

Hydric “Costs” of Reproduction: Pregnancy Increases Evaporative Water Loss in the Snake *Vipera aspis*

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ABSTRACT

q1 Water constraints can mediate evolutionary conflict either among individuals (e.g., parent-offspring conflict, sexual conflict) or within an individual (e.g., cost of reproduction). During pregnancy, water is of particular importance because the female provides all water needed for embryonic development and experiences important maternal shifts in behavior and physiology that, together, can compromise female water balance if water availability is limited. We examined the effect of pregnancy on evaporative water loss and microhabitat selection in a viviparous snake, the aspic viper. We found that both physiological (increased metabolism and body temperature) and morphological (body distension) changes contribute to an increased evaporative water loss in pregnant females. We also found that pregnant females in the wild select warmer and moister basking locations than nonreproductive females, likely to mitigate the conflict between thermal needs and water loss. Water resources likely induce significant reproductive constraints across diverse taxa and thus warrant further consideration in ecological research. From an evolutionary perspective, water constraints during reproduction may contribute to shaping reproductive effort.

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Keywords: physiological trade-off, water loss, viviparity, behavioral mitigation.

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Introduction

Resource-based trade-offs are central to life-history theory. Across taxa, water is a vital resource, with water shortage often compromising individual performance and survival (Kleiner 1999). Thus, water limitations must be considered to fully understand reproductive strategies. For instance, brood attendance and selection of nesting sites can be influenced by risks of egg desiccation in both vertebrates and invertebrates (Stahlschmidt et al. 2011; Delia et al. 2013; Poo and Bickford 2013; Touchon and Worley 2015). Similarly, brood attendance can also depend on risks of parental dehydration (Chelini and Machado 2012; Consolmagno et al. 2016). In insects, water restriction can influence female mating decisions (Ursprung et al. 2009), and nuptial gifts can contribute to female hydration and enhance reproductive output and longevity (Ivy et al. 1999). Therefore, water constraints can mediate resource-based evolutionary conflict either between individuals (e.g., parent-offspring conflict, sexual conflict) or within an individual (e.g., cost of reproduction).

Viviparity has evolved repeatedly in a diversity of species among invertebrates, fishes, amphibians, reptiles, and mammals (Clutton-Brock 1991; Blackburn 2000) because it provides multiple benefits, from enhanced control of developmental conditions to the transfer of a diversity of fetomaternal nutrients (Bernardo 1996; Shine 2005; Van Dyke et al. 2014; Ostrovsky et al. 2015). However, pregnancy also imposes significant constraints on the female, such as prominent physical burden (i.e., the need to accommodate the mass and volume of the offspring) and modified behavior (i.e., increased basking frequency), that can reduce mobility and increase vulnerability to predators (Shaffer and Formanowicz 1996; Viérin and Bouissou 2001; Shine 2003). Pregnancy also extends the duration of the reproduction event and the related mismatch between energy expenditures and energy intake, which can alter the body condition and survival of the female (Bonnet et al. 1999; Lourdais et al. 2004a; Van Dyke and Beupre 2011). Thus far, “costs” associated with pregnancy have been predominantly considered in the framework of heightened predation risks and energy constraints. However, potentially of equal importance is the overlooked fact that pregnant females must provide water to their developing embryos and are particularly exposed to water loss (Kleiner 1999; Faichney et al. 2004; Arnott et al. 2012).

Embryonic development requires a vast amount of water and is sensitive to water shortage, notably in terrestrial (i.e., desiccating) environments (Taigen et al. 1984; Packard 1991; Vleck 1991; Du and Shine 2008). In viviparous species, maternal body water is required to supply embryonic demand, which increases exponentially over pregnancy (Cheung and Brace 2005; Lourdais et al. 2015). Different physiological

adaptations have arisen to support this specific resource allocation. Pregnancy is associated with increased blood and extracellular-fluid volume, lowered osmolality (Lindheimer and Davison 1995), and lowered thirst threshold (Davison et al. 1984; Olsson 2005). Increased expression of aquaporin to support water allocation to reproduction has also been demonstrated in mammals and insects (Hua et al. 2013; Benoit et al. 2014; Zhu et al. 2015). Yet pregnancy appears to be particularly sensitive to water limitation. Water shortage during pregnancy can result in altered maternal survival and reproductive output (Dauphin-Villemant and Xavier 1986; Ross and Desai 2005). Offspring traits can also be profoundly affected (Perillan et al. 2008; Arnott et al. 2012).

A conflict between pregnancy and water balance is expected in water-limited environments for several reasons. Water demand by the embryos is related to fecundity (Lourdais et al. 2015; Webber et al. 2015), and, as with energy, a significant maternal-fetal conflict for water should arise when this resource becomes limiting (Dupoué et al. 2015a). Beside direct allocation of water to the embryos, pregnant females are also more prone to water loss to the environment. Physiological changes associated with pregnancy (e.g., elevated metabolism) may increase evaporative water loss through increased ventilation or perspiration (Kleiner 1999). In addition, the body distension imposed by pregnancy increases skin surface area, which can enhance cutaneous evaporative water loss (Lillywhite 2006; Webber et al. 2015). Thus, it is not surprising that high ambient temperature induces water loss and thereby reduces amniotic fluid volume (Luton et al. 2004). Together, these effects likely impose water-based constraints when water availability is limited and should favor specific maternal behavior to increase water acquisition or minimize water loss.

Viviparity has evolved repeatedly among squamate reptiles (Blackburn 2006), which have characteristic traits that make them excellent study systems for examining the impact of pregnancy on water balance. Pregnant squamates may have increased evaporative water loss because they prefer a relatively high body temperature, which increases metabolic rate and requires that they bask in warmer, possibly more dehydrating microenvironments (Köhler et al. 2011; Lorigou et al. 2013). In addition, reproductive effort is pronounced in viviparous species, and this affects maternal morphology and body distension (Qualls and Shine 1995).

We previously demonstrated that physiological state influences evaporative water loss in the viviparous aspic viper (*Vipera aspis*; Dupoué et al. 2015b). Here, our aim was to focus on specific consequences of pregnancy, testing the following predictions regarding evaporative water loss:

1. Pregnant females exhibit physiological changes (increased ventilation, increased preferred body temperature) that increase evaporative water loss.
2. Pregnant females exhibit morphological changes (body distension) that increase evaporative water loss.
3. Pregnant females select warm and moist basking locations to mitigate the conflict between their thermal needs and water balance.

Material and Methods

Study Species

The aspic viper *Vipera aspis* is a small, viviparous snake of the western Palearctic region, and the thermal and reproductive physiology of this species has been intensely studied (Saint Girons 1952; Bonnet et al. 2001; Lourdais et al. 2002; Dupoué and Lourdais 2014). Gestation begins in early June (Naulleau 1981), and pregnant females have a higher preferred body temperature and bask more than nonreproductive females (Lorigou et al. 2013; Stahlschmidt et al. 2013). Parturition occurs in late August to early September (Lourdais et al. 2004b).

Capture and Maintenance

In spring 2010, we caught 39 female aspic vipers in west-central France from the neighboring districts of Loire Atlantique, Vendée, and Maine et Loire to examine evaporative water loss. In this area, there is no color polymorphism. Each female underwent an ultrasonographic examination (Micromaxx, SonoSite, Bothell, WA) to assess reproductive status (18 vitellogenic and 21 nonreproductive females). In spring 2012, from the same localities, we captured 8 reproductive and 8 nonreproductive females to evaluate cutaneous water loss.

At the lab, each female was measured (snout-vent length [SVL] \pm 0.5 cm), weighed (body mass [BM] \pm 0.1 g), and marked by clipping 2 mm of keratinized tissue from one to three ventral scales. Snakes were housed in experimental cages (100 cm \times 30 cm \times 35 cm). A thermal gradient (18°–41°C) was created within the cage by placing a 75-W light bulb over one side of the cage. Snakes were allowed to bask for 6 h per day, between 10 a.m. and 4 p.m. Room temperature was held constant at 18°C. Because vipers are low-energy specialists and infrequent feeders, long-term fasting is common, especially during pregnancy (Lourdais et al. 2002a). Thus, to avoid confounding effects of nutritional balance, no female (either pregnant or nonreproductive) was fed until the completion of the experiment. For each pregnant female, we recorded parturition date and metrics of reproductive output (litter size, litter mass; see details in Lourdais et al. 2002). After the completion of the experiment, each female was fed a mouse and released with its offspring at the exact site of capture. All procedures were in accordance with the ethical standards of the national research committee. Experiments were performed under the local permit A79-001 (Préfecture des Deux-Sèvres).

Evaporative Water Loss

We tested the impact of pregnancy on both total evaporative water loss (TEWL) and cutaneous evaporative water loss (CEWL). Measurements were made at late stages of gestation (on average, 55.9 \pm 2.1 days after the snakes' periovulatory ecdysis date, 78.6% \pm 2.2% through gestation). This period corresponds to a period of fetal life when physiological and morphological constraints are presumably highest (Dupoué et al. 2015a; Lourdais et al. 2015).

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TEWL. We used flow-through respirometry to measure TEWL (mg h^{-1}) and \dot{V}_{O_2} (mL h^{-1} ; see details on methods in Dupoué et al. 2015) at three trial temperatures: 15.0°C (assumed body temperature during inactivity), 25.0°C (body temperature frequently achieved by nonreproductive female aspic vipers; Ladyman et al. 2003; Lorigoux et al. 2013), and 33.0°C (mean preferred body temperature, T_{set} , of pregnant female aspic vipers; Lorigoux et al. 2013). We previously demonstrated that females shift their thermal preferences when pregnant and that this shift is independent of fecundity but related to embryonic thermal requirements (Lorigoux et al. 2013).

We placed each snake in a test chamber (500-mL glass jar, to minimize hygroscopicity of the respirometric system), which was housed in a temperature-controlled climatic chamber ($\pm 1^\circ\text{C}$; Cryosystem, air-conditioned system Carel MasterCella). Snakes were introduced into the chamber 3 h before testing to reach thermal equilibrium and avoid exploratory activity. Air supply to the test chambers was controlled by a mass flow controller (UFC-1100, Unit Instruments, Yorba Linda, CA). Air influx was $162.0 \pm 0.5 \text{ mL min}^{-1}$, which provided 99% air turnover within the test chamber every 15 min (Lasiewski et al. 1966). To obtain target humidity of the supply air, ambient air was first saturated by bubbling it through two serially connected, heated water columns and then through an empty column, all within a temperature-controlled chamber (400 DG, LMS, Sevenoaks, UK) set at 10.0°C. The empty column collected any water condensate due to initial supersaturation from the heated columns. The hydrated air, with a precise dew point of 10.0°C, then flowed to the test chamber. The dew point of the test chamber's effluent air stream was measured with a dew-point hygrometer (RH300, Sable Systems, Las Vegas, NV). The efflux was then passed through a column of desiccant (self-indicating Drierite) before entering the CO_2 analyzer (CA10, Sable Systems) and the O_2 analyzer (FC10A, Sable Systems). Minimally hygroscopic tubing (Bev-A-Line IV, Thermoplastic Processes, Stirling, NJ) was used throughout the system.

Measurements were made after stabilization of the dew point. Baseline values for the supply air were obtained at the beginning and end of each session by flowing the supply air through an empty test chamber. The lack of urine and feces production was confirmed at the end of each trial. We used hygrometric dew points to calculate vapor pressures with an eighth-order polynomial describing saturation vapor pressure as a function of air temperature (Flatau et al. 1992). Vapor pressures were used to calculate vapor densities with the ideal gas law (Campbell and Norman 1998). Finally, we used equations derived from Withers (1977) and Lighton (2008), and adapted to our setup to calculate TEWL and \dot{V}_{O_2} .

CEWL. We used a separate group of vipers (see above) to measure the rate of CEWL. We compared the rate of CEWL between 8 pregnant (mean \pm SE, BM = $88.7 \pm 4.6 \text{ g}$, SVL = $45.4 \pm 0.9 \text{ cm}$) and 8 nonreproductive (BM = $74.9 \pm 10.6 \text{ g}$, SVL = $46.2 \pm 2.6 \text{ cm}$) females. Measurements were carried out at room temperature (25°C), and snakes were acclimated to this temperature for at least 2 h before measurement.

We used an AquaFlux AF200 (Biox, London) and the Biox software AquaFlux 6.2 to calibrate and compute CEWL rate ($\text{g m}^{-2} \text{ h}^{-1}$) from a single dorsolateral location at the snake's mid-body (Guillon et al. 2014). An O-ring of closed-cell foam was attached around the AquaFlux probe's orifice to assure a complete seal between the device and the snake's skin. Contact was maintained by gently restraining the snake and applying steady but slight pressure to the probe against the skin. Trials continued until the CEWL reading stabilized ($\pm 0.02 \text{ g m}^{-2} \text{ h}^{-1}$) for 10 s. The CEWL reading was numerically and graphically displayed in real time, and equilibration typically took 35 min (as surface water was cleared and only water from CEWL contributed to the reading). If any movement (by the snake or the investigator) caused a leak in the seal between the probe and the snake, this was easily detected as a sudden change in the graphic display's clearance curve, and the trial was repeated. The AquaFlux unit was calibrated at the beginning of each trial.

Morphometric Measurements

We examined the influence of different allometric determinants on the rate of TEWL. Notably, we examined the relationship between water loss and skin distension imposed by pregnancy. For each individual, we collected pictures of the left side at mid-body (Panasonic DMC TZ2), and we used Inkscape software (ver. 0.15) to determine interscale distance. We measured the distance (mm) between two scale apices separated by a scale in the same scale row (length) and between two adjacent scale rows (width; Fig. 1). Each measure was collected in triplicate. We added the mean length (intraindividual variation: $7.4\% \pm 0.6\%$) and width (intraindividual variation: $7.3\% \pm 0.6\%$) as an estimate of interscale distance (mm). This measure integrates the size of the scales, and therefore the size of the individual, but also the interscale spaces. We derived the residuals from the relationship between the interscale distance and SVL ($F_{1,24} = 23.4, P < .001$) to estimate the interscale distension, which represents the residual variation in scale distance not explained by maternal body size.

Microclimatic Conditions of Basking Sites

The field component of this study was conducted at a long-term population monitoring site in west-central France (Loire Atlantique district; $47^\circ 29' \text{N}$, $1^\circ 45' \text{W}$; Guiller et al. 2012). The study site is approximately 200 ha and composed of pastures separated by a network of hedgerows. We wanted to determine whether pregnant females have specific microclimatic requirements for basking in open sunlight. We captured 29 reproductive and 35 nonreproductive females from May to August in 2009 and 2010. At capture, we recorded the temperature and relative humidity (RH) at the location (ground level) where each viper was detected, using a thermohygrometer (Votcraft HT-200; $\pm 0.5^\circ\text{C}$ and $\pm 2\% \text{ RH}$). We converted RH to absolute humidity (AH) using the equation $\text{AH} = \text{RH} \cdot 6.11 \cdot 10^{7.5 \cdot T / (237.7 + T)}$ (Flatau et al. 1992). While we did not acquire the full distribution of operative temperatures

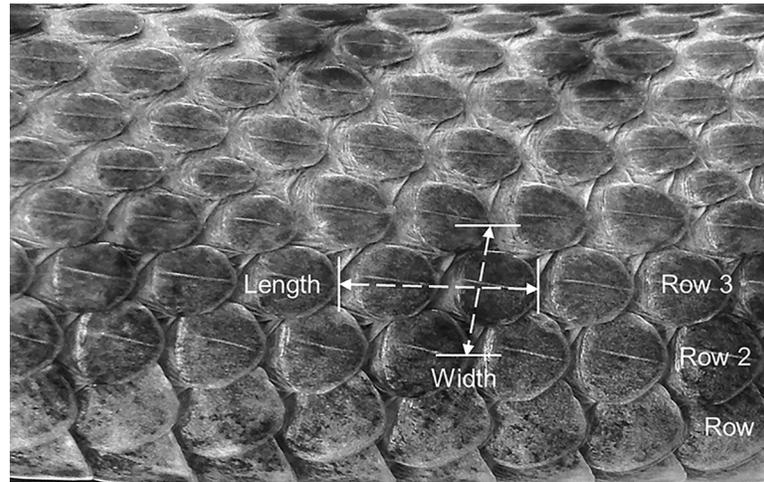


Figure 1. Method used to measure scale distention. We measured the distance (mm) between two scale apices separated by a scale in the same scale row (length) and that between two adjacent scale rows (width). See text for details.

and humidities, this approach still enabled us to compare selected basking microclimates between reproductive statuses.

Statistical Analyses

All statistical analyses were performed with R software (R Development Core Team, ver. 2.13.1). All data were \log_{10} transformed in analyses to achieve normality. We checked that the residuals of our models respected this condition (Shapiro-Wilk test; all $P > .05$).

First, we used a linear mixed model (package nlme) to examine the impact of test temperature (i.e., 15°, 25°, or 33°C) and reproductive status (i.e., pregnant or nonreproductive) on TEWL and $\dot{V}O_2$. Female SVL was included as a linear covariate to correct for body size variation. We did not use animal BM because of allometric contrast between pregnant and nonreproductive females. Notably, female BM changes dramatically over pregnancy, and we were not able to evaluate the respective masses of their metabolically active (maternal soma, developing embryos) versus metabolically inactive compartments (yolk, water incorporated in the fertilized ova). We included female identity as a random factor to account for repeated measures. We used pairwise post hoc Tukey tests (package lsmeans) to test for differences between reproductive statuses at each temperature. We used least squares means (package lsmeans) to adjust values of TEWL by SVL in the figure.

We used linear models to test the relationship between the rate of TEWL and $\dot{V}O_2$ in pregnant and nonreproductive females separately. We tested the influence of different allometric traits (i.e., SVL, interscale distension) on the rate of TEWL in pregnant females, using linear models. We considered only data collected at 33°C, which corresponds to pregnant-female thermal preference. We considered the model with the smallest Akaike information criterion (AIC) to be the best determinant of the rate of TEWL. When two models differed by less than 2 in AIC, they were

considered equivalent, and the model with the fewer variables was used.

We used linear models to test the effect of reproductive status on the rate of CEWL. We also used a linear model to test the relationship between CEWL or reproductive effort and interscale distension in pregnant females. Finally, we used linear models to analyze the effect of reproductive status on temperature and water-vapor pressure selected by vipers in the field with the reproductive status, the month (i.e., May, June, July, or August), and the interaction between these two parameters as factors. Because of limited sample size, we pooled the data from the two years together. Previous investigations on the data set revealed that there was no effect of year on selected temperature ($F_{1,62} = 2.3$, $P = .134$) or water-vapor pressure ($F_{1,62} = 0.1$, $P = .738$).

Results

TEWL

Pregnant vipers had higher BM than nonreproductive ones ($F_{1,37} = 5.4$, $P = .026$), but the two reproductive statuses had similar SVL ($F_{1,37} = 0.1$, $P = .739$). The TEWL was influenced by SVL ($F_{1,36} = 7.3$, $P = .011$), temperature ($F_{2,67} = 194.1$, $P < .0001$), reproductive state ($F_{1,36} = 31.0$, $P < .0001$), and the interaction between temperature and reproductive status ($F_{2,67} = 6.7$, $P = .002$). The TEWL of pregnant females was higher than that of nonreproductive females at all temperatures (Fig. 2).

When adjusted for SVL, $\dot{V}O_2$ of pregnant females was higher than that of nonreproductive females at 15°C (pregnant size-adjusted $\dot{V}O_2 = 3.57 \pm 0.62$ mL h⁻¹, nonreproductive size-adjusted $\dot{V}O_2 = 0.84 \pm 0.58$ mL h⁻¹; $F_{1,36} = 11.5$, $P = .002$), 25°C (pregnant size-adjusted $\dot{V}O_2 = 5.92 \pm 0.62$ mL h⁻¹, nonreproductive size-adjusted $\dot{V}O_2 = 3.85 \pm 0.66$ mL h⁻¹; $F_{1,31} = 4.2$, $P = .050$), and 33°C (pregnant size-adjusted

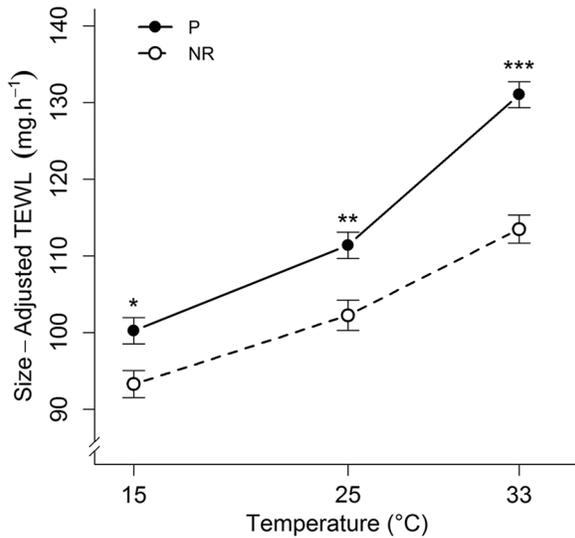


Figure 2. Total evaporative water loss (TEWL) of pregnant (P; $n = 18$) and nonreproductive (NR; $n = 21$) females at three temperatures. Points represent mean (\pm SE) TEWL (mg h^{-1}) adjusted by snake snout-vent length with residuals from a regression. Statistical effect of reproductive status: *** $P < .001$, ** $P < .01$, * $P < .05$.

$\dot{V}_{\text{O}_2} = 10.37 \pm 0.62 \text{ mL h}^{-1}$, nonreproductive size-adjusted $\dot{V}_{\text{O}_2} = 7.20 \pm 0.61 \text{ mL h}^{-1}$; $F_{1,34} = 11.1$, $P = .002$). We found a significant positive influence of \dot{V}_{O_2} on TEWL in pregnant and nonreproductive females (Table 1). When 33°C is considered, \dot{V}_{O_2} explains 21% and 48% of the variations of TEWL in pregnant and nonreproductive females, respectively (Table 1).

Focusing on pregnant females, we found that interscale distension (residuals) was the best determinant to explain the variation in TEWL (Table 2). The model including both animal size and interscale distension explains an equivalent degree of variation, but it includes two explanatory variables and therefore is less parsimonious than the model with only interscale distance (Table 2).

CEWL

The rate of CEWL was higher in pregnant than in nonreproductive females ($F_{1,14} = 60.9$, $P < .001$; Fig. 3). Pregnant females have a CEWL rate that is 46% higher than that of nonreproductive females. Furthermore, in pregnant females, CEWL is strongly related to the interscale distension ($F_{1,6} = 17.3$, $P < .01$, $r^2 = 0.74$).

Reproductive Effort and Scale Distension

Data on reproductive effort and scale distension were collected for 26 females. We found that variation in interscale distension was positively related to litter size ($F_{1,24} = 7.1$, $P = .013$, $r^2 = 0.23$). This relation was also found when accounting for female size (residuals of the linear relationship

between litter size and female SVL: $F_{1,24} = 11.7$, $P = .002$, $r^2 = 0.33$; Fig. 4).

Microclimate Conditions of Basking Sites

We found a significant effect of reproductive status on selected temperature ($F_{1,56} = 4.8$, $P = .033$; Fig. 5a) and on selected water-vapor pressure ($F_{1,56} = 9.0$, $P < .01$; Fig. 5b) of free-ranging asp viper. We also observed a significant effect of month on selected temperature ($F_{3,56} = 6.4$, $P < .001$) and water-vapor pressure ($F_{3,56} = 2.9$, $P = .044$). Selected water-vapor pressure is higher during July than during May (Tukey's post hoc test, $P = .039$). We found no interaction between reproductive status and month when considering selected temperature ($F_{3,56} = 0.8$, $P = .485$) or water-vapor pressure ($F_{3,56} = 0.1$, $P = .984$), highlighting a similar contrast in microclimate selection between pregnant and nonreproductive females throughout the summer.

Discussion

Allocation trade-offs are critical for understanding life-history strategies and reproductive modes (Clutton-Brock 1991; Crespi and Semeniuk 2004). While energy constraints are frequently considered a mediator of reproductive costs (Bergeron et al. 2011; Blount et al. 2016), water constraints have received limited attention (Ofstedal 2002; Ross and Desai 2005; Stein and Badyaev 2011). Our study of a viviparous snake highlights that physiological, morphological, and behavioral changes during pregnancy all contribute to increased TEWL. Our field data suggest that females mitigate these losses by selecting basking sites that have higher humidity.

Influence of Temperature and Reproductive Status

We found a strong influence of temperature and reproductive state on TEWL. In support of our first prediction, we found that pregnant females had higher size-adjusted TEWL rates than did nonreproductive females (7.4%, 8.9%, and 15.4% higher at 15°, 25°, and 33°C, respectively). Pregnant vipers select higher body temperatures and bask for prolonged pe-

Table 1: Correlation between total evaporative water loss and \dot{V}_{O_2} for each reproductive status and the three test temperatures

Status, temperature	r^2	P
Pregnant:		
33°C	.21	.001
25°C	.22	.012
15°C	.03	.518
Nonreproductive:		
33°C	.31	<.001
25°C	.47	>.001
15°C	.18	.043

Note. Boldface indicates statistically significant ($P < .05$).

Table 2: Model selection to compare the influence of different determinants of total evaporative water loss in pregnant females ($n = 18$)

Model	df	AIC	r^2	P
Snout-vent length (SVL; cm)	3	-59.8	.15	.112
Interscale distension (residuals)	3	-61.6	.23	.042
SVL + interscale distension (residuals)	4	-63.6	.38	.027

Note. This analysis was conducted at 33°C, which corresponds to female thermal preference during pregnancy. See text for details. AIC = Akaike information criterion.

riods when compared to nonreproductive ones (Lorioux et al. 2013). Combining the thermal sensitivity of TEWL, the direct effect of reproductive state on TEWL, and the thermal shift associated with pregnancy, the overall result is that TEWL in pregnant females at their preferred temperature (T_{set} , 33°C) is, on average, 28.1% higher than that in nonreproductive females at their typical body temperature (25°C). Therefore, pregnancy in aspic vipers has a substantial effect on fluid loss, as reported in mammals (Kleiner 1999).

Proximate Determinants of TEWL

Gestation is well known to increase metabolic rates in vertebrates (Kleiner 1999; Van Dyke and Beaupre 2011; Dupoué and Lourdais 2014). Likewise, we found that pregnant females had higher $\dot{V}O_2$ at all temperatures. We also found that $\dot{V}O_2$ and TEWL were correlated, supporting a proximate link between oxygen consumption and water loss. However, the proportion of variance explained by $\dot{V}O_2$ was lower for pregnant than for nonreproductive females, suggesting other influences. Skin surface exposure is an important component of TEWL (Dmi'el 1972, 1998; Lillywhite 2006), and pregnant females face significant body distension as a result of embryonic growth. When focusing on pregnant females, we detected a significant influence of interscale distension on TEWL. Body distension increases the surface area from which water can be lost and therefore increases absolute CEWL. The soft skin between scales may be more permeable to water exchange, although this idea has been questioned (Licht and Bennett 1972; Bennett and Licht 1975; Toni and Alibardi 2007). In support of our TEWL results, we found that pregnant females had higher surface area-specific CEWL than did nonreproductive females. We also found a close relationship between scale distension and CEWL among pregnant females, thereby supporting a proximate influence of interscale skin exposure on water loss. Increased transepidermal water loss may also be related to the alteration of skin structure and lipid barrier due to stretching, as reported in humans (Henry et al. 1997; Rawlings et al. 2012).

A recent study in the bark scorpion (*Centruroides sculpturatus*) demonstrated elevated water loss in pregnant females because of increased exposure of the pleural membrane and increased selected temperatures (Webber et al. 2015). Therefore, morphological and physiological changes associated with pregnancy may alter water loss in a diversity of taxa. The different

determinants of increased water loss may differ in their relation to fecundity. That is, increased metabolic rate and physical burden are closely related to reproductive effort (Dupoué and Lourdais 2014; Webber et al. 2015). In contrast, maternal shift in thermal preferences reflects optimal developmental temperature (Bernardo 1996; Lorioux et al. 2013) and is not related to the number of developing embryos (Bull and Shine 1979).

Evidence for Behavioral Mitigation

Microhabitat selection based on thermal properties has been largely demonstrated in ectotherms (Blouin-Demers and Weatherhead 2002). However, the integration of hydric properties of the environment is likely key to better understanding habitat use by both terrestrial ectotherms (Davis and DeNardo 2009; Peterman and Semlitsch 2014; Pintor et al. 2016) and endotherms (Dunkin et al. 2013). High ambient water-vapor pressure passively limits water loss (Mautz 1982; Dupoué et al. 2015b), which would provide females the opportunity to carefully select humid microclimates to address thermal requirements while limiting water loss (Stahlschmidt and DeNardo 2010; Guillon et al. 2014). In support of our last prediction, we found that pregnant females tend to select warmer and moister basking sites in the field when compared to nonreproductive ones. This result is consistent with previous studies that experimentally demonstrated the importance of humidity in microclimate selection by gravid snakes (Stahlschmidt et al. 2011; Dupoué et al. 2015b).

Evolutionary Implications

Water is critical for supporting embryonic growth (Brace and Cheung 2014; Lourdais et al. 2015). Yet water-based trade-offs during reproduction remain largely understudied from both eco-

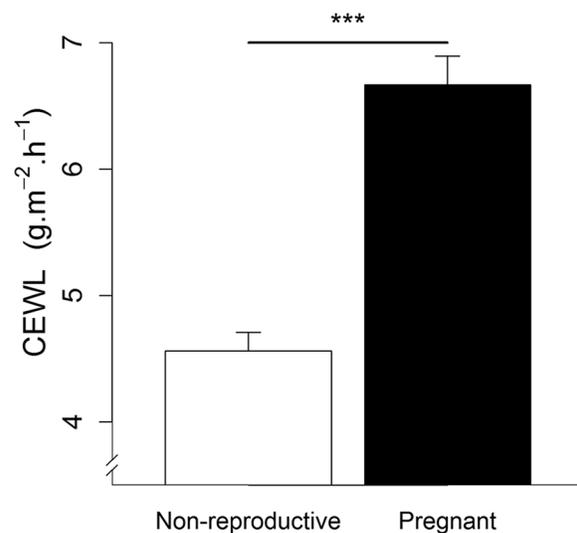


Figure 3. Cutaneous evaporative water loss (CEWL) of pregnant ($n = 8$, filled bar) and nonreproductive ($n = 8$, open bar) female vipers. Bars represent means (\pm SE) of CEWL ($\text{g m}^{-2} \text{h}^{-1}$). Statistical effect of reproductive status: $***P < .001$.

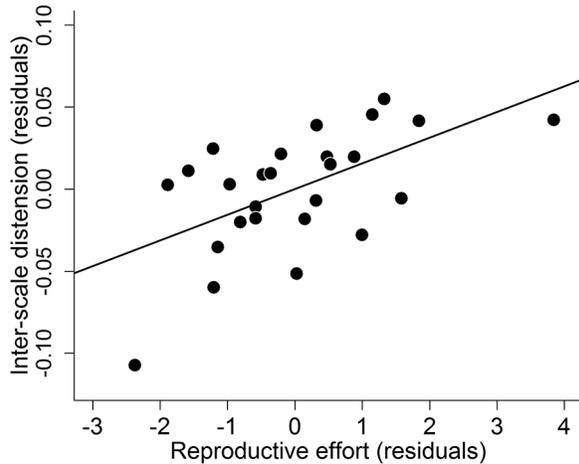


Figure 4. Relation between reproductive effort (residuals of the relationship between litter size and female snout-vent length [SVL]) and interscale distension (residuals from the relationship between the interscale distance and SVL). See text for statistics.

logical and evolutionary perspectives. Contrary to energy, where large amounts can be accumulated and stored in advance (as body fat), for most species there are fewer options for maintaining an endogenous water resource to anticipate and cope with an environmental shortage. While some species can use the urinary bladder as a centralized water reservoir (e.g., tortoises: Peterson 1996; Gila monsters: Davis and DeNardo 2007), most species are limited to more distributed and limited water resources, such as those associated with edema or the catabolism of body tissues. Despite the relatively small storage capability compared to the urinary bladder, the catabolism of tissues can provide significant quantities of water, and water limitation may dictate which tissues are catabolized (Rutkowska et al. 2016). Thus, a better understanding of the means of and potential for water storage is needed to fully understand how water constraints influence physiological processes such as reproduction.

The relation between reproductive effort and the various potential “costs” is critical for understanding life-history traits such as fecundity. Water allocation constraints may well contribute to shaping reproductive effort. For instance, we previously demonstrated (1) that pregnant vipers with larger reproductive effort must supply more water to their embryos (Lourdais et al. 2015) and (2) that a fetomaternal conflict for water exists under water restriction and is heightened by fecundity (Dupoué et al. 2015a). The current study demonstrates that females with larger reproductive effort also face greater loss of water to the environment. Water constraints are likely to exist even in temperate climates, notably during episodes of summer drought (Rasmont and Iserbyt 2012). While this concept must be addressed more thoroughly in the field, pregnant females caught in the wild often drink readily when water is provided in captivity (G. Guiller, personal observation), suggesting significant dehydration, as seen in other snake species (Lillywhite et al. 2015). We posit that water limitations must be considered, and, when present, must be incorporated into efforts to understand reproductive and allocation strategies.

Effects of water availability on reproduction are well known in human clinical medicine and animal science because it compromises fluid exchanges and blood flow (Kleiner 1999; Ciglenecki et al. 2013). For example, lowered amniotic volume (oligohydramnios) in women is more frequent during summer and is worsened by heat waves (Luton et al. 2004; Feldman et al. 2009). More generally in mammals (including humans), dehydration during pregnancy can result in higher fetal and/or maternal death and altered offspring traits (Hirschhorn et al. 1969; Ross and Desai 2005). Water limitations during reproduction likely induce significant reproductive costs across diverse taxa and thus warrant further consideration in ecological research.

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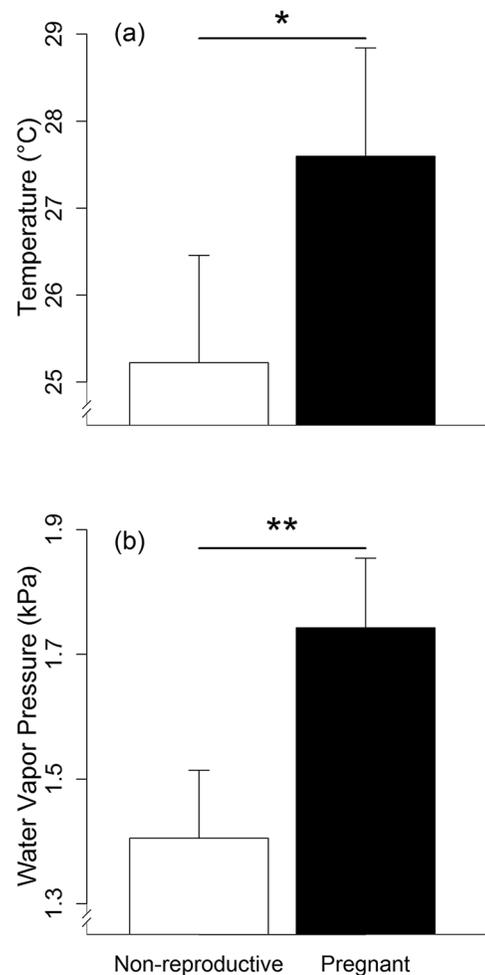


Figure 5. Microclimate conditions in basking sites of pregnant (filled bars) and nonreproductive (open bars) females. Bars represent mean (\pm SE) ground temperature ($^{\circ}$ C; a) and water-vapor pressure (kPa; b) of selected microclimates. Statistical effect of reproductive status: ** $P < .01$, * $P < .05$.

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