Multiscale Evaluation of Thermal Dependence in the Glucocorticoid Response of Vertebrates

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abstract: Environmental temperature has profound effects on animal physiology, ecology, and evolution. Glucocorticoid (GC) hormones, through effects on phenotypic performance and life history, provide fundamental vertebrate physiological adaptations to environmental variation, yet we lack a comprehensive understanding of how temperature influences GC regulation in vertebrates. Using field studies and meta- and comparative phylogenetic analyses, we investigated how acute change and broadscale variation in temperature correlated with baseline and stress-induced GC levels. Glucocorticoid levels were found to be temperature and taxon dependent, but generally, vertebrates exhibited strong positive correlations with acute changes in temperature. Furthermore, reptile baseline, bird baseline, and capture stress–induced GC levels to some extent covaried with broadscale environmental temperature. Thus, vertebrate GC function appears clearly thermally influenced. However, we caution that lack of detailed knowledge of thermal plasticity, heritability, and the basis for strong phylogenetic signal in GC responses limits our current understanding of the role of GC hormones in species' responses to current and future climate variation.

Keywords: physiological regulation, thermal dependency, steroid hormones, macrophysiology, vertebrates, performance.

Introduction

Environmental temperature is among the most significant agents of natural selection shaping global ecology (Johnston and Bennett 2008; Huey et al. 2009; Chown et al. 2010). From genes to whole organisms, complex physiologies have evolved to maintain homeostasis and optimize fitness across life histories in direct response to environmental temperature variation (Hochachka and Somero 1968, 2002; Cossins and Bowler 1987). To understand the acute effects of temperature at the whole-organism level, it is common to measure the thermal dependence (i.e., reaction norm) of single or multiple traits (e.g., growth, fecundity, metabolism, activity) using thermal performance curves (Huey and Stevenson 1979). Typically, whole-animal performance is maximal over a single intermediate modal temperature before decaying and being bounded to zero at extreme temperatures as physiological dysregulation causes death (Huey and Stevenson 1979; Angilletta 2006). Importantly, considerable genetic variance in thermal dependence curves exists within and among species (Angilletta et al. 2010; Latimer et al. 2011). Such variation is manifest across phenotypes due to differences in proximate mechanisms (e.g., cellular-through systems-level physiologies) and their trade-offs that lead to variation in whole-animal performance responses to temperature.

The thermal dependence of many biochemical and physiological processes and their effect on organismal performance has been well described (Hochachka and Somero 1980; Johnston and Bennett 2008), yet we know surprisingly little about the thermal dependence of high-order physiological regulators, such as steroid hormone activity, that have broad-acting influences on vertebrate function and fitness (Martin et al. 2011; Jessop et al. 2013*a*). For example, glucocorticoid hormones (hereon referred to as GC), notably cortisol and corticosterone, are key circulating steroid hormones of vertebrates synthesized by the adrenal cortex (Romero 2002, 2004). Glucocorticoids, via effects on gene transcription, can regulate up to 10% of an animal's genome, permitting diverse and complex control over behavior, metabolism, reproduction, growth, and immune functions (Wingfield and Sapolsky 2003; Romero 2004; Phuc Le et al. 2005). In particular, GCs are implicated in the

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physiological regulation of organismal tolerances to both capricious and seasonal environmental variation (Wingfield et al. 1988; Wingfield 2005, 2013). Specifically, circulating concentrations of GCs are extremely responsive to the complex interplay between internal and external environmental factors that govern organismal homeostasis (Wingfield et al. 1988; Romero 2004; Schoech et al. 2013). Further, because GCs regulate multiple physiologies/behaviors, they generate trade-offs in competing or correlated traits and, hence, mediate life-history schedules, another key response of organisms to environmental variation (Hau and Wingfield 2011; Crespi et al. 2013). Thus, understanding the thermal dependencies of GC function is important for evaluating physiological regulation and adaptation to a major component of abiotic environmental variation. However, our current understanding of relationships between environmental temperature and plasma GC levels in free-living vertebrates is limited.

Here we evaluate the thermal dependence of GCs in vertebrates using three approaches. First, we present four field case studies, using a lizard, crocodile, sea turtle, and amphibian, respectively, in which relationships between GC and short-term (i.e., intradaily) thermal variation were quantified. Ectothermic vertebrates are taxa considered especially sensitive to climate warming (Huey et al. 2009), yet there are relatively few field studies describing the relationships between GCs and temperature in free-living reptile and amphibian populations and none that describe how GC responses perform as body temperatures approach upper or lower critical limits (Cree et al. 1990; Romero and Wikelski 2006; Dupoué et al. 2013). How might vertebrate plasma GC levels respond to temperature variation? Here we predict that plasma GC levels will show positive correlations with the magnitude and rate of ambient temperature change (cooler or hotter) commensurate with homeostatic departure from an animal's preferred optimal temperature. However, once an animal's body temperature reaches its lower or upper lethal thermal limits, then a progressive loss of cellular function could contribute to alteration in hypothalamic-pituitary-adrenal axis function and lead to decreased plasma GC levels (Dupoué et al. 2013). This predicted relationship would be comparable to thermally dependent patterns observed for many physiological processes (e.g., metabolism; Johnston and Bennett 2008). In two of our case studies (green sea turtles and cane toads), we measured GC responses at operative temperatures that approached or reached the upper lethal thermal maximum, presenting a rare opportunity to evaluate thermal responses in plasma GC levels of free-living populations under natural conditions.

Second, we conduct a meta-analysis to evaluate the generality of GC responses in vertebrates to short-term changes in environmental temperature under experimental conditions. Animals are generally expected to elevate GC levels above baseline during episodes of increased environmental variation, a response often thought to be adaptive (Wingfield et al. 1988). This suggests that more extreme and variable temperature exposures, dictated by the duration and rate of acute temperature change, should positively correlate with GC levels across species. In addition, species- or taxon-specific aspects of organismal design and associated physiological architecture could further influence how GC levels of vertebrates respond to short-term exposure to temperature change (Finch and Rose 1995). Thus, we might predict that the nature of environmental temperature exposure alongside inherent differences among species may interact to influence the thermal dependence of vertebrate GC responses.

Third, we apply a macrophysiological approach, examining variation in physiological traits across large geographical and phylogenetic scales to understand how physiological traits can be used to evaluate global patterns in species tolerance limits (Chown et al. 2004; Chown and Gaston 2008; Gaston et al. 2009). Recently, several comparative studies have revealed the extent to which environmental or life-history variation can explain species differences in GC activity (Goymann et al. 2004; Garamszegi et al. 2005; Bókony et al. 2009; Hau et al. 2010; Eikenaar et al. 2012; Jessop et al. 2013*b*). However, these studies have focused on geographic variables (e.g., latitude and elevation) and have not considered the direct influence of broadscale environmental temperature variation as potential ecological or evolutionary mediators of GC responses in vertebrates. Here we evaluate whether environmental variation in thermal properties (e.g., annual temperature variation) correlates with reptile and bird baseline and stress-induced GC responses. We predicted that, like many physiological traits, GC responses should covary with broadscale environmental temperature parameters (e.g., elevational and latitudinal clines in mean annual and seasonal temperature variation; Janzen 1967; Addo-Bediako et al. 2000). For example, species that inhabit thermally benign versus thermally extreme environments may possess differences in baseline GC levels to facilitate different modes of thermoregulation, metabolic efficiency, and performance breaths or permit differential investment in life histories (e.g., pace of life). Theory predicts that species that experience greater temperature seasonality or more extreme summer or winter temperatures would have greater GC stress responsiveness (i.e., investment; Addo-Bediako et al. 2000). Such patterns could help to explain the potential role of GC physiology in the distribution, abundance, and adaptations of animals that are otherwise not obvious at local scales (Chown et al. 2004; Gaston et al. 2009) and even have important implications for how animals may fare with respect to global change (Angelier and Wingfield 2013).

Methods

Thermal Sensitivity of the GC Responses within Vertebrates: Case Studies

We examined relationships between plasma corticosterone, the primary GC in reptiles and amphibians, and body temperature in four ectothermic vertebrates under field conditions. To do so, we obtained plasma corticosterone levels and body temperature data sets from field studies on the tawny dragon (*Ctenophorus decresii*; Flinders Ranges, South Australia; lat. 33°2′S, long. 138°7′E; ~450 m asl), the Australian freshwater crocodile (*Crocodylus johnstoni*; Lynd River and Fossil-brook Creek in north central Queensland, Australia; lat. 17°50′S, long. 144°20′E; ~200 m asl), the green sea turtle (*Chelonia mydas*; Raine Island, Queensland; lat. 11° 37′S, long. 144°01′E; ~3 m asl), and the cane toad (*Rhinella marina*; Camfield Station, Northern Territory; lat. 17°04′S, long. 131°43′E; ~150 m asl). From these four field studies, concurrent blood and body temperature data were collected. All four species were sampled using consistent field methods and daily conditions (e.g., low cloud cover, low wind, and no precipitation) to rapidly obtain blood samples and body temperatures, so our measures can be considered ecologically relevant and consistent. Importantly, all samples were collected over short durations ranging between 1 and 5 days of field sampling. This narrow sampling duration was deemed important to prevent possible variation in plasma corticosterone levels due to changes in life-history state or potentially due to seasonal variation. All individuals had both their body temperature and blood sampled (200 μL–3 mL) within 30 s–5 min of capture. Body temperature of ectotherms, which reflect prevailing environmental temperatures, was measured using a thermocouple (YCT, Taiwan) or digital thermometer inserted into the cloaca to read core body temperature. All blood samples were placed into vials and stored on ice for up to 4 h before they were centrifuged for 5 min to separate the plasma from the red blood cells. Plasma samples were stored in a freezer at −20°C or liquid nitrogen until they were assayed. For each species, either a radioimmunoassay (RIA) or enzyme-immunoassay (EIA) technique was used to determine plasma corticosterone levels. The intra- and interassay coefficient of variation (%) was, respectively, 4.5 and 9.6 for tawny dragon (using EIA); 10.3 and 12.2 for freshwater crocodiles (using RIA); 8.7 and 10.3 for green turtles (using RIA); and 4.9 and 11.0 for cane toads (using EIA). In part, some data used here have been published elsewhere but have not been used or analyzed within the context of this study (Jessop et al. 2000, 2003, 2013*a*).

Generalized additive models (GAMs) were used to compare the effect of body temperature on plasma corticosterone concentrations. GAMs are flexible, nonparametric regression models, which can maximize model fit by using cubic smoothing splines to determine underlying linear and nonlinear trends in continuous variables. The nonparametric regression fitted flexible smoothing splines to model the relationship between body temperatures and corticosterone. All GAMs were fitted using the mgcv package (Wood 2010) in R, version 3.0.2 (R Core Team 2013). The flexibility of GAM was constrained to three knots to ensure fitting of data was constrained to a polynomial fit.

Meta-analysis of GC Responses of Vertebrates to Acute Changes in Temperature

We conducted a meta-analysis by obtaining data from literature investigating GC responses of vertebrates to acute changes in temperature. To identify appropriate studies, we searched Google Scholar and ISI Knowledge using the following keywords: GC, corticosterone, cortisol, temperature exposure, and thermal gradient. Published studies had to meet two main criteria for consideration in the analysis: first, each species must have been exposed to thermal variation (high or low temperature) and, second, the thermal exposure needed to involve a rapid change in environmental temperature so that the rate and magnitude of temperature change exceeded normal daily variation. We further restricted data selection to short-term experiments (less than a month) and considered only studies that measured adults outside the breeding season to avoid ontogenetic and reproductive variation in GC levels (Moore and Jessop 2003). We averaged data presented as sex-specific responses for males and females to calculate a mean species GC response value. A total of 88 studies, across all vertebrate taxa, met these criteria (tables 1, 2).

For each study, we calculated the log-response ratio (ln) by subtracting the plasma GC values collected at the first time and temperature interval from that obtained after change in temperature over time. Thus, each study provided a single datum constituting an effect size. A positive logit indicated an increased GC value associated with temperature change, and a negative logit indicated a decreased GC value associated with temperature change. We preferred this approach over commonly used effect sizes involving standardized mean differences (e.g., Hedge's *d*) because it does not require within-study variance, which could not be calculated for a large portion of our data set, for example, where such values are not reported (Winfree et al. 2009).

We considered eight covariates to evaluate thermal sensitivity in the GC response of vertebrates. These included: (1) Temperature rate of change (standardized to a per hour equivalent) over the experiment. (2) Initial experimental temperature: the holding temperature prior to commencement of acute temperature change. (3) Final experimental temperature: the absolute experimental maximal temperature

Table 1: Model ranking of the effects of different thermal parameters on baseline glucocorticoid variation in reptiles

Rank	Model	K	DIC	ΔDIC	w_i
1	Mean annual temp.	4	14	0	.2
2	Min. temp.	4	14.5	.43	.1
3	Max. temp. + min. temp. + seasonality	6	14.5	.44	.1
4	Min. temp. + seasonality	5	14.6	.61	.1
5	Mean annual temp. + seasonality	5	15.2	1.2	.1
6	Max. temp. + min. temp.	5	15.5	1.52	.1
7	Mean annual temp. + min. temp.	5	15.6	1.58	.1
8	Mean annual temp. + min. temp. + seasonality	6	15.7	1.66	.1
9	Seasonality	4	16.6	2.58	0
10	Mean annual temp. + max. temp.	5	16.6	2.59	0
11	Mean annual temp. + max. temp. + min. temp. + seasonality	7	17.1	3.1	0
12	Null	3	17.5	3.44	0
13	Max. temp.	4	17.6	3.55	0
14	Mean annual temp. + max. temp. + seasonality	6	17.7	3.69	0
15	Mean annual temp. + max. temp. + min. temp.	6	18.1	4.08	0
16	Max. temp. + seasonality	5	18.3	4.28	0

Note: The table reports reptile baseline corticosterone levels, including model in rank order determined by deviance information criteria (DIC), differences in model rank relative to the top-ranked model (ΔDIC), and the model weight (w_i). Min. = minimum; max. = maximum; temp. = temperature.

to which each species was exposed. (4) Total temperature change: the range of temperature exposure experienced during the experiment. (5) Environment: each animal was classified as belonging to a terrestrial or aquatic environment. Naturally, such environments expose animals to markedly different daily and seasonal variation in temperature that could influence how animals respond to experimental changes in ambient temperature (Wilson et al. 2000; Angilletta 2009). (6) Taxon: taxonomic class was used as a covariate to identify whether thermal sensitivity is conserved across different vertebrate taxa, namely, amphibians, birds, fish, mammals, and reptiles. This covariate represents a proxy for phylogenetic history and shared organismal design that could have direct bearing on how species respond to temperature. (7) Hormone type: Cortisol and corticosterone are the two GC produced by vertebrates, and they differ structurally (by a sin-

Table 2: Model ranking of the effects of different thermal parameters on capture stress–induced glucocorticoid variation in reptiles

Rank	Model	K	DIC	ΔDIC	w_i
1	Null	3	6.5	0	.35
2	Max. temp.	4	8.1	1.6	.16
3	Mean annual temp. + seasonality	5	8.3	1.8	.14
4	Mean annual temp. + max. temp. + min. temp.	6	9.9	3.45	.06
5	Mean annual temp. + min. temp.	5	10	3.56	.06
6	Mean annual temp. + max. temp. + min. temp. + seasonality	6	10.2	3.75	.05
7	Mean annual temp. + max. temp. + seasonality	4	11.1	4.66	.03
8	Min. temp.	4	11.3	4.85	.03
9	Mean annual temp. + min. temp. + seasonality	6	11.4	4.98	.03
10	Max. temp. + min. temp.	5	12.3	5.82	.02
11	Seasonality	4	12.4	5.98	.02
12	Mean annual temp. + max. temp.	5	12.6	6.18	.02
13	Max. temp. + seasonality	5	12.9	6.48	.01
14	Min. temp. + seasonality	5	14.4	7.92	.01
15	Max. temp. + min. temp. + seasonality	6	14.5	8.02	.01
16	Mean annual temp. + max. temp. + min. temp. + seasonality	7	15.7	9.2	.00

Note: The table reports reptile (30 min postcapture) capture stress–induced corticosterone levels, including model in rank order determined by deviance information criteria (DIC), differences in model rank relative to the top-ranked model (ΔDIC), and the model weight (w_i). Min. = minimum; max. = maximum; temp. = temperature.

gle hydroxyl group) due to different pathways of steroidogenesis. Different taxa tend to be dominant in one GC, and they are assumed to be functionally equivalent; however, to explicitly test this, we evaluated whether either hormone responded differently to temperature. (8) Sample source: GC levels are often analyzed in different biological samples (e.g., plasma, urine, and feces) that have different hormone concentration dynamics (e.g., levels in plasma change within minutes, while those in feces often change over hours to days). Hence, we considered sample type in our analysis.

Boosted regression trees (BRT; Elith et al. 2008) were used to quantify and illustrate the relative influence of these eight different variables on GC levels in vertebrates. BRT models iteratively develop a large collection of small regression trees constructed from random subsets of the data. They are capable of testing multiple types of predictor variables (including categorical and continuous), and their predictive capacity exceeds most traditional modeling methods (Elith et al. 2008). The BRT technique is especially advantageous for modeling animal responses to environmental parameters because such responses are often complex and nonlinear. Visualization of fitted functions in a BRT model was achieved using partial dependence plots that depict the effect of a predictor variable on the GC response after accounting for the average effects of all other variables in the model. As a selection criteria for identifying those variables considered to have meaningful inference, we present only partial dependent plots for all predictor variables that exceeded 5% influence on GC levels (Elith et al. 2008).

BRT models were fitted using a bag fraction of 0.5 and a learning rate of 0.001. Tree complexity, which determines the number of nodes in a tree and controls the interactions between variables, was set to 2 (following recommendations of Elith et al. 2008), so that at least 1,000 trees were produced for a small data set, which is considered slow enough for reliable estimates. BRT models were fit with Gaussian distribution. All models were built with tenfold cross-validation process, which identified the optimal number of trees. All BRT analyses were undertaken in R, version 3.0.2 (R Core Team 2013), using the gbm package (Ridgeway 2015).

Comparative Analyses of Relationships between Environmental Temperature Parameters and Baseline and Capture Stress–Induced GC Levels

Our third analysis used a phylogenetic comparative approach to evaluate the effects of different broadscale environmental temperature parameters on explaining variation in baseline and capture stress–induced GC data obtained from reptile and bird studies. This analysis would have benefited from consideration of all vertebrate taxa, however, studies from mammals, fish, and amphibians could not provide a sufficient or equivalent baseline capture stress–induced GC response to enable their inclusion. We identified appropriate reptile and bird studies (using databases as described above) by using the following keywords: corticosterone, acute response, stress-series protocol, captive-stress protocol, capture-handling stress, standardized stress-response protocol, and stress-response protocol. We further refined our search based on several criteria. The key criterion for all studies was that each species had been exposed to a standardized capture-stress protocol to ensure a consistent stressor among species. The capture-stress protocol involves the rapid capture of an animal, followed by restraint and collection of an initial first blood sample termed the baseline sample. The animal is subsequently stressed in response to being held captive and restrained for a relatively brief period of time (minutes to hours) where one or more blood samples are taken and subsequently analyzed via RIA or EIA protocols. The capture-stress protocol has been widely demonstrated to elicit increased levels of circulating GCs in birds and reptiles and is, therefore, comparable across a wide range of species (e.g., Wingfield et al. 1982; Romero 2002; Moore and Jessop 2003).

As for the meta-analysis, we considered only studies that measured adult animals outside the breeding season (from the nonreproductive period) and averaged male and female responses if these were presented separately to calculate a mean species GC stress value. Where multiple studies on the same species (particularly for birds) meeting the above criteria had been conducted, we arbitrarily selected only a single study. If data were obtained from individuals from several different locations, we used only these data if the locations were within a 50 km radius, with one of those locations specified as the study location and GC values averaged across individuals. Studies that combined data from individuals from locations that were over a greater distance than the 50 km radius were discarded. Though there are potentially hundreds of studies that have measured GC responses of birds and reptiles, using such criteria reduced the total number of studies to 22 reptile and 65 bird species.

We extracted two plasma GC values from each species, conditional on both being recorded within the same capture-stress protocol. The first value is considered the baseline sample, for which plasma GC levels must be obtained from blood samples collected under 3 min from initial capture (Romero 2002). However, this may be extended in some reptiles (e.g., turtles and crocodilians). Here we considered the baseline sample to represent GC levels measured within 3–5 min of capture for birds and reptiles, respectively. The second plasma GC value we evaluated was measured at 30 min postcapture (herein referred to as the T30 capture-stress sample). Although capture stress–response protocols can extend beyond 30 min (particularly in reptiles), the T30 capture-stress sample is one of the most common end-

points used to evaluate physiological stress responsiveness in vertebrates. These metrics represent two performance dimensions of GC regulation, with baseline samples being linked to homeostasis under normal conditions and T30 capture-stress levels to allostasis or the rate of responsiveness of organisms to homeostatic challenges (McEwen and Wingfield et al. 2003). A key assumption of our study is that these GC metrics are highly indicative of an animal's GC function and ensuing regulation of organismal function. We acknowledge that this may not always be the case, pending the influence of plasma-binding protein kinetics or receptor dynamics that also play a crucial part in regulating corticosterone abilities to induce the vertebrate stress response (Westphal 1983; Breuner 2002).

To evaluate relationships between temperature and variation in baseline and GC stress-response levels, we considered four parameters for each species at each study location: (1) average annual mean monthly temperature (°C), (2) minimum monthly temperature (i.e., peak winter temperature), (3) maximum monthly temperature (i.e., peak summer temperature), and (4) a measure of annual temperature variation (i.e., seasonality; calculated as the coefficient of variation in temperature by dividing the standard deviation of monthly mean temperature by the mean annual temperature). Temperature data for each study location was obtained using the closest weather station using NASA's Goddard Institute for Space Studies surface-temperature analysis website (http://data.giss.nasa.gov/gistemp/station_data/).

We used phylogenetic linear mixed models (PLMM) to account for the phylogenetic nonindependence in data. Specifically, a PLMM includes phylogenetic correlations derived from the corresponding phylogenetic tree as levels in a random factor. Thus, the total phenotypic variance (V_P) in the data is partitioned into phylogenetic variance (V_A) and residual variance (V_e). The ratio of V_A over V_P gives the phylogenetic heritability (H^2_P; estimated on a scale between 0 and 1), which indicates the degree to which related taxa provide phenotypic information about each other under a Brownian motion model of evolution.

We used a Bayesian information-theoretic approach to model the relationship between log-transformed baseline and capture stress–induced GC levels and putative large-scale environmental thermal covariates (Spiegelhalter et al. 2002). We considered 15 models incorporating combinations of the four temperature parameters as well as a null (i.e., an intercept-only model) model. These 16 models were fitted to the data using Bayesian Markov chain Monte Carlo (MCMC) methods through the use of the MCMCglmm package (Hadfield 2015*b*) in R, version 3.0.2 (R Core Team 2013). PLMMs were fit with a Gaussian error, and we assumed uniform prior distributions (Hadfield 2015*a*). Parameter estimates are based on 1,000 iterations subsampled from 106 iterations after a 1,000 sample burn-in and a thinning interval of 500, which was more than sufficient for the MCMC chain to reach stationarity. Effective sample sizes were close to 1,000, and autocorrelations were less than 0.1 for all random and fixed effects. We also visually inspected plots of traces and the posterior distributions to make sure that all models converged.

We used the deviance information criterion (DIC) to identify the relative support for each model (Spiegelhalter et al. 2002). The best-fitting model has the smallest DIC, and we ranked models from best to worst according to the differences between each model's DIC (Δi) values. Model weights (w_i) were computed from the DIC values following Burnham and Anderson (2002). Model weights can be interpreted as the probability that the model is the best model. We also considered that only the temperature models that exceeded the rank of the null model by ≥ 2 DIC units were biologically informative. To further assess the effects of the best-ranked temperature models, we calculated a pseudo-R^2 using Nagelkerke's modified statistic that estimates the variance explained by fixed effects in the PLMM for each of the top-ranked models.

The phylogeny used in the analysis of birds was derived by pruning the bird supertree phylogeny produced by Davis (2008). For reptiles, a composite phylogeny was constructed from several sources. Overall relationships between the major groupings of reptiles (Testudines, Crocodylia, Sphenodontida, Squamata) were derived from Werneburg and Sánchez-Villagra (2009). Because these phylogenies do not all have branch length estimates, we set branch lengths to equal length (=1) for our analysis. All meta- and comparative analysis data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.mb65n (Jessop et al. 2016).

Results

Thermal Sensitivity of the GC Responses within Vertebrates: Case Studies

In three of the four ectothermic vertebrates, acute increases in body temperature were significantly correlated with plasma corticosterone levels (fig. 1); tawny lizard (GAM: $F = 6.13$, $P = .006$, adj. $R^2 = 0.17$; fig. 1*a*), green sea turtle (GAM: $F = 38.92$, $P < .001$, adj. $R^2 = 0.45$; fig. 1*b*), and cane toad (GAM: $F = 15.97$, $P < .001$, adj. $R^2 = 0.43$; fig. 1*c*). For the cane toads (fig. 1*b*.) and green sea turtles (fig. 1*d*), as individuals approached sub- or lethal environmental temperatures, plasma corticosterone levels appeared to asymptote. No significant relationship between plasma corticosterone and body temperature was found for the freshwater crocodile (GAM: $F = 2.42$, $P = .12$, adj. $R^2 = 0.18$; fig. 1*c*); however, these reptiles were tested only across the narrow temperature range of 25°–29°C. For lizards, turtles,

and toads that experienced much hotter temperatures than crocodiles, it was also evident that interindividual variance in plasma corticosterone levels increased with body temperature.

Meta-analysis of GC Responses of Vertebrates to Acute Changes in Temperature

In boosted regression tree analysis of GC responsiveness in vertebrates, five of the eight parameters exceeded 5%, suggesting they have an important influence on GC responses. The most important temperature parameter was the rate of temperature change (°C/hour) that animals experienced during the experiment, and this explained 33.0% of variation in GC responses. The partial dependence plot (fig. 2*a*) indicates that GC responses increased with the rate of temperature change (hotter or colder). However, most sensitivity in GC responsiveness occurred during rapid increases in temperature rather than rapid decreases in temperature. Additionally, as the rate of temperature change increased—and, in particular, as it decreased—the predicted capacity for change in the GC response appeared highly attenuated and, hence, apparently unresponsive to more pervasive temperature exposure regimes.

Taxonomic group (fig. 2*b*) was the second-most influential predictor and explained 19.4% of GC effect size variation and showed considerable differences among vertebrate groups. Here it was evident that basal vertebrate taxa, especially fish and amphibians, had larger GC responses to temperature exposure compared to reptiles, mammals, and, especially, birds.

The temperature at which experiments commenced and concluded also explained 14.5%–17.6% of GC effect size variation. These temperature parameters resulted in U-shaped GC responses, with more extreme temperatures producing larger responses (fig. 2*c*, 2*d*). The magnitude of tempera-

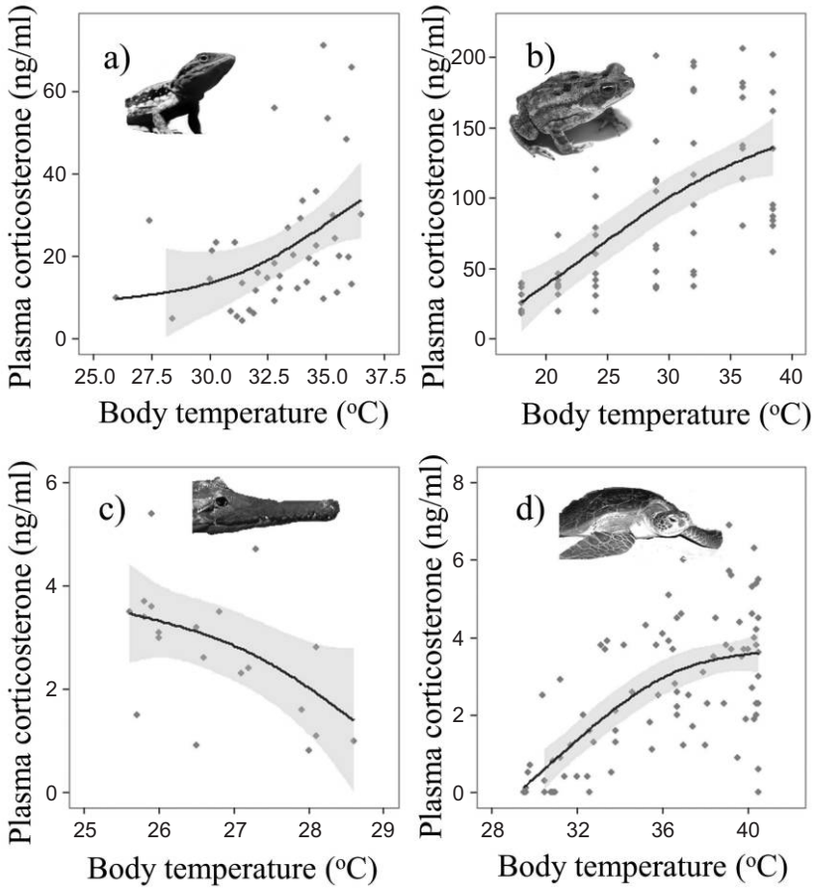

Figure 1: Fitted generalized additive models depicting significant relationships between plasma corticosterone levels (ng/mL) and body temperature (°C) for tawny dragon lizard (*a*), cane toad (*b*), freshwater crocodile (*c*), and green sea turtle (*d*). Error lines represent point-wise 95% confidence bands of fitted models.

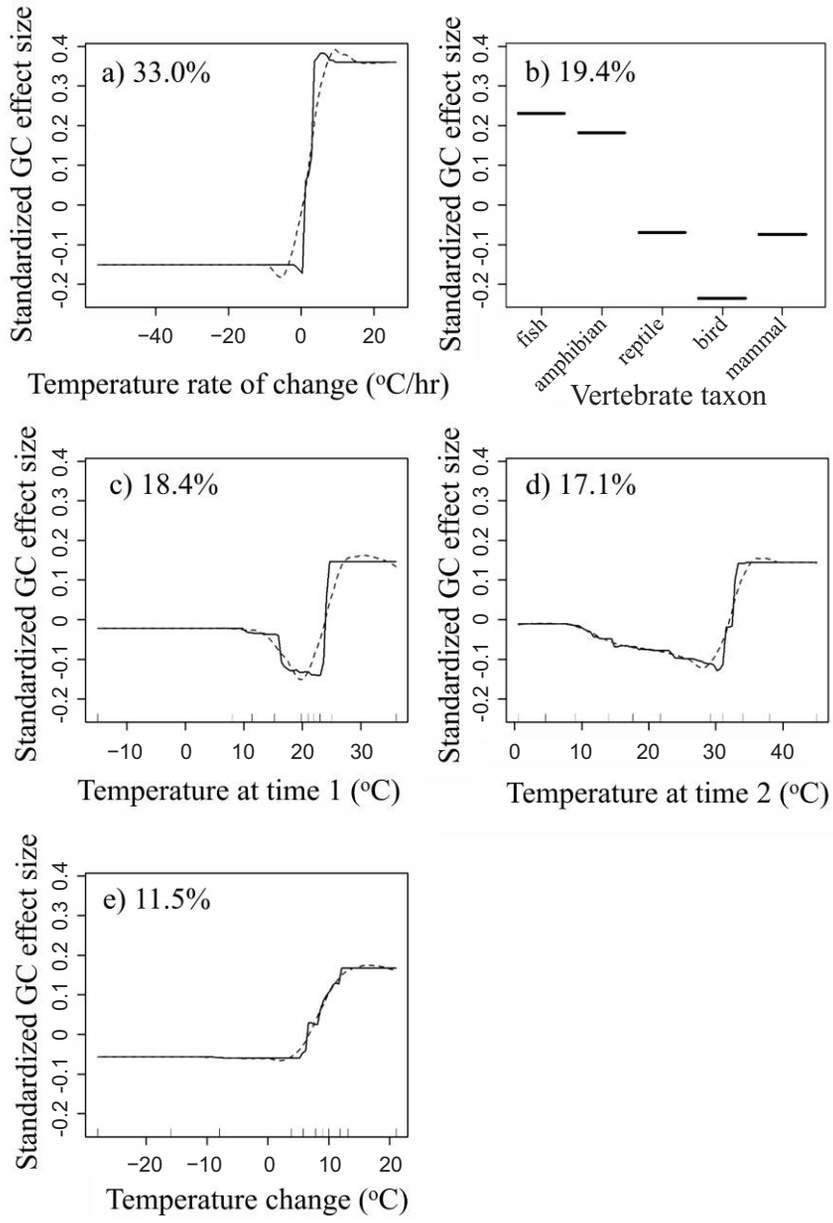

Figure 2: Partial dependence plots generated from boosted regression tree analyses show predicted relationships between glucocorticoid (GC) data of vertebrates and the five highest-ranked predictor variables. In order of influence are temperature rate of change (*a*), taxa (*b*), initial experimental temperature at time 1 (°C; *c*), final experimental temperature at time 2 (°C; *d*), and total temperature change experienced during the study (*e*). Use of straight lines (solid line) and smoothing splines (dashed line) depict predicted trends for the effects of temperature variables on GC data. Rug plots on the bottom horizontal axis of partial dependence plots show the distributions of the variables. The *Y*-axes values are presented on a uniform standardized logit scale that permits direct comparison of the relative influence of each predictor variable. The relative influence (%) of each predictor variable on the GC response is presented in the top left corner of each plot.

ture change was the only other parameter influencing GC response variation (fig. 2*e*). Again, hotter temperatures resulted in much larger GC responses than exposure to colder temperatures. Furthermore, outside a relatively narrow range of temperatures, GC response again appeared to be relatively invariant to additional temperature change. The remaining three predictors (i.e., environment, GC hormone type, and the biological sample that GC data was derived from) all had

very low influence as each accounted for <1% variation in GC responses.

The top two predictors, namely, temperature rate of change and taxon, produced the strongest combined effect on GC effect size responses in vertebrates (fig. 3). Here it was clearly evident that considerable variation existed among taxa in the GC responses to acute changes in temperature. Amphibians and fish exhibited the largest increase in GC effect size relative to mammals and reptiles, with birds having the lowest response, on average.

Comparative Analyses of Environmental Temperature Effects on Baseline and Stress GC Responsiveness

In three out of the four comparative analyses, environmental temperature parameters better explained variation in baseline or T30 capture stress–induced GC levels than an intercept-only model (i.e., null model; tables 1–4). For baseline GC levels in reptiles, the effects of mean annual temperature gave better model fit (ΔDIC $= 0.43$) than the second-ranked model of minimum annual temperature. For the best model, the posterior mode (i.e., analogous to a beta coefficient) for the effect of mean annual temperature (-0.040, lower and upper credible interval range $= -0.08$ to -0.001; adjusted $R^2 = 0.25$) indicated that baseline GC levels decreased with changes in mean annual temperature (table 1). Thus, reptiles that experienced warmer average annual temperatures (i.e., tropics) had lower baseline GC levels than reptiles inhabiting colder climates. With respect to explanatory power, this top model accounted for 25% of the variation in baseline GC levels. By contrast, the T30 capture stress–induced GC levels of reptiles appeared poorly explained by any broadscale temperature parameter, with the null model ranked highest (table 2).

For birds, variation in baseline GC levels was best explained by maximum annual temperature (highly supported model with $\omega_i = 0.79$; table 3). The posterior mode for the effect of maximum annual temperature (-0.007, lower and upper credible interval range $= -0.02$ to -0.001; adjusted $R^2 = 0.12$) indicates that bird species that experienced hotter summers maintained lower baseline GC levels. By contrast, variation in T30 capture stress–induced GC levels was best explained by minimum annual temperature (strongly supported model with $\omega_i = 0.62$; table 4). The posterior mode was again negative (-0.004, lower and upper credible interval range $= -0.01$ to -0.001; adjusted $R^2 = 0.10$), indicating that bird species that experienced colder winters produced lower stress-induced GC levels. These models explained between 8% and 14% of baseline and T30 capture stress–induced GC levels in birds, respectively.

In reptiles, baseline GC levels showed substantial phylogenetic heritability ($H^2_P = 0.97$). Similarly, in birds, the H^2_P for baseline and T30 capture stress–induced GC levels were high at 0.82 and 0.81, respectively.

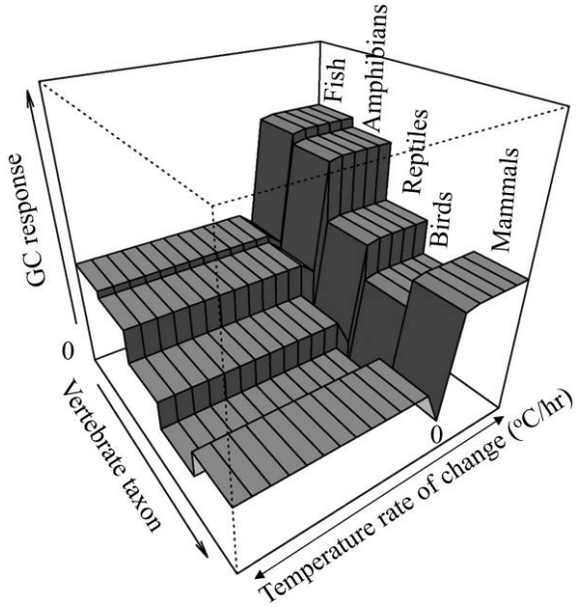

Figure 3: Two-factor partial dependence plot presenting the strongest interactive effect of vertebrate taxon and temperature rate of change on vertebrate glucocorticoid (GC) stress responsiveness.

Discussion

Temperature profoundly affects the phenotypic performance of all organisms by exerting strong selection across an organism's genome, culminating in the evolution of thermally dependent behavioral, physiological, and life-history traits (Angilletta 2009). Here we considered correlative relationships between GC variation in vertebrates both to acute temperature exposure and across broadscale environmental temperature variation using a multiscale analytical approach. Our results confirm that variation in GC levels are strongly thermally correlated but that the nature of the relationship varies among taxa. Taxonomic dependency is also evident in influencing relationships between GC responses and broadscale temperature variation.

Thermal Sensitivity of the GC Responses within Vertebrates: Case Studies

For three of four ectothermic vertebrates, acute increases in body temperature were strongly correlated with greater plasma GC levels. Our results from free-living populations are consistent with laboratory-based studies indicating similar associations (e.g., Cree et al. 1990). Positive correlations between temperature and GC levels are likely linked with

Table 3: Model ranking of the effects of different thermal parameters on baseline glucocorticoid variation in birds

Rank	Model	*K*	DIC	ΔDIC	w_i
1	Max. temp.	4	8.4	0	.7
2	Max. temp. + seasonality	5	10.9	2.5	.2
3	Mean annual temp. + max. temp.	5	14.34	5.94	0
4	Null	3	15.2	6.8	0
5	Max. temp. + min. temp.	5	17.3	8.9	0
6	Mean annual temp. + max. temp. + seasonality	6	18.5	10.1	0
7	Seasonality	4	18.6	10.2	0
8	Mean annual temp.	4	20.2	11.8	0
9	Max. temp. + min. temp. + seasonality	6	21	12.6	0
10	Min. temp.	4	23.2	14.8	0
11	Mean annual temp. + seasonality	5	25.04	16.64	0
12	Min. temp. + seasonality	5	25.8	17.4	0
13	Mean annual temp. + min. temp.	5	27.7	19.3	0
14	Mean annual temp. + max. temp. + min. temp. + seasonality	7	28.14	19.74	0
15	Mean annual temp. + max. temp. + min. temp.	6	28.2	19.8	0
16	Mean annual temp. + min. temp. + seasonality	6	31.4	23	0

Note: The table reports bird baseline corticosterone levels, including model in rank order determined by deviance information criteria (DIC), differences in model rank relative to the top-ranked model (ΔDIC), and the model weight (w_i). Min. = minimum; max. = maximum; temp. = temperature.

thermally dependent scaling of organismal metabolism, where elevated GC levels could promote intermediary metabolism, such as glucose mobilization (Romero 2004). Importantly, for the two species that approached (i.e., cane toad) or reached (i.e., sea turtle) critical thermal maxima, plasma GC levels of individuals appeared to begin to plateau. Thus, at extreme upper levels of temperature-induced physiological dysregulation (or stress), changes in rates of hormone synthesis or clearance may cause plasma GC levels to become unresponsive to further temperature increases. This result is

Table 4: Model ranking of the effects of different thermal parameters on capture stress–induced glucocorticoid variation in birds

Rank	Model	*K*	DIC	ΔDIC	w_i
1	Min. temp.	4	−1.3	0	.28
2	Min. temp. + seasonality	5	−.1	1.34	.15
3	Max. temp. + min. temp.	5	.6	2.12	.1
4	Mean annual temp. + min. temp.	5	1.2	2.61	.08
5	Max. temp.	4	1.9	2.83	.07
6	Max. temp. + min. temp. + seasonality	6	2.1	3.22	.06
7	Null	3	2.2	3.57	.05
8	Max. temp. + seasonality	5	2.7	3.97	.04
9	Mean annual temp. + min. temp. + seasonality	6	2.7	3.78	.04
10	Seasonality	4	3.4	4.39	.03
11	Mean annual temp.	4	4.3	5.35	.02
12	Mean annual temp. + max. temp.	5	4.3	5.7	.02
13	Mean annual temp. + max. temp. + min. temp.	6	4.4	4.48	.03
14	Mean annual temp. + max. temp. + min. temp. + seasonality	7	4.5	5.73	.02
15	Mean annual temp. + max. temp. + seasonality	6	5.3	6.43	.01
16	Mean annual temp. + seasonality	5	5.5	6.41	.01

Note: The table reports bird (30 min postcapture) capture stress–induced corticosterone levels, including model in rank order determined by deviance information criteria (DIC), differences in model rank relative to the top-ranked model (ΔDIC), and the model weight (w_i). Min. = minimum; max. = maximum; temp. = temperature.

different from what we predicted and is inconsistent with other organismal processes (e.g., metabolic rate), where responses at upper thermal limits decrease as individuals succumb to lethal temperature exposure. Understanding what mechanisms produce such trajectories is far from simple, as multiple pathways that mediate plasma GC responses to temperature could be at play. For example, neural and neurotransmitter regulation of the HPA axis and/or adrenal and hepatic enzymes involved in GC synthesis or degradation could influence plasma-response trajectories to temperature increases (Wingfield and Sapolsky 2003). Similarly, rates of production or binding characteristics of corticosteroid-binding globulin (CBG) and GC receptors, alongside sensitivity to negative feedback within the HPA axis, could all contribute to the observed response patterns (Breuner 2002; Dupoué et al. 2013). Further, each of these components of the HPA axis that regulate plasma GC levels could also be subject to independent genetic or environmental consequences arising from temperature. Thus, variation in plasma GC levels to temperature could be defined by extremely complex interactions (Dupoué et al. 2013). Further, these complexities could also help to explain the large interindividual variation in GC levels reported at high temperatures.

Clearly, because plasma GC levels of ectotherms can show strong associations with environmental temperature, there are at least two important implications for field endocrine studies. First, it appears that blood sampling protocols may need to consider (e.g., via methodological design) an animal's body temperature to reduce individual variation among samples that may confound detection of unrelated processes that are often of primary interest in understanding sources of GC level variation in free-living vertebrates. Second, as ectotherms approach upper temperature limits, increased individual variation in GC levels may compromise the use of plasma GC levels to accurately infer an animal's or a population's response to environmental stressors (Schoech et al. 2013).

Meta-analysis of GC Responses of Vertebrates to Acute Changes in Temperature

Our meta-analysis clearly indicated that temperature dependency in the GC response is highly conserved across vertebrates. Here the hourly rate of change in temperature (°C/h; ~33% of variation) was ranked the most important determinant of GC effect sizes in animals. Such a relationship is entirely consistent with temperature effects on organismal physiology (Angilletta 2009). Furthermore, variation in GC levels appeared to be much more influenced by increased, than decreased, rates of temperature change. Thus, exposure to hotter temperatures was associated with greater GC responsiveness than cold exposure. This dynamic is again consistent with often asymmetrical thermal tolerances in other physiologies (Hochachka and Somero 1968, 1980; Huey and Stevenson 1979). Here the upper thermal tolerance range in trait performance is reduced because cellular- and systems-level physiological deregulation occurs faster at increasing temperatures. Furthermore, GC responses appeared much more sensitive to increased, than decreased, rates of temperature change. Thus, exposure to hotter temperatures initiated greater GC responsiveness than cold exposure. This dynamic is again consistent with often asymmetrical thermal tolerances in other physiologies (Hochachka and Somero 1968, 1980; Huey and Stevenson 1979) because cellular- and systems-level physiological deregulation occurs faster at increasing temperatures. Furthermore, even sublethal exposure to high temperatures can lead to permanent damage, which has fitness implications (Hoffman 2010) and necessitates a greater response in physiology and behavior to maintain homeostasis or behavioral avoidance of thermal stress. However, an unexpected prediction arising from the BRT model indicated that as temperature change varied outside relatively narrow margins, responsiveness in GC levels become largely attenuated to increased, and especially decreased, temperature change. At present, we cannot offer a sound mechanistic explanation for why such invariance in GC responses might arise. However, observations of invariance in other thermally sensitive traits (e.g., heat tolerances in ectotherms) across different temperatures is also reported (Sunday et al. 2011). Clearly, better understanding these dynamics is critical for generalizing about how GC responses at upper and lower bounds of temperature change might influence attributes of vertebrate performance or fitness.

While temperature dependence of the GC response was conserved across vertebrates, there was evidence that different taxa exhibited different responses to acute temperature exposure. Several processes could account for different thermal dependencies among vertebrate groups. First, because species within each taxonomic group share similar thermal modalities and such modalities differ among taxa (e.g., fish, amphibians-thermoconformers; reptiles-thermoregulators, birds, and mammals-endotherms), we might expect taxa that are exposed to the least variation in environmental temperature to have narrower performance breadths and require increased investment in behavioral or physiological strategies to minimize changes to homeostasis and performance loss (Hoffman et al. 2013). Similarly, constraints imposed by temperature on other physiologies (e.g., oxygen-limitation mechanisms sensu Pörtner 2002) may predispose fish and potentially amphibians to require greater GC stress responsiveness relative to reptiles, birds, and mammals. Evolutionary constraints on organismal design and physiological architecture could further contribute to explaining differences among vertebrate clades in GC thermal responses (Clusella-Trullas et al. 2011; Hoffman et al. 2013).

Comparative Analyses of Environmental Temperature Effects on Baseline and Stress GC Responsiveness

The comparative macrophysiological analysis indicated that baseline and T30 capture stress–induced plasma GC concentrations of reptiles and birds had different sensitivities to environmental temperature parameters. Baseline corticosterone levels of reptiles covaried negatively with mean annual temperatures. This effect is intuitive for several reasons. From an annual activity perspective, colder climes (e.g., higher latitudes/elevations) would restrict reptile activity to a narrow summer period and may require them to increase daily activity to maximize energy intake during this period (Adolph and Porter 1993). However, elevated baseline GC levels could arise as a cause or consequence of persisting in colder climes. For example, increased daily activity during brief summer periods in colder climates would mean higher daily metabolic requirements that would require commensurate elevation in baseline GC levels (Hamann et al. 2007; Jessop et al. 2015). Alternatively, because GC can directly affect reptile metabolic rate (independent of temperature; e.g., DuRant et al. 2008), increased production of GC in colder climates could promote increased foraging and territorial defense during a contracted activity season.

For birds, baseline GC levels were negatively correlated with maximum annual temperatures. The higher environmental productivity in warmer climates (except in deserts) could provide bird species with increased energetic buffering and, ultimately, reduce the need for elevated baseline GC levels. This pattern is consistent with a similar trend in baseline metabolic rates of birds that are also reduced in warmer climates (White et al. 2007). The GC stress response of birds was positively correlated with minimum annual temperatures, suggesting that harsher, colder climates favor heightened stress responsiveness. In general, decreasing minimum temperatures are associated with increasing latitudes, with an increased frequency of severely challenging weather phenomena. Increased frequencies of extreme weather could, in turn, promote GC stress responsiveness, ultimately improving survival.

Environmental temperature parameters explained variation in baseline (in reptiles and birds) and capture stress–induced (in birds) GC metrics better than the null model. Thus, temperature might be the environmental factor responsible for the latitudinal patterns in both plasma GC and androgen levels in a variety of taxa (Goymann et al. 2004; Garamszegi et al. 2005; Bókony et al. 2009; Hau et al. 2010; Eikenaar et al. 2012; Jessop et al. 2013*b*). More broadly, our results, like those of other macrophysiology studies, suggest that environmental temperature can be an important determinant of global patterns of more general physiological variation (Chown et al. 2004; Clusella-Trullas et al. 2011). Because temperature has general effects on life-history variation and morphology (e.g., clines in body size or Bergmann's rule), covariation across traits is widely apparent (Blackburn et al. 1999). Thus, large-scale patterns in GC response variation could underlie life-history traits and trade-offs to optimize organismal responses across environmental gradients (Crespi et al. 2013).

Given that environmental temperature explained negligible (e.g., reptile capture-stress GC levels) through 25% of variation in baseline and GC responsiveness in reptile and bird species, other factors must account for residual variation. Life-history traits, social environments, and trophic interactions—attributes that can show relatively weak covariation with thermal clines—are known to have considerable influence on GC variation in vertebrates (e.g., Hau et al. 2010; Robert and Bronikowski 2010; Creel et al. 2013). Furthermore, animals possess diverse traits to enable them to cope with thermal stress, including other physiologies (e.g., heat shock proteins; hibernation and torpor), morphologies (e.g., body size, coloration, and insulation), and behaviors (e.g., microhabitat selection, seasonal or irruptive movements; Johnston and Bennett 2008). The relative importance of these other traits may well influence thermal selection on GC response and, therefore, its covariation with broadscale environmental temperature parameters. For example, animals inhabiting hash thermal environments (e.g., desert or polar regions) must rely extensively on non-GC related adaptations to persist, potentially weakening covariation between GC response and environmental temperature.

Ideally, future studies should consider additional large (e.g., productivity gradients) and local-scale (e.g., density dependence) processes to better evaluate the relative influence of different environmental and ecological phenomena on the GC responses of vertebrates (Jessop et al. 2013*a*). Additionally, the strong phylogenetic signal (i.e., >0.8) in both reptiles and birds could indicate that very different processes underlie the evolution of the GC response among vertebrate taxa. Strong phylogenetic signal could arise because of evolutionary constraints on physiological tolerances and, therefore, the environment in which species persist (Hoffman et al. 2013). Alternatively, a strong phylogenetic signal could result if related species co-occur in the same environment and therefore experience similar selection pressures (i.e., spatially covary; Losos 2008; Revell et al. 2008; Freckleton and Jetz 2009). Further research is needed to separate which of these processes (evolutionary constraint or selection) determines phylogenetic signal in the GC responses of vertebrates (Freckleton and Jetz 2009).

Conclusions

Glucocorticoid responses were found to be strongly temperature and taxon dependent, but generally, vertebrates ex-

hibited strong responses to acute increases in temperature, and to some extent, they covaried with environmental temperature gradients. Thus, vertebrate GC function, like other physiological processes, is thermally dependent. However, we caution that, unlike other general stress-resistance mechanisms (e.g., heat shock proteins in invertebrates) that confer general and temperature-specific tolerances, the adaptive and evolutionary potential of the vertebrate GC response to temperature variation, especially in wild populations, is little known. Here studies that evaluate phenotypic plasticity and heritability in—and natural selection on—thermal dependency of the GC response is needed to explain adaptive implications of covariation with environmental temperature (Hoffmann and Sgrò 2011; Angelier and Wingfield 2013). There are many examples of intra- and intergenerational phenotypic plasticity in the GC response in vertebrates (Wingfield and Sapolsky 2003; Jessop et al. 2004; Creel et al. 2013; Sheriff and Love 2013). Furthermore, baseline and stress-induced GC levels often have low to moderate heritability (realized heritability of ~0.15–0.35; Satterlee and Johnson 1988; Evans et al 2006; Jenkins et al. 2014). However, at present, we lack the direct experimental evidence regarding whether GC responses to short- or long-term changes in the thermal environment reflect selection or plasticity (Wilson et al. 2000; Piersma and Drent 2003; Angilletta 2006; Hoffman et al. 2013). Thus, future research investigating the capacity for adaptation in the GC response to environmental temperature is crucial and likely to have important implications for understanding how physiological processes influence species persistence under global change (Huey et al. 2009; Chown et al. 2010; Hoffman and Sgrò 2011; Angelier and Wingfield 2013).

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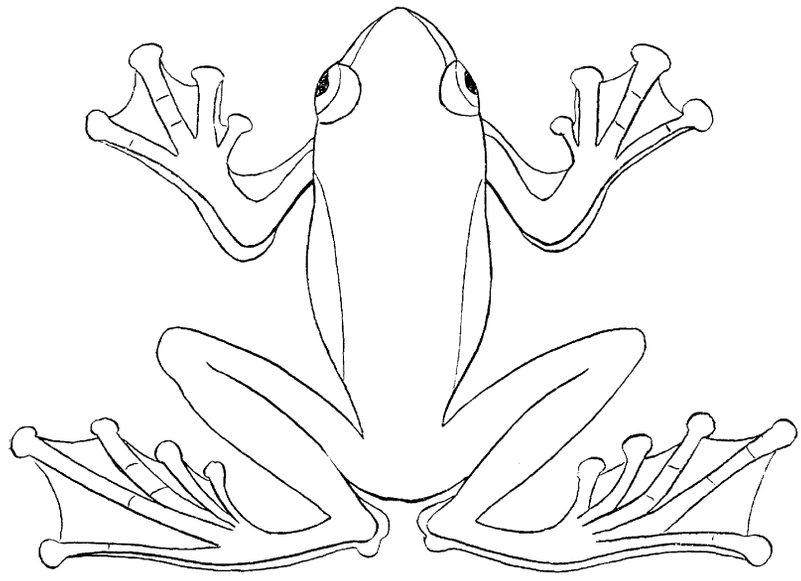

"The only volant adaptation among the Amphibia is that of the tree frog, Rhacophorus, in which the webbed feet bear the creature up during the prolonged leaps that it takes from tree to tree." From "Volant Adaptation in Vertebrates" by Richard S. Lull (*The American Naturalist* 1906, 40:537–566).