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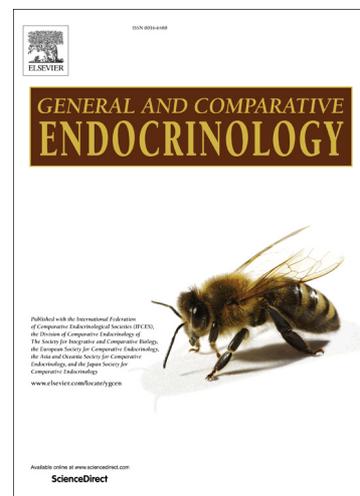
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Title: Reproductive state and water deprivation increase plasma corticosterone in a capital breeder.

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Abstract: Plasma corticosterone (CORT) concentrations fluctuate in response to homeostatic demands. CORT is widely recognized as an important hormone related to energy balance. However, far less attention has been given to the potential role of CORT in regulating salt and water balance or responding to osmotic imbalances. We examined the effects of reproductive and hydric states on CORT levels in breeding Children's pythons (*Antaresia childreni*), a species with substantial energetic and hydric costs associated with egg development. Using a 2×2 experimental design, we examined how reproduction and water deprivation, both separately and combined, impact CORT levels and how these changes correlate with hydration (plasma osmolality) and energy levels (blood glucose). We found that reproduction leads to increased CORT levels, as does dehydration induced by water deprivation. The combined impact of reproduction and water deprivation led to the largest increases in CORT levels. Additionally, we found significant positive relationships among CORT levels, plasma osmolality, and blood glucose. Our results provide evidence that both reproductive activity and increased plasma osmolality can lead to increased plasma CORT in an ectotherm, which could be explained by either CORT having a role as a mineralocorticoid or CORT being elevated as part of a stress response to resource imbalances.

Key words: Hydration; Squamate; Reproductive investment; *Antaresia childreni*; Osmotic stress

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1. Introduction

The endogenous secretion of glucocorticoids is one of the main mechanisms by which animals modulate their physiological and behavioral responses to unpredictable and predictable stimuli (Romero and Wingfield, 2016). The main glucocorticoid in rodents and non-mammalian terrestrial vertebrates (i.e., birds, amphibians, and reptiles) is corticosterone (CORT) (Sapolsky, 2000), and it can affect growth (Busch et al., 2008; Midwood et al., 2014), survival (O'Connor et al., 2010; Romero et al., 2009), and reproductive success (Robert et al., 2009; Wingfield and Sapolsky, 2003). There is an impressive body of work examining the elevation in CORT in response to unpredictable stressors and noxious stimuli (i.e., allostatic response, see Romero et al., 2009; Vera et al., 2017). However, baseline CORT levels are also important to consider because they may reflect the homeostatic demands faced by the organism (Landys et al., 2006; Romero, 2002). CORT is important for regulating shifts in metabolism and behavior (Landys, 2006) and it oscillates to meet demands associated with shifts in life-history stages (Romero, 2002). For example, baseline CORT levels are typically related to energy balance during reproduction and can either increase to support direct allocation to reproduction (i.e., energy model hypothesis) or decrease to minimize maintenance requirements (Bonier et al., 2009a,b).

However, important variations exist among and even within species in the directionality in which CORT levels respond (i.e., increase, decrease, or no change) to endogenous demands and predictable exogenous constraints (Dickens and Romero, 2013). For example, individuals can have dramatically different CORT responses to annual, expected events such as decreased temperature during winter (increase – Sheriff et al., 2012; decrease – Xu and Hu, 2017; no change – Khonmee et al., 2016) or seasonal precipitation (increase – Strier et al., 1999; decrease – Schoof et al., 2016; no change – Wilkening et al., 2016). These variable responses suggest that

changes in CORT levels are extremely context dependent (de Bruijn and Romero, 2018; Graham et al., 2017), emphasizing the importance of considering factors such as life-history stage, body condition, and environmental conditions when investigating the role of CORT within an organism. Despite these variable patterns, CORT is critical to survival, maintenance, and reproduction (Darlington et al., 1990), and CORT levels are modulated to adjust energy demands (Dallman et al., 1993; Ramage-Healey and Romero, 2001) and maintain homeostatic balance (Landys et al., 2006; Romero, 2002).

While energy is critical, water is another vital resource (Kleiner, 1999), and CORT has been shown to have important mineralocorticoid roles involved with water balance in rodents (Agarwal and Mirshahi, 1999; Bentley, 1970; Liu et al., 2010; Thunhorst et al., 2007). While more limited in number, several studies have examined the relationship between CORT and water balance in non-rodent taxa. For example, a link between CORT levels and hydration state has been demonstrated in water-constrained environments (Bradshaw, 1997). However, high variability in CORT levels suggests the response is context dependent. For example, recent studies found that baseline CORT levels are not affected by dehydration in animals with low metabolic demands (non-reproductive snakes and lizards, Dupoué et al., 2014; Moeller et al., 2017). However, in animals with higher metabolic requirements and greater water deficits (e.g., pelagic sea birds), the opposite has been documented (Brischoux et al., 2015). At least two studies have considered the additive challenges of reproduction and water deprivation on CORT levels (Dauphin-Villemant and Xavier, 1986; Dupoué et al., 2016). In the asp viper (*Vipera aspis*), CORT has been found to be closely related to the energy demands of pregnancy (Lorioux et al., 2016). However, Dupoué et al. (2016) found that changes in CORT levels were further increased when pregnant females faced water deprivation. In these studies, the study animals

were able to thermoregulate, possibly resulting in differing selected body temperatures and thermal depression (see Ladyman and Bradshaw, 2003) that, in turn, could have influenced the CORT response.

To further explore the influence that reproduction and water balance have on plasma CORT levels, we used Children's pythons (*Antaresia childreni*) in a two-by-two experiment where reproductive status and hydric states varied but body temperature was controlled to avoid confounding thermal effects on CORT levels. This capital-breeding, oviparous squamate faces considerable physiological and performance costs associated with reproduction, especially when considering that the energetic (Lourdais et al., 2013) and hydric (Brusch et al., 2017) demands of reproduction must be met when energy and water resources are limited in the environment (Taylor and Tulloch, 1985). We tested the hypothesis that plasma CORT concentration is influenced by both reproductive status and hydric state. We predicted that (1) reproduction will increase CORT; (2) water deprivation, leading to dehydration, will increase CORT; and (3) the combined challenges from reproduction and water deprivation will have an additive effect on CORT.

2. Material and methods

2.1 Study species

Children's pythons inhabit the wet-dry tropics of northern Australia (Wilson and Swan, 2013) where they experience substantial, natural fluctuations in water availability during the Austral dry-season, which frequently lasts 3-4 months (May-August, Taylor and Tulloch, 1985). Egg development and oviposition typically occurs towards the end of the dry season so that eggs hatch at the start of the wet season. As capital breeders, females accumulate energy resources

over a long duration (typically in excess of a year) and utilize the resultant lipid reserves to support the reproductive effort from vitellogenesis through egg brooding (Stephens et al., 2009). However, a lack of free-standing water during the period of egg development results in considerable challenges to female water balance (Brusch et al., 2017). Coupled with a lack of available water to support themselves, reproductive females face additional water requirements associated with egg development (Deeming, 2004; Lourdais et al., 2015). In the weeks just prior to oviposition, in the absence of free-standing water in the environment, females must transfer substantial amounts of body water to their eggs (Stahlschmidt et al., 2011) and this can dramatically challenge female water balance (Brusch et al., 2017).

2.2 Experimental design

All work was conducted under the oversight of the Arizona State University Institutional Animal Care and Use Committee (protocol # 17-1532R). Snakes used for this study were part of a long-term colony at Arizona State University, AZ, USA, and were housed individually in $91 \times 71 \times 46$ cm cages (Freedom Breeder, Turlock, CA, USA). Snakes were deprived of food from the onset of overwintering until oviposition (~5 months) because they typically do not eat when reproductively active. To stimulate the reproductive cycle, snakes were over-wintered for six weeks from mid-December through January (light-dark cycles of 6:18 h and a daily temperature cycle of 20:15°C that changed in conjunction with the light cycle). During the 6-hr light phase a supplemental sub-surface heat source was provided under one end of each cage to allow for thermoregulation. After overwintering, room temperature was increased to a constant 31.5°C, which is the preferred temperature of gravid Children's pythons (Lourdais et al., 2008), with a 12:12 h light-dark cycle and no supplemental heat provided. Females were held at a constant

temperature without the opportunity to thermoregulate to prevent temperature-based difference resulting from differential thermoregulation between treatment groups.

Females were randomly assigned to either the reproductive ($n = 19$) or non-reproductive groups ($n = 16$). Females in the reproductive group were exposed to a different male every 2-3 days, resulting in each reproductive female being sequentially housed with 6-8 males during this 18-day breeding period. Each non-reproductive female was yoked to a reproductive female of similar body mass so that the timing of its treatments, measurements, and sampling coincided with that of the reproductive female to which it was yoked. At ovulation (based on a peri-ovulation shed, Lourdais et al., 2008), half of the reproductive females, and their yoked partners, were deprived of water until the reproductive female laid her eggs (22 ± 1 d). Deprivation of water during this time leads to ecologically relevant levels of dehydration (Brusch et al., 2018). Treatment groups were: gravid with water provided *ad libitum* (number of females, mean mass \pm SEM, and plasma osmolality at the end of the experiment \pm SEM: $n = 9$, 464 ± 28 g, 324 ± 5 mOsm kg^{-1}), non-reproductive with water provided *ad libitum* ($n = 8$, 414 ± 18 g, 297 ± 4 mOsm kg^{-1}), gravid with no water provided ($n = 10$, 424 ± 41 g, 354 ± 3 mOsm kg^{-1}), and non-reproductive with no water provided ($n = 8$, 423 ± 33 g, 340 ± 4 mOsm kg^{-1}).

2.3 Blood sampling and analyses

We used heparinized 1 ml syringes with a 25 gauge X 1.6 cm (5/8 in) needle to collect a 0.8 ml blood sample via cardiocentesis. Blood samples were collected from females once the reproductive female of the yoked partners reached late vitellogenesis (20 mm follicles determined by ultrasonographic examination, Sonosite MicroMazz, Bothell, WA, USA) and again when it reached late gravidity (twenty days after periovulation ecdysis and 1-4 days prior

to oviposition). Total time for capture, restraint, and blood collection was typically less than three minutes and did not exceed seven minutes, which is less than the time required to detect measurable CORT concentration changes in squamates (Romero and Wikelski, 2001). To control for potential circadian fluctuations in CORT concentrations, all blood samples were collected between 0900-1100 hr. We immediately centrifuged the blood samples at 3000 rpm for three minutes to separate plasma from blood cells. We aliquoted plasma (~50 μ l) into separate vials that were then frozen at -80°C. From the collected blood samples, we measured plasma CORT concentration as well as plasma osmolality (based on triplicate analysis using a vapor pressure osmometer, #5600, Wescor Inc., Logan, UT, USA) and circulating concentrations of glucose as a metric of circulating energy resources (blood glucose meter, #EG220546, Medline Industries, Northfield, IL, USA).

Plasma CORT concentrations (ng ml^{-1}) were determined at the Centre d'Etudes Biologiques de Chizé (CEBC) following a well-established radioimmunoassay protocol (Lormée et al., 2003). The sample dilution curve in assay buffer was parallel to the standard curve, suggesting that the assay is specific for *A. childreni* with limited interference. We used a polyclonal anti-CORT antibody produced in rabbit whole antiserum (C 8784, Sigma-Aldrich, St Louis, MO, USA). Cross-reactions of the anti-CORT antibody were: 11-deoxycorticosterone 20%, progesterone 15.7%, 20 α -hydroxyprogesterone 8.8%, testosterone 7.9%, 20 β -hydroxyprogesterone 5.2%, cortisol 4.5%, aldosterone 4.4%, cortisone 3.2%, androstenedione 2.6%, 17-hydroxyprogesterone 1.8%, 5 α -dehydrotestosterone 1.4%, androsterone <0.1%, estrone <0.1%, and estriol <0.1%. The percent retrievable fraction of CORT after diethyl ether extraction was greater than 95% and all samples were run in duplicate (intra-assay variation: 8.05%, inter-assay variation: 9.79%).

2.4 Statistical Analyses

We performed all statistical analyses in R, version 3.5.0 (R Development Core Team 2018). We checked to ensure the data met the assumptions for parametric testing and used transformations where necessary. First, we examined the effect of reproductive status and water treatment on CORT values using repeated measures analysis of variance (rmANOVA). We tested for three-way interactions and used treatment (water or no water), status (reproductive or non-reproductive), and sampling period (late-vitellogenesis and late-gravidity bleeds) as fixed effects, and individual as a random effect. We included parameters addressing potential size effects by using a body condition index (BCI, standardized residuals from a linear regression using mass and SVL). However, this variable was removed from the final model as a result of stepwise removal using Δ AIC and model weights (Arnold, 2010; Zuur et al., 2010). We used an ANOVA to examine changes in CORT values between females in each of the four groups (reproductive or non-reproductive, with or without water from late-vitellogenesis to late-gravidity). We used a post-hoc Tukey's HSD test to determine which of the groups were significantly different within and between time.

We then used linear regressions comparing the profiles among individuals to explore the relationships between CORT and osmolality or glucose across both sampling periods. We first compared the relationship by grouping all females together, regardless of treatment or status, after which we separately explored the effects of treatment, status, and the combination of the two. We next used variance partitioning methods described by Anderson and Gribble (1998) to decompose our full response into orthogonal subsets to examine how treatment (water or no water) or status (reproductive or non-reproductive) affected the relationship between CORT and

osmolality or glucose and performed similar linear regressions. Using methods described by Nakagawa and Schielzeth (2013), we calculated a general measure of R^2 , marginal R^2 (R^2_{GLMM}), from our models to estimate the variance explained by CORT and treatment, status, or a combination of the two.

We used the packages “nlme” and “multcomp” (Hothorn et al., 2008; Pinheiro et al., 2018) for rmANOVA’s, “CAR” (Fox and Weisberg, 2011) for linear regressions, “MuMIn” (Barton, 2018) for estimates of marginal R^2 , and “agricolae” (de Mendiburu, 2017) for post-hoc tests. Significance was set at $\alpha = 0.05$.

3. Results

3.1 Variation in CORT levels

We found significant main effects of sampling ($F_{1,31} = 25.08$, $p < 0.001$), status ($F_{1,31} = 26.69$, $p < 0.001$), and treatment ($F_{1,31} = 4.41$, $p = 0.044$). We also found significant sampling-by-reproductive status ($F_{1,31} = 8.29$, $p = 0.007$) and sampling-by-water treatment ($F_{1,31} = 5.32$, $p = 0.028$) interactions (Table 1, Fig. 1A,B), but no three-way interaction ($F_{1,31} = 1.19$, $p = 0.283$). A Tukey’s HSD post-hoc test revealed that, while reproductive females initially had higher CORT (mean \pm SEM; 16.77 ± 1.67 ng mL⁻¹) compared to non-reproductive females (5.52 ± 1.46 ng mL⁻¹), the difference was more pronounced at the second sampling period (42.28 ± 6.72 ng mL⁻¹ and 11.75 ± 4.84 ng mL⁻¹, respectively; Fig 1A). At the start of the experiment, CORT values were comparable in females with (11.08 ± 2.16 ng mL⁻¹) and without (12.15 ± 2.06 ng mL⁻¹) water. However, water-deprived females had much higher CORT (36.56 ± 7.74 ng mL⁻¹) at the time of the second sampling period, compared to those who had water provided (19.61 ± 3.59 ng mL⁻¹; Fig 1B).

When examining changes in CORT, we found significant differences among groups ($F_{3,31} = 493.19$, $p = 0.006$). Specifically, a Tukey's HSD post-hoc revealed that water deprived reproductive females had significantly greater (all $p < 0.05$) changes in CORT (36.0 ± 6.29 ng mL⁻¹) when compared to other females (13.9 ± 6.66 ng mL⁻¹, 10.0 ± 6.84 ng mL⁻¹ and 2.5 ± 1.15 ng mL⁻¹ for reproductive with water, non-reproductive without water and non-reproductive with water, respectively). CORT change was not significantly different between these three groups (all $p < 0.05$).

3.2 Correlates of CORT levels

We found a significant positive relationship between CORT and plasma osmolality ($F_{1,34} = 34.20$, $p < 0.001$, $R^2_{\text{GLMM}} = 0.331$) when all females and sampling sessions were grouped together. When we isolated the effects of treatment and status, we found a significant positive relationship between CORT and osmolality in reproductive females with (Fig. 2A) and without access to water from late vitellogenesis to late gravidity ($F_{1,8} = 8.06$, $p = 0.022$, $R^2_{\text{GLMM}} = 0.321$; Fig. 2A) and without access to water from late vitellogenesis to late gravidity ($F_{1,9} = 22.05$, $p = 0.001$, $R^2_{\text{GLMM}} = 0.537$; Fig. 2B), and in non-reproductive females without access to water during a similar period ($F_{1,7} = 6.77$, $p = 0.035$, $R^2_{\text{GLMM}} = 0.311$; Fig. 2C). We did not detect a significant relationship in non-reproductive females with access to water ($p > 0.05$; Fig. 2D).

When evaluating CORT and blood glucose, we did not detect a significant relationship ($p > 0.05$) when all females were grouped together. However, when we isolated the effects of treatment and status, we found a significant positive relationship between CORT and blood glucose in reproductive females, both those with (Fig. 3A) and those without access to water during gravidity ($F_{1,8} = 8.45$, $p = 0.019$, $R^2_{\text{GLMM}} = 0.332$; Fig. 3A) and those without access to water during gravidity ($F_{1,9} = 7.41$, $p = 0.024$, $R^2_{\text{GLMM}} = 0.266$;

Fig. 3B). We did not detect a significant relationship in either of the non-reproductive groups ($p > 0.05$; Fig. 3C,D).

4. Discussion

Throughout an organism's lifetime, glucocorticoids play a fundamental role in responding to fluctuations in internal, biotic, and abiotic conditions (Landys et al., 2006; Sapolsky, 2000). CORT is widely recognized as an important hormone that mediates energy balance (Landys et al., 2006; Romero, 2002; Sapolsky, 2000). For example, growth (Belden et al., 2005; Cadby et al., 2010; Hayward and Wingfield, 2004), reproduction (Angelier et al., 2009; Love et al., 2014; Moore and Jessop, 2003), and migration (Cease et al., 2007; Eikenaar et al., 2014) are, in part, regulated through the dynamic actions of CORT. A large body of literature on rodents has also recognized mineralocorticoid-like effects of CORT (Bartter and Forman, 1962; Bidet et al., 1987; Schultz, 1998). The potential role of CORT in regulating salt and water balance has attracted far less attention in other taxa (Vera et al., 2017). Our results provide evidence that increased CORT is contextually linked to both reproduction and plasma osmolality in an ectotherm.

At the time of the first blood sampling, all females had access to water and the reproductive females were in late vitellogenesis, which is the period of peak energetic investment in oviparous taxa (Stephens et al., 2009). The reproductive females had higher CORT compared to their non-reproductive yoked partners (Fig. 1A), and this is not surprising when considering the classic, energy-centric view of CORT (Romero, 2002). As capital breeders, Children's pythons rely on internal reserves to fuel reproductive investment during vitellogenesis and increased CORT allows females to mobilize internal resources to fund the creation of energy-

rich vitellogenin (Jaatinen et al., 2013). As expected, non-reproductive females, which had relatively low energetic demands during the same time-period, had substantially lower CORT levels.

In contrast with the high energetic demands of vitellogenesis, reproductive females during late-gravidity had already completed a majority of their energetic investment but were divesting considerable amounts of internal water to their developing eggs (Stahlschmidt et al., 2011). Regardless of water availability, reproductive females had the highest CORT levels during late-gravidity, despite lower energetic requirements at this time compared to late vitellogenesis (Fig. 1A). These findings are in contrast with the CORT-energy allocation relationship (Romero, 2002), yet increased CORT has been associated with later stages of pregnancy in other species (Dauphin-Villemant and Xavier, 1985; Lind et al., 2010; Lориoux et al., 2016; Schuett et al., 2005). Increased CORT levels may also be associated with maternal physiological and behavioral shifts associated with gravidity (Lourdais et al., 2008). This period of embryonic development is particularly sensitive to thermal variation and females shift their activity to increase body temperatures (Lориoux et al., 2012). Additionally, CORT may serve as a hormonal stimulus for females to oviposit (Taylor et al., 2004), which may explain why CORT is at its highest in late gravid females.

We found significant positive relationships between CORT and blood glucose, which is also in line with the classic role of CORT as a mediator of energy levels (Romero, 2002). However, this relationship appears to be context dependent. It was only after parsing our data into treatment groups that we detected any significant relationships. We found significant relationships in reproductive females (Fig. 3A,B), but not in non-reproductive females (Fig. 3C,D), regardless of water availability. Previous research has found that female Children's

pythons are able to preferentially catabolize muscle during gravidity to sustain the demands of reproduction (Lourdais et al., 2013) and that muscle catabolism increases with water deprivation (Brusch et al., 2018). This, coupled with data from our study, emphasizes that these relationships (i.e., CORT and glucose levels or increased muscle catabolism) may only be detectable during periods of high physiological burden such as what occurs when an organism must face simultaneous challenges (e.g., reproduction and dehydration).

Reproductive females in our study with *ad libitum* access to water had significant increases in plasma osmolality (average osmolality increase: 37 mOsm kg⁻¹) compared to their non-reproductive yoked pairs (6 mOsm kg⁻¹) likely due to the considerable hydric demands of egg production. These increases were not significantly different from non-reproductive females without water access (44 mOsm kg⁻¹) that had the hydric challenge of living without water. Reproductive females without access to water during gravidity were faced with the combined hydric challenges of reproduction and water deprivation, and subsequently had the largest plasma osmolality increases during the experiment (66 mOsm kg⁻¹; Brusch et al., 2018). Increases in CORT throughout the experiment followed comparable trends with non-reproductive, water-provided females having the smallest change (average CORT increase: 2.5 ng mL⁻¹), non-reproductive without water and reproductive with water in the middle (10.0 ng mL⁻¹ and 13.9 ng mL⁻¹, respectively), and reproductive without water during gravidity having the largest change (36.0 ng mL⁻¹).

We found a strong relationship between CORT and plasma osmolality, although, as with the rest of our results, it appears to be context dependent. When we examined the influence of reproductive status and water availability on osmolality, we found that CORT explained 54% of the variance in reproductive females without access to water during gravidity. The low metabolic

demands of the non-reproductive females enabled us to examine the relationship between CORT and plasma osmolality (i.e., hydration) independent of the high energetic demands. Non-reproductive females showed increases in CORT when they were deprived of water, and we found a significant positive relationship between hyperosmolality (dehydration) and CORT (Fig. 2C). Juxtaposed against these results, we did not detect any similar increases or a significant relationship in non-reproductive females with water provided (Fig. 2D). While non-reproductive females in our study were exposed to a constant temperature approximately 2.5°C above their preferred body temperature (Lourdais et al., 2008), this was not enough to impact their CORT levels.

Our results from an ectotherm appear to be in congruence with decades of research in rodents that show CORT has important mineralocorticoid actions involved in water balance (see de Kloet et al., 2000 and Joëls, 1997 for review). Unlike previous studies (Dupoué et al., 2014; Moeller et al., 2017), we found that dehydration induced a CORT increase in non-reproductive females. Also in contrast with recent studies (Dupoué et al., 2016; Lorioux et al., 2016), our data suggest that CORT is linked to both energy and water balance. Our study controlled for a fundamental factor in heterothermic taxa, temperature, which may explain the discrepancy between previous results and ours. Organisms often alter their thermal preference based on their internal state. For example, dehydrated tiger snakes (*Notechis scutatus*) have reduced preferred temperatures, likely in an attempt to reduce water loss (Ladyman and Bradshaw, 2003). Such thermal adjustments may also impact other physiological functions including hormone production and release. Given that temperature and the availability of resources are predicted to dramatically fluctuate due to climate change, further work is needed to better understand the potential inter-relationship among CORT, energy, and water balance.

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Author contributions: GABIV, OL, and DFD designed the study and collected the samples. GABIV conducted a majority of the assays (except for CORT), performed the statistical analyses, and led the writing of the manuscript. DD and OL contributed to revisions and gave final approval for publication.

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Journal Pre-proofs

Figure Legends

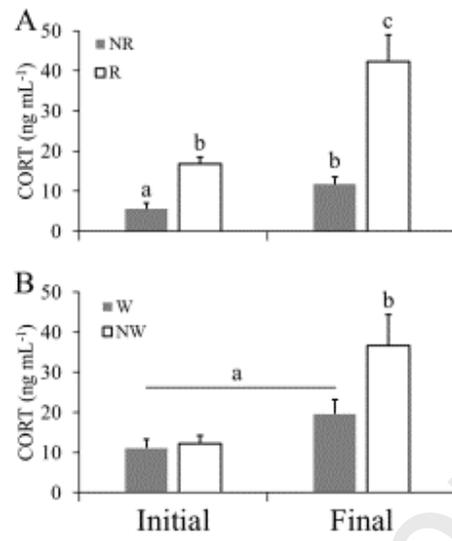
Figure 1 – Average plasma corticosterone (CORT, ng mL⁻¹) concentrations measured in non-reproductive (NR) and reproductive (R) female *Antaresia childreni* (A) that were held with (W) or without (NW) access to water (B) during the duration of gravidity or its equivalent for non-reproductive yoked females. Initial blood samples were collected when reproductive females were in late-vitellogenesis, prior to water deprivation, and final blood samples were collected at late-gravidity. Error bars represent ± 1 SEM. Different letters indicate significant differences between groups and blood samples (Tukey's HSD post-hoc test).

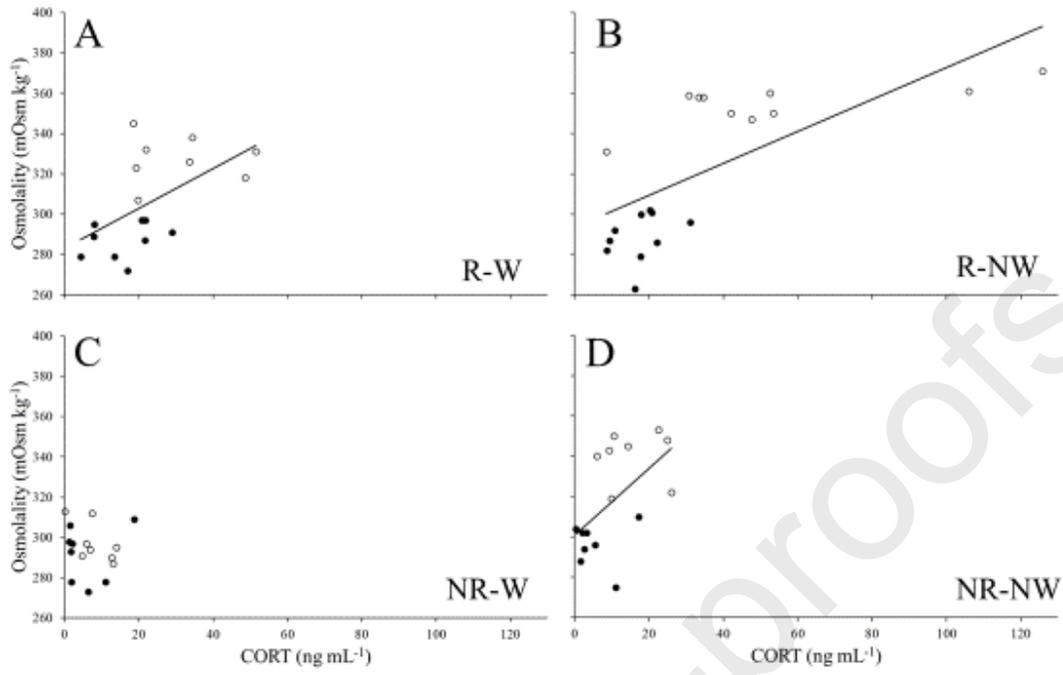
Figure 2 – Relationships between corticosterone (CORT, ng mL⁻¹) and plasma osmolality (mOsm kg⁻¹) measured in reproductive (R, n = 19; A,B) and non-reproductive (NR, n = 16; C,D) female *Antaresia childreni*. Females were held with (W) or without (NW) access to water during the duration of gravidity (i.e., prior to the second sampling point) or its equivalent for non-reproductive yoked females. Both sampling periods were combined for statistical analyses (see text for details) and a line of best fit is included for significant relationships ($p < 0.05$). Closed circles represent initial blood sampling, while open circles represent final blood sampling.

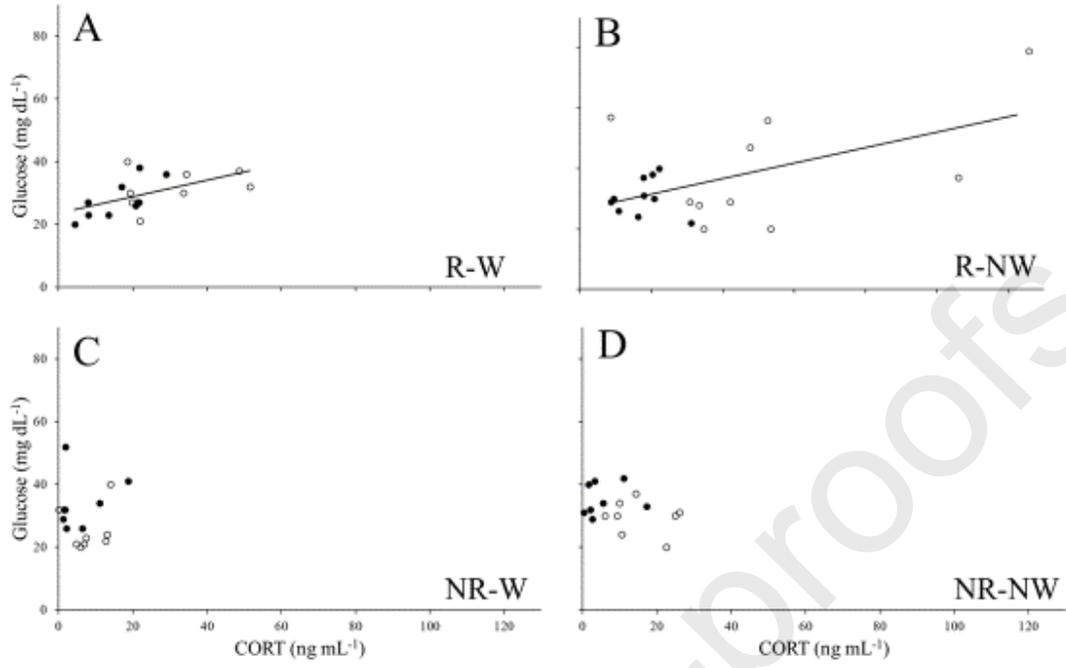
Figure 3 – Relationships between corticosterone (CORT, ng mL⁻¹) and glucose (mg dL⁻¹) measured in reproductive (R, n = 19; A,B) and non-reproductive (NR, n = 16; C,D) female *Antaresia childreni* during the experiment. Females were held with (W) or without (NW) access to water during the duration of gravidity (i.e., prior to the second sampling point) or its equivalent for non-reproductive yoked females. Both sampling periods were combined for statistical analyses (see text for details) and a line of best fit is included for significant relationships ($p < 0.05$). Closed circles represent initial blood sampling, while open circles represent final blood sampling.

Table 1 – Statistical models used to examine corticosterone concentrations (ng mL^{-1} , CORT) in female *Antaresia childreni* during initial and final blood samplings (session), that were either reproductive or non-reproductive (status) and had continuous access to water or were water-restricted (treat) during the time reproductive females were gravid.

Factors	d.f.	F ratio	P value
intercept	1, 31	98.29	< 0.001
status	1, 31	26.69	< 0.001
treat	1, 31	4.41	0.044
session	1, 31	25.08	< 0.001
status \times treat	1, 31	1.22	0.277
status \times session	1, 31	8.29	0.007
treat \times session	1, 31	5.32	0.028
status \times treat \times session	1, 31	1.19	0.283







Author contributions: GABIV, OL, and DFD designed the study and collected the samples. GABIV conducted a majority of the assays (except for CORT), performed the statistical analyses, and led the writing of the manuscript. DD and OL contributed to revisions and gave final approval for publication.

Highlights:

- Reproduction and water deprivation independently lead to elevated plasma corticosterone (CORT) levels.
- Plasma CORT is positively correlated with plasma osmolality and circulating energy levels.
- CORT may serve important roles in both energetic and hydric states.