

RESEARCH ARTICLE

Water deprivation increases maternal corticosterone levels and enhances offspring growth in the snake *Vipera aspis*

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ABSTRACT

Circulating glucocorticoid (GC) levels may increase as a result of reproductive effort or in response to unpredictable events. However, GC secretion can vary with the availability of vital trophic resources such as energy. While water represents another critical resource, the impact of water deprivation on GC secretion during reproduction has not yet been thoroughly investigated. Here, we examined the effects of water deprivation on plasma corticosterone (CORT) concentrations of female aspic vipers (*Vipera aspis*), and determined the impacts of water deprivation on offspring traits. We exposed both pregnant and non-reproductive females to a 20-day water deprivation and compared their pre- and post-deprivation CORT levels with those of control females. At the end of the treatment, only water-deprived pregnant females showed a significant increase in CORT levels. In pregnant females, changes in baseline CORT level were correlated with changes in female hydration state. Changes in baseline CORT levels were also negatively influenced by maternal reproductive effort in pregnant control females, while such a relationship was not apparent in pregnant water-deprived females. Finally, we found that offspring from water-deprived females had higher growth rates than offspring from control females. Offspring growth was also positively correlated with changes in both maternal osmolality and baseline CORT levels. Together, our results suggest that dehydration increases maternal CORT levels, which may subsequently influence offspring development. Further long-term field studies are therefore required to assess whether there is an adaptive significance of this response.

KEY WORDS: Allostasis, Dehydration, Ectotherms, Glucocorticoids, Maternal effects, Reproductive effort

INTRODUCTION

Allostasis integrates an organism's functional responses to predictable and/or unpredictable environmental conditions (Angelier and Wingfield, 2013; Landys et al., 2006; McEwen and Wingfield, 2003, 2010; Romero et al., 2009). In vertebrates, glucocorticoids (GCs) constitute the main mediators of allostasis because they are released in response to both natural physiological demands and unpredictable events (i.e. allostatic load or stress response) (McEwen and Wingfield, 2003, 2010; Romero et al., 2009; Wingfield et al., 1998). GCs are essential for immediate survival, although if over-secreted (i.e. allostatic overload or chronic stress), they may impair

other functions such as digestion, growth, immunity, and reproduction (Berger et al., 2005; Sapolsky et al., 2000; Wingfield et al., 1998). GC secretion has thereby been viewed as a context-dependent adaptive response with positive, negative, or neutral impacts on physiological performance and fitness (Breuner et al., 2008).

Reproduction can be particularly challenging because it induces several constraints leading to a potential trade-off with individual survival or lifetime reproductive success (i.e. the cost of reproduction) (Blount et al., in press; Bonnet et al., 1998; French et al., 2007; Magnhagen, 1991; Stier et al., 2012). For instance, investment in energy and time over reproduction (i.e. reproductive effort) is costly for individuals and can increase GC levels (Bonier et al., 2009a,b, 2011; Love et al., 2014). Concomitantly with predictable demands of reproductive effort, breeding individuals can also face unpredictable events that further amplify GC secretion (e.g. density, Dantzer et al., 2013; food shortage, Kitaysky et al., 2010; heat stress, Eriksen et al., 2007; predation, Coslovsky and Richner, 2011; Saino et al., 2005; anthropogenic disturbances, Ellenberg et al., 2007; Tartu et al., 2014). In addition, several studies have experimentally demonstrated that an increase in maternal levels of corticosterone (CORT; the main GC in birds, reptiles, and rodents) significantly influenced offspring phenotypes (Belliere et al., 2004; Hayward and Wingfield, 2004; Meylan and Clobert, 2004, 2005; Meylan et al., 2002; Warner et al., 2009; Zagron and Weinstock, 2006). Such influence of maternal hormones on offspring traits, also known as maternal effects, are widespread in all taxa and may subsequently impact offspring fitness (Mousseau and Fox, 1998; Mousseau et al., 2009).

To the best of our knowledge, the effects of water deprivation on CORT secretion in breeding wild vertebrates and consequences on offspring have not yet been investigated in this framework (but see Brischoux et al., 2015; Dupoué et al., 2014). CORT is presumed to be tightly linked to the water balance, considering its role in allostasis and because CORT has been demonstrated to act as a mineralocorticoid that is involved in water balance and osmoregulation (Bentley, 2002; Dauphin-Villemant and Xavier, 1986; Duggan and Lofts, 1978; McCormick and Bradshaw, 2006). In that context, our study aims to better understand the functional link between water demand, maternal CORT levels, and offspring development in wild vertebrates.

Squamate reptiles (lizards and snakes) are an excellent model system in which to investigate the impacts of water availability on CORT secretion during pregnancy and the resulting impacts on offspring development. Squamates display relatively limited dispersal capacities to evade environmental constraints and individuals are therefore directly exposed to local conditions (Aragón et al., 2010). Because pregnancy generally occurs during summer, reproductive females are particularly exposed to periods of water shortage, especially during associated drought periods.

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List of abbreviations

BM	body mass
CORT	corticosterone
GC	glucocorticoid
RFLM	relative fit litter mass
RFLS	relative fit litter size
RLM	relative litter mass
RLS	relative litter size
SVL	snout–vent length

Moreover, most viviparous species are lecithotrophic and embryonic energetic demands are supported by important yolk reserves. Therefore, embryos mainly rely on maternal water during their development (Stewart and Thompson, 2000). Embryonic water requirements may represent an important constraint because water demand increases exponentially with somatic growth during pregnancy (Dauphin-Villemant and Xavier, 1986; Lourdais et al., 2015). For instance, we recently documented an intergenerational trade-off for water (Dupoué et al., 2015a) in a viviparous snake, *Vipera aspis* (Linnaeus 1758). Suppressing access to water for 20 days, we found that pregnant females were twice as dehydrated as non-reproductive females. Water deprivation resulted in significant dehydration (i.e. increased mass loss and osmolality) that was amplified by fecundity. In contrast, reproductive performance and offspring traits at birth were not affected (Dupoué et al., 2015a).

The present study provides complementary analysis of the data collected in our previous experiment on *V. aspis* (Dupoué et al., 2015a) but focusing on CORT levels. Our general hypothesis is that CORT levels are closely correlated with hydration state and should be affected by the interaction between water deprivation and reproductive status. Specifically, we tested the following predictions: (1) water deprivation should increase baseline CORT levels notably among reproductive females, which are more prone to dehydration; (2) in response to a stress protocol, we expect a similar CORT stress response between control and water-deprived snakes, but higher stress-induced CORT levels in water-deprived snakes compared with control ones because of higher baseline CORT levels (Dupoué et al., 2014); and (3) CORT levels should be closely correlated with osmolality, thereby supporting the linkage with hydration state. Finally, we examined the influence of water deprivation and maternal CORT levels on offspring traits with a specific focus on early growth.

MATERIALS AND METHODS**Animals**

The aspic viper (*V. aspis*), a medium-size viviparous snake that is abundant in Western Europe (Naulleau, 1981), is a typical capital breeder. Females mobilize their energy reserves for yolk production during vitellogenesis. Pregnancy begins in early June after ovulation (Naulleau, 1981), which is associated with a peri-ovulatory ecdysis (Lorioux et al., 2013a). Parturition occurs 2 to 3 months later, from late August to early September (Lourdais et al., 2002b). Pregnant females often cease to feed (Bonnet et al., 1998; Lourdais et al., 2002a), mainly because of a behavioral shift that emphasizes thermoregulation over foraging (Lorioux et al., 2013b). During the entire study, we followed all laws and rules relating to the conservation and welfare of the animals (permit no. 792, Direction service vétérinaire des Deux-Sèvres).

This study was conducted simultaneously with a previously published study (see Dupoué et al., 2015a), and therefore animals and experimental design are as previously described. Briefly, we

caught 58 adult females (29 pregnant, 29 non-reproductive) in May and June 2012 from neighboring sites in western France (Vendée and Loire-Atlantique Districts). Reproductive status was determined by palpation in the field and then pregnancy was confirmed with high-resolution ultrasonography (SonoSite MicroMaxx, Bothell, WA, USA) when the animals were brought to the laboratory. All snakes were measured [snout–vent length (SVL) ± 0.5 cm] and weighed [body mass (BM) ± 1 g]. Husbandry followed the protocol described in Lorioux et al. (2013b). Individuals were fasted for 2 weeks prior to the start of the experiment and remained fasted throughout the experiment, to prevent meal consumption from changing the water balance of the snakes (Wright et al., 2013) and to avoid any influence of digestive activity on CORT levels.

Experimental design

Individuals were randomly assigned to the control (pregnant: $n=15$; non-reproductive: $n=15$) or water-deprived (pregnant: $n=14$; non-reproductive: $n=14$) treatments (Dupoué et al., 2015a). Water-deprived and control females did not differ in BM or SVL (all $P>0.117$) (Dupoué et al., 2015a). During the experiment, housing conditions remained as described above, except that females in the water-deprived treatment had their water removed for 20 days during mid-gestation (Dupoué et al., 2015a). This duration represents that of a typical summer drought in western France (Dupoué et al., 2015a).

Of the 29 pregnant females, 26 (13 controls, 13 water-deprived) successfully completed pregnancy, producing 125 neonates (60 neonates born from control females, 65 neonates born from water-deprived females). Immediately after birth, each neonate was individually housed in a box (30 \times 16 \times 10 cm) with a thermal gradient (20 to 33°C) 6 h per day (from 09:00 to 15:00 h), and water was provided *ad libitum* in a bowl. Data from the female and her offspring were collected on the day of birth and again 2 weeks later (see below). Estimates of maternal reproductive effort were assessed by considering absolute measures of reproductive output that are routinely used (Bonnet et al., 2001; Dupoué and Lourdais, 2014; Lourdais et al., 2002a); that is, the total litter size or litter mass including viable neonates, stillborn individuals and undeveloped ova, and the ‘fit’ litter size or ‘fit’ litter mass, which include only viable neonates. Although females likely invest similar amounts of yolk into each ovulated follicle, the water demands of each of these during pregnancy is likely highly dependent on whether the ovulated follicle becomes a live neonate or remains undeveloped. Because reproductive effort is largely driven by maternal size (Bonnet et al., 2003), we also examined relative (size-adjusted) litter size (RLS), relative fit litter size (RFLS), relative litter mass (RLM), and relative fit litter mass (RFLM), which were derived from the residuals of the linear relationships between reproductive effort and maternal SVL (all $P<0.003$). Once all measurements were collected, females were each fed a mouse and then released with their litters at their original capture sites.

Blood sampling

Blood samples were collected at the beginning and end of the water deprivation period (i.e. sampling session). Vipers were randomly bled in order to mix the hydric treatment and reproductive status. Because of communal housing and the potential disturbance of cagemates when taking an individual from its cage, we sampled only one female per cage in the morning or afternoon. All blood samples were collected via cardiocentesis, using a 1 ml heparinized syringe and a 27 gauge needle. Two blood samples were collected from each female during each sampling session. The first blood samples (i.e. baseline CORT level, 150 μ l) were collected within 3 min

(mean±s.e.m.=2.75±0.11 min) of the snake being picked up from its cage. This duration is less than that required for measurable changes in CORT concentration in squamates (Romero and Wikelski, 2001). This blood sample was also used to determine plasma osmolality (see details in methods from Dupoué et al., 2015a). After blood sampling, the snake was placed in an empty transparent box (30×20×10 cm), which induces an increase in plasma CORT concentrations in snakes (Dupoué et al., 2013). After 1 h, we collected a second blood sample (i.e. stress-induced CORT level, 100 µl). Collected blood was placed in a 0.675 ml microcentrifuge tube and centrifuged for 3 min at 2000 g. The plasma was separated and stored at –28°C. Plasma CORT concentration (ng ml⁻¹) was determined at the Centre d'Etudes Biologiques de Chizé (CEBC) following a well-established radioimmunoassay protocol (see Lormée et al., 2003 for details). 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%, 20α-hydroxyprogesterone 8.8%, testosterone 7.9%, 20β-hydroxyprogesterone 5.2%, cortisol 4.5%, aldosterone 4.4%, cortisone 3.2%, androstenedione 2.6%, 17-hydroxyprogesterone 1.8%, 5α-dehydrotestosterone 1.4%, androsterone <0.1%, estrone <0.1%, and estriol <0.1%. Samples were run in two assays (intra-assay variation: 7.07%, inter-assay variation: 9.99%).

We removed from the analyses two females from which we failed to collect blood during the second sampling session. We did not find any significant influence of time of day or body temperature on CORT levels (all $P>0.115$) and thus did not include these covariates in the analyses of CORT levels.

Offspring growth

On the day of birth, we measured offspring SVL (±0.1 cm) and jaw length (JL; ±0.1 mm) with a tape measure and digital caliper

(Digimatic caliper 150 mm, Mitutoyo, Japan), respectively. We did not observe any difference in offspring SVL or JL at birth (all $P>0.574$) (Dupoué et al., 2015a). Each neonate was singly housed in a plastic box (30×16×10 cm) with a natural thermal gradient (20–33°C) and water provided *ad libitum*. We then repeated the morphological measurements after 2 weeks (mean±s.e.m.=13.9±0.3 days) to determine offspring early growth, which is energetically supported solely by body reserves. We calculated body and jaw growth (mm day⁻¹) as the difference between final and birth measurements divided by the number of days between measurements. One offspring born from a control female displayed several morphological abnormalities and died 9 days after birth and was not included in the growth analyses.

Statistical analyses

All analyses were performed using R software (R Development Core Team, 2011). We checked that residuals of our models respected normality (Shapiro–Wilk test).

We analyzed the effect of water deprivation on baseline CORT level using a linear mixed model with baseline CORT level as the dependent variable, and sampling session (initial versus final), hydric treatment (control versus water-deprived), reproductive status (pregnant versus non-reproductive), and double and triple interactions between these terms as explanatory factors. Female identity was set as a random factor because measures were repeated between sampling sessions. We compared inter- and intra-group differences in baseline CORT level using Tukey *post hoc* tests on the triple interaction (package lsmeans). Stress-induced CORT level was analyzed with the same design as above. We used two sets of models: one in which we did not adjust stress-induced CORT with baseline CORT level, and another in which we added the baseline CORT level as a linear covariate.

We used linear models to test the relationship between changes from baseline or absolute stress-induced CORT level and changes in

Table 1. Mixed-effects models (individuals set as random factor) were used to determine the impact of sampling session, hydric treatment, reproductive status, and interaction terms on baseline, absolute, and adjusted stress-induced CORT levels in aspic vipers

Variable	Factor	d.f.	F	P
Baseline CORT	Intercept	1,52	291.2	<0.001***
	Session	1,52	7.00	0.011*
	Treatment	1,52	0.01	0.905
	Status	1,52	19.01	<0.001***
	Session×Treatment	1,52	1.88	0.176
	Session×Status	1,52	2.11	0.153
	Treatment×Status	1,52	0.02	0.877
	Session×Treatment×Status	1,52	4.47	0.039*
	Stress-induced CORT	Intercept	1,51	471.3
Session		1,51	4.66	0.036*
Treatment		1,51	0.24	0.624
Status		1,51	3.53	0.066
Session×Treatment		1,51	7.79	0.007**
Session×Status		1,51	0.44	0.509
Treatment×Status		1,51	1.51	0.225
Session×Treatment×Status		1,51	4.19	0.046*
Adjusted stress-induced CORT		Intercept	1,51	567.1
	Baseline CORT	1,50	19.50	<0.001***
	Session	1,50	1.26	0.268
	Treatment	1,51	0.48	0.492
	Status	1,51	0.43	0.515
	Session×Treatment	1,50	4.66	0.036*
	Session×Status	1,50	0.05	0.819
	Treatment×Status	1,51	1.51	0.225
	Session×Treatment×Status	1,50	1.65	0.205

In adjusted stress-induced CORT level analysis, baseline CORT is included as a linear covariate. Asterisks indicate a significant effect of the covariates, the factors or the interaction terms (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

Table 2. Comparisons of CORT levels between (inter-group) and within (intra-group) pregnant and non-reproductive (NR) asp viper females from the control and water-deprived (WD) treatments during initial and final sampling sessions

	Inter-group comparisons (control vs WD)				Intra-group comparisons (initial vs final)			
	Initial		Final		Pregnant		NR	
	Pregnant	NR	Pregnant	NR	Control	WD	Control	WD
Baseline CORT	$z=0.91$, $P=0.985$	$z=-0.30$, $P>0.999$	$z=-1.45$, $P=0.833$	$z=0.16$, $P>0.999$	$z=-0.39$, $P>0.999$	$z=-3.78$, $P=0.004$	$z=-0.97$, $P=0.979$	$z=-0.28$, $P>0.999$
Stress-induced CORT	$z=0.88$, $P=0.988$	$z=1.31$, $P=0.894$	$z=-1.89$, $P=0.556$	$z=0.86$, $P=0.990$	$z=0.97$, $P=0.978$	$z=-3.82$, $P=0.003$	$z=-0.38$, $P>0.999$	$z=-1.18$, $P=0.936$
Adjusted stress-induced CORT	$z=0.74$, $P=0.996$	$z=1.49$, $P=0.814$	$z=-1.50$, $P=0.806$	$z=0.87$, $P=0.988$	$z=0.93$, $P=0.983$	$z=-2.42$, $P=0.232$	$z=-0.10$, $P>0.999$	$z=-1.06$, $P=0.965$

Data are the results of *post hoc* Tukey's tests. In adjusted stress-induced CORT level analysis, baseline CORT is included as a linear covariate. Significant effects are in bold.

osmolality within pregnant and non-reproductive females. Hydric treatment significantly impacted plasma osmolality (Dupoué et al., 2015a), and we avoided a co-linearity issue by not including treatment in the model.

We used linear models to test the influence of the estimates of maternal reproductive effort (RLS, RFLS, RLM, and RFLM) on changes from baseline or absolute stress-induced CORT levels within each hydric treatment. Hydric treatment was treated as an explanatory factor and each estimate of maternal reproductive effort as a linear covariate.

We used linear mixed models to test the effect of female hydric treatment on offspring growth, with body or jaw growth as a dependent variable and maternal treatment as an explanatory factor. Female identity was set as a random factor because siblings are not statistically independent (Massot et al., 1994).

Finally, we used a linear mixed model to test the relationship between offspring body growth or jaw growth and changes in maternal osmolality or changes from baseline or absolute stress-induced CORT level. Hydric treatment significantly impacted offspring growth (see Results), and we avoided a co-linearity issue by not including treatment in the model. All data are presented as means \pm s.e.m.

RESULTS

Impact of water deprivation and reproductive status on plasma osmolality

As previously reported (Dupoué et al., 2015a), plasma osmolality increased in water-deprived pregnant (321.8 ± 2.4 to 385.1 ± 5.4 mOsm kg⁻¹) and non-reproductive females (307.2 ± 3.4 to 342.9 ± 6.0 mOsm kg⁻¹), while no significant change was observed in their respective controls (pregnant: 322.0 ± 3.6 to 324.8 ± 3.2 mOsm kg⁻¹; non-reproductive: 321.3 ± 3.6 to 324.1 ± 4.0 mOsm kg⁻¹).

Impact of water deprivation and reproductive status on CORT levels

Baseline CORT level was significantly influenced by sampling session and reproductive status, but not by hydric treatment (Table 1). We did not find a significant influence of any of the double interactions, but did find a significant influence of the triple interaction (Table 1), highlighting different baseline CORT levels between sampling sessions. Inter-group comparisons showed no effect of reproductive status or hydric treatment on baseline CORT level (Table 2). Intra-group analyses showed a significant increase in baseline CORT level through the treatment period in pregnant females from the water-deprived treatment (Table 2, Fig. 1A). Baseline CORT level did not significantly change through the treatment period in any other group (Table 2, Fig. 1).

Stress-induced CORT level was significantly influenced by sampling session and marginally by reproductive status, but it was not influenced by hydric treatment (Table 1). We found a significant impact of the interaction between sampling session and treatment on stress-induced CORT level (Table 1), suggesting that the sampling session differentially affected the stress responses of control and water-deprived females. The triple interaction significantly influenced stress-induced CORT level (Table 1), highlighting different baseline CORT levels between sampling sessions for pregnant and non-reproductive females from the control or the water-deprived treatment.

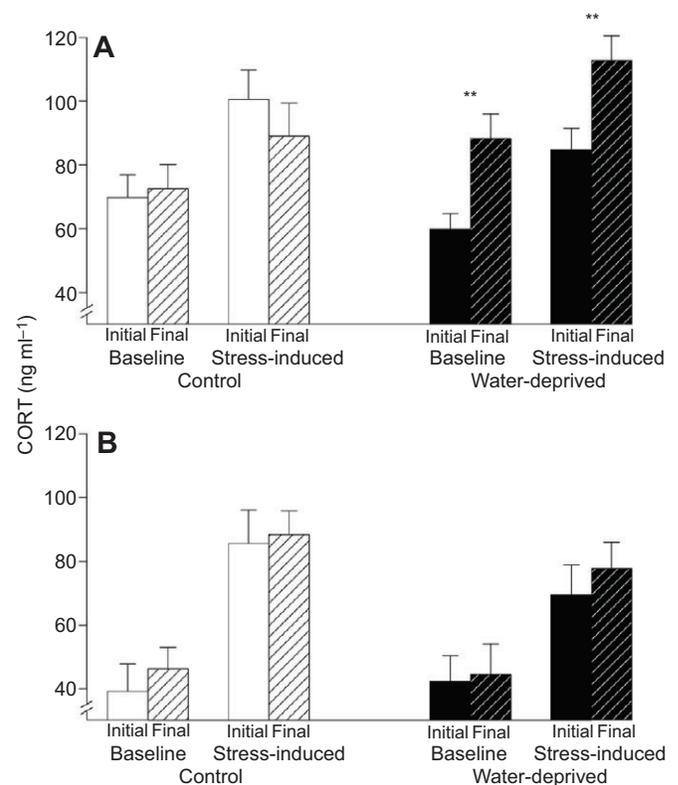


Fig. 1. Effect of water deprivation and reproductive status on asp viper CORT levels. Bars represent means (\pm s.e.m.) of baseline and stress-induced CORT levels during initial (full bars) and final (hatched bars) sampling sessions in (A) pregnant and (B) non-reproductive females from the control (white bars, $n=15$ pregnant, $n=14$ non-reproductive) and water-deprived treatments (black bars, $n=13$ pregnant, $n=14$ non-reproductive). Significant differences in CORT levels between initial and final sampling sessions are indicated by asterisks (** $P<0.01$).

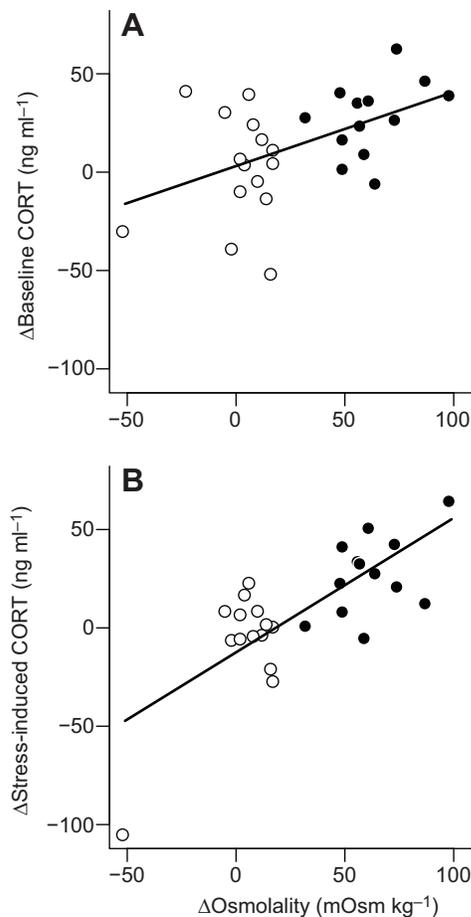


Fig. 2. Relationships between changes in CORT levels and changes in hydration state over the water deprivation period in pregnant asp viper. Changes in (A) baseline and (B) stress-induced CORT levels in females from the control (open circles, $n=15$) or water-deprived (filled circles, $n=13$) treatments. Correlation lines are included for the following significant relationship: changes in osmolality are positively correlated with changes in baseline CORT and with changes in stress-induced CORT.

Inter- and intra-group analyses on stress-induced CORT level revealed the same statistical differences as the analyses of baseline CORT level. That is, there were no differences at either the onset or the end of water deprivation between control and water-deprived females, regardless of reproductive status, and there was a significant increase of stress-induced CORT level only in water-deprived pregnant females (Table 2, Fig. 1). When adjusted for the baseline CORT level, stress-induced CORT level was not significantly influenced by any factor except the interaction between sampling session and treatment as above (Table 1). Similarly, inter- and intra-group analyses were all non-significant (Table 2).

Determinant of changes in CORT levels

In pregnant females, we found that changes in both baseline and stress-induced CORT levels were correlated with changes in osmolality (respectively, $F_{1,26}=8.4$, $P=0.007$, and $F_{1,25}=32.2$, $P<0.001$; Fig. 2). In non-reproductive females, no relationship was found (all $P>0.582$; Fig. 3).

When considering control pregnant females, we found that changes in baseline CORT level were negatively correlated with fit litter size ($t=-2.2$, $P=0.036$) or RFLS ($t=-2.1$, $P=0.045$; Fig. 4A), but not with other estimates of reproductive effort (all $P>0.127$). No

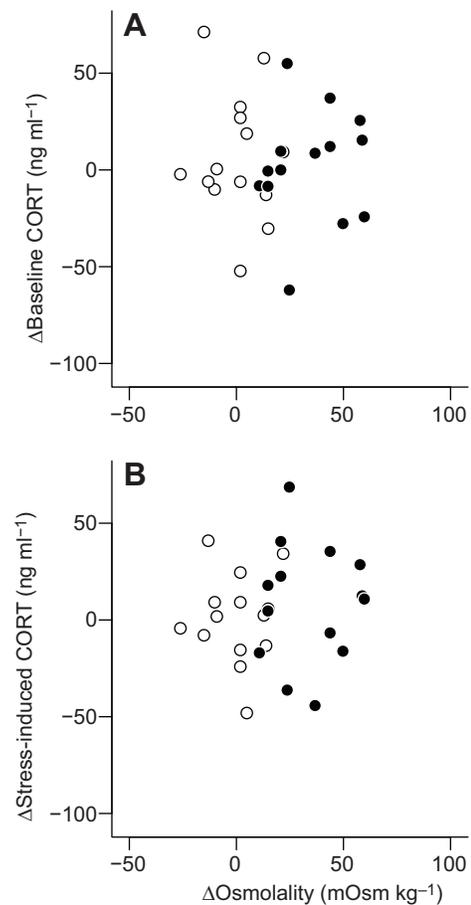


Fig. 3. Relationships between changes in CORT levels and changes in hydration state over the water deprivation period in non-reproductive asp viper. Changes in (A) baseline and (B) stress-induced CORT levels in females from the control (open circles, $n=14$) or water-deprived (filled circles, $n=14$) treatments.

relationship between changes in baseline CORT level and the different estimates of maternal reproductive effort was found among water-deprived females (all $P>0.232$). Changes in stress-induced CORT level were not correlated with the different estimates of maternal reproductive effort for control (all $P>0.126$) or water-deprived females (all $P>0.133$; Fig. 4B).

Impacts of water deprivation on offspring growth

We found a significant effect of water deprivation during pregnancy on both offspring body growth ($F_{1,23}=7.1$, $P=0.014$) and jaw growth ($F_{1,23}=7.8$, $P=0.010$; Fig. 5). Compared with offspring from control females, offspring from water-deprived females showed greater body and jaw growth (Fig. 5).

We found a positive relationship between changes in maternal osmolality and offspring body growth ($F_{1,22}=6.7$, $P=0.017$) or jaw growth ($F_{1,22}=8.1$, $P=0.009$; Fig. 6). We also found a positive relationship between changes in maternal baseline CORT levels and offspring body growth ($F_{1,22}=4.4$, $P=0.047$, Fig. 6). However, we did not find any relationships between changes in stress-induced CORT levels and offspring body growth or jaw growth (all $P>0.184$).

DISCUSSION

Water is a vital resource that can profoundly affect animal behavior and performance (Davis and DeNardo, 2009; Lorenzon et al., 1999; McKechnie and Wolf, 2010; Moeller et al., 2013; Prates et al.,

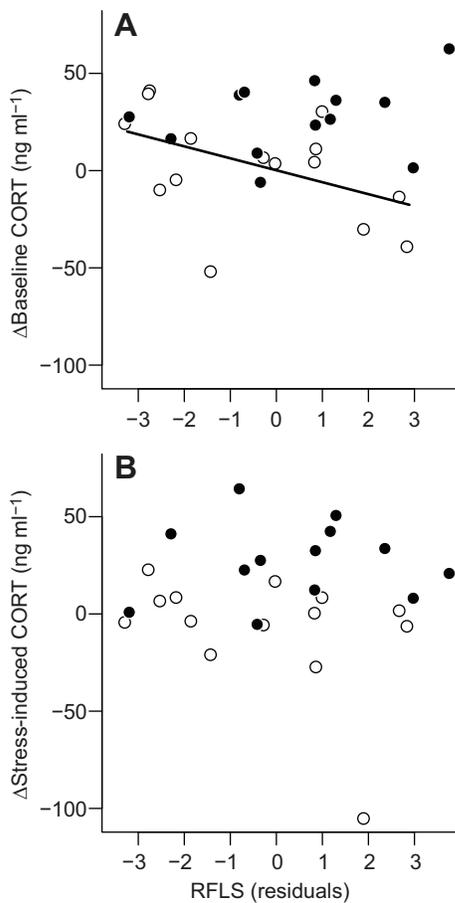


Fig. 4. Relationships between changes in CORT levels over the water deprivation period and maternal relative fit litter size (RFLS; residuals from the linear model of fit litter size against mother snout-vent length). Changes in (A) baseline or (B) stress-induced CORT levels in females from the control (open circles, $n=15$) or water-deprived (filled circles, $n=13$) treatments. The correlation line represents the only significant result, where RFLS was negatively correlated with changes in baseline CORT levels in control females.

2013), yet it is often overlooked in ecophysiological studies (Bonnet and Brischox, 2008). For instance, although CORT is a well-established mediator of allostasis, the effects of water restriction on CORT secretion remain relatively unknown. In this study, we investigated the effects of water deprivation on plasma CORT levels in pregnant and non-reproductive aspik vipers, as well as on offspring traits. We found that water deprivation led to higher CORT levels (both baseline and stress-induced levels) in pregnant females, and that CORT changes were correlated to changes in female plasma osmolality and maternal reproductive effort. We also found that water deprivation was correlated with greater offspring growth, which was positively correlated with maternal dehydration and changes in baseline CORT.

Water deprivation influences CORT levels in pregnant females

As pregnant females become dehydrated as a result of water deprivation, they experience a significant elevation in baseline and stress-induced CORT levels. This response was not observed in water-deprived non-reproductive females, even though they experienced a significant, but less dramatic, increase in osmolality (Dupoué et al., 2015a). This suggests increased physiological demands during pregnancy. Notably, pregnant females have an

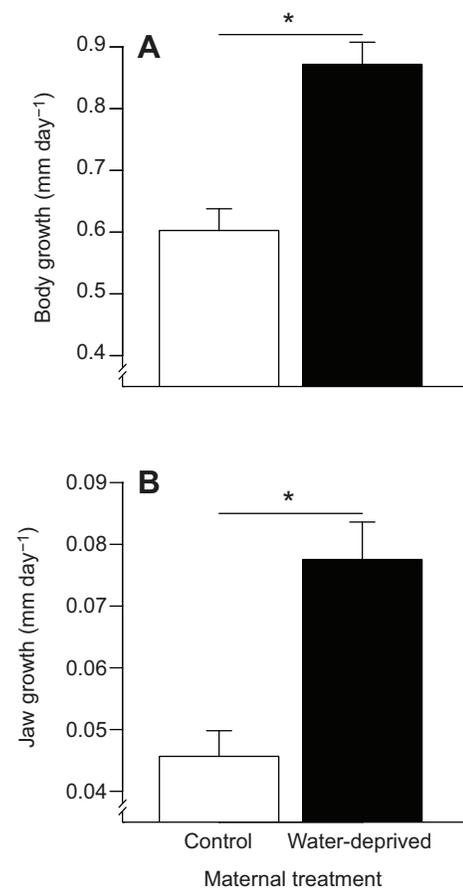


Fig. 5. Effect of maternal hydric treatment on offspring growth 2 weeks after birth. Bars represent means (\pm s.e.m.) of (A) body growth and (B) jaw growth of offspring born to mothers from control (white bars, $n=60$) and water-deprived (black bars, $n=65$) treatments. Significant differences between treatments are indicated by asterisks ($*P<0.05$).

increased rate of evaporative water loss (Dupoué et al., 2015b; Webber et al., 2015) and they also have to provide water to the developing embryos (Dauphin-Villemant and Xavier, 1986; Lourdais et al., 2015). These increased demands on water may lead to water constraints if water availability is limited. This could explain why the increase in osmolality of water-deprived pregnant females was twice that of water-deprived non-reproductive females (Dupoué et al., 2015a). Although we expected dehydration to increase CORT levels in non-reproductive females, this result is consistent with previous studies on non-breeding reptiles in which CORT does not appear to be involved in the regulation of water balance per se (Bradshaw and Rice, 1981; Dauphin-Villemant and Xavier, 1986; Dupoué et al., 2014). Supporting specific effects of dehydration on CORT levels, we found that changes in baseline CORT levels were positively correlated with changes in osmolality in pregnant females, but not in non-reproductive females.

We propose two non-exclusive hypotheses to explain the increase in baseline CORT level of water-deprived pregnant females. (1) CORT may be involved in the transfer of maternal body water to the developing embryos. For instance, at baseline levels CORT is involved in energy allocation (Landys et al., 2006; Romero, 2004; Wingfield et al., 1998) and might have the same function in water allocation. (2) Alternatively, the increase in baseline CORT level in response to water deprivation could reflect an acute physiological

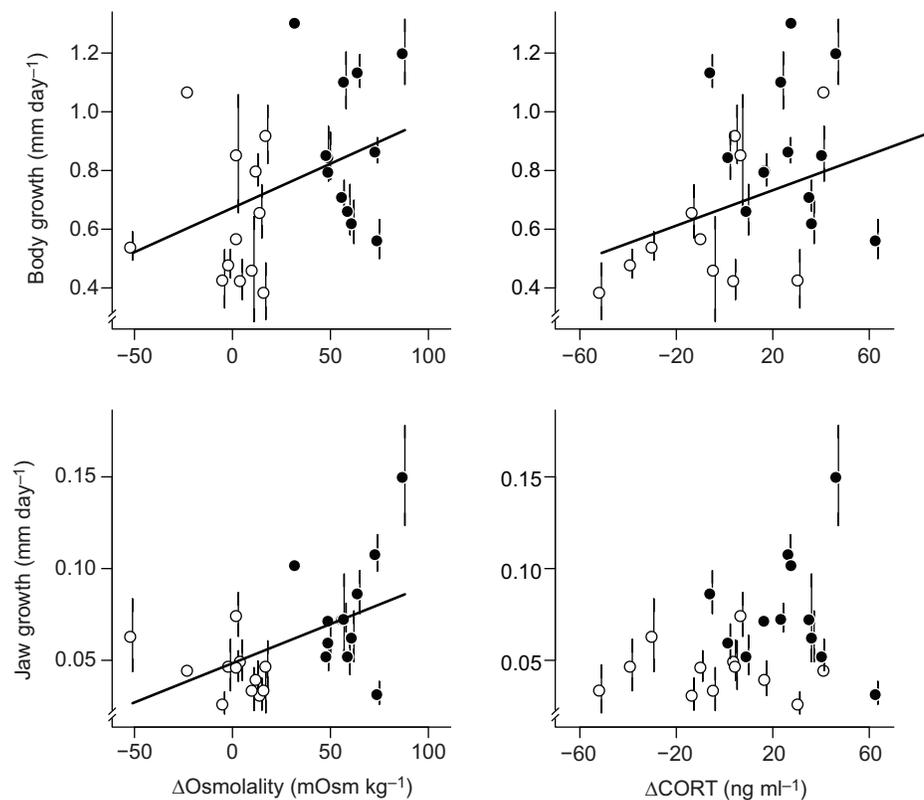


Fig. 6. Relationships between offspring body or jaw growth and changes in maternal osmolality or baseline CORT level. Offspring body and jaw growth data are means \pm s.e.m. per litter. Trend lines are included for significant relationships. Δ Osmolality was positively correlated with offspring body growth and jaw growth. Δ CORT was positively correlated with offspring body growth but not offspring jaw growth.

stress generated by high gestational water demand in this species (i.e. high allostatic load or allostatic overload). However, the lack of a significant CORT increase in non-reproductive water-deprived females suggests the need for a threshold of dehydration before the activation of the central nervous system (i.e. hypothalamo–pituitary–adrenal axis). Further experiments manipulating CORT levels are necessary to clarify the role of CORT in water transfer during pregnancy and to determine whether CORT is secreted at a given dehydration threshold.

CORT levels and maternal reproductive effort

In pregnant control females, changes in baseline CORT level were negatively correlated with two estimates of maternal reproductive effort (i.e. fit litter size and RFLS), whereas there was no relationship in water-deprived females. Previous studies have demonstrated either positive (Bonier et al., 2011) or negative relationships between CORT and reproductive effort (Angelier et al., 2010; Bonier et al., 2009b). A negative relationship between baseline CORT and reproductive effort is consistent with the CORT–fitness hypothesis (Bonier et al., 2009a,b), which posits that high baseline CORT level is associated with lower reproductive success. Alternatively, the negative relationship between baseline CORT level and maternal reproductive effort in control females might reflect a dilution of plasma CORT concentrations that is due to an increase of maternal body water. Female drinking behavior is correlated with fecundity in asp viper (Dupoué et al., 2015a; Lourdaï et al., 2015), and this might passively decrease baseline CORT concentration in fecund females. However, such a passive effect of maternal reproductive effort is unlikely here because it should have also impacted the stress-induced CORT level. Further experiments are required to investigate the long-term relationship of baseline CORT level and reproductive performance.

Water deprivation effect on offspring growth

We found significant effects of mother treatment on offspring growth and positive influences of both maternal hydration state and baseline CORT on offspring growth. Together, these results suggest that the dehydration induced by water deprivation might have increased maternal CORT levels, which influenced offspring developmental trajectories. In support of this, previous experimental studies on wild vertebrates have shown either positive (Chin et al., 2009; Dantzer et al., 2013) or negative (Hayward and Wingfield, 2004; Lesage et al., 2001; Meylan and Clobert, 2005) effects of increased maternal GC levels on offspring growth. CORT is typically involved in energy allocation (Landys et al., 2006; Romero, 2004; Wingfield et al., 1998). Therefore, maternal CORT might have favored residual yolk mobilization by offspring, resulting in increased growth. Maternal CORT may also have influenced offspring behavior (Robert et al., 2009; Wilcoxon and Redei, 2007), increasing, for example, their drinking behavior and enhancing yolk conversion in somatic tissues (Belinsky et al., 2004; Thompson and Speake, 2002). However, we did not monitor offspring drinking behavior and therefore we cannot determine whether this component was involved in accelerated growth.

In past decades, the early influence of the maternal environment on offspring phenotypes (i.e. maternal effects) was extensively documented (Mousseau and Fox, 1998; Mousseau et al., 2009). Importantly, depending on the environmental context, mothers may adjust the offspring phenotype in ways that favor (or not) its fitness (Grootuis and Schwabl, 2008; Grootuis et al., 2005; Marshall and Uller, 2007; Monaghan, 2008; Sinervo and DeNardo, 1996). Here, we cannot assess whether higher offspring growth is an adaptive response to perturbation of maternal water balance because we have not considered other indicators of fitness (e.g. survival, age and size at maturity; Roff, 1992; Stearns, 1992). Yet, accelerated juvenile growth could result in different selective advantages (anti-predator,

competitive and/or dispersal; reviewed in Arendt, 1997). Moreover, jaw size is a major trait that affects feeding performance in neonates (Forsman and Lindell, 1993). Previous studies have demonstrated that jaw growth can depend on developmental conditions such as temperature (Lorioux et al., 2012), and plasticity in jaw growth may be ecologically relevant (Aubret et al., 2004; Forsman, 1996). In a previous long-term field study, Marquis et al. (2008) demonstrated that level of precipitation (i.e. water availability) positively influences reproductive performance of female common lizard, *Zootoca vivipara* (i.e. litter size, offspring size and survival). However, fast growth can also mediate delayed costs (Gotthard, 2000; Metcalfe and Monaghan, 2001), and further studies are now required to model the effects of decreasing precipitation intensity and frequency on animal growth and survival.

Conclusions

Currently, a major objective of ecophysiologicalists is to determine the functional responses of organisms to rapid environmental changes (Bozinovic and Pörtner, 2015; Dillon et al., 2010; Todgham and Stillman, 2013). Drought frequency and intensity are expected to increase as a result of global climate change (IPCC, 2014), so investigations into the impacts of water restriction on animal physiological and behavioral adjustments is crucial. Such an understanding would allow the inclusion of species responses (behavioral adjustments, physiological limits, etc.) in predictive models (Huey et al., 2012; McCluney et al., 2012; McKechnie and Wolf, 2010). Although our results suggest that reproductively active individuals may be particularly impacted by dehydration, as demonstrated for other vertebrates (Hirschhorn et al., 1969; Rittenhouse et al., 2009), parental effects may influence offspring physiology and behavior (Lorenzon et al., 1999, 2001; Marquis et al., 2008). Future work could specifically investigate the potential role for parental effects in mediating the impacts of adverse hydric conditions on offspring fitness.

Acknowledgements

We thank Gaëtan Guiller for his help with snake captures. We thank Stéphanie Ruault for her significant help with the corticosterone assay. We are grateful to Christian Wright for performing the osmolality assays.

Competing interests

The authors declare no competing or financial interests.

Author contributions

A.D. contributed to all aspects of this study with conception, designing and executing experiments or analyses, and drafting and revising the article. F.A. contributed to the execution of experiments or analyses, and drafting and revising the article. F.B. contributed to the execution of experiments or analyses, and drafting and revising the article. D.F.D. contributed to the execution of experiments or analyses, and drafting and revising the article. C.T. contributed to the execution of experiments. C.P. contributed to the execution of experiments. O.L. contributed to all aspects of this study with conception, designing and executing experiments or analyses, and drafting and revising the article.

Funding

Financial support was provided by the Région Poitou-Charentes, the Département des Deux-Sèvres, the Projet BOCAGE, and the Centre National de la Recherche Scientifique (CNRS).

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