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SYMPOSIUM

IUCN Conservation Status Does Not Predict Glucocorticoid Concentrations in Reptiles and Birds

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Synopsis Circulating glucocorticoids (GCs) are the most commonly used biomarkers of stress in wildlife. However, their utility as a tool for identifying and/or managing at-risk species has varied. Here, we took a very broad approach to conservation physiology, asking whether International Union for the Conservation of Nature (IUCN) listing status (concern versus no obvious concern) and/or location within a geographic range (edge versus non-edge) predicted baseline and post-restraint concentrations of corticosterone (CORT) among many species of birds and reptiles. Even though such an approach can be viewed as coarse, we asked in this analysis whether CORT concentrations might be useful to implicate species at risk. Indeed, our effort, relying on HormoneBase, a repository of data on wildlife steroids, complements several other large-scale efforts in this issue to describe and understand GC variation. Using a phylogenetically informed Bayesian approach, we found little evidence that either IUCN status or edge/non-edge location in a geographic distribution were related to GC levels. However, we did confirm patterns described in previous studies, namely that breeding condition and evolutionary relatedness among species predicted some GC variation. Given the broad scope of our work, we are reluctant to conclude that IUCN status and location within a range are unrelated to GC regulation. We encourage future more targeted efforts on GCs in at-risk populations to reveal how factors leading to IUCN listing or the environmental conditions at range edges impact individual performance and fitness, particularly in the mammals, amphibians, and fish species we could not study here because data are currently unavailable.

Introduction

The first efforts in ecology were largely physiological (Cooke et al. 2013). The father of animal ecology, Victor Shelford (Shelford 1911), comprehensively identified factors affecting plant and animal distributions, predominantly focusing on energy balance, thermal relationships, and other physiological processes to explain why some species, and not others,

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thrived in some areas. In modern times, efforts to use physiology to understand geographic distributions have morphed into at least two subdisciplines. One is the description of basic physiological patterns at large spatiotemporal scales, a discipline termed macrophysiology. Macrophysiology has been very insightful for some plants, invertebrates and ectothermic vertebrates (Chown et al. 2004). For instance, by comparing rare plant species to common ones within the same genus, researchers revealed traits that make rare species distinctive as conservation priorities (Dunbar-Co et al. 2009). This approach for vertebrates, especially in the context of conservation, has been rare (Jessop et al. 2013b). Explicit focus on conservation, however, is the basis of a second subfield: conservation physiology (Wikelski and Cooke 2006). Conservation physiologists seek to use physiological data to predict how natural and anthropogenic stressors pose a challenge to population viability and animal welfare. To date, almost all research in the discipline has involved vertebrates (Cooke et al. 2013), and the majority of studies have relied on a particular group of steroid hormones, the glucocorticoids (GCs).

GCs have been favored in conservation physiology because they often relate to individual health and even fitness (Dantzer et al. 2014), making them potential predictors of population viability. These hormones are also quite simple to measure, being easily detectable in the blood or even feathers, fur, urine, blow (in the case of cetaceans), and feces. GCs also have many functions relevant to conservation interests (Angelier and Wingfield 2013; Sorenson et al. 2017). GCs are integral to water balance (McCormick and Romero 2017) and coordinating key life history stage transitions (Crespi et al. 2013), however, they have mostly been studied because of their impacts on energy metabolism (i.e., baseline GCs). In particular, GCs are critical for how individuals respond to unfamiliar, unpredictable, and often adverse conditions (i.e., responses to acute stressors such as severe weather or short-term food unavailability [Romero and Wingfield 2015]). In conservation physiology, GCs are typically portrayed as biomarkers of stress. The implicit assumption has been that GC disregulation implicates organisms in need of aid, although description of genuine disregulation is quite difficult (Tarlow and Blumstein 2007; Lindenmayer et al. 2013; Dantzer et al. 2014; Kilvitis et al. 2017). A prime recent example involves Cape mountain zebras (Lea et al. 2018). There, zebra fecal GCs were higher where grass forage was low and inversely related to female fecundity and subsequent population growth in the same sites. Overall, conservation physiology aspires to use GCs to indicate cause–effect relationship between stressors and population dynamics (Wikelski and Cooke 2006; Madliger et al. 2015), which could lead to suitable mitigation or remediation options (Cooke et al. 2013).

In many ways, a zeal for GCs as biomarkers of wildlife stress is justified (Martin et al. 2016a). GC measurements, in an appropriate context, have long been recognized as useful to determine exposure to various pesticides, herbicides, and other anthropogenic toxins (Martin et al. 2010; Rohr et al. 2013). Likewise, multiple large-scale analyses have revealed that anthropogenic disturbances are associated with altered GCs (Dickens and Romero 2013; Dantzer et al. 2014; Kleist et al. 2018). For instance, GC regulation in Magellanic penguins (*Spheniscus magellanicus*) (Walker et al. 2005), marine iguanas (*Amblyrhynchus cristatus* [Berger et al. 2007]), and hoatzins (*Opisthocomus hoazin* [Mullner et al. 2004]) is altered by tourism. Translocation into captive breeding programs or to more favorable parts of the range also affects GCs (Dickens et al. 2009), as do changes in conspecific density, with high densities and frequent territorial incursions increasing GCs in all vertebrate classes (Creel et al. 2013). The trouble with using GCs as biomarkers of stress for conservation aims is that relationships between GC concentrations and individual fecundity, survival, and recruitment are often complex (Breuner et al. 2008; Bonier et al. 2009; Sorenson et al. 2017). The implicit assumption of much conservation endocrinology, that "high" GCs indicate at-risk organisms (Dantzer et al. 2014; McCormick and Romero 2017), is probably untenable. Indeed, baseline GCs can increase, decrease, or remain unchanged in response to (chronic) stressors (Dickens and Romero 2013).

On the other hand, large-scale comparisons among species have revealed that simple descriptions of GC regulation can be insightful about challenges faced by populations. Hau et al. (2010) found that baseline GCs (i.e., those measured from blood taken quickly upon organism capture) were higher in species with shorter breeding seasons and smaller body mass. By contrast, GCs measured after a short physical restraint (i.e., stress responses) varied inversely with body mass and positively with annual adult survival rates. In another study, avian species that maintained high residual reproductive value after their first reproductive events mounted weaker GC responses than species with faster-paced reproductive life histories (Bokony et al. 2009). Finally, and most relevant to the present study, GCs have been revealed to have some value for understanding how species

might respond to global change (Jessop et al. 2013a): among 22 reptile and 66 bird species, combinations of a few variables (e.g., body mass, net primary productivity, and latitude) explained appreciable (14% and 33%, respectively) variation in GC responses to restraint.

The above broad comparative studies raise the possibility that a few measures of GCs might implicate at-risk species, which could help direct resource manager efforts to taxa most needing attention. Even though such a study could not indicate the causes of the distress that species experience, the availability of HormoneBase and the insight offered by prior broad comparative studies support effort to query the utility of GCs as a coarse bellwether of conservation risk. Ideally, as in the zebra study discussed above (Lea et al. 2018), one would characterize how individual variation in physiology predicts fitness in the same animals in response to adversity. However, if species prone to conservation concern generally regulate GCs differently than those that cope well with adversity, one might be able to implicate vulnerable species before concerted, expensive, and labor-intensive management efforts ensue.

To test the utility of GCs as such a proxy for conservation risk at the species level, we used HormoneBase (Vitousek et al. 2018), a database containing >6580 entries on glucocorticoid and androgen hormone levels in 474 species. First, we asked whether International Union for the Conservation of Nature (IUCN) status predicted baseline and post-restraint GCs in birds and reptiles, as these two vertebrate groups were the only groups with sufficient data for this analysis. The IUCN is a global organization charged with assigning a "concern" status to many of the world's species. It categorizes extinction risk, including "red list" assignment to some, depending on traits such as a geographic distribution, demographic structure, and population size and trend (Baillie et al. 2004). According to the IUCN, 86–88% of all birds, mammals, and amphibians assessed in 2010 were somehow threatened, usually by habitat alteration and/or loss (IUCN 2012). We asked whether GCs differ systematically between species of least concern (i.e., the IUCN category for species with no known threats) versus species in all "concern" categories. There were insufficient species in various categories of concern to make comparisons at a higher resolution (see below). Also, we felt that multiple forms of selection and/or phenotypic plasticity (Patterson et al. 2011; Wada 2014) made directional predictions between GCs and risk status difficult at the level of our analysis. The most straightforward expectation would be high GCs in species of concern. However, the reverse pattern was also plausible because (1) chronic stressors could lead to habituation of the hypothalamic–pituitary–adrenal axis (HPA), (2) exposure to adversity in early-life or selection could favor damped GC responses, or (3) adrenal insufficiency could lead to low GCs in at-risk populations. A third possibility was simply that phylogeny was the strongest driver of variation, an observation consistent with previous work (Jessop et al. 2013b). Our interest was solely to determine whether general patterns between IUCN status and GCs were detectable given the great enthusiasm for GCs in conservation physiology. Follow-up work involving data not available in HormoneBase would be necessary to discern why particular directional patterns occurred, which we describe in the "Discussion" section.

Our second interest was to reveal whether populations from the edges of geographic distributions regulate GCs differently than populations from range cores. This comparison was partly motivated by conservation interests, as at-risk populations commonly dwell at the margins of species distributions where habitat suitability is probably poor compared with the core. However, this part of our analysis was also motivated by work from some authors of the present paper (Martin et al. 2005; Liebl and Martin 2012, 2013; Martin and Liebl 2014; Martin et al. 2015, 2016a) and others (Atwell et al. 2012; Brown et al. 2015). Recurrently, GC responses to restraint have been found stronger at range edges than range cores. Although direct evidence does not yet exist regarding the eco-evolutionary reasons for these patterns, some evidence indicates that strong stress responses at range edges might facilitate particular behaviors conducive to success in novel and/or challenging areas (Martin and Fitzgerald 2005; Liebl and Martin 2012, 2014). In the present study, we expected post-restraint GCs to be higher if samples were collected from the margins versus the core of a species' range. We did not make directional predictions for baseline GCs, but we conducted the exploratory analysis to search for patterns that might be of future value to conservation biologists.

We, as others in conservation endocrinology, chose to focus on GCs because of their salience to homeostasis (Sapolsky et al. 2000). When GCs are disregulated, gluconeogenesis, water and electrolyte balance, and a host of other processes compromise nerve signaling, muscle contraction, immune defenses, and eventually reduce physical performance, often leading to death (McCormick and Romero 2017). We chose to study the two forms of GC data available in HormoneBase (baseline and

post-restraint), as GC effects vary depending on which receptors they engage. Baseline GC levels, the effects of which are predominantly mediated by mineralocorticoid receptors, are usually interpreted to reflect integrated energetic expenditures over long time periods (Crespi et al. 2013). That is, baseline GCs often increase moderately with energetic demands, including increases in physical workload, thermoregulatory, reproductive, or defensive (e.g., immunity) needs. Both high and low baseline GCs can therefore have negative health consequences depending on how long and by how much concentrations diverge from typical circadian/circannual rhythms (McEwen and Wingfield 2003; Romero et al. 2009; Martin et al. 2016c). By contrast, post-restraint GCs are mostly regulated through lower-affinity glucocorticoid receptors (GRs). Transient elevations of GCs work through GRs to facilitate emergency life processes that help animals avoid, endure, recover, or cope with stressors (Wingfield et al. 1998). If stressors continue or intensify, and individuals are not able to escape or habituate, persistent GC elevations can lead to pathology, as systems remain engaged beyond the point that they are protective (Korte et al. 2005; Martin 2009). Below, we describe how we used HormoneBase to discern whether IUCN status and point of sample collection within a species' range predict GCs in several species of wild birds and reptiles.

Methods

HormoneBase

Over the course of several years, we searched the literature to find all published accounts of circulating GC concentrations in wild organisms. The details about this search and the resultant database are explained in a recent publication (Vitousek et al. 2018). There as here, we analyzed only data on circulating corticosterone (CORT) concentrations, as comparisons of GC metabolites and/or fecal GCs were unavailable and would be difficult to interpret in any case due to interspecific variation in digestive physiology (Goymann 2012). We excluded mammal (M), amphibian (A), and fish (F) samples from our analysis for one or more of the following reasons: (1) too few species have an IUCN status (A), (2) marine/aquatic species ranges were impossible to describe in the same way as terrestrial birds and reptiles (F), and/or (3) entries in HormoneBase were too few to make meaningful comparisons (M and A). We also had to exclude some species of marine birds because their ranges could not be described in a manner comparable to non-marine species. We focused on CORT as it is the most widely-measured GC in birds and reptiles.

Statistical analysis

We used Bayesian-informed generalized linear mixed-effect models in the R package MCMCglmm (Hadfield 2010) to ascertain whether (1) IUCN status and (2) location within a species range where samples were collected predicted baseline and post-restraint concentrations of CORT. For IUCN status, we used the IUCN Red List website (http://www.iucnredlist.org) to assign all species for which we had data to a category. There are four categories of concern (near-threatened, vulnerable, endangered, and critically endangered) and one of "least concern" assigned by IUCN, but there were too few data for species in "concern" categories for us to analyze data based on original assignments. Subsequently, all species of any level of concern were collapsed into a single "concern" category and these species compared with the remaining "least concern" species. This approach resulted in 1153 measures of baseline glucocorticoids from 139 species assigned to "least concern," and 228 from 22 species assigned to "concern"(Supplementary Table S1). For post-restraint CORT we had 410 glucocorticoid measures from 79 species assigned to "least concern" and 78 measures from 13 species assigned to "concern" (Supplementary Table S2).

To categorize location of capture within each species' range, we used IUCN distribution maps in conjunction with Google Earth (https://earth.google.com/web) and geographic coordinates (from the original papers reported in HormoneBase) to assign each HormoneBase entry to one of three categories. For each species, we determined whether samples were collected within the inner 10% of the area of a species' range (i.e., core), the outer 10% of a species' range (i.e., edge), or the intervening 80% (i.e., intermediate). However, as our expectations were that range edges would be most different (and demanding) from other parts of the range (and to maximize statistical power), we collapsed the core and intermediate samples into a single category (i.e., non-edge), and used this binary predictor in all final analyses. We conducted separate analyses for IUCN status and site within the geographic distribution. Although many species of concern (in regards to IUCN status) have narrow distributions, our interests in the two factors were distinct. This approach resulted in 326 "edge" and 526 "non-edge" baseline CORT data points from 78 and 43 species,

respectively (Supplementary Table S3). For post-restraint CORT, we had 175 "edge" and 146 "non-edge" data points from 51 and 28 species, respectively (Supplementary Table S4).

As the broad comparative approach we took might obscure subtle relationships, we took several steps to disentangle focal predictors from other variables that might influence CORT. First, to account for variability in assay methodology (Fanson et al. 2017), we used laboratory identity as a random effect. Although this factor might not capture all potential sources of variation in CORT data, it should encompass substantial variation in many aspects of methodology and importantly it was the only relevant methodological variable we had available for the majority of data. Second, because our analyses often included multiple observations of the same species, we included species as a random effect. Third, we included several variables as covariates in models that are known or expected to affect GCs. We included altitude and latitude at the site of collection, mean body mass of individuals in the collected population (Haase et al. 2016), and breeding status (breeding versus non-breeding, with breeding being the reference state in models [Romero 2002]). HormoneBase was the source of all of these covariates for the sake of consistency in analytical efforts among projects (Johnson et al. 2018). We also attempted to use ambient temperature (mean and standard deviation) in the month in which samples were collected, but as that approach greatly diminished our sample sizes, we did not include climate variables in our analysis. We included all samples from HormoneBase in our analysis as long as authors identified them as baseline.

Finally, we used a tree developed specifically for analyses of HormoneBase data in this SICB symposium (Johnson et al. 2018) as our phylogenetic hypothesis to account for potential phylogenetic structure in the relationship between GCs and the predictor variables, which was converted into an inverted phylogenetic covariance matrix prior to analyses. We allowed the model to estimate Pagel's λ, and we report the posterior mode from the MCMC chain. Pagel's λ ranges from 0 to 1 and reflects no and high phylogenetic signal, respectively. We used uninformed priors in the models; default priors for all fixed effects and, reflecting an inverse-Gamma distribution, we used $V = 1$ and $\mu = 0.02$ for the variance components of each random effect. We scaled all continuous predictors (i.e., z-transformations) to facilitate direct comparisons of variable effects in models to each other. We conducted the IUCN and geographic distribution analyses separately, as inclusion of both predictors in models would have greatly reduced our sample sizes (due to missing IUCN or distribution data for some HormoneBase entries).

We used an all-subsets approach in model selection. Specifically, from our global model we ranked models using the Deviance Information Criteria (DIC) using the dredge function in the MuMIn R package (Barton 2009) and considered all models $\mathrm{DIC} \leq 2$ from the top ranked model as competitive. All model runs were based on 2000 samples drawn from 50,000 MCMC iterations, a burn-in of 10,000, and a thinning rate of 20. For each global model, plus all strongly ranked models (i.e., $\mathrm{DIC} \leq 2$), we verified that MCMC chains were mixing by visually inspecting trace and density plots and ensuring that autocorrelation of sampled iterations was less than 0.1. Finally, using the R package coda (Plummer et al. 2008), we verified that four independent chains converge using the Gelman–Rubin statistic. We also calculated marginal (fixed effects only) and conditional (fixed and random effects) R^2 for all posterior models (Nakagawa and Schielzeth 2013). We used two pieces of evidence to determine whether a predictor had a strong effect: (1) whether predictors occurred in well-supported models (i.e., $\mathrm{DIC} \leq 2$) and (2) whether the 95% credible intervals, calculated from the posterior means, excluded zero (bolded terms in relevant tables).

Results

IUCN status

The top models for baseline CORT always included breeding status and latitude, but none included IUCN status, and all marginal R^2 values for all models were very low (Table 1). In all models, breeding birds had higher baseline CORT than non-breeding birds and Pagel's λ values were high (Table 2). Latitude was negatively related to baseline CORT in only one model, and CIs overlapped zero for all other terms in all other models. Models for post-restraint CORT also always included breeding status, and two models included IUCN category (Table 3); again though, marginal R^2 was low for all models. Most models also included body mass. However, only breeding status CIs did not overlap zero in the top models (Table 4); again, breeding status was related to post-restraint CORT concentrations. Although IUCN was included in two of the five top models, its influence was very modest. Pagel's λ values were also high for all models.

Table 1 Top models predicting variation among avian and reptilian baseline corticosterone entries in HormoneBase (IUCN status)

Model	Terms in model	df	Loglikelihood	DIC	Delta	Weight	Marginal R^2	Conditional R^2
1	Breeding + latitude + body mass	7	−1223.29	2614	0	0.246	0.01	0.92
2	Breeding + latitude	6	−1223.67	2614.5	0.41	0.200	0	0.93
3	Breeding + latitude + altitude + body mass	8	−1223.63	2615.3	1.26	0.131	0.01	0.93
4	Breeding + latitude + altitude	7	−1224	2615.7	1.68	0.106	0.01	0.93

Table 2 Composition of top models for baseline corticosterone, IUCN analysis

Model	Predictor	Post.mean	Lower 95%	Upper 95%	Effective sample size
1	(Intercept)	1.363	−0.390	3.293	2000
(λ=0.93)	**Latitude (scaled)**	**−0.133**	**−0.244**	**−0.029**	**2000**
	Body mass (log, scaled)	0.098	−0.030	0.221	2000
	Breeding (±)	**−0.134**	**−0.228**	**−0.041**	**1936**
2	(Intercept)	1.464	−0.333	3.351	2000
(λ=0.92)	**Latitude (scaled)**	**−0.119**	**−0.236**	**−0.016**	**2000**
	Breeding (±)	**−0.134**	**−0.230**	**−0.041**	**2000**
3	(Intercept)	1.409	−0.416	3.221	2000
(λ=0.93)	**Latitude (scaled)**	**−0.113**	**−0.225**	**−0.010**	**2000**
	Body mass (log, scaled)	0.097	−0.013	0.228	2000
	Altitude (scaled)	0.044	−0.020	0.107	2000
	Breeding (±)	**−0.135**	**−0.225**	**−0.036**	**2276**
4	(Intercept)	1.478	−0.371	3.279	2000
(λ=0.91)	Latitude (scaled)	−0.097	−0.207	0.013	2182
	Altitude (scaled)	0.042	−0.022	0.109	2138
	Breeding (±)	**−0.134**	**−0.225**	**−0.039**	**2136**

Bolded text highlights terms with credible intervals that do not overlap zero.

Table 3 Top models predicting variation among avian and reptilian post-restraint corticosterone entries in HormoneBase (IUCN status)

Model	Terms in model	df	Loglikelihood	DIC	Delta	Weight	Marginal R^2	Conditional R^2
1	Breeding + body mass	6	−264.888	619	0	0.172	0.005	0.93
2	Breeding + IUCN + body mass	7	−264.681	619.3	0.32	0.146	0.006	0.93
3	Breeding + latitude + body mass	7	−264.687	620	1.03	0.103	0.005	0.93
4	Breeding + IUCN + latitude + body mass	8	−264.739	620.3	1.32	0.089	0.007	0.93
5	Breeding	5	−265.583	620.6	1.63	0.076	0.002	0.93

Geographic distribution

Location in the range was included in two of the top models for baseline CORT, but breeding status and latitude were included in all models, and body mass and altitude were included in many models (Table 5). All marginal R^2 values were low. Only breeding status and altitude affected baseline CORT (Table 6). Range location CIs always overlapped zero when it was included as a predictor; the same was true for all other variables in the best-fit models. Pagel's λ was also high in all four models. For post-restraint CORT, only two models were supported, both of which included breeding status and body mass (Table 7), but marginal R^2 values were again very low. Breeding animals had higher post-restraint CORT in all models, but no other effects, including range location, were strong based on CIs overlapping zero (Table 8). Pagel's λ values were high for both models.

Discussion

We investigated whether IUCN status and location within the geographic range predicted GC concentrations in birds and reptiles because GCs enable

Table 4 Composition of top models for post-restraint corticosterone, IUCN analysis

Model	Predictor	Post.mean	Lower 95%	Upper 95%	Effective sample size
1	(Intercept)	2.906	1.674	4.217	2000
(λ=0.93)	**Breeding (±)**	**−0.178**	**−0.280**	**−0.068**	**2000**
	Body mass (log, scaled)	0.085	−0.027	0.192	2000
2	(Intercept)	2.941	1.685	4.164	2000
(λ=0.93)	**Breeding (±)**	**−0.183**	**−0.299**	**−0.082**	**2000**
	IUCN	−0.102	−0.440	0.208	2146
	Body mass (log, scaled)	0.091	−0.023	0.198	2000
3	(Intercept)	2.883	1.529	4.152	2131
(λ=0.93)	Latitude (scaled)	−0.010	−0.104	0.078	2223
	Breeding (±)	**−0.178**	**−0.277**	**−0.062**	**2000**
	Body mass (log, scaled)	0.089	−0.026	0.194	2000
4	(Intercept)	2.896	1.639	4.262	2000
(λ=0.92)	IUCN	−0.112	−0.411	0.229	2000
	Latitude (scaled)	−0.013	−0.097	0.088	2000
	Breeding (±)	**−0.181**	**−0.287**	**−0.062**	**2000**
	Body mass (log, scaled)	0.097	−0.019	0.214	2000
5	(Intercept)	3.011	1.770	4.280	2000
(λ=0.92)	**Breeding (±)**	**−0.174**	**−0.280**	**−0.066**	**2000**

Bolded text highlights terms with credible intervals that do not overlap zero.

Table 5 Top models predicting variation among avian and reptilian baseline corticosterone entries in HormoneBase (geographic range)

Model	Terms in model	df	Loglikelihood	DIC	Delta	Weight	Marginal R^2	Conditional R^2
1	Breeding + range + latitude + altitude	8	−708.403	1534.6	0	0.239	0.01	0.94
2	Breeding + range + latitude + altitude + body mass	9	−707.89	1534.9	0.28	0.208	0.01	0.95
3	Breeding + latitude + altitude + body mass	8	−708.817	1535.5	0.83	0.157	0	0.95
4	Breeding + latitude + body mass	7	−709.065	1535.7	1.03	0.142	0	0.94

individuals to adjust their phenotypes to variation in environmental conditions (Hau et al. 2016). Unlike most other biomolecules (Martin et al. 2011), GCs can enduringly or reversibly modulate the properties of many tissues, giving them a molecular form of functional pleiotropy that orients organismal phenotypes to a shared life priority (McGlothlin and Ketterson 2008; Ketterson et al. 2009; Cohen et al. 2012; Martin and Cohen 2014). Such extensive effects of GCs make them both potential facilitators and impediments to conservation interests, depending on their collective effects across tissues and contexts. Overall, we found little evidence that IUCN status (some level of concern versus least concern) or location within the geographic range (edge versus non-edge) predicted baseline and post-restraint CORT concentrations of several bird and reptile species.

Null results are always difficult to discuss and interpret, but our rediscovery of previously described interspecific patterns (e.g., effects of breeding status on CORT) gives us confidence that our analysis was appropriately executed. As with previous work (Casagrande et al. 2018), we found seasonal changes in the regulation of GCs breeding (Romero 2002) even though we used a very coarse surrogate for breeding status (yes/no). Likewise, we have strong evidence for effects of phylogeny (all $\lambda > 0.90$) on both forms of CORT (Jessop et al. 2013b), and we found effects of latitude on BL CORT consistent with other studies (Hau 2010, 4767). In light of these rediscoveries and the exceptionally broad scope of our work, we think it is reasonable to conclude that IUCN listing for species and edge/non-edge status of populations are unrelated to CORT in birds and lizards. For these reasons, we discourage simple future efforts to use CORT as a biomarker of stress at such very broad scales. Below though, we discuss a few potential reasons why other studies of GCs in the service of conservation are warranted, and we propose some potentially useful ways forward at multiple levels of analysis.

Table 6 Composition of top models for baseline corticosterone, range analysis

Model	Predictor	Post.mean	Lower 95%	Upper 95%	Effective sample size
1	(Intercept)	2.204	0.189	4.160	2024
(λ=0.95)	Range	−0.083	−0.242	0.084	2000
	Latitude (scaled)	−0.053	−0.159	0.043	1859
	Altitude (scaled)	**0.092**	**0.018**	**0.166**	2000
	Breeding (±)	**−0.167**	**−0.277**	**−0.054**	**2150**
2	(Intercept)	2.156	0.084	4.256	2000
(λ=0.95)	Latitude (scaled)	−0.061	−0.165	0.039	2000
	Range	−0.091	−0.259	0.071	2000
	Altitude (scaled)	**0.091**	**0.017**	**0.165**	2000
	Breeding (±)	**−0.166**	**−0.275**	**−0.047**	**2000**
	Body mass (log, scaled)	0.060	−0.069	0.209	2000
3	(Intercept)	2.038	0.077	4.202	2023
(λ=0.94)	Latitude (scaled)	−0.060	−0.164	0.037	1847
	Altitude (scaled)	**0.104**	**0.031**	**0.179**	2000
	Breeding (±)	**−0.165**	**−0.282**	**−0.052**	**2000**
	Body mass (log, scaled)	0.057	−0.085	0.196	2000
4	(Intercept)	2.125	−0.085	3.991	2000
(λ=0.95)	Latitude (scaled)	−0.054	−0.143	0.051	2000
	Altitude (scaled)	**0.103**	**0.027**	**0.173**	1875
	Breeding (±)	**−0.163**	**−0.280**	**−0.041**	**2245**

Bolded text highlights terms with credible intervals that do not overlap zero.

Table 7 Top models predicting variation among avian and reptilian post-restraint corticosterone entries in HormoneBase (geographic range)

Model	Terms in model	df	Loglikelihood	DIC	Delta	Weight	Marginal R^2	Conditional R^2
1	Breeding + body mass	6	−170.157	406.5	0	0.244	0.01	0.94
2	Breeding + latitude + body mass	7	−170.26	407.1	0.65	0.176	0	0.94

Table 8 Composition of top models for post-restraint corticosterone, range analysis

Model	Predictor	Post.mean	Lower 95%	Upper 95%	Effective sample size
1	(Intercept)	3.747	2.037	5.595	2299
(λ=0.95)	**Breeding (±)**	**−0.247**	**−0.395**	**−0.121**	**2000**
	Body mass (log, scaled)	0.066	−0.040	0.165	2303
2	(Intercept)	3.694	1.974	5.440	2000
(λ=0.95)	**Breeding (±)**	**−0.259**	**−0.405**	**−0.137**	**2000**
	Latitude (scaled)	−0.031	−0.125	0.061	1829
	Body mass (log, scaled)	0.072	−0.030	0.177	2000

Bolded text highlights terms with credible intervals that do not overlap zero.

IUCN status

IUCN status did not predict baseline or post-restraint CORT in birds and reptiles well in spite of quite large sample sizes and phylogenetic coverage. Our care to include several covariates in our analyses also should have helped tease out any relationships between IUCN status and CORT variation if they exist. The most likely reasons we did not reveal appreciable effects of IUCN status on CORT is that (1) variation in CORT is strongly context-dependent (Busch and Hayward 2009) and (2) IUCN status is

too imprecise an indicator of stress experienced by wildlife. In regards to the former, context-dependence is the rule more than the exception for HPA function, the neuroendocrine axis regulating the release of CORT into the bloodstream. CORT and other HPA regulatory elements fluctuate over several timescales (Woods and Wilson 2014), and these fluctuations are integral to CORT achieving its physiological functions. Baseline CORT largely works in a permissive manner, enhancing the actions of catecholamines, preparing the immune system for insult, stimulating lipolysis and gluconeogenesis, and increasing food consumption and deposition of energy stores (Sapolsky et al. 2000). Conversely, post-restraint CORT mediates emergency life history responses, inducing rapid and transient changes that help an individual flee, endure, actively cope with, and recover from adversity (Wingfield et al. 1998). Perhaps had we included life history stage, health, sex, and variation, and other traits of individuals (Korte et al. 2005), we might have revealed effects of IUCN status on CORT. Repeatedly, breeding status was one of the best predictors of CORT variation. We were unable to include more precise variables because HormoneBase does not include data at the individual animal level and because of the exceptional inherent diversity in life history strategies among the species we considered.

Even had we individual-level data or the ability to describe species in a more specific way, we might not have detected effects of IUCN status on CORT. Others have used GCs for conservation pursuits because of their utility as proxies for individual health and fitness (Strasser and Heath 2013), and the most informative studies, in terms of linking physiology to conservation risk or mitigation opportunity, have been at the level of populations (Martínez-Mota et al. 2007; Homyack 2010). For instance, within species, individuals with lower body condition often have higher baseline GCs (Lindström et al. 2005; Angelier et al. 2009). However, relationships between CORT and aspects of fitness are complex (Schoenle et al. 2018), so even population-level comparative work risks imprecision. For example, baseline CORT in tree swallows (*Tachycineta bicolor*) was only elevated in some populations when food restriction was imposed on females during the period when offspring were being fed (Madliger and Love 2014); outside this period and/or in other populations, baseline CORT did not change with food restriction. In house sparrows (*Passer domesticus*), the direction of the relationship between baseline CORT and reproductive success flipped depending on the breeding stage; before egg-laying, the relationship was positive but during offspring provisioning, it was negative (Ouyang et al. 2011). These studies and the absence of intelligible patterns in our study highlight that for conservation purposes, we probably need to characterize better how HPA responses to adversity mediate fitness among individuals within populations (see below).

A second likely reason for no influence of IUCN status on CORT is the breadth of factors that leads to IUCN listing in the first place. Listing represents the integration of several different forms of information about a species as well as trends of focal populations (IUCN 2012). This complex algorithm for listing means that organisms can occupy the most dire rankings for quite different reasons. Many of the most threatened species are listed because of very narrow geographic ranges (i.e., endemics), whereas others are listed because their populations are declining over large spatial scales (e.g., habitat destruction), and still others are listed because of particularly vulnerable life stages (e.g., marine turtles). Conservation threats also equate to sources of selection in an evolutionary sense, so when selection acts at different points of life, CORT variation can be driven in a particular direction, obscuring any interspecific patterns. In white-crowned sparrows (*Zonotrichia leucophrys*), for example, survival selection favored high post-restraint CORT, but fecundity selection favored weaker CORT responses to restraint (Patterson et al. 2011). We focused on adult animals here to try to moderate age-dependency in our comparisons, but ideally, one would analyze data from multiple age classes (and other sensitive categories mediating listing) to reveal what about IUCN listing instigates GC variation.

Our perspective is that IUCN status, as a species-level designation, is probably just too coarse a category to relate to GCs or most any other form of physiological variation in the service of conservation. Moreover, as many data in HormoneBase will have come from samples available from the least sensitive parts of a species range (because managers are probably often reluctant to permit capture and handling of critically endangered species), it might be even less likely that coarse analyses would reveal patterns. By contrast, some populations of IUCN-listed species might have been restricted to protected but otherwise suboptimal habitats. Without sampling from multiple points across the range of an IUCN-listed species, it might be hard to link IUCN status and GC variation. Some populations will simply experience less stress than others. Although it is as yet unclear which of the two above factors predominate in our study, it is sensible that future conservation endocrinology efforts take a more focused approach.

Geographic range

Although the "edge" term appeared in the two top models for baseline CORT, its explanatory power was weak compared with other factors. Much like IUCN status, capture location (i.e., edge/non-edge) is probably too imprecise a way to describe adversity of conditions at a site. Our main motivation for conducting this comparison came from previous work on range-expanding populations; the roles of GCs in individual fitness is probably quite distinct in range-edge organisms relative to those enduring conditions for generations at the core. In light of the present results, if we are to discern whether and how location within a range influences GCs, or vice versa, we will need to investigate directly the forces inducing variation in the first place. Although repeatability of both stress-induced GC and baseline concentrations are high in birds (Taff et al. 2018), both measures are also strongly influenced by environmental conditions. Data in HormoneBase represent a mix of within- and among-individual variation, yet without repeated GC measures from individuals, one will remain unable to separate these sources of variation (Baugh et al. 2014). Environmentally-induced variation is important functionally, but it does not capture as well how GCs mediate fitness and thus success or failure in marginal areas (Hau et al. 2016). Moreover, because relationships between fitness and GCs can be non-linear and vary among populations (Martin et al. 2005; Busch and Hayward 2009; Crespi et al. 2013), it will be imperative to study some populations intensively to reveal how GCs mitigates population dynamics at various sites (Zanette et al. 2011; Dantzer et al. 2013; Kleist et al. 2018). Perhaps if we could have compared relationships between GCs and fitness among edge and non-edge sites, we would have revealed interesting patterns. Such data are not available in HormoneBase, however, largely because those datasets remain relatively rare. In the future, efforts to link GCs and geographic distribution should focus on how GCs support population viability, but perhaps expand their perspectives on the functions of GCs. So far, conservation and comparative endocrinologists have focused mostly on the role of CORT in energy balance (Vera et al. 2017). However, GCs have manifold effects (Dallman et al. 2007) including those that mitigate responses to infections (Gervasi et al. 2016; Martin et al. 2016b; Gervasi et al. 2017) and even water balance (Vera et al. 2017). These well-known effects of GCs have been little considered for affecting the viability or distribution of populations, even species in marginal habitats where desertification (Hofmeister and Rubenstein 2016), climate change, or other challenges to water balance are becoming more common.

Suggestions for future conservation physiology involving GCs

Our results suggest that future work attend to the particular pathways by which GCs affect individual fitness instead of taking coarse comparative approaches, even with large databases. We do not mean to disparage all future large-scale comparative conservation endocrinology efforts, as they could be insightful. For instance, because GCs are deposited into fur, feather, feces, and other tissues at slower rates than they are released and metabolized in the blood, it might be informative to compare GCs from other tissues as proxies of IUCN listing or other forms of conservation risk at broad (i.e., species) levels. Typically though, nuanced approaches to GC conservation physiology will probably be more insightful (Chown and Gaston 2016). The work of Valladares et al. (2014) is a great example; they studied how relationships between phenotypic plasticity and the thermal niche could be used to improve forecasts of species responses to environmental change. We encourage that researchers interested in GCs as a biomarker of wildlife stress also consider the physiological functions of GCs and the context in which it is measured.

As an example of a future promising approach, consider how habitat degradation and destruction could work through GCs to affect mammalian extinction risk. Habitat degradation and destruction are the main threats to extinction for most vertebrate species (Drake and Griffen 2010), but risk changes depending on variation in environmental factors, body size, and intrinsic traits (e.g., life history schedules) among and within species/populations. Large mammals, particularly those from the tropics (Fritz et al. 2009), tend to have higher extinction risks because of their low reproductive rates. However, species above a 3-kg body size threshold are disproportionately (negatively) impacted by environmental (and intrinsic) factors including human population density (Cardillo et al. 2004). To discern whether GCs can serve as biomarkers with genuine conservation value, one could track serum (or possibly fecal) GCs in at-risk populations, expecting GCs to become increasingly disregulated as populations experience critical slowing down, points on population growth trajectories at which rates of recovery from small perturbations decrease (Drake and Griffen 2010). These quite involved efforts would be prohibitive in many systems, but their execution in a

few species could provide valuable guidance for particularly threatened species.

Note too that for GCs to be supported as genuinely useful biomarkers, one will need to link concentrations in single measurements to the regulatory plasticity of the HPA that mediates GC effects on performance and fitness (Guindre-Parker 2018). Although aspects of GC regulation are sometimes inter-related (Liebl et al. 2013), one should not assume that single measures are proxies for the flexible changes in hormone concentrations that mediate function and fitness. GCs regulatory flexibility probably represents an important form of phenotypic flexibility (Martin and Cohen 2014; Martin and Liebl 2014; Martin et al. 2015; Hau et al. 2016; Taff and Vitousek 2016; Kilvitis et al. 2017), which is beneficial when it allows an individual to alter its phenotype to match a changing environment (DeWitt et al. 1998). In fluctuating environments flexible individuals may be more competitive and have higher reproductive success and survival than inflexible (canalized) individuals, although outcomes will be contingent on the time scale over which environments fluctuate and whether regulation can keep pace. Conversely, although more canalized individuals may have a lower potential to adapt to fluctuating environments, they may be more successful under stable conditions, especially if plasticity is costly (DeWitt et al. 1998). It would be insightful to determine whether HPA regulatory flexibility predicts stable and/or unstable population dynamics, ideally using state-based population models (Crespi et al. 2013).

A final lucrative consideration for conservation endocrinology is to account for the form of chronic stress that is leading populations or species to be of concern in the first place (Dickens and Romero 2013). In terms of their endocrinological effects, chronic stressors tend to take two forms (Martí and Armario 1998). In chronic continuous stress (e.g., some adverse social situations), stressor exposure is omnipresent. In these scenarios, HPA habituation often occurs and GC elevations subside or even decrease relative to levels measured prior to stressor exposure. The other form, chronic intermittent stress, involves exposure to a series of stressors on a consistent or rotating basis. Animals in these conditions do not habituate and maintain high levels of GCs for long periods. We already know from populations of no obvious concern that GCs can be low at certain times of year in challenging environments (e.g., high latitudes), presumably to allow individuals to continue to breed in spite of suboptimal conditions (Wingfield and Sapolsky 2003). Growing evidence also suggests that selection often favors dampened GC stress responses in human-disturbed areas too (Partecke and Gwinner 2007; Atwell et al. 2012). We recognize that it will be very difficult to conduct involved endocrinological studies on many threatened and endangered wildlife, but more physiologically-nuanced approaches are critical if GCs are to serve as a broadly-useful conservation tool. The distinct research methods and lexicons of physiology and conservation biology alone make collaborations challenging (Lennox and Cooke 2014), but the payoff is mutual awareness and, ideally, realized conservation aspirations (Redpath et al. 2013).

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Supplementary data

Supplementary data available at *ICB* online.

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