# Why not both? A case study measuring cortisol and corticosterone in poison frogs

Sarah E. Westrick [a], Ryan T. Paitz [b], and Eva K. Fischer [a]

[a] Department of Evolution, Ecology and Behavior, University of Illinois Urbana-Champaign, 505 S Goodwin Ave Urbana, IL 61801

[b] School of Biological Sciences, Illinois State University, Campus Box 4720 Normal, IL 61790

Corresponding author: Sarah E. Westrick, westse@illinois.edu

**Abstract**

A general tenet in stress physiology is that the hypothalamic-pituitary-adrenal (HPA) axis predominantly produces one glucocorticoid (GC) in response to stressors. However, the two main glucocorticoids produced by vertebrates – cortisol and corticosterone – show variation across species in absolute levels, relative proportions, and stress responsivity. Therefore, focusing on a single GC may not tell the whole story. In this study, we measured both cortisol and corticosterone in water samples collected from five species of Dendrobatid poison frogs. Understanding the full picture of the physiological stress response in poison frogs is of special interest due to their susceptibility to environmental stressors and conservation status. Based on pioneering studies of steroidogenesis in a select few species of amphibians, corticosterone is thought to be dominant and more abundant in all amphibians. Counter to this idea, we found water-borne cortisol was more abundant than corticosterone in three of the five species. *Dendrobates tinctorius* and *Phyllobates terribilis* released equal amounts of cortisol and corticosterone. When we stimulated the HPA axis in *D. tinctorius*, we found water-borne corticosterone was responsive to adrenocorticotropic hormone (ACTH), while cortisol did not change. Comparing plasma and water samples, we found water-borne cortisol was positively correlated with cortisol in plasma and the relationship between plasma and water-borne corticosterone was positive but not statistically significant. These results challenge the broadly accepted assumption that corticosterone is 'dominant' in amphibians and add to the growing literature highlighting the importance of measuring both GCs to understand (stress) physiology.

**Keywords:** ACTH challenge, anurans, corticosterone, cortisol, Dendrobatids, glucocorticoids

## 1. Introduction

Researchers across biology disciplines are interested in understanding stress. The ability to measure stress physiology non-invasively in the lab and in the wild is key for behavioral ecologist and conservation biologists seeking to understand animal behavior in a changing world. In studying stress physiology, researchers often measure glucocorticoids (GCs), most commonly cortisol and corticosterone, produced by the adrenal cortex (Sapolsky et al., 2000). Glucocorticoids are of keen interest not only as a readout of stress, but also because they elicit a variety of physiological, morphological, and behavioral responses (MacDougall-Shackleton et al., 2019). Glucocorticoids are often measured from plasma samples but can also be measured in many other sample types including fecal, urine, hair, feathers, baleen, eggs, and water (Palme, 2019). These methods of non-invasive sampling allow for repeated sampling from small individuals where repeated blood draws are not possible and can reduce the confounds of stress from sampling itself (Palme, 2019).

The majority of studies measure only a single GC, the one considered to be “dominant” (Hancock, 2010). The dominant GC is typically defined as the one released in response to adrenocorticotropin hormone (ACTH), though dominance is often assumed based on higher abundance. Which GC is dominant varies across species. Yet a key thing that is often overlooked in stress physiology is the fact that other glucocorticoids are present, often in substantial amounts, and can be produced independently of the HPA axis being activated during times of stress (Bornstein et al., 2008). Thus, to fully understand the role glucocorticoids play in behavior, development, and population health, we need to move beyond simply studying the glucocorticoid we assume to be dominant.

We set out to validate waterborne hormone measurements in our focal species, the dyeing poison frog *Dendrobates tinctorius*, by quantifying levels of corticosterone and cortisol. In pursuing this goal, we uncovered unexpected patterns within and across species of poison frogs. Below, we briefly review the literature comparing cortisol and corticosterone to provide context for our study and its implications and present our results as a case study in the value of measuring both GCs. Our findings highlight the importance of this question specifically in (poison) frogs and for those interested in behavior and conservation across vertebrates more broadly.

*1.1 Cortisol vs corticosterone –*

Cortisol and corticosterone are perhaps best known for their role in the "stress response". However they also change as a result of ACTH-dependent circannual and circadian rhythms (Dickmeis et al., 2013) and play a critical role in other physiological processes (MacDougall-Shackleton et al., 2019), including, but not limited to, energy metabolism (Vegiopoulos and Herzig, 2007), immune response (Cruz-Topete and Cidlowski, 2015), growth and development (Seckl and Meaney, 2004), reproduction (Whirledge and Cidlowski, 2017), and cardiovascular function (e.g., Cruz-Topete et al., 2016). Circulating GCs also impact many behaviors such as aggression (Haller, 2014), parental care (Bales et al., 2006; Crossin et al., 2012; Raulo and Dantzer, 2018), and feeding/foraging (Crespi and Denver, 2005; Crossin et al., 2012) to name a few. Because of their widespread influences across physiology and behavior, changes in GCs and/or their receptors can be immensely impactful on survival and fitness. While acutely elevated GC levels often have adaptive functions, in many species chronically elevated GCs can disrupt normal physiology and decrease fitness (reviewed in Bonier et al., 2009).

Most vertebrates produce both cortisol and corticosterone which are often assumed to be interchangeable in their physiological function, though they can elicit different effects due to different receptor binding affinities (Sutanto and De Kloet, 1987). Typically, either cortisol or corticosterone is considered the 'dominant' GC produced by a species because it is more abundant at baseline and increases in response to stressors or ACTH (Aerts, 2018). ACTH challenges are often used to measure the responsiveness of the adrenals, to identify the dominant GC, and to validate that the method of hormone measurement is detecting an increase in HPA axis activity (Palme, 2019; Touma and Palme, 2005). However, the GC that is not responsive or less responsive to ACTH can also play an important physiological role. For instance, in humans, cortisol is considered the primary effector hormone of the HPA (Brück, 1983); however, corticosterone plays a role in the central nervous system (Raubenheimer et al., 2006) and pre-natal responses to stressors (Wynne-Edwards et al., 2013). Corticosterone is also an intermediary in the production of aldosterone and the rate of conversion from corticosterone to aldosterone may vary resulting in more or less circulating corticosterone in cortisol-dominant species (Koren et al., 2012). Additionally, steroid transporters and receptors interact with cortisol and corticosterone differently. For example, in mice, P-glycoprotein transports cortisol, but not corticosterone, out of the brain (Karssen et al., 2001).

Despite large interspecific variation in GC levels and relative proportions of cortisol and corticosterone (Koren et al., 2012), we commonly make default assumptions about which GC is more abundant (and therefore assumed to be dominant) based on taxon. The consequence of this generalization is that many studies measure only the GC that is generally considered dominant, such as measuring only cortisol in fish and only corticosterone in birds. These simplifying assumptions are especially prevalent in studies using GCs as a readout of stress or correlate of behavior, rather than exploring GC physiology per se. Yet, these assumptions and generalizations can be misleading. GC dominance varies both across (Koren et al., 2012) and within species (e.g., Keogh et al., 2020; Koren et al., 2012; Larson et al., 2009), and a growing number of studies are also showing the importance of testing interspecific variation in GC responses even among closely related species. For instance, across mammals, we see all combinations of which GC responds to an acute stressor or exogenous ACTH, independent of relative abundance. Some species respond primarily with an increase in cortisol (Ganjam et al., 1972; Johnston et al., 2013; Van Mourik et al., 1985; Vera et al., 2012, 2011), while in others corticosterone responds but not cortisol (Young et al., 2004), and still in others *both* GCs respond (Rosenthal et al., 1993; Smith and Bubenik, 1990; Young et al., 2004). Sea otters (*Enhydra lutris*) are an interesting case where some studies document an increase in cortisol in response to ACTH (Murray et al., 2020; Wasser et al., 2000), while others find an acute stressor (catching and handling) causes an increase in corticosterone (Larson et al., 2009). These findings suggest a potential for non-ACTH-driven regulation of corticosterone, such as modulation by epinephrine or norepinephrine. This within and among species variance in abundance and responsiveness suggests that we should use caution when following traditional assumptions about which GC to measure based on order, family, or even population within the same species (Hancock, 2010). While in some cases there may be financial or logistical limitations preventing the measurement of both GCs, whenever possible we should explore both to discover potentially interesting and informative patterns.

*1.2 Glucocorticoids in amphibians –*

Environmental and anthropogenic changes are contributing to amphibian population decline and biodiversity loss. These stressors include global climate change, pesticides, pollution, and habitat loss or alteration (Blaustein and Kiesecker, 2002; Collins, 2010; Kiesecker, 2011;

Kiesecker et al., 2001; Ohmer et al., 2021; Stuart et al., 2004). Many amphibians are small bodied and repeated plasma sampling is not feasible. Developing non-invasive techniques to study the stress physiology of amphibians is instrumental in understanding how species respond to multifactor environmental stressors, such as habitat modification, climate variability, and disease, and can inform conservation strategies for at-risk species (Kumar and Umapathy, 2019; Narayan, 2013; Palme, 2019; Walls and Gabor, 2019). Corticosteroids also play critical roles in metamorphosis (Denver, 2009; Kulkarni and Buchholz, 2014) with size at metamorphosis having life-long consequences for survival and fitness (Glennemeier and Denver, 2002; Kulkarni and Gramapurohit, 2017).

Corticosterone is typically referenced as the dominant and more abundant glucocorticoid in amphibians (Moore and Jessop, 2003; Norris and Carr, 2013; Wingfield and Romero, 2011), and occasionally listed as amphibians' only glucocorticoid (Katsu and Iguchi, 2016). In the 1970s and 1980s, early studies about steroid synthesis reported production of corticosterone and little, if any, cortisol from isolated interrenal cells of frogs and toads *in vitro* (Chan and Edwards, 1970; Jolivet-Jaudet and Ishii, 1983; Jolivet-Jaudet and Leloup-Hatey, 1984; Mehdi and Carballeira, 1971). The results of these studies from just a few species of anurans (namely *Bufo bufo*, *Rana catesbeiana*, and *Xenopus laevis*) led to a strong focus on corticosterone in the majority of studies on stress physiology in all amphibians.

This bias towards studying corticosterone in amphibians prevails despite a history of cortisol being detected in several species of anurans (frogs and toads) (e.g., Dale, 1962; Krug et al., 1983) and a growing number of studies demonstrating that the assumption of corticosterone responsiveness to ACTH/acute stressors and/or greater abundance does not hold for all species of amphibians. In some species of amphibians, corticosterone is the dominant glucocorticoid, as canonically expected. For example, in water samples from Rio Grande Leopard tadpoles released more corticosterone than cortisol overall and increased corticosterone release rates in response to an ACTH challenge, but decreased cortisol release rates (Forsburg et al., 2019). Similarly, in American bullfrogs (*Rana catesbeiana*), corticosterone in plasma responded to ACTH whereas cortisol was undetectable (Johnston et al., 1967). In contrast, in fecal samples from two species of Panamanian harlequin frogs (*Atelopus certus* and *Atelopus glyphus*) both cortisol and corticosterone were responsive to ACTH, but cortisol metabolites were detected at much higher concentrations overall (Cikanek et al., 2014). In the common water frog (*Rana esculenta*),

cortisol and corticosterone are both detectable in plasma and both are elevated in ovulatory and postovulatory phases compared to the preovulatory phase (Gobbetti and Zerani, 1993).

In an attempt to resolve this confusion, some researchers have suggested aquatic amphibia may rely on cortisol as their primary GC, as many fishes do, whereas semi-terrestrial or terrestrial amphibia typically use corticosterone (Jungreis et al., 1970). For example, aquatic hellbender salamanders (*Cryptobranchus alleganiensis*) mainly produce cortisol in response to a stressor (Hopkins et al., 2020). Yet the vast majority of studies on a popular aquatic model species, the clawed frog (*Xenopus* sp.), measure corticosterone (e.g., Jaudet and Hatey, 1984; Kulkarni and Buchholz, 2012; Shewade et al., 2020), likely based on results from *in vitro* studies with *Xenopus laevis* interrenal cells (Chan and Edwards, 1970; Jolivet-Jaudet and Leloup-Hatey, 1984). Moreover, cortisol has been detected in multiple species of amphibians (terrestrial and aquatic) using dermal swabs (Santymire et al., 2018), and cortisol increases in response to ACTH in terrestrial green treefrogs (*Hyla cinerea*), American toads (*Anaxyrus americanus*), and red-spotted newts (*Notophthalamus viridescens*) (Santymire et al., 2018).

Further equivocal results about cortisol and corticosterone in amphibians, including contradictory results in the same species (such as *Xenopus laevis* and *Rana pipens*), are nicely summarized in Table 2 in Hopkins et al. (2020). In sum, the myriad of contradictory studies brings into question whether corticosterone can indeed be assumed to be the only GC with biological importance in amphibians as we expand our study of endocrinology to more non-traditional species. Measuring both GCs in more species of amphibians will help us discern the role of different GCs across different life histories and inform our general understanding of amphibian physiology.

*1.3 Stress physiology in Neotropical poison frogs –*

Many Neotropical poison frog (Dendrobatidae) species are threatened due to risks including agrochemicals (Angulo et al., 2019), climate change (Whitfield et al., 2007), smuggling (Auliya et al., 2016), and habitat destruction or fragmentation (Becker et al., 2007; Cushman, 2006). Due to their susceptibility to the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Courtois et al., 2015) and conservation status (Guillory et al., 2019), the development of non-invasive measurements of GCs is important for studying poison frog physiology and informing conservation efforts. Neotropical poison frogs are primarily diurnal and terrestrial, and have been

gaining attention in behavioral ecology and neuroethology due to their interesting parental behavior (Ringler et al., 2023; Roland and O'Connell, 2015; Westrick et al., 2023). In addition, poison frogs vary in size but generally are relatively small, with an average snout-vent length (SVL) of ~17-60 mm. Thus, repeated plasma sampling is not feasible, and we need to further explore non-invasive methods of studying the endocrinology of poison frogs.

Our goal for this study was to investigate which glucocorticoid is the primary hormone of the HPA axis in Dendrobatid poison frogs. We tested interspecific variation in the relative abundance of cortisol and corticosterone and focused on the response of both glucocorticoids to a physiological stressor in one focal species. We used non-invasive water-borne hormone sampling to allow for repeated measures from the same individuals (Baugh et al., 2018; Gabor et al., 2013; Rodríguez et al., 2022). Both cortisol (Fischer and O'Connell, 2020) and corticosterone (Nowicki et al., 2022) have been previously measured in poison frogs, albeit in different species and sample types (plasma from *Dendrobates tinctorius* and water from *Ranitomeya imitator*, respectively).

To examine species variation in glucocorticoid abundance among poison frogs, we took "baseline" water samples from five species of Dendrobatids: the golden poison frog (*Phyllobates terribilis*), mimic poison frog (*Ranitomeya imitator*), Zimmermann's poison frog (*Ranitomeya variabilis*), dyeing poison frog (*Dendrobates tinctorius*), and Anthony's poison frog (*Epipedobates anthonyi*). These species share many behaviors, such as parental care, and are endemic to tropical regions of Central and South America, but vary in body size and toxicity in the wild (Wells, 2007). *P. terribilis* and *D. tinctorius* are the largest of the group (~37-60 mm SVL), *E. anthonyi* are intermediate (~19 to 25 mm SVL), and both *Ranitomeya* are among the smallest poison frogs (~17-22 mm SVL). To confirm whether the more abundant GC is also dominant, i.e., elevates in response to an acute stress signal, we additionally performed an ACTH challenge in our primary focal species (*D. tinctorius*). In *D. tinctorius*, we then compared glucocorticoid concentrations between water and plasma samples from the same individuals to assess whether water samples are representative of circulating hormone levels in blood. All together this study informs our understanding of the 'stress' physiology of this particularly vulnerable clade of frogs and contributes important data to the puzzle of GC dominance and abundance in amphibians.

## 2. Materials and methods

*2.1 Animal husbandry –*

We housed all frogs in our breeding colony at University of Illinois Urbana-Champaign in glass terraria (18x18x18”, 12x12x18”, or 36x18x18” Exo Terra®) with soil, sphagnum moss, live tropical plants, and coconut shelters. We kept frogs on a 12:12 h light cycle and fed them flightless *Drosophila* fruit flies (*D. melanogaster* or *D. hydei*) dusted with vitamin supplements three times weekly. Our automated misting system kept humidity >75% and the room was kept at 70-76ºF. We housed adult frogs in groups of two to three individuals of the same species, either one or two males with one female (*E. anthonyi*, *D. tinctorius*, *R. variabilis*, *R. imitator*) or as a group of two to three juveniles that were too young to be sexed at the time of sampling (*P. terribilis*). All frogs were at least 1 year old. For the ACTH challenges, we weighed frogs prior to sampling by placing them in a plastic container on a scale and subtracting the weight of the container. All procedures were approved by UIUC Institutional Animal Care and Use Committee (protocol #20147).

*2.2 Hormone sampling –*

To collect water-borne hormones, we placed individual frogs in glass containers with RO water based on size. For the smaller poison frogs (*E. anthonyi*, *R, variabilis*, and *R. imitator*), we used 4cm x 4cm x 5cm hexagonal glass jars with lids filled with 15 mL of RO water. For the larger frogs (*D. tinctorius* and *P. terribilis*), we used 6.5cm x 6.5cm x 6cm glass tubs with lids and 40 mL of RO water. Our goal was to use enough water to cover frogs’ highly vascularized pelvic skin in a container small enough that the frogs would stay in the water and not climb the walls. We left frogs in the water for one hour before returning them to their home terraria or a new water tub for additional sampling. We collected all samples 4.5-8 hours into the frog’s 12 h light cycle. We filtered water samples with Whatman® 1 filter papers to remove any large particulates, such as fecal matter, prior to steroid hormone extraction (see Section 2.4). To compare across species, we collected n=84 water samples (*D. tinctorius* n = 6, *E. anthonyi* n = 20, *P. terribilis* n = 20, *R. imitator* n = 20, *R. variabilis* n = 18). With n=32 samples, we subsampled half of the water for additional projects and stored each subsample separately. We stored all water samples at -20ºC until extraction. For the plasma and water-borne hormone comparison, we collected water samples as described above for *D. tinctorius* (n = 10)

immediately prior to collecting plasma. For n=5 samples, we split water samples in half for additional projects. To collect plasma samples, we anesthetized frogs with benzocaine (Orajel™) and euthanized via rapid decapitation. We used heparinized microhematocrit capillary tubes to collect trunk blood from the body. We combined all the collected blood from an individual in a microcentrifuge tube and centrifuged for 5 min at 5,000 rpm to separate the plasma. Using a micropipette, we collected the plasma supernatant and stored all plasma samples at -20°C.

*2.3 ACTH hormone challenge –*

For the ACTH hormone challenges, we used a within-individual repeated measures design with 18 adult *D. tinctorius* (9 male, 9 female) for a total of 36 challenges (18 ACTH injections and 18 control injections). We injected frogs with 0.5 ug ACTH per g body weight (Sigma-Aldrich®, product no. A7075) diluted in 0.9% saline and a vehicle injection of only 0.9% saline with 6 days between injections. The order of injections was counterbalanced across individuals such that half of the frogs were first injected with vehicle, and half were first injected with ACTH. Prior to the start of trials, we dissolved the ACTH in saline at a concentration of 0.125 ug/µL and aliquoted the solution into insulin syringes which were frozen at -20°C until the day of injection, along with saline vehicle aliquots. On the day of the trial, we thawed the aliquots of saline and ACTH and calculated the volume needed to inject each frog with a dosage of 0.5 ug ACTH per g body weight (mean ± SD = 5.89 ± 1.5 g), based on previous work using ACTH challenges in Neotropical frogs (Baugh et al., 2018). Since the precision of the insulin syringes was limited, we rounded to the nearest 10 µL to get the volume for injection with both ACTH and saline. For each trial, we collected a water-borne hormone sample, as described above, for one hour prior to injection as our “pre-injection” sample. We then used insulin syringes to inject frogs intraperitoneally. After injecting, we immediately placed the frogs in a new water bath for one hour to collect the “post-injection” sample. After the post-injection sample, we returned frogs to their home terraria. We repeated this process the following week with the alternate treatment (ACTH or saline). We performed all trials between five and seven hours after the lights turned on. We split each water sample (four samples per 18 frogs = 72 samples total) in half for additional projects and stored subsamples separately.

*2.4 Steroid hormone extraction –*

For all water samples, we used C18 cartridges (Waters™ Corporation, Milford, MA) for solid-phase extraction to collect steroid hormones. With cartridges on a vacuum manifold, we used a vacuum pump to prime the filters with 6 mL 100% methanol. We then rinsed the cartridges with 6 mL RO water before passing the sample through the cartridge. We eluted the hormones from the cartridges with 4 mL of 100% methanol into 13x100mm borosilicate glass vials. We dried samples under a stream of nitrogen gas in a 37°C dry heating block until completely dry. We stored desiccated samples at -20°C until reconstituted with assay buffer for enzyme linked immunosorbent assay (ELISA) analysis (see Section 2.6).

To measure glucocorticoids from plasma samples, we used the dissociation reagent included in our ELISA kits (see Section 2.6). We chose to use the dissociation reagent over doing an extraction process to avoid losing sample through the extraction steps and due to the limited volume of plasma collected from each frog. We combined 5 µL of dissociation reagent and 5 µL of plasma, vortexed, and incubated for a minimum of 5 min at room temperature. We then added 450 µL of assay buffer to make a 1:92 dilution before running samples on ELISA plates.

*2.6 Enzyme linked immunosorbent assays –*

We used DetectX® ELISA kits from Arbor Assays™ (Ann Arbor, MI) to measure total cortisol and corticosterone concentration in plasma and water samples. We used each kit according to the manufacturer's instructions except for using the same assay buffer (catalog no. X065) for both kits to allow us to split samples across cortisol and corticosterone plates, following manufacturer recommendations. For the corticosterone kit, we followed instructions for the 100 µL format because the standard curve for the 100 µL format can measure smaller concentrations than the 50 µL format. For both cortisol and corticosterone, we measured the optical density at 450 nm. After removing samples with a coefficient of variation (CV) > 20% (n = 110 out of 352 total measurements), the average intra-assay CV across the remaining samples for the analyses was 8.4% for cortisol and 8.2% for corticosterone. The cross reactivity for the cortisol ELISA plate with corticosterone is reported by the manufacturer as 1.2% and for the corticosterone ELISA plate cross reactivity with cortisol is reported as 0.38%. Information about cross reactivity with other molecules can be found on the Arbor Assays™ website.

We used a four-parameter logistic curve (4PLC) regression to fit the respective standard curve on each plate using MyAssay software recommended by the manufacturer

(www.MyAssay.com). Based on this curve, we calculated the concentration of cortisol and corticosterone (pg/mL of assay buffer) for each sample and averaged across duplicates. Due to low concentrations of both GCs in some species, some samples were too low to fall on the standard curve (n=30 out of 352 total measurements), though some had concentrations of cortisol that were too high (n=12). To be conservative, for samples that were off the standard curve in either direction (too low or too high), we censored the data at the minimum or maximum detection limits of the assay, respectively. To confirm the censored data was not skewing our findings, we ran additional analyses with only samples measuring within the assay detection limits and CV < 20%, and the overall pattern of results remained the same (Supplemental Materials, Figure S1). We report release rate (pg/hr) for both cortisol and corticosterone from water samples (Gabor et al., 2013). For samples that were split in half prior to extraction, we multiplied the rate (pg/hr) by two to allow comparison with samples that were not split. We confirmed parallelism for each assay for parallelism using a highly concentrated pooled water sample from adult *D. tinctorius* (see Supplemental Materials for details).

*2.7 Statistical analysis –*

We used R v4.2.2 (R Core Team, 2022) in RStudio v3.1.446 (Posit Team, 2023) for all statistical analyses and ran all analyses on log transformed hormone release rates to normalize residuals. Across species, we were primarily interested in whether the release rates of the two GCs differed within species, rather than absolute GC differences between species. To this end, we fit a linear mixed model for log transformed hormone release rate with a fixed effect of the interaction between species and glucocorticoid type (corticosterone vs cortisol) with sample ID as a random effect. We ran a Type III ANOVA on the linear mixed model to assess the overall effect of the interaction term. We then ran post-hoc simultaneous pairwise comparisons using the multcomp package (Hothorn et al., 2008) to specifically look at the difference in release rate between cortisol and corticosterone within species (e.g., *D. tinctorius* cortisol vs. *D. tinctorius* corticosterone). We did not include sex as a covariate since not all individuals could be confidently sexed at time of sampling. However, in a reduced model with only individuals that we could confidently sex, we found no overall effect of sex on GC production (Table S1). To increase our sample size for *D. tinctorius* in the species comparison, we included samples on the first day of trials before the injection during ACTH challenges in the analysis. Our final species

comparison dataset included 29 measurements from *D. tinctorius* (15 corticosterone, 14 cortisol), 25 measurements from *E. anthonyi* (12 corticosterone, 13 cortisol), 23 measurements from *P. terribilis* (8 corticosterone, 15 cortisol), 22 measurements from *R. imitator* (8 corticosterone, 14 cortisol), and 19 measurements from *R. variabilis* (3 corticosterone, 16 cortisol).

For the ACTH challenges, we fit a linear mixed effects model to ask if there was an effect of treatment (ACTH or control) on either cortisol or corticosterone. The model had the following main effects: GC type (cortisol or corticosterone), time point (pre- or post-injection), and injection type (ACTH or saline). We also included a three-way interaction between GC type, time point, and injection type. This interaction indicated if the difference between pre- and post-injection varied by injection type and whether this relationship varied by GC type. In other words, is there a change in cortisol from pre- to post-injection based on injection type and is this change (or lack thereof) the same or different in corticosterone? We included trial ID as a nested random effect within frog ID to account for repeated measures across trials and individuals. We used post-hoc pairwise comparisons using the multcomp package to interpret interaction effects. After filtering for CV, our sample size was 26 pre- and 26 post-injection cortisol measurements and 27 pre- and 30 post-injection corticosterone measurements. Because our main interest was how the within-individual response to the injection varied across injection types, all four samples for each individual were run on the same plate to reduce possible confounding effects of inter-plate variation within individuals and trials.

We calculated the original plasma sample concentration by multiplying the concentration measured in the assay by the dilution factor. To examine the relationship between water-borne glucocorticoids and circulating glucocorticoids in plasma, we fit linear models (R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2016)) for both cortisol and corticosterone with plasma concentrations (log transformed pg/mL) as the fixed effect predicting water hormone release rate (log transformed pg/hr) for the matched plasma-water samples.

## 3. Results

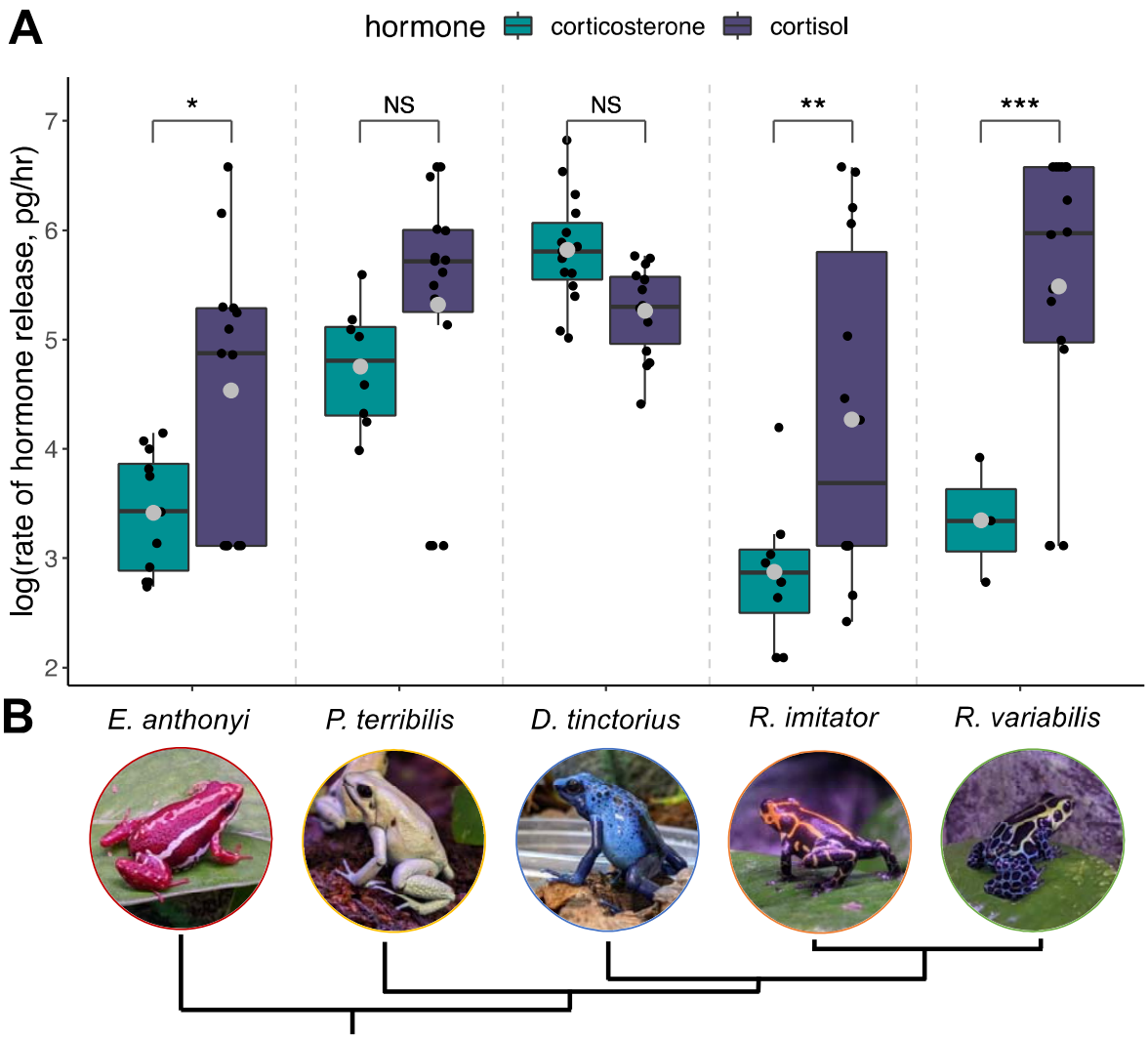
*3.1 Species variation in relative glucocorticoid abundance –*

Overall, we found species differed in which glucocorticoid was more prevalently released in the water ($F_{9,49.44}$=9.97, $p < 0.0001$; Fig 2). Specifically, pairwise comparisons within species

revealed *D. tinctorius* and *P. terribilis* released almost equal amounts of corticosterone and cortisol, while the other three species (*E. anthonyi*, *R. imitator*, and *R. variabilis*) released more cortisol (Table 1; Fig 2). If we exclude all samples that did not fall within the range of the assays (i.e., remove censored values), *D. tinctorius* released higher amounts of corticosterone while all other species released more cortisol (Table S2; Fig S1).

***Table 1.*** Results from simultaneous multiple pairwise comparisons of means for hormone release rate across species. P-values < 0.05 are in bold.

| | **log(release rate, pg/hr): cortisol - corticosterone** | | | |
|---|---|---|---|---|
| *species* | *estimates* | *std. error* | *z-value* | *p-value* |
| *D. tinctorius* | -0.57 | 0.33 | -1.73 | 0.35 |
| *E. anthonyi* | 1.07 | 0.36 | 3.02 | **0.013** |
| *P. terribilis* | 0.66 | 0.39 | 1.68 | 0.38 |
| *R. imitator* | 1.47 | 0.40 | 3.62 | **< 0.01** |
| *R. variabilis* | 2.28 | 0.60 | 3.78 | **< 0.001** |




species tested, the release rate of corticosterone was lower than the release rate of cortisol, suggesting cortisol is the dominant glucocorticoid in these species. In *D. tinctorius*, we found cortisol and corticosterone were released at the same rate. Boxplots for corticosterone are teal (left) and for cortisol are purple (right). Grey dots indicate the mean for each group. Black dots represent individual samples. (NS = not significant, ** = $p < 0.001$, *** = $p < 0.0001$) (B) The species names correspond to the respective photos beneath the x-axis labels. The tree below the photos indicates the phylogenetic relationships between species (Guillory et al., 2019).

*3.2 ACTH challenges in D. tinctorius –*

The effect of an injection (ACTH vs vehicle) varied by GC and also injection type (type of GC x injection type x time point: ß= -0.97, $p = 0.024$; Table 2; Fig 2). We found decreased GC

release rates post-injection (ß=-0.59, p = 0.007; Table 2) and this effect was driven by a decrease in corticosterone in saline injected frogs (pairwise comparison of corticosterone between pre- and post-injection saline: ß = 0.59, z = 0.21, p = 0.031; Fig 2). After an injection, frogs had higher corticosterone release rates when injected with ACTH compared to saline alone (pairwise comparison of corticosterone between ACTH and saline post-injection: ß = 1.36, z = 5.86, p <0.001; Fig 2). Cortisol release did not vary pre- and post-injection for either type of injection (pairwise comparisons of cortisol between pre- and post-injection: saline ß = -0.02, z = -0.09, p = 1.00, ACTH ß = -0.03, z = -0.03, p = 1.00; Fig 2).

***Table 2.*** Results from linear model for hormone release rate across injection type and time points. The reference group is corticosterone, control (saline) injection, and pre-injection time point. P values < 0.05 are listed in bold.

| | **log(release rate, pg/hr)** | | |
|---|---|---|---|
| *Predictors* | *Estimates* | *CI* | *p* |
| intercept | 5.30 | 4.95 – 5.65 | **<0.001** |
| glucocorticoid type (cortisol) | -0.44 | -0.87 – -0.01 | **0.043** |
| injection type (ACTH) | 0.40 | -0.08 – 0.89 | 0.098 |
| time point (post) | -0.59 | -1.02 – -0.17 | **0.007** |
| cortisol x ACTH | 0.06 | -0.54 – 0.66 | 0.842 |
| cortisol x post-injection | 0.61 | 0.00 – 1.22 | **0.048** |
| ACTH x post-injection | 0.95 | 0.37 – 1.53 | **0.002** |
| cortisol x ACTH x post-injection | -0.97 | -1.80 – -0.13 | **0.024** |
| **Random Effects** | | | |
| $\sigma^2$ | 0.29 | | |
| $\tau_{00\ trial:frogID}$ | 0.12 | | |
| $\tau_{00\ frogID}$ | 0.02 | | |
| ICC | 0.32 | | |
| $N_{trial}$ | 35 | | |

| | |
|---|---|
| $N_{frogID}$ | 18 |
| Observations | 109 |
| Marginal $R^2$ / Conditional $R^2$ | 0.314 / 0.536 |

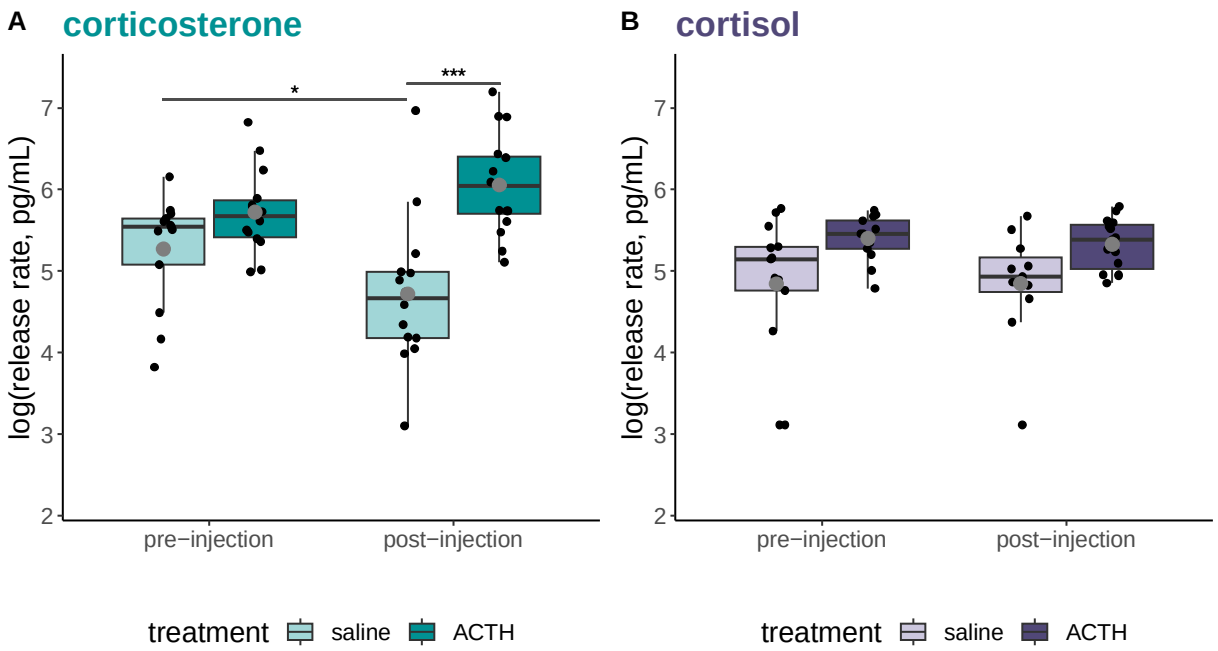



***Figure 2.*** Glucocorticoid response to an ACTH injection compared to a saline control injection. (A) Corticosterone release rate was higher after an ACTH injection than after a saline injection. (B) In contrast, we found no difference in cortisol release rate between samples taken before and after injection of either saline or ACTH. Purple boxplots indicate cortisol data. Boxplots for corticosterone data are in teal. Lighter colors (on the left within injection groups) indicate the control/saline injection for the respective glucocorticoid. Grey dots indicate the mean for each treatment group. Black dots are individual water samples. (* = $p < 0.05$, *** = $p < 0.001$)

*3.3 Plasma vs. water glucocorticoids –*

When we compared water glucocorticoids with plasma glucocorticoids in *D. tinctorius*, the concentration of cortisol in plasma was positively correlated with the release rate of cortisol in water (ß=1.15, CI=0.42 – 1.87, $R^2$/$R^2$ adjusted = 0.829/0.786, p = 0.012; Fig 3). We found a slightly positive but statistically non-significant relationship between corticosterone

concentration in plasma and corticosterone release rate in water (ß=0.19, CI = -0.38 – 0.76, $R^2/R^2$ adjusted = 0.100/-0.050, p = 0.445; Fig 3).

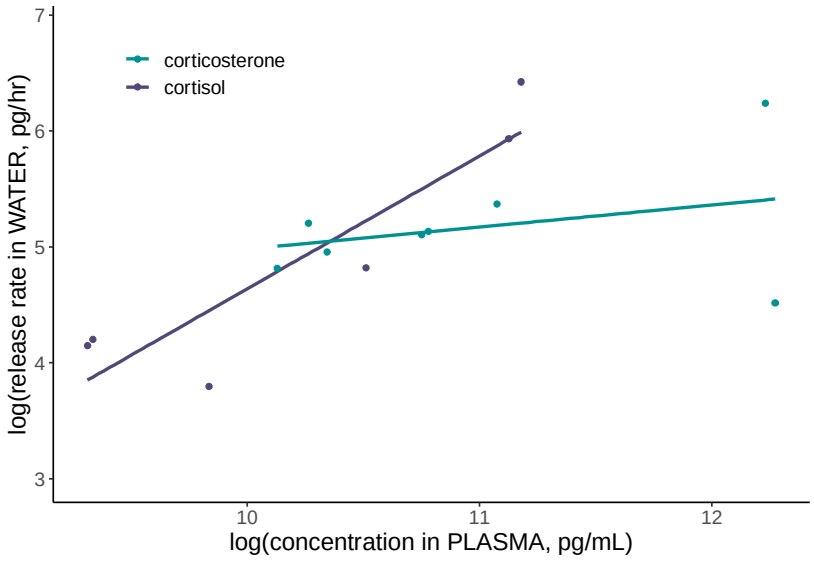



***Figure 3.*** Correlations between glucocorticoids in plasma and water. The concentration of cortisol, but not corticosterone, in plasma was positively correlated with the concentration in water samples from the same individuals. Grey shaded area is the confidence interval around the statistically significant linear regression for cortisol.

## 4. Discussion

Glucocorticoids are involved in many physiological and behavioral processes that are essential for survival and fitness (MacDougall-Shackleton et al., 2019; Nicolaides et al., 2015); therefore, understanding glucocorticoids and HPA axis dynamics can inform conservation efforts and improve our understanding of how animals are being impacted by environmental changes and threats (Busch and Hayward, 2009; Millspaugh and Washburn, 2004). To better understand the stress physiology of Dendrobatids, we measured water-borne glucocorticoids in multiple poison frogs and performed an ACTH hormone challenge and plasma-water correlations in our focal species, *D. tinctorius*. Our findings challenge the common assumption that corticosterone is

more abundant in frogs and – more broadly – the assumption that relative abundance predicts dominance. We found striking differences in relative abundance of glucocorticoids across closely related species and suggest that focusing only on the ACTH-responsive GC can overlook potentially important ACTH-independent regulation of other abundant GCs.

*4.1 Species variation in glucocorticoid dominance –*

Results from all five poison frog species across the *Dendrobatidae* family contradict the common assumption that corticosterone is the most abundant GC in amphibians (Moore and Jessop, 2003; Norris and Carr, 2013; Wingfield and Romero, 2011), and in particular in terrestrial amphibians (Jungreis et al., 1970). In water-borne hormone samples from five poison frog species, we found that three out of five species released more cortisol than corticosterone. On average, both *Ranitomeya* species released more 1.4x as much cortisol as corticosterone (Fig 1). The difference between cortisol and corticosterone was less dramatic for *E. anthonyi* but cortisol was still released at more than 1.25x the rate of corticosterone (Fig 1). In contrast, in *D. tinctorius* and *P. terribilis* we detected approximately equal amounts of both glucocorticoids (Fig 1). We opportunistically chose these five species of poison frogs representing four genera. More systematic sampling is needed to determine whether variance in relative GC abundance across Dendrobatids is associated with phylogeny, life history, or some combination. Nonetheless, we can confidently say that this group does not follow the assumption of higher corticosterone abundance in (terrestrial) amphibians.

Most studies comparing cortisol and corticosterone within-species are done in mammals with few systematic studies in other taxa (Vera et al., 2017). The few studies in amphibians that have looked at both cortisol and corticosterone report a range of empirical results across different sample types with no consistent pattern, as discussed above. These findings in amphibians and other taxa beg the question of what explains this variation in GC dominance. One idea is that the two GCs are functionally interchangeable, and variation is therefore random. However, given variation even within species, it has been suggested that cortisol and corticosterone may in fact have some different physiological functions and be differentially regulated. The two GCs could be playing different roles across age classes and metabolic functions. For instance, in a study on zebra finches (*Taeniopygia guttata*), the more abundant GC varied by tissue type with more corticosterone (the dogmatic dominant GC of birds) detected in plasma, but more cortisol

detected in immune tissues (Schmidt and Soma, 2008). Additionally, corticosterone and cortisol may differentially bind to species-specific corticosteroid-binding globulins resulting in differing amounts of bound and unbound corticosteroid available in circulation (Breuner and Orchinik, 2002; Martin and Ozon, 1975; Westphal, 1986). Pharmacological studies also suggest differential glucocorticoid receptor-mediated gene regulation based on which type of corticosteroid is available (e.g. Nixon et al., 2016; Pariante et al., 2003) and differential transport of each corticosteroid across the blood-brain barrier (e.g. Karssen et al., 2001). In sum, focusing only on the ACTH-responsive or "dominant" GC ignores ACTH-independent regulation or extra-adrenal production of the other GC, which may be just as important (Bornstein et al., 2008; Bornstein and Chrousos, 1999; Taves et al., 2011).

*4.2 Glucocorticoid response to adrenal activation in D. tinctorius –*

Our second goal in this study was to identify which glucocorticoid was responsive to activation of the adrenal glands via injection of ACTH, i.e., the dominant GC by the standard definition. In *D. tinctorius*, we found the release rate of corticosterone decreased following a saline injection, indicating frogs were habituating to the sampling process and no longer mounting a stress response, however an ACTH injection prevented this decrease in corticosterone, demonstrating a stress response relative to controls (Fig 2). Interestingly, cortisol did not respond to either an injection of ACTH or saline (Fig 2) suggesting cortisol is not responsive to acute adrenal activation and does not fluctuate across short time periods (i.e., does not show an increased stress response or habituation). In combination, these results indicate corticosterone is more responsive to acute adrenal activation than cortisol, despite approximately equal amounts of cortisol and corticosterone prior to injections. Often, the GC that responds most to adrenal activation through an ACTH challenge is also much more abundant (e.g., Forsburg et al., 2019; Hopkins et al., 2020), but this was not the case in *D. tinctorius*. Our finding that corticosterone and cortisol are similar in abundance yet differentially responsive to ACTH underscore the need to characterize both GCs and raise questions as to their primary roles in poison frog physiology and behavior.

Though speculative, we hypothesize that corticosterone and cortisol may serve distinct functions and may be released via distinct mechanisms depending on context, thus providing a means for modular regulation of behavioral and physiological functions of hormones

(McGlothlin and Ketterson, 2008). As discussed above, there are various non-ACTH routes of GC production, including regulation via neurotransmitters and neuropeptides involved in behavior (Bornstein and Chrousos, 1999). For example, our current study shows corticosterone responds to ACTH, however a previous study in *D. tinctorius* found an increase in cortisol in plasma during an energetically costly behavior, tadpole transport (Fischer and O'Connell, 2020). The parental state involves a myriad of physiological changes (Numan, 2020; Rosenblatt and Snowdon, 1996) and perhaps the long-term physiological versus short-term behavioral demands of parenting rely on the production of glucocorticoids through different routes. We suggest future studies explore the idea that ACTH and ACTH-independent mechanisms could provide modular regulation of responses to various stressors and stressful contexts, such as parental care.

Expanding on the above ideas, the functionality of cortisol and corticosterone within a species may be more distinct than we typically assume (Schmidt and Soma, 2008; Vera et al., 2017), leading to preferential expression of one GC over the other in some contexts and synchronized release in other contexts. In some species and contexts, cortisol is more responsive to acute stress than corticosterone (Koren et al., 2012; Venkataseshu and Estergreen, 1970; Vera et al., 2012, 2011), however the two GCs may also simultaneously elevate in more chronic states with high energetic burdens. For instance, both cortisol and corticosterone increase during pregnancy in humans (Wintour et al., 1978), little brown bats (*Myotis lucifugus*, Reeder et al., 2004), North Atlantic right whales (Hunt et al., 2017), and Asian elephants (*Elephas maximus*, Kajaysri and Nokkaew, 2014). In essence, the ratio of the two GCs in circulation may be dependent on physiological state. In the context of increased cortisol during parenting in *D. tinctorius* (Fischer and O'Connell, 2020), an increase in corticosterone, but not cortisol, following an ACTH injection suggests non-ACTH routes of GC production may be important in upregulating cortisol during behavioral states with higher energetic demand. Such modular regulation could occur through changes in the regulation of the enzyme required to produce cortisol (17α-hydroxylase). Further studies are needed to explore these intriguing possibilities.

*4.3 Plasma vs. water*

We note that many assumptions about GC abundance and dominance are based primarily on studies of plasma or adrenal/interrenal tissue. It is possible that these assumptions do not translate to water-borne hormone sampling techniques. For example, cortisol and corticosterone

release rates could differ across species such that waterborne levels of GCs are not representative of circulating levels of GCs in all species. We think it is unlikely that mechanism leading to a difference in release rates would be different among closely related species, thus the differences we see among species likely reflect actual physiological species differences. Additionally, there is precedent for our findings as previous studies examining ACTH responsiveness and relative abundance of GC across many different sample types in amphibians - including water, fecal, and skin swabs - have demonstrated variation in GC dominance across species. In any case, our results provide evidence that the Dendrobatid species we tested produce substantial amounts of cortisol, contrary to assumptions that amphibians only produce corticosterone.

Water-borne hormones are an integrative measure of hormone release rate over time, rather than measuring one moment in time as in plasma. In our current study, we did find a positive correlation between plasma concentration and water release rate for cortisol. Yet, the collection of water-borne hormones and plasma is inherently separated in time and therefore, perhaps our null expectation should not be a tight relationship between plasma and water-borne hormones. Alternatively, even when plasma and water hormone levels are correlated, GCs may differ in their release rates such that relative abundances differ between plasma and water. For example, in túngara frogs (*Physalaemus pustulosus*), cortisol was present in higher concentrations than corticosterone in water-borne samples, but corticosterone was higher in plasma samples (Baugh et al., 2018). However, there is evidence that water-borne samples are a still biologically meaningful method for non-invasively measuring glucocorticoids in anurans (Ruthsatz et al., 2023; Tornabene et al., 2021). Thus, while most studies of water-borne hormones in amphibians find a positive relationship between plasma and water measurements as we did here, the complexities of this relationship require further study.

### *4.4 Conclusions*

Altogether, our findings highlight the importance of measuring both cortisol and corticosterone when investigating the complex, multivariate roles of GCs in physiology, development, and behavior. Our results suggest poison frogs go against the textbook expectation of corticosterone being dominant and therefore more abundant in (terrestrial) amphibians. We found cortisol released at higher rates than corticosterone in multiple species. *D. tinctorius* and *P. terribilis* were distinctive in releasing nearly equal amounts of corticosterone and cortisol, yet

*D. tinctorius* follows the general assumption of corticosterone dominance by responding to ACTH with an increase in corticosterone. These findings inspire more questions than they answer. For instance, why does relative GC abundance vary across poison frogs? Are cortisol and corticosterone independently regulated through ACTH and non-ACTH routes? Are poison frogs unique among terrestrial frogs in producing more cortisol than corticosterone? We clearly have a lot left to learn about the stress physiology of this unique clade of frogs and amphibians in general. We can say with confidence that there is interesting, under-appreciated, and unexplored variation in HPA activity and glucocorticoids, and that simplifying assumptions about the relationship between GC abundance and dominance do not hold.

Ultimately, we need more studies measuring both GCs whenever possible. We encourage more researchers to investigate both GCs in their own focal species. Even in well-studies species, measuring both GCs can bring to light new information about the differential regulation and functionality of cortisol and corticosterone, which may not be as interchangeable as we assume (Hancock, 2010). With data across more species, we can look for broader patterns in GC dominance and abundance to investigate whether there are adaptive and/or ecological explanations for the diverse patterns emerging across species, populations, and life stages. We can also test hypotheses about potential unique physiological roles for cortisol versus corticosterone, which may have previously been obscured due to measurement of only a single GC. Additionally, measuring both cortisol and corticosterone may better inform conservation practices through improving our understanding of stress physiology across species and environments.

**Declarations of Competing Interest** None.

**CRediT authorship contribution statement**

**SEW:** conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review & editing, visualization, project administration, funding acquisition. **RTP:** conceptualization, methodology, writing – review & editing, supervision. **EKF:** conceptualization, methodology, writing – review & editing, supervision, funding acquisition.

**Acknowledgements** We thank members of the Fischer Lab for their feedback on the manuscript and for the caretaking of the frogs.

**Funding** This study was supported by the National Science Foundation [#2010714 to SEW; #2146058 to EKF] and University of Illinois Urbana-Champaign [start-up funds and RB21025 to EKF].

**Data statement** Data and code are available on FigShare (https://doi.org/10.6084/m9.figshare.23537409.v2).

**References**

Aerts, J., 2018. Quantification of a Glucocorticoid Profile in Non-pooled Samples Is Pivotal in Stress Research Across Vertebrates. Frontiers in Endocrinology 9.

Angulo, A., von May, R., Icochea, J., 2019. A reassessment of the extinction risk of the Critically Endangered Oxapampa poison frog *Ameerega planipaleae* (Dendrobatidae). Oryx 53, 557–560. https://doi.org/10.1017/S0030605318000571

Auliya, M., García-Moreno, J., Schmidt, B.R., Schmeller, D.S., Hoogmoed, M.S., Fisher, M.C., Pasmans, F., Henle, K., Bickford, D., Martel, A., 2016. The global amphibian trade flows through Europe: the need for enforcing and improving legislation. Biodivers Conserv 25, 2581–2595. https://doi.org/10.1007/s10531-016-1193-8

Bales, K.L., Kramer, K.M., Lewis-Reese, A.D., Carter, C.S., 2006. Effects of stress on parental care are sexually dimorphic in prairie voles. Physiology & Behavior 87, 424–429. https://doi.org/10.1016/j.physbeh.2005.11.002

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

Baugh, A.T., Bastien, B., Still, M.B., Stowell, N., 2018. Validation of water-borne steroid hormones in a tropical frog (*Physalaemus pustulosus*). General and Comparative Endocrinology 261, 67–80. https://doi.org/10.1016/j.ygcen.2018.01.025

Becker, C.G., Fonseca, C.R., Haddad, C.F.B., Batista, R.F., Prado, P.I., 2007. Habitat Split and the Global Decline of Amphibians. Science 318, 1775–1777. https://doi.org/10.1126/science.1149374

Blaustein, A.R., Kiesecker, J.M., 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5, 597–608. https://doi.org/10.1046/j.1461-0248.2002.00352.x

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

Bornstein, S.R., Chrousos, G.P., 1999. Adrenocorticotropin (ACTH)- and Non-ACTH-Mediated Regulation of the Adrenal Cortex: Neural and Immune Inputs. The Journal of Clinical Endocrinology & Metabolism 84, 1729–1736. https://doi.org/10.1210/jcem.84.5.5631

Bornstein, S.R., Engeland, W.C., Ehrhart-Bornstein, M., Herman, J.P., 2008. Dissociation of ACTH and glucocorticoids. Trends in Endocrinology & Metabolism 19, 175–180. https://doi.org/10.1016/j.tem.2008.01.009

Breuner, C.W., Orchinik, M., 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. Journal of Endocrinology 175, 99–112. https://doi.org/10.1677/joe.0.1750099

Brück, K., 1983. Functions of the Endocrine System, in: Schmidt, R.F., Thews, G. (Eds.), Human Physiology. Springer, Berlin, Heidelberg, pp. 658–687. https://doi.org/10.1007/978-3-642-96714-6_29

Busch, D.S., Hayward, L.S., 2009. Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biological Conservation 142, 2844–2853. https://doi.org/10.1016/j.biocon.2009.08.013

Chan, S.T.H., Edwards, B.R., 1970. Kinetic Studies on the Biosynthesis of Corticosteroids *in vitro* from Exogenous Precoursors by the Interrenal Glands of the Normal,

Corticotrophin-Treated and Adenohypophysectomized *Xenopus laevis* Daudin. Journal of Endocrinology 47, 183–195. https://doi.org/10.1677/joe.0.0470183

Cikanek, S.J., Nockold, S., Brown, J.L., Carpenter, J.W., Estrada, A., Guerrel, J., Hope, K., Ibáñez, R., Putman, S.B., Gratwicke, B., 2014. Evaluating Group Housing Strategies for the Ex-Situ Conservation of Harlequin Frogs (*Atelopus* spp.) Using Behavioral and Physiological Indicators. PLOS ONE 9, e90218. https://doi.org/10.1371/journal.pone.0090218

Collins, J., 2010. Amphibian decline and extinction: What we know and what we need to learn. Dis. Aquat. Org. 92, 93–99. https://doi.org/10.3354/dao02307

Courtois, E.A., Gaucher, P., Chave, J., Schmeller, D.S., 2015. Widespread Occurrence of Bd in French Guiana, South America. PLOS ONE 10, e0125128. https://doi.org/10.1371/journal.pone.0125128

Crespi, E.J., Denver, R.J., 2005. Roles of stress hormones in food intake regulation in anuran amphibians throughout the life cycle. Comp Biochem Phys A 141, 381–390. https://doi.org/10.1016/j.cbpb.2004.12.007

Crossin, G.T., Trathan, P.N., Phillips, R.A., Gorman, K.B., Dawson, A., Sakamoto, K.Q., Williams, T.D., 2012. Corticosterone Predicts Foraging Behavior and Parental Care in Macaroni Penguins. The American Naturalist 180, E31–E41. https://doi.org/10.1086/666001

Cruz-Topete, D., Cidlowski, J.A., 2015. One Hormone, Two Actions: Anti- and Pro-Inflammatory Effects of Glucocorticoids. NIM 22, 20–32. https://doi.org/10.1159/000362724

Cruz-Topete, D., Myers, P.H., Foley, J.F., Willis, M.S., Cidlowski, J.A., 2016. Corticosteroids Are Essential for Maintaining Cardiovascular Function in Male Mice. Endocrinology 157, 2759–2771. https://doi.org/10.1210/en.2015-1604

Cushman, S.A., 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation 128, 231–240. https://doi.org/10.1016/j.biocon.2005.09.031

Dale, E., 1962. Steroid excretion by larval frogs. General and Comparative Endocrinology 2, 171–176. https://doi.org/10.1016/0016-6480(62)90001-1

Denver, R.J., 2009. Stress hormones mediate environment-genotype interactions during amphibian development. General and Comparative Endocrinology 164, 20–31. https://doi.org/10.1016/j.ygcen.2009.04.016

Dickmeis, T., Weger, B.D., Weger, M., 2013. The circadian clock and glucocorticoids – Interactions across many time scales. Molecular and Cellular Endocrinology 380, 2–15. https://doi.org/10.1016/j.mce.2013.05.012

Fischer, E.K., O'Connell, L.A., 2020. Hormonal and neural correlates of care in active versus observing poison frog parents. Hormones and Behavior 120, 104696. https://doi.org/10.1016/j.yhbeh.2020.104696

Forsburg, Z.R., Goff, C.B., Perkins, H.R., Robicheaux, J.A., Almond, G.F., Gabor, C.R., 2019. Validation of water-borne cortisol and corticosterone in tadpoles: Recovery rate from an acute stressor, repeatability, and evaluating rearing methods. General and Comparative Endocrinology 281, 145–152. https://doi.org/10.1016/j.ygcen.2019.06.007

Gabor, C.R., Bosch, J., Fries, J.N., Davis, D.R., 2013. A non-invasive water-borne hormone assay for amphibians. Amphibia-Reptilia 34, 151–162. https://doi.org/10.1163/15685381-00002877

Ganjam, V.K., Campbell, A.L., Murphy, B.E.P., 1972. Changing Patterns of Circulating Corticosteroids in Rabbits Following Prolonged Treatment with ACTH. Endocrinology 91, 607–611. https://doi.org/10.1210/endo-91-2-607

Glennemeier, K.A., Denver, R.J., 2002. Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components. General and Comparative Endocrinology 127, 16–25. https://doi.org/10.1016/S0016-6480(02)00015-1

Gobbetti, A., Zerani, M., 1993. Prostaglandin E2 and prostaglandin F2α involvement in the corticosterone and cortisol release by the female frog, *Rana esculenta*, during ovulation. Journal of Experimental Zoology 267, 164–170. https://doi.org/10.1002/jez.1402670209

Guillory, W.X., Muell, M.R., Summers, K., Brown, J.L., 2019. Phylogenomic Reconstruction of the Neotropical Poison Frogs (Dendrobatidae) and Their Conservation. Diversity 11, 126. https://doi.org/10.3390/d11080126

Haller, J., 2014. The Glucocorticoid/Aggression Relationship in Animals and Humans: An Analysis Sensitive to Behavioral Characteristics, Glucocorticoid Secretion Patterns, and Neural Mechanisms, in: Miczek, K.A., Meyer-Lindenberg, A. (Eds.), Neuroscience of Aggression, Current Topics in Behavioral Neurosciences. Springer, Berlin, Heidelberg, pp. 73–109. https://doi.org/10.1007/7854_2014_284

Hancock, T.M., 2010. A duality in mammalian glucocorticoid signaling evidence against the dominant glucocorticoid/interchangeability assumption (M.Sc.). Queen's University (Canada), Ontario, Canada.

Hopkins, W.A., DuRant, S.E., Beck, M.L., Ray, W.K., Helm, R.F., Romero, L.M., 2020. Cortisol is the predominant glucocorticoid in the giant paedomorphic hellbender salamander (*Cryptobranchus alleganiensis*). General and Comparative Endocrinology 285, 113267. https://doi.org/10.1016/j.ygcen.2019.113267

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical Journal 50, 346–363. https://doi.org/10.1002/bimj.200810425

Hunt, K.E., Lysiak, N.S., Moore, M., Rolland, R.M., 2017. Multi-year longitudinal profiles of cortisol and corticosterone recovered from baleen of North Atlantic right whales (*Eubalaena glacialis*). General and Comparative Endocrinology 254, 50–59. https://doi.org/10.1016/j.ygcen.2017.09.009

Jaudet, G.J., Hatey, J.L., 1984. Variations in aldosterone and corticosterone plasma levels during metamorphosis in *Xenopus laevis* tadpoles. General and Comparative Endocrinology 56, 59–65. https://doi.org/10.1016/0016-6480(84)90061-3

Johnston, C., Davis, J., Wright, F., Howards, S., 1967. Effects of renin and ACTH on adrenal steroid secretion in the American bullfrog. American Journal of Physiology-Legacy Content 213, 393–399. https://doi.org/10.1152/ajplegacy.1967.213.2.393

Johnston, S., Booth, R., Pyne, M., Keeley, T., Mackie, J., Hulse, L., Ellis, W., 2013. Preliminary study of faecal cortisol and corticosterone as an index of acute cortisol secretion in the koala (Phascolarctos cinereus). Australian Veterinary Journal 91, 534–537. https://doi.org/10.1111/avj.12125

Jolivet-Jaudet, G., Ishii, S., 1983. Nature des produits de la synthèse interrénalienne in vitro chez *Bufo bufo formosus* adulte. C R Seances Acad Sci, III 296, 1059–1062.

Jolivet-Jaudet, G., Leloup-Hatey, J., 1984. Interrenal function during amphibian metamorphosis: In vitro biosynthesis of radioactive corticosteroids from (414C)-progesterone by interrenal in *Xenopus laevis* tadpoles. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 79, 239–244. https://doi.org/10.1016/0305-0491(84)90020-8

Jungreis, A.M., Huibregtse, W.H., Ungar, F., 1970. Corticosteroid identification and corticosterone concentration in serum of *Rana pipiens* during dehydration in winter and summer. Comparative Biochemistry and Physiology 34, 683–689. https://doi.org/10.1016/0010-406X(70)90293-8

Kajaysri, J., Nokkaew, W., 2014. Assessment of Pregnancy Status of Asian Elephants *(Elephas maximus)* by Measurement of Progestagen and Glucocorticoid and Their Metabolite Concentrations in Serum and Feces, Using Enzyme Immunoassay (EIA). J. Vet. Med. Sci. 76, 363–368. https://doi.org/10.1292/jvms.13-0103

Karssen, A.M., Meijer, O.C., van der Sandt, I.C.J., Lucassen, P.J., de Lange, E.C.M., de Boer, A.G., de Kloet, E.R., 2001. Multidrug Resistance P-Glycoprotein Hampers the Access of Cortisol But Not of Corticosterone to Mouse and Human Brain. Endocrinology 142, 2686–2694. https://doi.org/10.1210/endo.142.6.8213

Katsu, Y., Iguchi, T., 2016. Corticosterone, in: Handbook of Hormones. Elsevier, pp. 527-e95A-3. https://doi.org/10.1016/B978-0-12-801028-0.00228-2

Keogh, M.J., Gastaldi, A., Charapata, P., Melin, S., Fadely, B.S., 2020. Stress-related and reproductive hormones in hair from three north Pacific otariid species: Steller sea lions, California sea lions and northern fur seals. Conservation Physiology 8, coaa069. https://doi.org/10.1093/conphys/coaa069

Kiesecker, J.M., 2011. Global stressors and the global decline of amphibians: tipping the stress immunocompetency axis. Ecol Res 26, 897–908. https://doi.org/10.1007/s11284-010-0702-6

Kiesecker, J.M., Blaustein, A.R., Belden, L.K., 2001. Complex causes of amphibian population declines. Nature 410, 681–684. https://doi.org/10.1038/35070552

Koren, L., Whiteside, D., Fahlman, Å., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., Wynne-Edwards, K., 2012. Cortisol and corticosterone independence in cortisol-dominant wildlife. General and Comparative Endocrinology 177, 113–119. https://doi.org/10.1016/j.ygcen.2012.02.020

Krug, E.C., Honn, V., Battista, J., Nicoll, C.S., 1983. Corticosteroids in Serum of *Rima catesbeiana* during Development and Metamorphosis. General and Comparative Endocrinology 52, 232–241. https://doi.org/10.1016/0016-6480(83)90117-X

Kulkarni, P.S., Gramapurohit, N.P., 2017. Effect of corticosterone on larval growth, antipredator behaviour and metamorphosis of Hylarana indica. General and Comparative Endocrinology, Special issue on International Symposium on Comparative Endocrinology and Integrative Physiology - CEIP 2015 251, 21–29. https://doi.org/10.1016/j.ygcen.2016.09.001

Kulkarni, S.S., Buchholz, D.R., 2014. Corticosteroid signaling in frog metamorphosis. General and Comparative Endocrinology, 2nd Meeting of The North American Society for Comparative Endocrinology 203, 225–231. https://doi.org/10.1016/j.ygcen.2014.03.036

Kulkarni, S.S., Buchholz, D.R., 2012. Beyond Synergy: Corticosterone and Thyroid Hormone Have Numerous Interaction Effects on Gene Regulation in Xenopus tropicalis Tadpoles. Endocrinology 153, 5309–5324. https://doi.org/10.1210/en.2012-1432

Kumar, V., Umapathy, G., 2019. Non-invasive monitoring of steroid hormones in wildlife for conservation and management of endangered species — A review. Indian J Exp Biol.

Kuznetsova, A., Brockhoff, P., Christensen, R., 2016. lmerTest: Tests in linear mixed effects models. R package version 3.0.0, https://cran.r-project.org/package=lmerTest.

Larson, S., Monson, D., Ballachey, B., Jameson, R., Wasser, S.K., 2009. Stress-related hormones and genetic diversity in sea otters (*Enhydra lutris*). Marine Mammal Science 25, 351–372. https://doi.org/10.1111/j.1748-7692.2008.00260.x

MacDougall-Shackleton, S.A., Bonier, F., Romero, L.M., Moore, I.T., 2019. Glucocorticoids and "Stress" Are Not Synonymous. Integrative Organismal Biology 1, obz017. https://doi.org/10.1093/iob/obz017

Martin, B., Ozon, R., 1975. Steroid-Protein Interactions in Nonmammalian Vertebrates. II. Steroids Binding Proteins in the Serum of Amphibians; a Physiological Approach. Biology of Reproduction 13, 371–380. https://doi.org/10.1095/biolreprod13.4.371

McGlothlin, J.W., Ketterson, E.D., 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philosophical Transactions of the Royal Society B: Biological Sciences 363, 1611–1620. https://doi.org/10.1098/rstb.2007.0002

Mehdi, A.Z., Carballeira, A., 1971. Steroid biosynthesis by the American bullfrog (*Rana catesbeiana*) interrenals: I. In vitro studies with progesterone-4-14C and Δ5-pregnenolone-4-14C. General and Comparative Endocrinology 17, 1–13. https://doi.org/10.1016/0016-6480(71)90150-X

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138, 189–199. https://doi.org/10.1016/j.ygcen.2004.07.002

Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior 43, 39–47. https://doi.org/10.1016/S0018-506X(02)00038-7

Murray, M.J., Young, M.A., Santymire, R.M., 2020. Use of the ACTH challenge test to identify the predominant glucocorticoid in the southern sea otter (*Enhydra lutris nereis*). Conservation Physiology 8, coz116. https://doi.org/10.1093/conphys/coz116

Narayan, E.J., 2013. Non-invasive reproductive and stress endocrinology in amphibian conservation physiology. Conservation Physiology 1, cot011–cot011. https://doi.org/10.1093/conphys/cot011

Nicolaides, N.C., Kyratzi, E., Lamprokostopoulou, A., Chrousos, G.P., Charmandari, E., 2015. Stress, the Stress System and the Role of Glucocorticoids. Neuroimmunomodulation 22, 6–19. https://doi.org/10.1159/000362736

Nixon, M., Mackenzie, S.D., Taylor, A.I., Homer, N.Z.M., Livingstone, D.E., Mouras, R., Morgan, R.A., Mole, D.J., Stimson, R.H., Reynolds, R.M., Elfick, A.P.D., Andrew, R., Walker, B.R., 2016. ABCC1 confers tissue-specific sensitivity to cortisol versus corticosterone: A rationale for safer glucocorticoid replacement therapy. Science Translational Medicine 8, 352ra109-352ra109. https://doi.org/10.1126/scitranslmed.aaf9074

Norris, D.O., Carr, J.A., 2013. Chapter 9 - Comparative Aspects of Vertebrate Adrenals, in: Norris, D.O., Carr, J.A. (Eds.), Vertebrate Endocrinology (Fifth Edition). Academic Press, San Diego, pp. 291–315. https://doi.org/10.1016/B978-0-12-394815-1.00009-4

Nowicki, J.P., Rodríguez, C., Lee, J.C., Goolsby, B.C., Yang, C., Cleland, T.A., O'Connell, L.A., 2022. Evidence for Empathy in Pair Bonding Poison Frogs. https://doi.org/10.1101/2022.09.25.509360

Numan, M., 2020. The parental brain: mechanisms, development, and evolution. Oxford University Press, New York.

Ohmer, M.E.B., Alton, L., Cramp, R.L., 2021. Physiology provides a window into how the multi-stressor environment contributes to amphibian declines. Conservation Physiology: Applications for Wildlife Conservation and Management 165–182. https://doi.org/10.1093/oso/9780198843610.003.0010

Palme, R., 2019. Non-invasive measurement of glucocorticoids: Advances and problems. Physiology & Behavior 199, 229–243. https://doi.org/10.1016/j.physbeh.2018.11.021

Pariante, C.M., Kim, R.B., Makoff, A., Kerwin, R.W., 2003. Antidepressant fluoxetine enhances glucocorticoid receptor function in vitro by modulating membrane steroid transporters. British Journal of Pharmacology 139, 1111–1118. https://doi.org/10.1038/sj.bjp.0705357

Posit Team, 2023. RStudio: Integrated Development Environment for R.

R Core Team, 2022. R: A language and environment for statistical computing.

Raubenheimer, P.J., Young, E.A., Andrew, R., Seckl, J.R., 2006. The role of corticosterone in human hypothalamic– pituitary–adrenal axis feedback. Clinical Endocrinology 65, 22–26. https://doi.org/10.1111/j.1365-2265.2006.02540.x

Raulo, A., Dantzer, B., 2018. Associations between glucocorticoids and sociality across a continuum of vertebrate social behavior. Ecology and Evolution 1–20. https://doi.org/10.1002/ece3.4059

Reeder, D.M., Kosteczko, N.S., Kunz, T.H., Widmaier, E.P., 2004. Changes in baseline and stress-induced glucocorticoid levels during the active period in free-ranging male and female little brown myotis, Myotis lucifugus (Chiroptera: Vespertilionidae). General and Comparative Endocrinology 136, 260–269. https://doi.org/10.1016/j.ygcen.2003.12.020

Ringler, E., Rojas, B., Stynoski, J.L., Schulte, L.M., 2023. What Amphibians Can Teach us About the Evolution of Parental Care. Annu. Rev. Ecol. Evol. Syst. 54, annurev-ecolsys-102221-050519. https://doi.org/10.1146/annurev-ecolsys-102221-050519

Rodríguez, C., Fusani, L., Raboisson, G., Hödl, W., Ringler, E., Canoine, V., 2022. Androgen responsiveness to simulated territorial intrusions in *Allobates femoralis* males: Evidence supporting the challenge hypothesis in a territorial frog. General and Comparative Endocrinology 326, 114046. https://doi.org/10.1016/j.ygcen.2022.114046

Roland, A.B., O'Connell, L.A., 2015. Poison frogs as a model system for studying the neurobiology of parental care. Current Opinion in Behavioral Sciences 6, 76–81. https://doi.org/10.1016/j.cobeha.2015.10.002

Rosenblatt, J.S., Snowdon, C.T. (Eds.), 1996. Parental Care: Evolution, Mechanisms, And Adaptive Significance, Advances in the Study of Behavior. Academic Press.

Rosenthal, K.L., Peterson, M.E., Quesenberry, K.E., Lothrop, C.D., 1993. Evaluation of plasma cortisol and corticosterone responses to synthetic adrenocorticotropic hormone administration in ferrets. Am J Vet Res 54, 29–31.

Ruthsatz, K., Rico-Millan, R., Eterovick, P.C., Gomez-Mestre, I., 2023. Exploring water-borne corticosterone collection as a non-invasive tool in amphibian conservation physiology: benefits, limitations and future perspectives. Conservation Physiology 11, coad070. https://doi.org/10.1093/conphys/coad070

Santymire, R.M., Manjerovic, M.B., Sacerdote-Velat, A., 2018. A novel method for the measurement of glucocorticoids in dermal secretions of amphibians. Conservation Physiology 6, coy008. https://doi.org/10.1093/conphys/coy008

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21, 55–89. https://doi.org/10.1210/er.21.1.55

Schmidt, K.L., Soma, K.K., 2008. Cortisol and corticosterone in the songbird immune and nervous systems: local vs. systemic levels during development. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 295, R103–R110. https://doi.org/10.1152/ajpregu.00002.2008

Seckl, J.R., Meaney, M.J., 2004. Glucocorticoid Programming. Annals of the New York Academy of Sciences 1032, 63–84. https://doi.org/10.1196/annals.1314.006

Shewade, L.H., Schoephoerster, J.A., Patmann, M.D., Kulkarni, S.S., Buchholz, D.R., 2020. Corticosterone Is Essential for Survival Through Frog Metamorphosis. Endocrinology 161, bqaa193. https://doi.org/10.1210/endocr/bqaa193

Smith, J.H., Bubenik, G.A., 1990. Plasma concentrations of glucocorticoids in white-tailed deer: the effect of acute ACTH and dexamethasone administration. Can. J. Zool. 68, 2123–2129. https://doi.org/10.1139/z90-295

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and Trends of Amphibian Declines and Extinctions Worldwide. Science 306, 1783–1786. https://doi.org/10.1126/science.1103538

Sutanto, W., De Kloet, E.R., 1987. Species-Specificity of Corticosteroid Receptors in Hamster and Rat Brains. Endocrinology 121, 1405–1411. https://doi.org/10.1210/endo-121-4-1405

Taves, M.D., Gomez-Sanchez, C.E., Soma, K.K., 2011. Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. American Journal of Physiology-Endocrinology and Metabolism 301, E11–E24. https://doi.org/10.1152/ajpendo.00100.2011

Tornabene, B.J., Hossack, B.R., Crespi, E.J., Breuner, C.W., 2021. Evaluating corticosterone as a biomarker for amphibians exposed to increased salinity and ambient corticosterone. Conservation Physiology 9, coab049. https://doi.org/10.1093/conphys/coab049

Touma, C., Palme, R., 2005. Measuring Fecal Glucocorticoid Metabolites in Mammals and Birds: The Importance of Validation. Annals of the New York Academy of Sciences 1046, 54–74. https://doi.org/10.1196/annals.1343.006

Van Mourik, S., Stelmasiak, T., Outch, K.H., 1985. Changes in plasma levels of cortisol and corticosterone after acute ACTH stimulation in rusa deer (*Cervus rusa timorensis*). Comparative Biochemistry and Physiology Part A: Physiology 81, 545–549. https://doi.org/10.1016/0300-9629(85)91023-0

Vegiopoulos, A., Herzig, S., 2007. Glucocorticoids, metabolism and metabolic diseases. Molecular and Cellular Endocrinology 275, 43–61. https://doi.org/10.1016/j.mce.2007.05.015

Venkataseshu, G.K., Estergreen, V.L., 1970. Cortisol and Corticosterone in Bovine Plasma and the Effect of Adrenocorticotropin. Journal of Dairy Science 53, 480–483. https://doi.org/10.3168/jds.S0022-0302(70)86234-8

Vera, F., Antenucci, C.D., Zenuto, R.R., 2011. Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*). General and Comparative Endocrinology 170, 550–557. https://doi.org/10.1016/j.ygcen.2010.11.012

Vera, F., Zenuto, R., Antenucci, C.D., 2017. Expanding the actions of cortisol and corticosterone in wild vertebrates: A necessary step to overcome the emerging challenges. General and Comparative Endocrinology 246, 337–353. https://doi.org/10.1016/j.ygcen.2017.01.010

Vera, F., Zenuto, R.R., Antenucci, C.D., 2012. Differential Responses of Cortisol and Corticosterone to Adrenocorticotropic Hormone (ACTH) in a Subterranean Rodent (*Ctenomys talarum*). Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 317, 173–184. https://doi.org/10.1002/jez.1711

Walls, S.C., Gabor, C.R., 2019. Integrating Behavior and Physiology Into Strategies for Amphibian Conservation. Frontiers in Ecology and Evolution 7.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A Generalized Fecal Glucocorticoid Assay for Use in a Diverse Array of Nondomestic Mammalian and Avian Species. General and Comparative Endocrinology 120, 260–275. https://doi.org/10.1006/gcen.2000.7557

Wells, K., 2007. The Ecology and Behavior of Amphibians. The University of Chicago Press, Chicago.

Westphal, U., 1986. Steroid-Protein Interactions Revisited, in: Westphal, U. (Ed.), Steroid-Protein Interactions II, Monographs on Endocrinology. Springer, Berlin, Heidelberg, pp. 1–7. https://doi.org/10.1007/978-3-642-82486-9_1

Westrick, S.E., Moss, J.B., Fischer, E.K., 2023. Who cares? An integrative approach to understanding the evolution of behavioural plasticity in parental care. Animal Behaviour 200, 225–236. https://doi.org/10.1016/j.anbehav.2022.10.005

Whirledge, S., Cidlowski, J.A., 2017. Glucocorticoids and Reproduction: Traffic Control on the Road to Reproduction. Trends in Endocrinology & Metabolism 28, 399–415. https://doi.org/10.1016/j.tem.2017.02.005

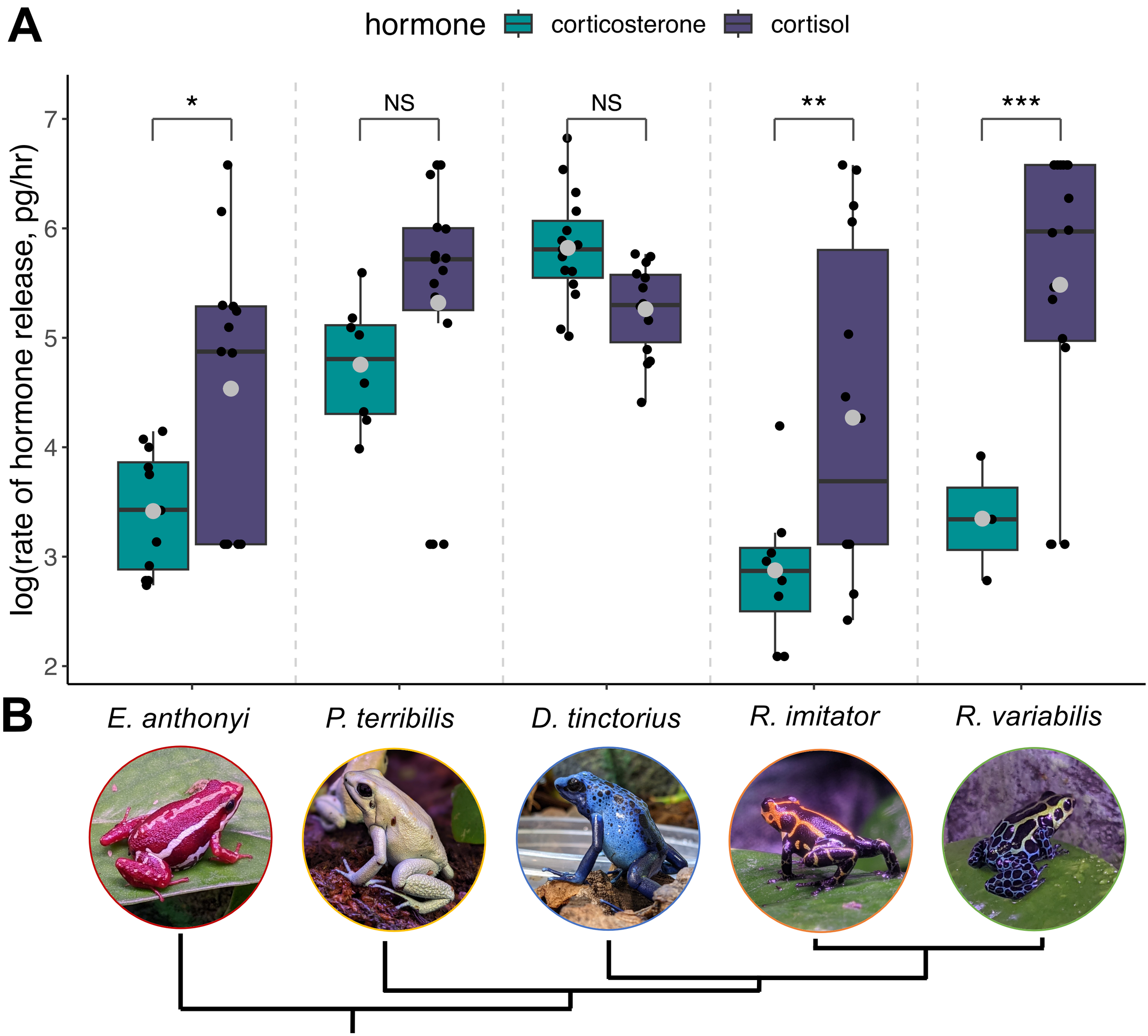
Whitfield, S.M., Bell, K.E., Philippi, T., Sasa, M., Bolaños, F., Chaves, G., Savage, J.M., Donnelly, M.A., 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. Proceedings of the National Academy of Sciences 104, 8352–8356. https://doi.org/10.1073/pnas.0611256104

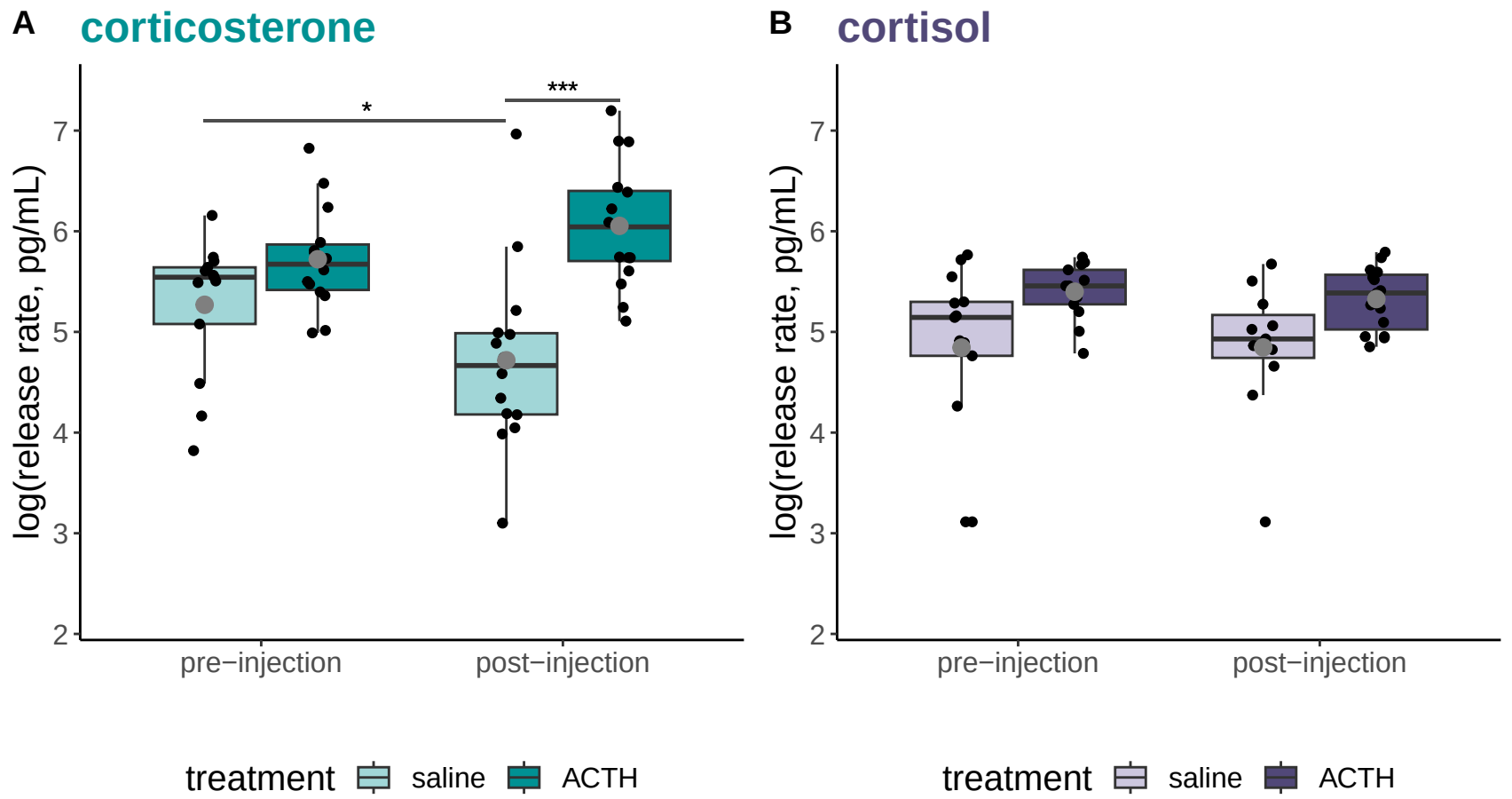
Wingfield, J.C., Romero, L.M., 2011. Adrenocortical Responses to Stress and Their Modulation in Free-Living Vertebrates, in: Comprehensive Physiology. John Wiley & Sons, Ltd, pp. 211–234. https://doi.org/10.1002/cphy.cp070411

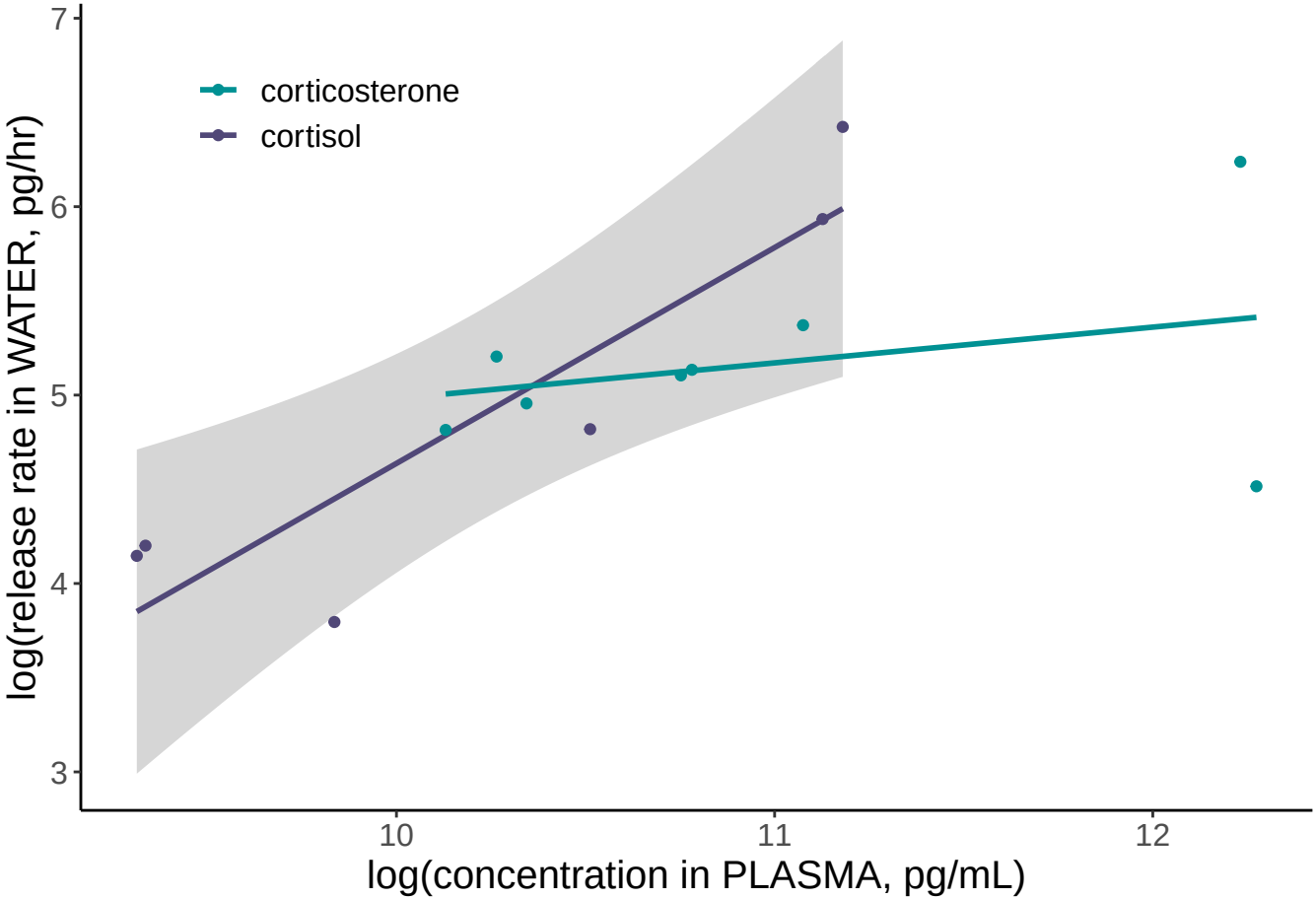
Wintour, E.M., Coghlan, J.P., Oddie, C.J., Scoggins, B.A., Walteis, W. a. W., 1978. A Sequential Study of Adrenocorticosteroid Level in Human Pregnancy. Clinical and Experimental Pharmacology and Physiology 5, 399–403. https://doi.org/10.1111/j.1440-1681.1978.tb00690.x

Wynne-Edwards, K.E., Edwards, H.E., Hancock, T.M., 2013. The Human Fetus Preferentially Secretes Corticosterone, Rather than Cortisol, in Response to Intra-Partum Stressors. PLOS ONE 8, e63684. https://doi.org/10.1371/journal.pone.0063684

Young, K.M., Walker, S.L., Lanthier, C., Waddell, W.T., Monfort, S.L., Brown, J.L., 2004. Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. General and Comparative Endocrinology 137, 148–165. https://doi.org/10.1016/j.ygcen.2004.02.016


A
hormone
corticosterone
cortisol
log(rate of hormone release, pg/hr)
7
6
5
4
3
2
*
NS
NS
**
***
B
E. anthonyi
P. terribilis
D. tinctorius
R. imitator
R. variabilis


A
corticosterone
B
cortisol
log(release rate, pg/mL)
7
6
5
4
3
2
*
***
pre−injection
post−injection
treatment
saline
ACTH


corticosterone
cortisol
log(release rate in WATER, pg/hr)
7
6
5
4
3
10
11
12
log(concentration in PLASMA, pg/mL)