DOI: 10.4025/actascibiolsci.v30i3.5030

The influence of circadian rhythms on the metabolism of the snake *Bothrops jararaca* (Serpentes, Viperidae)

José Geraldo Pereira da Cruz*, Camila Grimes, Daiane Luchetta Ronchi and Vanessa Pecini da Cunha

*Departamento de Ciências Naturais, Centro de Ciências Exatas e Naturais, Universidade Regional de Blumenau, Rua Antônio da Veiga, 140, 89010-971, Blumenau, Santa Catarina, Brazil. *Author for correspondence. E-mail: jgcruz@furb.br*

ABSTRACT. The thermoregulatory activity has led to an extensive search for correlations between physiological variables, including metabolic functions, and the ideal level of body temperature. Snakes were also often seen basking, when their body temperatures were relatively independent of ambient temperature, indicating successful thermoregulation. *Bothrops jararaca* were exposed to two different ambient temperatures (20 and 30ºC) over a time course of three weeks and oxygen consumption and body temperature were measured. The snakes exhibited a freerunning rhythm of body temperature. Metabolic rate was increased at the same circadian phase as the increase in body temperature in the 30ºC. The increase of body temperature and oxygen consumption of *B. jararaca* occurs in the scotophase of the photoperiod, consistent with that of nocturnal species. However, prior to a scotophase period the snakes under 20ºC maintain body temperature and oxygen consumption at higher levels during the day. These results demonstrate for the first time that ectothermic animals may display physiologically generated circadian rhythms of body temperature similar to those recorded in endotherms. Circadian rhythms allow animals to anticipate environmental changes: physiological parameters such as body temperature and mobilization of energy reserves have to be adjusted before the expected environmental changes actually take place.

Key words: oxygen consumption, reptile, thermoregulation.

RESUMO. A influência dos ritmos circadianos no metabolismo da serpente *Bothrops jararaca* (Serpentes, Viperidae). A atividade termorreguladora conduziu a uma busca extensiva para o entendimento das correlações entre as variáveis fisiológicas, incluindo as funções metabólicas e a temperatura corporal. Frequentes observações mostram que algumas serpentes podem se aquecer, sendo este aumento de temperatura independente da temperatura ambiente, indicando a termorregulação bem sucedida. *Bothrops jararaca* foram expostas a dois ambientes com diferentes temperaturas (20 e 30ºC) durante três semanas, sendo mensuradas a temperatura corporal e o consumo de oxigênio. O aumento da temperatura corporal e consumo de oxigênio de *Bothrops jararaca* ocorreram na fase de escuro do fotoperíodo, consistente para espécies noturnas. Entretanto, antecedendo a fase de escuro, as serpentes em 20ºC apresentaram os níveis mais elevados durante o dia para temperatura corporal e consumo de oxigênio. Estes resultados indicam pela primeira vez que animais termodependentes podem controlar a temperatura corporal por meio de ritmos fisiológicos circadianos, semelhante aos observados em termoindependentes. Os ritmos circadianos permitem que os animais antecipem as mudanças no ambiente: parâmetros fisiológicos como a temperatura corporal e as reservas de energia ou sua mobilização podem ser ajustadas antes que as mudanças ambientais previstas ocorram realmente.

Palavras-chave: consumo de oxigênio, répteis, termorregulação.

Introduction

Studies of metabolism are important to physiological ecology as they can suggest potential energetic constraints that operate on individual organisms. Temperature profiles of organism metabolic rates can be scaled to the population level and used to predict the effects of the inevitable global climate change. In addition, species-specific metabolic relationships are important for comparative studies of metabolic adaptation.

Temperature effects on snake ecology and physiology have been well documented (Andrews and Pough, 1985; Huey *et al.*, 1989; Peterson *et al.*, 1993). Metabolic rates of ectotherms typically increase with increasing temperature (Bennett and Dawson, 1976). Low temperature may considerably reduce the metabolic rates of snakes and consequently constrain their activity (Marques *et al.*, 2006). Because of the limited potential for thermoregulation at night, nocturnal ectotherms often experience low body temperatures during activity (Huey *et al.*, 1989). However, exceptions do exist, primarily with species that exhibit plateaus in their temperature response curves (Pough and Gans, 1982).

In the case of metabolic performance at low temperatures, one can argue that different selective pressures have shaped the organism metabolic rate accordingly. However, it is unknown whether the differences are genetically fixed or the result of a genotype-environment interaction. The interesting information lies in the significant temperature interaction with latitude of origin. In the northern part of their geographic range, the Arkansas and Missouri *Agkistrodon piscivorus* show an elevated resting metabolic rate at low temperatures. In contrast, Louisiana snakes showed practically no increase in resting metabolic rate at low temperatures (Zaidan, 2003).

Circadian rhythms in metabolic rates may vary among species (Bennett and Dawson, 1976). However, few recent studies have addressed the possibility of temporal variation in metabolism. Blem and Killeen (1993) reported that *Agkistrodon piscivorus* and *Nerodia taxispilota* exhibited higher scotophase metabolic rates in snakes that were acclimated to a reverse photoperiod and tested in complete darkness. Blem and Killeen (1993) believed that movement in the chambers was partially responsible for the observed temporal variation. In rattlesnakes (*Crotalus lepidus*, *C. molossus*, *C. atrox* and *C. horridus*), the lowest oxygen consumption or carbon dioxide production rates typically occurred between 08:00 and 11:00 CST/MST (Beaupre, 1993), which closely matched their early photophase period (07:00-10:00 CST). In contrast, the lowest Vco_2 in *Agkistrodon piscivorus* occurred earlier during the late scotophase period (01:00-06:00 CST). The highest Vco_2 in *Agkistrodon piscivorus* occurred during the late photophase period (13:00-18:00 CST). The maximum during late photophase and the minimum during the late scotophase demonstrated real circadian variation in the metabolism of *Agkistrodon piscivorus* (Zaidan, 2003).

Reptile species that are active diurnally tend to exhibit relatively high and constant body temperature, whereas nocturnal taxa display lower and more variable temperatures (Angilletta *et al.*, 2002). This difference is not a simple consequence of environmental conditions that the animals experience during their periods of activity, because the same pattern is seen when animals are confined to thermal gradients in the laboratory, where the usual diurnal rhythm in thermal conditions is eliminated (Huey *et al.*, 1989). Most studies of reptilian thermoregulation have been based on lizards or large diurnal snakes and, therefore, are not representative of all reptiles.

The thermoregulatory activity has led to an extensive search for correlations between physiological variables, including metabolic functions, and the preferred level of body temperature. Therefore, in the present study, we investigated whether snakes could use this heat source. Specifically, we examined whether the body temperature of the *B. jararaca* is affected by the increased metabolic rate experienced when maintained at 20 or 30°C in a light/dark cycle for 21 days in summer.

Material and methods

B. jararaca were maintained under captivity in the serpentarium of the Universidade Regional de Blumenau, Santa Catarina State, Brazil (900238/Ibama). *B. jararaca* (mean weight 312.5 ± 23.9 g) were housed in an environmental chamber at 24 ± 2°C under 12:12 light/dark cycles and then subdivided into two groups. One group (N = 4) was maintained at 20°C in light/dark; the other (N = 4) was moved to 30°C in light/dark for 21 days in summer. Following each thermal acclimation, oxygen consumption and body temperature were measured at the same temperature as acclimation. Studied individuals were obtained in northeastern Santa Catarina State, 26º55'26" latitude and 49º03'22" longitude.

A double-chambered, volumetric system was used for the measurement of oxygen consumption at 20 to 30°C, as described by Al-Saddon and Spellerberg (1985; 1987). Hourly resting oxygen consumption was expressed as mL O_2 g^{-1} body weight. To avoid circadian effects on oxygen consumption, experiments were always conducted at the same time of the day. Cloacal temperature was measured immediately after each experiment using a quick-reading cloacal thermometer (shaded bulb) to the nearest 0.2ºC, and snakes cloacal temperatures were always recorded within 30 sec.

The results were analyzed by parametric descriptive statistics analysis and expressed as mean ± standard deviation (0 ± SD). Comparison between groups was performed by one-way Anova test and Tukey's multiple comparison as post-Anova test. The rejection level of statistical significance adopted was $p > 0.05$.

Results

The analysis showed a statistically significant circadian rhythmicity in oxygen consumption in snakes (Anova; $F = 60.531$; $p < 0.0001$). Resting oxygen consumption by the snakes was determined at 10°C intervals from 20 to 30°C and their oxygen consumption curves were plotted (Figure 1). Mean Vo_2 reached peaks of 0.13 ± 0.001 O_2 (mL g^{-1} h^{-1}) between 12-18h of day, and decreased to 0.03 ± 0.002 O_2 (mL g^{-1} h^{-1}) between 6-12h of day, under 20°C. Mean Vo_2 reached peaks of 0.22 ± 0.002 O_2 (mL g^{-1} h^{-1}) between 18-24h of day, and decreased to 0.04 ± 0.002 O_2 (mL g^{-1} h^{-1}) between 6-12h of day, under 30°C. In general there was an increase in oxygen consumption in one instance where the rate of oxygen consumption of *B. jararaca* was nearly constant between 0-12h ($p > 0.05$). Post-hoc analysis showed an increase ($p < 0.001$) in the oxygen consumption between 12-18h under 20°C and 18-24h for 30°C.

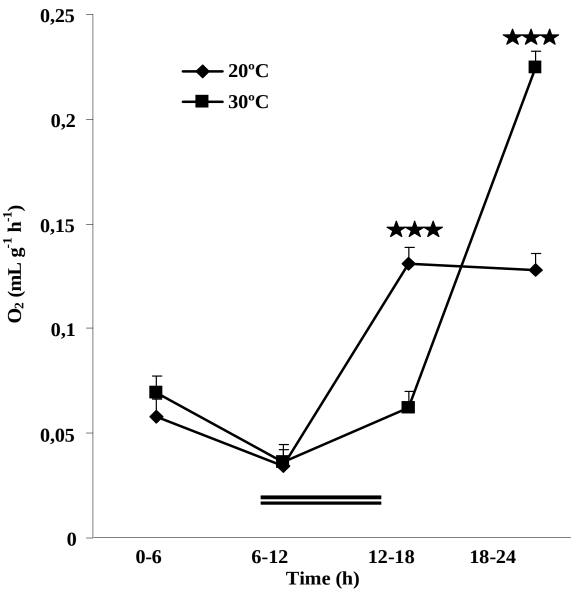

Figure 1. Oxygen consumption measurements of *Bothrops jararaca* measured at 20 and 30°C using the volumetric system. Each symbol indicates a mean for period and standard errors are shown by the vertical lines. Each horizontal black bar indicates the photophase of the photoperiod. N = 4 snakes per group. ***$p < 0.001$ 20°C versus 30°C (Tukey's test).

Temperature positively affected oxygen consumption and the temperature effect was also dependent on time of day. The analysis showed a statistically significant circadian rhythmicity in body temperature in snakes (Anova; $F = 86.698$; $p < 0.0001$). Mean temperature reached peaks of 29.25 ± 0.2°C under 20°C and 31.5 ± 0.2°C for 30°C between 18-24h of day, and decreased to 23.67 ± 0.1°C under 20°C between 6-12h and 28.25 ± 0.2°C under 30°C between 0-12h of day (Figure 2). Body temperature did not differ significantly between 12-18h ($p > 0.05$). However, post-hoc analysis showed an increase ($p < 0.001$) in the body temperature between 12-18h under 20°C and 12-24h for 30°C ($p < 0.001$).

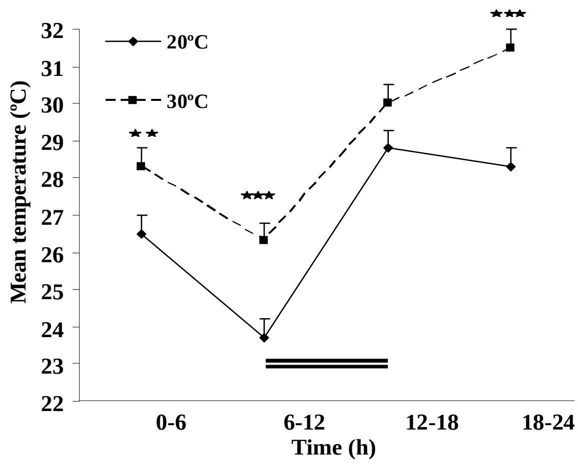

Figure 2. Body temperature at 20 to 30°C under a 12:12 light/dark cycles for a 24 h observation period. Each symbol indicates a mean for period and standard errors are shown by the vertical lines. Each horizontal black bar indicates the photophase of the photoperiod. N = 4 snakes per group. **$p < 0.01$ and ***$p < 0.001$ 20°C versus 30°C (Tukey's test).

Discussion

Circadian systems are believed to have evolved because they provide organisms with the ability to anticipate, and thus prepare for, relatively predictable environmental changes that are associated with the light/dark cycle (Goldman, 1999).

Thermoregulatory tactics in such animals have attracted intense scientific scrutiny, with many studies emphasizing the precision and subtlety of temperature regulation (Peterson *et al.*, 1993). Many habitats, however, impose substantial impediments to behavioral thermoregulation. For example, thermal heterogeneity may be very limited for animals that are active nocturnally, or occupy dense forests where direct sunlight rarely penetrates to the forest floor (Vitt *et al.*, 1997). A high proportion of snake species are nocturnal, or spend much of their time hidden within retreat-sites that typically offer only limited thermal variation (Kearney, 2002). More generally, much of the interspecific variation in thermoregulatory tactics among squamate reptiles may reflect differences in species-specific costs and benefits of thermoregulation rather than variation among habitats. Thus, it is important to understand how temperature influences specific physiological functions in relation to the preferred temperature of a species performing specific biological tasks.

Many ectothermic vertebrates undergo large diurnal and seasonal fluctuations in body temperature with associated changes in metabolism and in cardio-respiratory function (Wang *et al.*, 1998). Metabolic rate is the most fundamental biological rate as it represents the rate of energy uptake, transformation, and allocation (Brown *et al.*, 2004). Knowledge of an organism's metabolic rate at different temperatures is important as it provides a basic indication of its energy requirements in different environments. Assessing the effect of temperature on metabolism is complicated by the simultaneous influence of temperature on several, inter-related endogenous parameters. Elevated levels of activity, for example, are associated with increasing temperatures, which in turn further accelerate an organism's metabolic rate.

Although there is a difference in body temperature selection between laboratory and field in reptiles (Huey, 1982), our data indicate that a snake's selected body temperature in the laboratory reflects its actual needs for their normal activity period. Two of the most basic biological attributes for any ectothermic animal are the times of day that it is active and the body temperatures that it exhibits. In the nocturnal *B. jararaca*, oxygen consumption and body temperature reach a maximum during scotophase and a minimum during the photophase, demonstrating real circadian variation (Figures 1 and 2). The wide range of temperatures that snakes can acquire during their activity period is beneficial in terms of minimizing energy expenditure and indicates thermal adaptation of these snakes to the environment in which they live.

The literature on reptile thermoregulation has identified many strong interspecific correlations among traits, such as mean selected temperatures, thermoregulatory precision, and daily patterns of activity. For example, reptile species that are active diurnally tend to exhibit relatively high and constant body temperatures, whereas nocturnal taxa display lower and more variable temperatures (Huey *et al.*, 1989; Autumn *et al.*, 1999; Angilletta *et al.*, 2002). This difference is not a simple consequence of environmental conditions that the animals experience during their periods of activity, because the same pattern is seen when animals are confined to thermal gradients in the laboratory, where the usual daily rhythm in thermal conditions is eliminated (Huey and Bennett, 1987; Huey *et al.*, 1989).

As with any other ectothermic organism, the snake, *B. jararaca*, depends on external heat sources to regulate body temperature. Although this type of thermoregulatory strategy conserves energy by avoiding the use of metabolism for heat production (Pough, 1983), it requires that the animals inhabit a suitable thermal environment to sustain activity.

The presence of daily or circadian metabolic cycles in *B. jararaca* and other snakes suggests temporal Vo_2 variation may be common in snakes as a group (Beaupre and Zaidan, 2001; McCue and Lillywhite, 2002; Zaidan, 2003; Roe *et al.*, 2004). Thus, our results suggest that cyclic Vo_2 variation is due to increased nocturnal activity and/or cyclic changes in other physiological or behavioral processes such as ventilatory patterns (Hicks and Riedesel, 1983) or state of alertness (Feder and Feder, 1981). Uniquely among snake use metabolic heat production (thermogenesis) to maintain high and stable body temperatures occurs in the scotophase of the photoperiod, consistent with that of nocturnal species (Figure 1 and 2). This may be particularly true in ectothermic taxa because they often exhibit a stronger physiological link to temperatures in their habitat than do endotherms.

Metabolic cold adaptation appears to exist, with cottonmouths from northern populations having higher low temperature metabolic rates. Calculations suggest that Arkansas cottonmouths (*Agkistrodon piscivorus leucostoma*) allocate almost twice as much energy to resting metabolism during non-feeding periods (brumation) as Louisiana cottonmouths. While maintenance metabolism alone during brumation is more costly near the northern range limit, it is most likely not a limiting factor in geographic distribution and may be used to fuel important processes other than activity metabolism (Zaidan, 2003). Levels of resting oxygen consumption and characteristic of the metabolic rate curves would seem to be explained in part by the differences in behavior and ecology of the species.

The results of this investigation underscore the importance of identifying metabolic variation associated with circadian rhythms, yet such variation is rarely considered in studies of body temperatures in snakes. However, the study of ectothermic vertebrates might provide a fruitful alternative for such studies, providing a system where one can potentially separate the consequences of altering metabolism and body temperature, since these two functions are not as intricately intertwined in ectotherms as they are in endotherms. Thus, it seems possible that the beneficial effects of metabolic thermogenesis on body temperature may assume a greater importance during the night.

Conclusion

Environmental temperature varies at different time-scales; thus, organisms are continually challenged to maintain their thermoregulatory homeostasis. The presence of daily or circadian metabolic cycles in *B. jararaca* suggests temporal Vo_2 variation may be common in snakes as a group. Uniquely among snake use metabolic heat production (thermogenesis) to maintain high and stable body temperatures occurs in the scotophase of the photoperiod, consistent with that of nocturnal species.

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Received on July 30, 2007.
Accepted on May 26, 2008.