



Cold climate specialization: Adaptive covariation between metabolic rate and thermoregulation in pregnant vipers



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HIGHLIGHTS

- Climatic specialization is a critical question in ectotherms.
- We studied thermoregulation and metabolic rate during pregnancy in two vipers.
- The cold specialist is a more efficient thermoregulator with higher metabolic rate.
- Covariation between physiology and behavior may be a key component of climatic adaptations.

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ABSTRACT

We compared thermoregulatory strategies during pregnancy in two congeneric viperid snakes (*Vipera berus* and *Vipera aspis*) with parapatric geographic ranges. *V. berus* is a boreal specialist with the largest known distribution among terrestrial snakes while *V. aspis* is a south-European species. Despite contrasted climatic affinities, the two species displayed identical thermal preferences (T_{set}) in a laboratory thermal gradient. Under identical natural conditions, however, *V. berus* was capable of maintaining T_{set} for longer periods, especially when the weather was constraining. Consistent with the metabolic cold adaptation hypothesis, *V. berus* displayed higher standard metabolic rate at all temperatures considered. We used the thermal dependence of metabolic rate to calculate daily metabolic profiles from body temperature under natural conditions. The boreal specialist experienced higher daily metabolic rate and minimized gestation duration chiefly because of differences in the metabolic reaction norms, but also superior thermoregulatory efficiency. Under cold climates, thermal constraints should make precise thermoregulation costly. However, a shift in the metabolic reaction norm may compensate for thermal constraints and modify the cost–benefit balance of thermoregulation. Covariation between metabolic rate and thermoregulation efficiency is likely an important adaptation to cold climates.

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1. Introduction

Environmental temperature is especially important in shaping the distribution of ectotherms because of their reliance on behavioral mechanisms for thermoregulation [1–4]. Unraveling the proximate mechanisms that enable climate specialization is vital to better understand thermoregulatory strategies. Such an understanding is important because the response of particular species to climate change will likely depend on their physiological tolerances [5]. For example, tropical ectotherms are supposedly particularly at risk from climate change because of their narrow thermal tolerances, which confer a reduced “thermal safety margin” [4,6]. Cold-adapted species, however,

may also be particularly vulnerable to anticipated climate change for at least two reasons: (i) the magnitude of predicted changes in temperature increases with latitude, and (ii) adaptations specific to cold temperature (i.e., higher metabolic rate) will be disadvantageous because the predicted temperature changes will result in further increased maintenance costs [7].

Thermoregulatory strategies of ectotherms vary from active thermoregulation to thermal conformity, and theoretical models predict that the optimal investment in thermoregulation should balance costs and benefits [8]. The cost–benefit model of thermoregulation predicts limited thermoregulatory effort (thermal conformity) in habitats with low thermal quality [8], but empirical data provide only ambiguous support for this prediction [9,10]. One reason for the limited support might be that specific shifts in thermal requirements may enable the exploitation of a given habitat [11]. Specifically, the thermal sensitivity of metabolic rate constitutes an important aspect of thermal adaptation

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[12], since most physiological rates, including those that influence digestion, growth, physical performances, and activity patterns, are directly dependent upon metabolism.

To improve our understanding of thermoregulatory decisions in cold climates, we need to focus on the interaction between physiology and thermoregulatory behavior. According to the metabolic cold adaptation (MCA) hypothesis, metabolic rate should be higher in ectotherms living in cold environments compared to closely related individuals living in warmer environments [13] because a higher temperature-specific metabolic rate compensates for the effects of colder temperature on metabolism. An upward shift in standard metabolic rate (SMR) will influence thermal performance curves in cold-adapted species. Despite this adaptation, ectotherms in cold climates face thermal constraints that likely require them to actively thermoregulate to achieve a preferred body temperature range to optimize performance [9].

Embryonic life is particularly thermally sensitive, and cold temperatures slow development and alter reproductive success [14,15]. Females that maintain developing embryos typically show considerable behavioral shifts, notably intense basking and modified thermal preferences [16]. Maternal thermoregulation is beneficial by hastening development (“cold climate model”, [17–19]) and improving offspring quality (“maternal manipulation hypothesis” [20,21]). Phenological maternal effects are especially relevant under cold climates where activity time is constrained [22–24]. Enhanced maternal thermoregulation may be a critical facet of climatic specialization and has even been viewed as a driving force for the repeated evolution of viviparity among cold climate squamates [18,19].

We compared thermoregulation during gestation in two congeneric vipers with contrasted parapatric distributions [25,26]. We studied the common adder (*Vipera berus*), a boreal species with the largest known distribution among terrestrial snakes, and its southern European relative, the aspic viper (*Vipera aspis*), with a more restricted range. While we acknowledge the inherent limits of two species comparison [27], the species considered are congeners with similar morphology and ecology [28] which should alleviate some of these limits.

Specifically, we addressed the following questions:

- (i) Does the boreal specialist display lower thermal preferences (T_{set}) during gestation and higher temperature-specific SMR?
- (ii) In the same constraining environment, is the boreal specialist a more efficient thermoregulator during gestation?
- (iii) To what extent does thermoregulation influence metabolic performance during gestation?

2. Materials and methods

2.1. Study animals

V. berus and *V. aspis* have been intensely studied and their comparative distributions have attracted considerable interest [29,30]. They are closely related viviparous snakes of the western Palearctic region [25], but their ranges overlap in western France where the northern extreme of the range of *V. aspis* meets the southern distribution of *V. berus* [29]. Both species are relatively small (mean SVL ≤ 50 cm), rely on sit-and-wait foraging, and accumulate vast amounts of energy before reproduction (capital breeding) resulting in low reproductive frequency [31–33]. During pregnancy, thermal needs are elevated and females actively bask [34–36]. Food intake during pregnancy is often reduced or absent, probably because of a trade-off with thermoregulatory activities [37,38].

In the spring of 2008, we captured 24 reproductive females (12 *V. berus* and 12 *V. aspis*) in neighboring districts of west-central France. Reproductive status (late stages of vitellogenesis) was confirmed by gentle palpation of the abdomen. In the spring of 2010, we captured 15 (9 males, 6 females) non-reproductive *V. berus* and 13 (7 males, 6 females) non-reproductive *V. aspis* to assess standard

metabolic rate. Body mass (± 1 g) and snout vent length (± 5 mm) were recorded, and scale clipping was used to identify individuals.

2.2. Surgical procedure

In late June 2008, we surgically implanted a miniature temperature data logger (Thermochron iButton, Maxim, Dallas, TX) into each reproductive female. The loggers were programmed to record temperature every 15 min, coated with Plasti-Dip (PDI Inc., Circle Pines, MN), and then disinfected in a solution of benzalkonium chloride for 12 h prior to implantation. Each snake was anesthetized using isoflurane and the skin was locally disinfected using povidone-iodine (Betadine, Purdue Products, Stamford, CT). An approximately 1.5 cm ventro-lateral incision was made through the skin and body wall, and the logger was then placed intracoelomically between the gall bladder and the first embryo. The incision was closed with everting mattress sutures (3-0 Vicryl, Ethicon, Somerville, NJ). Recovery from anesthesia was rapid (<30 min) with no mortality. A single 10 mg/kg intramuscular injection of enrofloxacin (Baytril 22.7%, Bayer, Wayne, NJ) was administered post-surgically to prevent infection. At the end of an animal's participation in the study, its temperature logger was surgically removed using a similar procedure.

2.3. Thermal preferences in captivity

Twelve pregnant, temperature-logger-implanted females, 6 *V. berus* (SVL = 46.3 ± 3.2 cm; BM = 101.0 ± 30.0 g) and 6 *V. aspis* (SVL = 46.8 ± 2.3 cm; BM = 93.4 ± 5.3 g) were kept in captivity for the entire gestation period. Individuals were maintained in the laboratory in separate cages ($40 \times 20 \times 20$ cm) that provided access to a heat source (temperature range in the cage: 28 to 33 °C). Water was available ad libitum in a bowl.

In mid-July 2008, we measured preferred body temperature (T_{set}) in a thermal gradient devoid of thermoregulatory costs [39,8]. The gradient was a 3 m² concrete box. A heat source at one end and cooled air at the other produced a smooth gradual temperature gradient ranging from 25 to 45 °C during the photophase. The room was lit with ceiling mounted fluorescent fixtures to approximate the natural day length (14L:10D). The heat source in the thermal gradient was off during the scotophase, leading to a temperature of 22.0 ± 1.1 °C throughout the enclosure during this period. Females were allowed to acclimate in the thermal gradient for two days prior to a 3-day data collection period. Body temperature (T_b) data were collected from the explanted temperature loggers, and we calculated hourly body temperature for each animal. For each species, we calculated T_{set} by averaging the hourly T_b values during 12:00–17:00, a time period which allowed the animals ample time to heat up to their chosen temperature. $T_{set\ low}$ and $T_{set\ high}$ were derived from the bounds of the central 50% of selected T_b .

2.4. Thermoregulation under semi-natural conditions

To examine the influence of weather conditions on thermoregulation, we used an outdoor enclosure (10 × 10 m) at the “Terrarium of Kerdanet” snake facility, Côtes d’Armor (48° 30’ N, 03° 01’ W) which is located in the geographic range of *V. berus* and close (<200 km) to the area of sympatry with *V. aspis*. Vegetation in the enclosure provided a mosaic of basking sites, shade, and shelters. No food was provided during gestation.

Using the implanted temperature loggers, body temperature (T_b) was measured in 6 pregnant *V. berus* (SVL = 48.7 ± 3.2 cm; BM = 106.7 ± 18.0 g) and 6 pregnant *V. aspis* of similar mass and size (SVL = 46.6 ± 4.7 cm; BM = 110.7 ± 21.5 g) every 15 min for 38 days between 7 July and 13 August 2008. Simultaneously, we measured operative environmental temperatures (T_e) in the various microhabitats available to the snakes [40] with nine physical models (copper tubes filled with water) reproducing the thermal characteristics

of the study species. We ensured the realism of our models by calibrating them against two fresh snake carcasses. We placed the models in each of three microhabitats: on the ground under shrubs ($N = 3$), on the ground in the open ($N = 3$), and in an underground retreat ($N = 3$).

Days were classified as hot, medium, or cold based on the amount of time that the maximal T_e ($T_{e \max}$) was higher than the lower bound of T_{set} ($T_{\text{set low}}$) for each species. Days were considered “cold” when $T_{e \max}$ was above $T_{\text{set low}}$ for <4 h/day, “medium” when $T_{e \max}$ was above $T_{\text{set low}}$ for 4–8 h/day, and “hot” when $T_{e \max}$ was above $T_{\text{set low}}$ for >8 h/day. We used previously established indices to measure both the accuracy and the effectiveness of thermoregulation in reptiles [39,41,42]. Because active thermoregulation occurs only during the day in these species, indices were calculated using only temperature data collected during daylight hours (08:00–21:00). We calculated separate indices for each type of day. We calculated the accuracy of T_b as the mean of the deviations of T_b from $T_{\text{set low}}$ (individual deviation, d_b). We measured the extent of thermoregulation as the time spent with T_b above $T_{\text{set low}}$. We measured thermal exploitation (Ex) using a modification of the index proposed by Christian & Weavers [41]: the proportion of time spent above $T_{\text{set low}}$ expressed as a percentage of the time available to do so (i.e., when $T_{e \max} > T_{\text{set low}}$). We measured the thermal quality of each microhabitat by the daily duration when T_e was above $T_{\text{set low}}$ and within T_{set} of each species.

2.5. Parturition dates

Parturition dates were recorded in 2008 from females maintained in the outdoor enclosure (6 pregnant *V. berus* and 6 pregnant *V. aspis*) and in the laboratory (6 pregnant *V. berus* and 6 pregnant *V. aspis*). Because it was impossible to observe each female in the enclosure on each day, we moved all females in the enclosure to the lab once the first female in the lab gave birth. This enabled us to check each female daily for parturition.

2.6. Standard metabolic rate and metabolic performance estimates

We used closed system respirometry to measure SMR in non-reproductive, post-absorptive snakes. Measurements were performed on 15 (9 males, 6 females) *V. berus* (SVL = 45.1 ± 4.6 cm; BM = 62.1 ± 23.3 g) and 13 (7 males, 6 females) *V. aspis* (SVL = 47.8 ± 4.7 cm; BM = 74.7 ± 23.3 g). We measured rates of oxygen consumption (analyzed in ml h^{-1} with body mass as a cofactor, but presented in $\text{ml h}^{-1} \text{g}^{-1}$ in figures and tables) at T_b s of 10, 20, and 30 °C. We randomized the order of trials, and provided at least 10 h between each trial. Trials were conducted in opaque test chambers (internal volume 3230 ml) covered with an air-tight glass lid. The individual was placed into the test chamber and maintained in a temperature-controlled environmental chamber (± 1 °C) 3 h prior to testing. Outside air was drawn into each chamber using a Bioblock Scientific 55 l air pump for 5 min assuring 99+% air turnover [43]. Baseline samples of outside air were collected at the onset of each trial. Snakes could be observed during the trial through a small viewing port. Snakes showed limited exploratory movements, rapidly coiled, and stayed inactive during the entire trial. Trial duration was determined in a pilot study for each test temperature (2–10 h) to ensure sufficient O_2 suppression (0.5–1.0%). At the end of each trial, an end air sample was collected using two 150-ml syringes connected to the chamber via a three-way stopcock. O_2 concentration was then determined using an oxygen analyzer (FC10A, Sable Systems, Las Vegas, NV). Each air sample was stripped of water by passing it through Drierite (W. A. Hammond Drierite Co., Xenia, OH) and then sent at a controlled rate to the analyzer using an infusion pump (KDS 210, KD Scientific Inc., Holliston, MA). Oxygen consumption rate was calculated as: $(\text{O}_2 \text{ initial}) - (\text{O}_2 \text{ final}) \times (\text{chamber volume} - \text{snake volume}) / (\text{trial duration})$. Snake volume was determined based on a mass density of 1 ml/g . The analyzer was calibrated each day using outside air.

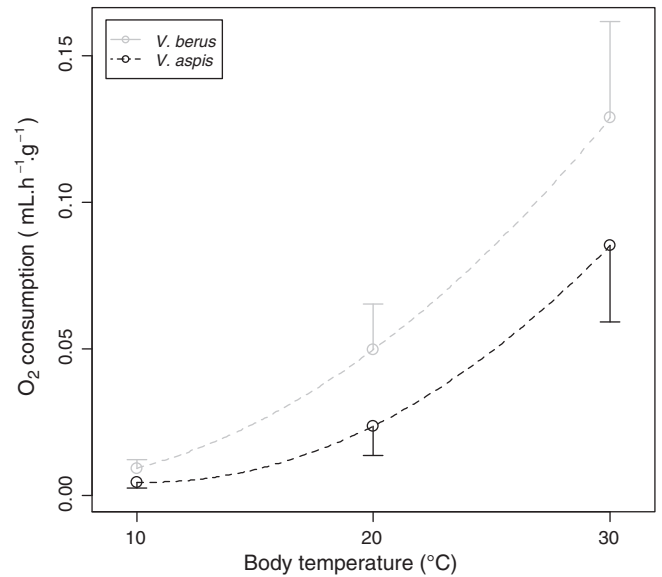


Fig. 1. Metabolic reaction norms in *V. berus* (9 males, 6 females) and *V. aspis* (7 males, 6 females). Individuals were non-reproductive and post-absorptive and were previously acclimated to standard laboratory condition. Data points are means \pm 1 standard deviation, and dashed lines are quadratic regressions.

Overall metabolic rate is a product of SMR thermal reaction norms and behavioral thermoregulation. Under constraining (cold) conditions higher metabolic rate is advantageous leading to enhanced physiological performances. We used our SMR data at three temperatures to estimate the thermal reaction norms for metabolic rate for each species. We used these equations along with T_b data from pregnant females in the enclosure to calculate cumulative daytime SMR (e.g., total oxygen consumption for the daylight hours of a given day, 08:00–21:00) for each day of the monitoring period. While SMR measures were made using males and non-reproductive females, these reaction norms likely apply to pregnant females. A previous study reported a prevalent effect of body temperature, but no effect of reproductive status, on metabolic rate [44]. Pregnant females' cumulative SMR were then compared for the two species and day type. We also wanted to quantify the effect that thermoregulation had on metabolic difference. Therefore we calculated, for each day, species difference in cumulative SMR (D1). Then we calculated, for the species that was the more efficient thermoregulator, the difference (D2) between its cumulative daytime SMR and an alternate calculation that used its own SMR thermal reaction norm but the thermal profile of the other species. Relative contribution (%) of thermoregulation to SMR difference was then derived from the ratio: (D2/D1).

2.7. Statistical analyses

All data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett test), and transformed when necessary to achieve the conditions for using parametric tests. All statistical comparisons were performed in R software (R Development Core Team, 2007). For thermoregulation, we used mixed models (nlme package) with individuals as the random effect (log-likelihood maximization). We used 5 models to test for the effects of species and type of day with the thermoregulation indices as dependent variables (Y) from constant model ($Y \sim 1$) to complete model ($Y \sim \text{Species} + \text{Type Day} + \text{Interaction}$). We used stepwise model selection based on corrected Akaike information criterion (AICc) to select the most appropriate model (Burnham & Anderson, 2002). We then used this model to estimate parameters. Pairwise comparisons for species were realized using Tukey *post hoc* tests (package multcomp). Means are provided \pm 1SD. We accepted significance at an alpha level of 0.05.

3. Results

3.1. Thermal preference during pregnancy

The two species selected similar preferred body temperatures during pregnancy: *V. berus* $T_{set} = 33.47 \pm 1.69$ °C ($T_{set\ low} = 32.64$ °C; $T_{set\ high} = 34.64$ °C) and *V. aspis* $= 33.21 \pm 2.12$ °C ($T_{set\ low} = 32.69$ °C; $T_{set\ high} = 34.66$ °C) ($F_{1,9} = 0.137$, $P = 0.72$, linear mixed effects model).

3.2. Thermal sensitivity of metabolism

Oxygen consumption was positively influenced by temperature (linear mixed-effects model; $F_{2,53} = 181.9$; $P < 0.0001$; Fig. 1).

V. berus had a higher SMR at each of the test temperatures (linear mixed effects model; $F_{1,28} = 10.5$; $P = 0.003$), but there was no interaction between species and temperature (linear mixed effects model; $F_{2,53} = 2.3$; $P = 0.112$). For both species, the best fit was a quadratic regression (*V. berus* O_2 consumption = $0.00742 - 0.00173 T_b + 0.00019 T_b^2$, adjusted $R^2 = 0.85$ and *V. aspis* O_2 consumption = $0.02797 - 0.00445 T_b + 0.00021 T_b^2$, adjusted $R^2 = 0.83$).

3.3. Thermoregulation in semi-natural conditions

Under the same environmental conditions, *V. berus* on average exhibited higher daytime T_b than *V. aspis*: 26.41 ± 6.60 °C (13.06–35.32) vs. 25.35 ± 6.47 °C (range 14.25–35.17) for *V. berus*

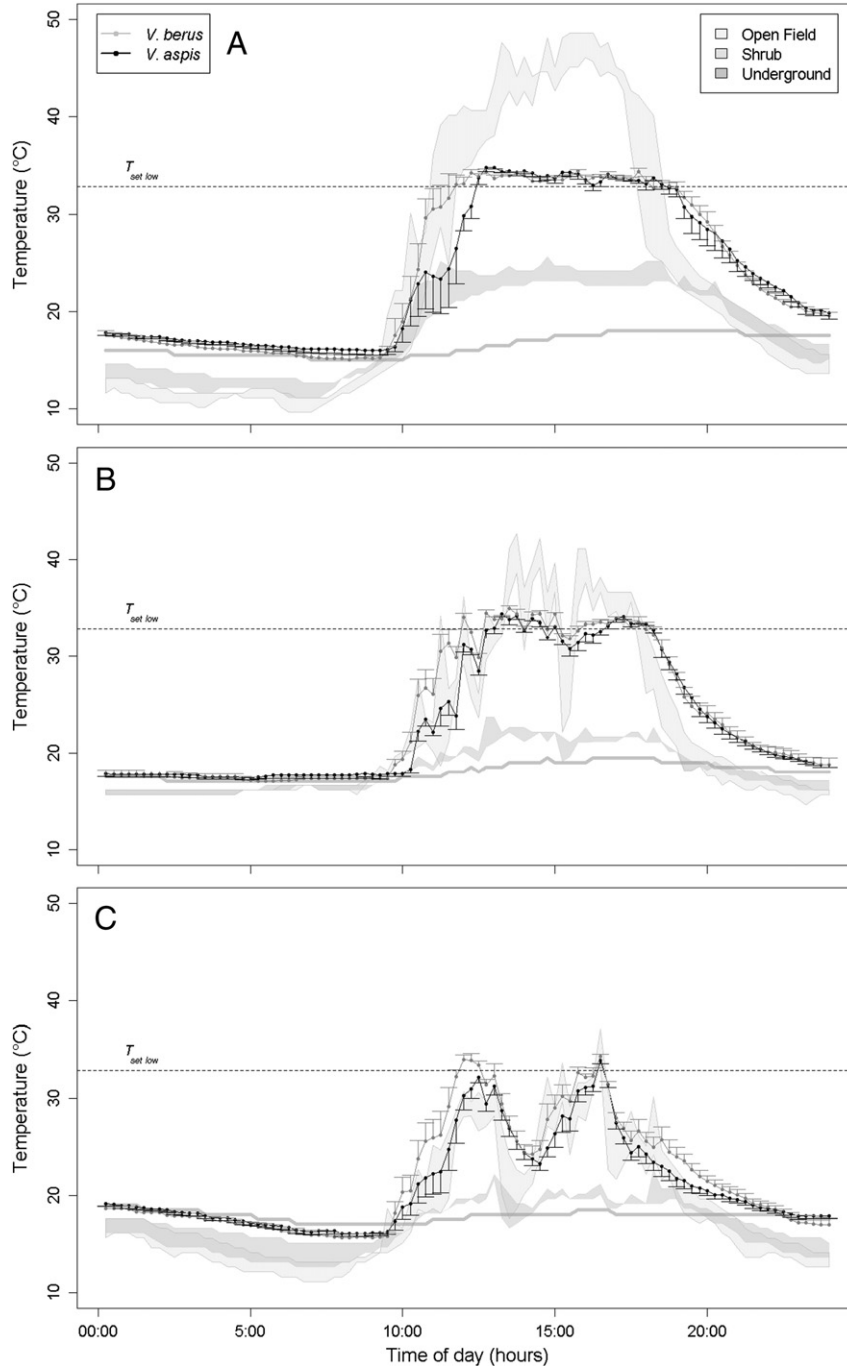


Fig. 2. Thermoregulation in pregnant *V. berus* (gray symbols) and *V. aspis* (black symbols) during 3 contrasted days (A: hot, B: medium and C: cold). Points represent mean body temperature ± 1 standard errors. Dashed lines represent the lower bound of the range of preferred body temperature selected in a laboratory thermal gradient ($T_{set\ low}$). Shaded polygons represent the range of environmental temperatures (T_e) in 3 microhabitat types.

Table 1

Model selection used to describe thermoregulation of *V. berus* and *V. aspis* using corrected Akaike information criterion (AICc). K = number of parameters in the function, w_i = Akaike weight. Models in boldface were selected for analyses.

	K	AICc	Δ_{AICc}	w_i AICc
<i>Mean T_b</i>				
Y ~ Species + Type Day	6	1644.935	0.00	0.788
Y ~ Species + Type Day + Interaction	8	1647.593	2.71	0.203
Y ~ Type Day	5	1653.919	8.96	0.009
<i>Ex</i>				
Y ~ Species + Type Day	6	-126.617	0.00	0.717
Y ~ Species + Type Day + Interaction	8	-124.891	1.86	0.283
Y ~ Type Day	5	-110.211	16.36	0.0002
<i>T_e within T_{set} (h)</i>				
Y ~ Species + Type Day + Interaction	8	1396.241	0.00	0.565
Y ~ Species + Type Day	6	1396.762	0.52	0.435
Y ~ Type Day	5	1413.072	16.83	0.0001
<i>d_b</i>				
Y ~ Species + Type Day	6	1622.663	0.00	0.742
Y ~ Species + Type Day + Interaction	8	1624.895	2.23	0.243
Y ~ Type Day	5	1630.480	7.82	0.015

and *V. aspis*, respectively (linear mixed effects model, $F_{1, 2023} = 825.64$, $P < 0.0001$). Because the two species had similar T_{set} , our day classifications were the same for the two species: nine days were cold, 12 were medium, and 13 were hot. The model with species and day category was the best for explaining mean T_b variation during the day (Table 1). *V. berus* showed higher T_b and *Ex* than *V. aspis* for all day categories, but this difference was particularly marked during cold days (24.13 ± 2.00 °C vs. 22.81 ± 2.53 °C; Fig. 2, Table 2). The best model for time spent above $T_{set\ low}$ included species and type of day (Table 1). Time spent above $T_{set\ low}$ was strongly linked to thermal conditions for both species (Table 1) and *V. berus* was always able to maintain T_{set} for longer periods than *V. aspis* (Table 2). Thermoregulation accuracy was highly dependent on daily thermal conditions and species, with a significant interaction between the two factors (Table 1). *V. berus* showed more precise thermoregulation than *V. aspis* (lower d_b) under constraining conditions (cold and medium days) but the difference was less dramatic during hot days (Table 2).

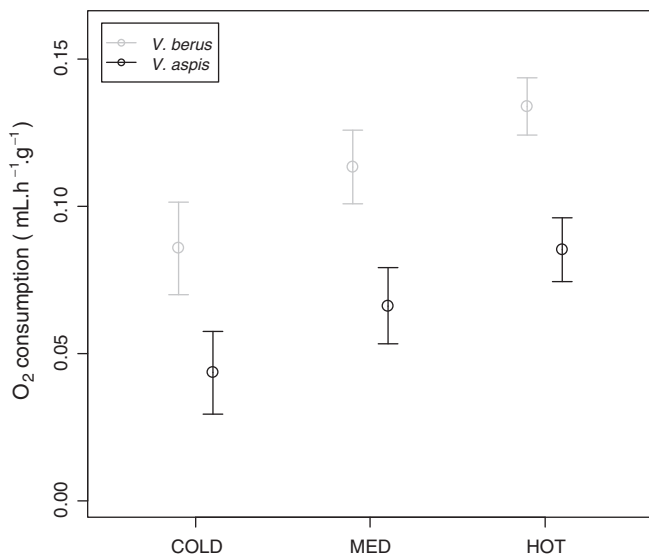


Fig. 3. Estimate of cumulative daytime SMR ($\text{ml}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$) in pregnant *V. berus* (gray symbols) and *V. aspis* (black symbols) for the three day classes. Values were derived from metabolic reaction norm and thermal profiles restricted to the basking period (08:00–21:00). Circles represent mean values \pm one standard deviation.

Table 2

Thermoregulatory differences in pregnant *V. berus* and *V. aspis* maintained in an outdoor enclosure under variable weather. Body temperatures (\pm standard deviation) were recorded simultaneously at the same time interval (15 min). Analyses were limited to the basking period (08:00–21:00). Z and P values represent differences between species. See text for detailed information on each indices and day classification.

Indices	Day type	Species		Tukey
		<i>V. berus</i>	<i>V. aspis</i>	Post hoc tests
Mean T_b °C	Cold	24.13 \pm 2.00	22.81 \pm 2.53	Z = 3.005 P < 0.001
	Medium	27.16 \pm 1.52	26.02 \pm 1.95	Z = 2.823 P < 0.001
	Hot	29.42 \pm 1.18	28.61 \pm 1.70	Z = 3.479 P < 0.001
<i>Ex</i> (%)	Cold	41.15 \pm 28.41	21.28 \pm 25.12	Z = 3.85 P < 0.001
	Medium	66.40 \pm 19.55	46.60 \pm 23.61	Z = 4.559 P < 0.001
	Hot	70.35 \pm 10.54	56.73 \pm 15.52	Z = 5.912 P < 0.001
Time within T_{set} (h)	Cold	1.14 \pm 0.93	0.58 \pm 0.71	Z = 3.512 P < 0.001
	Medium	3.95 \pm 1.54	2.80 \pm 1.66	Z = 4.288 P < 0.001
	Hot	6.46 \pm 1.08	5.23 \pm 1.53	Z = 5.816 P < 0.001
d_b	Cold	8.83 \pm 1.96	10.09 \pm 2.50	Z = -2.918 P < 0.001
	Medium	6.04 \pm 1.45	7.07 \pm 1.87	Z = -2.673 P < 0.001
	Hot	3.96 \pm 1.16	4.64 \pm 1.65	Z = -2.978 P = 0.003

3.4. Parturition date

V. berus gave birth earlier than *V. aspis* ($F_{1, 18} = 7.92$, $P = 0.011$) with a significant effect of housing conditions ($F_{1, 18} = 9.72$, $P = 0.005$). Ordinal parturition date for animals held in captivity was 228 ± 4 vs. 241 ± 14 for *V. berus* and *V. aspis*, respectively. Parturition occurred later in individuals housed in the outdoor enclosure (ordinal date 241 ± 5 vs. 249 ± 7 for *V. berus* and *V. aspis* respectively).

3.5. Daily metabolic rate

V. berus exhibited a higher cumulative daily SMR than *V. aspis* for all day categories (average oxygen consumption for all days was 0.114 vs. 0.068 $\text{ml}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$, for *V. berus* and *V. aspis*, respectively; linear mixed effects model, $F_{1, 10} = 578.193$; $P < 0.0001$; Fig. 3). The model with species and day category was the best to explain daily SMR variation. Daily SMR in *V. berus* was significantly elevated by higher thermoregulatory efficiency notably when conditions were constraining. For instance, the relative contribution of thermoregulation to SMR differences was negatively linked to mean daily temperature ($F_{1, 32} = 26.08$, $P < 0.001$, R^2 adjusted = 0.43; Fig. 4).

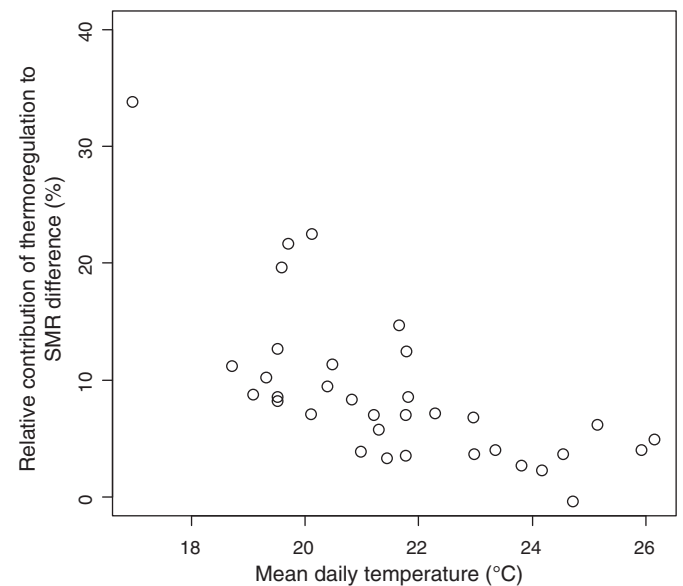


Fig. 4. Relative contribution of thermoregulation to species difference in SMR and its thermal dependence during the 38 days of monitoring. See text for detailed information on calculations.

4. Discussion

The goal of this study was to test for adaptive covariation between metabolism and thermoregulation by comparing a boreal specialist to its southern European relative. We emphasized gestation, which is a highly temperature-sensitive performance both in terms of offspring quality and phenology [45,23,46]. Moreover, we considered metabolic rate since it is a major component of climatic adaptation [47].

Lower thermal preferences should be advantageous under constraining (cold) climatic conditions [48,49]. Indeed, poor weather may dramatically reduce the opportunity to reach and maintain T_{set} and lower thermal requirements may thus compensate thermal constraints. Still, we found that pregnant females selected relatively high and precise body temperatures in a cost-free environment, and T_{set} during pregnancy was similar in the two species. Thermal preferences obtained in the gradient were consistent with maximum body temperature recorded in the external enclosure. For instance, during hot days, we found that pregnant females maintained stable T_b very close to the thermal preference over most of the basking period (Fig. 2).

Our results in semi-natural conditions supported the prediction of higher thermal efficiency in the boreal specialist. Under variable weather conditions, *V. berus* consistently maintained higher body temperatures, minimized deviation from T_{set} and was able to maintain T_{set} longer under each day category when compared to *V. aspis*. This difference was most marked when weather conditions were unfavorable and access to T_{set} limited. For instance, E_x was 92.95, 42.48 & 23.98% higher in *V. berus* for cold, medium, and hot days, respectively. Examination of daily thermal profiles suggests that *V. berus* was able to reach T_{set} earlier in the day than *V. aspis* (Fig. 2). Previous work has emphasized the influence of thermal conditions on reproduction in squamates [50,45] and the significance of maternal thermal shift [21]. However, recent studies indicated that the duration of exposure to T_{set} is also a crucial parameter (independently of mean temperature) in improving offspring quality (phenotypical effects) and hastening development (phenological effects) [51,52,46]. High thermoregulatory efficiency may be especially important under thermally limiting conditions [45,53] and likely involves several proximate mechanisms [29,34,54,55]. Pronounced thermophilic behavior has been previously reported in *V. berus* with conspicuous exposure most notably during cool weather. In addition, *V. berus* is unique in the genus for its ability to flatten the body and increase the surface area receiving solar radiation [35,30]. Physiological thermoregulatory mechanisms (changes in heat transfer) have been reported in viviparous squamates [56], but have not been investigated in *V. berus*. Finally, melanism is frequent in *V. berus*, notably in northern populations [57]. Melanism likely increases thermoregulatory efficiency beyond what we report here for normally colored (cryptic) individuals [58].

Our results on SMR support the metabolic cold adaptation hypothesis with markedly higher standard metabolic rates in *V. berus* for all temperatures considered. These findings are consistent with previous work on neonates [59] and help explain the unusually effective physiological performance at low temperatures in this species. For instance, a comparative study of five European vipers showed that digestion is fastest in *V. berus* with a unique ability to completely digest prey at low temperatures [60]. Using metabolic reaction norms, we estimated cumulative daily SMR in pregnant females based on T_b selected under semi-natural conditions. We revealed an important metabolic contrast between the two species. *V. berus* always had a higher daily metabolic rate, because of the difference in metabolic thermal reaction norms combined with superior thermoregulatory efficiency.

Higher metabolic rate is beneficial under cold conditions in that it ensures faster physiological processes [13] and embryonic development [47,61,62]. It may also influence maternal behavior by allowing refuge emergence and the onset of basking while thermal conditions are still constraining for other species [30]. The metabolic contrast was

illustrated by earlier parturition date in *V. berus*. In natural situations where the two species are syntopic, parturition also occurs sooner in *V. berus* than in *V. aspis* [29,30,63]. Therefore, higher metabolic rate and thermal efficiency are likely crucial to the ecological success of *V. berus* and its ability to minimize gestation duration [45,53]. Such phenological advantage should enhance offspring survival [23] as well as female ability to recoup energy before wintering [33].

Overall, our results suggest consistent covariation between thermoregulatory behavior, metabolic rate and reproductive performance. According to optimality models, precise thermoregulation is considered costly under cold conditions due to high costs of exposure (predation risks) and reduced physiological benefits [8]. However, higher SMR may compensate for thermal constraints [64,65,13] and therefore modify the cost–benefit balance of thermoregulation [46] by enhancing physiological performances (e.g. gestation duration). Viviparity appears to be a major contributor to the ecological radiation of viperid snakes [19]. Specific behavioral and metabolic adaptations in relation to reproduction may explain the distribution patterns displayed in the genus *Vipera*. Understanding coadaptation between metabolic rate and thermoregulation efficiency is critical for unraveling climatic adaptation and better defining the costs and benefits of thermoregulation [62,61].

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