

# Daily and annual cycles in thermoregulatory behaviour and cardio-respiratory physiology of black and white tegu lizards

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**Abstract** This study was designed to determine the manner in which metabolism is suppressed during dormancy in black and white tegu lizards (*Tupinambis merianae*). To this end, heart rate ( $f_H$ ), respiration rate ( $f_R$ ), and deep body temperature ( $T_b$ ) were continuously monitored in outdoor enclosures by radio-telemetry for nine months. There was a continuous decline in nighttime breathing and heart rate, at constant  $T_b$ , throughout the late summer and fall suggestive of an active metabolic suppression that developed progressively at night preceding the entrance into dormancy. During the day, however, the tegus still emerged to bask. In May, when the tegus made a behavioural commitment to dormancy,  $T_b$  (day and night) fell to match burrow temperature, accompanied by a further reduction in  $f_H$  and  $f_R$ . Tegus, under the conditions of this study, did arouse periodically during dormancy. There was a complex interplay between changes in  $f_H$  and  $T_b$  associated with the direct effects of temperature and the indirect effects of thermoregulation, activity, and changes in metabolism. This

interplay gave rise to a daily hysteresis in the  $f_H/T_b$  relationship reflective of the physiological changes associated with warming and cooling as preferred  $T_b$  alternated between daytime and nighttime levels. The shape of the hysteresis curve varied with season along with changes in metabolic state and daytime and nighttime body temperature preferences.

**Keywords** Reptiles · Tegu lizards · Torpor · Dormancy · Hibernation · Cardiorespiratory control · Seasonal adjustments

## Introduction

Hibernation is employed by many animals as a strategy to survive periods of limited energy availability in the environment (Carey et al. 2003). Some of the hallmark traits of endothermic hibernation are a depression of body temperature ( $T_b$ ) and a reduction of basal metabolic rate, accompanied by falls in ventilation and heart rate (Lyman 1982; Willis 1982; Carey et al. 2003; Tøien et al. 2015). While the magnitude and mechanism of metabolism and  $T_b$  depression in hibernating endotherms have been well studied (Lyman 1982; Nedergaard et al. 1990; Storey and Storey 1990; Carey et al. 2003), the same aspects of dormancy have not been extensively studied in ectothermic hibernators. It is clear that many overwintering ectotherms are capable of similar reductions in metabolism, often independent of changes in  $T_b$ ; Mayhew (1965) referred to this as brumation, in order to distinguish ectothermic winter dormancy from endothermic hibernation.

Reduction in metabolic rate at the initiation of hibernation involves both lowering of the hypothalamic set point for body temperature regulation and active metabolic

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suppression (mammals: Heller et al. 1977; Nedergaard et al. 1990; Heldmaier et al. 1992). This is also true of amphibians and reptiles; however, these groups rely on behavioural rather than physiological methods to reduce  $T_b$  (Rollinson et al. 2008; Tattersall and Boutilier 1997; Donohoe et al. 1998; Tattersall and Boutilier 1999; Huey and Pianka 1977; Guppy and Withers 1999; Glanville and Seebacher 2006). The manner in which these behavioural and physiological strategies are integrated and employed by ectotherms during entrance into dormancy, however, is not well understood.

The black and white tegu (*Tupinambis merianae*) is a large, diurnal lizard of South America that undergoes winter dormancy in its southern range (Milstead 1961; Abe 1995; Ávila-Pires 1995). During dormancy, tegus retreat into their burrows where they fast and remain inactive from May to August (Abe 1995; Andrade et al. 2004). Preparation for dormancy begins well before environmental conditions become adverse and tegus can depress metabolism to dormant levels at any time of the year when inactive in constant cold, darkness and deprived of food (Milsom et al. 2008). By the end of the autumn/beginning of the winter, the final steps leading to dormancy appear to be a behavioural decision to retreat into the burrow, let  $T_b$  equilibrate with the surroundings, and abandon behavioural thermoregulation. There also appears to be 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 reduction in  $Q_{10}$  has been proposed to be an advantage to maintaining extremely low metabolic rates even when burrow temperatures fluctuate (Toledo et al. 2008).

The previous studies on hibernating tegus have given rise to several questions. First, under natural conditions, can nightly metabolic depression be detected in advance of winter dormancy while the animals are still active during the day? As soon as tegus commit to dormancy and retreat to the burrow they generally remain there for the season, but it is not known whether they immediately enter dormancy or if the degree of metabolic suppression increases as dormancy progresses. The latter has been reported to occur in *Lacerta vivipara* (Patterson and Davies 1978), although the metabolic suppression was not sustained throughout the entire season. Finally, it is not clear whether the period of dormancy is always one prolonged bout or whether these animals undergo periodic arousals during which they remain relatively inactive within their burrows, as is seen in other dormant lizards, such as *Varanus rosenbergi* (Rismiller and McKelvey 2000).

To understand the natural mechanisms involved in winter dormancy requires continuous monitoring of behaviour,  $T_b$  and, ideally, metabolism throughout the year. Here, we record continuously behaviour and  $T_b$  along with heart and

breathing rates as physiological surrogates for metabolism (Zaar et al. 2004; Butler et al. 2000, 2002; Clark et al. 2004, 2006; Green et al. 2008; Piercy et al. 2015), in a group of black and white tegus, *T. merianae*, housed outdoors under semi-natural conditions. We hypothesized that metabolic suppression (as indicated by changes in heart rate and breathing frequency) would not be evident until the tegus remained in the burrows for extended periods but that metabolism would then progressively fall and be sustained throughout the dormant period.

## Methods

Our study was conducted at the Jacarezario, UNESP Bela Vista Campus, Rio Claro, SP, Brazil. Tegus were captive bred and reared for scientific study and conservation ex situ. The study cohort consisted of 2 males and 2 females. Only 4 animals could be recorded from concurrently as the telemetry base station could only receive and decode 4 signals at the same time. Animals were weighed each month except during dormancy to reduce interference with the dormant state. The tegus weighed  $3.2 \pm 0.3$  kg at the start of the study and  $3.5 \pm 0.4$  kg by the end. All surgeries and experiments were conducted under animal care approval from both the UBC animal care committee and the Universidade Estadual Paulista-Rio Claro (Protocol #A09-0232).

## Surgery

Animals were anaesthetized using Halothane vapour. An incision was made mid-ventrally from just below the sternum to just anterior to the post-hepatic septum. A second incision (~3 cm) was then made through the post-hepatic septum and the body of the T29F-7B implantable biopotential/temperature amplifier/encoder ( $5.7 \times 2.8 \times 0.92$  cm; (60 g or roughly 2 % of body weight)) (Konigsberg Instruments, Inc., Pasadena, CA, USA) was inserted through the opening to lie between the fat bodies ventral to the digestive tract in the abdominal cavity. To monitor heart rate, ECG leads were affixed to the medial pleuroperitoneal membrane along the body wall with PeriAcryl glue and mersiline mesh so that the negative (–) contact lead rested near the apex of the heart and the positive (+) contact lead was near the conus arteriosus. To monitor respiration rate, Biopotential leads were sutured into the intercostal muscles on the left side, about 1 cm apart vertically, in the area between the fourth and fifth ribs of the lateral body wall. The underlying muscle layers and integument were sutured closed independently, and artificial ventilation with air was continued until the animals regained consciousness. The tegus were treated post-surgery with Baytril (0.1 ml/kg IM,

every other day) and housed in indoor enclosures for at least a week to ensure full recovery.

### Study enclosures

Outdoor enclosures measuring 2.5 m × 3.5 m enclosed with 1 m high walls were planted with local short blade grass and each enclosure housed a small tree (*Eugenia uniflora*). Each enclosure also contained a rectangular burrow (60 cm wide × 80 cm long × 50 cm deep) constructed of brick and cement half buried in the ground with an opening 30 cm × 10 cm.

These artificial burrows resemble refuges used in nature; tegus choose to hibernate in concrete, man-made structures (such as under houses) as opposed to under rocks (Winck and Cechin 2008). Over the top of the burrows were pyramidal lids (60 cm × 80 cm base, 1 m height) constructed of plywood and internally insulated with 2 cm thick Styrofoam insulation. These unorthodox lids reduced solar heating of burrows as well as accommodated infrared cameras to monitor activity in the burrows. Four StowAway TidBit temperature data loggers (Digi-Key Corp., Thief River Falls, MN, USA) programmed to take a reading of the local ambient temperature every 15 min were placed around the enclosures, one in each burrow, one affixed on the north facing wall of one enclosure and one on the opposite south-facing side of the wall, about 75 cm above the ground. The data logger on the north face recorded temperatures in direct sunlight while the south-facing data logger recorded ambient temperatures in the shade.

The TidBit data loggers and telemetry implants were calibrated in water baths set at four temperatures (7.2, 23, 29, and 39.7 °C) for at least 30 min and compared against a precision mercury thermometer both before and after the study. Data loggers were placed on a backing of 2 cm thick Styrofoam to insulate them from conductive heat transfer from underlying structures. Antennae to receive telemetric signals from the implants were suspended 1 m above the enclosures to maximize receptivity.

### Data acquisition

Environmental data (rainfall, atmospheric pressure, relative humidity) were collected from the local UNESP meteorological station (22°23'S, 47°32'W, 626.5 m altitude). Daily mean atmospheric pressure and relative humidity were calculated by UNESP personnel from three daily readings taken at 09:00, 15:00 and 21:00.

The T29F-7B implantable biopotential/temperature amplifier/encoders (Konigsberg Instruments, Inc.) were configured to continuously receive and broadcast the electrocardiogram (ECG), a biopotential recording of chest wall impedance associated with intercostal muscle movements, and body core

temperature. Telemeter signals were received and decoded with a TR8-2-2/TD14-10 telemetry signal processor and demodulator (Konigsberg Instruments, Inc.) and the raw decoded voltage signals were collected with a Dataq Instruments DI-720 data acquisition system at 250 Hz per channel. These files were later processed with custom-designed Matlab scripts configured to full-wave rectify (only the breathing biopotentials), digitally filter (low pass), and detect peaks (using peakdetect.m from Matlab Central) to detect instantaneous heart ( $f_H$ ), and respiration rates ( $f_R$ ). Automated peak detection was visually verified for accuracy, and to account for any irregularities or interference in electrical signals.

### Experimental protocol

The tegus were housed as pairs (one male and one female) and allowed to roam freely in their enclosures. Active lizards were fed to satiation on average every 3 days, their diet consisting of meat mixed with vegetables and fruit with added multivitamin supplement. In the months prior to dormancy, the tegus consumed progressively less food and eventually stopped for the duration of the dormancy period. Water was available at all times. Continuous data recording began on the first of January and continued through to the end of September for all lizards. Recordings ceased at various times throughout October as the life span of the batteries in the telemetry units was reached.

### Data analysis

Average values were calculated for each variable [heart rate ( $f_H$ ), breathing rate ( $f_R$ ), deep body temperature ( $T_b$ ), burrow temperature ( $T_{\text{burrow}}$ ), and the temperature in direct sunlight and shade] for each individual for each 15 min time period for the entire study period. These 15 min averages were subsequently averaged over each day, week, and month for each tegu. Daily maximum and daily minimum values were also extracted from each individual for subsequent comparisons and averaged over each week, and month for each tegu. Seasonal comparisons were made by comparing critical months corresponding to the: active period (February), dormant period (May), and the post-arousal reproductive period (September).

Monthly nighttime minimum levels of oxygen consumption were calculated from the formula derived by Piercy et al. (2015) from the relationship between heart rate and metabolic rate for this species of tegu lizard under quiescent conditions. The equation used was:  $\log_{10}(\text{O}_2 \text{ consumption}) = -1.47 + 0.67(\log_{10}(\text{heart rate}))$ .

Statistical analysis between averaged values was done by repeated measures one-way ANOVA followed by a Student–Newman–Keuls post hoc test, unless normalcy tests failed, when a non-parametric repeated measures one-way

ANOVA on ranks (Kruskal–Walis test) was used. Within month, data were compared by paired *t* test. All values are presented as mean  $\pm$  standard error of the mean (s.e.m). Differences were considered to be statistically significant at the level of  $P < 0.05$ .

## Results

### Meteorological data

In general, the summer time (December to February) in Rio Claro is warm and wet while the winter (June to August) is cool and dry. During this study, the lowest mean daily temperatures occurred from May to August ( $\sim 15$  °C) and corresponded to the periods of highest barometric pressure (716 mmHg) while the highest mean daily temperatures occurred from November to January ( $\sim 27$  °C) and corresponded to the periods of lowest barometric pressure ( $\sim 705$  mmHg). In this particular year, August and September were the driest months (virtually no rainfall) and were the months with the lowest relative humidity ( $\sim 50$  %). At this latitude, however, seasonal differences in all these variables, except rainfall, are modest.

### Seasonal patterns of behaviour

#### January–July

Throughout this period, there were days when animals remained in their burrows and did not emerge. Such events were rare from January to March (2–4 events/month of 1–2 days each), and associated with inclement weather. In April, these periods were common (6–8 periods) and lasted  $\sim 2$ –3 days each. During late April/early May, the lizards began block the entrance to the burrows with vegetation and remained inactive in their burrows marking the start of the dormancy period that continued through June and July. Tegus still emerged periodically throughout the dormancy period (3–4 times/month on average for usually 1 day each).

#### August–September

Starting in August, the tegus began to emerge from their burrows every day indicating the end of the dormancy period. Animals never remained inactive in their burrows during the daytime in September.

### Seasonal patterns of physiological change

#### January–March

From January to March, daytime temperatures in the enclosures ( $T_a$ ) often rose to 40 °C or higher and nighttime

temperatures fell to below 20 °C (Fig. 1a). Burrow temperatures ( $T_{\text{burrow}}$ ) fluctuated little over the day, ranging between 23 and 26 °C (Fig. 2a). Tegus went out to bask each day at roughly the time that  $T_a$  rose above  $T_{\text{burrow}}$  (as indicated by the red dotted line and the rise in body temperatures for the tegus in February in Fig. 1a). During this period, maximum daytime  $T_b$  ( $\sim 32$ –35 °C) was remarkably uniform in all animals (Fig. 2b). The tegus entered the burrows in the evening well before  $T_a$  began to approach  $T_{\text{burrow}}$  (Fig. 1a) and their  $T_b$  fell very slowly, equilibrating with burrow temperature by the middle of the night (Fig. 1a). Nighttime  $T_b$  minima were also relatively uniform throughout this period (Fig. 2b).

In the morning during this period,  $f_H$  and  $f_R$  began to rise at a constant  $T_b$  an hour or more before the tegus left their burrows to bask, while in the evening  $f_H$  and  $f_R$  began to fall in advance of  $T_b$  declining (Fig. 1b). Maximum daily  $f_H$  and  $f_R$  began to fall significantly ( $P = 0.031$  and  $< 0.05$ , respectively) in March (Fig. 2c, d). Similarly, minimum values of  $f_H$  and  $f_R$  during the night fell progressively ( $P < 0.05$ ) over the three months (Fig. 2c, d) (by 60–75 %) despite the fact that mean  $T_b$  was relatively constant.

#### April

Although  $T_{\text{burrow}}$  in April still fluctuated over the same range as the preceding months, nighttime minimum  $T_b$  continued to fall ( $P = 0.007$ ) (Fig. 2). Maximum daily voluntary temperature also declined ( $P < 0.001$ ) (Fig. 2), despite the sustained and elevated ambient temperatures in the sun. During April, when animals remained in their burrows, daytime maximum  $T_b$  remained at the previous night's minimum  $T_b$ .

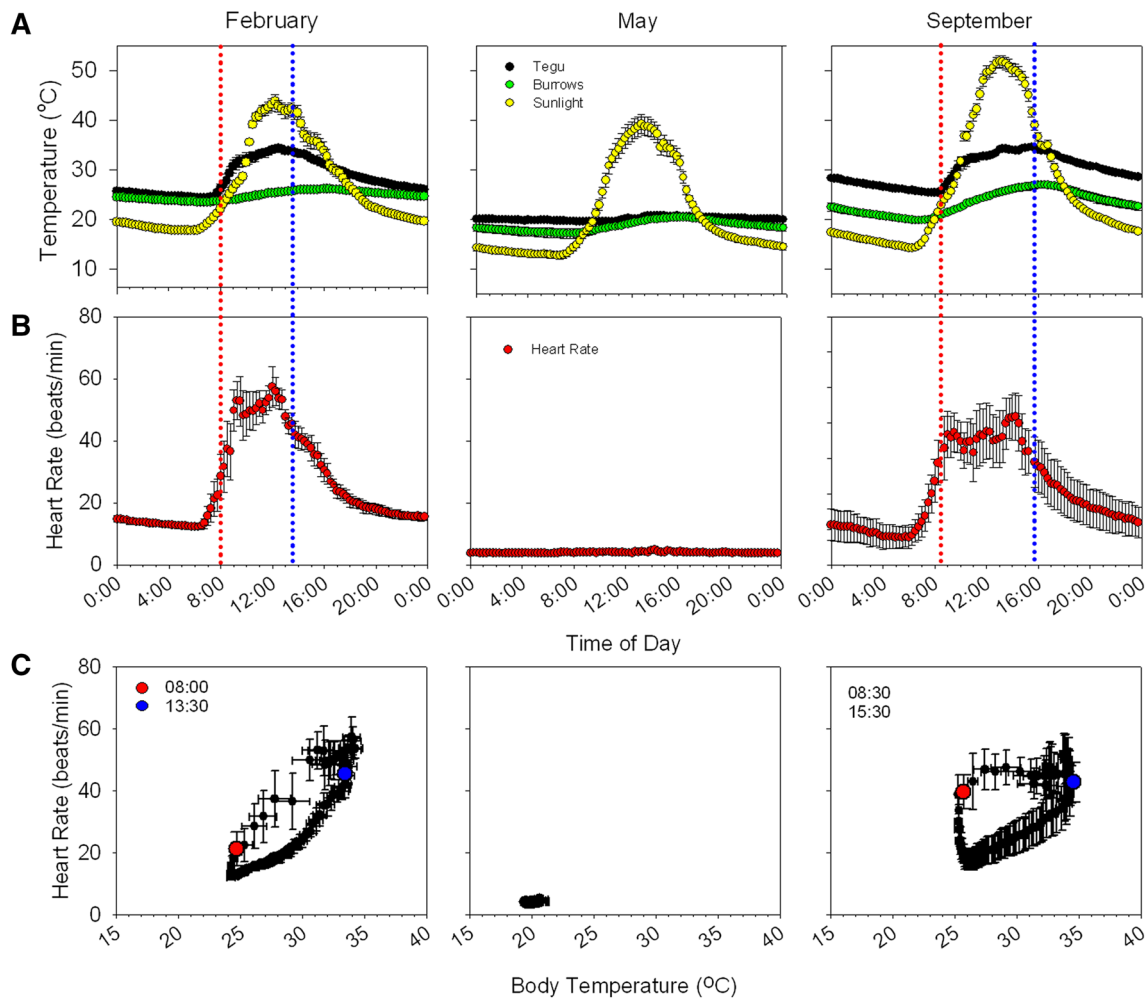
During April,  $f_H$  and  $f_R$  no longer began to rise in the morning before  $T_b$ , but rose only when the tegus left their burrows to bask. Both maximum daytime and minimum nighttime rates were lower in April than in March (for  $f_H$  min  $P < 0.05$ , and for max  $P = 0.031$ ; for  $f_R$  both min and max  $P < 0.05$ ) and the magnitude of the daily changes in  $T_b$ ,  $f_H$ , and  $f_R$  was reduced.

#### May–July

During the dormancy period, the tegus largely remained in their burrows that were at their lowest temperatures for the year. At this time,  $T_b$  equalled  $T_{\text{burrow}}$ . When a tegu did emerge, it was late in the day and while  $T_b$  at such times did rise, it was only to moderate levels (18–22 °C) and for brief amounts of time. Once the animals entered dormancy, mean  $f_H$  and  $f_R$  remained low and relatively constant throughout the day and night (Fig. 2).

#### August–September

In August, animals began frequently to emerge from their burrows and by September they were emerging every day.



**Fig. 1** Mean ( $\pm$ SEM) values for **a** ambient temperature, burrow temperature, tegu temperatures, and **b** heart rate for all tegus for each 15 min period on all days during February (the active season), May (the dormancy season) and September (the post-arousal, reproductive season). The *dotted lines* represent the average time when tegus left their burrows in the morning (*red*) and retreated into their burrows

for the evening (*blue*) during days of emergence. **c** The relationships between mean values of  $T_b$  and heart rate for all tegus for all days of each of these months. Times when tegus, on average, emerged to bask (*red dot*) or retreated to their burrows to rest for the night (*blue dot*) are indicated in the *upper left corner* of each graph

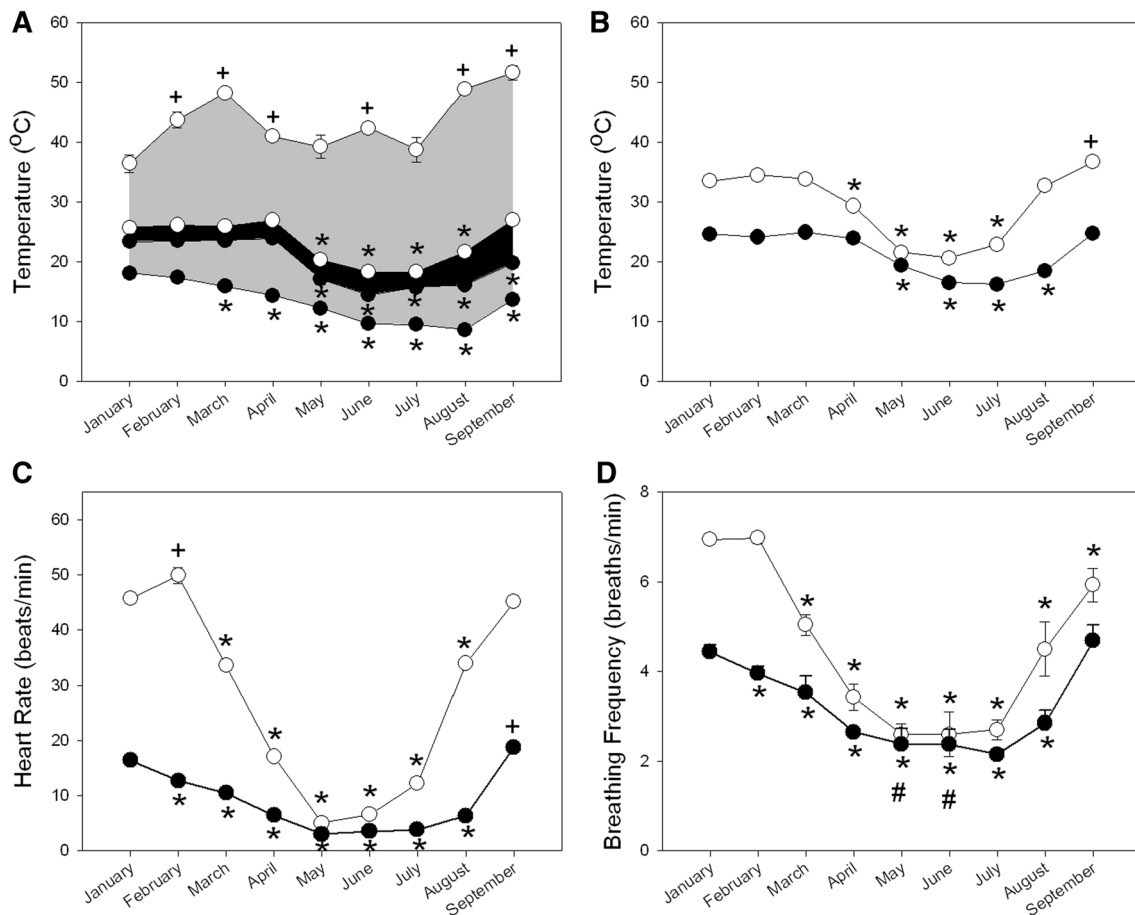
Again, emergence occurred only once  $T_a$  exceeded  $T_{burrow}$  (as indicated by the rise in body temperatures for the tegus in September in Fig. 1). Periods of basking were longer and maximum daytime  $T_b$  increased to 33–37 °C (Fig. 2). Animals entered the burrows after  $T_a$  began to fall, but while  $T_a$  was still well above  $T_{burrow}$  (as indicated by the blue dotted line for September in Fig. 1a). Mean  $T_{burrow}$  was beginning to increase at night and during the day ( $P < 0.001$ ) (Fig. 2). During this period,  $T_b$  never fell to the level of  $T_{burrow}$  during the night (Figs. 1, 2).

In the morning during this period,  $f_H$  and  $f_R$  again began to rise at a constant  $T_b$  before the tegus left their burrows to bask and began to fall in advance of  $T_b$  in the evening (Fig. 1). Maximum daily  $f_H$  and  $f_R$  began to increase progressively ( $P = 0.011$  and

$<0.05$ , respectively) through August and September, as did nighttime  $f_H$ ,  $f_R$  and  $T_b$  ( $P < 0.05$ ,  $P < 0.05$  and  $P = 0.002$ , respectively) (Fig. 2).

### Heart rate hysteresis

From January through March, the rate of increase (with respect to  $T_b$ ) in  $f_H$  during warming exceeded the rate of decrease during cooling and thus there was a large hysteresis in the correlation between  $f_H$  and core  $T_b$  (Fig. 3). During the dormancy period, there was less hysteresis in the relationship between  $f_H$  and  $T_b$  (Fig. 3). Beginning in August, but most evident in September, are dramatic increases in  $f_H$  before  $T_b$  rises in the morning, and falls in heart rate before  $T_b$  falls in the evening (Figs. 1, 3).



**Fig. 2** Mean ( $\pm$ SEM; error bars may be smaller than symbols) monthly values for maximum and minimum **a** ambient temperatures and burrow temperatures, **b** tegu body temperatures, **c** heart rate, and **d** breathing rate for all tegus over the entire recording period. Maximum values are indicated by open symbols while minimum values are indicated by filled symbols. In **a** the grey shading links the maximum and minimum ambient temperatures while the black shading links

the maximum and minimum burrow temperatures. Note how well the burrows are buffered from ambient temperature swings. Asterisk indicates values that are significantly lower than January values. + indicates values that are significantly elevated compared to January values. All minimum values are lower than maximum values except for those indicated with a #

### Nighttime metabolic rate

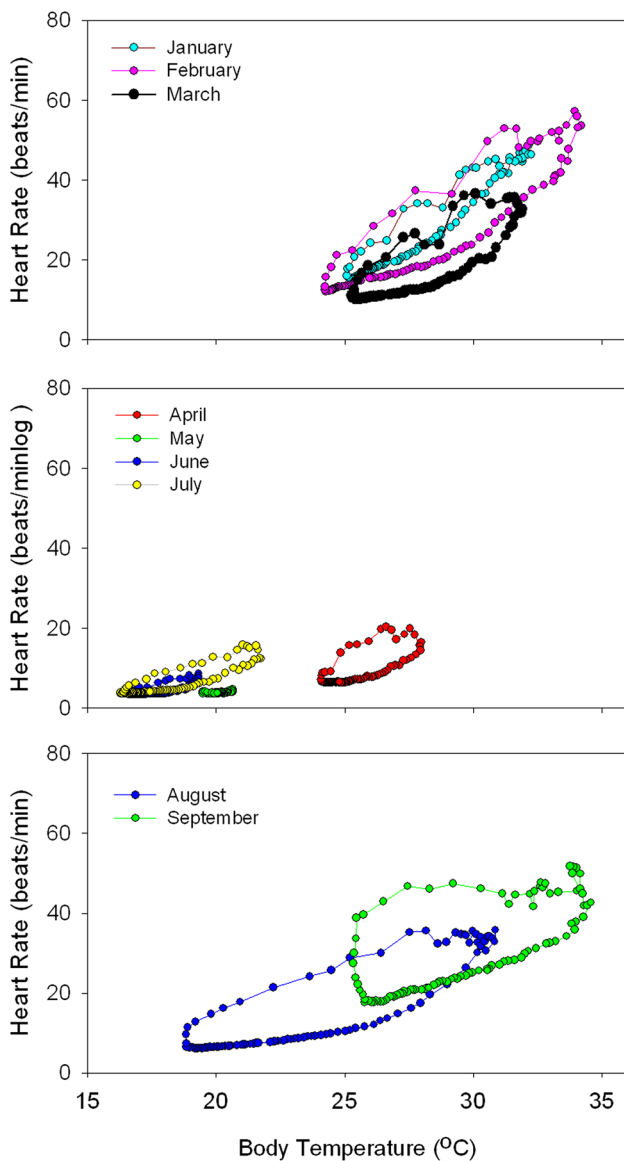
In Fig. 4, the monthly nighttime minimum values of  $T_b$ ,  $T_{\text{burrow}}$ ,  $f_H$  and  $f_R$  have been re-plotted along with rates of oxygen consumption calculated from the formula derived by Piercy et al. (2015) from the relationship between heart rate and metabolic rate for this species of tegu lizard under quiescent conditions. From this figure, it is clear that  $f_H$ ,  $f_R$  and calculated levels of  $O_2$  consumption fell progressively at night from January through April ( $P < 0.001$ , 0.05 and 0.001, respectively) despite the fact that  $T_b$  was constant. From April into May, however, there was a further progressive drop in  $f_H$ ,  $f_R$  and estimated  $O_2$  consumption (only the latter was significant  $P = 0.037$ ) at night but this was associated with a further, non-significant drop in  $T_b$ . In June and July, all variables remained relatively constant while in August and September, all variables increased ( $P = 0.003$

for  $O_2$  consumption,  $<0.001$  for  $f_H$ ,  $<0.05$  for  $f_R$ ,  $<0.05$  for  $T_b$  and  $<0.001$  for  $T_{\text{burrow}}$ ).

### Discussion

#### The pattern of metabolic rate reduction leading into dormancy

One of the goals of this study was to describe the pattern by which metabolism falls during the autumn under natural conditions. Does it occur progressively during the day and night, does it occur only at night, or does it occur only during multi-day periods of inactivity in the burrow? Previous studies have shown that tegu lizards depress metabolism in advance of dormancy (Abe 1983, 1993, 1995). In all these studies, measurements were made on animals confined in



**Fig. 3** The relationships between mean values of body temperature and heart rate for all tegus for all days of all months (*error bars* are omitted for clarity)

dark for several days, usually at constant temperature (Abe 1983, 1993, 1995; de Souza et al. 2004; Andrade and Abe 1999; Milsom et al. 2008; Toledo et al. 2008). By contrast, in nature during this period tegus are still active and warm themselves to active temperatures during the day while being exposed to progressive changes in photoperiod and ambient temperature (Köhler and Langerwerf 2000).

We found that from January to March, tegus regulated their maximum daily  $T_b$  from 33 to 37 °C, except on days with inclement weather when  $T_a$  did not permit behavioural thermoregulation to this extent. Minimum nighttime  $T_b$  and  $T_{\text{burrow}}$  from January to April also remained constant. By contrast, nighttime values of  $f_H$  and  $f_R$  declined

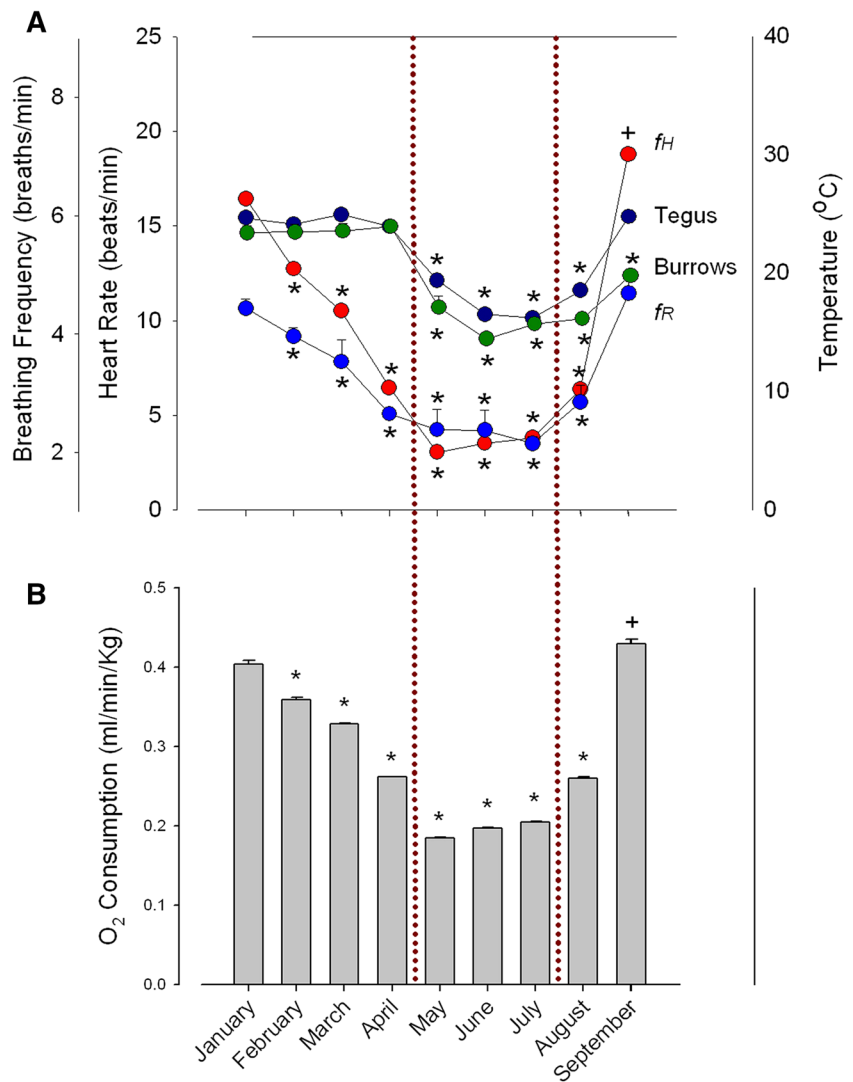
progressively over this period and daytime maximum levels also began to decline in March. The declines in nighttime  $f_H$  and  $f_R$  at constant  $T_b$  suggest that metabolic rate was being suppressed actively and progressively over this period of time. Based on the calculations in Fig. 4, nighttime metabolic rate appears to have been suppressed by approximately 45 % from January to April. This is similar to the progressive decline in metabolism seen in *L. vivipara*, although for this species the decline occurred during the dormancy only and not preceding it (Patterson and Davies 1978). While we do not have data that reveal the mechanism underlying this reduction, altered right-to-left intra-cardiac shunting, reducing  $O_2$  delivery to the tissues, has previously been implicated in metabolic suppression (Hicks and Wang 2004) and is a definite possibility.

Daytime  $f_H$  fell in March, suggesting that daytime metabolic rate may also have begun to fall prior to entrance into dormancy. However, the animals were active to varying degrees during the day and not in a steady state; therefore, heart rate could not be used to estimate metabolic rate.

The decline in maximum  $T_b$  that occurred in April–May was indicative of an endogenous seasonal rhythm of body temperature and metabolism. Although it was possible for the tegus to achieve higher maximum  $T_b$  through behavioural thermoregulation, that they did not suggest an endogenous seasonal rhythm, similar to that seen in the sleepy lizard, *T. rugosa* (Firth and Belan 1998; Ellis et al. 2008). From April into May, there was a further significant nighttime drop in  $f_H$  and  $f_R$  indicating a further suppression in metabolism, but this was associated with a significant fall in  $T_b$ . This amounted to a 30 % decrease in  $O_2$  consumption (a  $Q_{10}$  of 2.1) bringing the metabolic rate to levels that were 45 % of those calculated in January. As indicated by the low but consistent levels of  $T_b$ ,  $f_H$  and  $f_R$  (day and night) from May through July, metabolism was relatively uniform during dormancy. The values of metabolic rate estimated for the tegus in dormancy (0.18–0.21 ml  $O_2$ /min/kg) are similar to those measured in previous studies on dormant tegus (0.15–0.30 ml  $O_2$ /min/kg; Abe 1995; Andrade and Abe 1999; Milsom et al. 2008; de Souza et al. 2004; Toledo et al. 2008), indicating that our methods for estimating metabolic rate were consistent with previous studies.

In August and September, all variables increased to levels significantly greater than those recorded from tegus at similar body temperatures in May and April, suggesting that they were due not only to the increases in  $T_b$ , but also due to removal of the active metabolic suppression. The increasing incidence of arousals associated with slowly increasing nighttime heart rate and breathing in August is also suggestive that the degree of metabolic suppression was decreasing as the period of arousal progressed, which has been documented in other lizard species as they approach arousal (Patterson and Davies 1978).

**Fig. 4 a** Mean ( $\pm$ SEM; error bars may be smaller than symbols) monthly values for heart rate ( $f_H$ ), breathing rate ( $f_R$ ) and minimum burrow and tegu body temperatures for all tegus over the entire recording period. **b** Resting levels of estimated oxygen consumption over the same period (derived from the equation of Piercy et al. 2015). Note the fall in heart rate, breathing rate and rate of oxygen consumption at constant burrow and tegu temperatures from January to April. Asterisk indicates values that are significantly lower than January values. + indicates values that are significantly elevated compared to January values. Vertical dotted lines indicate the entrance into and the emergence from dormancy



### Arousal during dormancy

While species of reptiles that undergo dormancy are not likely to emerge from their burrows in mid-winter when environmental conditions are extreme, they may still arouse from dormancy and remain within the burrow. To date, however, there is no documentation that this occurs. Species of reptiles that go dormant in subtropical regions should be less constrained to remain in their burrows during periods of arousal and it has been shown that *Varanus rosenbergi* spontaneously arouse frequently during dormancy (Ris-miller and McKelvey 2000). In the present study, *T. meri-anae* also exhibited periodic bouts of arousal accompanied by short bouts of emergence. Amongst the four individuals in this study, there was a wide range of variability in the occurrence of this behaviour, both in the number of times an individual aroused over the period of dormancy and in the phase of the dormant period (early versus late) during which these events occurred. There was no synchrony

to the occurrence of arousals in tegus inhabiting the same burrow suggesting that they were not tightly correlated to local factors such as temperature change, noise or disturbance. Arousals appeared to occur randomly, with no distinct pattern in any animal, suggesting that they were not the consequence of an underlying biological rhythm. This does not preclude the existence of an internal clock controlling arousal from dormancy or the onset of reproduction. Many species that are arrhythmic in winter can be rhythmic at other seasons (see Revel et al. 2007; Ellis et al. 2008).

It is possible that these arousals were the consequence of the experimental design. The artificial burrows were designed for ease of access and to allow infra-red recording of activity within the burrow and were thus spacious and left the animals relatively exposed. Natural burrows tend to be more constrictive and possibly deeper in the substrate where daily fluctuations in temperature would be absent. Animals rarely, if ever, leave them during the dormancy season. A more constant temperature and tactile stimulation



may promote dormancy and eliminate periods of arousal. At present, the underlying cause of the arousals seen in this study is not clear.

Periodic arousals are a hallmark of most mammalian hibernation (Willis 1982) and here too it is not clear what the underlying cause is (Barnes et al. 1993; Wang 1993; Carey et al. 2003). One hypothesis is that transcription and translation of genetic material are inhibited by low temperatures and that animals must arouse periodically to undertake essential maintenance activities (Van Breukelen and Martin 2002; Carey et al. 2003). The occurrence of periodic arousals is normally rhythmic in mammals (Twente and Twente 1967), but at present there is no consensus on what triggers these arousals. The incidence and role of periodic arousals in both mammalian and ectothermic hibernation are, therefore, areas that require further study.

### Heart rate hysteresis and implications for body temperature regulation

Heart rate hysteresis has been well described in reptiles, and its role in temperature regulation has received much attention. To maximize the period where body temperature exceeds ambient temperature, many reptiles increase cutaneous blood flow in the morning to maximize heat gain. A concomitant rise in heart rate due to the baroreflex leads to an appropriate increase in cardiac output that maintains blood pressure constant (Bartholomew and Tucker 1963; Galli et al. 2004; Crossley et al. 2015). A decrease in cutaneous blood flow and heart rate in the evening conserves heat by reducing the rate of heat loss (Morgareidge and White 1969; Langille and Crisp 1980; Galli et al. 2004; Clark et al. 2006). This gives rise to hysteresis in the relationship between  $f_H$  and  $T_b$  in which the rate of change in  $f_H$  reflects the effects of temperature on  $f_H$  and metabolic rate ( $Q_{10}$  effects), the effects of activity and feeding (Zaar et al. 2004), and the effects of thermoregulatory processes associated with reaching/retaining preferred  $T_b$  (Seebacher 2000; Seebacher and Franklin 2001, 2005).

From January to March, and again in August and September, on days when the tegus emerged from their burrows to bask,  $f_H$  and  $f_R$  began to rise in the morning, at constant  $T_b$ , even before the tegus left their burrows. The most extreme case was in September when  $f_H$  more than doubled, reaching almost maximum daytime levels over a two hour period before the tegus emerged from their burrows. This correlated with the period of greatest reproductive mating activity, highest daytime  $T_b$  and heart rate, and longest periods spent active. This suite of changes is not uncommon in reptiles during mating season and has been attributed to “mating unrest,” which can be accompanied by an elevation in preferred  $T_b$  (Huey and Bennett 1987; Rismiller and Heldmaier 1982, 1991; Luiselli and Akani 2002; Seebacher and

Franklin 2005). Once mating occurs, preferred  $T_b$  in pregnant females may increase (*Hoplodactylus maculatus*, Werner and Whitaker 1978; *Thamnophis sirtalis*, Stewart 1965; *Gerrhonotus coeruleus*, Stewart 1984) or decrease (Lacerta vivipara, Patterson and Davies 1978; Scleroperuscyanogenys, Garrick 1974; Scleroporopus jarrovi, Beuchat 1986).

This rapid initial increase in  $f_H$  was most likely due to changes in activity state (sleep to alert) and activity in the burrow. Throughout the fall, this pre-emergence increase in  $f_H$  slowly decreased and by April,  $f_H$  and  $f_R$  no longer began to rise in the morning before  $T_b$ , instead only rising when the tegus left their burrows to bask.

In all seasons, once tegus left the burrow and began to warm,  $f_H$  and  $f_R$  increased further, with the rates of these changes varying across the seasons (Fig. 3). Not surprisingly, the higher the  $f_H$  at the time of emergence from the burrow, the lower the rate of rise until the maximum daily  $f_H$  and  $T_b$  were reached. The rate of rise at this time must reflect the effects of temperature on  $f_H$  and metabolic rate ( $Q_{10}$  effects), the effects of activity, and the effects of thermoregulatory processes associated with reaching preferred  $T_b$ .

In the evenings of the non-dormant periods,  $f_H$  and  $f_R$  began to fall in advance of  $T_b$  with the greatest changes occurring in September. These rapid changes most likely reflect increases in total peripheral vascular resistance associated with vasoconstriction of peripheral beds for heat retention as described above (Seebacher 2000; Clark et al. 2004, Galli et al. 2004; Seebacher and Franklin 2005). This rapid fall in  $f_H$  was absent during dormancy when tegu  $T_b$  fell rapidly to approximate  $T_{\text{burrow}}$  within hours of entering the burrows, suggesting that peripheral vascular resistance was not increased and, therefore, heat retention was not actively occurring as it was during the non-dormant periods. The abandonment of heat retention strategies during dormancy may be a strategy to maintain a reduced metabolic rate, as much as it may be related to the reduction in preferred body temperature.

There were seasonal differences in the complex interplay between changes in  $f_H$  due to the direct effects of temperature and the indirect effects of thermoregulation, activity, and changes in metabolism. The base hysteresis (i.e. that in each monthly loop) is reflective of the physiological changes associated with daily warming and cooling as preferred  $T_b$  alternated between day time and nighttime levels. Changes in the shape and position of the hysteresis curves reflect the fact that these daytime and nighttime preferences change with the seasons.

### Conclusion

While this study was largely observational, the continuous recording of body temperature along with heart rate

and breathing rate in black and white tegu lizards provides insight into the physiological correlates of changes in behaviour patterns. In particular, the data suggest that there was a continuous decline in nighttime metabolic rate, at constant  $T_b$  throughout the late summer and fall during the lead up to the dormancy period. This is indicative of an active metabolic suppression that develops progressively, but only at night in the early stages. Although lizards dedicate shorter periods of daytime to basking during the late summer and fall, they still reach the same  $T_b$  values seen in spring and early summer. In May, when the tegus made a behavioural commitment to dormancy, there was a decrease in  $T_b$  associated with a decrease in  $T_{\text{burrow}}$  and accompanied by a further reduction in heart rate, breathing rate and metabolic rate. Dormancy was a fairly uniform state from which the tegus, under the conditions of this study, did arouse periodically. The sum of the data suggests that tegu lizards can actively suppress metabolism in a complex and temperature independent fashion for which the underlying mechanism remains to be explored.

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