

Dedicated mothers: predation risk and physical burden do not alter thermoregulatory behaviour of pregnant vipers



Sophie Lorionx^{a,b,c,*}, H el ene Lisse^a, Olivier Lourdais^{a,b}

^a Centre d'Etudes Biologiques de Chiz e, CNRS UPR 1934, Villiers en Bois, France

^b School of Life Sciences, Arizona State University, Tempe, AZ, U.S.A.

^c Universit e de Poitiers, Poitiers, France

ARTICLE INFO

Article history:

Received 4 October 2012

Initial acceptance 2 January 2013

Final acceptance 6 May 2013

Available online 2 July 2013

MS. number: 12-00768R

Keywords:

aspic viper
predation
pregnancy
prenatal care
refuge use
thermoregulation
Vipera aspis

Escape tactics and optimal refuge use have attracted considerable interest, but the influence of pregnancy on escape tactics remains understudied. For instance, embryonic sensitivity to environmental conditions and maternal constraints vary dramatically across pregnancy, and these dynamics should modulate the cost–benefit balance of refuge use. We experimentally studied thermoregulation and refuge use in pregnant and nonreproductive aspic vipers, *Vipera aspis*, at different stages of gestation (first, middle and last third). We determined preferred body temperatures (T_{set}) in a cost-free environment to test for fecundity and stage dependencies of maternal T_{set} . Then, we examined behavioural responses to repeated simulated predatory attacks. Pregnant females were extremely precise thermoregulators compared with nonreproductive females, and T_{set} was independent of litter size or gestation stage. After simulated attacks, pregnant females limited their time spent in the refuge and thereby their deviation from T_{set} . By contrast, nonreproductive females had a greater response to predation exposure and adjusted their response to risk level. Contrary to our predictions, pregnant females did not vary their behaviour based on gestation stage, despite increasing physical burden as pregnancy progressed. Overall, our results illustrate that pregnant females alter their behaviour to benefit their developing embryos thermally despite risk to themselves (increased exposure). By doing so, however, the female realizes a benefit by accelerating temperature-sensitive developmental time and thus reducing the duration of pregnancy and associated physical burden.

  2013 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Predation is a major selective and ecological pressure that influences exposure strategy, activity budget and behaviour. Behavioural decisions should balance the fitness costs and benefits of predator avoidance (Sih 1980; Cooper 2009). Although predation risk can be reduced by occupying refuges that offer effective protection (Cooper et al. 1999; Goldsborough et al. 2004; Cooper & Wilson 2008), some life history stages, as well as some physiological and behavioural processes, require significant exposure (Schwarzkopf & Shine 1992; Webb & Whiting 2005). For instance, in ectotherms, behavioural thermoregulation, which often requires direct exposure to the sun, is essential to maintain body temperature within an optimal range (Huey & Slatkin 1976) and enhance major physiological processes and performances (Angilletta 2009). Terrestrial squamates usually escape from predators by fleeing into the nearest refuge, which, in mild climates, typically provides suboptimal temperature for performance (Mart ın 2001). Therefore,

refuge use is often associated with thermal costs, as it induces a time-dependent decrease in body temperature. In that context, emergence behaviour should balance a decrease in predation risk over time against thermal costs and the opportunity costs for other activities (Mart ın 2001; Amo et al. 2007).

Although the determinants of escape tactics and refuge use in ectotherms have attracted considerable interest, few studies have addressed the effect of reproductive status and pregnancy on these variables (Bauwens & Thoen 1981; Amo et al. 2007). In viviparous squamates, pregnant females display prolonged basking behaviours, modified thermal preference (either higher or lower than that of nonreproductive females), and more stable body temperatures (Shine 1980, 2006). Maternal thermoregulation is thought to be beneficial in that it accelerates developmental rate (Shine & Bull 1979; Tinkle & Gibbons 1979) and improves offspring quality (Shine 1995; Lorionx et al., in press). Contrary to most postnatal maternal care, the intensity of which depends on fecundity, prenatal thermoregulation should be driven by embryonic thermal sensitivity and therefore be independent of the number of developing offspring (Bernardo 1996; Angilletta 2009). Yet, to our knowledge this simple assumption has never been thoroughly tested.

* Correspondence: S. Lorionx, CEBC-CNRS UPR 1934, 79360 Villiers en Bois, France.

E-mail address: sophie.lorionx@gmail.com (S. Lorionx).

Although thermal requirements are likely to be fecundity independent, the ability of the female to thermoregulate and the risks associated with thermoregulation may be more sensitive to reproductive condition. Pregnancy is associated with significant physical burden (Shine 1980; Seigel & Ford 1987) and impaired locomotor capacities (Brodie 1989; Le Galliard et al. 2003; Shine 2003), both of which are accentuated with litter size and progressing reproductive stage. The combination of increased exposure in order to meet thermoregulatory requirements and impaired locomotor effort associated with pregnancy may lead to considerably greater predation risk for pregnant females, which could be fecundity dependent.

Because of the substantial implications of predation risk, predation avoidance tactics may influence maternal behaviour (Kurdiková et al. 2011). In this context, Schwarzkopf & Andrews (2012) recently proposed the selfish mother hypothesis (SMH), stating that behavioural and physiological changes associated with reproduction may reflect benefits to the female more than to the developing offspring. Alternatively, changes in body temperature associated with reproduction may be a passive reflection of the burden of pregnancy and be dependent on reproductive load (Schwarzkopf & Andrews 2012). Although stimulating, these interpretations have been challenged, and only limited supporting evidence exists (see DeNardo et al. 2012; Shine 2012).

To understand maternal response to predation risks better, the dynamic nature of pregnancy must be considered. Embryonic development is a multiphase process, and early embryonic stages are particularly susceptible to environmental perturbations as a result of strong developmental constraints (Andrews 2004; Lorioux et al. 2012, in press). Meanwhile, physical burden increases as gestation progresses because of (1) embryonic water uptake that occurs during late stages (Dauphin-Villemant & Xavier 1986) and (2) progressive protein mobilization that alters maternal musculature and performance (Lourdais et al. 2004a, 2013). Based on these dynamics, it is reasonable to postulate that pregnant females adjust their predation avoidance behaviours over the course of development, with females favouring thermoregulation over refuge use during early stages of development when embryos are more sensitive (Lorioux et al. 2012, in press) and females are more agile.

By assessing thermoregulation and escape tactics in pregnant and nonreproductive aspik vipers, *Vipera aspis*, at different stages of gestation (first, mid and last third), we addressed the following two predictions: (1) in a cost-free environment, pregnant females thermoregulate more precisely than nonreproductive ones and selected body temperature should be fecundity independent; (2) under the risk of predation, pregnant females should favour thermoregulation at early (i.e. thermally sensitive) embryonic stages, whereas predator avoidance should increase at later pregnancy stages because of a reduced thermal sensitivity and an increased physical burden.

METHODS

Study Species and Breeding

We used a long-term captive colony of aspik vipers. The aspik viper is a small venomous snake inhabiting the Western Palearctic region. This species is a typical capital breeder (storage is the main source of energy for reproduction) and accumulates energy over an extended period before engaging in reproduction (Bonnet et al. 2002; Lourdais et al. 2002). Gestation is associated with an increase in thermal preference and precision, and this necessitates an increase in thermoregulatory activities (Saint Girons 1952; Naulleau 1979; Ladyman et al. 2003). Gestation is also associated

with a decrease in movement and food intake (Saint Girons 1952). Reproduction in females has high energetic demands (Bonnet et al. 2002; Ladyman et al. 2003), which lead to substantial emaciation at parturition (Lourdais et al. 2002) and low reproductive frequency (typically biennial to triennial).

After a 2-month wintering period at 8 °C, 30 males and 30 female vipers were transferred to experimental cages (100 × 30 cm and 35 cm high). The front of each cage was made of glass, which enabled observation of the snake (see below). A thermal gradient (18–41 °C) was created within the cage by placing a 75 W incandescent light bulb over one side of the cage. The light was on for 6 h per day, enabling basking from 1000 to 1600 hours. Room temperature was held constant at 18 °C. Two males and two females were housed in each experimental cage. Each day for 4 weeks, one male was replaced with a new male to encourage courtship and mating. We observed no agonistic behaviour between males; nor were any males injured as a result of co-housing males. None of the males were fed during this period, as they are typically anorectic during the greater part of the breeding season (Saint Girons 1986; Naulleau et al. 1987).

Reproductive Status and Housing Conditions

At the end of the breeding period, males were removed, and we assessed female reproductive condition using ultrasonography (MicroMaxx, SonoSite, Inc., Bothell, WA, U.S.A.). Nineteen females were nonreproductive (NR). Embryonic development was monitored by regular ultrasonography of the 11 pregnant (P) females, with embryonic stage estimated based on the amount of yolk present and on developmental tables (Hubert & Dufaure 1968). All ultrasonic observations were performed within 10 min to reduce disturbance and stress. At parturition, we determined, for each pregnant female, the 'fit litter size', which is the number of healthy offspring born.

All females were then randomly assigned to one of the 12 experimental cages, some housing two females and some housing three because of space limitations. As a result of the random assignment: five enclosures contained one pregnant female and one nonreproductive female; six enclosures contained one pregnant female and two nonreproductive females; and one enclosure contained two nonreproductive females. Importantly, no agonistic behaviour was observed in co-housed individuals. Females were maintained throughout gestation in the experimental cages and under the same conditions as described above (100 × 30 cm and 35 cm high), but with a couple of modifications. The front of each cage was covered to avoid visual disturbances and thus limit perceived threats. On the side opposite that with the light bulb, we provided an opaque, half-cylinder PVC shelter (diameter = 15 cm, length = 37 cm) with two 3 cm circular openings facing the light source. This refuge allowed snakes to escape disturbances and was also used as a nocturnal shelter (S. Lorioux, personal observations). Additional shelters (clay roof tiles) were provided to facilitate movement between the main shelter and the basking area. As vipers are low-energy specialists and infrequent feeders, long-term fasting is a natural event, notably during pregnancy (Lourdais et al. 2002). Thus, to avoid confounding effects of nutritional balance, none of the females was fed until after the experiment.

Body Temperature

Temperature profiles were recorded using miniaturized iButton data loggers (DS1922L, Dallas Semiconductor, Dallas, TX, U.S.A.; modification as described in Robert & Thompson 2003). All data loggers were previously calibrated in climatic chambers (Vötsch Industrietechnik, VP600, Balingen, Germany) using a mercury

thermometer (Fisher Scientific, Illkirsch, France). We used four calibration temperatures (15, 20, 25 and 33 °C) that spanned the range selected by females and produced a specific calibration equation for each logger. The loggers were programmed to record temperature every 2 min for 6 days. One day before an experimental trial, each female was anaesthetized (5% isoflurane in air) and a data logger was placed into the throat and gently palpated down into the stomach. After the procedure, each snake was individually monitored through recovery until voluntary movements (tongue flicking) appeared. Air was administered via an endotracheal tube to hasten recovery, which took 10 to 20 min. No adverse effects were observed as a result of the procedure. Data loggers were either regurgitated (40%, average of 15 days postplacement) or, if regurgitation did not occur, retrieved by gentle manual palpation up the oesophagus (60%).

Study Design

Thermoregulation and behavioural trials were conducted in the first, middle and last third of pregnancy ($N = 11$ females). These periods are relevant, as they match important steps in embryonic life, including organization, growth and fetal life. NR females ($N = 19$) were used as a control group and tested at the same three time points (designated as sessions S1 to S3). On the first 2 days, we assessed the response to simulated attacks. During the last 4 consecutive days, females were left undisturbed in a cost-free environment to assess thermal preference (T_{set}).

Thermoregulation in a cost-free environment

We compared the body temperatures (T_b) of pregnant females and nonreproductive females in cost-free conditions (see housing conditions described above). For each session and each individual, we calculated T_{set} by averaging all T_b values collected during the basking period (1200 and 1600 hours) and pooling the 4 days of monitoring (Hertz et al. 1993; Christian & Weavers 1996). $T_{set\ low}$ and $T_{set\ high}$ were derived from the bounds of the central 50% of selected T_b for each individual.

We also considered two derived variables to assess within- and between-female variation in T_b . First, we determined the extent of within-female daily T_b variation by examining daily T_b standard deviation calculated during the basking period of each day and for each session. Second, to measure the influence of reproductive status on between-female daily T_b variation, we calculated for each individual the absolute deviation between mean daily T_b and the grand mean of daily T_b derived from all females with the same reproductive status.

Response to simulated predator attack

Repeated predatory attacks are known to influence behavioural decisions (Martín & López 2001), so we simulated such a scenario. We again used the same experimental cages, but the front was not covered and only the PVC refuge was provided at the side opposite the light source. The light source came on 1 h before the start of the trial to allow the enclosure to reach thermal stability.

To control for initial body temperature, the female was cooled for 1 h at 16 °C in a climatic chamber (LMS LDT, Sevenoaks, Kent, U.K.; stability ± 1 °C), and then placed into the experimental cage, where recording (Sony, HDR XR100, Sony corporation, Tokyo, Japan) started. After 90 min in the experimental cage, the operator (the same operator conducted all behavioural trials using a standardized methodology) simulated a first attack with brief, repeated contacts (one contact every 0.5 s) with the posterior half of the female's body using 60 cm surgical forceps (Endo Babcock, Autosuture, Covidien, Mansfield, MA, U.S.A.). This manipulation

typically led to the female swiftly retreating into her refuge. In cases where animals were hidden at the time of an attack, they were not subjected to a simulated attack and they were left undisturbed. All animals were then left for another 60 min, during which time the animal typically re-emerged to thermoregulate (94.8%). A second simulated attack was then performed using the same procedure and the trial was stopped 60 min later (total recording time 210 min). Between trials, cages and shelters were cleaned with 70% alcohol to remove residual odours.

Variables examined

For each individual, we assessed the proportion of time spent in exploration, basking and under the shelter during each trial. We measured shelter time (time between entering the refuge and emergence of the whole body). We also examined scanning time at the time of emergence, as this variable is an important component in squamate escape tactics (Avery et al. 1993). When aspik vipers first emerge from a refuge, they usually elevate their head 5–10 cm above the ground and maintain this upright posture. Although no studies have experimentally evaluated the value of raising the head, increased ability to view one's surroundings (i.e. scanning) is the most likely role for this, and we thus refer to the duration of this behaviour as 'scanning time'. We determined the proportion of animals staying in the refuge after an attack (no emergence) and the proportion of individuals already hidden at the time of the simulated attacks. We assessed thermal costs of refuge use by calculating the difference between body temperature at the time of the attack and minimal value subsequently recorded while in the refuge to evaluate thermal costs experienced by the females. We also calculated mean T_b after a predatory attack using temperatures 30–60 min after an attack. We ignored the first 30 min postattack to provide the female time to re-emerge. For this analysis, we removed individuals that remained undercover for the entire 60 min after the attack.

Body size is usually a proximate determinant of reproductive effort in snake (Bonnet et al. 2003), and it was also the case in this study (correlation between litter size and mother's snout–vent length (SVL): $R^2 = 0.45$, $P < 0.001$). To address the effects of fecundity on thermoregulatory behaviour, we needed to control for allometric influences, so we calculated size-adjusted litter size by extracting residuals of the regression of litter size on mother's SVL.

Statistical Analyses

All statistical analyses were performed using R software (version 2.12.1; R Development Core Team 2010). As the same individuals were tested for each session, we used linear mixed models for longitudinal data (lme models, nlme package), with reproductive status and session (effect of time) as co-factors, and day and mother identity as random effects. Attack repetition (first versus second attack) was also included in the models for analyses of emergence behaviour to account for the effects of repeated predatory attacks. Proportional data were first logit-transformed (Warton & Hui 2011), and this transformation ensured normality assumptions. As a result of the non-normality of the residuals, shelter time and scanning time were log transformed. However, this transformation did not satisfy normality assumptions, so we used a Box–Cox transformation of the data (Peltier et al. 1998) for linear models. Optimal λ was assessed using the 'boxcox' procedure (package MASS). Data were then transformed using the 'bcPower' procedure (package car), and analysed using linear models for longitudinal data. At the time of each simulated attack, we scored whether the female was already under cover (1) or was out of the refuge (0). These data were analysed using generalized linear mixed models for binomial distribution (Warton & Hui 2011; glmer

model; package lme4). As no P values are available in glmer models, we used a model selection procedure based on the Akaike information criterion to test for the effect of reproductive status, session and the interaction term. To test for the effects of physical burden on thermoregulatory behaviour in pregnant females, we calculated the residuals of the regression of litter size on mother's SVL. Post hoc paired t tests with Bonferroni correction were used for all pairwise comparisons. Significance was determined at $\alpha < 0.05$ for all tests. Values are presented as mean \pm SE.

RESULTS

Thermoregulation in a Cost-free Environment

Selected body temperature during the basking period was influenced by reproductive status, but not session (Table 1, Fig. 1). T_{set} was higher in pregnant females (mean \pm SE = 33.46 ± 0.71 °C; $T_{\text{set low}} = 33.15$ °C; $T_{\text{set high}} = 33.82$ °C) compared with nonreproductive ones (mean \pm SE = 30.63 ± 4.22 °C; $T_{\text{set low}} = 29.73$ °C; $T_{\text{set high}} = 32.94$ °C). T_{set} was not related to adjusted litter size in pregnant females ($R^2 = 0.003$, $P = 0.557$).

Pregnant females showed less daily T_b variation compared with nonreproductive females (means \pm SE 0.50 ± 0.17 versus 1.70 ± 1.24 °C; RMANOVA: $F_{1,28} = 46.989$, $P < 0.001$; Table 1). Similarly, absolute between-female T_b variation was influenced by reproductive status (Table 1), and was significantly higher for nonreproductive females (means \pm SE = 2.73 ± 0.14 and 0.43 ± 0.07 for nonreproductive and pregnant females, respectively).

Behaviour Under the Risk of Predation

Activity budget was dependent on reproductive status (Table 2). Relative time spent in exploration was higher in nonreproductive females and was also influenced by session (Table 2; post hoc t tests with Bonferroni correction: S1 versus S2: $P = 0.015$; S1 versus S3: $P < 0.001$) with a significant decrease over time (Fig. 2). By contrast, pregnant females spent more time basking compared with nonreproductive females (Table 2, Fig. 2). Time spent basking increased over the sessions for nonreproductive females (post hoc t tests with Bonferroni correction: S1 versus S3: $P = 0.011$; S2 versus S3: $P = 0.012$), but was session independent for pregnant females (all pairwise comparisons: $P > 0.05$). Reproductive status also influenced the relative time spent hidden (Table 2). Pregnant females spent less time hidden than nonreproductive females (10% versus 40%; Fig. 2), with no difference between sessions detected (Table 2). In pregnant females, activity was not related to adjusted litter size whether considering time basking ($R^2 = 0.022$,

Table 1
Effects of reproductive status and session on body temperature (T_b) variation in *Vipera aspis*

Traits	Factors	df	Sum of squares	F	P
T_{set} (°C)	Reproductive status (A)	1	28	38.518	<0.001
	Session (B)	2	307	1.562	0.211
	A*B	2	307	0.357	0.700
Daily T_b standard deviation (°C)	Reproductive status (A)	1	28	49.118	<0.001
	Session (B)	2	336	0.867	0.421
	A*B	2	336	0.348	0.706
Between-females T_b variation (°C)	Reproductive status (A)	1	28	136.031	<0.001
	Session (B)	2	336	1.892	0.153
	A*B	2	336	2.788	0.063

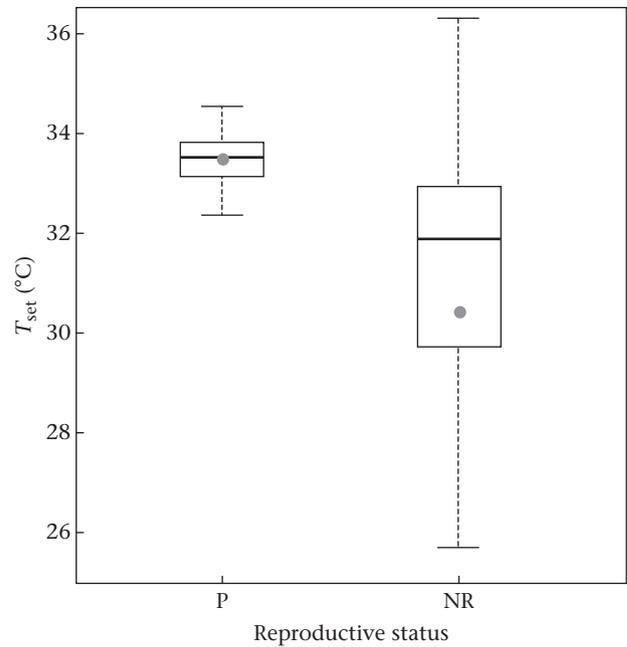


Figure 1. Effects of reproductive status in the asp viper on female preferred body temperature (T_{set}) in a cost-free environment and across sessions. The boxes represent the interval between the 25% and 75% quartiles and the whiskers represent the range. The grey circles represent the mean values. The middle horizontal line in the box plot represents the median. P = pregnant females ($N = 11$); NR = nonreproductive females ($N = 19$). Female identity was treated as a random factor.

$F_{1,127} = 0.425$, $P = 0.516$), in exploration ($R^2 = 0.001$, $F_{1,127} = 1.730$, $P = 0.191$) or hidden ($R^2 = 0.069$, $F_{1,127} = 0.213$, $P = 0.645$).

Response to Simulated Predatory Attacks

The proportion of females that were hidden at the time of the attack was always higher in nonreproductive than pregnant females regardless of the session (glmer: $P < 0.05$; S1: 26.3% versus 7.7%; post hoc t test with Bonferroni correction: $P < 0.05$; S2: 42.1% versus 7.7%; post hoc t test with Bonferroni correction: $P < 0.001$; S3: 21.1% versus 0: $P = 0.018$). After a simulated attack, all pregnant females emerged from the refuge, whereas some nonreproductive females remained under cover (16.4, 6.8 and 8.3%, respectively, for sessions 1, 2 and 3).

Scanning time was influenced by reproductive status with a significant interaction between attack number and reproductive status (Table 2, Fig. 3). That is, scanning was longer after the second attack for nonreproductive females (post hoc t test with Bonferroni correction: $P = 0.005$), but not for pregnant females ($P > 0.05$). Shelter time was much shorter in pregnant females (mean \pm SE = 5.7 ± 0.9 min) compared with nonreproductive females (mean \pm SE = 22.4 ± 3.5 min; Table 2) and was not variable across sessions (Table 2). Emergence behaviour was not influenced by adjusted litter size (scanning time: $R^2 = 0.078$, $P = 0.453$; shelter time: $R^2 = 0.136$, $P = 0.670$).

Thermal Costs of Refuge Use

Refuge use caused a significant decrease in T_b that was influenced by the time spent under the refuge (RMANOVA: $F_{1,176} = 299.863$, $P < 0.001$) and initial T_b ($F_{1,176} = 100.787$, $P < 0.001$). Temperature reduction was greater when initial T_b was high and time spent in the refuge increased. We found a significant effect of reproductive status ($F_{1,29} = 69.419$, $P < 0.001$), session ($F_{2,176} = 28.256$, $P = 0.002$) and the interaction term ($F_{2,215} = 3.180$,

Table 2

Effects of reproductive status and session on activity budget and of status, session and simulated attack repetition on emergence behaviour in a context of predation risk in *Vipera aspis*

Behaviour	Factors	df	Sum of squares	F	P
Time in exploration (%)	Reproductive status (A)	1	30	14.362	<0.001
	Session (B)	2	344	11.959	<0.001
	A*B	2	344	0.881	0.416
Time hidden (%)	Reproductive status (A)	1	30	35.938	<0.001
	Session (B)	2	344	1.357	0.259
	A*B	2	344	2.968	0.053
Time in thermoregulation (%)	Reproductive status (A)	1	28	86.672	<0.001
	Session (B)	2	322	4.953	0.002
	A*B	2	322	3.359	0.036
Scanning time (min)	Reproductive status (A)	1	29	47.051	<0.001
	Session (B)	2	243	0.348	0.707
	Attack (C)	1	243	2.87	0.092
	A*B	2	243	7.318	0.765
	A*C	1	243	2.87	0.007
	B*C	2	243	1.8	0.167
	A*B*C	2	243	0.017	0.983
	A*B*C	2	243	0.147	0.864
Shelter time (min)	Reproductive status (A)	1	29	47.8	<0.001
	Session (B)	2	242	0.327	0.721
	Attack (C)	1	242	3.797	0.052
	A*B	2	242	1.189	0.306
	A*C	1	242	2.567	0.110
	B*C	2	242	1.486	0.228
	A*B*C	2	242	0.147	0.864
	A*B*C	2	242	0.147	0.864

$P = 0.044$) on thermal costs (Fig. 4). Despite having a lower initial T_b at the time of the attack (33.22°C versus 29.68°C for pregnant and nonreproductive females, respectively: $F_{1,27} = 42.576$, $P < 0.001$), nonreproductive females experienced greater thermal costs (mean \pm SE = $2.83 \pm 1.96^\circ\text{C}$) than pregnant females (mean \pm SE = $1.34 \pm 0.93^\circ\text{C}$). Thermal costs were more variable for nonreproductive females with an increase in the last session (post hoc t tests with Bonferroni correction: S2 versus S3: $P < 0.001$; all other pairwise comparisons: $P > 0.05$). No variation between sessions was found in pregnant females (post hoc t tests with Bonferroni correction, all pairwise comparisons: $P > 0.05$). Lastly, we found that mean T_b after a predation threat was influenced by reproductive status (RMANOVA: $F_{1,26} = 60.644$, $P < 0.001$), but not

by session ($F_{2,220} = 0.916$, $P = 0.401$). That is, mean T_b 30–60 min after an attack was similar to T_{set} in pregnant females (mean \pm SE = $33.44 \pm 0.08^\circ\text{C}$) but significantly lower than T_{set} in nonreproductive females (mean \pm SE = $26.75 \pm 0.34^\circ\text{C}$).

DISCUSSION

Behavioural decisions regarding exposure reflect a balance between the resulting benefits (e.g. foraging, thermoregulation) and costs (e.g. predation risk; Cooper & Frederick 2007; Cooper 2009). Herein, we provided a thorough assessment of maternal thermoregulation and escape tactics in a viviparous snake. We found that pregnant females were extremely precise thermoregulators, and that changes in thermoregulation that are associated with reproduction are fecundity independent. Pregnant females clearly adopted a risk-prone strategy to limit thermal costs of refuge use. In turn, nonreproductive females adjusted their response to predation risk and over time. Our results underline the extent of maternal dedication towards thermoregulation.

Temperature Regulation of the Embryos

Although a shift in thermal preference during reproduction has been reported in a number of species (see Lourdaï et al. 2008), the components of maternal precision (daily T_b variation, between-female differences) have seldom been addressed (Lourdaï et al. 2008; Schwarzkopf & Andrews 2012). We reveal that pregnant females are extremely precise thermoregulators, selecting a narrow T_{set} range (33.15 – 33.82°C). Such a range lies within the highest level of thermal precision reported in squamates (Angilletta 2009). Daily temperature variations were much smaller in pregnant females, indicating stable thermal profiles. Between-female variation was reduced, suggesting a convergence towards a similar 'set point'. Maternal changes in thermoregulation (T_{set}) were not correlated with reproductive effort, therefore challenging the physical burden hypothesis (DeNardo et al. 2012). Fecundity is a classic component of parental care (Clutton-Brock 1991). Yet, thermoregulation of the developmental environment differs from other parental influences (Bernardo 1996) because all embryos simultaneously benefit from maternal thermoregulation rather than compete for it. Hence, changes in maternal preference are likely to reflect a narrow embryonic thermal tolerance (Angilletta 2009; Shine 2012; Lориoux et al., in press) rather than an encumbrance or selfish maternal tactic. For instance, pregnant females

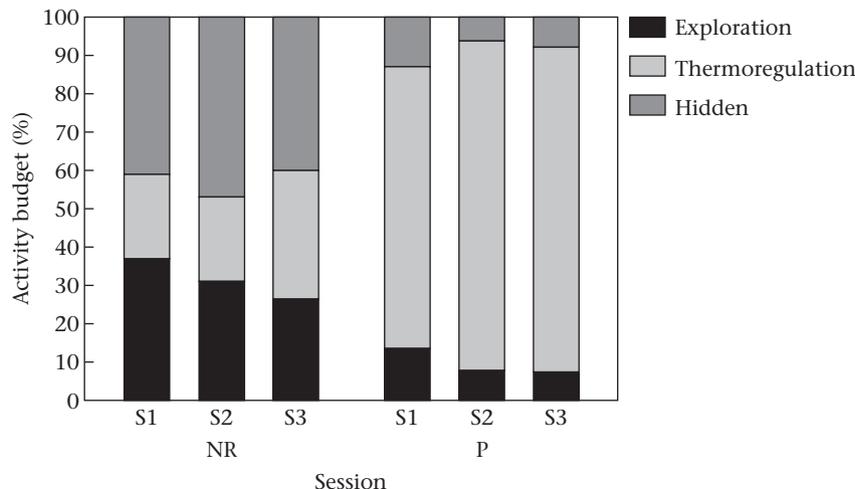


Figure 2. Effect of reproductive status and session on activity budget in the aspic viper. P = pregnant females ($N = 11$); NR = nonreproductive females ($N = 19$).

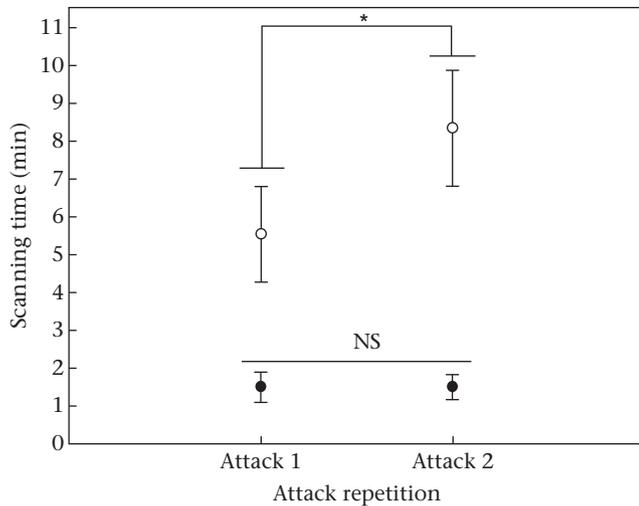


Figure 3. Effects of reproductive status and attack repetition on scanning behaviour. Pregnant females (P; $N = 11$) are represented by black circles and nonreproductive females (NR; $N = 19$) by open circles. Error bars represent SE. * $P < 0.05$.

maintained their elevated thermal preference even when exposed to predation risk (see below).

Heat for Safety? Pregnant Females Minimize Thermal Costs

Pregnancy had a strong impact on activity budget and response to predator attack. In accordance with field observations, pregnant females dedicated most of their time to thermoregulation (Saint Girons 1952; Naulleau 1979; Ladyman et al. 2003), and thereby increased their exposure. In turn, nonreproductive females spent most of their time hidden and were often under cover at the time of a simulated attack. After a simulated attack, refuge use time was considerably shorter in pregnant females and associated with reduced scanning before emergence. Scanning behaviour is a crucial component of risk assessment (Avery et al. 1993; Hollen et al. 2011; Fernandez-Juricic 2012) but has not been studied in snakes. Nonreproductive females adjusted their behaviour to risk level with longer scanning behaviour after the second attack, as has

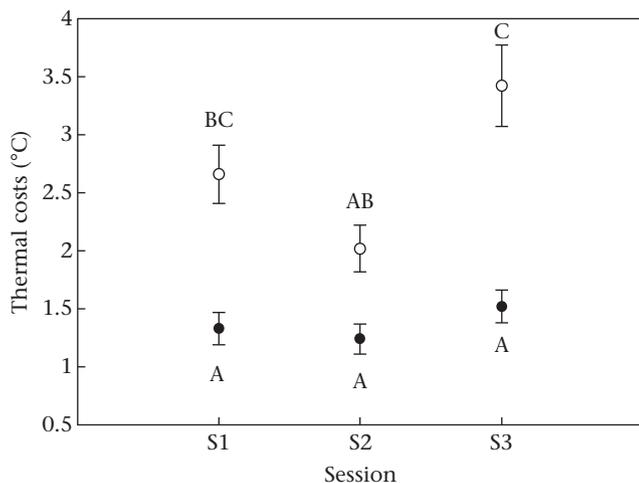


Figure 4. Effects of reproductive status and session on the thermal costs associated with refuge use. Black circles represent pregnant females (P; $N = 11$) and open circles represent nonreproductive females (NR; $N = 19$). Error bars represent SE. Levels connected with the same letter are not significantly different.

been previously demonstrated in lizards (Amo et al. 2004). Our results underline the idea that pregnancy modifies behavioural decisions and the integration of environmental cues.

Pregnant females tolerated only a small decrease in body temperature before emerging compared with nonreproductive ones. Therefore, pregnant females always minimized deviation from their thermal preference, and consequently time spent in the refuge. Although such a behavioural shift appears costly (increases exposure to potential predators), it is supported by previous observations (Lourdais et al. 2002, 2003). In addition, field observations suggest that females select microhabitats for thermoregulating that also favour swift retreat to a nearby refuge (< 1 m). Hence, careful microhabitat selection may be a response to the conflicting needs of exposure and predator evasion. Finally, vipers are venomous snakes and potentially dangerous to predators. Pregnant females may rely on camouflage to limit detection by a predator, as already suggested by other studies (Wüster et al. 2004; Valkonen et al. 2011). Hence, ecological risks may be offset by specific behavioural strategies and appropriate habitat use.

Why Do Females Not Adjust Behaviour to Changing Gestational Constraints?

Overall, our findings contradict the SMH, as we demonstrate a risk-prone tactic in pregnant females. Also, pregnant females did not adjust their behaviour and thermal preferences over the course of gestation. Maternal burden and associated locomotor impairment increase over time (Le Galliard et al. 2003) because of substantial embryonic water uptake (Dauphin-Villemant & Xavier 1986) combined with maternal protein mobilization resulting in altered performance (Lourdais et al. 2004a). Regardless, pregnant vipers maintained their behavioural effort at all stages considered.

Understanding the exposure trade-off requires an assessment of the benefits derived from maternal behaviour (Shine 2004). In an experimental study on asp viper (Lorioux et al., in press), we demonstrated contrasted stage dependence of maternal thermal effects. For example, maternal thermoregulation is critical at early developmental stages to optimize offspring phenotypic quality. In turn, reaching T_{set} is important throughout gestation to reduce gestation duration and optimize reproductive phenology. Date of birth is a critical life history parameter (Uller & Olsson 2010), notably in temperate climates, where low temperatures delay parturition (Lourdais et al. 2002, 2004b) and influence offspring survival (Warner & Shine 2007; Le Henaff et al. 2012). Hence, unchanging maternal thermoregulation over gestation may be primarily linked with phenological effects (Uller & Olsson 2010; Wapstra et al. 2010). That is, pregnant females may value any opportunity to reach their thermal preference to speed up developmental rate and enhance offspring viability. Shorter gestation might also benefit the mother (therefore supporting the SMH), by reducing the duration of physical impairment (Shine & Olsson 2003; Lorioux et al. 2012) and associated vulnerability.

Acknowledgments

We thank Dale DeNardo and Frédéric Angelier for providing valuable comments on the manuscript. Mathieu Authier helped with the statistical analyses. This research was made possible by the financial support of the 'Programme opérationnel plurirégional Loire FEDER' (no. PRESAGE 30810), the 'Etablissement Public Loire', the FYSSEN Foundation and the Centre National de la Recherche Scientifique.

References

- Amo, L., López, P. & Martín, J. 2004. Multiple predators and conflicting refuge use in the wall lizard, *Podarcis muralis*. *Annales Zoologici Fennici*, **41**, 671–679.
- Amo, L., López, P. & Martín, J. 2007. Pregnant female lizards *Iberolacerta cyreni* adjust refuge use to decrease thermal costs for their body condition and cell-mediated immune response. *Journal of Experimental Zoology*, **307A**, 106–112.
- Andrews, R. M. 2004. Embryonic development. In: *Reptilian Incubation: Environment, Evolution, and Behaviour* (Ed. by D. C. Deeming), pp. 75–102. Nottingham: Nottingham University Press.
- Angilletta, M. J. 2009. *Thermal Adaptation: a Theoretical and Empirical Synthesis*. New York: Oxford University Press.
- Avery, R., Basker, A. & Corti, C. 1993. 'Scan' behaviour in *Podarcis muralis*: the use of vantage points by an active foraging lizard. *Amphibia-Reptilia*, **14**, 247–259.
- Bauwens, D. & Thoen, C. 1981. Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology*, **50**, 733–743.
- Bernardo, J. 1996. Maternal effects in animal ecology. *American Zoologist*, **36**, 83–105.
- Bonnet, X., Lourdais, O., Shine, R. & Naulleau, G. 2002. Reproduction in a typical capital breeder: costs, currencies and complications in the aspic viper. *Ecology*, **83**, 2124–2135.
- Bonnet, X., Shine, R., Lourdais, O. & Naulleau, G. 2003. Measures of reproductive allometry are sensitive to sampling bias. *Functional Ecology*, **17**, 39–49.
- Brodie, E. D. 1989. Behavioural modifications as a means of reducing the cost of reproduction. *American Naturalist*, **134**, 225–238.
- Christian, K. A. & Weavers, B. W. 1996. Thermoregulation of monitor lizards in Australia: an evaluation of methods in thermal biology. *Ecological Monographs*, **66**, 139–167.
- Clutton-Brock, T. H. 1991. *The Evolution of Parental Care*. Princeton, New Jersey: Princeton University Press.
- Cooper, W. E. 2009. Fleeing and hiding under simultaneous risks and costs. *Behavioral Ecology*, **20**, 665–671.
- Cooper, W. E., Jr. & Frederick, W. G. 2007. Optimal time to emerge from refuge. *Biological Journal of the Linnean Society*, **91**, 375–382.
- Cooper, W. E. & Wilson, D. S. 2008. Thermal costs of refuge use affects refuge entry and hiding time by striped plateau lizards *Sceloporus virgatus*. *Herpetologica*, **64**, 406–412.
- Cooper, W. E., Wyk, J. H. & Mouton, P. L. N. 1999. Incompletely protective refuges: selection and associated defences by a lizard, *Cordylus cordylus* (Squamata: Cordylidae). *Ethology*, **105**, 687–700.
- Dauphin-Villemant, C. & Xavier, F. 1986. Adrenal activity in the female *Lacerta vivipara* Jacquin: possible involvement in the success of gestation. In: *Endocrine Regulation as Adaptive Mechanism to Environments* (Ed. by I. Assenmacher & J. Boissin), pp. 241–250. Paris: Editions du CNRS.
- DeNardo, D. F., Lourdais, O. & Stahlschmidt, Z. R. 2012. Are females maternal manipulators, selfish mothers, or both? Insight from pythons. *Herpetologica*, **68**, 299–307.
- Fernandez-Juricic, E. 2012. Sensory basis of vigilance behavior in birds: synthesis and future prospects. *Behavioural Processes*, **89**, 143–152.
- Goldsbrough, C. L., Hochuli, D. F. & Shine, R. 2004. Fitness benefits of retreat site selection: spiders, rocks and thermal cues. *Ecology*, **85**, 1635–1641.
- Hertz, P. E., Huey, R. B. & Stevenson, R. D. 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of inappropriate question. *American Naturalist*, **142**, 796–818.
- Hollen, L. I., Bell, M. B. V., Wade, H. M., Rose, R., Russell, A., Niven, F., Ridley, A. R. & Radford, A. N. 2011. Ecological conditions influence sentinel decisions. *Animal Behaviour*, **82**, 1435–1441.
- Hubert, J. & Dufaure, J. P. 1968. Table de développement de la vipère aspic: *Vipera aspis* L. *Bulletin de la Société Zoologique de France*, **93**, 135–148.
- Huey, R. B. & Slatkin, M. 1976. Costs and benefits of lizard thermoregulation. *Quarterly Review of Biology*, **51**, 363–384.
- Kurdiková, V., Smolinsky, R. & Gvozdík, L. 2011. Mothers matter too: benefits of temperature oviposition preferences in newts. *PLoS One*, **6**, e23842, <http://dx.doi.org/10.1371/journal.pone.0023842>.
- Ladyman, M., Bonnet, X., Lourdais, O., Bradshaw, D. & Naulleau, G. 2003. Gestation, thermoregulation and metabolism in a viviparous snake, *Vipera aspis*: evidence for fecundity-independent costs. *Physiological and Biochemical Zoology*, **76**, 497–510.
- Le Galliard, J. F., Le Bris, M. & Clobert, J. 2003. Timing of locomotor impairment and shift in thermal preferences during gravidity in a viviparous lizard. *Functional Ecology*, **17**, 877–885.
- Le Henanff, M., Meylan, S. & Lourdais, O. 2012. The sooner is better: reproductive phenology drives ontogenetic trajectories in a temperate squamate (*Podarcis muralis*). *Biological Journal of the Linnean Society*, **108**, 384–395.
- Lorigou, S., DeNardo, D. F., Gorelick, R. & Lourdais, O. 2012. Maternal influences on early development: preferred temperature prior to oviposition hastens embryogenesis and enhances offspring traits in the Children's python, *Antaresia childreni*. *Journal of Experimental Biology*, **215**, 1346–1353.
- Lorigou, S., Vaugoyeau, M., DeNardo, D. F., Clobert, J., Guillon, M. & Lourdais, O. In press. Stage-dependence of phenotypical and phenologicalmaternal effects: insight into squamate reptile reproductive strategies. *American Naturalist*.
- Lourdais, O., Bonnet, X., Shine, R., DeNardo, D. F., Naulleau, G. & Guillon, M. 2002. Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *Journal of Animal Ecology*, **71**, 470–479.
- Lourdais, O., Bonnet, X., Shine, R. & Taylor, E. 2003. When does a reproducing female viper (*Vipera aspis*) 'decide' on her litter size? *Journal of Zoology*, **259**, 123–129.
- Lourdais, O., Brischoux, F., DeNardo, D. F. & Shine, R. 2004a. Protein catabolism in pregnant snakes (*Epicrates cenchria maurus* Boidae) compromises musculature and performance after reproduction. *Journal of Comparative Physiology*, **174**, 383–391.
- Lourdais, O., Shine, R., Bonnet, X., Guillon, M. & Naulleau, G. 2004b. Climate affects embryonic development in a viviparous snake, *Vipera aspis*. *Oikos*, **104**, 551–560.
- Lourdais, O., Heulin, B. & DeNardo, D. F. 2008. Thermoregulation during gravidity in the Children's python (*Antaresia childreni*): a test of the pre-adaptation hypothesis for maternal thermophily in snakes. *Biological Journal of the Linnean Society*, **93**, 499–508.
- Lourdais, O., Lorigou, S. & DeNardo, D. F. 2013. Structural and performance costs of reproduction in a pure capital breeder, the Children's python, *Antaresia childreni*. *Physiological and Biochemical Zoology*, **86**, 176–183.
- Martín, J. 2001. When hiding from predators is costly: optimization of refuge use in lizards. *Ethologia*, **9**, 9–13.
- Martín, R. & López, P. 2001. Repeated predatory attacks and multiple decisions to come out from a refuge in an alpine lizard. *Behavioural Ecology*, **12**, 386–389.
- Naulleau, G. 1979. Etude biotéléométrique de la thermorégulation chez *Vipera aspis* (L.) élevée en conditions artificielles. *Journal of Herpetology*, **13**, 203–208.
- Naulleau, G., Fleury, F. & Boissin, J. 1987. Annual cycles in plasma testosterone and thyroxine for the male aspic viper *Vipera aspis* L. (Reptilia, Viperidae), in relation to the sexual cycle and hibernation. *General and Comparative Endocrinology*, **65**, 254–263.
- Peltier, M. R., Wilcox, C. J. & Sharp, D. C. 1998. Technical note: application of the Box-Cox data transformation to animal science experiments. *Journal of Animal Science*, **76**, 847–849.
- R Development Core Team. 2010. *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Robert, K. A. & Thompson, M. B. 2003. Reconstructing the DS1921 Thermochron iButtons to reduce size and weight as a new technique in the study of small animal thermal biology. *Herpetological Review*, **34**, 130–132.
- Saint Girons, H. 1952. Ecologie et éthologie des vipères de France. *Annales des Sciences Naturelles Zoologiques*, **14**, 263–343.
- Saint Girons, H. 1986. Caractères écophysiologicals du cycle sexuel des serpents dans les régions tempérées. In: *Endocrine Regulations as Adaptive Mechanisms to the Environment* (Ed. by I. Assenmacher & J. Boissin), pp. 63–77. Paris: Editions du CNRS.
- Schwarzkopf, L. & Andrews, R. M. 2012. Are moms manipulative or just selfish? Evaluating the maternal manipulation hypothesis and implications for life-history studies in reptiles. *Herpetologica*, **68**, 147–159.
- Schwarzkopf, L. & Shine, R. 1992. Costs of reproduction in lizards. Escape tactics and susceptibility to predation. *Behavioural Ecology and Sociobiology*, **31**, 17–25.
- Seigel, R. A. & Ford, N. B. 1987. Reproductive ecology. In: *Snakes: Ecology and Evolutionary Biology* (Ed. by R. A. Seigel, J. T. Collins & S. S. Novak), pp. 210–252. New York: Macmillan.
- Shine, R. 1980. Costs of reproduction in reptiles. *Oecologia*, **46**, 92–100.
- Shine, R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *American Naturalist*, **145**, 809–823.
- Shine, R. 2003. Locomotor speeds of gravid lizards: placing 'costs of reproduction' within an ecological context. *Functional Ecology*, **17**, 526–533.
- Shine, R. 2004. Incubation regimes of cold-climate reptiles: the thermal consequences of nest-site choice, viviparity and maternal basking. *Biological Journal of the Linnean Society*, **83**, 145–155.
- Shine, R. 2006. Is increased maternal basking an adaptation or a pre-adaptation to viviparity in lizards? *Journal of Experimental Zoology Part A: Comparative Experimental Zoology* **305A**, **6**, 524–535.
- Shine, R. 2012. Manipulative mothers and selective forces: the effects of reproduction on thermoregulation in reptiles. *Herpetologica*, **68**, 289–298.
- Shine, R. & Bull, J. J. 1979. The evolution of live-bearing in lizards and snakes. *American Naturalist*, **113**, 905–923.
- Shine, R. & Olsson, M. 2003. When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (*Scincidae*). *Journal of Evolutionary Biology*, **16**, 823–832.
- Sih, A. 1980. Optimal behaviour: can foragers balance two conflicting demands? *Science*, **210**, 1041–1043.
- Tinkle, D. W. & Gibbons, J. W. 1979. *The Distribution and Evolution of Viviparity in Reptiles*. Ann Arbor, Michigan: Museum of Zoology, University of Michigan.
- Uller, T. & Olsson, M. 2010. Offspring size and timing of hatching determine survival and reproductive output in a lizard. *Oecologia*, **162**, 663–671.
- Valkonen, J., Niskanen, M., Björklund, M. & Mappes, J. 2011. Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. *Evolutionary Ecology*, **25**, 1047–1063.
- Wapstra, E., Uller, T., While, G. M., Olsson, M. & Shine, R. 2010. Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *Journal of Evolutionary Biology*, **23**, 651–657.
- Warner, D. A. & Shine, R. 2007. Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia*, **154**, 65–73.

- Warton, D. I. & Hui, F. K. C.** 2011. The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, **92**, 3–10.
- Webb, J. K. & Whiting, M. J.** 2005. Why don't small snakes bask? Juvenile broad-headed snakes trade thermal benefits for safety. *Oikos*, **110**, 515–522.
- Wüster, W., Allum, C. S. E., Bjargardóttir, B. I., Bailey, K. L., Dawson, K. J., Guenioui, J., Lewis, J., McGurk, J., Moore, A. G., Niskanen, M., et al.** 2004. Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proceedings of the Royal Society B*, **271**, 2495–2499.