

Intergenerational trade-off for water may induce a mother–offspring conflict in favour of embryos in a viviparous snake

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Summary

1. Parent–offspring conflicts are likely to occur when resources are limiting either at pre- or post-natal stages due to intergenerational trade-offs over resources. Current theory posits that such conflicts may influence the evolution of parental allocation as well as reproductive modes. While energy allocation to the offspring has received considerable attention, the distribution of water – another potentially limited vital resource to both the mother and offspring – and the resulting outcomes remain grossly understudied.

2. Here, we explored the intergenerational trade-off related to water resources in the viviparous aspic viper (*Vipera aspis*) by examining the effects of water deprivation on female physiology (body mass, haematocrit and osmolality), water transfer to developing embryos and reproductive performance.

3. As a result of water deprivation, females became dehydrated, with the effects more pronounced in pregnant compared to nonreproductive females. Among pregnant females, the impacts of water deprivation on water balance were correlated with fecundity. In contrast, water deprivation had no effect on water transfer to the offspring or on reproductive performance.

4. Our results demonstrate that, under water-constraining conditions, female water balance is compromised in favour of the developing embryos, highlighting a significant intergenerational trade-off for water. Although ectothermic reptiles are particularly tolerant in water balance perturbations, our results suggest that, like energy, water can be a conflicting resource between mother and offspring. Parent–offspring conflict over water should therefore be further investigated to better understand reproductive modes and reproductive trade-offs in terrestrial organisms.

Key-words: aspic viper, dehydration, fecundity, parent–offspring conflict, pregnancy, reproductive mode, trade-off

Introduction

The parent–offspring conflict theory (Trivers 1974) aims at clarifying the determinants of the balance between parental investment and offspring requirements. Conflict occurs because parents and offspring are not genetically identical, and therefore, selection acts on each protagonist to favour its own needs (Trivers 1974). Several studies have provided empirical support to this theory (Parker & Mock 1987;

Kölliker & Richner 2001), mainly in species with post-natal parental care (Parker & Mock 1987; Clutton-Brock 1991; Kölliker & Richner 2001). Surprisingly, much less attention has been paid to conflicts at prenatal stages (Haig 1993; Crespi & Semeniuk 2004) even though prenatal parent–offspring conflict may be a major selective pressure in the evolution of reproductive mode (i.e. the viviparity-conflict hypothesis, hereafter “VCH”; Crespi & Semeniuk 2004). According to the VCH, a tug-of-war for resources exists between mother and developing embryos, and there is selection for offspring traits to draw maternal

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resources (Crespi & Semeniuk 2004). Supporting this hypothesis, manipulations of the mother by offspring have previously been reported in humans (Haig 1993) and non-mammalian vertebrates (Crespi & Semeniuk 2004). While offspring are expressing traits to draw maternal resources, females adjust their resource allocation to optimize their lifetime reproductive success (Schwarzkopf & Andrews 2012), which may result in resource allocation that is suboptimal for current offspring development (Crespi & Semeniuk 2004).

While not explicit to resource type, maternal-offspring conflicts are typically evaluated in terms of energy resource allocation (i.e. nutrient supply; Crespi & Semeniuk 2004). Conflicts between a mother and offspring may occur whenever there is an intergenerational trade-off for a limited resource (Charnov 1982; Godfray & Parker 1991; Godfray 1995). Several examples of energy-based trade-offs or conflicts between mother and offspring have been previously reported in invertebrates (Mas, Haynes & Kölliker 2009), fishes (Hussey *et al.* 2010), amphibians (Kupfer *et al.* 2006), birds (Müller, Korsten & von Engelhardt 2007) and mammals (Capellini, Venditti & Barton 2011). In comparison, while water represents another depreciable resource for organisms, water allocation has not yet been considered in the framework of parent-offspring trade-offs or conflicts. Water is oftentimes readily available, but this is not the case in environments where seasonal or prolonged droughts are common.

Studies of the impact of water limitation on the parent and its offspring are challenging because water is typically a significant component of the consumed meal, and thus it is characteristically difficult to separate the impacts of water constraint from those of energy constraint. However, squamate reptiles (lizards and snakes) provide an excellent opportunity to examine water limitations free of influence from energy conflicts. Many squamates energetically support reproduction by utilizing previously accumulated energy stores (i.e. capital breeding; Bonnet, Bradshaw & Shine 1998), and these energy stores are then invested into reproduction through yolk deposition into the developing follicles (i.e. lecithotrophy; Blackburn 1993). As a result, the allocation of energy by the female to the offspring is completed prior to ovulation, and therefore, the embryos cannot influence this investment.

Developing embryos require a considerable amount of water, especially during the later foetal life stage when somatic growth is exponential (Andrews 2004; Lourdais unpublished data). In viviparous species, this water must be provided by the female. The water demands of the foetuses are especially challenging for many squamates, because pregnancy is a lengthy process that generally occurs in the summer when water resources can be most limited. Additionally, pregnant female squamates typically increase their thermal preferences during pregnancy (Lourdais *et al.* 2002a, 2004; Lorient, Lisse & Lourdais 2013a), resulting in a concomitant increase in the rate of

evaporative water loss (Mautz 1982; Guillon *et al.* 2014). Collectively, these challenges make pregnant females particularly vulnerable to water limitations. Regardless, failure to provide sufficient water to the embryos can greatly compromise their development, as water content of the litter increases from 40 to 50% at ovulation to 80% at the end of gestation (Thompson 1981). In oviparous species, experimental and correlative studies suggest that water constraints during development alter yolk mobilization, affect offspring morphology and performance, and can result in embryo death (Shine & Brown 2002; Aubret *et al.* 2005; Lourdais, Hoffman & DeNardo 2007). Additionally, both experimental and field studies conducted in a viviparous species (*Zootoca vivipara*) suggest that water restriction and rainfall regime during pregnancy can alter offspring size and survival (Dauphin-Villemant & Xavier 1986; Marquis, Massot & Le Galliard 2008).

Given the maternal and embryonic sensitivities to water limitation, a significant mother-offspring conflict for water should exist when water resources are limited. Accordingly, we manipulated access to water (control vs. water deprived for 20 days) for pregnant and nonreproductive female aspic vipers (*Vipera aspis*). We considered the impact of water deprivation on (i) the physiology of females (body mass, haematocrit and plasma osmolality), (ii) the transfer of water to the embryos during pregnancy and (iii) the reproductive performance (reproductive success, litter traits and offspring quality at birth).

We hypothesized that a significant intergenerational trade-off for water may exist and induce a mother-offspring conflict when access to water is limited. We tested the following predictions:

1. Water deprived pregnant females should be more dehydrated than water deprived nonreproductive females as a result of water demands of the embryos and physiological changes associated with reproduction (e.g. increased body temperature).
2. The number of developing embryos (i.e. fecundity) should be correlated with the effects of water deprivation on female physiology.
3. Water deprivation should alter water transfer to the embryos and therefore, compromise reproductive performance.

Material and methods

STUDY SPECIES AND HOUSING

We studied the aspic viper (*Vipera aspis*), a medium-size viviparous snake that is abundant in Western Europe (Naulleau 1981). The aspic viper is a typical capital breeder, and females mobilize their energy reserves to support yolk production during vitellogenesis. Pregnancy begins after ovulation in early June (Naulleau 1981), which is associated with a pre-ovulatory ecdysis (Lorient *et al.* 2013b). Parturition occurs 2–3 months later from late August to early September (Lourdais *et al.* 2002a). Pregnant females often cease to feed (Bonnet, Bradshaw & Shine 1998; Lourdais, Bonnet & Doughty 2002b), mainly because of an

important behavioural shift that emphasizes thermoregulation over foraging (Lorioux, Lisse & Lourdais 2013a).

In May and June 2012, we caught 58 females (29 pregnant, 29 nonreproductive) from neighbouring sites in western France (Vendée and Loire-Atlantique Districts). Reproductive status was determined by palpation in the field and then confirmed with high-resolution ultrasonography (Sonosite microMaxx, Inc., Bothell, WA, USA) when the animals were brought to the laboratory where all snakes were measured (snout-vent length, SVL \pm 0.1 cm) and weighed (body mass, BM \pm 0.1 g). Husbandry followed the protocol described in Lorioux, Lisse & Lourdais (2013a). Individuals were housed 3–4 per cage in 16 cages (100 \times 30 \times 35 cm). Room temperature was held constant at 20 °C. To mimic natural conditions and allow for thermoregulation, heat was provided at one end of each cage with a 75W incandescent light bulb for 5 h per day (from 1000 to 1500 h), creating a thermal gradient (20–40 °C). Water was available *ad libitum*. Individuals were fasted 2 weeks prior to the start of the experiment and remained fasted throughout the experiment, thus preventing meal consumption from contributing dietary water to the snakes' water balance (Wright, Jackson & DeNardo 2013). During the entire study, we followed all laws and rules relating to the conservation and welfare of the animals (Permit #792, Direction service vétérinaire des Deux-Sèvres).

EXPERIMENTAL PROTOCOL

Individuals from each reproductive status were randomly assigned to the control (pregnant: $n = 15$; nonreproductive: $n = 15$) or water deprived (pregnant: $n = 14$; nonreproductive: $n = 14$) hydric treatments. Within each reproductive status, water deprived individuals and controls did not differ in BM or SVL (all $P > 0.117$). During the experiment, housing conditions remained as described above, except that water was removed for 20 days in the water deprived treatment. Water deprivation occurred at the time when pregnant females were in mid-gestation, and the duration of the deprivation represents the typical duration of a summer drought in western France.

At the end of the treatment period, water intake and mass recovery were assessed by placing the females in individual boxes (30 \times 20 \times 10 cm) with a thermal gradient (similar to that described earlier), a water bowl and a shelter. Pregnant females were checked daily for parturition (Lorioux *et al.* 2013b), and female and offspring traits were measured on the day of birth. Once all measurements were collected, females were fed and released with their litters at their original capture sites.

PHYSIOLOGICAL PARAMETERS

Body mass

Change in BM (Δ BM) has been well-established as an estimator of water loss in squamate reptiles (DeNardo, Zubal & Hoffman 2004; Lillywhite *et al.* 2008; Dupoué *et al.* 2014a). BM was collected after each blood sample. To account for changes in BM associated with reproductive state (pregnant or nonreproductive), we also determined Δ BM of water deprived females relative to the mean Δ BM of control females of the same reproductive state over the same period (relative Δ BM = Δ BM minus the mean Δ BM of relevant control animals).

Blood parameters

We measured the change in haematocrit (Δ Hct) and the change in osmolality (Δ Osm), as both parameters are effective indicators

of hydration state (Peterson 2002). Blood samples were collected at the beginning and end of the water deprivation period. Females were sampled in random order for both blood sampling points. Blood was immediately collected upon removing the female from its cage. We collected blood samples (150 μ L) via cardiocentesis using a heparinized 1-ml syringe with a 27-gauge needle. Immediately after collection, we allocated two replicates of blood into 10- μ L micro-capillary tubes to measure Hct. The microcapillary tubes were centrifuged for 3 min at 5500 \times g, and we then measured the lengths of the column of cells and the total sample (i.e. cells + plasma) with a digital calliper (\pm 0.01 mm). Hct (%) was determined as the proportion of cells to total sample. For each blood sample, we averaged the two Hct values (intra-individual CV: 3.82%). The remaining blood from each sample was placed into a 0.675 mL microcentrifuge tube and centrifuged for 3 min at 2000 \times g. The plasma was separated from the cells and stored in airtight tubes at -28 °C until laboratory analyses. Plasma osmolality (mOsm.kg $^{-1}$) was measured from 10- μ L triplicates (intra-individual CV < 1%) as described in Wright, Jackson & DeNardo (2013). In one female (pregnant from the water deprived treatment), blood collection failed during the final sampling session, so this female was not included in the analyses of maternal blood parameters.

WATER TRANSFER TO THE EMBRYOS

Because water deprivation likely impacts the transfer of water to the embryos, we monitored the change in volume of the embryonic unit (i.e. embryo, yolk and extra-embryonic membranes) using high-resolution ultrasonography (see Lorioux *et al.* 2013b). We determined total volume at three different stages of embryonic development (Hubert & Dufaure 1968): early pregnancy (mean \pm SE: 12.3 \pm 1.5 days since ovulation; stages 30–33), mid-pregnancy (58.8 \pm 1.6 days since ovulation; stages 37–39) and late pregnancy (78.7 \pm 1.4 days since ovulation; stages 42–43). The second, mid-pregnancy measurement was specifically performed at the end of the hydric treatment to assess the impact of water deprivation on water transfer to the embryos. For each female, we collected images in a sagittal view of the most cranial and caudal embryonic units and measured their heights (H), lengths (L) and form. We estimated embryonic volume (cm 3) following the method described in Maritz & Douglas (1994) which considers embryonic unit L, H and a coefficient associated with its form (F_{emb}): embryonic volume = $\pi \times L \times H^2 \times F_{emb}$. For analysis, we averaged the volume of the two embryonic units measured at each stage for each female (intra-individual variation: 15.3%).

REPRODUCTIVE PERFORMANCE

We determined the effect of hydric treatment on reproductive success (i.e. number of females with at least one viable offspring) and the duration of pregnancy. The duration of pregnancy (days) was derived from the difference between parturition and ovulation dates (Lorioux *et al.* 2013b). Additionally, we measured litter size (number of undeveloped ova, stillborn and neonates), fit litter size (number of neonates only), litter mass (mass of undeveloped ova, stillborn, and neonates) and fit litter mass (mass of neonates only) (see details in methods of Lourdais, Bonnet & Doughty 2002b). We also calculated embryonic fluid mass (g), which corresponds to the difference between mass lost by the female at parturition and total mass of the litter.

To assess offspring quality, we collected previously described morphometric traits (Lorioux *et al.* 2013b). Briefly, each individual was sexed by attempting to manually evert hemipenes. Shortly after birth (<1 day), we collected neonate BM (\pm 0.01 g) and SVL (\pm 0.1 cm), and we measured neonate jaw length (JL) using digital

callipers (± 0.01 mm). We collected all values in triplicate and used the mean values in analyses (intra-individual variation $< 1\%$). We estimated body condition (BC) as the residuals from the relationship of BM against SVL ($F_{1,123} = 69.9$, $P < 0.001$).

STATISTICAL ANALYSES

All analyses were performed using R software (R Development Core Team, 2011). We used the Shapiro–Wilk test to determine whether residuals of our models significantly differed from a normal distribution (all $P > 0.05$).

We built linear models with initial values of physiological parameters (BM, Hct and osmolality) as dependent variables and with treatment assignment, reproductive status and their interaction as fixed factors (Table 1). We used pairwise *post hoc* tests of Tukey (lsmeans, package lsmeans) on the interaction to determine statistical differences between groups. We used the same analytic design to test the effects of water deprivation and reproductive status on changes in BM (Δ BM), Hct (Δ Hct) and osmolality (Δ Osmo) using the initial value as a linear covariate.

We used linear models to test the relationship between Δ BM and Δ Osmo, or between Δ Hct and Δ Osmo. Fecundity effects on physiological parameters were analysed with linear models with Δ BM, Δ Hct or Δ Osmo as the dependent variable and fecundity as the explained variable.

We used linear mixed models (lme, package nlme) to determine the effect of water deprivation on embryonic volume with embryonic volume as the dependent variable, and hydric treatment, reproductive status, stage of pregnancy, and their interaction as fixed factors. Female identity was set as a random factor considering the females' repeated contribution over gestation.

For analyses of reproductive performance, we used different type of models systematically with hydric treatment as a fixed factor. Reproductive success of the female was analysed with binomial models (i.e. success = 1; fail = 0). Duration of pregnancy, litter size, litter mass, fit litter size, fit litter mass and embryonic fluid mass were analysed with linear models. Offspring BM, SVL, BC and JL were analysed with linear mixed models with mother identity as a random factor since siblings are statistically nonindependent.

Results

FEMALE PHYSIOLOGY

Body mass

At the onset of the water deprivation period, BM was not influenced by treatment assignment, reproductive status or their interaction (Table 1). Δ BM was not influenced by ini-

tial BM, but was significantly impacted by hydric treatment ($F_{1,53} = 96.3$, $P < 0.001$, Fig. 1a), reproductive status ($F_{1,53} = 4.5$, $P = 0.039$, Fig. 1a) and their interaction ($F_{1,53} = 9.6$, $P = 0.003$, Fig. 1a). Water deprivation induced a significant loss in BM for both pregnant females (*post hoc*, control vs. water deprived, $t = 9.1$, $P < 0.001$, Fig. 1a) and nonreproductive females (*post hoc*, control vs. water deprived, $t = 4.7$, $P < 0.001$, Fig. 1a). Absolute BM loss was similar between pregnant and nonreproductive water deprived females (*post hoc*, pregnant vs. nonreproductive, $t = 0.7$, $P = 0.897$, Fig. 1a). Within the control group, pregnant females gained mass while nonreproductive females lost mass (*post hoc*, pregnant vs. nonreproductive, $t = -3.7$, $P = 0.003$, Fig. 1a). Consequently, relative BM loss in water deprived females (i.e. Δ BM minus the mean Δ BM of the control group) was 89% higher in pregnant females compared to nonreproductive females (*post hoc*, pregnant vs. nonreproductive, $t = 4.1$, $p < 0.001$, Fig. 1a).

In water deprived pregnant females, Δ BM was negatively influenced by fecundity (i.e. females with higher fecundity lost more mass; $F_{1,11} = 4.9$, $P = 0.049$, Fig. 2a). Conversely, Δ BM was positively influenced by fecundity in control females (i.e. females with higher fecundity gained more mass; $F_{1,11} = 9.7$, $P < 0.01$, Fig. 2a).

The day after re-exposure to water, females from the water deprived treatment gained mass and attained BM similar to that of controls in both pregnant (*post hoc*, control vs. water deprived, $t = -0.7$, $P = 0.890$) and nonreproductive females (*post hoc*, control vs. water deprived, $t = -2.2$, $P = 0.143$).

Blood parameters

At the onset of the water deprivation period, Hct was significantly lower in pregnant females than in nonreproductive females (Table 1) and was similar between treatment assignments within each reproductive status (Table 1). Δ Hct was significantly influenced by initial value ($F_{1,52} = 4.2$, $P = 0.046$) and was not influenced by hydric treatment ($F_{1,52} = 3.4$, $P = 0.072$), reproductive status ($F_{1,52} = 0.7$, $P = 0.403$) or their interaction ($F_{1,52} = 0.04$, $P = 0.842$). Δ Hct was not affected by hydric treatment in pregnant females (*post hoc*, control vs. water deprived, $t = -1.4$, $P = 0.492$, Fig. 1b) or in nonreproductive

Table 1. Body mass (BM), haematocrit (Hct) and plasma osmolality of pregnant and nonreproductive female aspik vipers (*Vipera aspis*) at the onset of the water deprivation period. Females are represented by treatment assignment, although the water deprivation period treatment had not yet begun

	Nonreproductive		Pregnant		Statistical effect of		
	Control	Water deprived	Control	Water deprived	Assignment	Status	Assignment \times Status
BM (g)	83.5 \pm 8.6	90.3 \pm 9.5	98.5 \pm 6.7	101.2 \pm 6.7	n.s.	n.s.	n.s.
Hct (%)	28.2 \pm 1.1	28.4 \pm 1.5	21.7 \pm 0.7	19.9 \pm 0.9	n.s.	***	n.s.
Osmolality (mOsm.kg ⁻¹)	321.3 \pm 3.6	307.2 \pm 3.4	322.0 \pm 3.6	321.8 \pm 2.4	*	*	*

Significant differences are symbolized: * $P < 0.05$, *** $P < 0.001$, and n.s. (non significant). See text for details.

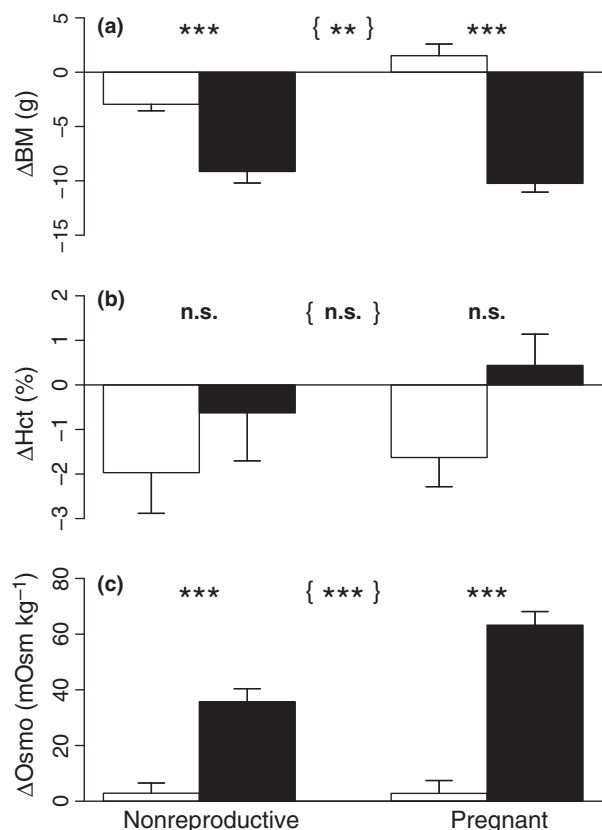


Fig. 1. Effects of hydric treatment on changes in (a) body mass (Δ BM), (b) haematocrit (Δ Hct) and (c) osmolality (Δ Osmo) between initial and final sampling points for pregnant and nonreproductive female asp viper (*Vipera aspis*). Bars represent means \pm SE for control (open bars) and water deprived (filled bars) females. Significant differences between control and water deprived females are symbolized: *** $P < 0.001$, and n.s. (nonsignificant), while significant effect of the interaction between hydric treatment and reproductive status are symbolized: {**} $P < 0.01$, {***} $P < 0.001$, and n.s.; non significant.

females (*post hoc*, control vs. water deprived, $t = -1.2$, $P = 0.643$, Fig. 1b). Δ Hct was not influenced by fecundity for either water deprived ($F_{1,10} = 0.3$, $P = 0.595$) or control females ($F_{1,11} = 0.0$, $P = 0.841$).

At the onset of the water deprivation period, plasma osmolality was influenced by treatment assignment, reproductive status and their interaction (Table 1) due to a lower value in nonreproductive females that were scheduled to start their water deprivation treatment (Table 1). Δ Osmo was significantly influenced by initial value ($F_{1,52} = 11.1$, $P = 0.002$) and was significantly impacted by hydric treatment ($F_{1,52} = 100.4$, $P < 0.001$, Fig. 1c), reproductive status ($F_{1,52} = 15.1$, $P < 0.001$, Fig. 1c) and their interaction ($F_{1,52} = 13.9$, $P < 0.001$, Fig. 1c). Osmolality significantly increased after water deprivation in pregnant females (*post hoc*, control vs. water deprived, $t = -9.8$, $P < 0.001$, Fig. 1c) and nonreproductive females (*post hoc*, control vs. water deprived, $t = -4.2$, $P < 0.001$, Fig. 1c). In water deprived females, Δ Osmo was 77% greater in pregnant than in nonreproductive females (*post hoc*, $t = -5.0$, $P < 0.001$, Fig. 1c).

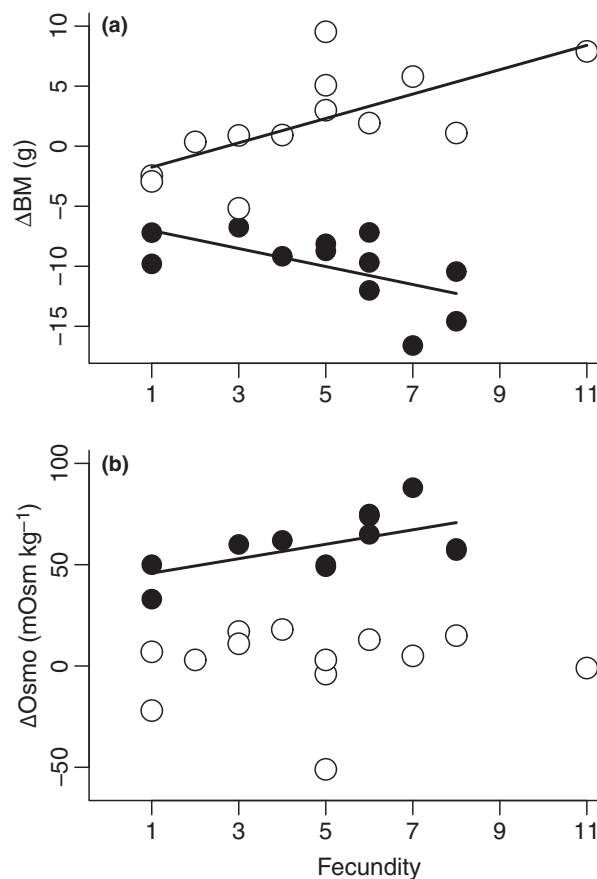


Fig. 2. Influence of fecundity on changes in (a) body mass (Δ BM) and (b) osmolality (Δ Osmo) of control (open circles) and water deprived (filled circles) pregnant female asp viper (*Vipera aspis*). Trend lines are included for significant relationships. Fecundity negatively influenced Δ BM of water deprived females ($r^2 = 0.31$), but positively influences Δ BM of control females ($r^2 = 0.47$). Fecundity positively influences Δ Osmo ($r^2 = 0.34$) in water deprived females, but not controls ($r^2 = 0.00$).

When combining treatments, we found a significant negative relationship between Δ BM and Δ Osmo for pregnant (i.e. females with greater increases in osmolality had greater BM loss, $F_{1,26} = 90.6$, $P < 0.001$, $r^2 = 0.78$) and nonreproductive females ($F_{1,27} = 24.0$, $P < 0.001$, $r^2 = 0.47$). We did not find a significant relationship between Δ Hct and Δ Osmo for either pregnant or nonreproductive females (all $P > 0.246$).

Finally, Δ Osmo was positively influenced by fecundity for water deprived females ($F_{1,10} = 5.2$, $P = 0.045$, Fig. 2b), but not for control females ($F_{1,11} = 0.04$, $P = 0.829$, Fig. 2b).

REPRODUCTION

Water transfer to the embryos

Embryonic volume increased significantly throughout development ($F_{2,48} = 105.7$, $P < 0.001$; Fig. 3), but it was not influenced by treatment ($F_{1,24} = 0.2$, $P = 0.644$; Fig. 3) or the interaction between treatment and stage of

development ($F_{2,48} = 0.6$, $P = 0.544$; Fig. 3). Mothers from the control and the water deprived treatments had similar embryonic volumes at all stages considered including the end of water deprivation (*post hoc*, control vs. water deprived, early pregnancy: $z = -1.0$, $P = 0.920$, mid-pregnancy: $z = 0.2$, $P > 0.999$, late pregnancy: $z = -0.3$, $P > 0.999$; Fig. 3).

Reproductive performance

The percentage of females that produced at least one viable offspring was equivalent between the control ($n = 13/15$; 87%) and water deprived ($n = 13/14$; 93%) treatments ($z = 0.0$, $P > 0.999$). Similarly, the duration of pregnancy was not impacted by hydric treatment (control: 99.9 ± 3.6 days, water deprived: 99.5 ± 2.6 days; $F_{1,24} = 0.01$, $P = 0.932$).

Water deprivation did not have a significant effect on litter size (control: 5.9 ± 0.5 , water deprived: 6.0 ± 0.3), fit litter size (control: 5.2 ± 0.7 , water deprived: 5.2 ± 0.6), litter mass (control: 28.9 ± 3.7 g, water deprived: 30.3 ± 3.0 g), fit litter mass (control: 27.4 ± 4.1 g, water deprived: 28.0 ± 3.8 g) or embryonic fluid mass (control: 16.7 ± 1.4 g, water deprived: 16.9 ± 1.8 g) (all $P > 0.774$).

Finally, hydric treatment did not significantly affect offspring BM (control: 5.48 ± 0.10 g, water deprived: 5.53 ± 0.10 g), SVL (control: 16.22 ± 0.11 cm, water deprived: 16.37 ± 0.10 cm), BC (control: 0.02 ± 0.08 , water deprived: -0.01 ± 0.08), or JL (control: 12.78 ± 0.07 mm, water deprived: 12.59 ± 0.10 mm) (all $P > 0.574$).

Discussion

In this study, we show for the first time in a viviparous vertebrate that an intergenerational trade-off exists over a limited water resource between the mother and her developing embryos. We predicted water deprived pregnant

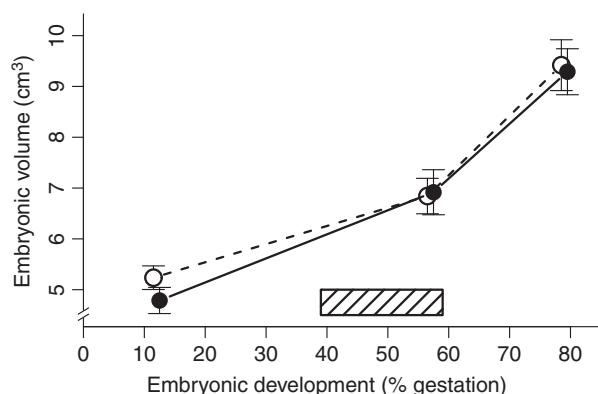


Fig. 3. Change in embryonic volume (cm^3) during development in control (open circle, dashed line) and water deprived (filled circle, solid line) pregnant female asp viper (*Vipera aspis*). Points represent means \pm SE. Hatched space corresponds to the timing of the water deprivation. While embryonic volume increased over time, there was no effect of treatment.

females to be more dehydrated than water deprived nonreproductive females as a result of water demands of the embryos. Accordingly, we found that a 20-day water deprivation had a greater negative impact on water balance of pregnant females compared to nonreproductive ones. We found that the number of developing embryos amplified physiological impacts of water deprivation on females' hydration state (greater loss of BM and increased plasma osmolality), thereby supporting our second prediction. Finally, we predicted water deprivation would alter water transfer to the embryos and therefore, compromise reproductive performance. Conversely, we found that water transfer and reproductive performance were not affected by water deprivation, suggesting that the females' hydration state is compromised in favour of embryos water balance and development. Even though female asp vipers tolerated dehydration, our results suggest that water is likely a limiting resource between mother and offspring and thus a likely target of conflict.

INCREASED SENSITIVITY TO WATER DEPRIVATION IN PREGNANT VIPERS

In the water deprived treatment, both pregnant and nonreproductive females showed a significant loss in BM. However and importantly, relatively to control individuals, pregnant females experienced 89% greater mass loss than nonreproductive females when water deprived. Similarly, water deprived females showed a sharp increase in plasma osmolality that was 77% higher in pregnant females compared to that of nonreproductive females. Although haematocrit is usually considered a good indicator of hydration state (Peterson 2002), there was no influence of treatment on changes in Hct or a significant relationship between changes in Hct and osmolality, thereby challenging this generality. Regardless, the patterns of BM and osmolality changes underline differing water requirements and constraints between pregnant and nonreproductive females. Indeed, during pregnancy, females actively drink to remain normosmotic (Lourdais unpublished data, this study). Water uptake is associated with an increase of BM that is positively related to fecundity (Lourdais unpublished data, this study). When exposed to water deprivation, we found that fecundity directly influenced changes in BM and osmolality in pregnant females. Therefore, when access to water is restricted, developing offspring have an additive, negative effect on water balance of pregnant females. Our results therefore suggest that, under water deprivation, female body water is transferred to developing offspring causing the female to become increasingly dehydrated. It is possible that females allocate water to their embryos and therefore, compromise their own water balance to support offspring survival. An alternative explanation is that embryos are actively acquiring water from the mother. For instance, during incubation, embryos of oviparous squamates are able to control aquaporin function or regulate the osmotic gradient to

efficiently extract water from the nest environment (Packard 1991; Brown & Shine 2005; Shine & Thompson 2006). These observations underline significant embryonic adaptations to accumulate water from their surrounding environment and therefore, provide support for a foeto-maternal conflict. Unfortunately, the physiological mechanisms used by females or embryos of viviparous species to control the transfer of water is not yet understood like they are for the transfer of energy (e.g. hormones, cytokines, growth factor, etc.) (Crespi & Semeniuk 2004). Clearly, further work on this topic is needed.

EFFECTS OF WATER DEPRIVATION ON WATER TRANSFER AND REPRODUCTIVE PERFORMANCE

The water deprivation period had no effect on reproductive success, gestation duration and litter or offspring traits, supporting the idea that body water from the female is transferred to the developing offspring to buffer the offspring at the expense of female water balance. Amniotic fluid might have buffered the transfer of female body water, but measuring fluid volume immediately after dehydration would have required invasive techniques. To indirectly address this question, we ultrasonographically estimated embryonic volume before and after dehydration, and we estimated non-embryonic fluid volume at parturition (prepartum mass of female minus litter mass and post-partum mass). We found no difference in either of these estimates between water deprived and control females, suggesting that amniotic fluid volume was not affected by dehydration. Importantly, water deprivation in this study was imposed during mid-pregnancy, and embryo water requirements are known to dramatically depend on developmental stage. Specifically, water demands increase exponentially throughout development following the pattern of somatic growth (Dauphin-Villemant & Xavier 1986). Therefore, any foeto-maternal conflict for water would likely be stage dependent, and it would be valuable to investigate the impact of water deprivation on reproductive performance at different stages, especially during late gestation (see Lorigou et al. 2013b). As embryonic demand for water increases, water deprivation in late-term pregnancy could result in severe dehydration of the female that may switch the mother-offspring conflict in favour of the mother (i.e. 'selfish mother hypothesis'; Schwarzkopf & Andrews 2012).

Our study highlights an intergenerational trade-off between mother and offspring over water resources. Parent-offspring conflicts can arise when resource optimums for the different protagonists are not the same (Trivers 1974). Parents have to limit the cost of reproduction (i.e. the trade-off between current reproductive effort and survival or future reproductive success; Stearns 1989), while offspring favour their own survival (Trivers 1974). Although ectothermic reptiles are particularly tolerant to water balance perturbations, dehydration is known to affect individual performances (Wilson & Havel 1989; Lorenzon et al. 1999; Moeller, Butler & DeNardo 2013).

Over the water deprivation period, osmolality of pregnant females raised from 321.8 ± 2.4 to 384.9 ± 5.4 mOsmol.kg⁻¹, and these females immediately drank when re-exposed to freshwater, suggesting that they were highly dehydrated (Moeller, Butler & DeNardo 2013; Lillywhite et al. 2014). Data on optimum water requirements are currently missing in the studied species, either for females during pregnancy or for developing embryos. However, the alteration of physiological performances is known to entail significant costs of reproduction (Shine 1980, 2003). Therefore, dehydration during pregnancy is likely an important constraint for females, and our findings advocate for a significant conflict between mother and offspring over water resource. Future studies need to investigate female and offspring optimums over water requirements and the effects of dehydration during pregnancy on female survival and future reproduction (Bonnet et al. 2000; Bleu et al. 2011).

PARENT-OFFSPRING WATER CONFLICT AND REPRODUCTIVE TRADE-OFFS

Considering the predictions of the VCH (Crespi & Semeniuk 2004), a water conflict could be, at least in squamate reptiles, an important influence during the transition to viviparity. Constraints on gas and water requirements have previously been hypothesized as a key factor in limiting egg retention in oviparous species to typically no more than the first-third of embryonic development (Shine & Thompson 2006). Here, we have demonstrated a significant foeto-maternal conflict for water when water restriction was imposed in the middle third of embryonic development. Because embryonic demand for water increases dramatically during the last third of development, we posit that water availability and foeto-maternal conflict for water may constitute important selective limitations to viviparity.

Parent-offspring conflict theory has been empirically demonstrated for parental investment of energy and time (see Kölliker & Richner 2001 for a review). To our knowledge, no study has examined parental-offspring conflict for water. Water is a critical, often unpredictable resource, and water restriction can affect both survival and reproductive success (McKechnie & Wolf 2010). Water-based parent-offspring conflict may be taxonomically widespread. For example, water is a critical resource in mammals, since pregnancy alters water balance and decreases the osmotic thresholds for thirst and antidiuretic hormone release (Davison et al. 1984). Importantly, in humans, severe dehydration highly increases the risk of foetal death (Hirschhorn, Chowdhury & Lindenbaum 1969). In altricial species, water might represent a conflicting resource between parents and offspring. However, water conflicts are difficult to dissociate from energy conflicts, since water is typically provided concurrently with energy (e.g. in the form of milk for mammals) (Williams & Nagy 1985). Nonetheless, some species display specific adaptations that exclusively provide water to their offspring (Mougeot et al.

2014), and these species could be excellent study organisms for evaluating post-natal water-based parent–offspring conflict. Interestingly, parent–offspring conflict also applies in plants (Uma Shaanker, Ganeshiah & Bawa 1988), and water is likely a transgenerational conflicting resource especially during fruit formation. Therefore, while energy and time resources have attracted considerable interest over past decades, we emphasize that water demands may mediate a critical parent–offspring conflict influencing reproductive trade-offs and optimal reproductive effort and thus deserve greater attention.

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Data accessibility

Data from this paper are deposited in the Dryad Digital Repository, doi: 10.5061/dryad.mn40k. Dupoué *et al.* (2014b).

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