

Long-Term Measures of Climate Unpredictability Shape the Avian Endocrine Stress Axis

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ABSTRACT: The vertebrate glucocorticoid stress response is an important mechanism facilitating pleiotropic phenotypic adjustments for coping with environmental change and optimizing fitness. Although circulating glucocorticoid hormones are mediators of plasticity that individuals can adjust rapidly in response to environmental challenges, they are also shaped by ecological selection. It remains unclear, however, how environmental variation on different timescales influences glucocorticoids. Here, we use an intraspecific comparative approach to determine how variation in precipitation on different timescales (months, years, decades) shapes distinct components of the glucocorticoid response. We sampled superb starlings (*Lamprolornis superbus*) at eight sites across Kenya in multiple years that differed in precipitation. Among-population variation in baseline glucocorticoids was shaped by both short- and long-term precipitation, whereas variation in stress-induced levels was poorly explained by precipitation on any timescale. Adrenal sensitivity, quantified via adrenocorticotrophic hormone injections, was shaped by long-term precipitation and was highest in unpredictable environments. Together, these results suggest that variation in glucocorticoids can be best explained by environmental variation at timescales that extend beyond the lives of individuals, although baseline glucocorticoids also reflect short-term environmental conditions. Patterns of long-term precipitation may represent a microevolutionary selective pressure shaping the endocrine stress axis across populations and influencing how individuals cope with environmental change.

Keywords: corticosterone, environmental change, climatic variation, comparative physiology, evolutionary endocrinology, Colwell's P.

Introduction

Since no habitat on earth is entirely constant, organisms must respond to environmental changes and challenges throughout their lives in order to optimize their fitness

(Charmantier et al. 2008; Canale and Henry 2010). Responding appropriately to environmental change can be difficult, however, and doing so requires an intricate adjustment of multiple phenotypes (Langerhans and DeWitt 2002; Piersma and Drent 2003). The vertebrate hypothalamic-pituitary-adrenal (HPA) axis is an endocrine cascade that regulates glucocorticoid responses to stressors, which in turn coordinates pleiotropic phenotypic adjustments under challenging environmental conditions (Hau and Goymann 2015; Hau et al. 2016; Wingfield 2018). Glucocorticoids (e.g., cortisol or corticosterone) are central regulators of several critical functions in vertebrates (Wingfield and Kitaysky 2002; Nicolaides et al. 2014). For example, glucocorticoids circulating at relatively low baseline concentrations regulate daily metabolic requirements (Chmura et al. 2016; Hennin et al. 2016) and can be upregulated rapidly and drastically for coping with sudden perturbations or stressors (Angelier and Wingfield 2013). In this way, glucocorticoids are thought to be critical for both maintaining homeostasis and returning to homeostasis following intrinsic or extrinsic challenges (Wingfield and Kitaysky 2002). Since glucocorticoids represent a key link between changing environmental conditions and optimal phenotypic adjustments that promote fitness (Kitaysky et al. 2007; Bonier et al. 2009; Wingfield 2013, 2018; Patterson et al. 2014), they are considered to be a plastic trait themselves (Piersma and Drent 2003)—an individual can adjust circulating levels rapidly in response to environmental change (Hau and Goymann 2015; Guindre-Parker 2020). Despite their plasticity on relatively short timescales—for example, a glucocorticoid stress response lasts only hours before returning to baseline—like all traits, glucocorticoids are also shaped by natural selection and are thus known to be influenced by longer-term patterns of environmental variation (Patterson et al. 2014; Cox et al. 2016; Guindre-Parker 2018).

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Yet it remains largely unknown how different timescales of environmental variation influence glucocorticoid variation and shape the evolution of the vertebrate HPA axis.

Although a number of interspecific comparative studies have shown that glucocorticoids are shaped by ecological selection, patterns of long-term variation in abiotic conditions explain only a small fraction of among-species variation in circulating hormone concentrations relative to the effects of life history (Vitousek et al. 2019a). Since both environmental conditions and life-history traits tend to covary with latitude on a global scale (Cardillo 2002)—and since both covary with glucocorticoids (Crespi et al. 2013)—it remains difficult to disentangle the relative strengths of their contributions to shaping glucocorticoid variation. One promising but rarely used approach to circumvent this issue is to study intraspecific variation in glucocorticoids across an environmental gradient, since doing so can better control for life history and isolate the role of environmental conditions across different timescales in shaping endocrine variation (Boonstra 2005; Guindre-Parker 2018).

Here, we examined whether glucocorticoids reflect variation in precipitation across different ecological timescales within a single species sampled across a broad environmental gradient ranging from desert to savanna to forest habitats. By surveying glucocorticoids across different populations of the same species that experience different precipitation regimes, we aimed to identify the extent to which environmental conditions that vary on different timescales shape different components of the vertebrate glucocorticoid stress response (Guindre-Parker 2018; Vitousek et al. 2018). We sampled superb starlings (*Lamprotornis superbus*) at eight locations in Kenya that spanned a gradient of variation in total precipitation as well as in the temporal variability of precipitation over both short (intra- and interannual) and long (decadal) timescales. Therefore, in addition to identifying the temporal scale over which precipitation shapes glucocorticoids most strongly, our data set also allows us to determine the extent to which glucocorticoids are shaped by mean differences in precipitation across sites compared with differences in fluctuations in precipitation, which represent alternative types of selection pressures that may shape endocrine traits differently (Fokidis et al. 2012; Wingfield 2013; Taborsky et al. 2021). We focused specifically on precipitation because it is the primary driver of variation in food availability for insectivorous organisms in East Africa (i.e., insect prey emergence increases after precipitation; Denlinger 1980) and because precipitation variation has been shown to influence HPA activity in this species (Rubenstein 2007). In addition to shaping variation in prey availability, precipitation may also shape the prevalence of parasites or predators (McCauley et al. 2006; Byrom et al. 2014; Titcomb et al. 2017; Weinstein et al.

2017), which could in turn shape glucocorticoid stress responses (Fischer et al. 2014; O'Dwyer et al. 2020).

The lack of consensus on what timescale environmental conditions most strongly shape variation in the HPA axis may stem from the fact that glucocorticoids control several critical functions in vertebrates (Wingfield and Kitaysky 2002). We expected that different components of the glucocorticoid stress response would respond to precipitation on distinct timescales because of their discrete downstream pleiotropic effects. Since baseline glucocorticoids regulate daily metabolic demand and energy acquisition (Landys 2004; Landys et al. 2006), where elevated baseline glucocorticoids support increased foraging and the acquisition/deposition of fat reserves (Dallman et al. 1993; Hennin et al. 2016), we hypothesized that baseline glucocorticoids would more strongly reflect short-term patterns of precipitation, as seasonal plasticity in baseline glucocorticoids is thought to be critical to optimize resources across life-history stages and throughout the year (Romero et al. 2009). Starlings experiencing higher short-term precipitation—which increases food availability—should show reduced baseline glucocorticoids. In contrast, stress-induced glucocorticoids initiate an emergency life-history stage following a perturbation or departure from homeostasis (Wingfield and Kitaysky 2002; McEwen and Wingfield 2003) that results in the reallocation of resources to promote survival (although often at the expense of reproduction; Almasi et al. 2013). Similarly to baseline glucocorticoids, we therefore hypothesized that stress-induced glucocorticoids would show high seasonal plasticity and would more strongly reflect short-term patterns of precipitation (Romero et al. 2008, 2009). Individuals from sites that are more variable or seasonal in the short term should mount elevated glucocorticoid stress responses. Finally, the HPA axis secretes glucocorticoids in response to adrenocorticotrophic hormone (ACTH), such that flooding the HPA axis using a standardized injection of ACTH can experimentally upregulate glucocorticoid production. Glucocorticoids secreted in response to an ACTH injection provide additional insight, as they can differ from stress-induced glucocorticoids. That is, while stress-induced glucocorticoids can vary within and among individuals according to stressor type (Canoine et al. 2002) or life-history stage (e.g., during reproduction; Vitousek et al. 2019b), ACTH-induced glucocorticoids should represent a more standardized measure of adrenal sensitivity that is not dependent on context. We hypothesized that ACTH-induced glucocorticoids would more strongly reflect long-term patterns of precipitation, since this represents a physiological ceiling that would be less plastic than the glucocorticoid stress response itself. Animals experiencing more variable or unpredictable long-term patterns of precipitation should have elevated ACTH-induced glucocorticoids.

Material and Methods

Field Sampling

We sampled glucocorticoids from nonbreeding adult superb starlings across eight sites in Kenya (for a map of study sites, see fig. S1; figs. S1–S4 are available online), targeting locations that differed in both their mean annual precipitation and their interannual variability in precipitation (fig. 1). While our predictions could be tested at any life-history stage, we restricted our sampling to the nonbreeding season, when precipitation is relatively low (Rubenstein 2011). To disentangle the influence of short- versus long-term precipitation on glucocorticoids, we revisited each site from two to four times across different years that varied in annual precipitation from 2014 to 2017. Sampling took place between June and September, depending on the year. We expected that individuals sampled at these sites have experienced broadly similar environmental conditions over their entire lives because superb starlings are nonmigratory and live year-round on territories defended cooperatively by a social group (Rubenstein 2016). Furthermore, while many adults remain at their natal site for their entire lives (7% of females and 41% males; Guindre-Parker and Rubenstein 2020), any individuals that disperse from their natal territory do so in a single short-distance dispersal event. For example, the nearest pair of sites were 43 km apart (Mpala and Nanyuki), which is more than twice the distance of the longest known dispersal event for a superb starling over

a 20-year study (<20 km; W. Nderitu, personal observation). Thus, both short- and long-term measures of precipitation at these sites should reflect the conditions experienced by individual starlings sampled in our study accurately.

We captured 342 birds using minimally food-baited ground traps over the course of this study (a breakdown of sample sizes are available in table S1; tables S1–S3 are available online) and followed a serial blood sampling protocol (see fig. S2). From each individual, we collected a first blood sample from the brachial vein rapidly after capture to measure baseline glucocorticoids (mean time to collection \pm SE was 133 ± 1.7 s, ranging from 58 to 210 s; all but four samples were collected within 180 s). A subset of this sample was preserved in queen's lysis buffer for genetic sexing as described previously (Rubenstein 2005). Birds were then restrained in cloth bags for 30 min before a second blood sample was collected to measure stress-induced glucocorticoids. Next, we injected birds with 5 mg/kg dexamethasone (DEX; product D2915, Sigma-Aldrich, Saint Louis, MO) intramuscularly and restrained individuals in the cloth bag for 30 min before a third blood sample was collected to measure DEX-induced glucocorticoids, a measure of the HPA axis's negative feedback efficiency (Lattin and Kelly 2020). Finally, we injected birds intramuscularly with 100 IU/kg ACTH (product A6303, Sigma-Aldrich) and restrained animals in the cloth bag for 15 min before a fourth blood sample was collected to measure ACTH-induced glucocorticoids, a standardized index of adrenal sensitivity. Although we were

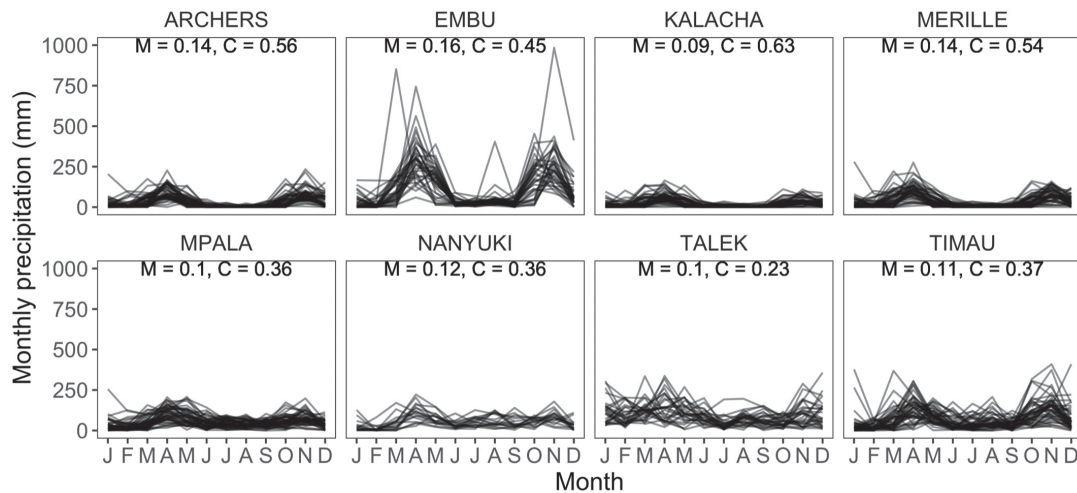


Figure 1: Monthly precipitation across the eight Kenyan study sites, where each line represents one year. Contingency (M) and constancy (C) values are included at the top of each panel. Higher contingency values indicate more seasonal environments (e.g., Embu, the wettest site, is highly seasonal, with two peaks in precipitation around April and November of every year). Higher constancy values indicate a more constant environment from year to year (e.g., Kalacha, the driest site, is very constant from year to year and the lines overlap much more closely compared with Talek, where precipitation changes often among years and precipitation across years does not follow a similar pattern).

unable to fully validate this protocol because of sample size constraints, pilot data collected in 2010 revealed a significant increase in circulating glucocorticoids from stress-induced to ACTH-induced titers following our protocol (mean increase in glucocorticoids = 16.2 ± 3.5 ng/mL; paired *t*-test comparing pre- and postinjection titers: $t = -4.6$, $df = 19$, $P = .0002$).

For both types of hormone injections, the correct dose for a 65-g bird (mean mass of superb starlings) was diluted in saline, and 220 μ L was injected in the pectoral muscle (on opposite sides for DEX and ACTH). Our preliminary results indicated that superb starlings did not respond to the DEX injection within 30 min, despite being given a relatively high dose (Fokidis and Deviche 2011). We note that the time interval between injection and blood sample collection is on the shorter end of typical DEX-injection protocols in birds, and while negative feedback can be observed within 30 min for many species (Fokidis and Deviche 2011; Lattin et al. 2012), others require a longer interval (Baugh et al. 2017), which we were unable to test. An alternative possibility is that superb starlings may be DEX resistant, as has been found in a several other species (Brooke et al. 1994; Hik et al. 2001). Importantly, we confirmed experimentally that the DEX injection did not influence the results of the subsequent ACTH injection (see the supplemental PDF, available online). Blood samples were centrifuged in the field, and plasma was flash-frozen at -80°C . After the serial blood col-

lection protocol, each animal was marked with a unique numbered metal leg band, and we measured tarsus length and body mass to assess body condition (residuals of mass on tarsus). Overall, we sampled 13.2 ± 0.46 individuals per site per year (mean \pm SE) at an approximately even sex ratio ($56\% \pm 2.6\%$ of birds were male; mean \pm SE).

Precipitation Measurements

Monthly precipitation data were obtained from several sources, including the Kenyan Meteorological Department, local weather stations (permanent or volunteer run; Caylor et al. 2017), or the East African Livestock Early Warning System (satellite data processed via the National Oceanic and Atmospheric Administration's African rainfall estimation algorithm [ver. 2.0]; Stuth et al. 2003; Matere et al. 2019). When precipitation data were available from more than one source during the same month and for the same site, the data sets were averaged. Importantly, we found that different data sets for the same site were tightly correlated (see the supplemental PDF).

From these monthly precipitation data sets, we characterized indices of (i) total precipitation, (ii) variation in precipitation, and (iii) predictability in precipitation across multiple timescales (i.e., monthly, annual, decadal) for each site, as described in table 1. Monthly measures of precipitation included monthly precipitation and deviation in monthly

Table 1: Glossary of the precipitation measurements calculated for all eight Kenyan study sites

Timescale, precipitation variable	Acronym	Definition
Monthly:		
Monthly precipitation	TMP	Total monthly precipitation during the calendar month of sampling
Deviation in monthly precipitation	TMP deviation	Difference between total monthly precipitation and the long-term mean monthly precipitation during the month of sampling calculated across all years of precipitation data
Annual:		
Total annual precipitation	TAP	Total annual precipitation during the calendar year of sampling
Variation in annual precipitation	Variation in TMP	Coefficient of variation in total monthly precipitation during the year of sampling (i.e., month-to-month variation in precipitation during the year of sampling)
Deviation in annual precipitation	TAP deviation	Difference between total annual precipitation and the long-term annual precipitation calculated across all years of precipitation data
Decadal:		
Mean annual precipitation	MAP	Long-term mean annual precipitation across all years of precipitation data
Long-term variation in precipitation	Variation in TAP	Coefficient of variation in total annual precipitation across all years (i.e., year-to-year variation in annual precipitation)
Contingency (Colwell's <i>M</i>)	Contingency	Degree to which precipitation depends on earlier state (i.e., seasonality)
Constancy (Colwell's <i>C</i>)	Constancy	Uniformity in state across years (i.e., inverse of year-to-year variability)

precipitation from the long-term mean. Annual measures of precipitation included total annual precipitation, annual variation in precipitation, and deviation in annual precipitation from the long-term mean. Decadal measures of precipitation included mean total annual precipitation and variation in precipitation among years (where data sets ranged from 17 to 47 years for different sites) as well as two measures that make up the predictability of precipitation (Colwell 1974): (i) contingency (M), a measure of precipitation seasonality, and (ii) constancy (C), a measure of precipitation uniformity among years or the inverse of year-to-year variability (fig. 1). Each of these measures is explained in greater detail in table 1. When monthly precipitation measurements were missing for one or more months in a year, we calculated the long-term average precipitation for that month at that site and input it in place of the missing value in order to be able to calculate short-term measures of precipitation.

Glucocorticoid Assay

We measured glucocorticoids using a commercially available enzyme immunoassay (Enzo Life Sciences, Farmingdale, NY). We followed the kit protocol that has been validated previously for this species (Guindre-Parker and Rubenstein 2018). In brief, we added equal parts of 1% steroid displacement buffer for 5 min to thawed plasma samples before diluting them 1:10 in assay buffer. Each plate included a standard curve (range = 32 to 20,000 pg/mL) and two blank controls. We ran samples in duplicate within 6 months of field collection, randomizing positions on the plate. We read the absorbance at 405 nm using a plate reader and calculated glucocorticoids in each sample relative to the standard curve in nanograms per milliliter. The intra- and interassay coefficients of variation were 3.1% and 5.0%, respectively. Baseline, stress-induced, and ACTH-induced glucocorticoids covaried positively (ρ ranged between 0.55 and 0.66 in pairwise Spearman's rank correlation tests). We log transformed baseline, stress-induced, and ACTH-induced glucocorticoids for all statistical analyses.

Statistical Analyses

To identify the top environmental predictors of different glucocorticoid measures (i.e., baseline, stress induced, or ACTH induced), we used an information theoretic approach to compare five candidate models. The first candidate model was a null model controlling for time of day of sampling, Julian date of sampling, year of sampling, sex, and body condition (residuals of mass on tarsus). The null model did not include any measures of precipitation. In the baseline glucocorticoid null model, the time it took to collect the first blood sample since capture was also in-

cluded as a covariate, since glucocorticoids begin to increase after capture even in samples collected in under 3 min (Small et al. 2017). The next four candidate models built on the null model by adding the following environmental predictor variables: (i) a monthly model included monthly precipitation and deviation in monthly precipitation; (ii) an annual model included total annual precipitation, annual variation in precipitation, and deviation in annual precipitation; (iii) a decadal model included mean total annual precipitation and long-term variation in precipitation; and (iv) a predictability model included contingency (seasonality) and constancy (inverse of year-to-year variability), both long-term measures of climate variability (Colwell 1974; Rubenstein and Lovette 2007). We identified the top model(s) on the basis of the Akaike information criterion correcting for small sample sizes (AICc; Akaike et al. 1973). We considered all models within 2 AICc values from the top-ranked model to fit our data set equally well. For each identified top model, we present the estimates and confidence intervals for each predictor variable via a coefficient plot. All continuous variables were standardized using z -scores prior to analysis. Variance inflation factors (VIFs) indicated that multicollinearity was unlikely to be an issue (VIFs ≤ 3.2 ; although see O'Brien 2007). Diagnostic plots indicated that model residuals appeared normally distributed. All analyses were performed in RStudio (ver. 1.2.1335); the package hydrostats (ver. 0.2.7) was used to calculate Colwell's contingency and constancy, and the package bbmle (ver. 1.0.20) was used to compute AICc scores and model weights.

Results

Our information theoretic approach indicated that three candidate models explained variance in baseline glucocorticoids, with a combined weight of 84%: the decadal model had the lowest AICc score, but the predictability and monthly models were within 2 AICc values of the top model (table 2; fig. 2A). This result suggests that short- and long-term patterns of precipitation contribute to shaping baseline glucocorticoids similarly well. Baseline glucocorticoids were positively correlated with deviation in total monthly precipitation and the long-term mean annual precipitation but were negatively correlated with long-term constancy in precipitation from year to year (fig. 3). Additionally, baseline glucocorticoids were higher when sample collection took more time, occurred earlier in the day, occurred later in the nonbreeding season, and varied among years but were unrelated to body condition or sex (fig. 2A). These results suggest that measures of both short- and long-term precipitation contribute to shaping among-population variation in baseline glucocorticoids.

Table 2: Comparison of candidate linear models to identify environmental predictors of three endocrine traits: baseline glucocorticoids, stress-induced glucocorticoids, and adrenocorticotropic hormone (ACTH)-induced glucocorticoids

Dependent variable, model name	Fixed effects in model	AICc	Δ AICc	Weight	df
Baseline glucocorticoids:					
Null	Julian date + year + capture time + body condition + sex + time to sample	902.8	2.3	.131	10
Monthly	Null + TMP + TMP deviation	902.0	1.4	.236	12
Annual	Null + TAP + variation in TMP + TAP deviation	905.9	5.3	.029	13
Decadal	Null + MAP + variation in TAP	900.6	.0	.406	12
Predictability	Null + contingency + constancy	901.7	1.1	.236	12
Stress-induced glucocorticoids:					
Null	Julian date + year + capture time + body condition + sex	515.5	.5	.274	9
Monthly	Null + TMP + TMP deviation	515.7	.8	.241	11
Annual	Null + TAP + variation in TMP + TAP deviation	520.1	5.2	.027	12
Decadal	Null + MAP + variation in TAP	515.0	.0	.354	11
Predictability	Null + contingency + constancy	517.4	2.4	.104	11
ACTH-induced glucocorticoids:					
Null	Julian date + year + capture time + body condition + sex	423.9	7.3	.024	9
Monthly	Null + TMP + TMP deviation	424.7	8.0	.017	11
Annual	Null + TAP + variation in TMP + TAP deviation	424.2	7.6	.021	12
Decadal	Null + MAP + variation in TAP	422.8	6.2	.041	11
Predictability	Null + contingency + constancy	416.7	.0	.898	11

Note: For each candidate model, we outline the predictors included as fixed effects (where continuous variables were standardized), and we present the Akaike information criterion correcting for small sample sizes (AICc) value, the difference in AICc value relative to the top model (Δ AICc), the model weight, and degrees of freedom. All models within 2 AICc scores of the top model are shown in boldface and can be interpreted to fit the data set equally well. TMP is the total monthly precipitation during the calendar month of sampling. TAP is the total annual precipitation during the calendar year of sampling. MAP is the mean annual precipitation. Contingency is the degree to which precipitation depends on an earlier state (i.e., seasonality). Constancy is the uniformity in state across years (i.e., the inverse of unpredictability in among-year precipitation).

We also found that multiple models explained stress-induced glucocorticoids equally well (i.e., within 2 AICc values from the top-ranked model) but that the null model was included in these top-ranked models. In other words, while the monthly and decadal precipitation models were among the top models—with model weights of 24.1% and 35.4%, respectively—the null model explained variance in our data set equally well with a weight of 27.4%. This suggests that our selected measures of mean short-term precipitation, mean long-term precipitation, and predictability in precipitation do not adequately explain among-population stress-induced glucocorticoids even if some of the individual predictors from these models are significantly correlated with stress-induced glucocorticoids (see fig. 2B). Stress-induced glucocorticoids were higher earlier in the day and were positively correlated with long-term mean annual precipitation.

Finally, we identified a single top-ranked model that best explained variance in ACTH-induced glucocorticoids. The precipitation predictability model had a weight of 89.9% and the lowest AICc score (table 2), suggesting that

long-term patterns of variation in precipitation shape adrenal sensitivity. Specifically, ACTH-induced glucocorticoids were positively correlated with precipitation contingency, such that individuals from more seasonal habitats produced higher concentrations of circulating glucocorticoids in response to the ACTH injection (figs. 2C, 3B). ACTH-induced glucocorticoids were also negatively correlated with precipitation constancy, such that individuals in less predictable environments where precipitation varies more among years produced elevated glucocorticoids following the ACTH injection (figs. 2C, 3C). Additionally, ACTH-induced glucocorticoids were higher earlier in the day but unrelated to date, year, body condition, or sex (fig. 2C).

Discussion

Glucocorticoids serve as a critical link between the environment and an animal's phenotype. Circulating concentrations of glucocorticoids should reflect long-term conditions due to ecological selection but also short-term variation in the abiotic environment as a result of being

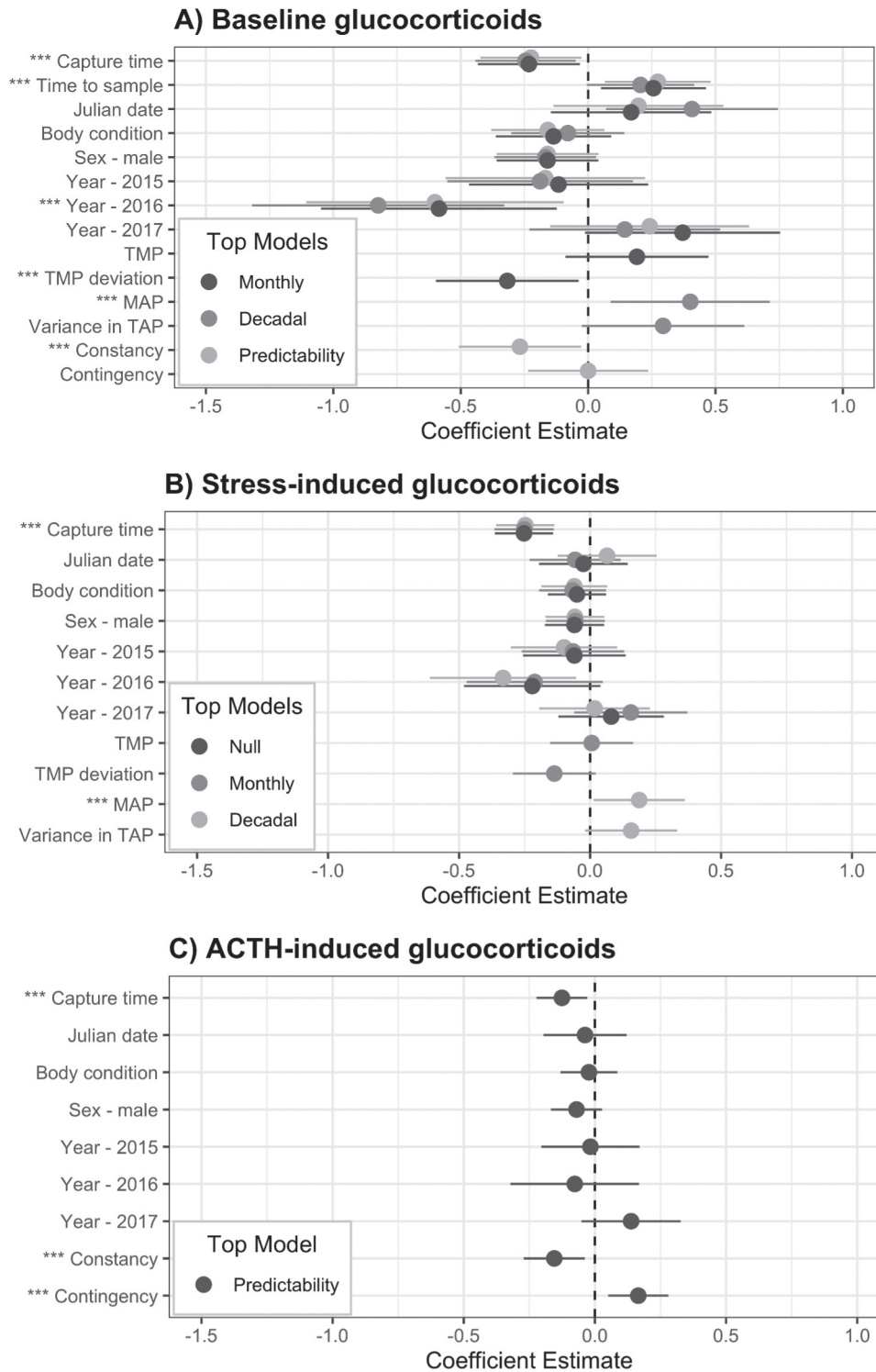


Figure 2: Coefficient estimates with 95% confidence intervals for the top model(s) identified via an information theoretic approach for each of the three dependent variables in our analyses: baseline glucocorticoids (A), stress-induced glucocorticoids (B), and adrenocorticotrophic hormone (ACTH)-induced glucocorticoids (C). Instances where the 95% confidence interval does not overlap with zero indicate significant predictors of each glucocorticoid trait and are noted with asterisks. MAP = mean annual precipitation; TAP = total annual precipitation; TMP = total monthly precipitation.

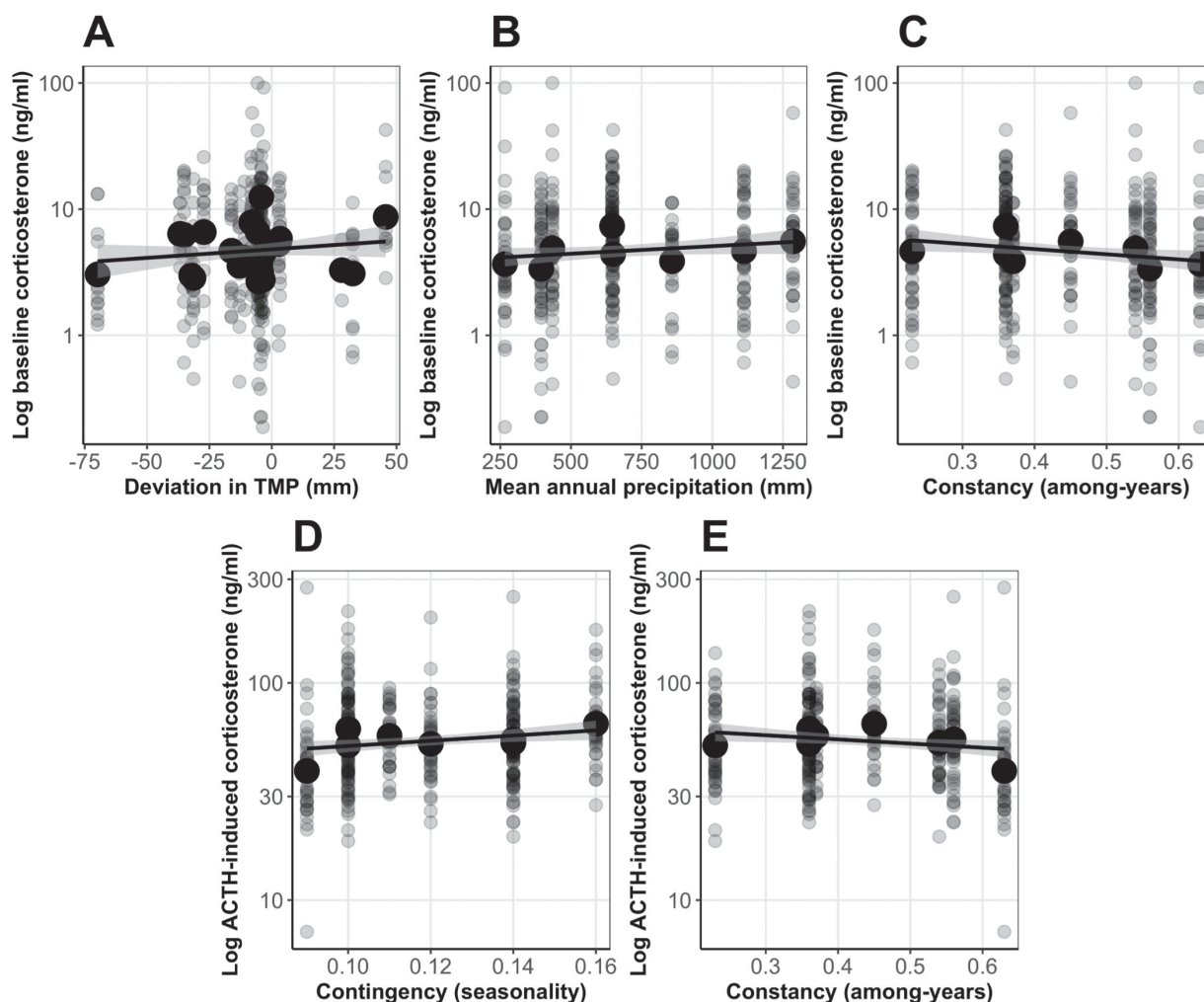


Figure 3: Multiple measures of precipitation predicted glucocorticoids in superb starlings. Baseline glucocorticoids were positively correlated with deviation in total monthly precipitation (TMP; *A*), positively correlated with mean annual precipitation across sites (*B*), and negatively correlated with constancy among years (*C*). Adrenocorticotropic hormone (ACTH)-induced glucocorticoids were positively correlated with seasonality (*D*) but negatively correlated with constancy (*E*) among years. In other words, mean ACTH-induced glucocorticoids are higher for populations that experience more variable precipitation (i.e., more seasonal precipitation within a year or more unpredictable precipitation among years).

such a plastic trait. Here, we investigated the timescale on which glucocorticoids reflect among-population differences in precipitation most strongly. We predicted that baseline and stress-induced glucocorticoids would reflect precipitation on recent timescales, while ACTH-induced glucocorticoids would reflect precipitation over longer periods. We found that both short- and long-term precipitation explained variation in baseline glucocorticoids, while long-term precipitation shaped ACTH-induced concentrations across starling populations. Conversely, precipitation on any timescale was not a better predictor of stress-induced glucocorticoids than the null model in our study. Interestingly, baseline glucocorticoids were correlated with monthly

precipitation, decadal precipitation, and predictability of precipitation, whereas ACTH-induced glucocorticoids were most strongly correlated with the predictability of precipitation across decades.

Superb starlings are long-lived for a passerine of their size, with the oldest known individual from our long-term study site reaching 15 years of age (mean life span is closer to 8 years; Guindre-Parker and Rubenstein 2020). Our results suggest that precipitation patterns over many decades—spanning multiple generations of starlings—shape variation in glucocorticoids among populations more strongly than precipitation experienced within the life span of an individual. While daily (Romero et al. 2000; Henderson et al. 2017;

de Bruijn and Romero 2018) or seasonal (Rubenstein 2007) precipitation certainly shape variation in glucocorticoids within individuals or among individuals of the same population, among-population intraspecific variation in glucocorticoids may be best explained by environmental conditions on longer timescales. Patterns of long-term precipitation may thus represent a microevolutionary selective pressure shaping glucocorticoids in nonbreeding males and females across populations. While we did not study superb starlings during the breeding season, it is possible that different ecological selective pressures would shape glucocorticoids in breeding versus nonbreeding individuals (Vitousek et al. 2019a).

Few other intraspecific comparative studies have explored the relationship between abiotic environmental conditions and among-population variation in glucocorticoids across timescales. Zimmer et al. (2020) showed that female tree swallows (*Tachycineta bicolor*) from populations facing increased among-year variability in breeding temperatures had elevated stress-induced glucocorticoids. This finding is similar to the pattern observed in ACTH-induced glucocorticoids in our study, where both superb starling and tree swallow individuals from populations found in more variable or less predictable environments are able to mount stronger responses. To our knowledge, the tree swallow study examined the effect of environmental conditions on glucocorticoids using a single timescale, so it remains unclear whether mean or variability in temperature on shorter timescales would also explain glucocorticoid variation among swallow populations. Similarly, fecal glucocorticoid metabolites were elevated in alpine chipmunk (*Tamias alpinus*) populations facing higher daily maximum temperatures at capture (Hammond et al. 2018). Interannual differences in glucocorticoids, likely caused by among-year variation in snow depth or median annual temperatures, suggest that abiotic conditions on both daily and annual timescales shape glucocorticoids in alpine chipmunks, although the relative importance of each timescale remains unknown (Hammond et al. 2018).

Our study adds an important and overlooked perspective on intraspecific variation in HPA axis function to a growing body of interspecific comparative work in evolutionary endocrinology. Interspecific comparative studies in select taxonomic groups—primarily amphibians, reptiles, and birds, for which glucocorticoid data are most readily available—have uncovered important links between glucocorticoids and environmental variation across taxa. For example, glucocorticoids were positively correlated with latitude and negatively correlated with net primary productivity across species of birds (Jessop et al. 2013). Similarly, glucocorticoids in reptiles were positively correlated with net primary productivity (Jessop et al. 2013) but did not covary with latitude or elevation (Eikenaar et al. 2012). In amphibians, gluco-

corticoids were negatively correlated with elevation and positively correlated with latitude (Eikenaar et al. 2012). Finally, glucocorticoids were positively correlated with temperature across species of both reptiles and birds (Jessop et al. 2016). In an interspecific comparative study across vertebrates, glucocorticoids were negatively correlated with precipitation and positively correlated with temperature, although they were not correlated with measures of variability in environmental conditions (Vitousek et al. 2019a). Thus, while environmental conditions and vertebrate life-history traits tend to covary with latitude on a global scale (Cardillo 2002), our study provides important evidence to support the idea that long-term precipitation also shapes variation in glucocorticoids across populations (a result that in our case is not confounded by variation in life-history traits).

Our finding that baseline glucocorticoid concentrations are shaped by short- as well as long-term measures of precipitation supports the roles of both immediate and lasting food availability in shaping intraspecific variation in baseline glucocorticoids. Baseline glucocorticoid concentrations are highly plastic to regulate daily metabolic demands (Landys 2004; Landys et al. 2006), and elevated baseline glucocorticoids are thought to increase the acquisition or deposition of fat reserves (Dallman et al. 1993; Hennin et al. 2016). Our study is consistent with the idea that populations experiencing short-term elevations in monthly precipitation (relative to expected precipitation for that month) have elevated baseline glucocorticoids, which could favor increased foraging during periods of high prey availability. Nevertheless, long-term precipitation also contributed to shaping differences in baseline glucocorticoids among populations, suggesting that mean baseline glucocorticoids may scale with the relative availability and predictability of insect prey items over multiple timescales.

Perhaps one of the most surprising findings of our study was that among-population variation in stress-induced glucocorticoids was not strongly correlated with precipitation. While mean annual precipitation was positively correlated with stress-induced glucocorticoids, this model fit our data set similarly well to the null model that included no measures of precipitation. This result is in contrast to studies of within-individual variation and interspecific variation in stress-induced glucocorticoids, which have both shown that abiotic factors like precipitation or temperature shape the stress response (Jessop et al. 2016; de Bruijn and Romero 2018). While precipitation was correlated with stress-induced glucocorticoid responses in superb starlings (e.g., from looking at individual models), results from our model selection approach suggest that factors beyond those measured in our study may be stronger predictors of stress responses compared with precipitation. This finding is similar to that of a recent interspecific study across all vertebrates

where stress-induced glucocorticoids were correlated with variation in breeding behavior but not with abiotic conditions (Vitousek et al. 2019a). Since superb starlings are highly social animals that breed cooperatively and live year-round in large groups, the social environment may contribute to shaping variation in stress-induced glucocorticoids more strongly than abiotic factors (Creel et al. 2013; Raulo and Dantzer 2018). Unfortunately, we cannot test this hypothesis because we were unable to quantify potential differences in social behavior at each of the study sites. An alternative is that stress-induced glucocorticoids measured via capture and restraint do not reflect precipitation, whereas the response to a different stressor—perhaps a sudden storm or drought—may more strongly reflect environmental conditions across sites (Romero et al. 2000; Canoine et al. 2002). This could explain why stress-induced glucocorticoids were unrelated to precipitation, whereas adrenal sensitivity does reflect long-term precipitation.

Finally, ACTH-induced glucocorticoids were elevated in populations that showed greater unpredictability in precipitation among years as well as greater seasonality in precipitation within a year. Although there remains uncertainty in whether ACTH-induced glucocorticoids in our study are indicative of maximum glucocorticoid secretion, these results suggest that while the general sensitivity of the adrenal glands may be constrained to secrete lower glucocorticoids for individuals living in predictable environments, unpredictability in precipitation may have selected for increased adrenal sensitivity. This supports recent theoretical work finding that the predictability of stressors may shape the evolution of stress responses even more strongly than stressor risk (Taborsky et al. 2021).

In summary, this study examined how precipitation across different temporal scales (monthly, annual, decadal) shaped among-population variation in glucocorticoids in superb starlings. We found that long-term variation in precipitation spanning multiple generations was the strongest predictor of baseline and ACTH-induced glucocorticoids—although shorter-term measures of precipitation also shaped baseline glucocorticoids—which suggests that precipitation and its associated link to food availability in this tropical region is a selective pressure that shapes intraspecific variation in the vertebrate HPA axis. Although researchers often assume that glucocorticoids reflect recent environmental stressors, we must remember that like all traits, glucocorticoids are also shaped by patterns of ecological selection at multiple timescales. To fully understand how organisms will cope in an era of rapid anthropogenic climate change, we must consider the microevolutionary processes that have shaped adaptive coping if we are to predict how organisms will respond physiologically to environmental change.

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Statement of Authorship

S.G.-P. and D.R.R. designed the study and collected the data. S.G.-P. performed laboratory analyses and analyzed the data, and S.G.-P. wrote the manuscript with input from D.R.R.

Data and Code Availability

Data are accessible in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.qz612jmfmm>; Guindre-Parker and Rubenstein 2021).

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