GAS EXCHANGE AND VENTILATION DURING DORMANCY IN THE TEGU LIZARD *TUPINAMBIS MERIANAE*

DENIS VIEIRA DE ANDRADE* AND AUGUSTO SHINYA ABE

Departamento de Zoologia, Universidade Estadual Paulista, Caixa Postal 199, 13506-900 Rio Claro, SP, Brazil

*e-mail: denis@ms.rc.unesp.br

Summary

The tegu lizard *Tupinambis merianae* exhibits an episodic ventilatory pattern when dormant at 17 °C but a uniform ventilatory pattern when dormant at 25 °C. At 17 °C, ventilatory episodes were composed of 1–22 breaths interspaced by non-ventilatory periods lasting 1.8–26 min. Dormancy at the higher body temperature was accompanied by higher rates of O_2 consumption and ventilation. The increase in ventilation was due only to increases in breathing frequency with no change observed in tidal volume. The air convection requirement for O_2 did not differ at the two body temperatures. The respiratory quotient was 0.8 at 17 °C and 1.0 at 25 °C. We found no consistent relationship between expired gas composition and the start/end of the ventilatory period during episodic breathing at 17 °C. However, following non-ventilatory periods of increasing duration, there was an increase in the pulmonary O_2 extraction that was not coupled to an equivalent increase in elimination of CO_2 from the lungs. None of the changes in the variables studied could alone explain the initiation/termination of episodic ventilation in the tegus, suggesting that breathing episodes are shaped by a complex interaction between many variables. The estimated oxidative cost of breathing in dormant tegus at 17 °C was equivalent to 52.3 % of the total metabolic rate, indicating that breathing is the most costly activity during dormancy.

Key words: gas exchange, ventilation, breathing pattern, metabolic rate, cost of breathing, dormancy, tegu lizard, *Tupinambis merianae*.

Introduction

Unlike the continuous breathing pattern generally observed in fishes, birds and mammals, respiration in reptiles can be characterized by the presence of apnoeic periods of variable duration interspaced between breaths (Glass and Wood, 1983; Wang et al., 1998). This intermittent breathing pattern can be split into two basic types: one with evenly spaced respiratory movements, and a second with respiratory movements occurring in episodes (ventilatory periods, VPs) separated by long-duration non-ventilatory periods (NVPs). Both patterns have been extensively reported for different species of reptile but seem to be rather constant for a given species (Wood and Lenfant, 1976; Glass and Wood, 1983; Shelton et al., 1986; Milsom, 1988). This notwithstanding, the breathing pattern in reptiles may be very labile, being influenced by such factors as activity and temperature (Cragg, 1978; Clark et al., 1978; Ackerman and White, 1979; Bickler, 1981; Milsom, 1991; Wang et al., 1998). As in other ectotherms, an increase in body temperature is usually followed by ventilatory adjustments required to attend to increased metabolic demands (Glass and Wood, 1983).

Ventilatory and metabolic responses to altered temperature have been well documented for reptiles, but only rarely have studies focused on seasonal effects (Abe, 1993). Such an approach, however, could render valuable data since a seasonal pattern of activity is a widespread feature among reptiles (Gregory, 1982). Because of their dependence upon external heat sources, reptiles may not be able to be active during cold periods (Bartholomew, 1982) and, therefore, many species engage in a seasonal dormancy that can extend for many months (Gregory, 1982). In these long periods of inactivity, spent in a hypometabolic state (Bennett and Dawson, 1976; Pough, 1983), one would expect, as reported for mammals (Milsom, 1992), the occurrence of adjustments in the gas transport cascade, especially in pulmonary ventilation.

The tegu lizard (*Tupinambis merianae*) is a large active predator widespread throughout South America (Avila-Pires, 1995). During the dry and cold winter in south-eastern Brazil, they stop eating, hide in burrows in the ground and hibernate for 4–5 consecutive months at temperatures around 17 °C. During this period, even an occasional increase in environmental temperature does not give rise to activity, indicating the occurrence of true metabolic depression, rather than a simple effect of cold on metabolic rate. The rate of oxygen consumption ($\dot{V}_{O_2}$) of tegus at this time is reduced to approximately 32 % of the value reported for resting lizards during the active season at the same temperature (Abe, 1995).

Furthermore, $\dot{V}_{O_2}$ in dormant tegus is relatively insensitive to temperature between 17 and 25 °C (Abe, 1995). In contrast, during the active season, ventilation, which occurs in evenly spaced breaths, is strongly influenced by body temperature (Abe, 1993). Moreover, at low temperatures during the active season, some individuals assume an episodic breathing pattern (A. S. Abe, unpublished data), reflecting the flexible nature of the tegus' ventilatory pattern. In this context, it seems plausible that the hypometabolic state experienced by the tegus during hibernation may lead to ventilatory adjustments. Accordingly, in the present study, we addressed how ventilation is adjusted to match the low metabolic demand observed in dormant tegus and how this ventilatory adjustment is influenced by temperature. Further, we investigated the possible factors that could be related to the control and generation of the ventilatory pattern of the tegus; in particular, critical values of lung P_{O_2} and P_{CO_2}, which may determine the beginning/end of a VP/NVP cycle. We also examined how ventilation and gas exchange were adjusted to compensate for the oxygen debt that arose during NVP and, since, dormancy implies metabolic depression to save energy reserves, we estimated how much energy was allocated for respiration.

Materials and methods
Animals

We used captive-bred juvenile *Tupinambis merianae* (Duméril & Bribon), 2–3 years old, with body mass varying from 525 to 2140 g (1351±734 g; mean ± S.D., N=6) kept in outdoor pens in Rio Claro, São Paulo State, southeastern Brazil. At the beginning of the inactivity season, characterized by spontaneous aphagia after a progressive reduction of food intake and activity, the lizards were placed individually in plastic boxes filled with moist wood shavings and kept in a climatic chamber (BOD-37 CD, Fanem) at 17±1 °C. The animals were maintained in these conditions for five consecutive months, and measurements were made during the second and third month.

Ventilation and gas exchange

Ventilation was studied using the mask technique described by Glass et al. (1978) and modified by Wang and Warburton (1995). For each lizard, we constructed an individual plastic mask attached to a resistor (Fleish tube) connected by Tygon tubes to a pressure transducer (Sable; PT-100) for ventilatory recordings, and to CO_2 and O_2 analyzers (Licor LI-6251 and Applied Electrochemistry S-3 A/L, respectively) for analysis of the expired gas. The gas analyzers were connected in line, and the airflow through them was produced by a suction pump (Sable; gas analyzer sub-sampler 1.0) placed downstream from the gas analyzers. The flow rate was kept constant at 200 ml min^{-1}, which was sufficiently fast to allow a breath-by-breath analysis. The signals from the CO_2 and O_2 analyzers and pressure transducer were sent to an analog-to-digital convertor and recorded through a computerized data-acquisition system (DAC, Sable System).

Experimental protocol

The masks were glued to the snouts of the lizards at least 48 h before experimentation, an interval sufficient for the lizards to return to a quiescent state after handling. The first measurements were made at the tegus' maintenance temperature, i.e. 17 °C, from midnight to 04:00 h. On the following morning (08:00–12:00 h) the chamber temperature was gradually increased to 25 °C, and then, in the following 00:00–04:00 h interval, the measurements were repeated at this new higher temperature.

All masks were calibrated individually with simulated breaths of known volume and gas composition. In all cases, the relationship between the electrical signal generated and volume and gas composition could be accurately described by a linear regression (r^2>0.9; fiducial standard error <0.005 in all cases).

Data handling and statistical analyses

For each lizard, we analyzed the data collected in four consecutive hours of recording at each temperature to determine the following physiological variables: respiratory frequency (f_R), the duration of the ventilatory period (t_{VP}), number of breaths in a ventilatory period, VP (N_b), breathing frequency during the VP (f_{VP}), duration of the non-ventilatory period (t_{NVP}), tidal volume (V_T), expired gas fractional O_2 and CO_2 concentration (F_{EO_2} and F_{ECO_2}, respectively), oxygen uptake (V_{bO_2}) and CO_2 output (V_{bCO_2}) of each breath, oxygen extraction coefficient (E_{O_2}), duration of the total respiratory movement (t_{TOT}), of inspiration (t_{INSP}) and of expiration (t_{EXP}). From these measurements were calculated the rate of oxygen consumption ($\dot{V}_{O_2}$), the rate of CO_2 production ($\dot{V}_{CO_2}$), respiratory quotient (RQ), total ventilation rate ($\dot{V}_e$) and the air convection requirement for O_2 ($\dot{V}_e/\dot{V}_{O_2}$) and CO_2 ($\dot{V}_e/\dot{V}_{CO_2}$). Except where otherwise specified, gas volumes are reported for STPD conditions and calculations follow Dejours (1975) and Glass and Wood (1983).

All data are presented as means ± S.D. unless stated otherwise. Comparisons among physiological variables between experimental temperatures were made using a paired t-test, with differences considered significant at P=0.05. The analysis of the respiratory variables within the ventilatory episodes was based on least-square regressions (Jandel Scientific). In this analysis, the correlation between the changes seen in any given variable and the position of that breath within the episode was determined by a Pearson product moment correlation test. In such correlations, $P \leq 0.001$ was considered significant. Variables that were found to change significantly within a ventilatory episode were submitted to a piecewise linear regression. This procedure allowed us to identify the breathing movements responsible for most of the variation (>50 %) found within the ventilatory episodes.

The possible relationships existing between physiological variables in a given ventilatory episode and the duration of the preceding/following NVP were tested using the Pearson product moment correlation, adopting a significance level of $P \leq 0.001$. Descriptive equations relating the variation of a

Table 1. *Ventilatory and gas exchange variables in* Tupinambis merianae *during dormancy at 17 and 25 °C*

Variable	17 °C	25 °C
f_R (breaths min^{-1})	0.52±0.19	1.43±0.42***
t_{TOT} (s)	11.38±1.63	8.91±0.54*
t_{INSP} (s)	9.51±2.6	7.44±2.08
t_{EXP} (s)	4.32±0.82	3.27±0.24*
V_T (ml kg^{-1})	7.89±1.62	7.11±1.93
F_{EO_2} (%)	16.83±1.14	16.82±0.64
E_{O_2} (%)	19.63±5.43	19.68±3.06
F_{ECO_2} (%)	3.61±1.33	4.25±1.06
V_{bO_2} (ml O_2 kg^{-1})	0.288±0.12	0.244±0.08
$\dot{V}_{O_2}$ (ml O_2 kg^{-1} h^{-1})	8.15±2.2	20.27±7.01**
V_{bCO_2} (ml CO_2 kg^{-1})	0.251±0.13	0.261±0.13
$\dot{V}_{CO_2}$ (ml CO_2 kg^{-1} h^{-1})	6.95±2.33	21.76±4.42**
$\dot{V}_e$ (ml kg^{-1} min^{-1})	4±1.04	9.9±2.69***
$\dot{V}_e/\dot{V}_{O_2}$	30.35±8.74	30.38±5.83
$\dot{V}_e/\dot{V}_{CO_2}$	37.16±13.71	30.51±8.35

For abbreviations, see List of symbols.

* indicates a significant difference between temperatures ($P \leqslant 0.05$); **$P \leqslant 0.01$; ***$P \leqslant 0.001$.
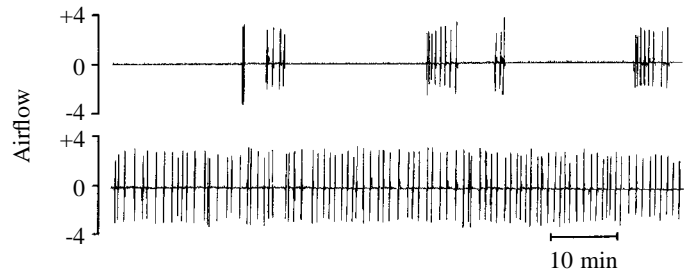
Values are means ± S.D., $N=6$.

Fig. 1. Ventilatory recordings from *Tupinambis merianae* showing the change in ventilatory pattern from episodic at 17 °C (upper trace) to evenly spaced at 25 °C (lower trace). Arbitrary units.

given respiratory variable to NVP duration were determined by least-square regressions.

Results

Ventilatory pattern and the effects of temperature

The ventilatory and gas exchange variables for hibernating tegus at 17 and 25 °C are presented in Table 1. All tegus showed an episodic ventilatory pattern at 17 °C, which changed to an evenly spaced pattern at 25 °C (Fig. 1). Thus, at 25 °C, f_R was significantly higher than at 17 °C ($P=0.001$), mainly due to a reduction in t_{NVP} which represented 39–78 % of the respiratory period at 17 °C (56.2±0.14 %). There was no significant difference ($P=0.3$) in the time between breaths (i.e. f_R) at 25 °C and the time between breaths in a VP (f_{VP}) at 17 °C. However, the mean time between two consecutive breaths was significantly different ($P=0.004$) between the two experimental temperatures, being approximately three times longer at 17 °C (1.93±0.78 min) than at 25 °C (0.6±0.22 min). At 25 °C, the length of the total breath (t_{TOT}) was shorter than at 17 °C ($P=0.02$), mainly due to faster expiration (t_{EXP}; $P=0.03$). t_{INSP} did not differ significantly between temperatures, but t_{INSP} was longer than t_{EXP} ($P=0.0001$) at both experimental temperatures.

Ventilatory episodes at 17 °C consisted of 1–22 breaths (5.41±4.8 breaths) interspaced by NVPs varying from 1.8 to 26.2 min (5.82±0.95 min). Ventilatory periods were considered as distinct ventilatory episodes where they were separated by NVPs longer than 2.5 times the mean pause between two breaths within a typical ventilatory episode. Every ventilatory episode started with an expiration and ended with an inspiration.

Changes in temperature did not resulted in changes in V_T ($P=0.26$), F_{ECO_2} ($P=0.31$), F_{EO_2} ($P=0.97$) or E_{O_2} ($P=0.97$). In the same way, V_{bO_2} and V_{bCO_2} did not change significantly with temperature ($P=0.36$ and $P=0.89$, respectively); V_{bO_2} showed a tendency to be lower at 25 °C than at 17 °C. Nevertheless, because of the increased f_R at 25 °C, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were significantly higher at 25 °C than at 17 °C ($P=0.002$ and $P=0.01$, respectively). Calculated values of Q_{10} were 3±0.5 for $\dot{V}_{O_2}$ and 4±1.5 for $\dot{V}_{CO_2}$. Accordingly, the calculated RQ values tended to be higher at 25 °C (RQ=1.0) than at 17 °C (RQ=0.8), although this difference was not significant ($P=0.11$). $\dot{V}_e$ was significantly higher at 25 °C than at 17 °C ($P=0.001$), once again as a result of the higher f_R at 25 °C, since V_T did not change with temperature. The concurrent increase in $\dot{V}_e$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ with an increase in temperature resulted in no difference in the air convection requirement for O_2 or CO_2 between experimental temperatures.

Effects of the duration of the non-ventilatory period

These data were derived from measurements taken at 17 °C since the ventilatory pattern was not episodic at 25 °C. For six tegus, we analyzed 155 NVPs and correlated their duration with all the ventilatory and gas-exchange variables studied (see above). The equations describing the variation of these variables with NVP duration are presented in Table 2.

The number of breaths in each ventilatory episode (N_b), as well as the duration of the episode (t_{VP}) increased with the length of the previous NVP ($P<0.00001$, in both cases). However, the same relationships did not occur between the following NVP and the number of breaths ($P=0.03$) or the duration ($P=0.002$) of a given ventilatory episode. V_T of the first breath in an episode ($P=0.0006$) and the total air volume ventilated during an episode ($N_b V_T$; $P<0.00001$) showed a positive correlation with the duration of the NVP preceding each breathing bout. The mean V_T of all breaths in an episode was not correlated with the duration of the previous NVP ($P=0.07$), and neither this nor the V_T of the first breath was correlated with the length of the NVP subsequent to the ventilatory episode ($P>0.002$).

The F_{EO_2} and F_{ECO_2} of the first breath in each ventilatory episode changed significantly with the duration of the previous NVP ($P<0.00001$ and $P=0.001$, respectively). However, F_{EO_2} and F_{ECO_2} showed different patterns with increasing NVP (Fig.

Table 2. *Statistics, constants and descriptive equations for ventilatory and gas-exchange variables as a function of non-ventilatory period (NVP) duration in dormant* Tupinambis merianae *at 17 °C*

	a	b	c	r^2	F	P
$N_b = a + bx^c$	−3.82	5.98	0.27	0.13	11.35	<0.00001
$t_{VP} = a + bx^c$	−5.82	6.42	0.28	0.14	11.95	<0.00001
First breath in a ventilatory episode						
$V_T = a + bx$	7.43	0.15	–	0.0734	12.13	<0.0006
$F_{EO_2} = a + b^{0.5}\log_e x$	17.26	−0.54	–	0.55	184.6	0.00001
$F_{ECO_2} = a + [b/(x)^{1.5}]$	5.07	−4.98	–	0.102	17.36	0.001
$E_{O_2} = a + bx^c$	3.77	11.46	0.44	0.466	66.38	0.0001
$V_{bO_2} = a + bx^c$	0.26	0.034	0.99	0.56	114.9	0.0002
$V_{bCO_2} = a + bx^c$	0.1	0.109	0.47	0.217	21.09	0.0001
Mean values averaged in a ventilatory episode						
$V_{bO_2} = a + x^b$	0.27	0.15	–	0.085	16.82	0.0002
Total values for the entire ventilatory episode						
$V_{bO_2} = a + bx^c$	0.054	0.46	0.63	0.377	54.17	0.0002
$V_{bO_2} = \log[(y-a)-b]^{-c}$	13.35	−10.48	4.87	0.094	9.25	0.0001
$V_{bCO_2} = a + bx^c$	−0.52	0.904	0.42	0.271	28.32	0.0001
$V_{bCO_2} = a + by$	3.65	1.61	–	0.122	20.95	0.0001

x and y are the duration of the NVP preceding or following a ventilatory episode, respectively.
For abbreviations and units, see List of symbols.

2). While F_{EO_2} decreased in a nearly continuous fashion as t_{NVP} increased, F_{ECO_2}, after an initial increase, reached a plateau at approximately 5 %. F_{ECO_2} began to stabilize at 4.35 %, i.e. it changed relatively little after t_{NVP} exceeded 3.7 min. In fact, variation in F_{ECO_2} after this point was responsible for less than 45 % of the total data variation (piecewise regression). Mean F_{EO_2} and F_{ECO_2}, averaged for a given ventilatory episode, showed no correlation with the preceding or following NVP ($P<0.05$, in all cases). Similarly, we found no correlation between F_{EO_2} and F_{ECO_2} for the first breath of an episode and the following NVP ($P>0.002$, for both cases).

The V_{bO_2} of the first breath, the mean V_{bO_2} and the sum of all V_{bO_2} values of each ventilatory episode were all greater after long NVPs ($P<0.0002$ in all cases). Accordingly, the E_{O_2} of the first breath in a ventilatory episode increased with the length of the preceding NVP ($P<0.0001$). However, mean E_{O_2} did not increase significantly with the duration of the previous NVP ($P=0.002$). V_{bCO_2} of the first breath and the total V_{bCO_2} of each ventilatory episode increased concurrently with the

duration of the preceding NVP ($P<0.0001$ for both). The same was not observed for the mean V_{bCO_2} in each episode ($P=0.052$). The greater the total V_{bO_2} and V_{bCO_2} of a given
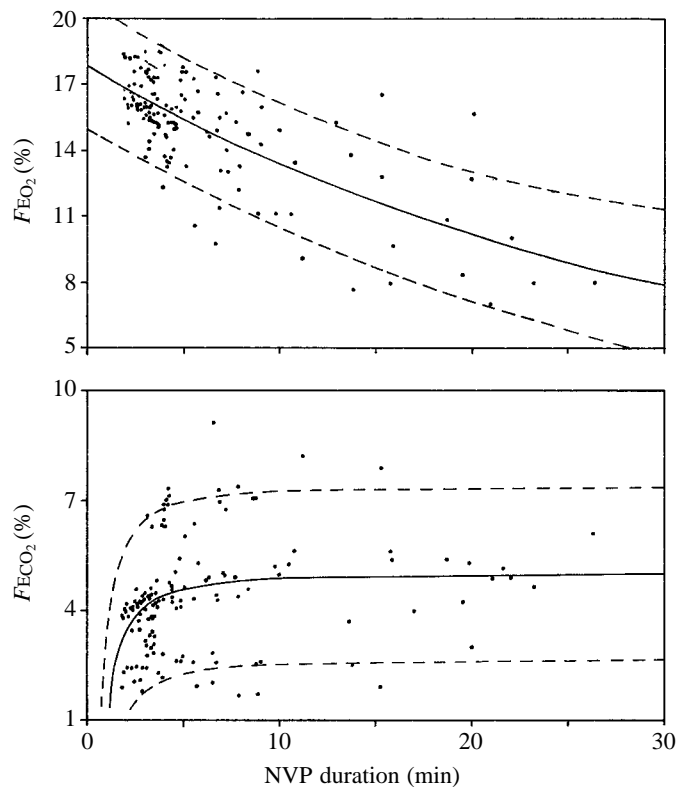

Fig. 2. The changes in expired gas composition (fractional concentrations of O₂ and CO₂) observed in the first breath of each ventilatory period as a function of non-ventilatory period (NVP) duration in dormant *Tupinambis merianae* at 17 °C. Note that, while the fractional concentration of O₂ (F_{EO_2}) decreases almost continuously with increasing NVP duration, the fractional concentration of CO₂ (F_{ECO_2}), after an initial increase, tends to level off. Solid and dashed lines denote the regression line and the 90 % confidence intervals, respectively. $N=6$ in both cases.

Table 3. *Statistics, constants and equations describing ventilatory and gas-exchange variations within ventilatory periods for dormant* Tupinambis merianae *at 17 °C*

	a	b	c	r^2	F
$t_{TOT}=a+bx^c$	8.37	3.69	−0.23	0.087	40.3
$V_T=a+b^{(-x/c)}$	6.2	2.32	6.73	0.055	24.5
$F_{ECO_2}=a+bx^c$	2.27	2.05	0.63	0.174	88.9
$F_{EO_2}=a+(b/x)$	18.32	−3.37	–	0.314	386.1
$V_{bCO_2}=a+bx^c$	0.023	0.29	0.31	0.166	83.8
$V_{bO_2}=a+bx^c$	0.079	0.34	−0.58	0.285	167.6

x denotes the location of an individual breath within a breathing episode.

For abbreviations and units, see List of symbols.

$P<0.0001$ in all cases.

ventilatory episode, the longer was the length of the NVP after the episode ($P<0.0001$ for both). No correlation was found between the duration of the following NVP and E_{O_2} ($P=0.003$), V_{bO_2} ($P=0.002$) and V_{bCO_2} ($P=0.001$) of the first breath of a ventilatory episode. In the same way, mean E_{O_2} ($P=0.87$), V_{bO_2} ($P=0.074$) and V_{bCO_2} ($P=0.05$) of a given ventilatory episode were not related to the duration of the NVP following it.

Regulation of ventilatory episodes

Ventilatory episodes lasted 0.14–24 min each (4.22±5.3 min) and were composed of 1–22 breaths. To analyze the changes in gas exchange and ventilatory variables within the ventilatory episodes, we grouped all the individual breaths by their position in the episode, aligning them by the first ventilatory movement of the breathing episode. Similar to the analysis of NVP, we identified 155 ventilatory episodes for the six tegus studied.

Most of the variables were significantly correlated with their location in the episode (see Table 3 for the equations used to describe such relationships) ($P<0.0001$, in all cases). Variations in V_T, F_{EO_2}, F_{ECO_2}, E_{O_2}, V_{bO_2} and V_{bCO_2} as a function of their position within a ventilatory episode are shown in Fig. 3. V_T and t_{TOT} decreased slowly over the course of a breathing bout. F_{ECO_2} was significantly greater in the first and second breath of each ventilatory episode, levelling off after reaching 3.3 %. The variation before this point was responsible for 60 % of the total variance. F_{EO_2} was significantly lower in the two first breaths of each ventilatory episode, stabilizing after reaching a value of 17.03 %. The variation before this point was responsible for 56.7 % of the total variance. E_{O_2} was significantly greater in the two first breaths of an episode, stabilizing after decreasing to 18.1 %; variation until this point accounted for 59 % of total variance. V_{bO_2} was greater in the first two breaths of each episode, while V_{bCO_2} remained elevated for the three initial breaths of the episode.

The values after which V_{bO_2} and V_{bCO_2} levelled off were 0.259 ml O_2 kg^{-1} and 0.219 ml CO_2 kg^{-1}, respectively. The variation before those points, in both cases, accounted for 60 % of the total data variance.

Discussion

Breathing pattern and temperature effects

The metabolic rate of dormant tegus has been reported to be weakly influenced by temperature in the range 17–25 °C, a feature reflected by a low Q_{10} value (1.5) compared with that of active lizards ($Q_{10}=3.2$) (Abe, 1995). In the present study, $\dot{V}_{O_2}$ was lower at 17 °C and higher at 25 °C than the values reported by Abe (1995). This resulted in a greater Q_{10} value (3.0), a value similar to that reported for active lizards. The discrepancy between these two studies is probably related to methodological differences. In our study, $\dot{V}_{O_2}$ was calculated from the expired gas concentration of all breaths included in a given interval, whereas Abe (1995) used only the lowest O_2 uptake values provided by an intermittently closed respirometry system for $\dot{V}_{O_2}$ calculation. Nevertheless, both studies concur that $\dot{V}_{O_2}$ of the dormant tegus was significantly lower than the values reported for awake animals at the same temperature. For example, at 25 °C, the $\dot{V}_{O_2}$ values of dormant tegus were 32 % (Abe, 1995) and 44 % (present study) of the values reported during the active season.

The increased metabolic rate following a temperature increase was paralleled by a rise in $\dot{V}_e$, mainly due to an increase in f_R, since V_T did not change substantially. The simultaneous increase in $\dot{V}_e$ with metabolic demand meant that $\dot{V}_e/\dot{V}_{O_2}$ and $\dot{V}_e/\dot{V}_{CO_2}$ remained unchanged between 17 and 25 °C. As a consequence, the mean values of F_{EO_2} and F_{ECO_2} did not change significantly following the temperature increase. Thus, despite transitory variations in the expired gas composition, particularly for animals breathing episodically at 17 °C, it seems that the tegus managed to defend a constant average lung gas composition at both temperatures. This pattern is different from that of most reptiles in which $\dot{V}_e/\dot{V}_{O_2}$ decreases with increasing temperature (Glass et al., 1985; Abe, 1995).

The increase in f_R following the increase in body temperature from 17 °C to 25 °C changed the tegus' ventilatory pattern from episodic to uniform. Since we found no significant differences between f_{VP} at 17 °C and f_R at 25 °C, the shift in respiratory pattern induced by the rise in temperature in *T. merianae* can be attributed exclusively to the shortening of the NVP. This observation agrees with the ventilatory adjustments, triggered by increased metabolic demand, usually found in animals with intermittent breathing (Milsom, 1991; Wang et al., 1998). V_T, the other variable that could be adjusted to increase $\dot{V}_e$, did not vary with temperature, as has been observed for active lizards (Abe, 1993).

Regulation of the non-ventilatory period

Reptiles that breathe episodically typically end each breathing bout with an inspiration (Glass and Wood, 1983; Milsom, 1991); hence, the expired gas concentration of the first breath in a given ventilatory episode reflects the magnitude of gas exchange during the preceding NVP. In the tegus, E_{O_2} increased with an increase in NVP duration, a pattern reflected in the F_{EO_2} and V_{bO_2} values. In contrast, F_{ECO_2} and V_{bCO_2}, after an initial increase, rapidly became independent of NVP
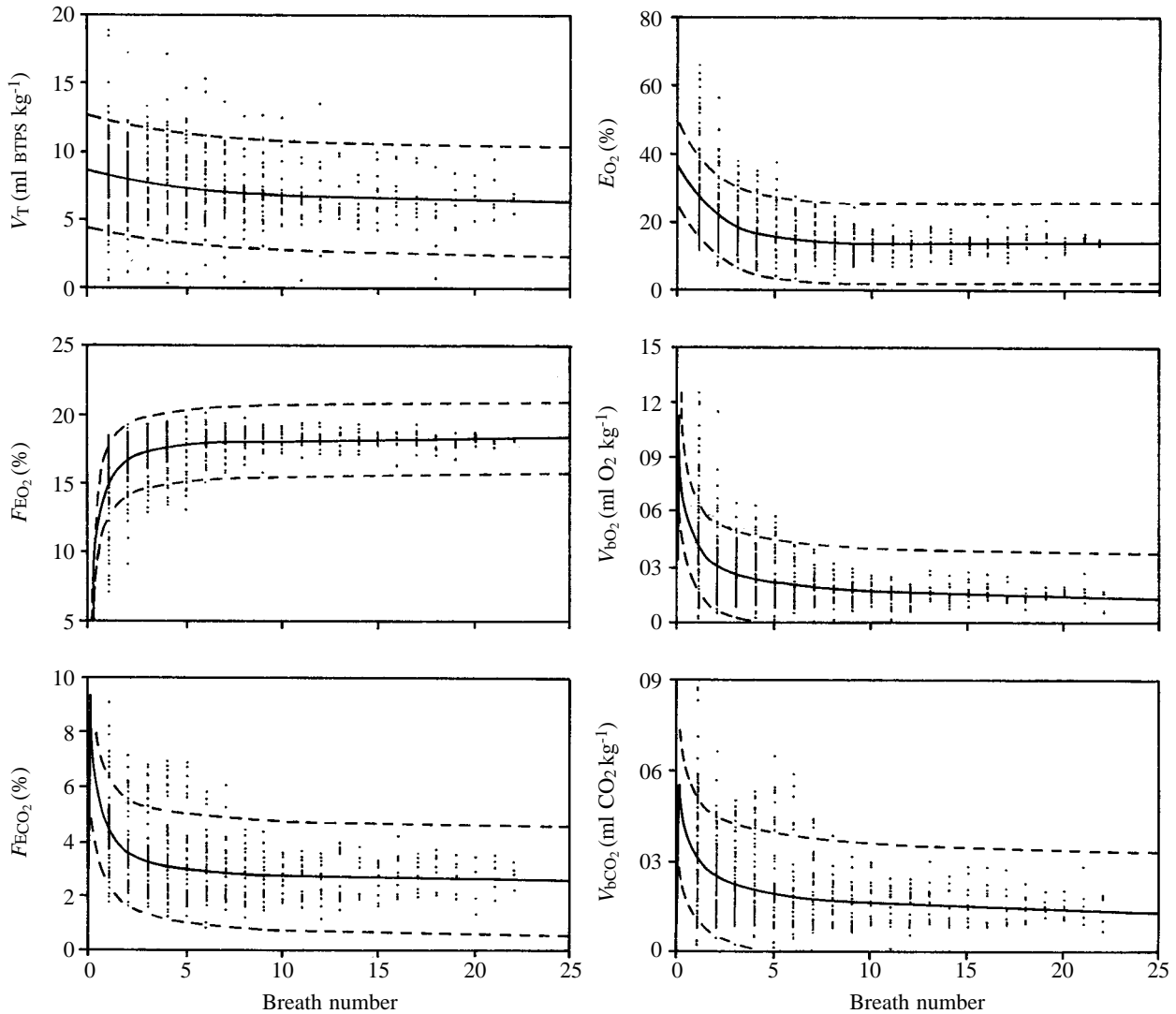
Fig. 3. The changes in ventilatory and gas-exchange variables within a ventilatory period in dormant *Tupinambis merianae* at 17 °C as a function of the breath number following the first breath in a breathing episode. Solid and dashed lines denote the regression line and the 90 % confidence intervals, respectively. $N=6$ in all cases. (A) Tidal volume, V_T; (B) fractional O_2 concentration, F_{EO_2}; (C) fractional CO_2 concentration, F_{ECO_2}; (D) oxygen extraction coefficient, E_{O_2}; (E) oxygen uptake of individual breath, V_{bO_2}; (F) carbon dioxide released by individual breath, V_{bCO_2}.

duration. This suggests that some of the CO_2 produced during an NVP may remain stored in the tissues and blood, which have a high CO_2 capacitance (Dejours, 1975). Thus, the main stimulus eliciting the end of an NVP and the beginning of a VP may be related to changes in lung volume (transitory changes in RQ), rather than to the attainment of an F_{EO_2} and/or F_{ECO_2} threshold value. This finding agrees with other studies in reptiles (Ackerman and White, 1979; Burggren and Shelton, 1979; Milsom and Chan, 1986; Milsom, 1988) and in hibernating mammals (Garland and Milsom, 1994).

The lack of data on the potential role of O_2 and CO_2 in the regulation of NVP length of the dormant tegus should be considered with caution, since it is well known that changes in P_{O_2} and P_{CO_2} in inspired air as well as in the arterial blood may strongly modify ventilation and gas exchange in reptiles

(Burggren and Shelton, 1979; Abe and Johansen, 1987; Wood et al., 1987; Wang et al., 1998). In *T. merianae*, although threshold values for F_{EO_2} and F_{ECO_2} could not be related to NVP duration, the total V_{bO_2} and V_{bCO_2} of a breathing bout significantly affected the duration of the following NVP. This observation indicates that the amounts of O_2 taken in and of CO_2 eliminated during a ventilatory episode are monitored and are involved in the control of NVP duration. The location of such monitoring and the reason why it is not reflected in clear P_{O_2} and P_{CO_2} threshold values remains unclear.

Ventilatory adjustments in response to NVP duration were particularly conspicuous in the first breath of each ventilatory episode but, in most cases, they could also be distinguished in the second breath of the episode. The lung volume as well as its gas composition appeared to be restored rapidly at the

Fig. 4. Schematic representation of an ideal respiratory cycle used to calculate the cost of breathing of a dormant *Tupinambis merianae* at 17 °C. The area above the line labelled *a* represents the O_2 debt built up during the preceding non-ventilatory period (NVP). The area bellow this line indicates the metabolic demand during the ventilatory period (VP) which, after subtracting the metabolic maintenance cost (black area), gives the oxidative cost of breathing (hatched area). See text for further details.
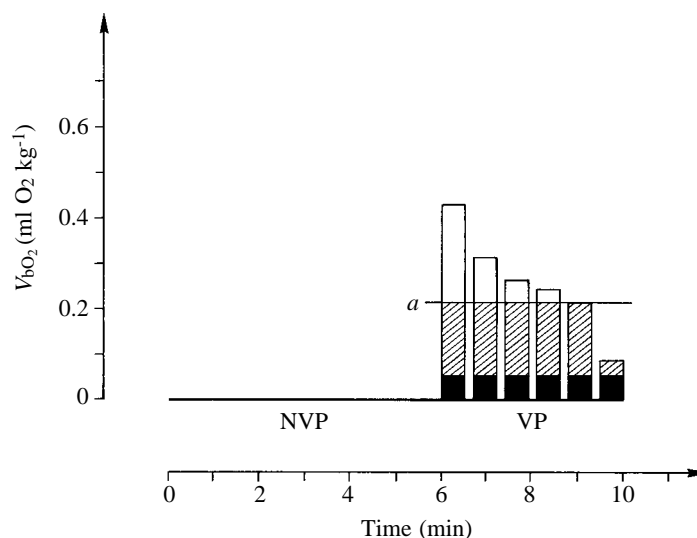
beginning of the ventilatory episode. Nevertheless, as the NVP gets longer, there is an increase in the number of breaths in an episode and in the duration of the breathing bout, which result in a constant f_{VP}. The mean values of the physiological variables examined did not vary significantly in response to NVP duration but, because of the increased number of breaths in an episode, there were increases in total V_{bO_2}, V_{bCO_2} and volume of air ventilated during each VP. Alterations in the number of breaths in an episode and VP duration in response to NVP length appear to be widespread among vertebrates (Milsom, 1988, 1991).

Regulation of the ventilatory period

During the course of a breathing bout, F_{EO_2} and F_{ECO_2} tended to level off. However, even after F_{EO_2} and F_{ECO_2} became stable, the tegus often continued to breathe for a considerable period. Thus, it was not possible to identify clear threshold values of F_{EO_2} and F_{ECO_2} associated with the termination of a VP.

All variables studied showed great variation in the first two breaths of each VP. However, V_T did not vary to the same degree as observed for expired gas composition. This agrees with the fact that variations in F_{EO_2} and F_{ECO_2} depend only on the washout of the lung gases exchanged during the previous NVP, while changes in V_T are the result of complex integrative adjustments (Milsom, 1991). Just as with V_T, t_{TOT} decreased very slightly as the VP proceeded, indicating that there were no significant changes in the air flow rate through the respiratory system during the course of a breathing bout.

V_{bO_2} and V_{bCO_2} decreased during the course of a ventilatory episode, but the decline in V_{bCO_2} occurred more gradually than that of V_{bO_2}. This pattern appears to be common in vertebrates breathing episodically (Milsom, 1991; Garland and Milsom, 1994) and could be attributable to the high tissue capacitance for CO_2 (Dejours, 1975). Thus, as mentioned above for the regulation of NVP, changes in lung volume could also play a role in the regulation of VP. Nonetheless, the relative contribution of changes in lung volume to the regulation of VP

is unclear since this difference between O_2 uptake and CO_2 elimination was only found in the first three or four breaths of the VP and, thus, could not function as a signal to finish a breathing bout.

Oxidative cost of breathing

One complete idealized respiratory cycle of a dormant *T. merianae* at 17 °C is shown in Fig. 4. In these calculations, we used the mean values of VP and NVP duration and the mean number of breaths in a VP. The values of V_{bO_2} were calculated from the equation given in Table 3. The total V_{bO_2} of the ventilatory episode pictured represents the amount of O_2 needed to meet the metabolic requirement during a complete respiratory cycle (i.e. the preceding NVP plus the VP). Line *a* represents the grand mean of all values after V_{bO_2} stabilized. Assuming that the O_2 consumed above that line (open columns) represents the metabolic debt accumulated during the preceding NVP, we can calculate the rate of O_2 consumption during the NVP (non-breathing $\dot{V}_{O_2}$). Subtraction of the amount of O_2 needed to pay back the metabolic debt of the preceding NVP (area above line *a*) and the maintenance rate of O_2 consumption (calculated from the non-breathing $\dot{V}_{O_2}$, dark shaded area in Fig. 4) from the total V_{bO_2} of the ventilatory episode will give the amount of O_2 required for breathing (hatched area in Fig. 4); in *T. merianae*, this is 52.3 % of the total metabolic rate.

The mechanical work of the muscles associated with lung ventilation decreases as f_R increases and V_T decreases. As a consequence, there is an optimal match between f_R and V_T, at a given level of $\dot{V}_e$, for which the cost of respiration is minimized (Milsom, 1991). For animals with low metabolic demands, it has been suggested that the best way to reduce the cost of breathing is to interspace ventilatory periods, in which f_R and V_T are at optimal values, with non-ventilatory pauses. Moreover, this model predicts that an increase in metabolic demand would be better met by an increase in $\dot{V}_e$ through a reduction in the duration of the NVP (Milsom, 1991). Since we did not find differences between f_{VP} at 17 °C and f_R at 25 °C,

and V_T remained unchanged, the increase in $\dot{V}_e$ was entirely achieved by the reduction in NVP. Thus, the change in ventilatory pattern found in the dormant tegus seems to suggest optimization in the use of the energy devoted to breathing.

For terrestrial vertebrates, the relative cost of breathing has been measured in a few species and accounts for 1–15 % of total metabolic rate (Milsom, 1995; Wang and Warburton, 1995). In turtles, because of morpho-functional constraints, such costs can be as high as 30 %, although this seems to be an exception among terrestrial vertebrates (Vitalis and Milsom, 1986; Milsom, 1989). For hibernating *T. merianae*, breathing cost accounted for 52.3 % of total metabolic rate, a value that seems unexpectedly high. However, Garland and Milsom (1994) found that the relative cost of breathing in hibernating squirrels is also high, accounting for 90 % of total metabolic rate. These observations indicate that the mechanical work associated with breathing is one of the most energy-demanding activities for animals experiencing hypometabolic states. The high breathing cost found in these cases could be related to a possible inverse relationship between breathing cost and total metabolic rate, a possibility that remain to be tested.

List of symbols

E_{O_2}	O_2 extraction coefficient (%)
F_{ECO_2}	fractional CO_2 concentration in expired gas (%)
F_{EO_2}	fractional O_2 concentration in expired gas (%)
f_{VP}	breathing frequency during VP (breaths min^{-1})
f_R	breathing frequency (breaths min^{-1})
N_b	number of breaths in a ventilatory period
P_{CO_2}	partial pressure of CO_2
P_{O_2}	partial pressure of O_2
RQ	respiratory quotient
t_{EXP}	duration of expiration (s)
t_{INSP}	duration of inspiration (s)
t_{NVP}	duration of non-ventilatory period (min)
t_{TOT}	duration of total respiratory movement (s)
t_{VP}	duration of ventilatory period (min)
V_{bCO_2}	CO_2 production of an individual breath (ml CO_2 kg^{-1})
V_{bO_2}	O_2 consumption of an individual breath (ml O_2 kg^{-1})
$\dot{V}_{CO_2}$	CO_2 production rate (ml CO_2 kg^{-1} h^{-1})
$\dot{V}_e$	total ventilation rate (ml BTPS kg^{-1} min^{-1})
$\dot{V}_e/\dot{V}_{CO_2}$	air convection requirement for CO_2
$\dot{V}_e/\dot{V}_{O_2}$	air convection requirement for O_2
$\dot{V}_{O_2}$	O_2 consumption rate (ml O_2 kg^{-1} h^{-1})
V_T	tidal volume (ml kg^{-1})

References

Abe, A. S. (1993). Effect of the environment on ventilation in reptiles. In *The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life* (ed. J. E. P. W. Bicudo), pp. 87–93. Boca Raton, FL: CRC Press.

Abe, A. S. (1995). Estivation in South American amphibians and reptiles. *Brazil. J. Med. Biol. Res.* **28**, 1241–1247.

Abe, A. S. and Johansen, K. (1987). Gas exchange and ventilatory response to hypoxia and hypercapnia in *Amphisbaena alba* (Reptilia: Amphisbaenia). *J. Exp. Biol.* **127**, 159–172.

Ackerman, R. A. and White, F. N. (1979). Cyclic carbon dioxide exchange in the turtle, *Pseudemys scripta*. *Physiol. Zool.* **52**, 378–389.

Avila-Pires, T. C. (1995). *Lizards of Brazilian Amazonia (Reptilia: Squamata)*. Leiden: Zoologische Verhandelingen. 706pp.

Bartholomew, G. A. (1982). Physiological control of body temperature. In *Biology of the Reptilia, Physiology C, Physiological Ecology*, vol. 12 (ed. C. Gans and F. H. Pough), pp. 167–211. London: Academic Press.

Bennett, A. F. and Dawson, W. R. (1976). Metabolism. In *Biology of the Reptilia, Physiology A*, vol. 5 (ed. C. Gans and W. R. Dawson), pp. 127–224. London: Academic Press.

Bickler, P. E. (1981). Effects of temperature on acid–base balance and ventilation in desert iguanas. *Exerc. Physiol.* **51**, 452–460.

Burggren, W. W. and Shelton, G. (1979). Gas exchange and transport during intermittent breathing in chelonian reptiles. *J. Exp. Biol.* **82**, 75–92.

Clark, B. D., Gans, C. and Rosenberg, H. I. (1978). Air flow in snake ventilation. *Respir. Physiol.* **32**, 207–212.

Cragg, P. A. (1978). Ventilatory patterns and variables in rest and activity in the lizard, *Lacerta*. *Comp. Biochem. Physiol.* **60**A, 399–410.

Dejours, P. (1975). *Principles of Comparative Respiratory Physiology*. New York, Amsterdam: North-Holland/American Elsevier. 253pp.

Garland, R. J. and Milsom, W. K. (1994). End-tidal gas composition is not correlated with episodic breathing in hibernating ground squirrels. *Can. J. Zool.* **72**, 1141–1148.

Glass, M. L., Boutilier, R. G. and Heisler, N. (1985). Effects of body temperature on respiration, blood gases and acid–base status in the turtle *Chrysemys picta bellii*. *J. Exp. Biol.* **114**, 37–51.

Glass, M. L. and Wood, S. C. (1983). Gas exchange and control of breathing in reptiles. *Physiol. Rev.* **68**, 232–260.

Glass, M. L., Wood, S. C. and Johansen, K. (1978). The application of pneumotachography on small unrestrained animals. *Comp. Biochem. Physiol.* **59**A, 425–427.

Gregory, P. T. (1982). Reptilian hibernation. In *Biology of the Reptilia, Physiology D, Physiological Ecology*, vol. 13 (ed. C. Gans and F. H. Pough), pp. 53–154. London: Academic Press.

Milsom, W. K. (1988). Control of arrythmic breathing in aerial breathers. *Can. J. Zool.* **66**, 99–108.

Milsom, W. K. (1989). Comparative aspects of vertebrate pulmonary mechanics. In *Comparative Pulmonary Physiology* (ed. S. C. Wood), pp. 587–619. New York: Marcel Dekker.

Milsom, W. K. (1991). Intermittent breathing in vertebrates. *Annu. Rev. Physiol.* **53**, 87–105.

Milsom, W. K. (1992). Control of breathing in hibernating mammals. In *Physiological Adaptations in Vertebrates: Respiration, Circulation and Metabolism* (ed. S. C. Wood, R. E. Weber, A. R. Hargens and R. W. Millard), pp. 119–148. New York: Marcel Dekker, Inc.

Milsom, W. K. (1995). Regulation of respiration in lower vertebrates: role of CO_2/pH chemoreceptors. In *Advances in Comparative and Environmental Physiology*, vol. 21 (ed. R. G. Boutilier), pp. 61–104. Berlin: Springer-Verlag.

Milsom, W. K. and Chan, P. (1986). The relationship between lung volume, respiratory drive and breathing pattern in the turtle, *Chrysemys picta*. *J. Exp. Biol.* **120**, 233–247.

Pough, F. H. (1983). Amphibians and reptiles as low energy systems. In *Behavioral Energetics: Vertebrates Costs of Survival* (ed. W. P. Aspey and S. Lustick), pp. 141–188. Columbus: Ohio State University Press.

Shelton, G., Jones, D. R. and Milsom, W. K. (1986). Control of breathing in ectothermic vertebrates. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol. 2, *The Control of Breathing*, part 2 (ed. A. P. Fishman, N. S. Cherniak, J. G. Widdicombe and S. R. Geiger), pp. 857–909. Bethesda: American Physiological Society.

Vitalis, T. Z. and Milsom, W. K. (1986). Pulmonary mechanics and the work of breathing in the semi-aquatic turtle, *Pseudemys scripta*. *J. Exp. Biol.* **125**, 137–155.

Wang, T., Smiths, A. W. and Burggren, W. W. (1998). Pulmonary function in reptiles. In *Biology of the Reptilia, Morphology G, Visceral Organs*, vol. 19 (ed. C. Gans and A. S. Gaunt), pp. 297–374. Athens, OH: SSAR Press.

Wang, T. and Warburton, S. J. (1995). Breathing pattern and cost of ventilation in the American alligator. *Respir. Physiol.* **102**, 29–37.

Wood, S. C., Hicks, J. W. and Dupré, R. K. (1987). Hypoxic reptiles: blood gases, body temperature and control of breathing. *Am. Zool.* **27**, 21–29.

Wood, S. C. and Lenfant, C. (1976). Respiration: mechanics, control and gas exchange. In *Biology of the Reptilia*, vol. 5 (ed. C. Gans and W. R. Dawson), pp. 225–274. New York: Academic Press.