

# Effect of Ambient Temperature in Neonate Aspic Vipers: Growth, Locomotor Performance and Defensive Behaviors



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## ABSTRACT

The impact of temperature during incubation and gestation has been tested in various reptiles; the postnatal period has been rarely investigated however. Three groups of newborn aspic vipers (*Vipera aspis*) were placed under contrasted thermal regimes during 7 months: (1) a cool 23°C constant regime, (2) a warm 28°C constant regime, and (3) an optimal regime with free-access to a wide range of temperatures. Later, all the snakes were placed under hibernation conditions (6°C) during 3 months. Finally all the snakes were placed in the optimal thermal regime during 2 additional months. The total duration of the experiment was of 12 months. Body mass and feeding rates were recorded weekly, body size was measured monthly. We also assessed locomotor performance and recorded several behavioral traits (e.g., defensive and predatory behaviors). As expected, snakes raised under cool temperatures exhibited low feeding rate, growth rate, body condition, and they exhibited poor locomotor performance; they also displayed marked defensive behaviors (e.g., high number of defensive bites) whilst hesitating during longer periods to bite a prey. Such behavioral effects were detected at the end of the experiment (i.e., 5 months after exposure to contrasted thermal treatments [3 months of hibernation plus 2 months of optimal regime]), revealing long term effects. Surprisingly, growth rate and locomotor performance were not different between the two other groups, warm constant 28°C versus optimal regimes (albeit several behavioral traits differed), suggesting that the access to a wide range of ambient temperatures was not a crucial factor. *J. Exp. Zool.* 9999A:1–9, 2013. © 2013 Wiley Periodicals, Inc.

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Ambient temperatures influence many life history traits (Flatt et al., 2001; Forsythe et al., 2001; Gignac and Gregory, 2005; Angilletta, 2009), notably in ectotherms (Huey, '82; Lillywhite, '87; Peterson et al., '93; Radder and Shine, 2006; Vickers et al., 2011). For instance, they determine activity levels, metabolism, reproduction rate, and influence phenotypes notably during early development (Peterson et al., '93; Deeming, 2004; Zani, 2008; Wang et al., 2009). Likely, these effects favored the evolution of behavioral and physiological thermoregulation. Although physiological thermoregulation is important, numerous ectotherms, including reptiles, reach, and maintain appropriate body temperatures principally through behavioral means (Seebacher and Franklin, 2005; Angilletta, 2009).

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Many reptiles use a complex repertoire of behaviors to regulate their body temperature depending upon environmental conditions and in relation to their physiological status (Huey, '74; Werner et al., 2005; McConnachie et al., 2011; Bonnet et al., 2013). For example, gravid or pregnant females tend to select high, precise, and stable body temperatures to provide optimal conditions to developing embryos (Li et al., 2009; Lориoux et al., 2012) whereas non-reproductive females tend to select cooler temperature to save energy and limit predation cost (Bonnet, 2011). Ontogenetic shifts in thermal preference have also been documented in reptiles (Xu and Ji, 2006). The selection of specific body temperatures promotes essential functions such as digestion, somatic growth, and embryonic development (Blouin-Demers et al., 2000; Lориoux et al., 2012). Optimal thermoregulation is associated to the notion that in thermally contrasted environments, individuals attempt to select their preferred body temperature, PBT (Reynolds and Casterlin, '79). PBT should represent the net outcome of complex interactions between numerous physiological factors and, as such, no body temperature could maximize all functions. However, PBT has been associated to elevated body temperature, usually ranging between 30 and 35°C (Angilletta, 2001). For instance, in temperate and cool climates, many reptiles (notably reproductive and recently fed individuals) bask in the sun to elevate their body temperature; this classical observation likely promoted the commonly held view that PBTs correspond to high and presumably optimal body temperatures for a wide range of physiological performance (e.g., digestion, gametogenesis; Reynolds and Casterlin, '79; Huey and Bennett, '87; Angilletta, 2001; Edwards and Blouin-Demers, 2007). A recent study on the aspic viper (*Vipera aspis*) challenged one tenet of the PBT-concept. Although it has been widely acknowledged that PBT corresponds necessarily to the most efficient body temperature to achieve a given function, experimental results suggested that digestion efficiency was not compromised when the snakes were maintained under a cool constant temperature of 23°C, markedly lower than the documented 30°C-PBT of the species (Michel and Bonnet, 2010). In other words, the vipers maintained under a constant cool temperature regime (23°C) converted similar proportions of the ingested prey into somatic tissues compared to the snakes maintained under various fluctuating thermal regimes (18–28°C) that allowed the vipers to reach only periodically their PBT. Importantly all the regimes were characterized by identical mean temperature (23°C). The unexpected absence of difference was likely explained by parasite energy expenditure associated to high body temperature (see Michel and Bonnet, 2010 for a discussion of this issue). These results were obtained using sub-adults and adults. However, neonates are most sensitive to environmental conditions compared to older (larger) individuals, notably because most of their development until adulthood is still to come and thus various growth trajectories are possible leading to possible important variations in the adult phenotype (Gilbert, 2005). Consequently,

the impact of temperature during the crucial postnatal stage was not examined by Michel and Bonnet (2010), limiting the extent of their conclusions.

In the current study, we assessed the impact of contrasted ambient temperatures during prolonged time periods (7 months) on growth rate in neonate snakes. We also assessed locomotor performance and defensive behaviors. These traits are essential after birth because neonate *V. aspis* do not benefit from postnatal parental care. Consequently, newborns are under strong selection to acquire resources to reach maturity, relying exclusively on their own behavioral and locomotor performance to hunt and escape predation. Many studies performed during incubation and on adults reported strong effects of temperature on predatory and defensive behaviors in snakes, (Fox et al., '61; Hailey and Davies, '86; Gutzke and Packard, '87; Burger, '89; Shine et al., '97; Brodie and Russel, '99; Lourdaïs et al., 2004). To our knowledge, the influence of contrasted thermal regimes on growth rate, locomotor performance, and behaviors has been rarely investigated during the postnatal period.

We placed two groups of neonate vipers under constant cool (23°C) versus warm (28°C) thermal conditions; a third group was maintained under a thermal gradient (18–32°C) where the snakes could select their PBT. To assess possible persistence of the effects, following artificial hibernation (6°C, 3 months), we placed all the snakes under optimal conditions during 2 additional months. We hypothesized that the snakes maintained under optimal temperatures would exhibit higher growth rate and thus higher locomotor performance compared to the snakes maintained under the cool regime; finally, the snakes maintained under intermediate conditions (warm) were expected to exhibit intermediate growth rates. We were not able to predict the direction of the possible effects of thermal regimes on predatory and defensive behaviors; indeed complex (Shine et al., 2000) and sometimes contradictory effects have been reported (e.g., Schieffelin and de Queiroz, '91 versus Whitaker et al., 2000).

## MATERIALS AND METHODS

### Studied Species

Growth rate and reproduction of the aspic viper (*V. aspis*) have been studied in the field and under controlled conditions (Naulleau, '97; Bonnet et al., '99a,b, 2000, 2001; Aubret et al., 2002; Zuffi et al., 2009). In its natural habitat, this species faces important fluctuations of ambient temperature (–20.0 to 45.0°C), and such fluctuations determine the activity period (Naulleau, '97). Digestion and reproduction are associated with a marked thermophily: individuals usually select high PBT of approximately 30°C during these metabolically demanding periods (Naulleau, '97; Ladyman et al., 2003).

### Thermal Regimes and Sample Size

The neonates used in the current experiment were born in the colony of the Centre d'Etude Biologique de Chizé from 10

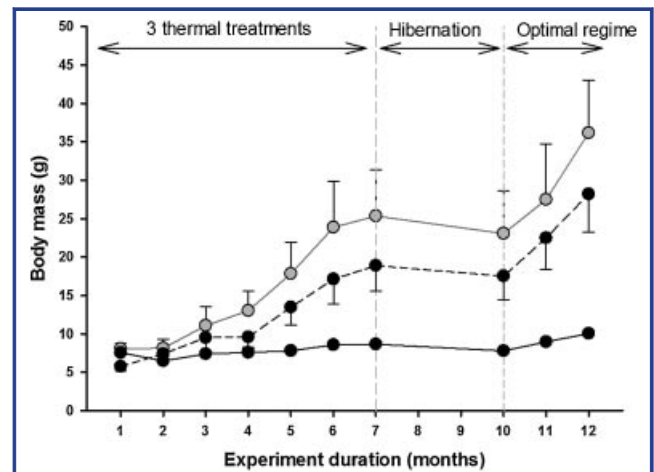
reproductive females (mated in the laboratory and maintained under similar controlled conditions during gestation, climatic chamber set at a constant temperature of 28°C). Seven females gave birth to 36 offspring. Immediately after birth, each newborn was placed into an individual transparent plastic box (33 cm × 19 cm × 11 cm, with artificial grass substratum, a shelter, and a water dish). The snakes were then randomly allocated to one of three thermal regimes: a cool constant thermal regime (23°C,  $N = 11$  snakes); a warm constant thermal regime (28°C,  $N = 14$  snakes); and an optimal thermal regime (temperature gradient ranging from 18 to 32°C across the length of the box,  $N = 11$  snakes). Fourteen neonates (39%) did not survive more than 6 months after birth; they did not accept the food presented (laboratory euthanatized pinky mice) due to unexplained reasons and they died from anorexia. The sex ratio was balanced (12 females and 10 males). The mean body mass of the neonates was  $7.1 \pm 1.7$  g (3.7–11.0 g) and the mean snout-to-vent length (SVL) was  $17.2 \pm 1.2$  cm (14.5–18.5 cm); these values are typical for this species (Bonnet et al., 2000).

The two constant thermal regimes were obtained using climatic chambers (Voetsch, Pharmaclim 500L, temperature 10–50°C, stability  $\pm 0.5^\circ\text{C}$ , homogeneity  $\pm 1.0^\circ\text{C}$ ). The optimal thermal regime was achieved by placing the boxes in a specifically designed rack with a set of heat cables running in the rear of the shelves creating a thermal gradient (18–32°C): this enabled the snake to select their PBT. The climatic chambers and the optimal thermal regime rack were placed into two air-temperature-controlled rooms set up respectively at 23°C and 18°C. Although the individual boxes were standardized for the three groups, the immediate environment surrounding the boxes in the climatic chambers versus rack were not similar. This difference, however, did not influence our main conclusions (see Results Section). The snakes were maintained under the three contrasted thermal regimes during 7 months. Later, all the young vipers were put in artificial hibernation: they were directly transferred from their respective thermal regimes to four climatic chambers set at 6°C (65% relative humidity, obscurity) during 3 months. The snakes were not fed at least 1 week before hibernation and thus none had a prey in the stomach during hibernation. This procedure mimicked natural situation: in the field the vipers rapidly undertake hibernation following the first brutal drop of ambient temperatures. The rationale for this treatment is that hibernation is a critical period for young snakes that takes place roughly 7 months after births in this species, like in other species from temperate climates (Naulleau, '97; Kissner and Weatherhead, 2005). Following hibernation, all the snakes were placed in the optimal regime during 2 additional months, thereby mimicking favorable thermal conditions during spring emergence. During all the experiment (except hibernation) the photoperiod was not manipulated and corresponded to the daylight of the natural activity period (the climatic chambers were fitted with large glass doors). Each snake was kept individually in the same type of

plastic box during the whole experiment (12 months, rapidly cleaned when necessary).

#### Morphological Measurements

The snakes were measured (SVL, snout vent length,  $\pm 0.5$  cm) and weighted ( $\pm 0.1$  g) every month and every week respectively. Except during hibernation, the vipers were fed following a quasi ad libitum design: they were offered food (euthanatized mice) every 2 weeks (adjusted to the snake body mass;  $24.6 \pm 2.6\%$ ); a quantity above the average amount of prey consumed in the field (Bonnet et al., 2000). At the beginning we proposed the same amount of food to the three groups of snakes. As the three groups exhibited different growth trajectories (see Results Section), the relative quantity of food was adjusted to individual body mass (one mouse per snake adjusted to the mass of the snake); thus the absolute mass of eaten prey diverged between treatments. In other words, the cool vipers digested slowly compared to the warm and optimal regime vipers, and hence they ate less often. The warm and optimal regime vipers consumed larger mice (i.e., absolute mass) as they became larger over time as relative prey mass was held constant. This means that the amount of food ingested varied among the three groups, but not the relative size of each meal (one mouse per meal). Body condition was calculated as the residual value of the regression between body mass (with no prey in the stomach) against body size. Because we observed long term differences in body size among the three groups of snakes (Fig. 1),



**Figure 1.** Changes in body mass (BM, Y-axis) over time in 22 neonate *Vipera aspis* subjected to three thermal treatments during 7 months: a cool constant regime (23°C,  $N = 7$ , black line), a warm constant regime (28°C,  $N = 6$ ; gray line), and an optimal thermal regime (18–32°C,  $N = 9$ , dashed line). Later the 22 snakes were placed in hibernation during 3 months. Finally, the 22 snakes were placed in the optimal thermal regime during 2 months. The total duration of the experiment was of 12 months. Means are expressed with their SE. See Statistics Section.

we decided to explore locomotor performance, defensive, and feeding behaviors. Consequently, such further assessments were performed later.

#### Locomotor Performance

Locomotor performance was recorded at the end of the 7 months period during which neonates were exposed to the three thermal treatments. We used swimming speed to assess locomotor performance. Indeed, swimming speed provides a reliable index to appreciate the physical condition of the propulsive structures in both aquatic and terrestrial snakes (Shine et al., 2003; Aubret and Bonnet, 2005). Indeed, when placed in the water, the snakes are forced to move forward (swimming at the surface is a reflex movement in vipers). By comparison, terrestrial crawling performance is usually more complex to assess as the snakes can stop, turn back, attempt to bite, etc. In practice, we placed each individual at one extremity of a long swimming pool (550 cm × 32 cm width × 25 cm depth, water temperature 25°C) fitted with red mark lines on the bottom every 50 cm. The snakes swam in the opposite direction of the release point in an attempt to escape the observer. When the snake reached the extremity of the pool, it was gently threatened (approaching the hand covered with a glove) by the observer in order to force it to turn back and to swim in the other direction: the snake covered 11 m (return trip in the pool) per trial. Each 11-m trial was repeated three times with a resting period (1 day) between two trials and the score was averaged to provide one value per individual. Swimming speed was expressed in meter traveled per second. A digital video camera (camcorder HVR-Z7E Sony®) hooked on a mobile track system attached to a metallic wire upon the pool allowed us to record the swimming characteristics. The high quality video data (50 frames/sec, HDV 1080i) were analyzed using the frame × frame reading option of the software Edius4® (Edius.4, 2006, Canopus Co. Ltd., Grass Valley, USA) that provides the exact timing (0.1 sec) for each frame. The swimming speed was calculated each time the snout of the snake crossed one of the 22 red mark lines (no lines at the extremities of the pool), hence every 25 cm swam. The video recording enabled us to discard imprecise data (e.g., a few times the snakes briefly ceased to swim). One snake refused to swim (i.e., simply floated at the surface) and thus provided no swimming speed.

#### Behavioral Measurements

Behavioral tests were performed only at the end of the experiment after the time period during which all the snakes were placed under similar optimal conditions (18–32°C gradient). We assessed anti-predator behavior by moving a leather glove in front of each viper, and then the middle of the body was touched (with the glove) in order to trigger defensive behaviors (Bonnet et al., 2005, 2013). We touched each individual 15 times. The time elapsed to elicit defensive behaviors (e.g., strike) was recorded. We observed two types of behaviors: some launched defensive bites whereas others refused to bite and fled.

For predatory behavior, we slowly presented a prey (dead pinky mice) to unfed snakes with a pair of forceps (30 cm) to elicit tongue flicking; we measured the latency period before the first predatory bite if any. We waited for a maximum period of 60 sec and considered that the snake refused to catch the prey in case of a total absence of strike. We estimated that the reaction time when a prey is close to the snake was an indicator of foraging ability (rapidity to catch a prey). Five individuals did not respond to the presence of the prey during the 60 sec test; nevertheless they eventually ate it later, for simplicity we arbitrarily attributed a reaction time of 60 sec. The room temperature was set at 23°C during behavioral measurements.

#### Statistics

Each individual is represented only once in analyzes and we found no litter effect (using maternal identity as a random factor in the analyses). The data were normally distributed (Shapiro–Wilk tests no significant). The time elapsed to strike under threat (“glove attack”) versus prey (“mice presentation”) stimulus diverged markedly (see Results Section), thereby validating our measurements: anti-predator and predatory behaviors were clearly distinguishable. We did not observe any differences between sexes in the analyses, thus, we pooled them in the analyses. Statistical analyzes were performed with Statistica7.1® (StatSoft, 2005). The alpha level for significance was 0.05.

## RESULTS

#### Neonate Survival

Among the 36 offspring, 14 did not survive: four, eight, and two snakes raised at 23, 28°C and under the optimal thermal regime, respectively. Apparently mortality was more pronounced at 28°C. However, a contingency table analysis suggests that such mortality rate did not differ among the three treatments ( $\chi^2 = 4.00$ ,  $df = 2$ ,  $P = 0.137$ , the result was close to significance however). Because we focused on long term effects, the results presented in the current experiment are consequently essentially based on 22 individuals ( $N = 7$ , 6, and 9, respectively, snakes maintained under the cool, warm, and optimal regimes) that did not present any sign of disorder and that accustomed well to captivity.

#### Effect of the Thermal Regimes on Morphological Traits

Although the neonates were randomly allocated to the three thermal regimes at the onset of the experiment ( $N = 36$ ), the early postnatal mortality introduced a weak difference in body mass between the groups (Table 1;  $N = 22$  snakes involved in the long-term experiment). However, a close inspection of the growth trajectories enabled us to discard this weak bias compared to the strong influence of the thermal treatments (Fig. 1). Indeed, after 7 months all the snakes increased in size, mass, and body condition ( $N = 22$ ; all  $P < 0.001$ ) and we found a strong effect of the temperature regimes on body size (SVL), body mass (BM), and

**Table 1.** Comparison of body mass (BM), body size (SVL), and body condition (BCI) among neonate *V. aspis* placed under three thermal treatments: "cool vipers" (23°C), "warm vipers" (28°C), and "optimal regime vipers" (18–32°C). Means are expressed with their SD. The snakes were measured at birth, before hibernation (BH, after 7 months of thermal treatment) and following 2 months of hibernation plus a 2 months period under a similar optimal thermal regime (H + Opt). The last column provides the statistics (ANOVAs with thermal treatment as a factor; see text for details).

Trait	Period	"Cool vipers" N = 7	"Warm vipers" N = 6	"Optimal regime vipers" N = 9	Statistics
BM (g)	Birth	7.5 ± 61.04	8.07 ± 1.67	6.05 ± 1.71	$F_{2, 19} = 3.678, P = 0.045$
	BH	8.66 ± 1.11	25.35 ± 14.82	18.91 ± 9.96	$F_{2, 19} = 4.671, P = 0.020$
	A + Opt	10.06 ± 1.08	36.67 ± 16.73	28.21 ± 14.89	$F_{2, 19} = 7.170, P = 0.005$
SVL (cm)	Birth	17.50 ± 1.00	17.92 ± 0.58	16.56 ± 1.33	$F_{2, 19} = 3.219, P = 0.062$
	BH	21.14 ± 0.99	30.58 ± 5.43	27.94 ± 4.71	$F_{2, 19} = 9.181, P = 0.001$
	A + Opt	22.71 ± 1.22	33.83 ± 6.27	31.56 ± 4.79	$F_{2, 19} = 11.557, <0.001$
BCI	Birth	0.01 ± 0.95	0.37 ± 1.67	-0.25 ± 0.59	$F_{2, 19} = 0.597, P = 0.560$
	BH	0.09 ± 2.95	2.79 ± 4.01	-1.93 ± 3.39	$F_{2, 19} = 5.578, P = 0.012$
	A + Opt	-1.27 ± 1.09	-0.11 ± 2.31	1.07 ± 2.03	$F_{2, 19} = 3.087, P = 0.069$

body condition (BCI) (Table 1). Thermal treatments influenced the increase in SVL and BM (ANOVA with repeated measures with respectively SVL and BM as the repeated dependent variables and the three thermal groups as a factor: Wilk-λ = 0.035 and 0.033;  $P < 0.001$  in both cases;  $N = 22$ ); but not the changes in BCI (Wilk-λ = 0.186,  $P = 0.490$ ). Moreover, the "warm vipers" and "optimal regime vipers" were not statistically different in terms of changes in BM, SVL, BCI (ANOVA with repeated measures with respectively BM, SVL, and BCI as the repeated dependent variables and the three thermal groups as a factor:  $F_{9, 99} = 0.542, F_{9, 117} = 0.231$ , and  $F_{8, 80} = 1.862$ , all  $P > 0.05$ ). All the 22 individuals survived to hibernation. Following emergence, the "cool vipers" did not catch up with the two other groups and remained smaller (SVL,  $F_{2, 19} = 7.170, P = 0.005$ ; BM,  $F_{2, 19} = 11.557, P < 0.001$ ).

**Effect of the Thermal Regimes on Swimming Performance**

Body size positively influenced mean swimming speed ( $F_{1, 19} = 29.579, r^2 = 0.608, P < 0.001; N = 21$ ), whilst body condition had no effect ( $F_{1, 19} = 0.171, r^2 = 0.008, P = 0.684$ ). The

"cool vipers" exhibited lower swimming speed compared to the other groups ( $F_{2, 18} = 5.669, P = 0.012$ ). However such effect was not apparent when swimming performance were adjusted to SVL (general linear model with mean swimming speed as a dependent variable, SVL as a co-variable and thermal treatment as factor:  $F_{2, 17} = 0.026, P = 0.974; N = 21$ ). Overall, the differences between the swimming performance of the three groups of vipers were driven by the divergence in SVL induced by the thermal treatments.

**Effect of the Thermal Regimes on Behavioral Traits**

After 5 months under similar thermal treatment (3 months of hibernation plus 2 months under optimal thermal regime), we found significant effects of the initial thermal regimes on anti-predator behaviors (Table 2). The "cool vipers" and "optimal regime vipers" tended to be more defensive compared to the "warm vipers" (Table 2): they struck sooner (LSD post hoc tests  $P < 0.001$  between "cool or optimal" versus "warm" vipers, no differences between "cool" and "optimal" vipers,  $P = 0.837$ ), and the "cool vipers" struck more often (LSD post hoc tests  $P < 0.010$

**Table 2.** Comparison of behavioral traits among neonate *Vipera aspis* placed under three thermal treatments: "cool vipers" (23°C), "warm vipers" (28°C) and "optimal regime vipers" (18–32°C). Means are expressed with their SD. The snakes were tested after 12 months of experiment, see text for details. The last column provides the statistics (ANOVAs with thermal treatment as a factor), post hoc tests are provided in Result Section.

	"Cool vipers" N = 7	"Warm vipers" N = 6	"Optimal regime vipers" N = 9	Statistics
Time before defensive striking (sec)	6.4 ± 5.02	17.34 ± 6.49	6.89 ± 2.99	$F_{2, 18} = 9.989, P = 0.001$
Number of strikes	4.86 ± 3.58	0.83 ± 0.41	3.89 ± 2.32	$F_{2, 19} = 4.452, P = 0.025$
Escape attempts	0.14 ± 0.38	0.83 ± 0.41	0.11 ± 0.33	$F_{2, 19} = 8.078, P = 0.002$
Time elapsed to predatory bite (sec)	40.71 ± 24.82	21.17 ± 21.20	12.67 ± 8.48	$F_{2, 19} = 4.603, P = 0.023$



between “cool” versus “warm” vipers,  $P = 0.033$  between “cool” and “optimal” vipers, and  $P = 0.455$  between “optimal” versus “warm” vipers). Considering escape behavior, we observed a complementary trend, the “warm vipers” fled more often than retaliated (Table 2; LSD post hoc tests  $P < 0.004$  between “cool or optimal” versus “warm” vipers, no difference between “cool” versus “optimal” vipers,  $P = 0.866$ ). The thermal treatments also influenced predatory behavior: the “cool vipers” hesitated during longer periods before they decided to bite the prey, the “optimal vipers” were faster and the “warm vipers” displayed intermediate reaction time (Table 2; LSD post hoc tests  $P < 0.001$  between “cool” versus “optimal” vipers,  $P = 0.073$  between “cool” versus “warm” vipers,  $P = 0.395$  between “optimal” versus “warm” vipers). Body size influenced the time elapsed before biting the prey ( $F_{1, 20} = 4.680$ ,  $r^2 = 0.189$ ,  $P = 0.043$ ), but the effect of the thermal treatments on this trait remained significant when body size was taken into account (general linear model with reaction time as the dependent variable, SVL as a co-variable and thermal treatment as a factor,  $F_{2, 19} = 4.603$ ,  $P = 0.023$ ).

## DISCUSSION

Our results show that in neonate vipers, divergent thermal treatments strongly influenced growth trajectories, locomotor performance and behavioral traits. After 7 months, individual body mass ranged between 7.7 and 63.7 g for the extreme cases whereas the initial body mass ranged between 7.04 and 8.21 g. As expected, the neonates exposed to cool ambient temperatures exhibited slow growth rate, and hence were smaller compared to the snakes placed under warmer conditions. Low temperature entailed slow digestion speed; consequently the snakes refused more often their prey; indeed the snakes with a prey in the stomach usually refuse to take another meal. The “cool vipers” accepted their meal less often and received a smaller number of smaller prey (in terms of absolute prey mass, not relative prey mass; see Methods Section). Overall, ambient temperature influenced a set of traits involved in somatic growth: digestion, metabolism, satiety, and feeding behaviors. Such temperature effects have been largely documented in ectotherms (Seebacher, 2005). The fact that larger snakes swam faster than the smaller ones as a by product of divergent growth trajectories affecting body size was also expected. These results reinforce the notion that high growth rate associated with high locomotor performance and favorable environmental conditions are determinant of somatic growth with possible consequence for the survival of young snakes (Arnold, '88; Burger and Zappalorti, '88; Forsman, '93).

Other results were more unexpected however. For instance, the “warm vipers” (constant 28°C regime) had no access to a wide range of temperatures, they nonetheless displayed similar growth trajectories compared to the snakes placed in the optimal thermal gradient. Moreover, their body condition was relatively high before hibernation (Table 1). We rather expected that the “warm vipers” would have exhibited intermediate growth performance.

Indeed, the “warm vipers” could not select their supposedly optimal PBT. More precisely, they could not escape continuous high temperatures entailing high metabolism, and thus they were subjected to an important waste of energy. Perhaps the “optimal regime vipers” selected high body temperature most of the time due to captivity stress and thus were also subjected to important energy expenditure (Bonnet et al., 2013). On the other hand, the “cool vipers” survived well to hibernation and exhibited a significant growth rate when placed under the optimal regime (Fig. 1). Therefore, a prolonged period (7 months) of unfavorable conditions did not eliminate the snake's capacities to develop rapidly under more favorable conditions, this phenomenon of growth compensation is known in juvenile lizards (Radder et al., 2007). Taken together, these results challenge the widely accepted notion that temperature fluctuations and access to high-PBT (i.e., generally above 30°C during digestion) are essential for reptiles living in temperate climates (Reynolds and Casterlin, '79); they rather suggest that the benefit of high-PBT is mainly represented by an acceleration of metabolically demanding processes (digestion and somatic growth notably).

We hypothesize that in the field, under fluctuating conditions (spring-early summer for the asp viper) the snakes select high-PBT to accelerate crucial and temperature dependent processes such as digestion; thereby increasing foraging rate, growth rate, and ultimately reproductive success. However, under natural conditions, various parameters (climatic factors notably) prevent the individuals to reach high-PBT. Furthermore during food shortage, we suggest that voluntary episodes of energy saving through the selection of low-PBT could be beneficial (Bonnet, 2011). Therefore, depending upon their physiological status, in combination with environmental conditions, the snakes can obtain major benefits through the selection of low-PBT; but this does not mean that temperature fluctuations are beneficial per se. This is illustrated by the data collected in “warm vipers:” food was offered almost ad libitum, the snakes had no difficulties digesting (28°C is a temperature only slightly lower than the PBT) and their performance did not differ significantly from that of snakes in the optimal thermal gradient. Overall, as proposed by Michel and Bonnet (2010) through a different experimental design, high-PBT may not necessarily provide the most efficient conditions regarding food conversion into somatic tissues but rather a mean to resume digestion rapidly in order to decrease the handicap caused by the presence of large prey in the stomach, and to assimilate resources as fast as possible. In this context, digestion speed should be distinguished from assimilation efficiency. Such a notion fits well with the fact that snakes living in warm climates do not select high-PBT (Shine and Madsen, '96; Luiselli and Akani, 2002) likely because they do not have to catch up with cool nocturnal episodes or cold seasonal periods. In addition, the elevation of body temperature through intensive sun basking increases conspicuousness and thus predation risks (Shine, '80; Bonnet and Naulleau, '96).

Our results on behavioral tests can be partly explained by the divergent growth trajectories and resulting divergent body sizes due to the thermal treatments. The “cool” smaller vipers responded more vigorously (struck more often) when threatened compared to the “warm” larger snakes (Table 2). In a parallel way, the larger “warm” and less defensive vipers decided more often to flee. These patterns suggest that the smallest snakes compensated for their size handicap through enhanced anti-predator displays. Small (young) snakes usually display stronger defensive behaviors than (larger) adults (Bonnet et al., 2005). We also observed significant differences in predatory responses: the cool and small snakes hesitated longer before deciding to catch their prey. Perhaps the “warm vipers” that consumed larger mice during the experiment were better accustomed to the type of food proposed. Alternatively, perhaps the “cool vipers” were slower as a direct effect of their cooler body temperature? The difference in body size generated by the three treatments could not explain entirely the pattern observed however: indeed the “optimal regime vipers” exhibited intermediate defensive and predatory behaviors, but they were not different in terms of body size compared to the “warm vipers.” This suggests that the thermal treatment per se affected the behaviors; yet we cannot explain the difference between the “warm vipers” versus “optimal regime vipers.” Interestingly, such behavioral differences that could not totally be explained by body size were observed after several months of common conditions (3-month of hibernation and 2-month of optimal regime). Thus, the difference induced by the “warm” versus “optimal regime” treatments persisted during a prolonged time period.

In conclusion, young snakes exposed to contrasted thermal regimes exhibited different growth trajectories that resulted into divergent body sizes. Such differences influenced locomotor performance, defensive, and predatory behaviors (some divergences persisted after the end of the thermal treatment). Importantly, our results suggest that constant ambient temperatures were not necessarily associated to inferior physiological performance compared to an optimal thermal gradient that allowed snakes to select their PBT; yet key processes of reproduction (e.g., gestation, embryo development) were not investigated, limiting the span of our conclusions. Finally, prolonged (7 months) low body temperatures slowed down growth rate but did not stop food assimilation. Our study thus provides another example of slow albeit regular growth occurring under environmental conditions usually considered as unsuitable for thermophilic ectotherms (Radder et al., 2007). Such phenomenon has been neglected in snakes. Further experimental investigations are needed to tease apart the importance of thermal conditions on survival and growth rate considering different developmental stages.

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