1 **Daily and Annual Cycles in Thermoregulatory Behaviour and Cardio-**

2 **Respiratory Physiology of Black and White Tegu Lizards**

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5 Colin E. Sanders[1], Glenn J. Tattersall[2], Michelle Reichert[1], Denis V. Andrade[3], Augusto

6 S. Abe[3] and William K. Milsom[1]

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9 [1]Dept. Zoology, Univ. British Columbia, Vancouver, Canada

10 [2]Dept. Biology, Brock University, St. Catharines, Ontario, Canada

11 and

12 [3]Instituto Nacional de Ciência e Tecnologia em Fisiologia Comparada, Depto de

13 Zoologia, Univ Estadual Paulista - Rio Claro, SP 13506-900, Brazil.

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21 Corresponding Author:

22 W.K. Milsom

23 Department of Zoology

24 University of British Columbia

25 6270 University Blvd.

26 Vancouver, British Columbia

27 Canada, V6T 1Z4

28 e-mail: milsom@zoology.ubc.ca

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31 Running Title: Torpor and dormancy in lizards

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1 **ABSTRACT:**

2 This study was designed to determine the manner in which metabolism is suppressed

3 during dormancy in black and white tegu lizards (*Tupinambis merianae*). To this end,

4 heart rate (f_H), respiration rate (f_R), and deep body temperature (T_b) were continuously

5 monitored in outdoor enclosures by radio-telemetry for nine months. There was a

6 continuous decline in nighttime breathing and heart rate, at constant T_b, throughout the

7 late summer and fall suggestive of an active metabolic suppression that developed

8 progressively at night preceding the entrance into dormancy. During the day, however,

9 the tegus still emerged to bask. In May, when the tegus made a behavioural commitment

10 to dormancy, T_b (day and night) fell to match burrow temperature, accompanied by a

11 further reduction in f_H and f_R. Tegus, under the conditions of this study, did arouse

12 periodically during dormancy. There was a complex interplay between changes in f_H and

13 T_b associated with the direct effects of temperature and the indirect effects of

14 thermoregulation, activity, and changes in metabolism. This interplay gave rise to a daily

15 hysteresis in the f_H/T_b relationship reflective of the physiological changes associated with

16 warming and cooling as preferred T_b alternated between daytime and nighttime levels.

17 The shape of the hysteresis curve varied with season along with changes in metabolic

18 state and daytime and nighttime body temperature preferences.

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20 Keywords: Reptiles, tegu lizards, torpor, dormancy, hibernation, cardiorespiratory

21 control, seasonal adjustments

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1   INTRODUCTION:

2   Hibernation is employed by many animals as a strategy to survive periods of

3   limited energy availability in the environment (Carey *et al.*, 2003). Some of the hallmark

4   traits of endothermic hibernation are a depression of body temperature (T_b) and a

5   reduction of basal metabolic rate, accompanied by falls in ventilation and heart rate

6   (Lyman 1965, Wilis, 1982; Carey *et al.*, 2003; Tøien et al. 2015). While the magnitude

7   and mechanism of metabolism and T_b depression in hibernating endotherms have been

8   well studied (Lyman, 1982; Nedergaard *et al.*, 1990; Storey and Storey, 1990; Carey *et*

9   *al.*, 2003), the same aspects of dormancy have not been extensively studied in

10   ectothermic hibernators. It is clear that many overwintering ectotherms are capable of

11   similar reductions in metabolism, often independent of changes in T_b; Mayhew (1965)

12   referred to this as brumation, in order to distinguish ectothermic winter dormancy from

13   endothermic hibernation.

14   Reduction in metabolic rate at the initiation of hibernation involves both lowering

15   of the hypothalamic set point for body temperature regulation and active metabolic

16   suppression (mammals: Heller et al. 1977; Nedergaard *et al.,* 1990; Heldmaier et al.

17   1993). This is also true of amphibians and reptiles, however these groups rely on

18   behavioural rather than physiological methods to reduce T_b (Rollinson et al., 2008;

19   Tattersall and Boutilier, 1997; Donohoe et al., 1998; Tattersall and Boutilier, 1999; Huey

20   *et al.*, 1977; Guppy and Withers, 1999; Glanville and Seebacher 2006). The manner in

21   which these behavioural and physiological strategies are integrated and employed by

22   ectotherms during entrance into dormancy, however, are not well understood.

1   The black and white tegu (*Tupinambis merianae*) is a large, diurnal lizard of

2   South America that undergoes winter dormancy in its southern range (Abe, 1995; Avila-

3   Pires, 1995). During dormancy, tegus retreat into their burrows where they fast and

4   remain inactive from May to August (Abe, 1995; Andrade *et al*., 2004). Preparation for

5   dormancy begins well before environmental conditions become adverse and tegus can

6   depress metabolism to dormant levels at any time of the year when inactive, in constant

7   cold, darkness and deprived of food (Milsom, *et al*. 2008). By the end of the

8   autumn/beginning of the winter, the final steps leading to dormancy appear to be a

9   behavioral decision to retreat into the burrow, let T_b equilibrate with the surroundings,

10   and abandon behavioural thermoregulation. There also appears to be a progressive

11   reduction in thermal sensitivity (Q_{10}) from summer to winter such that the metabolic rate

12   of dormant lizards becomes relatively temperature independent (Abe, 1983; 1993; 1995;

13   Souza *et al*., 2004). This reduction in Q_{10} has been proposed to be an advantage to

14   maintaining extremely low metabolic rates even when burrow temperatures fluctuate

15   (Toledo et al., 2008).

16   The previous studies on hibernating tegus have given rise to several questions.

17   First, under natural conditions, can nightly metabolic depression be detected in advance

18   of winter dormancy while the animals are still active during the day?   As soon as tegus

19   commit to dormancy and retreat to the burrow they generally remain there for the season,

20   but it is not known whether they immediately enter dormancy or if the degree of

21   metabolic suppression increases as dormancy progresses. The latter has been reported to

22   occur in *Lacerta vivipara* (Patterson & Davies, 1978), although the metabolic suppression

23   was not sustained throughout the entire season. Finally, it is not clear whether the period

1 of dormancy is always one prolonged bout or whether these animals undergo periodic

2 arousals during which they remain relatively inactive within their burrows, as is seen in

3 other dormant lizards, such as *Varanus rosenbergi* (Rismiller & McKelvey, 2000).

4   To understand the natural mechanisms involved in winter dormancy requires

5 continuous monitoring of behaviour, T_b and, ideally, metabolism throughout the year.

6 Here we record continuously behaviour and T_b along with heart and breathing rates as

7 physiological surrogates for metabolism (Zaar et al., 2004, Butler *et al*., 2000, 2002;

8 Clark *et al*., 2004; Clark et al., 2006; Green et al., 2008; Piercy et al., 2015), in a group of

9 black and white tegus, *T. merianae*, housed outdoors under semi-natural conditions. We

10 hypothesized that metabolic suppression (as indicated by changes in heart rate and

11 breathing frequency) would not be evident until the tegus remained in the burrows for

12 extended periods but that metabolism would then progressively fall and be sustained

13 throughout the dormant period.

14

15 METHODS:

16   Our study was conducted at the Jacarezario, UNESP Bela Vista Campus, Rio

17 Claro, SP, Brazil. Tegus were captive bred and reared for scientific study and

18 conservation *ex-situ*. The study cohort consisted of 2 males and 2 females. Only 4

19 animals could be recorded from concurrently as the telemetry base station could only

20 receive and decode 4 signals at the same time. Animals were weighed each month except

21 during dormancy to reduce interference with the dormant state. The tegus weighed 3.2 ±

22 0.3 kg at the start of the study and 3.5 ± 0.4 kg by the end. All surgeries and experiments

1   were conducted under animal care approval from both the UBC animal care committee

2   and the Universidade Estadual Paulista - Rio Claro (Protocol #A09-0232).

3   **Surgery:**

4   Animals were anaesthetized using Halothane vapour. An incision was made mid-

5   ventrally from just below the sternum to just anterior to the post-hepatic septum. A

6   second incision (~3cm) was then made through the post-hepatic septum and the body of

7   the T29F-7B implantable biopotential/temperature amplifier/encoder (5.7 X 2.8 X 0.92

8   cm; (60 g or roughly 2% of body weight)) (Konigsberg Instruments, Inc., Pasadena, CA,

9   USA) was inserted through the opening to lie between the fat bodies ventral to the

10   digestive tract in the abdominal cavity. To monitor heart rate, ECG leads were affixed to

11   the medial pleuroperitoneal membrane along the body wall with PeriAcryl glue and

12   mersiline mesh so that the negative (-) contact lead rested near the apex of the heart and

13   the positive (+) contact lead was near the conus arteriosus. To monitor respiration rate,

14   Biopotential leads were sutured into the intercostal muscles on the left side, about 1 cm

15   apart vertically, in the area between the fourth and fifth ribs of the lateral body wall. The

16   underlying muscle layers and integument were sutured closed independently, and

17   artificial ventilation with air was continued until the animals regained consciousness. The

18   tegus were treated post-surgery with Baytril (0.1ml/kg IM, every other day) and housed

19   in indoor enclosures for at least a week to ensure full recovery.

20   **Study Enclosures:**

21   Outdoor enclosures measuring 2.5 m X 3.5 m enclosed with 1 m high walls were planted

22   with local short blade grass and each enclosure housed a small tree (*Eugenia uniflora*).

23   Each enclosure also contained a rectangular burrow (60 cm wide X 80 cm long X 50 cm

1   deep) constructed of brick and cement half buried in the ground with an opening 30 cm X

2   10 cm.

3   These artificial burrows resemble refuges used in nature; tegus choose to

4   hibernate in concrete, man-made structures (such as under houses) as opposed to under

5   rocks (Winck & Cechin, 2008). Over the top of the burrows were pyramidal lids (60 cm

6   X 80 cm base, 1 m height) constructed of plywood and internally insulated with 2cm

7   thick Styrofoam insulation. These unorthodox lids reduced solar heating of burrows as

8   well as accommodated infrared cameras to monitor activity in the burrows. Four

9   StowAway TidBit temperature data loggers (Digi-Key Corp., Thief River Falls, MN,

10 USA) programmed to take a reading of the local ambient temperature every 15 minutes

11 were placed around the enclosures, one in each burrow, one affixed on the north facing

12 wall of one enclosure and one on the opposite south facing side of the wall, about 75 cm

13 above the ground. The data logger on the north face recorded temperatures in direct

14 sunlight while the south-facing data logger recorded ambient temperatures in the shade.

15   The TidBit data loggers and telemetry implants were calibrated in water baths set

16 at four temperatures (7.2, 23, 29, and 39.7°C) for at least 30 min and compared against a

17 precision mercury thermometer both before and after the study. Data loggers were placed

18 on a backing of 2 cm thick Styrofoam to insulate them from conductive heat transfer from

19 underlying structures. Antennae to receive telemetric signals from the implants were

20 suspended 1 m above the enclosures to maximize receptivity.

21 **Data Acquisition**

22   Environmental data (rainfall, atmospheric pressure, relative humidity) was

23 collected from the local UNESP meteorological station (22°23'S, 47°32'W, 626.5m

1   altitude). Daily mean atmospheric pressure and relative humidity were calculated by

2   UNESP personnel from three daily readings taken at 09:00, 15:00 and 21:00.

3   The T29F-7B implantable biopotential/temperature amplifier/encoders

4   (Konigsberg Instruments, Inc.) were configured to continuously receive and broadcast the

5   electrocardiogram (ECG), a biopotential recording of chest wall impedance associated

6   with intercostal muscle movements, and body core temperature. Telemeter signals were

7   received and decoded with a TR8-2-2/TD14-10 telemetry signal processor and

8   demodulator (Konigsberg Instruments, Inc.) and the raw decoded voltage signals were

9   collected with a Dataq Instruments DI-720 data acquisition system at 250 Hz per channel.

10   These files were later processed with custom-designed MatLab scripts configured to full-

11   wave rectify (only the breathing biopotentials), digitally filter (low pass), and detect

12   peaks (using peakdetect.m from Matlab Central) to detect instantaneous heart (f_H), and

13   respiration rates (f_R). Automated peak detection was visually verified for accuracy, and to

14   account for any irregularities or interference in electrical signals.

15   **Experimental Protocol**

16   The tegus were housed as pairs (one male and one female) and allowed to roam

17   freely in their enclosures. Active lizards were fed to satiation on average every three

18   days, their diet consisting of meat mixed with vegetables and fruit with added

19   multivitamin supplement. In the months prior to dormancy, the tegus consumed

20   progressively less food and eventually stopped for the duration of the dormancy period.

21   Water was available at all times. Continuous data recording began on the first of January

22   and continued through to the end of September for all lizards.   Recordings ceased at

1   various times throughout October as the life span of the batteries in the telemetry units

2   was reached.

3   **Data Analysis**

4   Average values were calculated for each variable [heart rate (f_H), breathing rate

5   (f_R), deep body temperature (T_b), burrow temperature (T_{burrow}), and the temperature in

6   direct sunlight and shade] for each individual for each 15 min time period for the entire

7   study period. These 15 min averages were subsequently averaged over each day, week,

8   and month for each tegu. Daily maximum and daily minimum values were also extracted

9   from each individual for subsequent comparisons and averaged over each week, and

10   month for each tegu. Seasonal comparisons were made by comparing critical months

11   corresponding to the: active period (February), dormant period (May), and the post-

12   arousal reproductive period (September).

13   Monthly nighttime minimum levels of oxygen consumption were calculated from

14   the formula derived by Piercy et al. (2015) from the relationship between heart rate and

15   metabolic rate for this species of tegu lizard under quiescent conditions. The equation

16   used was: $\log_{10}(O_2 \text{ consumption}) = -1.47 + 0.67 (\log_{10}(\text{heart rate}))$.

17   Statistical analysis between averaged values was done by repeated measures one-

18   way ANOVA followed by a Student-Newman-Kuels post-hoc test, unless normalcy tests

19   failed, when a non-parametric repeated measures one-way ANOVA on ranks (Kruskal-

20   Walis test) was used. Within month data were compared by paired t-test. All values are

21   presented as mean ± standard error of the mean (s.e.m). Differences were considered to

22   be statistically significant at the level of $P < 0.05$.

23

1   RESULTS:

2   **Meteorological Data**

3   In general, the summer time (December to February) in Rio Claro is warm and

4   wet while the winter (June to August) is cool and dry. During this study, the lowest mean

5   daily temperatures occurred from May to August (~15ºC) and corresponded to the

6   periods of highest barometric pressure (716 mmHg) while the highest mean daily

7   temperatures occurred from November to January (~27ºC) and corresponded to the

8   periods of lowest barometric pressure (~705 mmHg). In this particular year, August and

9   September were the driest months (virtually no rainfall) and were the months with the

10   lowest relative humidity (~50%). At this latitude, however, seasonal differences in all of

11   these variables, except rainfall, is modest.

12   **Seasonal Patterns of Behaviour**

13   *January-July:* Throughout this period there were days when animals remained in their

14   burrows and did not emerge. Such events were rare from January to March (2-4

15   events/month of 1-2 days each), and associated with inclement weather. In April these

16   periods were common (6-8 periods) and lasted ~2-3 days each. During late April / early

17   May, the lizards began block the entrance to the burrows with vegetation and remained

18   inactive in their burrows marking the start of the dormancy period that continued through

19   June and July. Tegus still emerged periodically throughout the dormancy period (3-4

20   times/month on average for usually 1 day each).

21   *August-September:* Starting in August the tegus began to emerge from their burrows

22   every day indicating the end of the dormancy period. Animals never remained inactive in

23   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 40ºC or higher and nighttime temperatures fell to below 20ºC (Fig. 1A). Burrow temperatures (T_{burrow}) fluctuated little over the day, ranging between 23 and 26ºC (Fig. 2A). Tegus went out to bask each day at roughly the time that T_a rose above T_{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 day-time T_b (~32-35ºC) were remarkably uniform in all animals (Fig. 2B). The tegus entered the burrows in the evening well before T_a began to approach T_{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 to 75%) despite the fact that mean T_b was relatively constant.

April: Although T_{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.

1   During April, f_H and f_R no longer began to rise in the morning before T_b, but rose

2   only when the tegus left their burrows to bask. Both maximum daytime and minimum

3   nighttime rates were lower in April than in March (for f_H min P<0.05, and for max

4   P0.031; for f_R both min and max P<0.05) and the magnitude of the daily changes in T_b, f_H,

5   and f_R were reduced.

6   *May-July:* During the dormancy period, the tegus largely remained in their burrows that

7   were at their lowest temperatures for the year. At this time, T_b equaled T_{burrow}. When a

8   tegu did emerge, it was late in the day and while T_b at such times did rise, it was only to

9   moderate levels (18-22 ºC) and for brief amounts of time. Once the animals entered

10   dormancy, mean f_H and f_R remained low and relatively constant throughout the day and

11   night (Fig. 2).

12   *August-September:* In August, animals began frequently to emerge from their burrows

13   and by September they were emerging every day. Again, emergence occurred only once

14   T_a exceeded T_{burrow} (as indicated by the rise in body temperatures for the tegus in

15   September in Fig. 1). Periods of basking were longer and maximum daytime T_b increased

16   to 33-37ºC (Fig. 2). Animals entered the burrows after T_a began to fall, but while T_a was

17   still well above T_{burrow} (as indicated by the blue dotted line for September in Fig. 1A).

18   Mean T_{burrow} was beginning to increase at night and during the day (P<0.001) (Fig. 2).

19   During this period, T_b never fell to the level of T_{burrow} during the night (Fig. 1,2).

20   In the morning during this period, f_H and f_R again began to rise at a constant T_b

21   before the tegus left their burrows to bask and began to fall in advance of T_b in the

22   evening (Fig. 1). Maximum daily f_H and f_R began to increase progressively (P = 0.011 and

1   <0.05 respectively) through August and September, as did nighttime f_H, f_R and T_b

2   (P<0.05, P<0.05 and P=0.002 respectively) (Fig. 2).

3   ***Heart Rate Hysteresis***

4   From January through March, the rate of increase (with respect to T_b) in f_H during

5   warming exceeded the rate of decrease during cooling and thus there was a large

6   hysteresis in the correlation between f_H and core T_b (Figure 3). During the dormancy

7   period there was less hysteresis in the relationship between f_H and T_b (Fig. 3). Beginning

8   in August, but most evident in September, are dramatic increases in f_H before T_b rises in

9   the morning, and falls in heart rate before T_b falls in the evening (Figs. 1,3).

10 ***Nightime Metabolic Rate:***

11   In Figure 4, the monthly nighttime minimum values of T_b, T_{burrow}, f_H and f_R have

12 been re-plotted along with rates of oxygen consumption calculated from the formula

13 derived by Piercy et al. (2015) from the relationship between heart rate and metabolic rate

14 for this species of tegu lizard under quiescent conditions. From this figure it is clear that

15 f_H, f_R and calculated levels of O_2 consumption fell progressively at night from January

16 through April (P<0.001, 0.05 and 0.001 respectively) despite the fact that T_b was

17 constant. From April into May, however, there was a further progressive drop in f_H, f_R

18 and estimated O_2 consumption (only the latter was significant P=0.037) at night but this

19 was associated with a further, non-significant drop in T_b. In June and July all variables

20 remained relatively constant while in August and September, all variables increased (P =

21 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

22 T_{burrow}).

23

1

2 DISCUSSION:

3 **The Pattern of Metabolic Rate Reduction Leading into Dormancy**

4   One of the goals of this study was to describe the pattern by which metabolism

5 falls during the autumn under natural conditions. Does it occur progressively during the

6 day and night, does it occur only at night, or does it occur only during multi-day periods

7 of inactivity in the burrow? Previous studies have shown that tegu lizards depress

8 metabolism in advance of dormancy (Abe, 1983; 1993; 1995). In all of these studies,

9 measurements were made on animals confined in dark for several days, usually at

10 constant temperature (Abe, 1983; 1993; 1995; Souza *et al*., 2004; Andrade and Abe,

11 1999; Milsom *et al*., 2008; Toledo et al., 2008). By contrast, in nature during this period

12 tegus are still active and warm themselves to active temperatures during the day while

13 being exposed to progressive changes in photoperiod and ambient temperature (Köhler

14 and Langerwerf, 2000).

15   We found that from January to March, tegus regulated their maximum daily T_b

16 from 33 to 37°C, except on days with inclement weather when T_a did not permit

17 behavioural thermoregulation to this extent. Minimum nighttime T_b and T_{burrow} from

18 January to April also remained constant. By contrast, nighttime values of f_H and f_R

19 declined progressively over this period and daytime maximum levels also began to

20 decline in March. The declines in nighttime f_H and f_R at constant T_b suggest that

21 metabolic rate was being suppressed actively and progressively over this period of time.

22 Based on the calculations in Fig. 4, nighttime metabolic rate appears to have been

23 suppressed by approximately 45% from January to April. This is similar to the

1   progressive decline in metabolism seen in *L. vivipara*, although for this species the

2   decline occurred during the dormancy only and not preceding it (Patterson & Davies,

3   1978). While we do not have data that reveals the mechanism underlying this reduction,

4   altered right-to-left intra-cardiac shunting, reducing O_2 delivery to the tissues, has

5   previously been implicated in metabolic suppression (Hicks and Wang, 2004) and is a

6   definite possibility.

7   Daytime f_H fell in March, suggesting that daytime metabolic rate may also have

8   begun to fall prior to entrance into dormancy. However, the animals were active to

9   varying degrees during the day and not in a steady state, therefore heart rate could not be

10   used to estimate metabolic rate.

11   The decline in maximum T_b that occurred in April may be indicative of an

12   endogenous seasonal rhythm of body temperature and metabolism. Although it was

13   possible for the tegus to achieve higher maximum T_b, that they did not is suggestive of an

14   endogenous seasonal rhythm, like that seen in the sleepy lizard, *T. rugosa* (Firth & Belan,

15   1998; Ellis *et al.,* 2008). From April into May there was a further significant nighttime

16   drop in f_H and f_R indicating a further suppression in metabolism, but this was associated

17   with a significant fall in T_b. This amounted to a 30% decrease in O_2 consumption (a Q_{10}

18   of 2.1) bringing the metabolic rate to levels that were 45% of those calculated in January.

19   As indicated by the low but consistent levels of T_b, f_H and f_R (day and night) from May

20   through July, metabolism was relatively uniform during dormancy. The values of

21   metabolic rate estimated for the tegus in dormancy (0.18 -0.21 ml O_2/min/kg) are similar

22   to those measured in previous studies on dormant tegus (0.15- 0.30 ml O_2/min/kg; Abe,

23   1995; Andrade and Abe, 1999; Milsom et al., 2008; Souza et al., 2004; Toledo et al.,

1 2008), indicating that our methods for estimating metabolic rate were consistent with

2 previous studies.

3   In August and September, all variables increased to levels significantly greater

4 than those recorded from tegus at similar body temperatures in May and April, suggesting

5 that they were due not only to the increases in T_b, but also due to removal of the active

6 metabolic suppression. The increasing incidence of arousals associated with slowly

7 increasing nighttime heart rate and breathing in August is also suggestive that the degree

8 of metabolic suppression was decreasing as the period of arousal progressed, which has

9 been documented in other lizard species as they approach arousal (Patterson & Davies,

10 1978).

11 **Arousal During Dormancy**

12   While species of reptiles that undergo dormancy are not likely to emerge from

13 their burrows in mid-winter when environmental conditions are extreme, they may still

14 arouse from dormancy and remain within the burrow. To date, however, there is no

15 documentation that this occurs. Species of reptiles that go dormant in subtropical regions

16 should be less constrained to remain in their burrows during periods of arousal and it has

17 been shown that *Varanus rosenbergi* spontaneously arouse frequently during dormancy

18 (Rismiller & McKelvey, 2000). In the present study, *T. merianae* also exhibited periodic

19 bouts of arousal accompanied by short bouts of emergence. Amongst the four individuals

20 in this study there was a wide range of variability in the occurrence of this behaviour,

21 both in the number of times an individual aroused over the period of dormancy and in the

22 phase of the dormant period (early versus late) during which these events occurred. There

23 was no synchrony to the occurrence of arousals in tegus inhabiting the same burrow

1   suggesting that they were not tightly correlated to local factors such as temperature

2   change, noise or disturbance. Arousals appeared to occur randomly, with no distinct

3   pattern in any animal, suggesting that they were not the consequence of an under-lying

4   biological rhythm. This does not preclude the existence of an internal clock controlling

5   arousal from dormancy or the onset of reproduction. Many species that are arrhythmic in

6   winter can be rhythmic at other seasons (see Revel et al., 2007; Ellis et al., 2008).

7   It is possible that these arousals were the consequence of the experimental design.

8   The artificial burrows were designed for ease of access and to allow infra-red recording

9   of activity within the burrow and were thus spacious and left the animals relatively

10   exposed. Natural burrows tend to be more constrictive and possibly deeper in the

11   substrate where daily fluctuations in temperature would be absent. Animals rarely, if

12   ever, leave them during the dormancy season. A more constant temperature and tactile

13   stimulation may promote dormancy and eliminate periods of arousal. At present the

14   underlying cause of the arousals seen in this study is not clear.

15   Periodic arousals are a hallmark of most mammalian hibernation (Willis, 1982)

16   and here too it is not clear what the underlying cause is (Barnes et al., 1993; Wang, 1993;

17   Carey *et al*., 2003). One hypothesis is that transcription and translation of genetic

18   material are inhibited by low temperatures and that animals must arouse periodically to

19   undertake essential maintenance activities (Van Breukelen and Martin, 2002; Carey *et al*.,

20   2003). The occurrence of periodic arousals is normally rhythmic in mammals (Twente

21   and Twente, 1967), but at present there is no consensus on what triggers these arousals.

22   The incidence and role of periodic arousals in both mammalian and ectothermic

23   hibernation are therefore areas that require further study.

Heart Rate Hysteresis and Implications for Body Temperature Regulation

1
2 Heart rate hysteresis has been well described in reptiles, and its role in

3 temperature regulation has received much attention. To maximize the period where body

4 temperature exceeds ambient temperature, many reptiles increase cutaneous blood flow in

5 the morning to maximize heat gain. A concomitant rise in heart rate due to the baroreflex

6 leads to an appropriate increase in cardiac output that maintains blood pressure constant

7 (Galli et al., 2004; Crossley et al., 2015). A decrease in cutaneous blood flow and heart

8 rate in the evening conserves heat by reducing the rate of heat loss (Morgareidge and

9 White, 1969; Langille and Crisp; 1980; Galli *et al.,* 2004; Clark and Frappell, 2006). This

10 gives rise to hysteresis in the relationship between f_H and T_b in which the rate of change

11 in f_H reflects the effects of temperature on f_H and metabolic rate (Q_{10} effects), the effects

12 of activity and feeding (Zaar et al., 2004), and the effects of thermoregulatory processes

13 associated with reaching/retaining preferred T_b (Seebacher, 2000; Seebacher and

14 Franklin, 2001, 2005).

15 From January to March, and again in August and September, on days when the

16 tegus emerged from their burrows to bask, f_H and f_R began to rise in the morning, at

17 constant T_b, even before the tegus left their burrows. The most extreme case was in

18 September when f_H more than doubled, reaching almost maximum daytime levels over a

19 two hour period before the tegus emerged from their burrows. This correlated with the

20 period of greatest reproductive mating activity, highest daytime T_b and heart rate, and

21 longest periods spent active. This suite of changes is not uncommon in reptiles during

22 mating season and has been attributed to "mating unrest," which can be accompanied by

23 an elevation in preferred T_b (Huey and Bennett, 1987; Rismiller and Heldmaier, 1982;

1   Rismiller and Heldmaier, 1991; Luiseli and Akani, 2002; Seebacher and Franklin,2005).

2   Once mating occurs, preferred T_b in pregnant females may increase (*Hoplodactylus*

3   *maculatus*, Werner and Whitaker, 1978; *Thamnophus sirtalis,* Stewart, 1965;

4   *Gerrhonotus coeruleus,* Stewart, 1984) or decrease (Lacerta vivipara, Paterson and

5   Davies, 1978; Scleroperuscyanogenys, Garrick, 1974; Scleroporus jarrovi, Beuchat,

6   1986)

7   This rapid initial increase in f_H was most likely due to changes in activity state

8   (sleep to alert) and activity in the burrow. Throughout the fall this pre-emergence

9   increase in f_H slowly decreased and by April, f_H and f_R no longer began to rise in the

10   morning before T_b, instead only rising when the tegus left their burrows to bask.

11   In all seasons, once tegus left the burrow and began to warm, f_H and f_R increased

12   further, with the rates of these changes varying across the seasons (Fig 3). Not

13   surprisingly, the higher the f_H at the time of emergence from the burrow, the lower the

14   rate of rise until the maximum daily f_H and T_b were reached. The rate of rise at this time

15   must reflect the effects of temperature on f_H and metabolic rate (Q_{10} effects), the effects

16   of activity, and the effects of thermoregulatory processes associated with reaching

17   preferred T_b.

18   In the evenings of the non-dormant periods, f_H and f_R began to fall in advance of

19   T_b with the greatest changes occurring in September. These rapid changes most likely

20   reflect increases in total peripheral vascular resistance associated with vasoconstriction of

21   peripheral beds for heat retention as described above (Seebacher, 2000; Clark *et al.*, 2004,

22   Galli et al., 2004; Seebacher and Franklin, 2005). This rapid fall in f_H was absent during

23   dormancy when tegu T_b fell rapidly to approximate T_{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 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

1   dormancy, there was a decrease in T_b associated with a decrease in T_{burrow} and

2   accompanied by a further reduction in heart rate, breathing rate and metabolic rate.

3   Dormancy was a fairly uniform state from which the tegus, under the conditions of this

4   study, did arouse periodically. The sum of the data suggest that tegu lizards can actively

5   suppress metabolism in a complex and temperature independent fashion for which the

6   underlying mechanism remains to be explored.

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Figure Captions:

Fig 1 Mean (±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

Fig 2 Mean (±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. * 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 #

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)

Fig 4 A. Mean (±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).

1   Note the fall in heart rate, breathing rate and rate of oxygen consumption at constant

2   burrow and tegu temperatures from January to April. * indicates values that are

3   significantly lower than January values. + indicates values that are significantly elevated

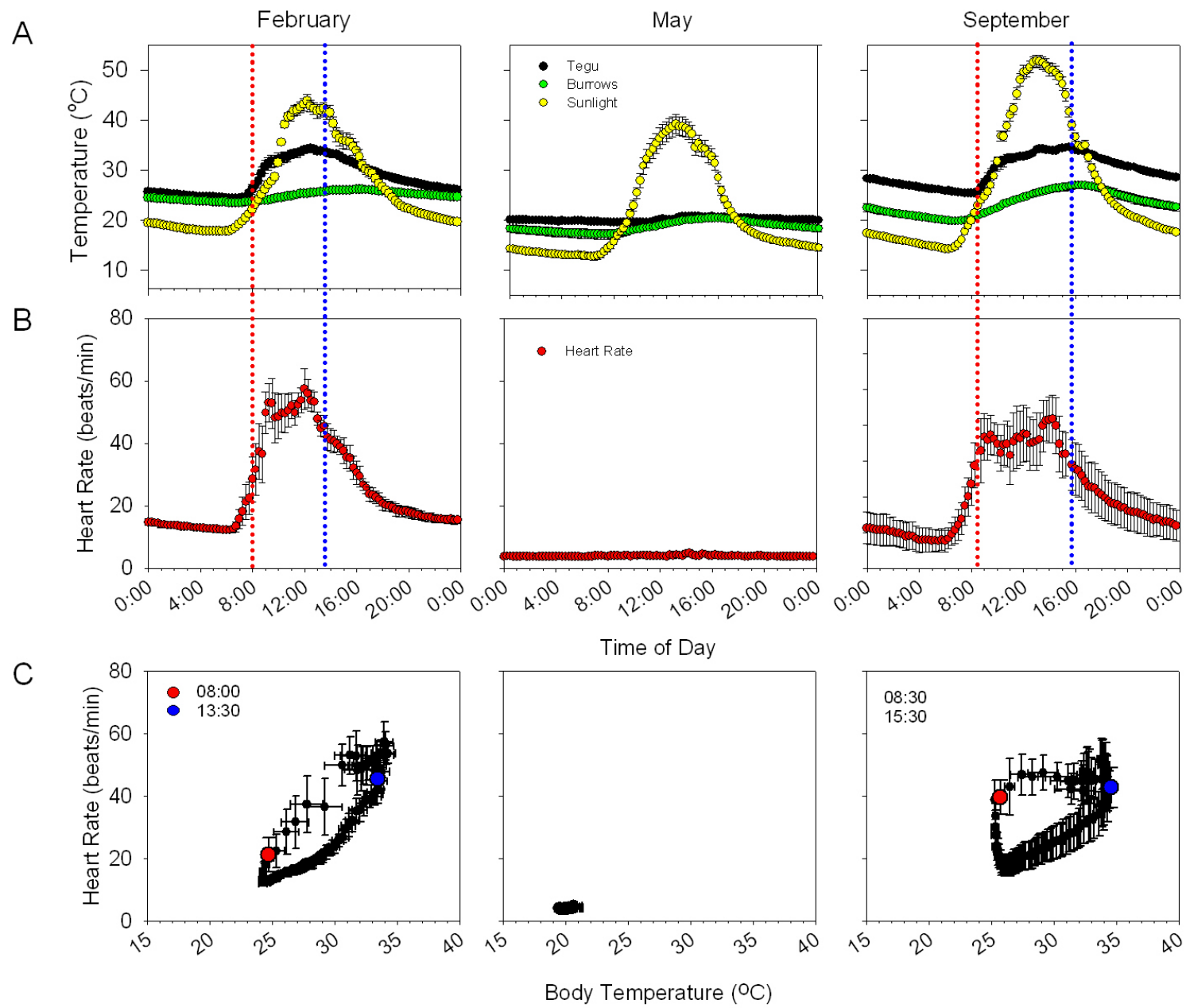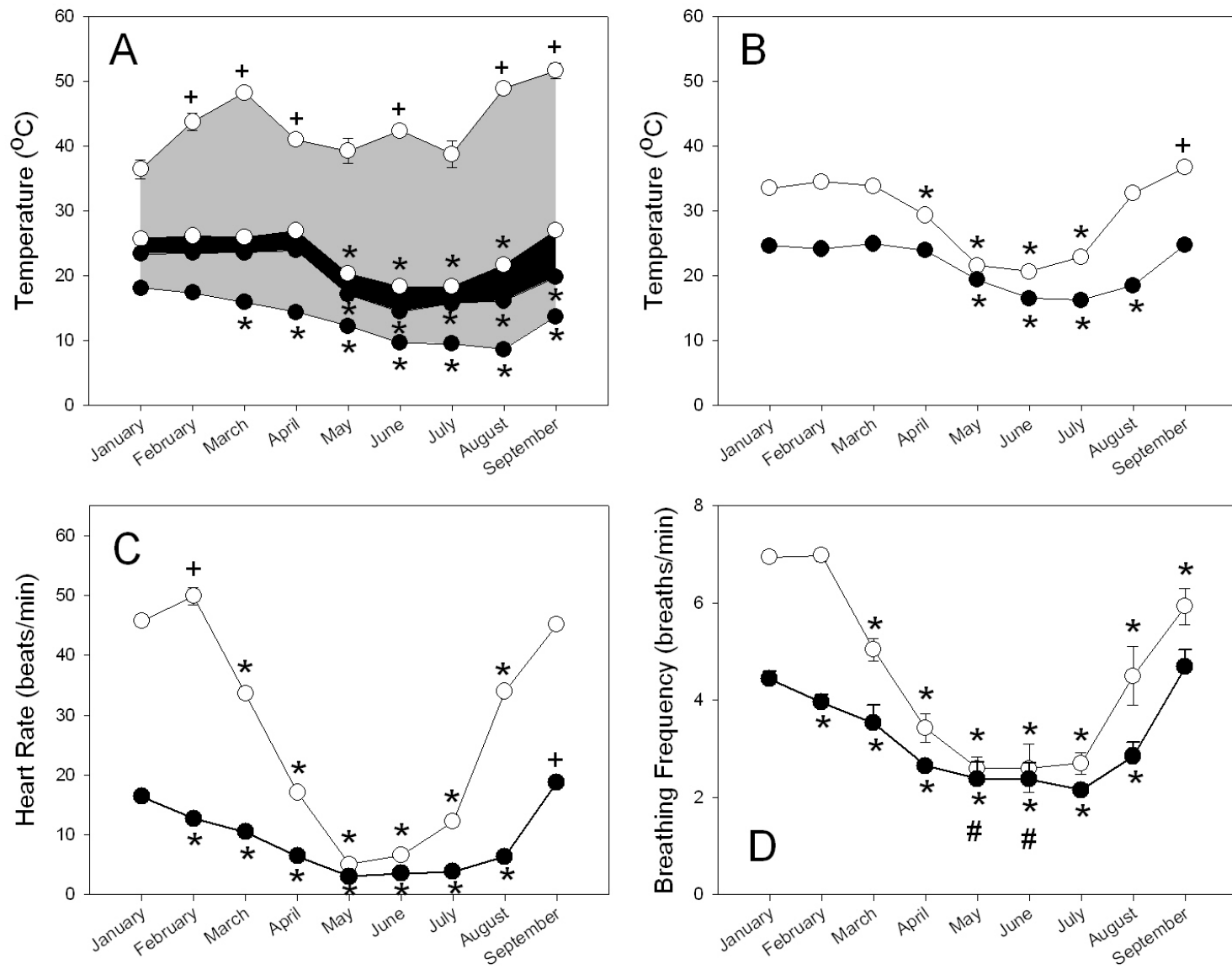4   compared to January values. Vertical dotted lines indicate the entrance into and the

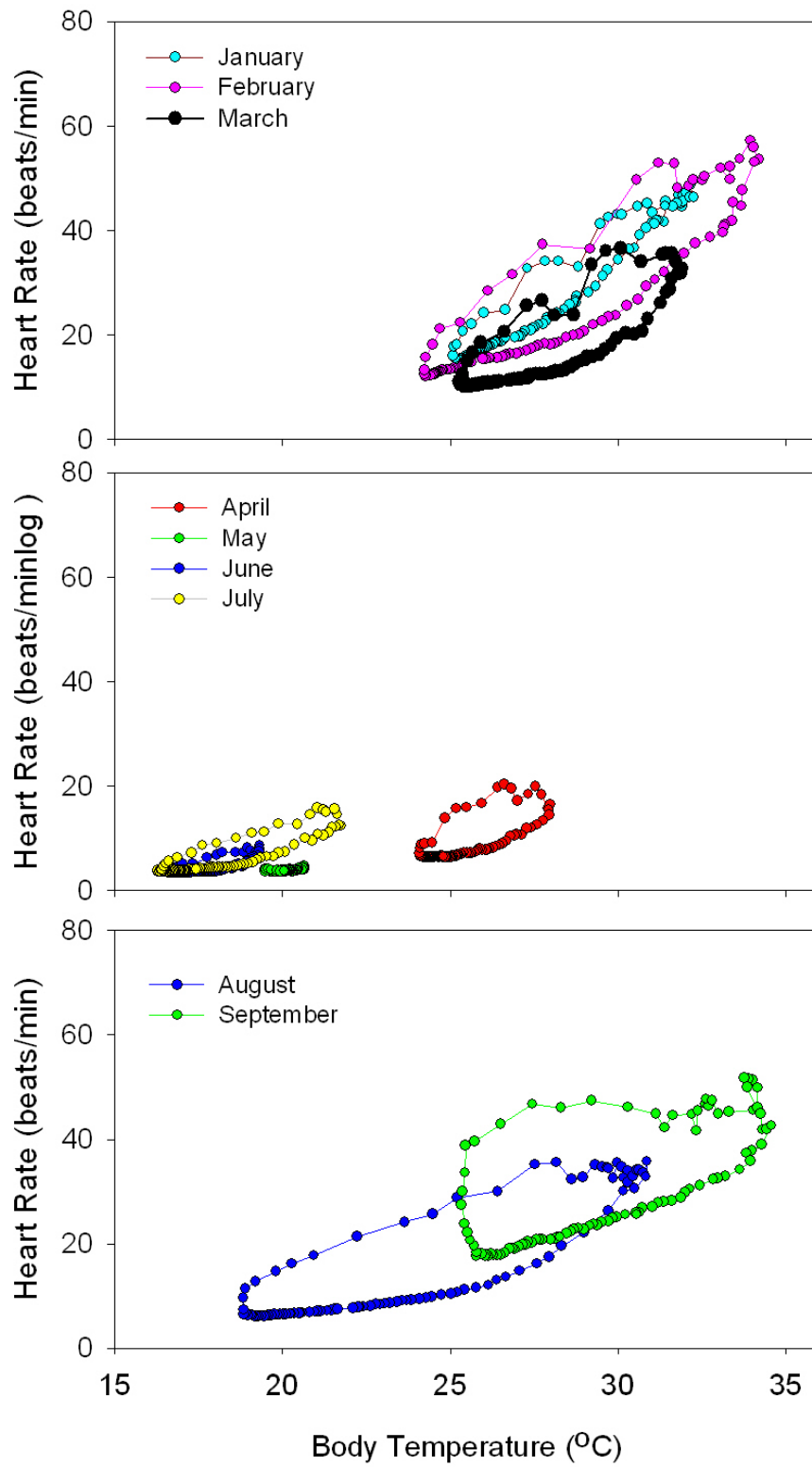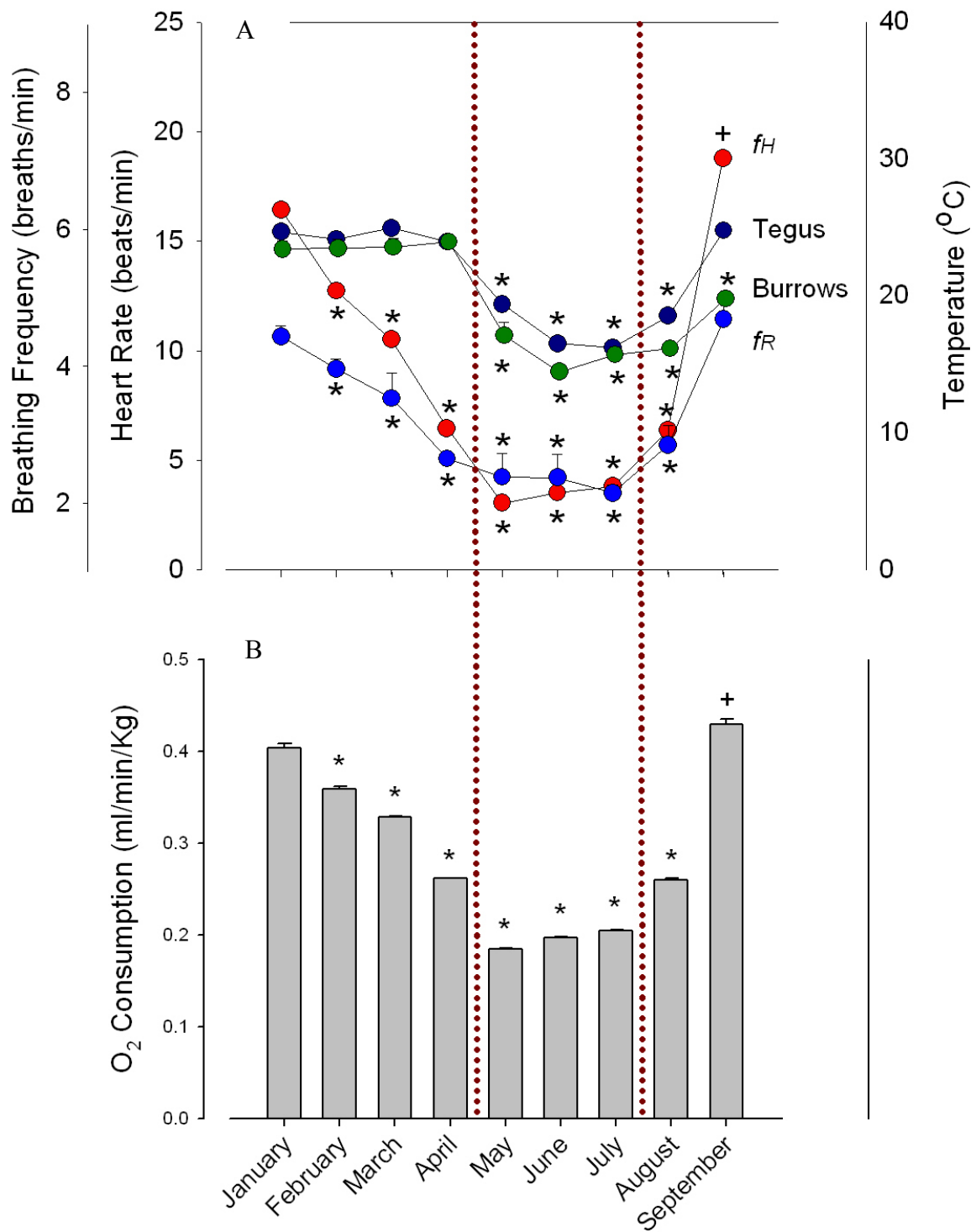5   emergence from dormancy.

6

7

Figure 1

Figure 2

Figure 3

Figure 4