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SYMPOSIUM

# Metabolic Scaling of Stress Hormones in Vertebrates

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**Synopsis** Glucocorticoids (GCs) are stress hormones that can strongly influence physiology, behavior, and an organism's ability to cope with environmental change. Despite their importance, and the wealth of studies that have sought to understand how and why GC concentrations vary within species, we do not have a clear understanding of how circulating GC levels vary within and across the major vertebrate clades. New research has proposed that much interspecific variation in GC concentrations can be explained by variation in metabolism and body mass. Specifically, GC concentrations should vary proportionally with mass-specific metabolic rates and, given known scaling relationships between body mass and metabolic rate, GC concentrations should scale to the −1/4 power of body mass and to the power of 1 with mass-specific metabolic rate. Here, we use HormoneBase, the newly compiled database that includes plasma GC concentrations from free-living and unmanipulated vertebrates, to evaluate this hypothesis. Specifically, we explored the relationships between body mass or mass-specific metabolic rate and either baseline or stress-induced GC (cortisol or corticosterone) concentrations in tetrapods. Our phylogenetically-informed models suggest that, whereas the relationship between GC concentrations and body mass across tetrapods and among mammals is close to −1/4 power, this relationship does not exist in amphibians, reptiles, and birds. Moreover, with the exception of a positive association between stress-induced GC concentrations and mass-specific metabolic rate in birds, we found little evidence that GC concentrations are linked to metabolic rate, although the number of species sampled was quite limited for amphibians and somewhat so for reptiles and mammals. Nevertheless, these results stand in contrast to the generally accepted association between the two and suggest that our observed positive association between body mass and GC concentrations may not be due to the well-established link between mass and metabolism. Large-scale comparative approaches can come with drawbacks, such as pooling and pairing observations from separate sources. However, these broad analyses provide an important counterbalance to the majority of studies examining variation in GC concentrations at the population or species level, and can be a powerful approach to testing both long-standing and new questions in biology.

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## Introduction

Allometry has a long history in biology (reviewed in Gayon 2000). The term was originally used to describe organ growth relative to body size (Huxley and Teissier 1936); however, it is now used to reflect scaling relationships at all levels of biological organization. Although widely applied to evolutionary studies of variation in morphology, and body size in particular (Gould 1971; Lande 1979; Emlen 1996), interest in allometric scaling has intensified over the last two decades with a central focus on metabolism (e.g., West et al. 1997; Brown et al. 2004; Glazier 2005). Much of the recent debate has centered around determining the value of and variation around the scaling exponent of metabolism with body mass (Bokma 2004; Uyeda et al. 2017). The value of the scaling exponent aside, by focusing on energy as a fundamental currency of life, with metabolism at its core, metabolic scaling theories have become useful frameworks for evaluating a variety of biological and ecological phenomena spanning rates of molecular evolution (Gillooly et al. 2005) to population growth rates (Savage et al. 2004) and global carbon cycles (Allen et al. 2005).

Recently, metabolic theories have also been applied to understanding variation in trait expression, such as the properties of acoustic signals within (Ziegler et al. 2016) and across species (Gillooly and Ophir 2010) or interspecific variation in dive duration among foraging animals (Halsey et al. 2006; Hayward et al. 2012). These studies are suggestive of the power of metabolic scaling theories for explaining trait expression more broadly. After all, the development, maintenance, and expression of all traits require energy and, hence, metabolism.

Metabolic rates are determined by many physiological processes. Glucocorticoid (GC) hormones, i.e., cortisol and/or corticosterone (henceforth GCs), should be tightly linked to metabolism given their function to convert stored energy into glucose to respond to challenges. Indeed, GC concentrations increase and decrease with energy expenditure (Welcker et al. 2015), and increase for routine energetic demands (Romero et al. 2009) or for high-energy life stages, such as reproduction (Bonier et al. 2011). Thus, metabolism and GC concentrations are generally assumed to covary positively (McEwen and Wingfield 2003; Romero et al. 2009). However, there have been surprisingly few explicit empirical tests of relationships between GC concentrations and metabolism, with, to our knowledge, the exception of several intraspecific studies (e.g., Buehler et al. 2012; Jimeno et al. 2017) and only one interspecific study of mammals (Haase et al. 2016). In the latter, Haase et al. (2016) adopted a metabolic scaling approach to determine whether cortisol concentration (GC) is related to mass-specific metabolic rate ($B/M$, mW $g^{-1}$) and body mass ($M$, g) as follows:

$$[\mathrm{GC}] = a\frac{B}{M} = ab\ M^{-1/4}, \quad (1)$$

where $a$ is a constant that delineates plasma GC concentration (ng $mL^{-1}$) to metabolic energy flux ($mW^{-1}$) per gram of tissue ($g^{-1}$), and $b$, a normalization constant, relates the rate of mass-independent energy flux per gram of tissue (mW $g^{0.25}$). These authors found broad support for cortisol concentrations scaling to the $-1/4$ power of body mass (i.e., $M^{-1/4}$) and proportional to mass-specific metabolic rate (i.e., $B/M$). These scaling exponents are consistent with the statistical expectations of metabolic theory of ecology (MTE) (Brown et al. 2004) and provide not only the best evidence to date that GC concentrations are linked to metabolism, but also suggest MTE is a suitable theoretical framework for predicting how plasma GC concentrations should vary across species.

Here, we sought to broaden the focus of Haase et al. (2016) and explore the relationship between dominant vertebrate GCs and body mass and metabolic rate using HormoneBase, a recently assembled database reporting hormone levels in unmanipulated wild vertebrate populations throughout the globe (Vitousek et al. 2018; Johnson et al. 2018). Our first objective was to determine whether GC concentrations are associated with body mass and metabolic rate across tetrapod animals and within Classes Amphibia, Reptilia, Aves, and Mammalia. We focused on un-adjusted basal metabolic rate (BMR) values from mammals and birds and standard metabolic rate (SMR) values adjusted to remove the influence of temperature for amphibians and reptiles (see "Methods" section). Although fish are present in HormoneBase, records with observations of body mass, GC concentrations, and metabolic rates were too few for formal analyses (Johnson et al. 2018) and thus are not considered here.

We then used the metabolic scaling framework articulated by Haase et al. (2016) to test whether baseline GC concentrations scale to the $-1/4$ power with body mass and are proportional to mass-specific metabolic rate (i.e., power of 1) in an independent mammal data set and among the other three classes of vertebrates while controlling for the potential influence of body temperature. These

allometric scaling exponents are those expected from MTE (e.g., Brown et al. 2004) and are based on scaling of blood volume and branching networks (Banavar et al. 1999, 2010). Because biological rates are closely linked to temperature (e.g., Gillooly et al. 2001; Brown et al. 2004), evaluating scaling relationships with metabolism (or possibly due to metabolism) must account for variation in body temperature, which can be quite narrow in some taxa, such as most mammals and birds, or as variable as the environment in which they live for others (i.e., most amphibians and reptiles). In general, we expected associations between baseline GC concentrations and metabolic rate, and baseline GC concentrations and body mass, because baseline GC concentrations should be associated with physiological demands under most conditions. However, because Haase et al. (2016) found a strong relationship between baseline and stress-induced GC concentrations in mammals, and that baseline GC concentrations were linked to mass and metabolic rate, we also tested for associations between stress-induced GC concentrations and body mass and stress-induced GC concentrations and metabolic rate. A potential explanation for an association between BMR or SMR and stress-induced GC concentrations is based on the possibility that capacity for activity should be positively associated with BMR or SMR (i.e., increased intake model; Careau and Garland 2012). Although stress-induced GC concentrations are not a measure of activity *per se*, GC concentrations increase with energetically demanding activities (Romero et al. 2009; Welcker et al. 2015). Thus, increases in BMR or SMR, which could reflect an organism's capacity for increased activity, could be positively related to stress-induced GC concentrations. Alternatively, because elevated concentrations of GCs have long been known to decrease peripheral update of glucose (reviewed in Munck 1971), the relationship between stress-induced GC concentrations and metabolic rate may be obscured or nonexistent.

## Methods

Hormone data used in this study are available in HormoneBase (HormoneBase.org; Vitousek et al. 2018). Details regarding body mass measures, temperature, metabolic rate, and our phylogenetic hypothesis can be found in Johnson et al. (2018). Briefly, GC measurements in our database included cortisol and corticosterone. For taxa where corticosterone is the main GC (i.e., amphibians, reptiles, rodents, birds), we assumed it is related to metabolism in the same way as is cortisol. In all cases, we only used GC concentrations obtained from plasma and assigned GC concentrations as baseline or stress-induced based on descriptions in the original studies. In preliminary analyses, we restricted record inclusion to those where baseline GC concentrations had been obtained <3 min following capture for mammals and birds and <5 min following capture for reptiles and amphibians. The results were qualitatively identical to analyses including the full data set and are not presented here.

Because temperature is linked to metabolic rate (Brown et al. 2004) and GCs relate to metabolism (McEwen and Wingfield 2003; Romero et al. 2009), we sought to account for the influence of temperature on the relationships between body mass and GCs, and mass-specific metabolic rate and GCs, in two ways. First, for analyses involving body mass and GCs, we calculated temperature-corrected GC concentrations by adding the Boltzmann–Arrhenius factor (i.e., $e^{-E/kT}$) to Equation (1), which reflects the universal temperature dependence of biological processes (Gillooly et al. 2001):

$$[\mathrm{GC}] = a\frac{B}{M} = ab\ M^{-1/4}e^{-E/kT}, \qquad (2)$$

where $E$ denotes the mean activation energy for biochemical reactions of metabolism, and here is assumed to be 0.65 eV, $k$ is Boltzmann's constant ($8.62 \times 10^{-5}$ $\mathrm{eVK}^{-1}$), and $T$ is the temperature in degrees K. Thus, if GC concentrations scale across vertebrates as documented in mammals by Haase et al. (2016), the natural logarithm of temperature-corrected baseline and stress-induced GC concentrations i.e., $\ln(\mathrm{GC}e^{E/kT})$ should be linear functions of the natural logarithm of body mass. Moreover, reflecting the dependence of metabolic rate on size, the slopes should be −0.25. Because variation in body temperature among birds and mammals is quite small (Clarke et al. 2010) and often treated as a class-level constant in scaling studies (Gillooly and Ophir 2010; Isaac and Carbone 2010), for the temperature correction we treated bird and mammal body temperature as 38.5°C and 36.2°C, the mean body temperatures for these classes, respectively (based on Prinzinger et al. 1991; White and Seymour 2003). For the temperature correction for amphibians and reptile GC concentrations, we used mean temperature values from the season in which hormone samples were collected in the original study (e.g., Johnson et al. 2018). Briefly, seasons were considered 3 months long. If hormone collection occurred during the months of a single season, we

used the mean temperature for that season. When hormone collection spanned multiple seasons, we used the average of all seasons. Although measured body temperature or ambient temperature for the location on the day of hormone collection would be preferable for this correction, original authors of records included in HormoneBase reported neither body temperature nor day of sample collection. Moreover, to our knowledge, global daily temperature time series datasets spanning all of the study years included in HormoneBase are not available, thus temperature of the season in which the hormones were sampled represented our best approximation for the temperature correction.

We also compiled metabolic rate data to be used in conjunction with HormoneBase (Johnson et al. 2018). These data include un-adjusted BMR values from mammals and birds and SMR values adjusted to remove the influence of temperature for amphibians and reptiles. For our analyses involving GC concentrations and mass-specific metabolic rate, we used BMR and uncorrected GC concentrations for birds and mammals. For amphibians and reptiles, we removed the potential influence of temperature on GC concentrations using the same approach by which temperature was removed from BMR values. Specifically, we used the following linear model:

$$\ln GC = \ln a + c(1000/T), \quad (3)$$

where GC is the baseline or stress-induced GC concentration, $a$ is the intercept, $T$ is the mean environmental temperature of the season in which the GC measurement was collected in K, and $c$ is the slope of the line relating the inverse of temperature to GC concentration. Residuals from these models were back-transformed and used as temperature-independent GC concentrations. Class-specific linear models were as follows:

$$\text{Reptile baseline GC}: \quad \ln GC = -2.086 - 1.368(1000/T). \quad (4)$$

$$\text{Reptile stress-induced GC}: \quad \ln GC = -45.80 + 13.99(1000/T). \quad (5)$$

$$\text{Amphibian baseline GC}: \quad \ln GC = 19.274 - 5.058(1000/T). \quad (6)$$

There was only one record in the database for amphibians where the stress-induced GC concentration and SMR were known, thus we were not able to calculate temperature-independent GC concentrations for amphibians for taxon-specific analyses. Finally, whether using uncorrected GC concentrations and BMR for mammals and birds, or temperature-independent GC concentrations and SMR for amphibians and reptiles, GC concentrations are expected to scale with body mass in the same way as mass-specific metabolic rate (Haase et al. 2016). Thus, the natural logarithm of baseline and stress-induced GC concentrations should be linear functions of the natural logarithm of mass-specific metabolic rate with a slope of 1. Because of differences in GC and metabolic rate data among groups, we were only able to evaluate taxon-specific, mass-specific metabolic rate–GC concentration relationships (i.e., within amphibians, reptiles, birds, or mammals, but not across Classes).

We tested for GC–mass and GC–metabolic rate relationships, and evaluated whether they conformed to expectations from MTE using best practices that account for shared evolutionary history among species in our analyses (White et al. 2012). We used Bayesian generalized linear mixed-effect models in the R package MCMCglmm (Hadfield 2010) to evaluate how GC concentrations relate to body mass and mass-specific metabolic rates. Prior to analyses, body mass and temperature-corrected, temperature-independent, or unadjusted GC concentration and mass-specific metabolic rate were natural log-transformed. To control for inter-laboratory variation in GC concentration measurements (Fanson et al. 2017), we included the laboratory identity as a random effect in all models. Additionally, because of variation in shared evolutionary history among species included in our analyses, we included species-level inverted phylogenetic covariance matrices as a random effect and estimated Pagel's $\lambda$ (i.e., estimated here as the posterior mode from MCMC chain) for each model. Pagel's $\lambda$ ranges from 0 to 1, where high values of $\lambda$ suggest strong phylogenetic structure in the relationship between the response and predictor variables (i.e., the relationship between predictor and response tends to be similar for closely related taxa) and 0 reflects no phylogenetic structure in the relationship between the response and predictor variables (Revell 2010). Finally, because we often included more than one record per species in our analyses, we also included species as a random effect. We used default priors for fixed effects and $V = 1$, $nu = 0.02$ for the variance components of each random effect to correspond to an inverse-Gamma distribution. Unless otherwise noted, all estimates were based on 50,000 MCMC iterations, a burn-in of 10,000, and a thinning rate of 20, which produced posterior estimates based on 2000 samples. For each model, we inspected trace and density plots to verify adequate mixing in the MCMC chain, only accepted models if the autocorrelation of stored iterations was $<0.1$ and assessed convergence of four independent

**Table 1** Model results relating baseline plasma GC concentrations (ng $mL^{-1}$) to body mass (g) and mass-specific metabolic rate (mW $g^{-1}$)

| Model | Species *n* | Record *n* | Slope | 95% CI | | Intercept | 95% CI | | *P* | $\lambda$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Temperature-corrected baseline GC and body mass | | | | | | | | | | |
| All | 167 | 1011 | −0.16 | −0.27 | −0.05 | 28.54 | 26.76 | 30.38 | **0.01** | 0.93 |
| Amphibians | 25 | 118 | −0.15 | −0.38 | 0.03 | 28.29 | 27.35 | 29.24 | 0.14 | 0.05 |
| Reptiles | 37 | 242 | −0.19 | −0.44 | 0.07 | 28.58 | 25.05 | 31.88 | 0.13 | 0.86 |
| Birds | 83 | 562 | −0.06 | −0.27 | 0.15 | 26.65 | 20.97 | 32.99 | 0.57 | 0.98 |
| Mammals | 22 | 89 | −0.21 | −0.42 | −0.02 | 30.87 | 27.27 | 34.44 | **0.04** | 0.94 |
| Baseline GC and mass-specific metabolic rate | | | | | | | | | | |
| Temperature-independent GC and mass-specific metabolic rate | | | | | | | | | | |
| Amphibians[a] | 12 | 53 | 0.025 | −0.16 | 0.21 | 0.02 | −0.76 | 0.92 | 0.80 | 0.08 |
| Reptiles[a] | 18 | 101 | 0.23 | −0.28 | 0.71 | 0.38 | −3.90 | 0.71 | 0.36 | 0.91 |
| Unadjusted GC and mass-specific metabolic rate | | | | | | | | | | |
| Birds | 40 | 292 | 0.25 | −0.14 | 0.63 | 3.08 | −2.26 | 7.82 | 0.20 | 0.95 |
| Mammals[a] | 14 | 74 | 0.11 | −0.39 | 0.66 | 5.70 | 1.08 | 10.85 | 0.72 | 0.95 |

Species *n* and record *n* reflect the number of species and records in each analysis. Slope, intercept, their 95% credible intervals (95% CI), and *P* denote posterior means. $\lambda$, degree of phylogenetic structure between the response and predictors, is calculated as the posterior mode.
[a]Iterations = 420,000, burn-in = 20,000, thin = 200. Bold values indicate *P* values $\leq$ 0.05.

chains using the Gelman–Rubin statistic (Gelman and Rubin 1992) through the R package *coda* (Plummer et al. 2006). We concluded that GC concentration was related to body mass or mass-specific metabolic rate if $P \leq 0.05$. Additionally, we concluded that a scaling exponent was different from the expected value of slope (i.e., −0.25 for temperature-corrected GC concentration as a function of body mass and 1 for temperature-independent [amphibians and reptiles] or unadjusted GC [birds and mammals] as a function of temperature-independent or unadjusted mass-specific metabolic rate) if the expected value fell outside of the slope's 95% credible interval (95% CI).

## Results

Baseline GC concentrations were related to body mass in the model considering all taxa and in the mammal-specific model, but not for amphibians, reptiles, or birds (Table 1 and Fig. 1). Additionally, in all cases, the expected slope of −0.25 fell within the 95% CI. The estimated slope for mammals (−0.21) was closest to the expected value. Reptiles (−0.19) and amphibians (−0.15) had slopes close to the expected value, but also had wide 95% CIs that also encompassed zero. We failed to find a relationship between baseline GC concentrations and mass-specific metabolic rate for all taxa. Only in birds did the expected slope of 1 fall within the 95% CI, but the 95% CI was large (Table 1). For most models, Pagel's $\lambda$ was >0.86, suggesting strong phylogenetic structure between body mass and baseline GC concentrations or mass-specific metabolic rate and baseline GC concentrations. The exception to this trend was in amphibians, where $\lambda = 0.05$ and 0.08 suggested almost no phylogenetic structure between body mass and baseline GC concentrations and mass-specific metabolic rate and baseline GC concentrations, respectively (Table 1). However, a low number of sampled species likely accounts for small $\lambda$ values for models restricted to amphibians.

In contrast to baseline GCs, we found no evidence for a relationship between stress-induced GC concentrations and body mass; estimated slopes ranged from negative among reptiles (−0.18) to near zero for all taxa combined (0.03), birds (0.04), and mammals (0.03) to positive in amphibians (0.19). Moreover, low confidence in estimates resulted in 95% CI overlapping the expected slope of −0.25 for amphibians, reptiles, and mammals, all of which had small sample sizes (Table 2). We failed to find a relationship between mass-specific metabolic rate and stress-induced GC concentrations in all taxa with estimated slopes for reptiles, birds, and mammals all near zero (Table 2). Estimated values of Pagel's $\lambda$ suggested strong phylogenetic structure (i.e., all $\lambda > 0.93$) between stress-induced GC concentrations and body mass or mass-specific metabolic rate in all cases except the stress-induced GC concentration and mass-specific metabolic rate in reptiles where $\lambda = 0.03$, suggesting a near absence of phylogenetic structure in the relationship.

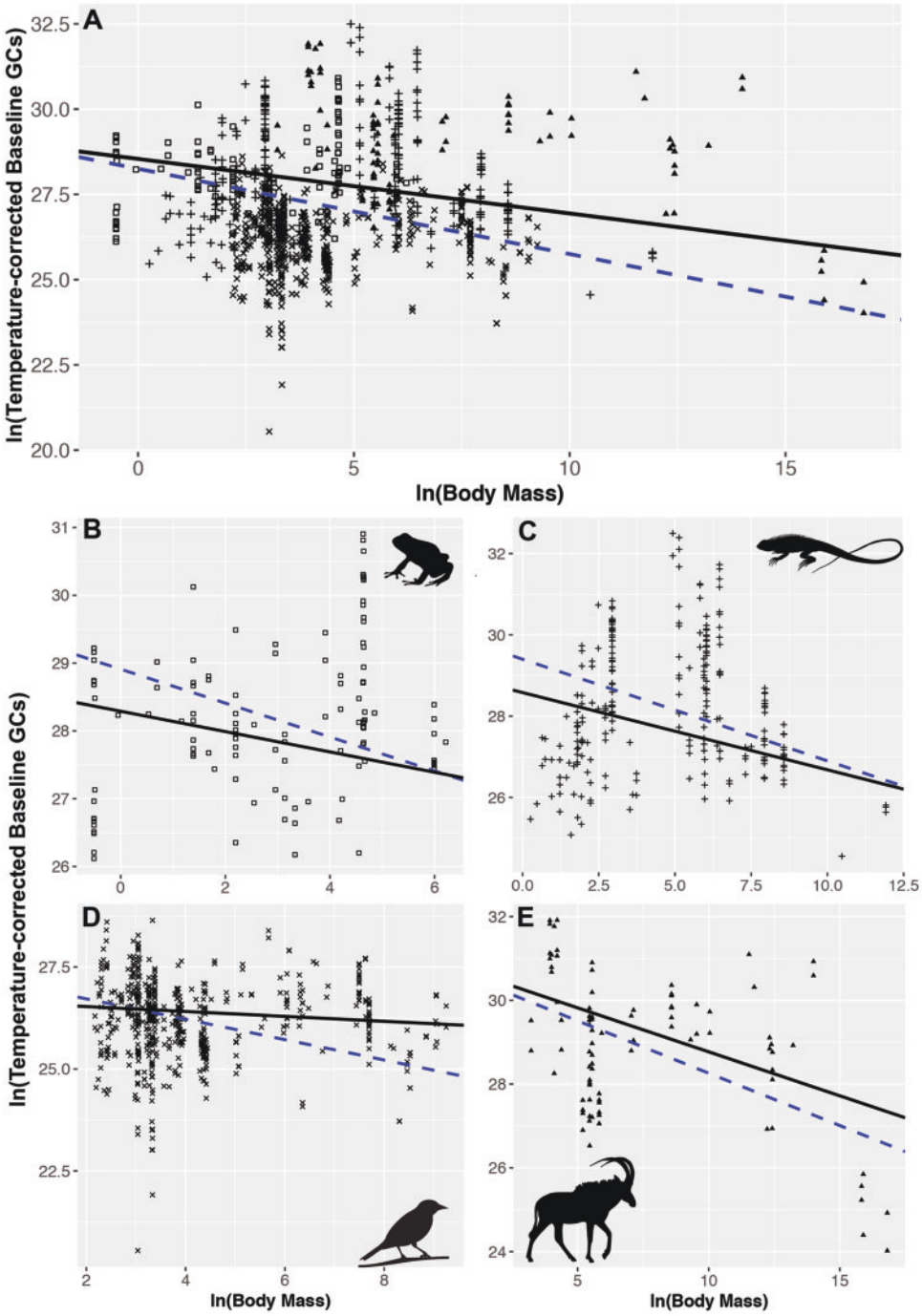


**Fig. 1** Relationship between the natural logarithm of temperature-corrected baseline glucocorticoid (GC) concentrations (ng $mL^{-1} * e^{E/kT}$) and the natural logarithm of body mass (g). (**A**) Reflects all taxa where open squares = amphibians, crosses = reptiles, "x" = birds, and triangles = mammals. Panels illustrate data and class-specific relationships for (**B**) amphibians, (**C**) reptiles, (**D**) birds, and (**E**) mammals. For all panels, the solid line is the estimated slope from the models and the dashed line denotes the expected slope of −0.25 with the intercept fitted empirically from (A) all data or (B–E) class-specific data.

## Discussion

Here we add to the limited number of studies that have explicitly evaluated the relationships between metabolic rate and GC concentrations (Buehler et al. 2012; Jimeno et al. 2017) and among metabolic rate, body mass, and GC concentrations (Haase et al. 2016). Using a similar approach to the phylogenetically-informed comparative analyses used by Haase et al. (2016), our model including amphibians, reptiles, birds, and mammals provide

**Table 2** Model results relating stress-induced plasma GC concentrations (ng $mL^{-1}$) to body mass (g) and mass-specific metabolic rate (mW $g^{-1}$)

| Model | Species *n* | Record *n* | Slope | 95% CI | | Intercept | 95% CI | | *P* | $\lambda$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Temperature-corrected stress-induced GC and body mass | | | | | | | | | | |
| All | 105 | 545 | 0.03 | −0.02 | 0.08 | 28.78 | 26.85 | 30.34 | 0.23 | 0.95 |
| Amphibians[a] | 4 | 22 | 0.19 | −0.52 | 0.88 | 27.79 | 22.35 | 31.90 | 0.41 | 0.99 |
| Reptiles[a] | 10 | 31 | −0.18 | −0.82 | 0.41 | 30.63 | 23.44 | 36.84 | 0.51 | 0.93 |
| Birds | 82 | 455 | 0.04 | −0.01 | 0.09 | 27.77 | 24.93 | 30.81 | 0.10 | 0.95 |
| Mammals[a] | 9 | 37 | 0.03 | −0.33 | 0.36 | 29.48 | 23.56 | 37.01 | 0.89 | 0.96 |
| Stress-induced GC and mass-specific metabolic rate | | | | | | | | | | |
| Temperature-independent GC and mass-specific metabolic rate | | | | | | | | | | |
| Reptiles[a] | 5 | 22 | −0.02 | −0.47 | 0.37 | −0.13 | −3.21 | 3.59 | 0.85 | 0.03 |
| Unadjusted GC and mass-specific metabolic rate | | | | | | | | | | |
| Birds[a] | 45 | 191 | −0.03 | −0.11 | 0.05 | 3.55 | 0.37 | 7.34 | 0.53 | 0.95 |
| Mammals[a] | 8 | 36 | 0.08 | −0.23 | 0.39 | 5.62 | −1.87 | 13.71 | 0.60 | 0.97 |

[a]Species *n* and record *n* reflect the number of species and records in each analysis. Slope, intercept, their 95% credible intervals (95% CI), and *P* denote posterior means. $\lambda$, degree of phylogenetic structure between the response and predictors, is calculated as the posterior mode. Iterations = 420,000, burn-in = 20,000, thin = 200.

general support for an association between baseline GC concentrations and body size, as does our model specific to mammals. However, we failed to find support for a strong positive association between GC concentrations and mass-specific metabolic rate. Thus, our results suggest that the relationship between body mass and GC concentrations may not be a consequence of the well-established body mass–metabolic rate relationship (reviewed in Brown et al. 2004; Glazier 2005, 2010). Moreover, we failed to reveal a general association between mass-specific metabolic rate and GC concentrations, although a relationship between the two is generally assumed, at least within individuals and for intraspecific comparisons (reviewed in McEwen and Wingfield 2003; Romero et al. 2009). It is possible that the lack of a relationship in our comparative analysis could be due to other physiological regulatory mechanisms that vary across species.

Our analyses of baseline GCs and body mass confirm and extend results from Haase et al. (2016), who reported that baseline GCs scale to the $-1/4$ power with body mass in mammals, with evidence for a similar scaling relationship in models for mammals and all taxa combined. In the latter analysis, the range of body mass values spanned seven orders of magnitude, which is consistent with the coarse scale where MTE usually performs well (Sibly et al. 2012). However, baseline GCs were unrelated to body mass for amphibians, reptiles, and birds, suggesting that, like metabolic rate itself, $-1/4$ power scaling of GCs with body mass may be the central tendency across the superclass Tetrapoda, but it is not universal (Bokma 2004; Uyeda et al. 2017). A smaller range of body mass variation could potentially explain our failure to find a baseline GC–body mass relationship in groups other than mammals, which span (25.24–20,000,000 g). For example, amphibian body mass only spanned 0.6–500 g and that of birds was also quite narrow (8.8–10,400 g). Additionally, even though a scaling exponent close to $-1/4$ power for baseline GCs with body mass appears to be the central tendency for all taxa combined, we found no evidence that GCs are linked to mass-specific metabolic rate, which conflicts with Haase et al.'s (2016) study that was restricted to mammals.

Our failure to find a baseline GC–metabolic rate association seems to support the findings of Buehler et al.'s (2012) intraspecific study that failed to find a relationship between baseline GC concentrations and BMR or basal mass-corrected metabolic rate both within and across individual red knots (*Calidris canutus*). However, our results, and those of Buehler et al. (2012) appear to conflict with those of Jimeno et al. (2017), who found a strong positive relationship between baseline GC concentration and metabolic rate in captive zebra finches (*Taeniopygia guttata*). Jimeno et al. (2017) suggested that their ability to detect a baseline GC–metabolic rate relationship was due to their synchronized measurements of GC concentrations and metabolic rate, which was not done in the Buehler et al. (2012) study.

Our comparative approach was also limited by an absence of temporal synchrony in measurements of GC concentrations and metabolic rate that Jimeno et al. (2017) suggest may be necessary to reveal the linkage between the two. Because GC concentrations can fluctuate with energy demands (McEwen and Wingfield 2003; Romero et al. 2009), it is possible that pairing measurements from the same individual during different energetic demands could mask any GC–metabolic rate association, as could pairing measurements from studies involving different individuals or populations, which is what we have done in this study. An assumption implicit to this approach is that trait variation among species will be much greater than variation within species. Considerable evidence suggests that this approach is fruitful for many traits (Pennell and Harmon 2013); however, intraspecific variation in trait expression can be problematic for comparative approaches (reviewed in Garamszegi and Møller 2010). GC concentrations and body mass can be quite variable within species. Whenever appropriate data were available, body mass and GC measurements included here were from the same individuals, but all metabolic rate measurements were from separate sources. To what degree this limitation can explain the absence of a relationship between GC concentrations and mass-specific metabolic rate is unknown. However, the Haase et al. (2016) study suffered from the same potential limitation, but found a strong association between GCs and mass-specific metabolic rate among mammals, suggesting that this limitation may not explain the general absence of this relationship in our analyses. Regardless, comparative studies stand to benefit from studies that synchronize GC concentration and metabolic rate measurements, especially because of well-established daily and seasonal cycles in both measurements (reviewed in Romero 2002; Touma and Palme 2005).

The ability to detect GC concentration allometries with mass or metabolic rate could also be influenced by interspecific variation in GC binding capacity and receptor types and densities. Corticosteroid-binding globulin (CBG) occurs in plasma and, by binding to GCs, could render GCs unavailable for tissue uptake (reviewed in Breuner et al. 2013). Recent comparative work based on 91 vertebrate species suggests that CBG concentrations and unbounded or "free" GCs can vary widely among vertebrates, including substantial variation within major vertebrate groups (i.e., reptiles, birds, mammals; Desantis et al. 2013). Despite this variation, whether total or "free" plasma GCs is more useful for most ecological questions is still debated (reviewed in Schoech et al. 2013). Regardless, as additional data become available for species-specific concentrations of free and CBG-bound GCs, new comparative analyses will be able to determine whether GC concentration allometries are restricted to free GCs or if accounting for CBG concentrations resolves any scaling relationships.

Despite the thousands of GC concentrations from free-living vertebrate populations across the world within the HormoneBase dataset, some of our analyses suffer from low power due to small sample sizes. This limitation was most acute for analyses involving metabolic rate and stress-induced GC concentrations. Additionally, while the number of total records in each analysis was always >20, the number of species represented was quite low in some models, such as 22 records for 4 amphibian species in the stress-induced GC and body mass analysis. In this particular case, *Desmognathus ochrophaeus*, the Allegheny Mountain dusky salamander, represented over 80% of the records. Thus, this model more accurately reflects a test of whether stress-induced GC concentrations are related to body mass in this species rather than amphibians broadly. More field measurements of GC concentrations and measures of metabolic rates will alleviate this shortcoming for comparative studies and will also make possible analyses that can test scaling at more than one level of biological organization (i.e., within and among species).

One potential power of metabolic theories and other allometric relationships is their ability to present *a priori* expectations for trait expression values (Gillooly and Ophir 2010; Haase et al. 2016), which can be compared with observed values to gain better insights on the ecological and evolutionary forces that shape the astounding variability among traits across the diversity of life. In essence, if a trait scales as expected from metabolic theories, one can use deviations from the scaling theory's expectations to test ideas about selective agents or forces that limit and promote expression of plastic traits. Our results suggest that it would be premature to evaluate the forces shaping GC concentrations through this approach based on available data, as only in our all-taxa model and the model restricted to mammals did we find general support for $-1/4$ power scaling of baseline GC concentrations with body mass. However, because individual researchers can add to HormoneBase, and publication of hormone data in a format suitable for synthesis appears to be increasing (Fig. 2), follow-up studies seeking to determine whether GC concentrations scale with metabolism

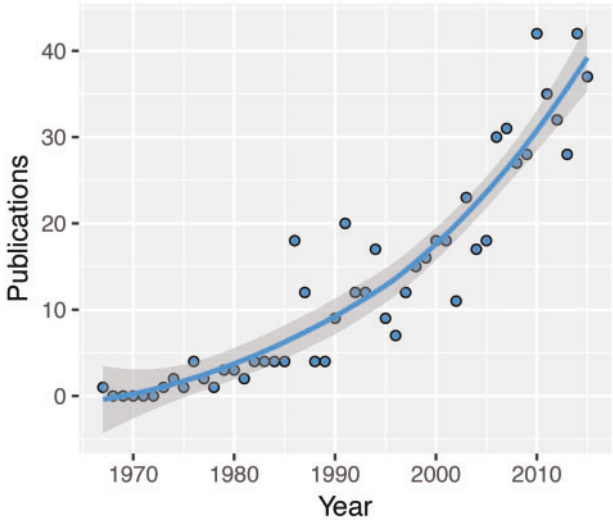


**Fig. 2** The number of publications reporting hormone measurements from unmanipulated, wild populations based on studies included in HormoneBase.

and body size may have robust sample sizes in the not-so-distant future.

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