

Contrasted Thermal Regimes Do Not Influence Digestion and Growth Rates in a Snake from a Temperate Climate

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ABSTRACT

Temperature influences almost all life-history traits. For a period of 3 mo, we placed four groups of snakes under four contrasted thermal treatments: (1) a natural regime (NR), based on daily variations (24-h cycle); (2) an accelerated regime (AR), where the thermoperiod fluctuated rapidly (12-h cycle); (3) a slow regime (SR; 48-h cycle); and (4) a cool stable regime (ZR; no fluctuation). The mean temperature, set at 23°C, was identical for the four groups. For the first three groups (NR, AR, SR), ambient temperature fluctuated between 18°C and 28°C. Relative humidity and photoperiod were constant. We recorded feeding success, digestion efficiency, growth rate, activity, and ecdysis events. Differences between groups were expected because of varied exposure to the optimal temperatures, most notably in the ZR group, where the preferred body temperature for digestion (~30°C) would not be reached. Surprisingly, there was no significant effect of the experimental treatment on feeding rate, digestion, body mass increase, and growth rate. Our results do not conform to the paradigm stipulating that maximal body temperature selected by ectotherms necessarily corresponds to the most efficient for resource assimilation and that temperature fluctuations are essential. We propose that increasing the digestive tract's performance through body-temperature elevation trades off against elevated (parasite) energy expenditure from the rest of the body. The main advantage of high body temperatures would be to reduce the amount of time necessary to assimilate prey rather than to improve the net mass gain during digestion.

Introduction

In most habitats, environmental temperature fluctuates and thus poses challenges to organisms, notably in temperate and cool climates (Huey 1982; Lillywhite 1987; Peterson et al. 1993; Row and Blouin-Demers 2006). Indeed, optimal body temperatures are required to achieve high physiological performances (Blouin-Demers et al. 2000; Seebacher and Wilson 2007; Clark et al. 2008). Some lineages of ectothermic vertebrates essentially rely on behaviors to attain optimal body temperatures. They can select their body temperature when a wide gradient of environmental temperatures is available; this notion generated the term "preferred body temperature" (PBT), which represents a major paradigm of thermal ecology (Reynolds and Casterlin 1979; Huey and Bennett 1987; Angilletta 2001; Edwards and Blouin-Demers 2007). The PBT paradigm was originally based on experimental studies showing that lizards and snakes spent most of their time in a relatively narrow range of temperatures when placed into thermal gradients (Reynolds and Casterlin 1979). The concept evolved, and currently the coadaptation hypothesis offers a more complex and realistic conceptual framework that notably considers a range of relationships between PBTs, life-history traits, and phylogeny (Huey and Bennett 1987; Angilletta et al. 2006). However, the PBT paradigm integrated into the broader coadaptation hypothesis is still useful and widely used (Angilletta 2001; Edwards and Blouin-Demers 2007). For instance, temperature-sensitive neurons involved in the precise selection of preferred temperature in relation with optimal performances have been recently localized in the *Drosophila* brain, providing strong neurophysiological support for the central role of PBT, at least in some insects (Hamada et al. 2008).

Importantly, the PBT paradigm should be considered differently with respect to broad climatic conditions, taxonomic group, individuals, and physiological state. As expected, in tropical areas thermoregulation is easier operationally; for instance, most tropical reptiles are able to maintain high and stable T_b (~30°C) throughout the year without overt thermoregulatory activities. The PBT concept is often irrelevant for these animals (Shine and Madsen 1996; Luiselli and Akani 2002). On the contrary, in temperate or cold-climate areas, individuals regularly forsake their refuge for basking in order to reach high body temperatures (Avery 1979). It is generally assumed that the maximal T_b selected by reptiles, usually expressed through a plateau following a phase of rapidly increasing T_b (e.g., during basking), also corresponds to the PBT of the organism (Dawson 1975; Jobling 1981; Bennett and Beiting 1997; Blouin-Demers et al. 2000; Angilletta et al. 2002). Under cool ambient temperatures, major functions such as digestion, ecdysis, foraging,

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mate searching, and pregnancy indeed induce a marked sun-exposure thermophily (Dawson 1975; Bennett and Dawson 1976; Slip and Shine 1988; Gibson et al. 1989; Gregory et al. 1999; Vincent and Mori 2008). These long periods of sun-basking behaviors are essential to elevate body temperature in order to perform main major physiological functions but also for the production of vitamin D in some species and for ectoparasite control (Peterson et al. 1993). Although often irrelevant for several ectotherms (e.g., fish; Feldmeth et al. 1974), temperature fluctuations are particularly important for many reptiles. The benefit of T_b fluctuations in reptiles living in thermally variable conditions is another well-established paradigm (Peterson et al. 1993) with important applications for captive maintenance (Gavaud 1983; Fleury and Naulleau 1987; Murphy and Campbell 1987; Radder and Shine 2006). We frame our study in this “temperate climate” context where regular episodes of sun basking are required to reach PBT and where body temperature fluctuations are assumed to be crucial.

Recent studies pointed out that the correspondence between a given T_b and a particular physiological function is insufficient to encompass the range of variations of life-history traits and to understand interindividual differences (Helmuth et al. 2005; Angilletta et al. 2006; Tewksbury et al. 2008). Investigating how organisms perform under marked thermal heterogeneity and under various environmental conditions is therefore essential. One option is to explore poorly studied aspects such as the effect of fluctuating temperatures on organisms.

Among reptiles living in temperate or cool areas, snakes can maintain relatively high and stable temperatures in thermally contrasted environments (Peterson et al. 1993); therefore, they represent a suitable model to assess the importance of PBT and fluctuating ambient temperatures. In the current study, we experimentally examined the influence of contrasted thermal regimes on an ectothermic vertebrate living in a temperate climate, the aspic viper (*Vipera aspis*). In this species, digestion and reproduction are associated with a marked thermophily during which the vipers bask intensively and select a T_b close to 30°C (Bonnet and Naulleau 1996; Naulleau 1997). Because 30° ± 1°C corresponds to the T_b selected by individuals observed in the field in the course of metabolically demanding processes (Naulleau 1997) and because digestion rate is elevated around such values (Naulleau 1979, 1983), we considered that the PBT of the aspic viper was approximately 30° ± 1°C. This notion is reinforced by the fact that pregnant females maintain their T_b to a plateau close to 30°C (G. Naulleau, X. Bonnet, and O. Lourdais, unpublished field telemetry and captivity data).

The main novelty of our work was to impose different thermal rhythms on the snakes using identical mean ambient temperatures, thereby exploring the effect of temporal fluctuations per se (i.e., thermoperiod) rather than testing the influence of different average temperatures or of thermal instability around mean values. Surprisingly, there are very few experimental studies dealing specifically with thermoperiod independent of photoperiod and average ambient temperature. Some studies assessed the influence of temperature cycle on melatonin synthesis (Valenciano et al. 1997). Other studies examined the

influence of acclimation to constant or cycling temperature on temperature tolerance (Feldmeth et al. 1974; Hutchison and Maness 1979; Currie et al. 2004). However, to our knowledge, none examined the effect of thermoperiod (i.e., different from a differential access to heat source) on feeding or growth rate, for instance. Under natural conditions, however, species are exposed to different thermal cycles. Importantly, we also suppressed the possibility of a group of individuals reaching their PBT by using a cool and stable thermal regime to test the paradigms that both PBT and thermal fluctuations are essential. We focused our study on feeding, digestion, growth rates, activity, and ecdysis. All of these parameters are influenced by temperature and represent the first steps that an animal must achieve before reproduction.

The main objectives of our experiments were (1) to examine the effect of major disturbances in the periodicity during which PBT is maintained and (2) to quantify the consequences of preventing an ectothermic vertebrate reaching its PBT. We predicted that long time periods during which T_b is established substantially below the PBT (e.g., using a fluctuating regime with long cold periods) would result in major physiological complications, especially for digestion. Similarly, individuals exposed to constant low ambient temperature should exhibit markedly degraded growth rates. Indeed, previous experiments showed that various constant body temperatures markedly influence digestion in the aspic viper (Naulleau 1983, 1997). Answering these questions is an essential element to identify which factors (such as mean temperatures, thermoperiod, etc.) are the most constraining in natural situations.

Material and Methods

Studied Species

The aspic viper (*Vipera aspis*) is a viviparous snake of the western Palearctic region. Snout-to-vent length (SVL) for mean adult body size is approximately 55 cm, and body mass averages 100 g (Naulleau 1997). Field and captivity studies provide backgrounds on life cycle, growth rate, and reproduction (Saint Girons 1957, 1978; Naulleau 1975, 1979, 1983; Naulleau and Bonnet 1996; Bonnet et al. 1999b, 2000, 2003; Zuffi et al. 1999, 2009). In the distribution area of the aspic viper, ambient temperature fluctuates between -20.0°C and 45.0°C, generating important variations of T_b (Naulleau 1997). In western central France, following long winter hibernation, the activity period usually extends from spring to late summer (6 mo). During this active period, T_b generally oscillates from 15°C to 31°C (Saint Girons 1978; Naulleau 1997). During the most intensive phase for foraging (late April–late June), the vipers often bask in the sun, especially in low air-temperature (T_a) conditions (5° < T_a < 25°C), to elevate their T_b substantially above T_a (Naulleau 1997). For the current experiment, we adopted a 3-mo-period design that corresponds broadly to the duration of the main phase of foraging activity.

The aspic viper is easily raised in captivity and is therefore suitable for long-term experimental studies. The individuals used in the current experiment originate from a single study

site located in a natural reserve (western Europe, France, Integral Biological Reserve of the forest of Chizé, 46°09'N, 0°24'W, 80 m elevation) and constitute a homogenous group of animals. The total sample size was 32 vipers: 8 adult females, 8 adult males, 8 juvenile females, and 8 juvenile males. In order to focus on resource assimilation, both adult males and females were unmated during the experiment. Several (6–24) months before the beginning of the experiment, the snakes were kept under identical conditions (individual boxes 42 cm × 34 cm × 19 cm, with artificial grass substratum, a shelter, a water dish, and a thermal gradient 20°–38°C during the day and 18°–20°C at night). They were weighed every week and measured once a month. The snakes adapted well to their environmental conditions as indicated by their regular increase in size and mass along with a total absence of difficulties during skin sloughing.

Experimental Design

Experimental Groups. We used four contrasted thermal treatments. Thermoperiod was the only variable parameter, fluctuating in three groups and stable in the fourth group but with an identical mean value for all the four groups (details are provided below). Consequently, we used four groups of snakes (8 snakes per group, total = 32 vipers) composed of an equivalent number of juvenile males, juvenile females, adult males, and adult females. Snakes from each age and sex category were randomly selected among more than 100 captive snakes and allocated among these groups; sex and age ratio were thus equally distributed. There were no differences between the four groups in terms of mean body mass (ANOVA with snake body mass as the dependent variable and the four groups as the factor: $F_{3,28} = 0.003$, $P = 0.999$), body size (same design ANOVA: $F_{3,28} = 0.001$, $P = 0.999$), and body condition (mass scaled by size [residuals]; same design ANOVA: $F_{3,28} = 0.335$, $P = 0.800$). At the beginning of the experiment, the mean characteristics of the snakes were consequently indistinguishable between the four groups. Each snake was placed into a new plastic box fitted with an artificial grass substratum, a shelter (refuge is essential for snakes), a water dish, but no heater. The size of the box was adjusted to the size of the snake: snakes smaller than 40 cm (SVL) were placed into 33 × 19 × 11-cm boxes; larger snakes were placed into 40 × 34 × 14-cm boxes. The snakes were offered food (killed mice every 2 wk adjusted to the size of the snake; 24.6% ± 2.6% of the snake mass), and their reaction was recorded (acceptance vs. refusal).

Climatic Chambers and Thermal Regimes. To manipulate thermal conditions, we used four large climatic chambers that enabled the independent control of temperature and humidity (Voetsch, Pharmaclim 500l, four large glass-door-equipped shelves with magnetic door seals, internal dimensions 58.5 cm × 65.5 cm × 133.5 cm, temperature 10°–50°C, stability ± 0.5°C, homogeneity ± 1.0°C; humidity 20%–90% RH, stability ± 3% RH). The four climatic chambers were placed into an air-temperature-controlled room set up at 23°C. Each climatic chamber received eight plastic boxes with one snake

in each and was set to one of the four climatic regimes over 3 mo (Fig. 1). The four climatic regimes were as follows.

1. A natural regime (NR), where ambient temperature shifted every 12 h. This regime mimicked daily fluctuations: a 12-h hot phase (sunny day) followed by a 12-h cool phase (night). The fluctuations imposed from 18°C to 28°C, mean 23.0° ± 0.5°C, were thus maintained within the range experienced by snakes in their natural environment.

2. An accelerated regime (AR), where the thermoperiod fluctuated rapidly, every 6 h. Mean temperature and the amplitude of variations were similar (23.0° ± 0.5°C; 18°–28°C) compared with the previous group, but the thermoperiod was half the thermoperiod experienced in natural conditions.

3. A slow regime (SR), where mean ambient temperature and the amplitude of variations remained unchanged but with only one shift every 24 h, generating a slow cycle (48 h).

4. A stable regime (ZR), characterized by an absence of cycle and a mean ambient temperature of 23.0° ± 0.5°C.

The climatic chambers were automatically synchronized and driven by a PC (using Simpati software). The humidity was maintained at 65% (RH) in all the climatic chambers. The photoperiod was a constant 12D : 12L, using external lamps to avoid temperature interference. These settings corresponded broadly to natural values during the peak period of activity (April–June). We did not use an ambient temperature of 30°C (supposedly the PBT) because preliminary tests showed that the snakes became agitated after several hours at this temperature but that they remained calm at 28°C. In our experimental design, the number of temperature shifts varied from 2 to 8 per 48 h depending on the group (AR, NR, SR). We selected a procedure to impose identical total shift duration for all individuals: the sum of the shifts was always 6 h per 48 hrs. The rest of the time (42 h) was characterized by thermal stability (± 0.5°C).

Data Collection

The boxes were examined at least once a day; the possible feces were immediately collected, weighed, dried (7 d in a 60°C autoclave), and then reweighed. The snakes were measured (SVL to the nearest 0.5 cm, body mass to the nearest 0.1 g) every week. Growth rates (increase in size), body mass, and body condition changes were then calculated. Skin sloughing status was recorded (change of eye color, sloughed skin) every day. To limit temperature perturbations provoked by door openings, all the snake manipulations occurred when the internal temperature of the climatic chambers was of 23°C, hence similar to the room temperature. After completion of measurements, the boxes were put back into their climatic chamber but randomly positioned among the shelves. This later procedure enabled us to control for a possible “position-in-the-chamber” effect.

To estimate the effect of temperature regime on activity level, we quantified snake behavior for 15 min/d. We used only two main types of behaviors: motionless and moving. We discarded the first 5 min after the arrival of the observer to limit the possible effect of movements in the air-temperature-controlled room and thus based the analyses on the following 10 min

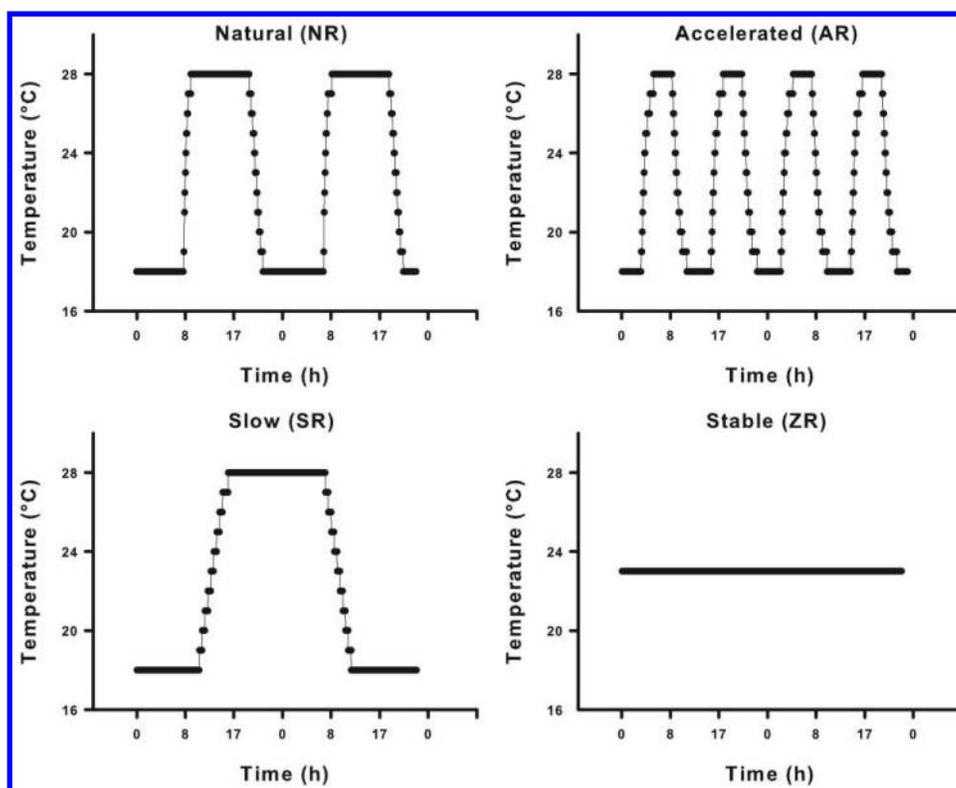


Figure 1. Four groups of snakes were placed in different climatic chambers under four contrasted thermal treatments: a natural regime (NR), based on daily variations (24-h cycle); an accelerated regime (AR), where the thermoperiod fluctuated rapidly (12-h cycle); a slow regime (SR; 48-h cycle); and a cool stable regime (ZR; no fluctuation). Each graph presents the temperature data recorded using four snake models per climatic chamber (see text for details). Each dot provides a mean value; the SDs were too small to be visible.

with a precision of 1 s; results are therefore presented in number of seconds (motionless vs. moving) during a 600-s period.

Analyses

The simple experimental design allowed for straightforward analyses. We considered body size, body mass, and body condition as different variables. Although interdependent (e.g., size and mass are correlated), they do not provide similar biological information, and they do not react identically under experimental conditions (Bonnet et al. 2001b; Aubret et al. 2004). Body condition, calculated as the residuals from the regression between body mass (log) against body size (log), is a precise indicator of body reserves in the asp viper (Naulleau and Bonnet 1996). Asp vipers are typical infrequent feeders; they were fed every 2 wk in the current design, and digestion plus the production of feces usually required 1 wk. Consequently, it was possible to calculate an index of digestion efficiency as follows. We divided the dry mass of the feces by the estimated dry mass of the corresponding ingested mouse (the dry mass was derived from the tight correlation between dry and fresh mass: $r = 0.99$). Such an index cannot provide the absolute value of digestion efficiency, but it can be used for intergroup comparisons: a low value of the ratio indicates a more complete

assimilation of the prey. Most of the data were normally distributed (all Shapiro-Wilk tests were nonsignificant), but activity and behavioral data were not, and both parametric and nonparametric tests were consequently employed. All statistics were performed with Statistica 7.1 (StatSoft, Tulsa, OK).

Results

Behavioral Response of the Snakes Placed in the Climatic Chambers

As no equivalent experiment has been previously performed on snakes (at least to our knowledge), it was important to ensure that the particular conditions experienced by the snakes during a long 3-mo period did not generate undesirable effects. Captive snakes that feel uncomfortable often scratch the tip of their snout through incessant attempts to escape from their cage; such individuals are also usually anorexic. These vipers were well accustomed to the artificial conditions of the climatic chambers; no snake exhibited any perceptible sign of behavioral or physical disorder. They all exhibited substantial growth rates and sloughed their skin in entirety. Importantly, the snakes spent a significantly greater amount of time visible and motionless in the open (mean = 436 ± 262 s/600 s; $72.7\% \pm 43.7\%$ of the time) than hidden under their shelter (mean =

164 ± 262 s/600 s; 27.3% ± 43.7%; $\chi^2 = 64.94$, $df = 1$, $P < 0.001$) regardless of gender, body size, and body condition. Such conspicuous behavior implied that the snakes were not particularly alarmed and suggested a low level of stress. Overall, these factors indicated that the vipers tolerated the experimental conditions well, enabling us to focus on the thermal treatment.

Long-Term Effects of the Four Thermal Regimes

Body Size, Body Mass, and Body Condition. The snakes accepted their meals almost every time, and consequently they all exhibited a significant somatic growth. Body size, body mass, and body condition increased over time (repeated-measure ANOVAs, $F_{3,28} = 59.308$, $P < 0.001$; $F_{3,28} = 58.299$, $P < 0.001$; and $F_{3,28} = 58.823$, $P < 0.001$, respectively; Fig. 2). Unexpectedly, there was no significant effect of the experimental treatment on these traits: change of body mass (Wilks's $\lambda = 0.962$, $F_{3,28} = 0.177$, $P = 0.982$), body size (Wilks's $\lambda = 0.993$, $F_{3,28} = 0.034$, $P = 0.999$), and body condition (Wilks's $\lambda = 0.955$, $F_{3,28} = 0.210$, $P = 0.972$) all increased at a regular rate independent of the thermal regime. The mean growth rate of the snakes was 2.85 ± 1.86 cm in the course of the 3-mo experiment.

Digestion Efficiency Index. There were no significant effects of the four thermal regimes on the mean mass of the feces ($F_{3,28} = 0.098$, $P = 0.961$), the mean number of feces produced by an individual ($F_{3,28} = 0.031$, $P = 0.993$), and the index of assimilation calculated over the entire study period ($F_{3,28} = 0.265$, $P = 0.850$). These results mirror the previous ones about growth rates. Overall, despite contrasted treatments, snakes ate similar amounts of food, produced an equivalent quantity of feces of the same mass that also contained an identical proportion of dry materials (an index of assimilation), and allocated similar amounts of resources into somatic growth.

Ecdysis. Under natural conditions, during the 6–7 mo of activity per year, ecdysis is observed one to three times (Nauulleau 1997). During the 3 mo of the experiment, most of the snakes shed their skin at least once ($N = 24$), sometimes twice ($N = 7$), and few individuals did not shed at all ($N = 8$). Thus, the ecdysis rate conformed well to what is observed in natural conditions.

There was no significant effect of thermal regime on the date for the first ecdysis (mean number of days elapsed between the beginning of the experiment and the first ecdysis, $F_{3,20} = 0.825$, $P = 0.495$) or on the number of ecdyses per individual ($F_{3,28} = 1.311$, $P = 0.290$). Although nonsignificant, it is interesting to note that all eight snakes subjected to the accelerated thermoperiod regime (AR) shed their skin at least once, while only half of the individuals placed under constant temperature (ZR) and two-thirds of the snakes of the natural (NR) or slow (SR) thermoperiod did.

Activity. As expected, snake T_b (determined by T_a) and feeding status positively influenced activity. Snakes were more active at 23°C than at 18°C, and were even more active at 28°C. After

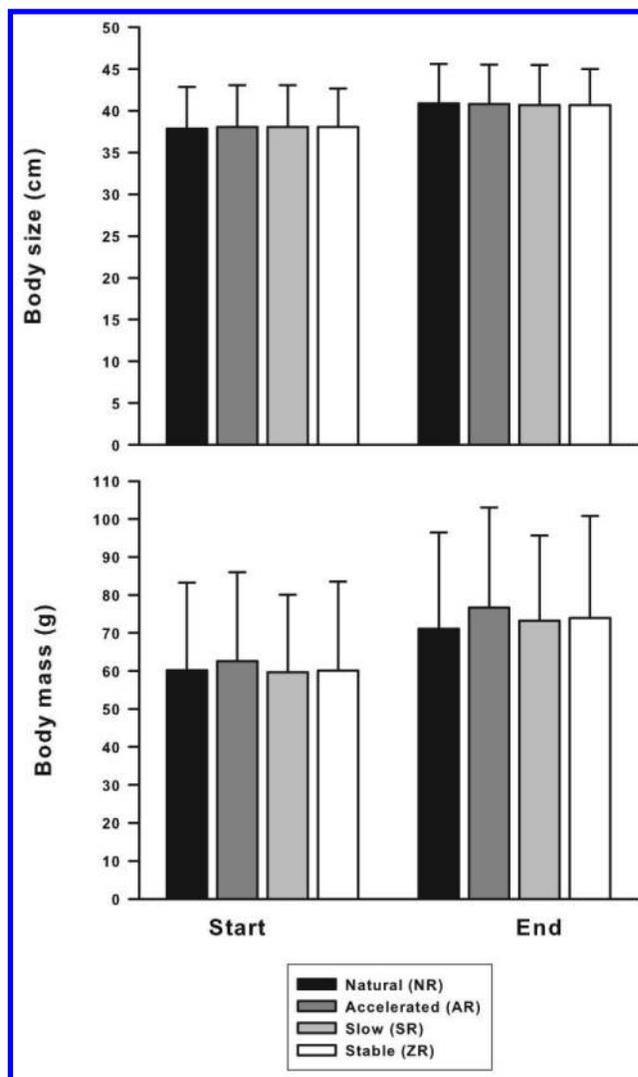


Figure 2. Body size (*top*) and body mass (*bottom*) of the snakes from each of the four thermal treatments (natural regime [NR], accelerated regime [AR], slow regime [SR], and cool stable regime [ZR]) at the beginning of the experiment (*Start*) and 3 mo later (*End*). Each bar provides mean value (\pm SE, $N = 8$). All snakes increased in size and mass, but we found no difference between the groups (see text for statistics).

a meal, hence during digestion, the snakes were more often motionless compared with unfed snakes (roughly 1 wk after ingestion). Such typical results are not presented in detail, but they suggest that using our method, it was possible to estimate the activity level of the monitored snakes.

On average, there was no significant difference of activity between the snakes from the four different experimental conditions (using the mean activity value calculated with all the data available for each individual: Kruskal-Wallis, $H_{3,28} = 5.111$, $P = 0.163$). However, in the climatic chambers the snakes were active at different time periods of the day because temperature variations did not occur simultaneously. The snakes in the stable climatic chamber (ZR) exhibited an average activity

level undistinguishable from the average level observed in the three other chambers. Because feeding rates were similar, the influence of feeding status on activity was equally distributed among treatments.

Discussion

Our experiment lasted 3 mo. However, because prey was offered on a regular basis to the snakes, growth-rate values corresponded to those recorded during an entire active season in the field (6 mo activity/yr), where important fluctuation in food availability occur (Bonnet et al. 2001a). We base this statement on the comparison of our experimental snakes with free-ranging aspik vipers, where both feeding and growth rates have been assessed (Bonnet et al. 1999a, 2000, 2002). Under natural conditions the aspik vipers are active during 6 mo on average and feed on voles (similar in size to laboratory mice); the mean annual growth rate of nonreproductive females monitored in the field over several years is 2.0 ± 2.3 cm/yr ($N = 206$; Bonnet et al. 2001a). Therefore, our experimental values correspond to the average annual absolute increase of size of the species. Considering a 3-mo timescale, growth rate was significantly greater for the females placed into the climatic chambers compared with females monitored in the field (t -test, $P < 0.001$). Therefore, the duration of the experiment combined with the elevated growth rate we recorded enables us to derive conclusions applicable to an entire annual activity of the species under temperate climate.

Our main result, the absence of effect of contrasted thermal regimes on resource assimilation, was unexpected. We emphasize that our experimental design did not question the positive influence of temperature on activity and metabolism per se; similarly, we do not claim that temperature fluctuations are unimportant for reptiles living in temperate or cold climates. Rather, our results challenge the simplistic (but widespread) vision of the PBT paradigm stipulating that selected, generally high body temperatures (both in the field or using an experimental thermal gradient) necessarily correspond to the most efficient T_b for major biological processes, such as resource assimilation. Comparing different lizard species, Huey and Bennett (1987) also questioned a simplistic view of the relationships between selected body temperature and locomotor performances. Importantly, by the term "efficiency" we mean the proportion of ingested resources converted into somatic tissues (approximated by assimilation and growth rates). From this perspective, speed (the quantity of time necessary to convert a given amount of food resources into somatic tissues) is clearly a distinctive characteristic in the overall process for resource assimilation. We suggest that such a neglected distinction has important implications for better understanding the thermal ecology of reptiles, most notably because they experience important fluctuations of T_a associated with various metabolic rates. Our experiment demonstrates that organisms such as the aspik viper, considered a typical thermophilic snake (Saint Girons 1975, 1978), can withdraw resources from prey with an equivalent overall efficiency under a far wider spectrum of T_b

than previously assumed. This is mainly supported by the surprising elevated growth rate of the snakes maintained at 23°C in our experiment.

Mean temperature was the sole constant thermal parameter between the four groups of snakes; this factor was therefore a key element. Importantly, the average T_b of 23°C (markedly under PBT, ~30°C, for digestion) we generated through marked fluctuations (NR, AR, SR) or via a stable regime (ZR), always provided favorable thermal conditions to the snakes. Indeed, all the vipers grew rapidly in absolute terms. Clearly, providing regular meals (one every 2 wk) and stable ambient temperature of 23°C ($T_a = T_b$ in the chambers) were particularly favorable for growth. The precise underlying physiological mechanisms (e.g., kinematics of all the enzymes involved) are unknown. However, we can propose a crude explanation for our unexpected results. Hot temperatures accelerate assimilation processes but inevitably entail high energy expenditure because the metabolism of the whole organism increases, including the tissues not involved in digestion, such as locomotor muscles, which represent more than 35% of the total body mass (Bonnet 1996; Bonnet et al. 1998). Conversely, low temperatures slow digestion, assimilation, and growth but enable energy saving. Overall, ignoring the speed of the processes, various temperature regimes with identical means are associated with similar performances to convert food into somatic tissues probably because the temperature of the organs involved in digestion (e.g., stomach, gut, liver) cannot be set at a different temperature level from the organs not involved (e.g., locomotor muscles, skin, gonads). Improving the performances of the digestive tract (less than 20% of the body mass; Bonnet 1996) via behavioral positive thermoregulation automatically results in parasite energy expenditure (PEE) from the rest (80%) of the body.

If our hypotheses are correct, then thermoregulation in natural conditions should be partly reconsidered. Indeed, many snakes from temperate areas bask intensively in the sun during digestion to reach high PBT, typically around 30°C, even when T_a is not particularly low (e.g., $T_a \sim 20^\circ\text{--}25^\circ\text{C}$). Basking, however, is a very conspicuous behavior that exposes individuals to avian predation (Naulleau et al. 1997). Consequently, high PBTs are considered as essential for digestion, and this necessity is assumed to compensate for the predation cost generated by the marked thermophily of digestion. Our results suggest that in many cases, individuals could avoid such high survival costs, without strong negative consequences on their assimilation performances, by adopting lower T_b —for example, close to 25°C—simply by remaining partly sheltered in the bushes.

Why do snakes instead coil themselves in the sunrises and adopt such visible and perilous postures? Does a slight benefit (completion of digestion shortened by no more than a few days) justify the risk of being killed by a predator? The answer possibly relies on the cool nocturnal phases that considerably lower the mean integrated T_b over weeks or months. High T_b may serve to compensate cool nocturnal phases, typically largely below 20°C during the main feeding period that occurs in spring (Bonnet et al. 2001a).

One of the most important predictions that can be derived

from our results is that snakes from cool climates should exhibit more precise high preferred temperatures compared with snakes living in hot or tropical climates. Indeed, the former should be under strong selection to compensate the cool phases that occur at night, during rainfalls, and during cold-weather episodes. Snakes living in hot climates, where thermoregulation is not necessarily a major issue (Shine and Madsen 1996; Luiselli and Akani 2002), do not have to develop such putative compensating abilities. Similarly, individuals in high body condition should adopt less conspicuous thermoregulatory behaviors than individuals in low body condition, for which rapid body reserve restoration is vital.

An important limitation to our study is that it only involved nonreproductive individuals; the massive effect of reproduction on behaviors, physiology, and thermal requirements may well impose different constraints on thermoregulation than food resource assimilation and growth. For instance, the respective thermal ecological niches of closely related species might be more explained by embryonic requirements rather than digestion efficiency. Further experiments are required to test these hypotheses.

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Literature Cited

- Angilletta M.J. 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* 82:3044–3056.
- Angilletta M.J., A.F. Bennett, H. Guderley, C.A. Navas, F. Seebacher, and R.S. Wilson. 2006. Coadaptation: a unifying principle in evolutionary thermal biology. *Physiol Biochem Zool* 79:282–294.
- Angilletta M.J., P.H. Niewiarowski, and C.A. Navas. 2002. The evolution of thermal physiology in ectotherms. *J Therm Biol* 27:249–268.
- Aubret F., R. Shine, and X. Bonnet. 2004. Evolutionary biology: adaptive developmental plasticity in snakes. *Nature* 431:261–262.
- Avery R.A. 1979. *Lizards: A Study in Thermoregulation*. Arnold, London.
- Bennett A.F. and W.R. Dawson. 1976. Metabolism. Pp. 127–223 in C. Gans and W.R. Dawson, eds. *Biology of the Reptilia*. Vol. 5. Academic Press, New York.
- Bennett W.A. and T.L. Beitinger. 1997. Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. *Copeia* 1997:77–87.
- Blouin-Demers G., K.J. Kissner, and P.J. Weatherhead. 2000. Plasticity in preferred body temperature of young snakes in response to temperature during development. *Copeia* 2000: 841–845.
- Bonnet X. 1996. *Gestion des Réserves Corporelles et Stratégie de Reproduction de la Vipère Aspic*. PhD diss. University of Lyon.
- Bonnet X., O. Lourdais, R. Shine, and G. Naulleau. 2002. Reproduction in a typical breeder: costs, currencies, and complications in the aspic viper. *Ecology* 83:2124–2135.
- Bonnet X. and G. Naulleau. 1996. Catchability in snakes: consequences for estimates of breeding frequency. *Can J Zool* 74:233–239.
- Bonnet, X., G. Naulleau, O. Lourdais, and M. Vacher-Vallas. 1999a. Growth in the asp viper (*Vipera aspis* L.): insights from long term field study. Pp. 63–69 in C. Miaud and R. Guyétant, eds. *Current Studies in Herpetology*. Societas Europaea Herpetologica, Le Bourget du Lac.
- Bonnet X., G. Naulleau, R. Shine, and O. Lourdais. 1999b. What is the appropriate time scale for measuring costs of reproduction in a capital breeder such as the aspic viper? *Evol Ecol* 13:485–497.
- . 2000. Reproductive versus ecological advantages to larger body size in female snakes, *Vipera aspis*. *Oikos* 89:509–518.
- . 2001a. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos* 92:297–308.
- Bonnet X., R. Shine, O. Lourdais, and G. Naulleau. 2003. Measures of reproductive allometry are sensitive to sampling bias. *Funct Ecol* 17:39–49.
- Bonnet X., R. Shine, G. Naulleau, and C. Thibirce. 2001b. Plastic vipers: environmental influences on the size and shape of Gaboon vipers, *Bitis gabonica*. *J Zool (Lond)* 255:341–351.
- Bonnet X., R. Shine, G. Naulleau, and M. Vacher-Vallas. 1998. Sexual dimorphism in snakes: different reproductive roles favour different body plans. *Proc R Soc B* 265:179–183.
- Clark N.J., M.A. Gordos, and C.E. Franklin. 2008. Thermal plasticity of diving behavior, aquatic respiration, and locomotor performance in the Mary River turtle *Elusor macrurus*. *Physiol Biochem Zool* 81:301–309.
- Currie R., W. Bennett, T. Beitinger, and D.S. Cherry. 2004. Upper and lower temperature tolerances of juvenile freshwater game-fish species exposed to 32 days of cycling temperatures. *Hydrobiologia* 523:127–136.
- Dawson W.R. 1975. On the physiological significance of the preferred body temperatures of reptiles. Pp. 443–473 in D.M. Gates and R. Schmerl, eds. *Perspectives of Biophysical Ecology*. Springer, New York.
- Edwards A.L. and G. Blouin-Demers. 2007. Thermoregulation as a function of thermal quality in a northern population of painted turtles, *Chrysemys picta*. *Can J Zool* 85:526–535.
- Feldmeth R., E.A. Stone, and J.H. Brown. 1974. An increased scope for thermal tolerance upon acclimating pupfish (*Cyprinodon*) to cycling temperatures. *J Comp Physiol* 89:39–43.
- Fleury F. and G. Naulleau. 1987. Relations between overwin-

- tering and recovery of endocrine activities (testicle and thyroid gland) in *Vipera aspis* L. (Reptilia, Viperidae). *Gen Comp Endocrinol* 68:271–277.
- Gavaud J. 1983. Obligatory hibernation for completion of vitellogenesis in the lizard *Lacerta vivipara*. *J Exp Zool* 225:397–405.
- Gibson A.R., D.A. Smucny, and J. Kollar. 1989. The effects of feeding and ecdysis on temperature selection by young garter snakes in a simple thermal mosaic. *Can J Zool* 67:19–23.
- Gregory P.T., L.H. Crampton, and K.M. Skebo. 1999. Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic? *J Zool (Lond)* 248:231–241.
- Hamada F.N., M. Rosenzweig, K. Kang, S. Pulver, A. Ghezzi, T.J. Jegla, and P.A. Garrity. 2008. An internal thermal sensor controlling temperature preference in *Drosophila*. *Nature* 454:217–220.
- Helmuth B., J.G. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? *Annu Rev Physiol* 67:177–201.
- Huey R.B. 1982. Temperature physiology and the ecology of reptiles. Pp. 25–91 in C. Gans and F.H. Pough, eds. *Biology of the Reptilia*. Vol. 12. Academic Press, New York.
- Huey R.B. and A.F. Bennett. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* 41:1098–1115.
- Hutchison V.H. and J.D. Maness. 1979. The role of behavior in temperature acclimation and tolerance in ectotherms. *Am Zool* 19:367–384.
- Jobling M. 1981. Temperature tolerance and the final preferendum: rapid methods for the assessment of optimum growth temperatures. *J Fish Biol* 19:439–455.
- Lillywhite H.B. 1987. Temperature, energetics and physiological ecology. Pp. 422–477 in R.A. Seigel, J.T. Collins, and S.S. Novak, eds. *Snakes: Ecology and Evolutionary Biology*. Macmillan, New York.
- Lillywhite H.B., P. Licht, and P. Chelgren. 1973. The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology* 54:375–383.
- Luiselli L. and G.C. Akani. 2002. Is thermoregulation really unimportant for tropical reptiles? comparative study of four sympatric snake species from Africa. *Acta Oecol* 23:59–68.
- Murphy M.F. and M.J. Campbell. 1987. Sudden infant death syndrome and environmental temperature: an analysis using vital statistics. *J Epidemiol Community Health* 41:63–71.
- Naulleau G. 1975. Activity cycles of *Vipera aspis* and choice between natural and artificial climatic conditions. *Vie Milieu* 25:119–136.
- . 1979. Biotelemetric study of thermoregulation of *Vipera aspis* (L) raised under experimental conditions. *J Herpetol* 13:203–208.
- . 1983. The effects of temperature on digestion in *Vipera aspis*. *J Herpetol* 17:166–170.
- . 1997. La vipère aspic. *Eveil Nature*, Saint Yrieix.
- Naulleau G. and X. Bonnet. 1996. Body condition threshold for breeding in a viviparous snake. *Oecologia* 107:301–306.
- Naulleau G., C. Verheyden, and X. Bonnet. 1997. Prédation spécialisée sur la vipère aspic *Vipera aspis* par un couple de buses variables *Buteo buteo*. *Alauda* 65:155–160.
- Peterson C.R., A.R. Gibson, and M.E. Dorcas. 1993. Snake thermal ecology: the causes and consequences of body temperature variation. Pp. 241–314 in R.A. Seigel and J.T. Collins, eds. *Snakes: Ecology and Behavior*. McGraw-Hill, New York.
- Radder R. and R. Shine. 2006. Thermally induced torpor in fullterm lizard embryos synchronizes hatching with ambient conditions. *Biol Lett* 2:415–416.
- Reynolds W.W. and M.E. Casterlin. 1979. Behavioral thermoregulation and the “final preferendum” paradigm. *Am Zool* 19:211–224.
- Row J.R. and G. Blouin-Demers. 2006. Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. *Oecologia* 148:1–11.
- Saint Girons H. 1957. Le cycle sexuel chez *Vipera aspis* dans l’ouest de la France. *Bull Biol Fr Belg* 151:284–350.
- . 1975. Coexistence de *Vipera aspis* et de *Vipera berus* en Loire Atlantique: un problème de compétition interspécifique. *Terre Vie* 29:590–613.
- . 1978. Thermorégulation comparée des vipères d’Europe: étude biotéléométrique. *Terre Vie* 32:417–440.
- Seebacher F. and R.S. Wilson. 2007. Fighting fit: thermal plasticity of metabolic function and fighting success in the crayfish *Cherax destructor*. *Funct Ecol* 20:1045–1053.
- Shine R. and T. Madsen. 1996. Is thermoregulation unimportant for most reptiles? an example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiol Zool* 69:252–269.
- Slip D.J. and R. Shine. 1988. Thermophilic response to feeding of the diamond python, *Morelia s. spilota* (Serpentes: Boidae). *Comp Biochem Physiol A* 89:645–650.
- Tewksbury J.J., R.B. Huey, and C.A. Deutsch. 2008. Putting the heat on tropical animals. *Science* 320:1296–1297.
- Valenciano A.I., A.L. Alonso-Gómez, M. Alonso-Bedate, and M.J. Delgado. 1997. Effect of constant and fluctuating temperature on daily melatonin production by eyecups from *Rana perezi*. *J Comp Physiol B* 167:221–228.
- Vincent S.E. and A. Mori. 2008. Determinants of feeding performance in free-ranging pit-vipers (Viperidae: *Ovophis okinavensis*): key roles for head size and body temperature. *Biol J Linn Soc* 93:53–62.
- Zuffi M.A.L., A. Gentili, E. Cecchinelli, F. Pupin, X. Bonnet, E. Filippi, L.M. Luiselli, F. Barbanera, F. Dini, and M. Fasola. 2009. Geographic variation of body size and reproductive patterns in Continental versus Mediterranean asp vipers, *Vipera aspis*. *Biol J Linn Soc* 96:383–391.
- Zuffi M.A.L., F. Giudici, and P. Ioalé. 1999. Frequency and effort of reproduction in female *Vipera aspis* from a southern population. *Acta Oecol* 20:633–638.