength of the relationship between temperature and corticosterone levels using these coefficients and chose the single interval with the strongest relationship for each corticosterone measurement (baseline, stress-induced and post-dexamethasone) for use in subsequent analyses.

2.8 | Comparing recent ambient temperature, temperature change and prediction error

We then used the long-term dataset to test whether glucocorticoid levels were better predicted by ambient temperature, recent temperature change (the interaction between current temperature and temperature the day before capture) or a negative prediction error. Here we used a model selection approach in which all models included year and individual ID as random effects and day of year as a fixed effect. Day of year was not strongly correlated with daytime average temperature (correlation coefficient = 0.30). As noted above, models of stress-induced corticosterone included baseline levels as a covariate, and models of post-dexamethasone corticosterone included stress-induced levels as a covariate. Each candidate model set included six hypothesis-testing models plus the null model; the latter included the random and fixed effects described above, but no temperature variables. Two candidate models tested the effect of ambient temperatures (those identified in the sliding window analysis described above as most predictive of each measure of corticosterone): (a) predictive ambient temperature and (b) predictive ambient temperature × breeding stage (incubation or provisioning). We tested whether corticosterone levels were better predicted by recent temperature change with two additional models: (c) current temperature (from 06:00 to 08:00 on the morning of capture) × mean temperature the day before capture (06:00–20:00) and (d) current temperature × mean temperature the day before capture × breeding stage. Finally, we included two additional candidate models testing prediction error, measured as the difference between ambient temperature and the 38-year average temperature on the date of sampling in units of standard deviations: (e) prediction error and (f) prediction error × breeding stage.

For each corticosterone response variable, we compared the set of models described above using AICc values implemented in the R package 'MuMIn' version 1.43.17 (Bartoń, 2020). Model diagnostics were examined following the workflow suggested for GLMMs using the R package 'DHARMa' version 0.4.3 (Hartig, 2021). We considered models with delta AICc values <4 to have meaningful support, and when examining model estimates, we considered those with 95% confidence intervals that did not cross zero to have meaningful support.

3 | RESULTS

3.1 | Field experiments

Temperature manipulations decreased nest temperature by an average of 5.1°C below ambient temperature in control boxes (Figure 1a; treatment [cooled] = −5.1 [CI: −6.6, −3.6], $p < 0.001$). Female tree swallows captured on the morning of the third day of cooling (~48–52 hr after onset) had higher overall corticosterone levels than controls (Figure 1b; intercept = 30.5 [CI: 26.4, 34.5], $p < 0.001$, treatment [control] = −10.5 [−16.6, −4.1], $p = 0.001$, smooth term [cooled]: $p < 0.001$, smooth term [control]: $p < 0.001$, $R^2 = 0.58$). Corticosterone levels did not appear to differ between groups at initial capture, but birds from cooled nests had a faster initial increase in corticosterone and reached their maximum corticosterone levels sooner (Figure 1b; 22 min in cooled birds vs. 26 min in controls). Control birds' maximum levels remained lower overall than cooled birds. The corticosterone levels of cooled birds also began to decline

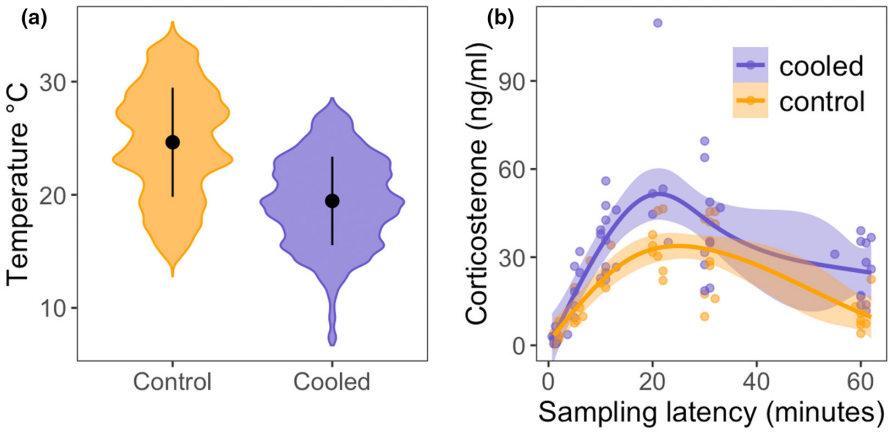

FIGURE 1 The results of nest temperature manipulation experiments. (a) The temperature of cooled vs. control nests. (b) The glucocorticoid stress response of female tree swallows at cooled vs. control nests; the line and shaded area represent the model predicted mean and 95% confidence interval. The *x*-axis represents the exact latency between initial disturbance and sample collection.

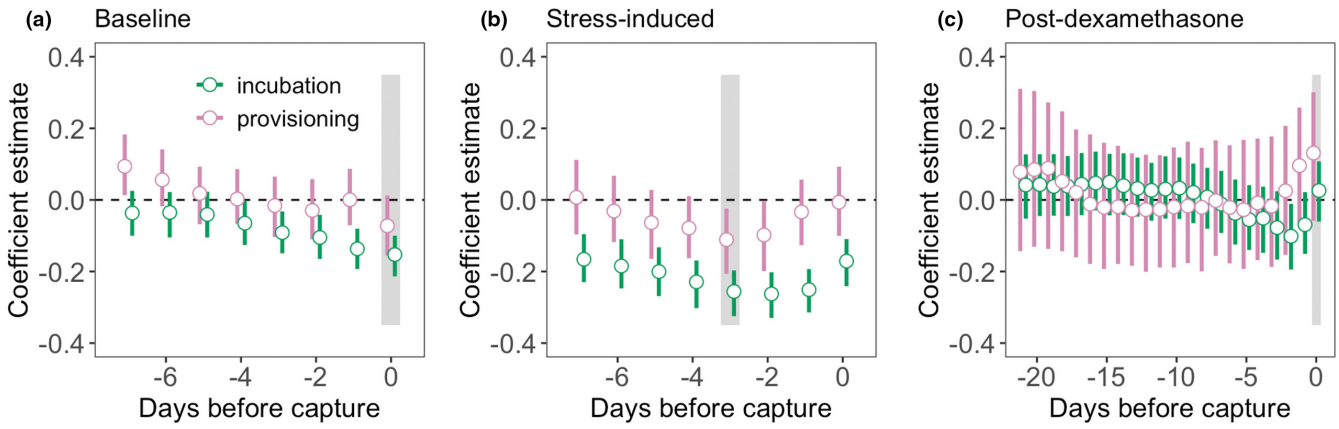

FIGURE 2 The coefficient estimates from sliding window analyses that illustrate the strength and direction of the relationship between ambient temperature over different time-scales and (a) baseline corticosterone, (b) stress-induced corticosterone and (c) post-dexamethasone corticosterone during incubation and provisioning. The *x*-axis represents the number of days preceding sampling over which ambient daytime temperatures were averaged. Day 0 measurements represent mean temperatures on the morning of capture. Bars represent the 95% confidence interval of the coefficient estimate. The grey shaded area represents the interval identified by the sliding window analysis as most predictive of corticosterone levels.

sooner than those of control birds. Although we were not able to determine the shape of the curve between 30 and 60 min post-stressor-onset due to a lack of sampling during this period, corticosterone levels appear to have remained higher in cooled birds than in control birds 60 min post-capture.

3.2 | The time-scale of the corticosterone response to ambient temperature

Sliding window analyses of the long-term dataset revealed that baseline corticosterone levels were negatively correlated with ambient temperature on the day of capture in both incubating and provisioning birds (Figure 2a). As predicted, this relationship was stronger during incubation than during provisioning, as illustrated by coefficient estimates that were generally further from zero. Ambient temperatures over longer time-scales had weaker negative or no relationship with baseline corticosterone levels, with the possible exception of a positive relationship with ambient temperatures averaged over the 7 days prior to capture during provisioning.

During incubation, birds that had recently experienced naturally occurring cold temperatures mounted stronger corticosterone responses to a standardized handling stressor (Figure 2b). This pattern was relatively consistent across all time-scales examined. During provisioning, a similar pattern was seen but the relationships were weaker (Figure 2b). Ambient temperatures 2–3 days prior to capture were negatively associated with stress-induced corticosterone during provisioning, but this pattern was not significant during the other windows examined.

The efficacy of negative feedback, measured as circulating corticosterone levels following a dexamethasone challenge, was not significantly predicted by ambient temperatures at capture, or during any of the other time windows examined (Figure 2c).

3.3 | Comparing recent ambient temperature, temperature change and prediction error

For both baseline and stress-induced corticosterone, models that included only recent temperatures (using the time-scales identified

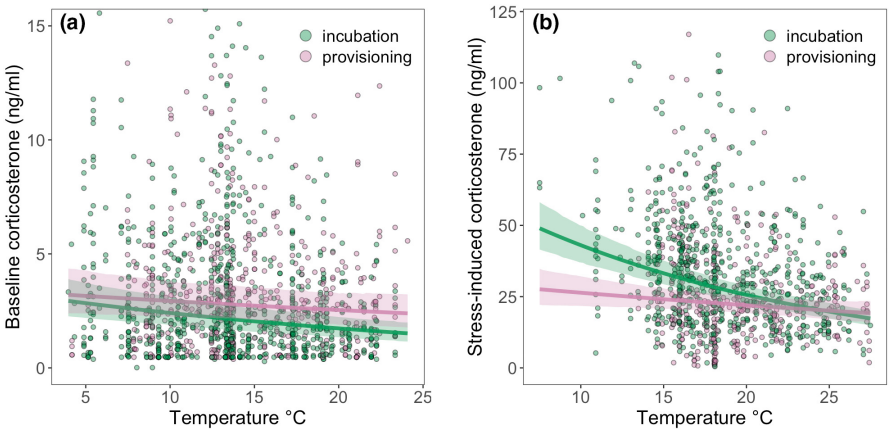

FIGURE 3 The best supported predictors of the effect of thermal environment on circulating corticosterone levels. (a) Baseline corticosterone was best predicted by ambient temperature on the morning of capture. (b) Stress-induced corticosterone was best predicted by average ambient temperature during the 3 days prior to sampling. The line and shaded area represent the model predicted mean and 95% confidence interval.

TABLE 1 A comparison of candidate models of baseline corticosterone

Model	*k*	Log likelihood	Delta AICc	Weight
Temp × Stage	8	−1,811.6	0.0	0.91
Temp	6	−1,816.5	5.8	0.05
PE × Stage	8	−1,814.8	6.4	0.04
Temp × Prev Temp × Stage	12	−1,812.5	9.9	0.01
Null	6	−1,819.0	10.7	0.00
PE	6	−1,819.4	11.6	0.00
Temp × Prev Temp	8	−1,818.7	14.1	0.00

Temp = ambient temperature on the morning of capture, Prev temp = mean ambient temperature on the previous day, PE = prediction error (offset). Stage = breeding stage. All candidate models also include day of year as a fixed effect, and year and individual ID as random effects

above) received more support than models that included either an interaction between current temperature and the previous day's temperature, or prediction error (the offset between experienced temperature and predicted temperature over the same time period) (Figure 3; Tables 1 and 2). The best supported models of both baseline and stress-induced corticosterone included predictive temperature, breeding stage and their interaction. Baseline corticosterone was significantly predicted by temperature and breeding stage, but not by their interaction (Figure 3a; intercept: 0.75 [CI = 0.45, 1.06], temperature: −0.14 [CI = −0.20, −0.09], breeding stage [provisioning]: 0.27 [CI = 0.13, 0.40], day of year: −0.07 [CI = −0.14, 0.00], temperature × stage: 0.08 [CI = −0.02, 0.18]; marginal R^2 = 0.03, conditional R^2 = 0.27). Baseline levels were higher in cold conditions, and during provisioning. Stress-induced corticosterone was significantly predicted by temperature, breeding stage and their interaction (Figure 3b; intercept: 3.31 [CI = 3.17, 3.46], temperature: −0.19 [CI = −0.24, −0.14], breeding stage [provisioning]: −0.20 [CI: −0.31, −0.09], day of year: −0.04 [CI: −0.10, 0.02], baseline cort: 0.11 [CI: 0.06, 0.15], temperature × stage: 0.12 [CI: 0.04, 0.20]; marginal R^2 = 0.10, conditional R^2 = 0.61). Birds that had experienced colder weather over the past 3 days secreted more corticosterone in response to the additional challenge of capture and handling; this relationship was more pronounced during incubation than during provisioning. The efficacy of negative feedback in the stress response was not predicted by recent temperatures, the change in temperatures or prediction error. Within this candidate model set, the null model was best supported, and no other models were within 4 ΔAICc of the null model. Thus, tree swallows do not appear to alter the effectiveness of negative feedback in response to cold.

4 | DISCUSSION

These results show that exposure to cold temperatures alters glucocorticoid regulation, and that this manifests primarily in terms of an upregulation in the sensitivity to *future* challenges, rather than a strong immediate glucocorticoid response to cold. Increasing the sensitivity to future challenges could prime individuals to respond more strongly or rapidly to worsening conditions, or to future challenges of a different type, without incurring the costs that can result from substantially elevated baseline glucocorticoid levels.

Findings from what was, to our knowledge, the first experimental test of the glucocorticoid response to cold in free-living adult birds paralleled the patterns seen in females sampled during naturally occurring cold snaps. Exposure to 2 days of decreased nest temperatures caused an elevation in the corticosterone response to a standardized acute stressor. Interestingly, a similar change in stress-induced corticosterone was seen in both the experimental and observational data, even though experimental nest cooling does not affect food availability. Experimental cooling is, however, expected to increase the thermoregulatory demands of both nestlings (Ardia et al., 2010; McCarty, 1995) and adult females (which often brood past days 6–7 in cold conditions: Winkler et al., 2020) similarly to naturally occurring cold snaps.

Most previous studies of the glucocorticoid response to cold in adults have focused on the immediate response (baseline glucocorticoids) rather than on whether cold exposure alters the sensitivity to future challenges. However, observational data show that several Arctic-breeding birds respond to snowstorms that occur

TABLE 2 A comparison of candidate models of stress-induced corticosterone

Model	*k*	Log likelihood	Delta AICc	Weight
Pred Temp × Stage	9	−1,033.8	0.0	0.91
Pred Temp	7	−1,039.4	7.1	0.05
PE × Stage	9	−1,037.6	7.5	0.04
PE	7	−1,042.9	14.2	0.00
Curr Temp × Prev Temp	9	−1,049.1	30.6	0.00
Curr Temp × Prev Temp × Stage	13	−1,045.1	30.8	0.00
Null	7	−1,057.9	44.1	0.00

Pred temp = mean ambient temperature for 3 days before capture (the best predictor of stress-induced corticosterone levels in the sliding window analysis), Curr temp = ambient temperature on the morning of capture, Prev temp = mean temperature on the previous day, PE = prediction error (offset), stage = breeding stage. All candidate models also include day of year and baseline corticosterone as fixed effects, and year and individual ID as random effects.

during moult by elevating the corticosterone response to the additional stressor of capture and handling (Lapland longspurs, common redpolls: *Acanthis flamea*, and snow buntings: *Plectrophenax nivelis*; Romero et al., 2000). Of these species, only Lapland longspurs show a similar response during breeding (Astheimer et al., 1995; Krause et al., 2018; Romero et al., 2000). It has previously been suggested that this kind of preparatory response could represent an evolved strategy for surviving in extreme environments. Here, however, we find a similar response in a temperate breeding bird that faces comparatively mild temperature fluctuations (very few days in our 8-year dataset had average temperatures below ~12°C). These findings suggest that increasing the sensitivity to future challenges may be a more common strategy than previously thought, including among temperate breeders.

We also show for the first time that temperature affects the speed of the glucocorticoid stress response. Experimentally cold-exposed birds showed a steeper initial increase in corticosterone in response to capture and handling than control birds and reached their maximum corticosterone levels sooner. This indicates that previously cold-exposed birds respond more quickly to future challenges, in addition to mounting a stronger overall response. Variation in the speed of the acute stress response has been suggested to be an overlooked target of selection (Luttbeg et al., 2021; Taff et al., 2021; Taff & Vitousek, 2016). We are not aware of previous tests of whether ecologically relevant challenges alter response speed, but body condition (Heath & Dufty Jr., 1998) and stressor exposure regime (Cockrem, 2013) have been linked with the speed of the glucocorticoid stress response in captive animals. The sampling regime that we currently use for non-experimental birds in our long-term study population is not conducive to testing individual differences in speed (and it is not clear whether it is possible to separately measure speed and scope at an individual level in most systems given sampling constraints: Taff, 2021). Thus, we could not test whether natural variation in temperature induced a similar increase in the speed of the response in our long-term dataset. However, our experimental results support the idea that the speed of the acute stress response is a flexible trait that can be adjusted based on perceived risk or prior exposure to challenges. Experimentally cooled birds also reached higher peak corticosterone levels than control birds and initiated negative feedback more quickly. Although our ability to discern the shape of the curve between 30 and 60 min post-stressor-exposure was limited by a lack of samples collected during this period, the slope of decline in corticosterone during negative feedback appeared to be shallower in experimentally cooled birds, whose corticosterone levels remained higher than controls 60 min post-capture. Overall, these patterns are consistent with the idea that many components of the glucocorticoid stress response can be rapidly adjusted based on current or recent exposure to challenges.

While stressors are frequently non-randomly distributed with respect to time, the real-life consequences of multiple stressors experienced in succession are generally poorly understood (Malkoc et al., 2021; Taff & Vitousek, 2016). At a physiological level, it has been widely demonstrated that exposure to even a single stressor can prompt a stronger response to future challenges of a different type—a phenomenon known as facilitation (e.g. Dallman et al., 1992; Romero, 2004). This strategy could be adaptive in some contexts, enabling organisms to sidestep the costs of continually elevated corticosterone while perhaps responding more rapidly or effectively to deteriorating conditions or compounding stressors. Yet in other contexts a heightened sensitivity to challenges could be costly—perhaps particularly when the secondary challenge is a chronic stressor. For example, if a cold-induced upregulation in the glucocorticoid stress response is followed by a lingering anthropogenic challenge, individuals may accrue more glucocorticoid-induced phenotypic damage than if they had not previously experienced a cold stressor. Thermally induced increases in the sensitivity to challenges could thus be an underappreciated cost of changing thermal regimes.

Analyses of the long-term data found that the acute stress response was more sensitive to ambient temperature during incubation than during provisioning. This pattern is consistent with the idea that the sensitivity to challenges is decreased in organisms engaged in particularly valuable reproductive attempts, and during life-history stages in which elevated glucocorticoids are likely to be particularly damaging to fitness (O'Reilly & Wingfield, 2001;

Wingfield & Sapolsky, 2003). Across vertebrates, the suppression of the glucocorticoid stress response during reproduction is common. This is often most pronounced in parents engaging in particularly valuable reproductive attempts—those that represent a high proportion of lifetime reproductive effort (Bókony et al., 2009; Vitousek et al., 2019)—and during breeding stages in which abandonment would have a particularly negative effect on fitness (Holberton & Wingfield, 2003). Tree swallows in our study population are almost exclusively single brooded. When cold snaps occur during incubation females frequently abandon nests, and often initiate a second reproductive attempt if conditions improve. However, if nests fail after eggs have hatched females almost never re-lay, and as annual survival rates are low (~35% in this population), most individuals have only a single season in which to reproduce. Thus, female tree swallows likely have more to gain from adjusting the sensitivity to environmental stressors (and concomitantly reproductive investment) during incubation than during provisioning. Unlike some other birds, tree swallows do not typically show a decline in the scope of the glucocorticoid response to acute stressors between incubation and provisioning (Zimmer et al., 2020); however, these findings indicate that they decrease the degree to which the scope of the stress response depends on the thermal environment.

Naturally occurring periods of cold weather induced a slight but significant elevation in baseline corticosterone in free-living female tree swallows. Across vertebrates, the effect of cold temperatures on baseline glucocorticoids varies. While experimental studies typically find that extreme cold or rapid temperature declines elicit a hormonal stress response (de Bruijn & Romero, 2018; Jessop et al., 2016), free-living animals do not always show a glucocorticoid response to the kinds of temperature challenges that they regularly experience (reviewed in de Bruijn & Romero, 2018). The slight but significant increase in baseline corticosterone in tree swallows on cold days could result from cold temperatures triggering a relatively mild stress response. Baseline corticosterone can also be upregulated to support energetically demanding periods, including outside of the context of traditionally 'stressful' stimuli (Bonier et al., 2009; Crossin et al., 2016; Landys et al., 2006; Ouyang et al., 2011; Patterson et al., 2014). For example, tree swallows whose broods are experimentally increased elevate baseline corticosterone in proportion to their increase in provisioning effort, a response that may facilitate the extra provisioning effort required to rear a larger brood (Bonier et al., 2011). Parental tree swallows facing reduced insect availability during cold snaps could similarly upregulate baseline corticosterone to support the additional foraging effort required to obtain sufficient food and to sustain reproduction under these conditions (Vitousek, Taff, Hallinger, et al., 2018).

Baseline corticosterone was, however, more strongly predicted by ambient temperature during incubation than during provisioning in the long-term dataset. This pattern is opposite of what would be predicted if the upregulation in baseline corticosterone was driven predominantly by the energetic demands of cold snaps, as these demands are greater during provisioning due to the elevated metabolic needs of cold-exposed nestlings coupled with the increased foraging effort required to provision when food availability declines (Winkler et al., 2013). Females faced with cold snaps during incubation must still contend with the increased difficulty of finding food for themselves; yet, tree swallows do not appear to respond to cold by increasing the time spent on incubation but instead tend to incubate less (Ardia et al., 2010; Coe et al., 2015). These findings are thus consistent with the possibility that the increase in baseline corticosterone induced by cold snaps is driven more by the perception of cold as a stressor than by the increased energetic demand it induces. Future studies that directly test this question will be important for illuminating the causes of thermally induced shifts in baseline corticosterone.

We did not see clear evidence that experimental nest cooling affected the baseline corticosterone levels of incubating females. However, because the specific sampling scheme that we used was not designed to explicitly test the effect of manipulation on baseline corticosterone, it is possible that the relatively subtle effect of natural cold snaps on baseline corticosterone was not detectable in the experimental study. Additionally, unlike naturally occurring cold snaps, experimental temperature manipulations affect nest temperature only, and thus the strength of the stimulus could be perceived as weaker. Alternatively, the natural upregulation in baseline corticosterone during cold snaps could be a direct result of reduced insect availability rather than a response to temperature alone. This raises the intriguing possibility that different types of cues associated with a cold snap could be differentially affecting various components of the hypothalamic–pituitary–adrenal (HPA) axis, which controls the secretion of glucocorticoids.

The time-scale over which temperature was most predictive varied for baseline and stress-induced corticosterone. Baseline corticosterone was best predicted by ambient temperature on the morning of capture, whereas stress-induced corticosterone was best predicted by temperatures over the preceding 3 days. While several studies have examined the relationship between cold exposure and baseline corticosterone levels on multiple time-scales (e.g. el-Halawani et al., 1973; Romero et al., 2000), we are not aware of other analyses that have used a similar approach to identifying the window of time in which ambient temperature is most predictive of glucocorticoid levels in a natural population. The observed discrepancy in the time-scale of effects could reflect different components of the HPA axis being modulated by differing cues, with baseline levels affected by current temperature and/or food availability (de Bruijn & Romero, 2018), and stress-induced levels altered by accumulating allostatic load (Wingfield & Ramenofsky, 2011). Acute responses to additional stressors could also be less flexible on shorter time-scales because of functional limitations—for example, if variation depends on changes in risk perception or on other mediators of the HPA axis that occur over longer time periods. This is consistent with some previous findings; for example, the temperature at which rock pigeons *Columbia livia* are held for 75 min between baseline and acute stress-induced sample collection does not affect the scope of the corticosterone stress response (Angelier et al., 2016). However,

there is also evidence (including from preliminary data in this species) that changes in context can result in the modulation of stress-induced corticosterone within minutes to hours (Dallman, 1993; Racic et al., 2020). Thus, we expect that the observed difference in the time-scale over which temperature best predicts baseline and stress-induced levels (hours vs. days) in tree swallows is not driven primarily by functional limitations in the speed of regulation of stress-induced corticosterone.

The efficacy of negative feedback, measured as post-dexamethasone corticosterone levels, was not strongly predicted by ambient temperatures within the past 30 days, recent temperature change or negative prediction error in the long-term dataset. However, while the experimental manipulation was not explicitly designed to test effects on negative feedback, the results suggested at least some rapid modulation of this response: experimentally cooled birds initiated negative feedback sooner than controls but feedback may have been weaker, as they appeared to be maintaining higher corticosterone levels 60 min after the onset of the stressor. Individual variation in the efficacy of negative feedback appears to be important for successfully responding to and recovering from challenges in tree swallows (Taff et al., 2018; Zimmer et al., 2019); yet when and how negative feedback is affected by environmental context remains poorly understood. Given the importance of negative feedback to stress coping capacity, determining when and how various environmental and genetic factors shape variation in this trait are important future directions.

Interestingly, while some conceptual models of the stress response emphasize unpredictability and/or a negative prediction error as defining characteristics of stressors (Del Giudice et al., 2011, 2018; Koolhaas et al., 2011; Romero et al., 2009; Ursin & Eriksen, 2004; Wingfield, 2003), both baseline and stress-induced corticosterone were better predicted by recently experienced ambient temperatures than by temperature change or negative prediction error (measured as the difference between experienced temperatures and the long-term average). Because all three measures were significantly correlated in this population during the years of study, it is not possible to fully separate their effects. However, our results do not support the idea that a sudden departure from previous thermal conditions triggers a greater hormonal response than more gradual changes in temperature. Similarly, we saw no evidence that negative prediction errors, at least for thermal challenges, are more salient stressors than experienced temperatures. These findings are also in accordance with the suggestion that at least in this population of tree swallows, daily fluctuations in ambient temperatures are a good proxy for the degree of environmental challenge faced during the breeding season (Shipley et al., 2020; Winkler et al., 2013).

5 | CONCLUSIONS

Like many species, tree swallows are facing increased cold exposure during critical periods of breeding (Shipley et al., 2020). Unlike captive vertebrates, which typically show a robust immediate increase in baseline corticosterone in response to cold (de Bruijn & Romero, 2018), free-living tree swallows showed only a weak up-regulation in baseline glucocorticoids during cold exposure. Instead, they increased the sensitivity to future stressors, altering both peak glucocorticoid levels and the speed of the initial response. Determining whether this kind of preparatory response is adaptive is an important direction for future research. Such a response could enable individuals to sidestep the costs of a longer term elevation in baseline corticosterone—which can direct resources away from important reproductive processes as well as cause lasting phenotypic damage—while preparing them to respond quickly and effectively to worsening conditions. However, these findings also suggest that even relatively mild thermal challenges can elevate the susceptibility to additional stressors. Determining whether thermal priming of the stress response increases the risk of reproductive failure or phenotypic damage, and is thus an overlooked cost of changing thermal regimes, is an important future direction.

It will also be valuable to determine whether cold exposure increases the glucocorticoid response to all future stressors (experienced within a given time), or whether this response is restricted to certain types of stressors. These results show that cold-exposed tree swallows were more sensitive to a future challenge of a different type (restraint stress), which suggests a generalized facilitation of the stress response. However, whether birds also show a heightened sensitivity to worsening stressors of the same type (cold), or to other ecologically relevant acute or chronic stressors is not known. Future studies that test the generality of this effect across populations and species that breed in different environments would also be valuable. To date, the effects of exposure to cold during key periods of development have been much more widely investigated than the effects of exposure in adulthood. Developmental cold exposure can cause immediate and sometimes lasting changes in the regulation of glucocorticoids (reviewed in Nord & Giroud, 2020), including in free-living birds (Lynn & Kern, 2017; Uehling et al., 2020). Determining the relative roles of developmental plasticity, adult experience and the potential interactions between them in shaping responses to future challenges will be important for elucidating the overall impacts of changing thermal regimes on natural populations.

Thermally induced changes in the sensitivity to threats could also affect the functioning of ecological communities—for example, by affecting where and on what birds forage, population growth or the rates of predation (Lima, 1998; Zanette & Clinchy, 2020). Determining when the thermal responses of individual species, clades or guilds affect ecological interactions is a promising future direction (Gilman et al., 2010; González-Tokman et al., 2020). Finally, there is mounting evidence that in addition to their short-term physiological effects, brief challenges can have lasting impacts on phenotype (e.g. Clinchy et al., 2011; Vitousek, Taff, Ardia, et al., 2018). Identifying how and when thermal challenges have such effects will help to reveal how climate change and other stressors affect the fitness of individuals, and ultimately the health of populations.

AUTHOR CONTRIBUTIONS

Maren N. Vitousek and Conor C. Taff conceived of the study and designed the methodology; All authors collected field data; Conor C. Taff and Cedric Zimmer conducted hormone assays; Conor C. Taff analysed data with feedback from Maren N. Vitousek; Maren N. Vitousek wrote the paper. All authors provided feedback on drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

The field and laboatory work would not have been possible without the help of many people; special thanks to P. Becker, N. Chamiraju, N. Faraco-Hadlock, G. Halterman, A. Injaian, T. Ryan, B. Laslow, A. Lazar, C. Miller, S. McNew, D. Preston, D. Scheck and J. Stedman. We are also grateful to M. Hau for helpful conversations about stressors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data and R-code associated with this manuscript are archived on Zenodo 10.5281/zenodo.6822541—https://zenodo.org/record/6822541#.Ys7fRHbMI2w (Vitousek et al., 2022).

ORCID

Maren N. Vitousek https://orcid.org/0000-0003-2855-0832
Jennifer L. Houtz https://orcid.org/0000-0002-1022-1398
Monique A. Pipkin https://orcid.org/0000-0002-9594-835X
David A. Chang van Oordt https://orcid.org/0000-0001-6505-2299
Jennifer J. Uehling https://orcid.org/0000-0003-2679-3996
Cedric Zimmer https://orcid.org/0000-0001-8160-2836
Conor C. Taff https://orcid.org/0000-0003-1497-7990

REFERENCES

Andreasson, F., Nilsson, J.-Å., & Nord, A. (2020). Avian reproduction in a warming world. *Frontiers in Ecology and Evolution*, *8*, 576331. https://doi.org/10.3389/fevo.2020.576331

Angelier, F., Parenteau, C., Ruault, S., & Angelier, N. (2016). Endocrine consequences of an acute stress under different thermal conditions: A study of corticosterone, prolactin, and thyroid hormones in the pigeon (*Columbia livia*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *196*, 38–45. https://doi.org/10.1016/j.cbpa.2016.02.010

Ardia, D. R., Perez, J. H., & Clotfelter, E. D. (2010). Experimental cooling during incubation leads to reduced innate immunity and body condition in nestling tree swallows. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1689), 1881–1888. https://doi.org/10.1098/rspb.2009.2138

Astheimer, L. B., Buttemer, W. A., & Wingfield, J. C. (1992). Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scandinavica*, *23*(3), 355. https://doi.org/10.2307/3676661

Astheimer, L. B., Buttemer, W. A., & Wingfield, J. C. (1995). Seasonal and acute changes in adrenocortical responsiveness in an Arctic-breeding birds. *Hormones and Behavior*, *29*(4), 442–457. https://doi.org/10.1006/hbeh.1995.1276

Bartoń, K. (2020). *MuMin: Multi-model inference*. R package version 1.43.17.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bókony, V., Lendvai, Á. Z., Liker, A., Angelier, F., Wingfield, J. C., & Chastel, O. (2009). Stress response and the value of reproduction: Are birds prudent parents? *The American Naturalist*, *173*(5), 589–598. https://doi.org/10.1086/597610

Bonier, F., Martin, P. R., Moore, I. T., & Wingfield, J. C. (2009). Do baseline glucocorticoids predict fitness? *Trends in Ecology & Evolution*, *24*(11), 634–642. https://doi.org/10.1016/j.tree.2009.04.013

Bonier, F., Moore, I. T., & Robertson, R. J. (2011). The stress of parenthood? Increased glucocorticoids in birds with experimentally enlarged broods. *Biology Letters*, *7*(6), 944–946. https://doi.org/10.1098/rsbl.2011.0391

Breuner, C. W., & Wingfield, J. C. (2000). Rapid behavioral response to corticosterone varies with photoperiod and dose. *Hormones and Behavior*, *37*(1), 23–30. https://doi.org/10.1006/hbeh.1999.1554

Burggren, W. (2018). Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *Journal of Experimental Biology*, *221*(9), jeb161984. https://doi.org/10.1242/jeb.161984

Clinchy, M., Schulkin, J., Zanette, L. Y., Sheriff, M. J., McGowan, P. O., & Boonstra, R. (2011). The neurological ecology of fear: Insights neuroscientists and ecologists have to offer one another. *Frontiers in Behavioral Neuroscience*, *4*, 1–6. https://doi.org/10.3389/fnbeh.2011.00021

Cockrem, J. F. (2013). Individual variation in glucocorticoid stress responses in animals. *General and Comparative Endocrinology*, *181*, 45–58. https://doi.org/10.1016/j.ygcen.2012.11.025

Coe, B. H., Beck, M. L., Chin, S. Y., Jachowski, C. M. B., & Hopkins, W. A. (2015). Local variation in weather conditions influences incubation behavior and temperature in a passerine bird. *Journal of Avian Biology*, *46*(4), 385–394. https://doi.org/10.1111/jav.00581

Conradie, S. R., Woodborne, S. M., Cunningham, S. J., & McKechnie, A. E. (2019). Chronic, sublethal effects of high temperatures will cause severe declines in southern African arid-zone birds during the 21st century. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(28), 14065–14070. https://doi.org/10.1073/pnas.1821312116

Cox, A. R., Robertson, R. J., Lendvai, Á. Z., Everitt, K., & Bonier, F. (2019). Rainy springs linked to poor nestling growth in a declining avian aerial insectivore (*Tachycineta bicolor*). *Proceedings of the Royal Society B: Biological Sciences*, *286*(1898), 20190018. https://doi.org/10.1098/rspb.2019.0018

Cox, A. R., Robertson, R. J., Rendell, W. B., & Bonier, F. (2020). Population decline in tree swallows (*Tachycineta bicolor*) linked to climate change and inclement weather on the breeding ground. *Oecologia*, *192*(3), 713–722. https://doi.org/10.1007/s00442-020-04618-8

Crossin, G. T., Love, O. P., Cooke, S. J., & Williams, T. D. (2016). Glucocorticoid manipulations in free-living animals: Considerations of dose delivery, life-history context and reproductive state. *Functional Ecology*, *30*, 116–125.

Dallman, M. F. (1993). Stress update: Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends in Endocrinology & Metabolism*, *4*(2), 62–69. https://doi.org/10.1016/S1043-2760(05)80017-7

Dallman, M. F., Akana, S. F., Scribner, K. A., Bradbury, M. J., Walker, C.-D., Strack, A. M., & Cascio, C. S. (1992). Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. *Journal of Neuroendocrinology*, *4*(5), 517–526. https://doi.org/10.1111/j.1365-2826.1992.tb00200.x

de Bruijn, R., & Romero, L. M. (2018). The role of glucocorticoids in the vertebrate response to weather. *General and Comparative Endocrinology*, *269*, 11–32. https://doi.org/10.1016/j.ygcen.2018.07.007

de Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joels, M. (1998). Brain corticosteroid receptor balance in health and disease 1. *Endocrine Reviews*, *19*(3), 269–301. https://doi.org/10.1210/edrv.19.3.0331

Del Giudice, M., Buck, C. L., Chaby, L. E., Gormally, B. M., Taff, C. C., Thawley, C. J., Vitousek, M. N., & Wada, H. (2018). What is stress? A systems perspective. *Integrative and Comparative Biology*, *58*(6), 1019–1032. https://doi.org/10.1093/icb/icy114

Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration model of stress responsivity. *Neuroscience & Biobehavioral Reviews*, *35*(7), 1562–1592. https://doi.org/10.1016/j.neubiorev.2010.11.007

el-Halawani, M., Waibel, P., Appel, J., & Good, A. (1973). Effects of temperature stress on catecholamines and corticosterone of male turkeys. *American Journal of Physiology-Legacy Content*, *224*(2), 384–388. https://doi.org/10.1152/ajplegacy.1973.224.2.384

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology & Evolution*, *25*(6), 325–331. https://doi.org/10.1016/j.tree.2010.03.002

González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A., & Villalobos, F. (2020). Insect responses to heat: Physiological mechanisms, evolution and ecological implications in a warming world. *Biological Reviews*, *95*(3), 802–821. https://doi.org/10.1111/brv.12588

Griebel, I. A., & Dawson, R. D. (2019). Predictors of nestling survival during harsh weather events in an aerial insectivore, the tree swallow (*Tachycineta bicolor*). *Canadian Journal of Zoology*, *97*(2), 81–90. https://doi.org/10.1139/cjz-2018-0070

Hartig, F. (2021). *DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models*. R package version 0.4.3.

Heath, J. A., & Dufty Jr., A. M. (1998). Body condition and the adrenal stress response in captive American kestrel juveniles. *Physiological Zoology*, *71*(1), 67–73. https://doi.org/10.1086/515888

Holberton, R., & Wingfield, J. C. (2003). Modulating the corticosterone stress response: A mechanism for balancing individual risk and reproductive success in arctic-breeding sparrows? *The Auk*, *120*(4), 1140–1150.

Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine Reviews*, *12*(2), 118–134. https://doi.org/10.1210/edrv-12-2-118

Jessop, T. S., Lane, M. L., Teasdale, L., Stuart-Fox, D., Wilson, R. S., Careau, V., Moore, I. T., Adkins-Regan, E., & Michalakis, Y. (2016). Multiscale evaluation of thermal dependence in the glucocorticoid response of vertebrates. *The American Naturalist*, *188*(3), 342–356. https://doi.org/10.1086/687588

Jimeno, B., Hau, M., & Verhulst, S. (2018). Glucocorticoid-temperature association is shaped by foraging costs in individual zebra finches. *Journal of Experimental Biology*, *221*(23), jeb187880. https://doi.org/10.1242/jeb.187880

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flügge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M., & Fuchs, E. (2011). Stress revisited: A critical evaluation of the stress concept. *Neuroscience & Biobehavioral Reviews*, *35*(5), 1291–1301. https://doi.org/10.1016/j.neubiorev.2011.02.003

Krause, J. S., Pérez, J. H., Chmura, H. E., Meddle, S. L., Hunt, K. E., Gough, L., Boelman, N., & Wingfield, J. C. (2018). Weathering the storm: Do arctic blizzards cause repeatable changes in stress physiology and body condition in breeding songbirds? *General and Comparative Endocrinology*, *267*, 183–192. https://doi.org/10.1016/j.ygcen.2018.07.004

La Sorte, F. A., Johnston, A., & Ault, T. R. (2021). Global trends in the frequency and duration of temperature extremes. *Climatic Change*, *166*(1–2), 1. https://doi.org/10.1007/s10584-021-03094-0

Landys, M. M., Ramenofsky, M., & Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, *148*(2), 132–149. https://doi.org/10.1016/j.ygcen.2006.02.013

Lattin, C. R., & Romero, L. M. (2014). Chronic stress alters concentrations of corticosterone receptors in a tissue-specific manner in wild house sparrows (*Passer domesticus*). *Journal of Experimental Biology*, *217*(14), 2601–2608. https://doi.org/10.1242/jeb.103788

Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. *Bioscience*, *48*(1), 25–34.

Luttbeg, B., Beaty, L. E., Ambardar, M., & Grindstaff, J. L. (2021). Mathematical modeling reveals how the speed of endocrine regulation should affect baseline and stress-induced glucocorticoid levels. *Hormones and Behavior*, *136*, 105059. https://doi.org/10.1016/j.yhbeh.2021.105059

Lynn, S. E., & Kern, M. D. (2017). Ecologically relevant cooling early in life alters prefledging adrenocortical response in free-living songbirds. *Physiological and Biochemical Zoology*, *90*(1), 118–123. https://doi.org/10.1086/688382

Malkoc, K., Mentesana, L., Casagrande, S., & Hau, M. (2021). Quantifying glucocorticoid plasticity using reaction norm approaches: There still is so much to discover! *Integrative and Comparative Biology*, icab196. https://doi.org/10.1093/icb/icab196

McCarty, J. P. (1995). Effects of short-term changes in environmental conditions on the foraging ecology and reproductive success of tree swallows, *Tachycineta bicolor*.

Nord, A., & Giroud, S. (2020). Lifelong effects of thermal challenges during development in birds and mammals. *Frontiers in Physiology*, *11*, 419. https://doi.org/10.3389/fphys.2020.00419

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), 1–11. https://doi.org/10.1006/gcen.2001.7676

Ouyang, J. Q., Sharp, P. J., Dawson, A., Quetting, M., & Hau, M. (2011). Hormone levels predict individual differences in reproductive success in a passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1717), 2537–2545. https://doi.org/10.1098/rspb.2010.2490

Paskitti, M. E., McCreary, B. J., & Herman, J. P. (2000). Stress regulation of adrenocorticosteroid receptor gene transcription and mRNA expression in rat hippocampus: Time-course analysis. *Molecular Brain Research*, *80*(2), 142–152.

Patterson, S. H., Hahn, T. P., Cornelius, J. M., & Breuner, C. W. (2014). Natural selection and glucocorticoid physiology. *Journal of Evolutionary Biology*, *27*(2), 259–274. https://doi.org/10.1111/jeb.12286

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Racic, A., Tylan, C., & Langkilde, T. (2020). Effects of temperature on plasma corticosterone in a native lizard. *Scientific Reports*, *10*(1), 16315. https://doi.org/10.1038/s41598-020-73354-z

Riddell, E. A., Iknayan, K. J., Wolf, B. O., Sinervo, B., & Beissinger, S. R. (2019). Cooling requirements fueled the collapse of a desert bird community from climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(43), 21609–21615. https://doi.org/10.1073/pnas.1908791116

Romero, L. M. (2004). Physiological stress in ecology: Lessons from biomedical research. *Trends in Ecology & Evolution*, *19*(5), 249–255. https://doi.org/10.1016/j.tree.2004.03.008

Romero, L. M., Dickens, M. J., & Cyr, N. E. (2009). The reactive scope model—A new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior*, *55*(3), 375–389. https://doi.org/10.1016/j.yhbeh.2008.12.009

Romero, L. M., Reed, J. M., & Wingfield, J. C. (2000). Effects of weather on corticosterone responses in wild free-living passerine birds. *General and Comparative Endocrinology*, *118*(1), 113–122. https://doi.org/10.1006/gcen.1999.7446

Rose, N. L., Yang, H., Turner, S. D., & Simpson, G. L. (2012). An assessment of the mechanisms for the transfer of lead and mercury from atmospherically contaminated organic soils to lake sediments with

particular reference to Scotland, UK. *Geochimica et Cosmochimica Acta*, *82*, 113–135. https://doi.org/10.1016/j.gca.2010.12.026

Ruuskanen, S., Hsu, B.-Y., & Nord, A. (2021). Endocrinology of thermoregulation in birds in a changing climate. *Molecular and Cellular Endocrinology*, *519*, 111088. https://doi.org/10.1016/j.mce.2020.111088

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89.

Shipley, J. R., Twining, C. W., Taff, C. C., Vitousek, M. N., Flack, A., & Winkler, D. W. (2020). Birds advancing lay dates with warming springs face greater risk of chick mortality. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(41), 25590–25594. https://doi.org/10.1073/pnas.2009864117

Stedman, J. M., Hallinger, K. K., Winkler, D. W., & Vitousek, M. N. (2017). Heritable variation in circulating glucocorticoids and endocrine flexibility in a free-living songbird. *Journal of Evolutionary Biology*, *30*, 1724–1735. https://doi.org/10.1111/jeb.13135

Taff, C. (2021). Functions for simulating data and designing studies of physiological flexibility in the acute glucocorticoid response to stressors [Preprint]. *EcoEvoRxiv*, https://doi.org/10.32942/osf.io/fk29q

Taff, C. C., & Vitousek, M. N. (2016). Endocrine flexibility: Optimizing phenotypes in a dynamic world? *Trends in Ecology & Evolution*, *31*(6), 476–488. https://doi.org/10.1016/j.tree.2016.03.005

Taff, C. C., Wingfield, J. C., & Vitousek, M. N. (2021). Environmental variability and longevity predict the speed of the acute glucocorticoid response across birds [preprint]. *Physiology*, https://doi.org/10.1101/2021.10.18.464833

Taff, C. C., Zimmer, C., & Vitousek, M. N. (2018). Efficacy of negative feedback in the HPA axis predicts recovery from acute challenges. *Biology Letters*, *14*(7), 20180131. https://doi.org/10.1098/rsbl.2018.0131

Taff, C. C., Zimmer, C., & Vitousek, M. N. (2019). Achromatic plumage brightness predicts stress resilience and social interactions in tree swallows (*Tachycineta bicolor*). *Behavioral Ecology*, *30*(3), 733–745. https://doi.org/10.1093/beheco/arz010

Uehling, J. J., Taff, C. C., Winkler, D. W., & Vitousek, M. N. (2020). Developmental temperature predicts the adult response to stressors in a free-living passerine. *Journal of Animal Ecology*, *89*(3), 842–854. https://doi.org/10.1111/1365-2656.13137

Ursin, H., & Eriksen, H. R. (2004). The cognitive activation theory of stress. *Psychoneuroendocrinology*, *29*(5), 567–592. https://doi.org/10.1016/S0306-4530(03)00091-X

Vitousek, M. N., Houtz, J. L., Pipkin, M. A., Chang van Oordt, D. A., Hallinger, K. K., Uehling, J. U., Zimmer, C., & Taff, C. C. (2022). Data from: Natural and experimental cold exposure in adulthood increase the sensitivity to future stressors in a free-living songbird. *Zenodo*, https://doi.org/10.5281/zenodo.6822541

Vitousek, M. N., Johnson, M. A., Downs, C. J., Miller, E. T., Martin, L. B., Francis, C. D., Donald, J. W., Fuxjager, M. J., Goymann, W., Hau, M., Husak, J. F., Kircher, B. K., Knapp, R., Schoenle, L. A., & Williams, T. D. (2019). Macroevolutionary patterning in glucocorticoids suggests different selective pressures shape baseline and stress-induced levels. *The American Naturalist*, *193*(6), 866–880. https://doi.org/10.1086/703112

Vitousek, M. N., Taff, C. C., Ardia, D. R., Stedman, J. M., Zimmer, C., Salzman, T. C., & Winkler, D. W. (2018). The lingering impact of stress: Brief acute glucocorticoid exposure has sustained, dose-dependent effects on reproduction. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1882), 20180722. https://doi.org/10.1098/rspb.2018.0722

Vitousek, M. N., Taff, C. C., Hallinger, K. K., Zimmer, C., & Winkler, D. W. (2018). Hormones and fitness: Evidence for trade-offs in glucocorticoid regulation across contexts. *Frontiers in Ecology and Evolution*, *6*, 1–14. https://doi.org/10.3389/fevo.2018.00042

Wingfield, J. C. (2003). Control of behavioural strategies for capricious environments. *Animal Behaviour*, *66*(5), 807–816. https://doi.org/10.1006/anbe.2003.2298

Wingfield, J. C., Kelley, J. P., & Angelier, F. (2011). What are extreme environmental conditions and how do organisms cope with them? *Current Zoology*, *57*(3), 363–374. https://doi.org/10.1093/czoolo/57.3.363

Wingfield, J. C., Pérez, J. H., Krause, J. S., Word, K. R., González-Gómez, P. L., Lisovski, S., & Chmura, H. E. (2017). How birds cope physiologically and behaviourally with extreme climatic events. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1723), 20160140. https://doi.org/10.1098/rstb.2016.0140

Wingfield, J. C., & Ramenofsky, M. (2011). Hormone-behavior interrelationships of birds in response to weather. *Advances in the Study of Behavior*, *43*, 93–188.

Wingfield, J. C., & Sapolsky, R. M. (2003). Reproduction and resistance to stress: When and how. *Journal of Neuroendocrinology*, *15*(8), 711–724. https://doi.org/10.1046/j.1365-2826.2003.01033.x/full

Winkler, D. W., Hallinger, K. K., Ardia, D. R., Robertson, R. J., Stutchbury, B. J., & Cohen, R. R. (2020). Tree swallow *Tachycineta bicolor*, version 1.0. In A. F. Poole (Ed.), *Birds of the world*. Cornell Lab of Ornithology.

Winkler, D. W., Luo, M. K., & Rakhimberdiev, E. (2013). Temperature effects on food supply and chick mortality in tree swallows (*Tachycineta bicolor*). *Oecologia*, *173*(1), 129–138. https://doi.org/10.1007/s00442-013-2605-z

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models: Estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*(1), 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Zanette, L. Y., & Clinchy, M. (2020). Ecology and neurobiology of fear in free-living wildlife. *Annual Review of Ecology, Evolution, and Systematics*, *51*(1), 297–318. https://doi.org/10.1146/annurev-ecolsys-011720-124613

Zimmer, C., Taff, C. C., Ardia, D. R., Rose, A. P., Aborn, D. A., Johnson, L. S., & Vitousek, M. N. (2020). Environmental unpredictability shapes glucocorticoid regulation across populations of tree swallows. *Scientific Reports*, *10*, 13682.

Zimmer, C., Taff, C. C., Ardia, D. R., Ryan, T. A., Winkler, D. W., & Vitousek, M. N. (2019). On again, off again: Acute stress response and negative feedback together predict resilience to experimental challenges. *Functional Ecology*, *33*(4), 619–628. https://doi.org/10.1111/1365-2435.13281

How to cite this article: Vitousek, M. N., Houtz, J. L., Pipkin, M. A., Chang van Oordt, D. A., Hallinger, K. K., Uehling, J. J., Zimmer, C., & Taff, C. C. (2022). Natural and experimental cold exposure in adulthood increase the sensitivity to future stressors in a free-living songbird. *Functional Ecology*, *00*, 1–13. https://doi.org/10.1111/1365-2435.14144