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

3 **Authors:** George A. Bruschi^{1,2*}, Dale F. DeNardo¹, Olivier Lourdais²

4 **Author affiliations:** 1 – School of Life Sciences, Arizona State University, 427 East Tyler Mall,
5 Tempe, AZ, 85281, USA

6 2 – Centre d’Etudes Biologiques de Chizé, CNRS, 79360 Villiers en Bois, France

7 *Author for correspondence: (bruschg@gmail.com)

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

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

25 **1. Introduction**

26 The endogenous secretion of glucocorticoids is one of the main mechanisms by which animals
27 modulate their physiological and behavioral responses to unpredictable and predictable stimuli
28 (Romero and Wingfield, 2016). The main glucocorticoid in rodents and non-mammalian
29 terrestrial vertebrates (i.e., birds, amphibians, and reptiles) is corticosterone (CORT) (Sapolsky,
30 2000), and it can affect growth (Busch et al., 2008; Midwood et al., 2014), survival (O'Connor et
31 al., 2010; Romero et al., 2009), and reproductive success (Robert et al., 2009; Wingfield and
32 Sapolsky, 2003). There is an impressive body of work examining the elevation in CORT in
33 response to unpredictable stressors and noxious stimuli (i.e., allostatic response, see Romero et
34 al., 2009; Vera et al., 2017). However, baseline CORT levels are also important to consider
35 because they may reflect the homeostatic demands faced by the organism (Landys et al., 2006;
36 Romero, 2002). CORT is important for regulating shifts in metabolism and behavior (Landys,
37 2006) and it oscillates to meet demands associated with shifts in life-history stages (Romero,
38 2002). For example, baseline CORT levels are typically related to energy balance during
39 reproduction and can either increase to support direct allocation to reproduction (i.e., energy
40 model hypothesis) or decrease to minimize maintenance requirements (Bonier et al., 2009a,b).

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

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

55 While energy is critical, water is another vital resource (Kleiner, 1999), and CORT has
56 been shown to have important mineralocorticoid roles involved with water balance in rodents
57 (Agarwal and Mirshahi, 1999; Bentley, 1970; Liu et al., 2010; Thunhorst et al., 2007). While
58 more limited in number, several studies have examined the relationship between CORT and
59 water balance in non-rodent taxa. For example, a link between CORT levels and hydration state
60 has been demonstrated in water-constrained environments (Bradshaw, 1997). However, high
61 variability in CORT levels suggests the response is context dependent. For example, recent
62 studies found that baseline CORT levels are not affected by dehydration in animals with low
63 metabolic demands (non-reproductive snakes and lizards, Dupoué et al., 2014; Moeller et al.,
64 2017). However, in animals with higher metabolic requirements and greater water deficits (e.g.,
65 pelagic sea birds), the opposite has been documented (Brischoux et al., 2015). At least two
66 studies have considered the additive challenges of reproduction and water deprivation on CORT
67 levels (Dauphin-Villemant and Xavier, 1986; Dupoué et al., 2016). In the asp viper (*Vipera*
68 *aspis*), CORT has been found to be closely related to the energy demands of pregnancy (Lorioux
69 et al., 2016). However, Dupoué et al. (2016) found that changes in CORT levels were further
70 increased when pregnant females faced water deprivation. In these studies, the study animals

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

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

86

87 **2. Material and methods**

88 2.1 Study species

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

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

103

104 2.2 Experimental design

105 All work was conducted under the oversight of the Arizona State University Institutional Animal
106 Care and Use Committee (protocol # 17-1532R). Snakes used for this study were part of a long-
107 term colony at Arizona State University, AZ, USA, and were housed individually in 91 × 71 ×
108 46 cm cages (Freedom Breeder, Turlock, CA, USA). Snakes were deprived of food from the
109 onset of overwintering until oviposition (~5 months) because they typically do not eat when
110 reproductively active. To stimulate the reproductive cycle, snakes were over-wintered for six
111 weeks from mid-December through January (light-dark cycles of 6:18 h and a daily temperature
112 cycle of 20:15°C that changed in conjunction with the light cycle). During the 6-hr light phase a
113 supplemental sub-surface heat source was provided under one end of each cage to allow for
114 thermoregulation. After overwintering, room temperature was increased to a constant 31.5°C,
115 which is the preferred temperature of gravid Children's pythons (Lourdais et al., 2008), with a
116 12:12 h light-dark cycle and no supplemental heat provided. Females were held at a constant

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

119 Females were randomly assigned to either the reproductive (n = 19) or non-reproductive
120 groups (n = 16). Females in the reproductive group were exposed to a different male every 2-3
121 days, resulting in each reproductive female being sequentially housed with 6-8 males during this
122 18-day breeding period. Each non-reproductive female was yoked to a reproductive female of
123 similar body mass so that the timing of its treatments, measurements, and sampling coincided
124 with that of the reproductive female to which it was yoked. At ovulation (based on a peri-
125 ovulation shed, Lourdais et al., 2008), half of the reproductive females, and their yoked partners,
126 were deprived of water until the reproductive female laid her eggs (22±1 d). Deprivation of water
127 during this time leads to ecologically relevant levels of dehydration (Brusch et al., 2018).

128 Treatment groups were: gravid with water provided *ad libitum* (number of females, mean mass ±
129 SEM, and plasma osmolality at the end of the experiment ± SEM: n = 9, 464±28 g, 324±5 mOsm
130 kg⁻¹), non-reproductive with water provided *ad libitum* (n = 8, 414±18 g, 297±4 mOsm kg⁻¹),
131 gravid with no water provided (n = 10, 424±41 g, 354±3 mOsm kg⁻¹), and non-reproductive with
132 no water provided (n = 8, 423±33 g, 340±4 mOsm kg⁻¹).

133

134 2.3 Blood sampling and analyses

135 We used heparinized 1 ml syringes with a 25 gauge X 1.6 cm (5/8 in) needle to collect a
136 0.8 ml blood sample via cardiocentesis. Blood samples were collected from females once the
137 reproductive female of the yoked partners reached late vitellogenesis (20 mm follicles
138 determined by ultrasonographic examination, Sonosite MicroMazz, Bothell, WA, USA) and
139 again when it reached late gravidity (twenty days after periovulation ecdysis and 1-4 days prior

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

151 Plasma CORT concentrations (ng ml^{-1}) were determined at the Centre d'Etudes
152 Biologiques de Chizé (CEBC) following a well-established radioimmunoassay protocol (Lormée
153 et al., 2003). The sample dilution curve in assay buffer was parallel to the standard curve,
154 suggesting that the assay is specific for *A. childreni* with limited interference. We used a
155 polyclonal anti-CORT antibody produced in rabbit whole antiserum (C 8784, Sigma-Aldrich, St
156 Louis, MO, USA). Cross-reactions of the anti-CORT antibody were: 11-deoxycorticosterone
157 20%, progesterone 15.7%, 20 α -hydroxyprogesterone 8.8%, testosterone 7.9%, 20 β -
158 hydroxyprogesterone 5.2%, cortisol 4.5%, aldosterone 4.4%, cortisone 3.2%, androstenedione
159 2.6%, 17-hydroxyprogesterone 1.8%, 5 α -dehydrotestosterone 1.4%, androsterone <0.1%, estrone
160 <0.1%, and estriol <0.1%. The percent retrievable fraction of CORT after diethyl ether extraction
161 was greater than 95% and all samples were run in duplicate (intra-assay variation: 8.05%, inter-
162 assay variation: 9.79%).

163

164 2.4 Statistical Analyses

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

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

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

190 We used the packages “nlme” and “multcomp” (Hothorn et al., 2008; Pinheiro et al.,
191 2018) for rmANOVA’s, “CAR” (Fox and Weisberg, 2011) for linear regressions, “MuMIn”
192 (Barton, 2018) for estimates of marginal R^2 , and “agricolae” (de Mendiburu, 2017) for post-hoc
193 tests. Significance was set at $\alpha = 0.05$.

194

195 **3. Results**

196 3.1 Variation in CORT levels

197 We found significant main effects of sampling ($F_{1,31} = 25.08$, $p < 0.001$), status ($F_{1,31} = 26.69$, $p <$
198 0.001), and treatment ($F_{1,31} = 4.41$, $p = 0.044$). We also found significant sampling-by-
199 reproductive status ($F_{1,31} = 8.29$, $p = 0.007$) and sampling-by-water treatment ($F_{1,31} = 5.32$, $p =$
200 0.028) interactions (Table 1, Fig. 1A,B), but no three-way interaction ($F_{1,31} = 1.19$, $p = 0.283$). A
201 Tukey’s HSD post-hoc test revealed that, while reproductive females initially had higher CORT
202 (mean \pm SEM; 16.77 ± 1.67 ng mL⁻¹) compared to non-reproductive females (5.52 ± 1.46 ng mL⁻¹),
203 the difference was more pronounced at the second sampling period (42.28 ± 6.72 ng mL⁻¹ and
204 11.75 ± 4.84 ng mL⁻¹, respectively; Fig 1A). At the start of the experiment, CORT values were
205 comparable in females with (11.08 ± 2.16 ng mL⁻¹) and without (12.15 ± 2.06 ng mL⁻¹) water.
206 However, water-deprived females had much higher CORT (36.56 ± 7.74 ng mL⁻¹) at the time of
207 the second sampling period, compared to those who had water provided (19.61 ± 3.59 ng mL⁻¹;
208 Fig 1B).

209 When examining changes in CORT, we found significant differences among groups ($F_{3,31}$
210 = 493.19, $p = 0.006$). Specifically, a Tukey's HSD post-hoc revealed that water deprived
211 reproductive females had significantly greater (all $p < 0.05$) changes in CORT (36.0 ± 6.29 ng
212 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
213 ng mL^{-1} for reproductive with water, non-reproductive without water and non-reproductive with
214 water, respectively). CORT change was not significantly different between these three groups
215 (all $p < 0.05$).

216

217 3.2 Correlates of CORT levels

218 We found a significant positive relationship between CORT and plasma osmolality ($F_{1,34} =$
219 34.20 , $p < 0.001$, $R^2_{\text{GLMM}} = 0.331$) when all females and sampling sessions were grouped
220 together. When we isolated the effects of treatment and status, we found a significant positive
221 relationship between CORT and osmolality in reproductive females with ($F_{1,8} = 8.06$, $p = 0.022$,
222 $R^2_{\text{GLMM}} = 0.321$; Fig. 2A) and without access to water from late vitellogenesis to late gravidity
223 ($F_{1,9} = 22.05$, $p = 0.001$, $R^2_{\text{GLMM}} = 0.537$; Fig. 2B), and in non-reproductive females without
224 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
225 not detect a significant relationship in non-reproductive females with access to water ($p > 0.05$;
226 Fig. 2D).

227 When evaluating CORT and blood glucose, we did not detect a significant relationship (p
228 > 0.05) when all females were grouped together. However, when we isolated the effects of
229 treatment and status, we found a significant positive relationship between CORT and blood
230 glucose in reproductive females, both those with ($F_{1,8} = 8.45$, $p = 0.019$, $R^2_{\text{GLMM}} = 0.332$; Fig.
231 3A) and those without access to water during gravidity ($F_{1,9} = 7.41$, $p = 0.024$, $R^2_{\text{GLMM}} = 0.266$;

232 Fig. 3B). We did not detect a significant relationship in either of the non-reproductive groups (p
233 > 0.05; Fig. 3C,D).

234

235 **4. Discussion**

236 Throughout an organism's lifetime, glucocorticoids play a fundamental role in responding to
237 fluctuations in internal, biotic, and abiotic conditions (Landys et al., 2006; Sapolsky, 2000).
238 CORT is widely recognized as an important hormone that mediates energy balance (Landys et
239 al., 2006; Romero, 2002; Sapolsky, 2000). For example, growth (Belden et al., 2005; Cadby et
240 al., 2010; Hayward and Wingfield, 2004), reproduction (Angelier et al., 2009; Love et al., 2014;
241 Moore and Jessop, 2003), and migration (Cease et al., 2007; Eikenaar et al., 2014) are, in part,
242 regulated through the dynamic actions of CORT. A large body of literature on rodents has also
243 recognized mineralocorticoid-like effects of CORT (Bartter and Forman, 1962; Bidet et al.,
244 1987; Schultz, 1998). The potential role of CORT in regulating salt and water balance has
245 attracted far less attention in other taxa (Vera et al., 2017). Our results provide evidence that
246 increased CORT is contextually linked to both reproduction and plasma osmolality in an
247 ectotherm.

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

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

258 In contrast with the high energetic demands of vitellogenesis, reproductive females
259 during late-gravidity had already completed a majority of their energetic investment but were
260 divesting considerable amounts of internal water to their developing eggs (Stahlschmidt et al.,
261 2011). Regardless of water availability, reproductive females had the highest CORT levels
262 during late-gravidity, despite lower energetic requirements at this time compared to late
263 vitellogenesis (Fig. 1A). These findings are in contrast with the CORT-energy allocation
264 relationship (Romero, 2002), yet increased CORT has been associated with later stages of
265 pregnancy in other species (Dauphin-Villemant and Xavier, 1985; Lind et al., 2010; Lorioux et
266 al., 2016; Schuett et al., 2005). Increased CORT levels may also be associated with maternal
267 physiological and behavioral shifts associated with gravidity (Lourdais et al., 2008). This period
268 of embryonic development is particularly sensitive to thermal variation and females shift their
269 activity to increase body temperatures (Lorioux et al., 2012). Additionally, CORT may serve as a
270 hormonal stimulus for females to oviposit (Taylor et al., 2004), which may explain why CORT is
271 at its highest in late gravid females.

272 We found significant positive relationships between CORT and blood glucose, which is
273 also in line with the classic role of CORT as a mediator of energy levels (Romero, 2002).
274 However, this relationship appears to be context dependent. It was only after parsing our data
275 into treatment groups that we detected any significant relationships. We found significant
276 relationships in reproductive females (Fig. 3A,B), but not in non-reproductive females (Fig.
277 3C,D), regardless of water availability. Previous research has found that female Children's

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

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

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

301 demands of the non-reproductive females enabled us to examine the relationship between CORT
302 and plasma osmolality (i.e., hydration) independent of the high energetic demands. Non-
303 reproductive females showed increases in CORT when they were deprived of water, and we
304 found a significant positive relationship between hyperosmolality (dehydration) and CORT (Fig.
305 2C). Juxtaposed against these results, we did not detect any similar increases or a significant
306 relationship in non-reproductive females with water provided (Fig. 2D). While non-reproductive
307 females in our study were exposed to a constant temperature approximately 2.5°C above their
308 preferred body temperature (Lourdais et al., 2008), this was not enough to impact their CORT
309 levels.

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

324

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328

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330

331 **Author contributions:** GABIV, OL, and DFD designed the study and collected the samples.
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333 analyses, and led the writing of the manuscript. DD and OL contributed to revisions and gave
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341

342 **Data availability:** The datasets supporting this article can be accessed at
343 <https://doi.org/10.6084/m9.figshare.7970819>

344

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580 **Figure Legends**

581

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

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

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

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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 × treat	1, 31	1.22	0.277
status × session	1, 31	8.29	0.007
treat × session	1, 31	5.32	0.028
status × treat × session	1, 31	1.19	0.283

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