

Seasonal Changes in Daily Metabolic Patterns of Tegu Lizards (*Tupinambis merianae*) Placed in the Cold (17°C) and Dark

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ABSTRACT

Oxygen consumption rate was measured continuously in young tegu lizards *Tupinambis merianae* exposed to 4 d at 25°C followed by 7–10 d at 17°C in constant dark at five different times of the year. Under these conditions, circadian rhythms in the rate of oxygen consumption persisted for anywhere from 1 d to the entire 2 wk in different individuals in all seasons except the winter. We also saw a progressive decline in standard oxygen consumption rate (at highly variable rates in different individuals) to a very low rate that was seasonally independent (ranging from 19.1 ± 6.2 to 27.7 ± 0.2 mL kg⁻¹ h⁻¹ across seasons). Although this degree of reduction appeared to take longer to invoke when starting from higher metabolic rates, tegu lizards reduced their metabolism to the low rates seen in winter dormancy at all times of the year when given sufficient time in the cold and dark. In the spring and summer, tegus reduced their standard metabolic rate (SMR) by 80%–90% over the experimental run, but only roughly 20%–30% of the total fall was due to the reduction in temperature; 70%–80% of the total fall occurred at constant temperature. By autumn, when the starting SMR on the first night at 25°C was already reduced by 59%–81% (early and late autumn, respectively) from peak summer values, virtually all of the fall (63%–83%) in metabolism was due to the reduction in temperature. This suggests that the temperature-independent reduction of metabolism was already

in place by autumn before the tegus had entered winter dormancy.

Introduction

It is well known that metabolic rate cycles dramatically over the course of the day during the active season in lizards (Kramer 1934; Roberts 1968; Songdahl and Hutchison 1972; Mautz and Case 1974; Rismiller and Heldmaier 1991; Klein et al. 2006). While lizards also demonstrate circadian rhythms in thermal preference and locomotor activity, metabolic rates in many still show circadian cycles even when temperature and activity remain constant (Kramer 1934; Roberts 1968; Rismiller and Heldmaier 1991). Tegu lizards appear to be no exception (Klein et al. 2006). Furthermore, some lizards also exhibit circadian rhythms in melatonin secretion and metabolism that persist, at least for several days, under conditions of constant temperature and darkness (Menaker and Wisner 1983; Rismiller and Heldmaier 1991; Tosini and Menaker 1996; Lutterschmidt et al. 2003) or following parietectomy and blinding (Songdahl and Hutchison 1972).

With their seasonal changes in behavior, many lizards also exhibit seasonal changes in metabolic rate. While some species show metabolic acclimation/acclimatization to compensate for seasonal changes in temperature (Roberts 1968), other species downregulate physiological and biochemical processes and become dormant. The metabolic rate of tegus, like other lizards in this latter group, measured at a constant temperature falls progressively from summer to winter, and the magnitude of this fall is proportionately less at lower temperatures (Weigmann 1932; Moberly 1963; Mayhew 1965; Gelineo 1967; Bennett and Dawson 1976; Abe 1983, 1993, 1995; de Souza et al. 2004). As a consequence, the effect of temperature on metabolic rate (the Q₁₀ effect) is progressively reduced from summer to winter, and thus the metabolic rate of dormant lizards is much less temperature dependent (Abe 1983, 1993, 1995; de Souza et al. 2004). Indeed, preparation for dormancy may begin well before winter, and the loss of temperature sensitivity may occur in midautumn and precede the general decrease in levels of metabolism (Gelineo 1967). Other studies, however, report that the Q₁₀ effect does not change with season (for review, see Bennett and Dawson 1976) or that Q₁₀ even increases during winter at higher temperatures in larger animals (Andrade and Abe 1999). Of particular note is that the effect of temperature on metabolic rate has been reported to be reduced, unchanged, or increased during winter all in the same species, *Tupinambis*

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merianae, in different studies (Abe 1983, 1993, 1995; Andrade and Abe 1999; de Souza et al. 2004; Toledo et al. 2008).

There are seven species of tegu lizard in the genus *Tupinambis*, with various distributions throughout South America. The three most southerly occurring species (*Tupinambis duseni*, *Tupinambis rufescens*, and *T. merianae*) belong to one clade that can undergo seasonal dormancy (Köhler and Langerwerf 2000; Manzani and Abe 2002). One of them, *T. merianae*, has been the subject of a variety of studies that have examined patterns of daily and seasonal change in activity, body temperature, and metabolic rate (Abe 1983, 1993, 1995; Andrade and Abe 1999; Andrade et al. 2004; de Souza et al. 2004; Klein et al. 2006; Toledo et al. 2008).

Tupinambis merianae are commonly found throughout south/southeastern Brazil and Argentina (Vanzolini et al. 1980; Avila-Pires 1995; Lopes and Abe 1999). They are active predators during the summer months (the hot, rainy season from October to April). At this time, they exhibit a pronounced circadian cycle of changes in activity, body temperature, and metabolic rate. These animals are diurnal and, in captivity, usually leave their burrows shortly after sunrise and bask until their body temperatures reach 35°–37°C. They remain active throughout the day and reenter their burrows during the late afternoon, where they remain inactive with body temperatures falling throughout the evening and night (Andrade et al. 2004; C. Sanders, personal observation). Active tegus will remain in their burrows throughout the day if the weather turns inclement and cool. During the winter months (the cold, dry season from May to September), *T. merianae* enter into dormancy, retreating permanently into their burrows where they remain inactive and fast for 3–4 mo (Abe 1995; Andrade et al. 2004).

It is clear that metabolic suppression in reptiles is a complex phenomenon and is regulated by both endogenous and exogenous factors and that these factors fluctuate on both a daily and a seasonal basis (Guppy and Withers 1999; de Souza et al. 2004). Given how little is known about these factors and their interactions, it is not surprising that such wide-ranging changes in oxygen consumption rate have been reported to occur as a function of season in different studies of the same species (Abe 1983, 1993, 1995; Andrade and Abe 1999; de Souza et al. 2004; Toledo et al. 2008). In a companion article (Toledo et al. 2008), we describe how the standard metabolic rate (SMR) of tegu lizards is affected by changes in body temperature, body mass, and season. In this article, we explore two of the proximate factors involved in inducing metabolic suppression. There are many factors that may be involved in inducing metabolic suppression in this species, including changes in food and water availability, temperature, and photoperiod. In this study, we wanted to examine the effects of season per se on resting metabolic rate in *T. merianae*. To do so, we placed animals under conditions of prolonged (at least 1 wk) low temperature (17°C) and darkness, which mimic the situation that tegus experience when they retreat into burrows at the beginning of the dormancy season (Andrade and Abe 1999). As such, each group was exposed to different conditions of food and water avail-

ability, temperature, and photoperiod before these changes, and we could compare the effects of enforced cold and dark on metabolic rate during the active period versus the seasonally induced effects of cold and behaviorally selected confinement to the (dark) burrow during the inactive period. While this would not reveal the cues that induce seasonal dormancy in this species, it would indicate whether seasonal changes were required to induce metabolic suppression or whether burrow confinement at lower temperatures alone could suppress metabolism regardless of season.

Material and Methods

Animals

All specimens of *Tupinambis merianae* used in this study were juvenile animals that had been captive bred at the Jacarezário, Universidade Estadual Paulista, in Rio Claro, São Paulo state, southeastern Brazil. Before experimentation, animals were group housed indoors in plastic boxes (74 cm × 56 cm × 41 cm). During the active season (October to April), the boxes were equipped with incandescent lights to provide a source of radiant heat for thermoregulation along with diffuse sunlight from outside. During the night, box temperature would fall to ambient temperature (20°–25°C). During the active season, all animals were fed two to three times a week with minced meat supplemented with fruit, minerals, and vitamins. Water was available ad lib. During the inactive season (May to September), animals were maintained in constant dark at room temperature (16°–21°C) without access to food or radiant heat. In all seasons, animals were fasted for at least 10 d before experimentation, and only individuals that seemed healthy and were not moulting were used.

Respirometry

Oxygen uptake rates ($\dot{V}O_2$) were measured using a computer-automated, open respirometry setup (TR-RM8, Sable System). This system was programmed to ventilate eight respirometers (1.0-L acrylic chambers) with fresh air (200 mL min⁻¹ per chamber) and to sample sequentially from each chamber for a 5-min period with an oxygen analyzer (PA-1, Sable System; 200 mL min⁻¹ flow). In this way, each chamber was sampled for 5 min every 40 min. The analyzer was calibrated frequently with gases that had been prepared by a precision gas mixing pump (model GF-3/MP, Cameron Instruments). Excurrent flow was passed through a drying column (Drierite) before entering the gas analyzer. The output from the gas analyzer was collected on a data acquisition system (DATACAN V, Sable System). The fractional composition of the incurrent air was checked regularly by drawing air through an empty chamber.

Experimental Protocol

Four separate experimental runs were performed with different groups of animals during the active season. Experiments were

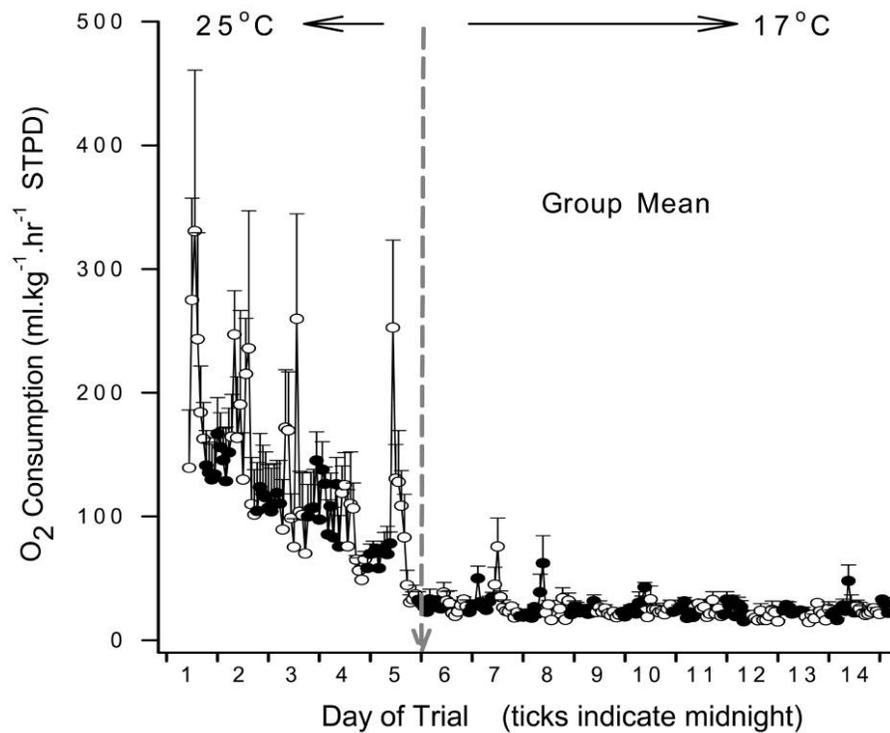


Figure 1. Relationship between oxygen consumption rate ($\text{mL kg}^{-1} \text{h}^{-1} \text{STPD}$) and time for the summer group. The vertical dashed line indicates when the chamber temperature was reduced from 25° to 17°C (after the fourth day in the chamber). Open symbols are data collected between 0600 and 1800 hours, while filled circles are data collected between 1800 and 0600 hours the next day. Animals were in constant dark, and data are presented in this format to indicate, roughly, the normal daytime and nighttime hours. Values are mean \pm SEM; $n = 5$.

performed in September (spring; $n = 8$, body mass 105–158 g, mean = 129 ± 6 g), February (summer; $n = 5$, body mass 340–620 g, mean = 423 ± 28 g), April (early autumn; $n = 7$, body mass 80–155 g, mean = 112 ± 16 g), and June (late autumn; $n = 6$, body mass 141–252 g, mean = 172 ± 5 g). The tegus were placed inside respirometry chambers of adequate volumes (0.3–1.5 L) and maintained within a climatic chamber at 25°C in constant dark. Tegus were allowed to acclimate to this temperature for 4 d before the temperature was reduced to 17°C overnight. Animals were then maintained at this lower temperature for 7–10 d. One additional group of animals was run during the winter season in August (winter; $n = 7$, body mass 146–440 g, mean = 301 ± 72 g). Because these animals were already dormant at 17°C, they were placed in the respirometry chambers and immediately placed within a climatic chamber at 17°C in constant dark for 6 d. Metabolic rate was monitored intermittently (5 min every 40 min for each animal) throughout all experimental series, and at the end of the series the animals were removed from the respirometers and returned to the colony.

The temperatures selected for this study were based on historical records of the mean temperatures measured in the artificial burrows in which the older animals resided year round (A. S. Abe, unpublished data). Unfortunately, we do not have access to records of temperatures in natural burrows, but we

believe the design of the artificial burrows was such that the temperature profiles should mimic those that would be seen in natural burrows. To illustrate the seasonal change in the thermal profile of the artificial burrows used by the tegus, in 2003–2004, the mean burrow temperature for two burrows during August was $19.04^\circ \pm 0.03^\circ\text{C}$, while that during December was $25.05^\circ \pm 0.02^\circ\text{C}$. The burrows are well insulated, and thus daily temperature fluctuations are well buffered. In general, daily fluctuations were $4.9^\circ \pm 0.4^\circ$ in winter and $4.0^\circ \pm 0.2^\circ$ degrees in summer. Outdoor temperatures for the same months fluctuated by $18.7^\circ \pm 0.9^\circ$ degrees in winter (mean night temperatures = 9.6°C , mean day temperatures = 28.3°C) and $25.0^\circ \pm 0.9^\circ$ in summer (mean night temperatures = 18.4°C , mean day temperatures = 42.6°C).

Data Analysis and Handling

The SMR was determined from the $\dot{V}\text{O}_2$ measurements. The $\dot{V}\text{O}_2$ was calculated from the difference in fractional concentration of oxygen in air entering and leaving each respirometer during the sampling period. The system was able to yield a $\dot{V}\text{O}_2$ measurement for each respirometer every 40 min. Barometric pressure and chamber temperature were used to convert the measures to STPD, and values are reported as milliliters per kilogram per hour STPD. Because circadian variations in me-

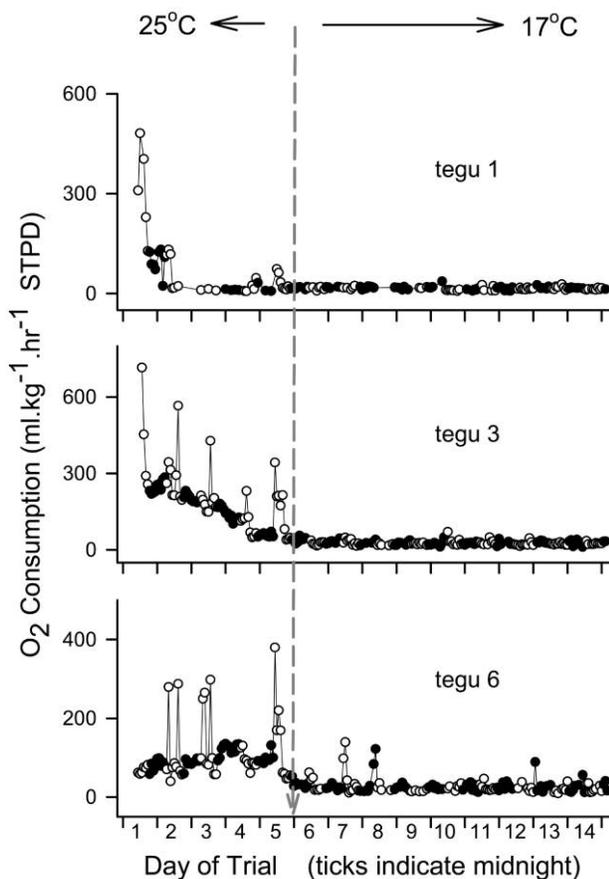


Figure 2. Relationship between oxygen consumption rate ($\text{mL kg}^{-1} \text{h}^{-1} \text{STPD}$) and time for three individual animals from the summer group to illustrate the individual variability in response to the protocol. The vertical dashed line indicates when the chamber temperature was reduced from 25° to 17°C (after the fourth day in the chamber). Open and filled symbols as in Figure 1.

metabolism were present for some animals, and because we had no way to verify whether there were circadian variations in activity associated with these, comparisons were made only between $\dot{V}\text{O}_2$ measurements taken during the period in which metabolism was lowest (usually at night), using a one-way ANOVA for repeated measures followed by a Tukey test for all pairwise multiple comparisons. All values are presented as mean \pm SEM. Differences were considered to be statistically significant at $P < 0.05$.

Results

In Figure 1, the mean oxygen consumption rates of all the animals recorded during the summer are shown to illustrate the general protocol. Note that during the first 4 d at 25°C in constant darkness, mean metabolic rate continued to exhibit circadian peaks at roughly midday to late afternoon and, as a rule, fell to progressively lower rates during each successive night. The time of maximal and minimal oxygen consumption rate shifted slightly each day as the circadian period became

“free running.” In this particular group, circadian peaks in activity continued to occur for the first few days after the temperature was reduced to 17°C. The data for three individual animals were selected to demonstrate the extent of individual variability that was seen in this response, and these are presented in Figure 2. In some animals (as in the case of tegu 1), metabolic rate fell rapidly, reaching low rates by the second night at 25°C. Others (as in the case of tegu 6) showed very little change in metabolic rate until the chamber temperature was reduced. These were the extremes, and tegu 3 demonstrates a response much like the mean for all animals.

Figure 3 presents mean oxygen consumption rates (\pm SEM) for each of the five groups for all days at 17°C, while Figure 4 presents similar data for three individual animals during the late autumn. There are noticeable circadian rhythms in the levels of oxygen consumption rate for all groups except the winter group, albeit these rhythms are larger in some groups than others. As can be seen from Figure 4, however, even in a group where there is a substantial rhythm in the group mean data, there is much individual variability. Some animals show no circadian peaks in oxygen consumption rate throughout the entire period at 17°C, some show sporadic peaks, and some show consistent daily increases in oxygen consumption rate. Given the low rates of oxygen consumption in these animals, one or two individuals with such significant daily peaks bias the group mean data. At least one individual was found in every group, except the winter group, that continued to show circadian cycles in oxygen consumption rate under these conditions of constant temperature and dark. It should be remembered, however, that the winter animals had been inactive in dark boxes at 17°C for at least 1 mo before recordings and, thus, were well acclimatized to these conditions.

The daily minimum levels in the rate of oxygen consumption for all individuals were used to estimate the effects of time and temperature on metabolism in each group, and these data are presented in Table 1. From this table, it can be seen that in spring and summer, when the resting metabolic rates of the lizards were high when they were first placed in the chamber, there was a significant (43%–55%) drop in metabolic rate over the first 4 d at 25°C. In the autumn, when starting metabolic rates were lower, this decrease was not large. During the first night that the temperature was reduced from 25° to 17°C, there was an additional 33%–63% fall in metabolic rate. The subsequent additional fall in metabolic rate during the 7 d at 17°C was significant only in the spring and summer. Overall, these data suggest that while the fall in metabolic rate over the entire course of a run was almost exclusively due to the change in temperature in animals in the autumn (early and late), most of the decrease was due to other factors in the spring and summer.

The effects of season on the metabolic rates recorded at night at 17°C are also presented in Figure 5. If the metabolic rates recorded during the first night at 17°C are compared, there is a progressive fall in oxygen consumption rate from $38 \pm 7 \text{ mL kg}^{-1} \text{h}^{-1}$ ($n = 8$) in spring to $22 \pm 6 \text{ mL kg}^{-1} \text{h}^{-1}$ ($n = 7$) in

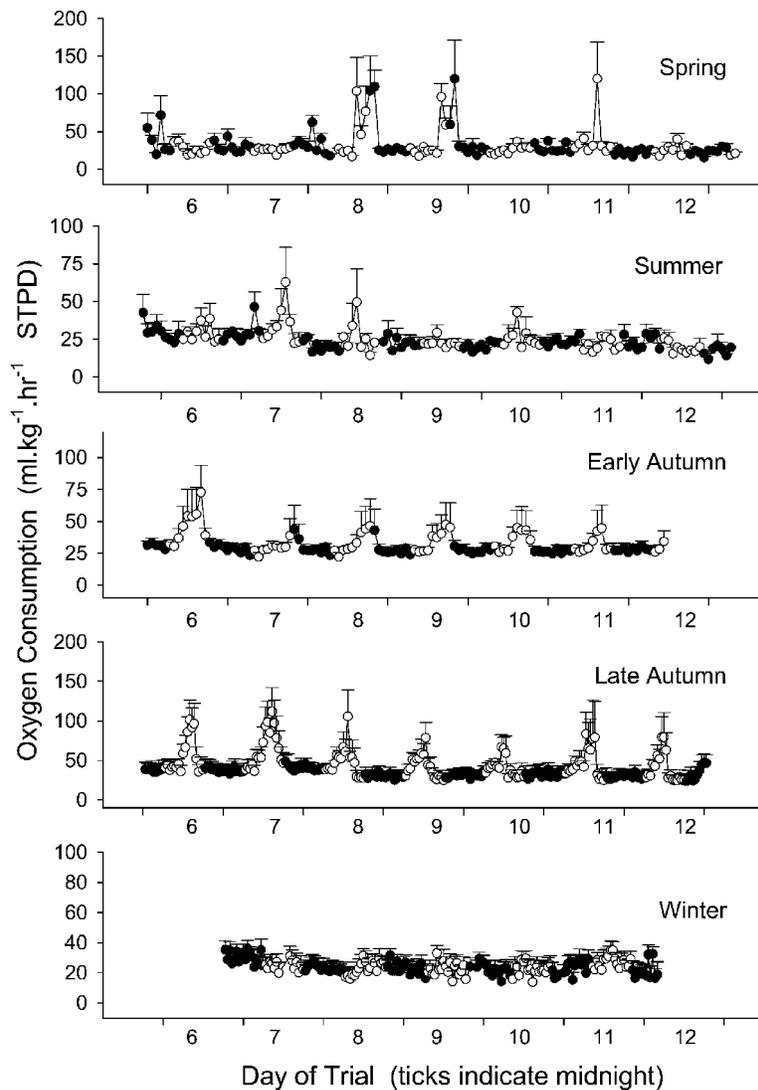


Figure 3. Relationship between oxygen consumption rate ($\text{mL kg}^{-1} \text{h}^{-1} \text{STPD}$) and time for five groups of animals recorded at different times of the year. In all cases, the chamber temperature was reduced from 25° to 17°C during the day before the data shown. Open and filled symbols as in Figure 1. Values are mean \pm SEM; $n = 8, 5, 7, 6,$ and 7 for spring, summer, early autumn, late autumn, and winter, respectively.

winter. The metabolic rate of the winter group is significantly lower than that of all other groups. When the metabolic rates recorded during the second night at 17°C are compared, however, the difference is reduced, and by the sixth night it is completely absent. Thus, if animals remain in the dark and the cold long enough, their metabolic rates fall to similar levels regardless of the season.

Discussion

On the basis of previous reports in the literature, we anticipated that with our protocol (4 d at 25°C followed by 7–10 d at 17°C , all in constant dark), we would see slow extinction of the circadian rhythms in oxygen consumption rate over many days accompanied by a relatively rapid decline in nighttime metabolic rate to a constant, seasonally dependent level (Menaker

and Wisner 1983; Rismiller and Heldmaier 1991; Abe 1995; de Souza et al. 2004). Instead, what we saw was tremendous variability in circadian behavior and a progressive decline in the oxygen consumption rate to a level that was seasonally independent but that occurred in seasonally specific ways.

Circadian Rhythms in the Rate of Oxygen Consumption

Numerous studies document daily variations in metabolic rates in reptiles. Much of this variation, however, is due to diel variations in body temperature and activity (Kramer 1934; Roberts 1968; Songdahl and Hutchison 1972; Mautz and Case 1974; Bennett and Dawson 1976; Rismiller and Heldmaier 1991; Klein et al. 2006). However, metabolic rates may still show circadian cycles even when temperature and activity remain constant,

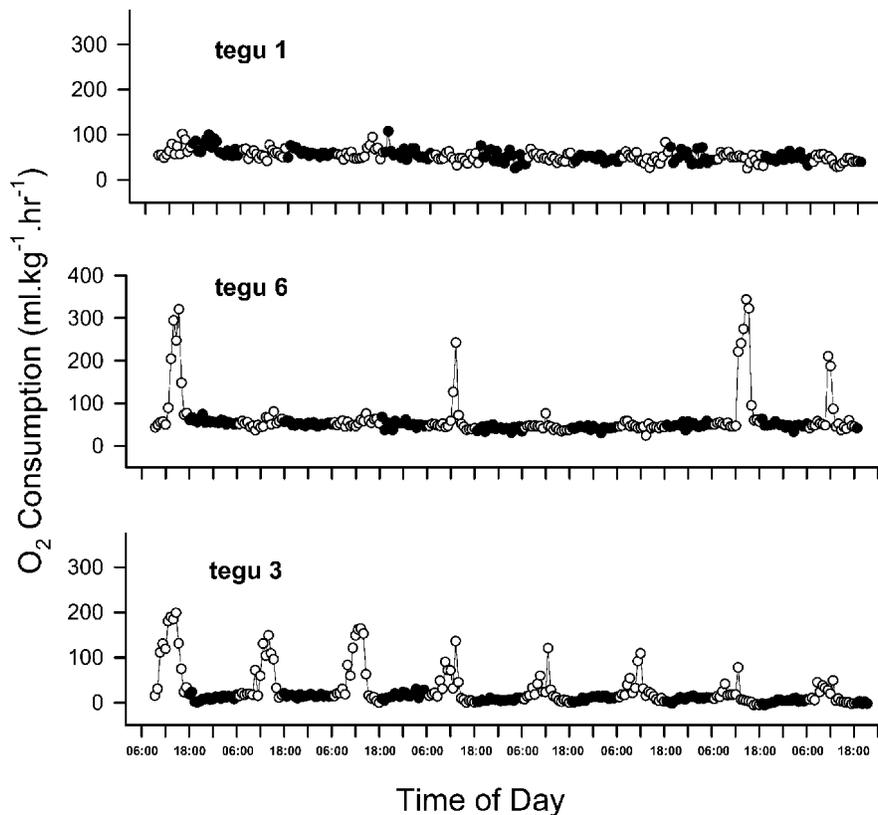


Figure 4. Relationship between oxygen consumption rate ($\text{mL kg}^{-1} \text{h}^{-1} \text{ STPD}$) and time for three individual animals from the late autumn group to demonstrate the individual variability in metabolic rate seen between individuals of the same group. In all cases, the chamber temperature was reduced from 25° to 17°C during the day before the data shown. Open and filled symbols as in Figure 1.

although the magnitude of the daily change may be reduced in winter and in any season at lower temperatures (Kramer 1934; Roberts 1968; Rismiller and Heldmaier 1991). Furthermore, some reptiles also exhibit circadian rhythms in melatonin secretion that persist, at least for several days, under conditions of constant temperature and darkness (Menaker and Wisner 1983; Underwood 1992; Tosini and Menaker 1996; Lutterschmidt et al. 2003). There is little data documenting the changes that occur in the diel cycle in metabolism under conditions of constant temperature and darkness, although the rhythmicity has been reported to persist for varying periods in several species (Kramer 1934; Roberts 1968; Hicks and Riedesel 1983; Rismiller and Heldmaier 1991; Klein et al. 2006).

In this study, we did not quantify the daily peaks in oxygen consumption rate or the magnitude of the rhythm, since we did not control for changes in activity level and cannot say whether they were activity dependent or independent. On the basis of observations of others (Rismiller and Heldmaier 1991; Klein et al. 2006), however, it is likely that most of the daily peaks in oxygen consumption rate represent activity-dependent increases in metabolism. What was most striking about the effects of constant dark and (after the first 4 d) cold was the tremendous individual variability in the persistence of this

rhythm. In some animals, it was extinguished after the first day, while in others, it lasted throughout the entire 14 d of the experiment. In most cases, the rhythm disappeared within the first week. If the peaks in daily metabolism do reflect daily cycles of activity, these data suggest that there is large variability in the tendency of these lizards to become and remain inactive. Some settle down immediately, some settle down progressively, and some sporadically become active again from time to time (see Fig. 4). We cannot say whether these daily peaks were reduced in winter since our winter animals had already been dormant at 17°C for 1–2 mo before being placed in the chambers. As a result, the winter animals had been in constant dark and low temperature for a prolonged period before recordings commenced. Given that this is their normal period of dormancy where activity is extremely rare, however, the lack of diel rhythms in metabolism was not surprising.

First 4 d in the Dark at 25°C

We saw large progressive nightly falls in metabolism over the first 4 d of darkness at 25°C in spring and summer, the seasons when SMR was most elevated. These were notably absent in the autumn. Again, what was striking about our results was

Table 1: Mean values of oxygen consumption rate ($\text{mL kg}^{-1} \text{h}^{-1} \text{STPD}$) for tegu lizards in each season

Night	Temperature ($^{\circ}\text{C}$)	Spring	Summer	Early Autumn	Late Autumn	Winter
$\dot{V}\text{O}_2$:						
1	25	121 ± 25^a	196 ± 32	81 ± 8^a	$38 \pm 1^{a,b}$...
4	25	55 ± 14^c	88 ± 26^c	75 ± 9	36 ± 1	...
5	25 \rightarrow 17	33 ± 6^c	33 ± 5^c	$31 \pm 3^{c,d}$	$24 \pm 2^{c,d}$...
6	17	30 ± 2^c	34 ± 5^c	$29 \pm 3^{c,d}$	$21 \pm 1^{a,c,d}$	22 ± 5^a
11	17	$23 \pm 2^{c,d}$	$20 \pm 3^{c,d}$	$28 \pm 3^{c,d}$	$19 \pm 1^{c,d}$	22 ± 6
Decrease (%):						
N1 \rightarrow N11	25 \rightarrow 17	$81.0^{a,c,d}$	89.8	65.4 ^a	50.0 ^{a,b}	...
Relative decrease:						
N1 \rightarrow N4	25 \rightarrow 25	67.4	61.4	11.3	10.5	
N4 \rightarrow N5	25 \rightarrow 17	22.4	31.3	83.0	63.2	
N5 \rightarrow N11	25 \rightarrow 17	10.2	7.3	5.7	26.3	
Total		100	100	100	100	
Q_{10} :						
N4 \rightarrow N5	25 \rightarrow 17	1.85	3.24	2.89	1.62	

Note. Data taken on the first and last nights of the protocol at 25°C , on the night of the switch to 17°C , and subsequently on the first and last nights at 17°C . Percent decrease in oxygen consumption rate between the first and last night at 25°C (N1–N4), between the last night at 25°C and the first night at 17°C (N4–N5), between the first and last full night at 17°C (N6–N11), and between the first night at 25°C and the last night at 17°C (N1–N11). Mean values \pm SEM; $n = 8, 5, 7, 6,$ and 7 for spring, summer, early autumn, late autumn, and winter, respectively.

^a Indicates significant difference from summer values on that night.

^b Indicates significant difference from spring values on that night.

^c Indicates significant difference from night 1 in that season.

^d Indicates significant difference from night 4 in that season.

the large individual variation in the falls that occurred in spring and summer. While some tegus (such as tegu 6 from the summer group in Fig. 2) maintained a constant nighttime SMR over this period, most did not. As a general rule, oxygen consumption rate fell progressively following each circadian cycle, although in some instances it fell to dramatically lower yet stable levels within 48 h (as in the case of tegu 1 in Fig. 2).

It is difficult to say whether the large falls in nighttime metabolism that occurred over the first 4 d during the spring and summer were due to initial stress (and hence high starting metabolic rates) or progressive metabolic suppression. We could find nothing specific in the literature for comparison since other studies measured values over the first 24 h either only in animals that were otherwise maintained under normal photoperiod (Rismiller and Heldmaier 1991) or in animals that were housed in the dark for extended times but in which metabolic rates were not measured continuously (Ragland et al. 1981). While several studies have shown that novel environments lead to elevated metabolic rates in reptiles (Snyder 1975; Bickler and Anderson 1986; Hare et al. 2004; Hopkins et al. 2004), there are no systematic studies indicating how long it takes to eliminate this effect. It is also possible that progressive fasting leads to reductions in metabolic rate, and these reductions may be larger in spring and summer, when animals are typically more active. All animals, however, had been fasted for 10 d before experiments began reducing (but not necessarily eliminating) the likelihood of this. On the other hand, two lines

of evidence suggest that the reductions observed in this study may have been true metabolic suppression. The first is that similar declines in metabolism did not occur over the first four nights at 25°C in the early or late autumn, despite the fact that all animals in all seasons should have been stressed to the same extent by our protocol. The second is that the decrease in metabolic rate that we saw over the first four nights in spring and summer reduced SMR to the same low levels seen naturally in the autumn. This would suggest that these lizards are capable of suppressing metabolism significantly in all seasons but normally do so only in the autumn. Removal of diel changes in photoperiod and temperature by experimental confinement in the dark at constant temperature in the spring and summer seems to elicit the same responses normally triggered by the physiological, behavioral, and meteorological changes that occur in the autumn (see further discussion below).

Switch from 25° to 17°C

The effect of temperature on metabolic rate (the Q_{10} effect) has been reported to be progressively reduced from summer to winter in many species of lizard (Abe 1983, 1993, 1995; Rismiller and Heldmaier 1991; Zari 1999; de Souza et al. 2004). Indeed, preparation for dormancy may begin well before winter, and the loss of temperature sensitivity may occur in midautumn and precede the general decrease in levels of metabolism (Gelineo 1967). Other studies, however, report that the Q_{10} effect

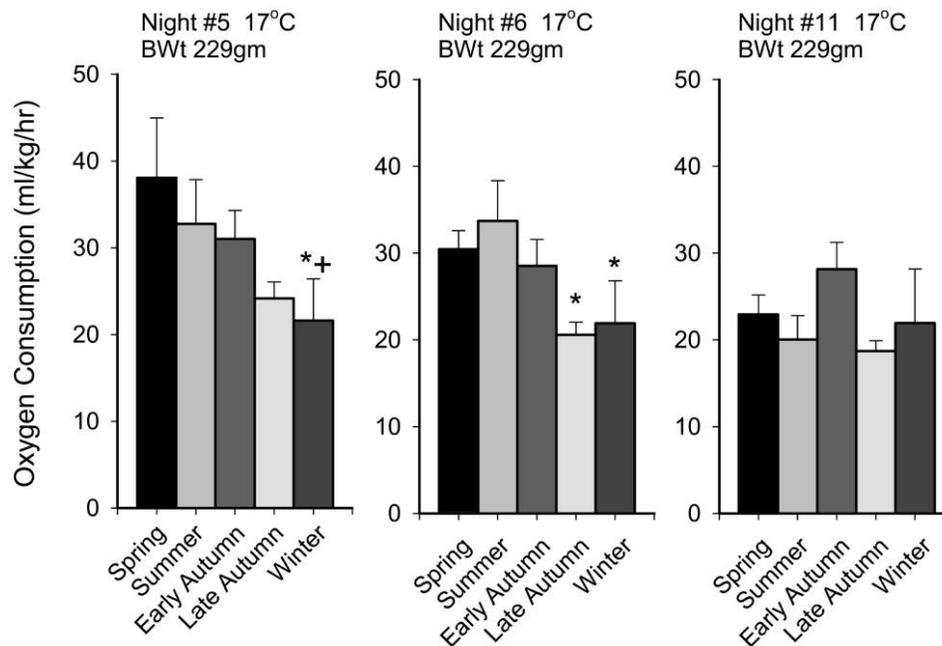


Figure 5. Levels of oxygen consumption rate (mean \pm SEM) recorded in the various groups during different nights over the period that animals were held at 17°C in constant dark. Values are mean \pm SEM; $n = 8, 5, 7, 6,$ and 7 for spring, summer, early autumn, late autumn, and winter, respectively. Asterisk indicates significant difference from summer values; plus sign indicates significant difference from spring values.

does not change with season (for review, see Bennett and Dawson 1976), that the Q_{10} increases during winter at higher temperatures in larger animals (Andrade and Abe 1999) as a strategy for stimulating arousal for reproduction in the spring, and that Q_{10} increases during winter at lower temperatures in some species as a mechanism to enhance metabolic suppression during dormancy (Zari 1996, 1999). In *Tupinambis merianae*, the effect of temperature on metabolic rate has been reported to be reduced, unchanged, or increased during winter in different studies, most likely reflecting differences in the size of the animals and the length of the acclimation period (Abe 1983, 1993, 1995; Andrade and Abe 1999; de Souza et al. 2004; for a more detailed discussion, see Toledo et al. 2008). In this study, Q_{10} values ranged from 1.62 to 3.24 across the seasons, leading to 30%–60% reductions in SMR. While the decrease was greatest in summer and early autumn, it was significant in all seasons.

Constant Dark at 17°C

As at 25°C, we also saw progressive nightly falls in metabolism over the first few days of darkness at 17°C. The falls were relatively small, however, and occurred in all seasons. We also found that the oxygen consumption rates measured during the first night following the reduction of chamber temperature to 17°C decreased progressively from spring through autumn, as did the fall that occurred over the next few days (Fig. 4). Many studies have documented such seasonal declines in oxygen consumption rate at constant temperature (Moberly 1963; Mayhew 1965; Rismiller and Heldmaier 1991; Zari 1999), and several

studies have documented this trend specifically in tegu lizards at 17°C (Abe 1983, 1993, 1995; de Souza et al. 2004; Fig. 6). However, since the oxygen consumption rate fell more in the active season over successive days in constant dark at 17°C, the metabolic rates in all seasons ultimately fell to the same low values. We interpret this as indicating that this species can suppress metabolism to the same absolute degree under appropriate conditions regardless of season. Although this degree of suppression appears to take longer to invoke when starting from higher metabolic rates during the active season, tegus can suppress their metabolism to these low levels at any time of the year if given sufficient time in the cold and dark.

Is This Metabolic Suppression?

This raises two questions: are the temperature-independent and seasonally dependent reductions in metabolism due to active suppression, and are the seasonally independent low metabolic rates seen in this study indicative of dormant animals? By the end of the six nights at 17°C, all groups in our study had an SMR equivalent to the winter group that had been dormant in their home boxes for 2 mo, which would suggest that they all were dormant by that time. The overall reductions in metabolic rate also compare favorably to values reported in the literature for metabolic depression in lizards during their inactive season expressed as a percentage of metabolic rate during their active season. In a recent review, these reductions have been reported to range from 25% to 69% for 11 species of temperate and

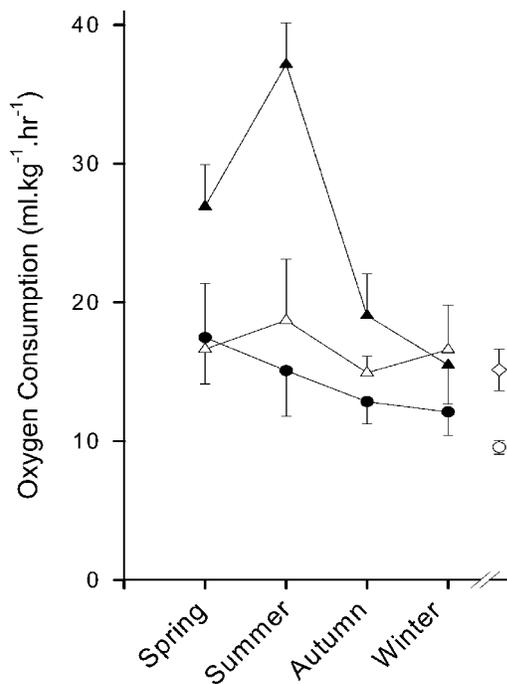


Figure 6. Relationship between oxygen consumption rate and season at 17°C from different studies on *Tupinambis merrianae*. Filled circle, data from Abe (1995; mean mass = 1,119 g; $n = 12$); filled triangle, data from Toledo et al. (2008; mass range 12–3,750 g; $n = 25$ –35); open triangle, data from this study (mass range 80–620 g; $n = 5$ –8). Data from de Souza et al. (2004; open diamond; mass range 92–129 g; $n = 12$) and Andrade and Abe (1999; open circle; mass range 525–2,140 g; $n = 6$) for winter animals only are also included for comparison. Note: for comparison, in all cases, the values of oxygen consumption rate have been converted to milliliters O_2 per hour normalized to 1 kg body mass using the scaling equations from Toledo et al. (2008).

tropical lizards (Christian et al. 1999), and our 50%–90% reductions fall within or exceed this range.

It should be noted that dormancy and depression of metabolism appear to be controlled independently in many lizards. In *Phrynosoma*, dormancy was reported to develop in response to decreased photoperiod, while metabolic depression was largely dependent on falls in temperature (Mayhew 1965). This was also true for our autumn animals. A fall in SMR under constant conditions (including constant temperature) provides the definition for true metabolic suppression (as opposed to cold-induced torpor) in reptiles (Patterson and Davies 1978; Gregory 1982), and in this context, if one only looks at the data for autumn animals, one would conclude that, in winter, our tegu lizards enter cold-induced torpor. The data when viewed across the seasons, however, suggest that they slowly enter a dormant state accompanied by true metabolic suppression.

It is interesting to note that the net fall in SMR during the different seasons was invoked in different ways. In the spring and summer, the animals in our study reduced their SMR by

80%–90% over the experimental run, but only roughly 20%–30% of the total fall was due to the reduction in temperature; 70%–80% of the total fall occurred at constant temperature. By autumn, the starting SMR on the first night at 25°C was already reduced by 59%–81% (early and late autumn, respectively) from peak summer values, and now virtually all of the fall (63%–83%) in metabolism over the experimental run was due to the reduction in temperature; only 17%–37% of the total fall occurred at constant temperature. The observation that, by autumn, the temperature-independent reduction of metabolism was already in place is consistent with several previous studies (Mayhew 1965; Abe 1983, 1993, 1995; de Souza et al. 2004; Toledo et al. 2008). However, our data further suggest that a metabolic decrease equivalent to that induced by seasonal change can also occur in spring and summer if animals are confined in the dark. It is not clear whether the seasonal changes in metabolism and the temperature-independent changes that can occur during the active season are induced by similar mechanisms.

The cues that initiate entrance into dormancy or other states of metabolic suppression in reptiles remain enigmatic (for reviews, see Gregory 1982; Christian et al. 1999). The mechanisms that underlie seasonal metabolic suppression also remain elusive. Metabolic acclimation/acclimatization may occur at the ultrastructural level (mitochondrial numbers or cristae density), the transcriptional or translational level (production of metabolic enzymes), as well as the posttranslational level (allosteric modulation of existing enzymes). Evidence for alterations at each of these levels have been demonstrated (Crawford and Powers 1992; Guderley and St. Pierre 2002; Seebacher et al. 2003). Perhaps most elusive of all, however, are the pathways that connect the proximate factors inducing states of metabolic suppression (whether they be environmental or endogenous) to the ultimate factors altering metabolic flux (whether they be mitochondrial or cellular) such that the activities of all cells in the bodies of these animals are regulated in a coordinated fashion.

Perspective

The picture that is emerging from this and other studies is that for *T. merrianae*, in spring and summer, the diel series of events (changing temperature, photoperiod, etc.) elevates metabolism at any constant temperature. As the autumn approaches, this effect is reduced (by unknown mechanisms), leading to a seasonal depression of metabolism (inverse acclimatization). This, combined with the fall in ambient and burrow temperatures, leads to very low rates of oxygen consumption. This is also accompanied by a progressive reduction in thermal sensitivity (Q_{10}) from summer to winter such that the metabolic rate of dormant lizards becomes relatively temperature independent (Abe 1983, 1993, 1995; de Souza et al. 2004). This suggests that metabolism is suppressed to the same minimum level in winter, regardless of the temperature, an observation that has also been made on some species of hibernating mammals (echidna: Nicol

et al. 1992; arctic ground squirrel: Buck and Barnes 2000; marmot: Ortmann and Heldmaier 2000). This has been proposed to have the selective advantage of maintaining extremely low metabolic rates even when burrow temperatures rise slightly.

Thus, our data indicate that, by autumn, the metabolic rate of the tegu is already depressed and the lizards are ready to enter dormancy, where a further minor temperature-dependent lowering of metabolism will occur. By the end of the autumn/beginning of the winter, the final step in the suite of events leading to dormancy is the behavioral decision to retreat into the burrow, let body temperature equilibrate with the surroundings, and abandon the daily increases in body temperature associated with active thermoregulation.

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Literature Cited

- Abe A.S. 1983. Observations on dormancy in tegu lizards, *Tupinambis teguixin* (Reptilia, Teiidae). *Naturalia* 8:135–139.
- . 1993. Effect of the environment on ventilation in reptiles. Pp. 87–93 in J.E.P.W. Bicudo, ed. *The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life*. CRC, Boca Raton, FL.
- . 1995. Estivation in South American amphibians and reptiles. *Braz J Med Biol Res* 28:1241–1247.
- Andrade D.V. and A.S. Abe. 1999. Gas exchange and ventilation during dormancy in the tegu lizard, *Tupinambis merianae*. *J Exp Biol* 202:3677–3685.
- Andrade D.V., S.P. Brito, L.F. Toledo, and A.S. Abe. 2004. Seasonal changes in blood oxygen transport and acid-base status in the tegu lizard, *Tupinambis merianae*. *Respir Physiol Neurobiol* 140:197–208.
- Avila-Pires T.C. 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zool Verh* 299:1–706.
- Bennett A.F. and W.R. Dawson. 1976. Metabolism. Pp. 127–224 in C. Gans and W.R. Dawson, eds. *Biology of the Reptilia*. Vol. 5. Academic Press, London.
- Bickler P.E. and R.A. Anderson. 1986. Ventilation, gas exchange, and aerobic scope in a small monitor lizard, *Varanus gilleni*. *Physiol Biochem Zool* 59:76–83.
- Buck C.L. and B.M. Barnes. 2000. Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *Am J Physiol* 279:R255–R262.
- Christian K.A., G.S. Bedford, and T.J. Schultz. 1999. Energetic consequences of metabolic depression in tropical and temperate-zone lizards. *Aust J Zool* 47:133–141.
- Crawford D.L. and D.A. Powers. 1992. Evolutionary adaptation to different thermal environments via transcriptional regulation. *Mol Biol Evol* 9:806–813.
- de Souza S.C.R., J.E. Carvalho, A.S. Abe, J.E.P.W. Bicudo, and M.S.C. Bianconcini. 2004. Seasonal metabolic depression, substrate utilization and changes in scaling patterns during the first year cycle of tegu lizards (*Tupinambis merianae*). *J Exp Biol* 207:307–318.
- Gelineo S. 1967. Oxygen consumption in lizards on the way to winter sleep. *Bull Acad Serbe Sci* 39:51–57.
- Gregory P.T. 1982. Reptilian hibernation. Pp. 53–154 in C. Gans and F.H. Pough, eds. *Biology of the Reptilia*. Vol. 13. Academic Press, London.
- Guderley H. and J. St. Pierre. 2002. Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *J Exp Biol* 205:2237–2249.
- Guppy M. and P. Withers. 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol Rev* 74:1–40.
- Hare K.M., S. Pledger, M.B. Thompson, J.H. Miller, and C.H. Daugherty. 2004. Conditioning reduces metabolic rate and time to steady-state in the lizard *Naultinus manukanus* (Reptilia: Gekkonidae). *Comp Biochem Physiol A* 139:245–250.
- Hicks J.W. and M.L. Riedesel. 1983. Diurnal ventilatory patterns in the garter snake, *Thamnophis elegans*. *J Comp Physiol B* 149:503–510.
- Hopkins W.A., J.H. Roe, T. Philippi, and J.D. Congdon. 2004. Standard and digestive metabolism in the banded water snake *Nerodia fasciata fasciata*. *Comp Biochem Physiol* 137:141–149.
- Klein W., S.F. Perry, A.S. Abe, and D.V. Andrade. 2006. Metabolic response to feeding in *Tupinambis merianae*: circadian rhythm and a possible respiratory constraint. *Physiol Biochem Zool* 79:593–601.
- Köhler G. and B. Langerwerf. 2000. *Tejus: Lebensweise, Pflege, Zucht*. Herpton, Offenbach.
- Kramer G. 1934. Der Ruheumsatz von Eidechsen und seine quantitative Beziehung zur Individuengrosse. *Z Vgl Physiol* 20:600–616.
- Lopes H.R. and A.S. Abe. 1999. Biología reproductiva e comportamento do teiú, *Tupinambis merianae*, em cativeiro (Reptilia, Teiidae). Pp. 259–272 in T.G. Fang, O.L. Montenegro, and R.E. Bodmer, eds. *Manejo y Conservación de Fauna Silvestre en América Latina*. Editorial Instituto e Ecología, La Paz, Bolivia.
- Lutterschmidt D.I., W.I. Lutterschmidt, and V.H. Hutchison. 2003. Melatonin and thermoregulation in ectothermic vertebrates: a review. *Can J Zool* 81:1–13.
- Manzani P.R. and A.S. Abe. 2002. A new species of *Tupinambis* Daudin, 1803 from southeastern Brazil (Squamata, Teiidae). *Arq Mus Nac Rio J* 60:295–302.
- Mautz W.J. and T.J. Case. 1974. A diurnal activity cycle in the granite night lizard, *Xantusia henshawi*. *Copeia* 1974:243–251.
- Mayhew W.W. 1965. Hibernation in the horned lizard, *Phrynosoma m'calli*. *Comp Biochem Physiol* 16:103–119.

- Menaker M. and S. Wisner. 1983. Temperature-compensated circadian clock in the pineal of *Anolis*. Proc Natl Acad Sci USA 80:6119–6121.
- Moberly W.R. 1963. Hibernation in the desert iguana, *Dipsosaurus dorsalis*. Physiol Zool 36:152–160.
- Nicol S., N.A. Andersen, and U. Mesch. 1992. Metabolic rate and ventilatory pattern in the echidna during hibernation and arousal. Pp. 150–159 in M.L. Augée, ed. Platypus and Echidnas. Royal Zoological Society of New South Wales, Sydney.
- Ortmann S. and G. Heldmaier. 2000. Regulation of body temperature and energy requirements of hibernating Alpine marmots (*Marmota marmota*). Am J Physiol 278:R698–R704.
- Patterson J.W. and P.M.C. Davies. 1978. Energy expenditure and metabolic adaptation during winter dormancy in the lizard, *Lacerta vivipara* Jacquin. J Thermal Biol 3:183–186.
- Ragland I.M., L.C. Wit, and J.C. Sellers. 1981. Temperature acclimation in the lizards *Cnemidophorus sexlineatus* and *Anolis carolinensis*. Comp Biochem Physiol 70A:33–36.
- Rismiller P.D. and G. Heldmaier. 1991. Seasonal changes in daily metabolic patterns of *Lacerta viridis*. J Comp Physiol B 161:482–488.
- Roberts L.A. 1968. Oxygen consumption in the lizard *Uta stansburiana*. Ecology 49:809–819.
- Seebacher F., H. Guderley, R.M. Elsey, and P.L. Trosclair III. 2003. Seasonal acclimatization of muscle metabolic enzymes in a reptile. J Exp Biol 206:1193–1200.
- Snyder G.K. 1975. Respiratory metabolism and evaporative water loss in a small tropical lizard. J Comp Physiol 104:13–18.
- Songdahl J.H. and V.H. Hutchison. 1972. The effect of photoperiod, parietectomy and eye enucleation on oxygen consumption in the blue granite lizard, *Sceloporus cyanogenys*. Herpetologica 28:148–156.
- Toledo L.F., S.P. Brito, W.K. Milsom, A.S. Abe, and D.V. Andrade. 2008. Effects of season, temperature, and body mass on the standard metabolic rate of tegu lizards (*Tupinambis merrianae*). Physiol Biochem Zool 81:158–164.
- Tosini G. and M. Menaker. 1996. The pineal complex and melatonin affect the expression of daily rhythm of behavioural thermoregulation in the green iguana. J Comp Physiol A 179:135–142.
- Underwood H. 1992. Endogenous rhythms. Pp. 229–298 in C. Gans and D. Crews, eds. Biology of the Reptilia. Vol. 18. University of Chicago Press, Chicago.
- Vanzolini P.E., A.M.M. Ramos-Costa, and L.J. Vitt. 1980. Répteis das Caatingas. Academia Brasileira de Ciências, Rio de Janeiro.
- Weigmann R. 1932. Jahreszyklische Veränderungen im Funktionszustand der Schilddrüse und im Stoffumsatz von *Lacerta vivipara* Jacq. Z Wiss Zool 142:491–509.
- Zari T.A. 1996. Seasonal acclimatization in resting metabolism of the Turkish gecko, *Hemidactylus turcicus*, in Jeddah, Saudi Arabia. Jap J Herpetol 16:129–133.
- . 1999. Seasonal acclimatization in metabolic rate of the fan-fingered gecko, *Ptyodactylus hasselquistii* (Reptilia: Gekkonidae). J Therm Biol 24:137–142.