

The Relationship Between Body Temperature, Heart Rate, Breathing Rate, and Rate of Oxygen Consumption, in the Tegu Lizard (*Tupinambis merianae*) at Various Levels of Activity

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## ABSTRACT:

The present study determined whether EEG and/or EMG recordings could be used to reliably define activity states in the Brazilian black and white tegu lizard (*Tupinambis merianae*) and then examined the interactive effects of temperature and activity states on strategies for matching  $O_2$  supply and demand. In a first series of experiments the rate of oxygen consumption ( $\dot{V}_{O_2}$ ), breathing frequency ( $f_R$ ), heart rate ( $f_H$ ), and EEG and EMG (neck muscle) activity were measured in different sleep/wake states (sleeping, awake but quiet, alert, or moving). In general, metabolic and cardio-respiratory changes were better indicators of the transition from sleep to wake than were changes in the EEG and EMG. In a second series of experiments, the interactive effects of temperature (17°C, 27°C and 37°C) and activity states on  $f_R$ , tidal volume ( $V_T$ ), the fraction of oxygen extracted from the lung per breath ( $FI_{O_2}-FE_{O_2}$ ),  $f_H$ , and the cardiac  $O_2$  pulse were quantified to determine the relative roles of each of these variables in accommodating changes in  $\dot{V}_{O_2}$ . The increases in oxygen supply to meet temperature- and activity-induced increases in oxygen demand were produced almost exclusively by increases in  $f_H$  and  $f_R$ . Regression analysis showed that the effects of temperature and activity state on the relationships between  $f_H$ ,  $f_R$  and  $\dot{V}_{O_2}$  was to extend a common relationship along a single curve, rather than separate relationships for each metabolic state. For these lizards, the predictive powers of  $f_R$  and  $f_H$  were maximized when the effects of changes in temperature, digestive state and activity were pooled. However, the best  $r^2$  values obtained were 0.63 and 0.74 using  $f_R$  and  $f_H$  as predictors of metabolic rate, respectively.

Key Words: Body Temperature, Heart Rate, Breathing Rate, Metabolic Rate, Tegu Lizard,

## INTRODUCTION:

The black and white tegu lizard (*Tupinambis merianae*) has been the focus of several metabolic studies, including studies of metabolic responses to exercise and feeding (Klein et al., 2006), the metabolic effects of changes in age, body size and season (Toledo et al., 2008), metabolic acclimation at constant temperature and photoperiod across seasons (Abe, 1995; Milsom et al., 2008), and metabolic depression during seasonal dormancy (Abe, 1983; Abe, 1995; Andrade & Abe, 1999; de Souza et al., 2004). Of the factors that influence metabolic rate, the ones of most interest and relevance for studies of dormancy in lizards in the field are the effects of temperature as well as the effects of reduced activity states.

In reptiles, it is often difficult to determine whether animals are dormant, asleep, or awake but quiet (eyes closed) without disturbing them. The use of EEG and EMG recordings in reptiles to distinguish activity states has produced conflicting results (Flanigan et al., 1973; Warner and Huggins, 1978; Meglasson and Huggins, 1979; Walker and Berger, 1973; Ayala-Guerrero et al., 1988; Tauber et al., 1968; Huntley, 1987; Flanigan, 1973; Ayala-Guerrero and Huitron-Resendiz, 1991). Although these different states may be difficult to distinguish visually or electrophysiologically, one would expect metabolism to be higher in animals when awake than when sleeping (Wood et al., 1978; Revell and Dunbar 2007), and to be suppressed below sleeping levels during dormancy. If this is true, one may be able to use metabolic rate (and potentially  $f_R$  or  $f_H$ ) to distinguish between different activity states. Our first objective, therefore, was to determine the extent to which electrophysiological, behavioural and metabolic changes could be used to determine changes in activity state.

The changes in metabolic demands for oxygen associated with changes in temperature and activity state may be met in a variety of ways. The two convective steps in the oxygen transport cascade are ventilation and perfusion. Thus changes in any of

ventilatory flow (altering breathing frequency and/or tidal volume), oxygen extraction from inspired air, cardiac output (altering heart rate and/or stroke volume) and oxygen extraction from the circulating blood may be recruited for this purpose. Our second objective was to quantify the interactive effects of temperature (17°C, 27°C and 37°C) and activity states on these various strategies used by tegu lizards for matching O<sub>2</sub> supply and demand.

## METHODS:

Two series of experiments were performed. The first used four juvenile tegu lizards (*Tupinambis meriannae*) <3 years of age ranging from 630-950 g, while the second series used six tegu lizards (three male and three female), each approximately 3-4 years of age with weights ranging from 2.2 to 3.9 kg. All lizards were bred in Brazil at the Jacarezario, UNESP, Bela Vista Campus, Rio Claro, SP. Animals were brought to the University of British Columbia within a few weeks of hatching and raised in captivity. All experimental procedures conformed to and were approved by the UBC animal care and use committee.

### **Series 1:**

#### *Instrumentation:*

In the first series of experiments animals were anesthetized with 2.5% halothane in air and four 0.65 mm holes were drilled bilaterally into the skull above the positions of the posterior telencephalon and the mid optic tectum. Four self-tapping screws (1.2 mm diameter, 6 mm length) were threaded into the holes and fixed to the skull with acrylic cement. Braided stainless steel coated wires (0.25 mm) housed in a thin silastic tube were passed externally to an amphenol pin strip sutured to the skin above the forelimbs to serve as EEG electrodes for chronic recording. EMG and ECG electrodes were passed subdermally to the amphenol pin strip. The EMG electrodes were attached bilaterally to the nuccal muscles

of the neck just beneath the skin. ECG electrodes were placed on the lateral thorax on either side of the heart. Following surgery the animals were given 10 days to recover and were individually housed under a 9:15 light-dark photoperiod. Instrumentation had no apparent effect on behaviour and the leads did not impede locomotion.

*Experimental Protocol:*

In this series of experiments, video recordings were used to score the behaviour of lizards (sleep, awake, alert and active, see below) while recording various physiological variables. During recordings, tegus were housed in a circular metabolic chamber (20 cm diameter x 10 cm high) placed in an insulated environmental chamber under a 9:15 h light-dark photoperiod at a constant temperature of 27°C. Red light was used during the scotopic period to permit digital video recording. Wire leads were connected from the amphenol pin strip to a commutator in the lid of the respirometer and then to a Grass polygraph recorder (model 79E) where signals were amplified and filtered (60 Hz notch filter). Air was drawn through the respirometer and then through a drying column to oxygen and carbon dioxide analyzers (Models OM-11 and LB-2, Beckman Instruments, USA) at a flow rate of 180 ml/min. These instruments were automatically calibrated every three hours.

Lizards were initially habituated to recording conditions for 48 hours. They were then removed from the recording chamber and given water in their home chambers. Twenty four hours later they were returned to the recording chamber and allowed a further 24 hours to settle before recording trials began. Recordings were carried out over a minimum 24 hour period beginning at 13:00 h.

**Series 2:**

*Instrumentation:*

In the second series of experiments custom-fitted masks were made for each lizard as described by Glass et al. (1978) and modified by Wang and Warburton (1995). A pneumotachograph was mounted on top of the mask and connected to the oxygen and carbon dioxide analyzers and a differential pressure transducer (Model DP103-18, Validyne Engineering Corp, USA) and amplifier (Model 7P122E, Grass Instruments, USA) for measuring tidal volume and breathing frequency. Three ECG electrodes (gold plated, Grass Instruments, USA) were implanted subcutaneously in the thorax/abdomen under anesthesia, triangulating the heart in order to monitor heart rate. The electrodes were sutured and glued in place and the lizards were allowed to recover for at least 24 hours before any experimental runs.

#### *Experimental Protocol*

Experiments involved four treatments: 17°C/fasting, 27°C/fasting, 37°C/fasting, and 37°C/digesting. Each trial lasted 3 hours during the active period (lights on), and was followed by a minimum 24 hour period as animals were acclimated to the next treatment temperature. Temperature treatments were randomized, but the digesting trial was always carried out as the final trial.

At the start of each trial, the outflow tubes from the pneumotachograph were connected to the gas analyzers and to the differential pressure transducer. Heart rate leads were connected to the amplifier (Grass Instruments, USA, Model 7P511K.). The dead space of the mask was small enough that individual breaths were resolvable and end tidal values for O<sub>2</sub> and CO<sub>2</sub> were easily observed. The lizard's posture and activities were recorded with a VCR, and were associated with the corresponding physiological data during analysis.

After each trial, the mask was removed and the lizard was offered water and kept in the environmental chamber while acclimating to the next treatment temperature. Prior to the 37°C/digesting trial the lizard was offered a standard meal of ground beef, vegetables, fruit and vitamin and mineral supplements and was allowed to feed to satiation; lizards never ate more than 4% of their body weight (roughly 100 to 150 g) despite being fasted for several weeks. Data collection typically began approximately 36 to 40 hours later as this is approximately when specific dynamic action reaches its peak in tegu lizards (Klein et al., 2006).

## **Data Analysis**

### *Series 1:*

The EEG, EMG, and ECG signals, along with the output of the O<sub>2</sub> and CO<sub>2</sub> analyzers, were recorded to computer at 240 Hz per channel using a Windaq data acquisition system (DI200; DataQ Instruments, Akron, OH, USA). Twenty one-minute epochs were randomly chosen from each behavioural state in each animal for analysis. From these traces we calculated EEG and EMG amplitude and performed a frequency analysis on the EEG signals using both visual analysis as well as a windowed fast-fourier transformation. Frequencies ranging from 1-33 Hz were analyzed in 1 Hz bands to assess the EEG frequencies associated with each state. Rates of oxygen consumption and CO<sub>2</sub> production were obtained by integrating the area under the O<sub>2</sub> and CO<sub>2</sub> curves and were corrected according to Withers (1977). Given the range in body mass of the lizards, to characterize the relationship between mass and resting oxygen consumption, all metabolic data were scaled to the power of 0.79 as

derived for tegu lizards by Toledo et al. (2008). Ventilation rate was determined from phasic EMG activity arising from the intercostal muscles and appearing on the ECG trace.

*Series 2:*

In these series, treatments were applied using a repeated-measures design. The output of the O<sub>2</sub> and CO<sub>2</sub> analyzers, the electrocardiogram (ECG) and the differential pressure across the pneumotachograph were also recorded to computer at 240 Hz per channel using the same Windaq data acquisition system. From these signals it was possible to calculate  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  (reported at STPD),  $f_H$ ,  $f_R$  and  $V_T$ .

Oxygen consumption and CO<sub>2</sub> production were obtained as described for Series 1. Ventilation volume ( $\dot{V}_E$ ) was calculated as the product of  $f_R$  and  $V_T$  and cardiac O<sub>2</sub> pulse was calculated as  $\dot{V}_{O_2}$  divided by  $f_H$ . Finally, Q<sub>10</sub> values were calculated for  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$ ,  $f_H$ , and  $f_R$ .

All data from the experiments in series 2 were analyzed using 5 minute samples of data taken approximately every 10 minutes. In both series, the first data sample was taken at least 30 minutes after commencing recording, and all samples were taken during steady state conditions.

*Statistical analysis*

In series 1, all variables in each state were compared using a repeated measures ANOVA and a student t-test was used for intergroup comparisons. All data reported as percent change were arcsin transformed prior to analysis. In series 2, for each protocol, the means for each variable were compared via a 2-way repeated measures ANOVA with treatment and activity as the two effects, followed by post-hoc Holm-Sidak tests to identify specific differences. The effect of temperature was tested using averages from 17°C/fasting,



27°C/fasting and 37°C/fasting experiments, while the effect of digestion was tested on averages from 37°C/fasting and 37°C/digesting experiments. Sleep data were excluded from statistical analysis of this series due to low sample size.

All statistics were performed using SigmaStat 3.1 and JMPIN 4.0.4. Results were considered significant if  $P < 0.05$ .

## RESULTS:

### **Series 1:**

Observations and measurements were made in Series 1 to determine whether objective electrophysiological criteria could be used to support identification of activity states based on behavioural criteria; in particular sleep and quiet wake.

Behavioural states were first classified based on the criteria proposed by Huntley (1987). During sleep, animals lay motionless with head and thorax in contact with the substrate, the palmar surfaces of the forelimbs facing up, and the palmar surfaces of the hindlimbs facing the body. During quiet wake, animals also lay motionless with their thorax and head on the substrate but with their forelimbs splayed laterally from the body with palmar surfaces down and hindlimbs extended to the side. We categorized animals as alert when their eyes were open and/or their body was held up off the floor, or when they were resting between periods of activity. All animals displaying any form of activity were categorized as “moving”.

Figure 1 illustrates EEG and EMG traces taken from one animal during two different episodes of alert wake (Fig. 1a,c) and sleep (Fig. 1b,d) while Figure 2 presents the EEG power spectral analysis for longer segments of these same four episodes from this animal. No distinct EEG frequency bands were associated with either state. In both states, periods

dominated by slower frequencies (Fig 1c,d and Fig 2c,d) alternated with periods dominated by higher frequencies (Fig 1a,b and Fig 2a,b). The only notable difference between states was in the absolute value of power spectral density reflecting the lower amplitude of the EEG in sleep (Fig. 2). Similar results were obtained from all other lizards.

Figure 3 presents the quantitative physiological data for all four animals. EEG amplitude fell significantly from alert to wake and from wake to sleep (Fig. 3). In contrast, EMG amplitude only fell from alert to awake activity states (Fig. 3).  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$ ,  $f_H$  and  $f_R$  all fell significantly with each step from alert to quiet wake to sleep (Fig. 3).

### **Series 2:**

Graphic representations of the mean values of  $\dot{V}_{O_2}$ ,  $f_H$ , and the cardiac  $O_2$  pulse are presented in Figure 4, illustrating the effects of temperature and activity. Oxygen consumption and  $f_H$  increased significantly as the animals progressed from quiet wake to alert and moving at all temperatures. Lizards also consumed more oxygen and increased heart rate with increasing temperature in all activity states (Figure 4, Table 1). The changes in mean values of the cardiac  $O_2$  pulse with increasing activity were qualitatively different depending on the temperature (Fig. 4, Table 1).

Graphic representations of the mean values of each variable in the Fick equation for oxygen transport by ventilatory convection are presented in Figure 5 and Table 1. Breathing frequency increased significantly with increasing activity at all temperatures and also increased with increasing temperature except going from 27 to 37°C during movement (Figure 5, Table 1). Mean values for  $V_T$  did not change significantly with increasing activity or temperature. Oxygen extraction generally decreased with increasing activity at all

temperatures (although this was only significant during movement) and there was no significant change with changing temperature within any activity state (Fig. 5, Table 1).

Table 2 depicts the effect of temperature on  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$ ,  $f_H$ , and  $f_R$ . As is apparent from Figures 4 and 5, the largest increases with temperature were seen in  $f_H$  and  $f_R$ . The  $Q_{10}$  values were generally close to 2 and similar at the transition from 17°C to 27°C and from 27°C to 37°C.

Mean values of each variable from digesting animals at 37°C were not statistically different from those of fasting animals at 37°C (Table 1).

#### *Heart rate vs. O<sub>2</sub> consumption*

Figure 6 shows all mass-specific O<sub>2</sub> consumption data grouped by treatment (Fig. 6a) and activity (Fig. 6b), plotted against heart rate. Independent regressions were calculated for each lizard at each temperature (not shown) and 22 out of 24 proved to be significant. All slopes were statistically different in all treatments except 17°C/fasting animals and intercepts were significantly different in all treatments. This allowed pooled regressions (i.e. the combination of data from multiple runs for a single individual) to be calculated for each treatment (as in Bevan et al., 1994; Bevan et al., 1995a). Pooled regressions for all lizards at each temperature (not shown) were significant in all cases, but there was still much variation not accounted for by the regressions (the  $r^2$  values ranged from 0.30 in 37°C/fasting animals, to 0.63 in 17°C/fasting animals). When pooled regressions were performed using activity as a grouping factor individual regressions were significant at each activity level (18 of 18 regressions had  $P < 0.05$ ) and values of  $r^2$  ranged from 0.47 to 0.66. Finally, all data (for all treatments and activities) were pooled together to perform one last regression analysis.

Linear regressions were carried out on untransformed and transformed data, and the primary

regression variables for each are shown in Table 3. All regressions were significant and transforming the data produced better regressions. When both  $f_H$  and  $O_2$  consumption were natural log-transformed, 74% of the variability in  $O_2$  consumption could be accounted for by changes in heart rate (Figure 6).

#### *Breathing frequency vs. $O_2$ consumption*

The same regression analysis was performed on  $f_R$  data as on the  $f_H$  data and the key results are presented in Figure 7 and Table 4. All regressions were significant, but in all cases,  $r^2$  values were lower compared with regressions using  $f_H$  (see Table 3). Again, transforming the data produced better regressions. When both  $f_R$  and  $O_2$  consumption were log-transformed, the regression could account for 63% of the variability in  $O_2$  consumption.

## DISCUSSION:

#### *Electrophysiological correlates of wake and sleep*

We found that the transition from quiet wake to sleep was accompanied by a significant, but small decrease in EEG amplitude (roughly 5%) and no change in EMG amplitude. These findings are in general agreement with previous studies of other reptilian species including crocodylians (Flanigan et al., 1973; Warner and Huggins, 1978; Meglasson and Huggins, 1979), chelonians (Walker and Berger, 1973; Ayala-Guerrero et al., 1988) and other species of lizards (Tauber et al., 1968; Huntley, 1987; Flanigan, 1973; Ayala-Guerrero and Huitron-Resendiz, 1991). We also did not find any distinct EEG frequency bands accompanying either the wake or sleep states. The hallmark of sleep in most mammals is the switch from a low amplitude, high frequency EEG pattern to a high amplitude pattern of slow waves (Rechtschaffen et al., 1968). While some researchers have reported a reduction in the predominant frequency band of the EEG with the transition from wake to sleep in reptiles

(Walker and Berger, 1973; Warner and Huggins, 1978; Meglasson and Huggins, 1979; Huntley, 1987) others, like us, have also reported the presence of slow waves in awake animals (Tauber et al., 1968; Rial et al., 1993; De Vera et al., 1994). High amplitude spikes (believed to be of cortical origin) have also been reported to be an indicator of behavioural quiescence and sleep in reptiles (Flanigan, 1973; Flanigan et al., 1973; Walker and Berger, 1973) while others report these spikes only during wake (Tauber et al., 1968; Huntley, 1987; De Vera et al., 1994). We observed them during both wake and sleep. In summary, our data suggest that while there is an overall decline in brain and muscle activity from the alert to sleep state, this is not accompanied by prolonged periods of synchronized activity, distinct EEG frequencies or unique high frequency spikes. As a result, we reasoned that using EEG/EMG criteria as markers of the wake-sleep transition did not contribute more than monitoring metabolic and cardio-respiratory variables alone (see next section).

#### *Metabolic and cardio-respiratory changes with activity and temperature*

Mass specific rates of O<sub>2</sub> consumption and CO<sub>2</sub> production in our study are in good agreement with values reported in the literature (Andrade & Abe, 1999; Skovgaard & Wang, 2004; Toledo et al., 2008; Milsom et al., 2008). The mean values of  $f_H$ ,  $f_R$ , and  $V_T$  presented here are also similar to those previously reported for tegu lizards at similar temperatures and activity states (Andrade & Abe, 1999; Klein et al., 2003; Skovgaard & Wang, 2004). The ranges reported in the literature are large, however, as rates of metabolism are seasonally dependent in this species, with standard metabolism in the spring and summer being significantly greater (up to three times) than in the autumn or winter (Andrade & Abe, 1999; de Souza et al., 2004; Toledo et al., 2008; Milsom et al., 2008).

#### *Effects of temperature*

Because tegu lizards are ectotherms, we had anticipated that all physiological rate processes, including  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$ ,  $f_H$ , and  $f_R$ , would increase with ambient temperature and this was the case. Our data show that there was no consistent difference between  $Q_{10}$  values calculated over the 17°C-27°C range, the 27°C-37°C range, or the full temperature range of 17°C-37°C. All values were close to 2.0. Reptiles in general have  $Q_{10}$  values ranging from 1.5 to 3.1 for metabolic processes (Bennett & Dawson, 1976).

#### *Effects of activity state*

Unfortunately, only the “quiet wake”, “alert” and “moving” states could be compared in Series 2 since no sleep occurred at the two higher temperatures and only a few lizards exhibited evidence of sleep at the lowest temperature. However, the trends seen in Series 2 were similar to those seen in Series 1 where data were obtained for “sleep”, “wake” and “alert” states. Analysis of both data sets suggests that while it is difficult to distinguish sleep from wake by monitoring electrophysiological variables (EEG and EMG) these behavioural states can be distinguished clearly based on metabolic and cardio-respiratory changes. Thus, even though lizards remain motionless in all of the behavioural states of “sleep”, “wake” and “alert”, each state is associated with significantly different levels of metabolism, heart rate and breathing frequency presumably associated with changes in resting muscle tone and other physiological changes.

#### *The correlation between heart rate and metabolic rate:*

Studies designed to address specific questions about metabolic depression during seasonal dormancy in animals under field conditions are challenged by the need to determine metabolic rates in unrestrained free ranging animals. The time energy budget method is one technique that may be used in the wild that estimates daily metabolic expenditure from the

proportion of time the animal spends performing different activities. This method of estimating metabolic rate requires energy costs of various activities to be predetermined by laboratory techniques and demands that the animal being studied is constantly monitored (Ettinger and King 1980; Bevan et al., 1994). In recent years, there have been numerous studies demonstrating that heart rate can be used as a reliable indicator of metabolic rate under field conditions as long as certain criteria are met (Butler, 1993; Bevan et al., 1995a; Green et al., 2001; Froget et al., 2002; Butler et al., 1992; Green and Frappell, 2007; Green et al., 2008), thus offering an alternative to the time energy budget method. The heart rate method has been best studied in mammals and birds and faces additional challenges in ectothermic animals, such as large fluctuations in body temperature and modifications in heart rate for thermoregulatory purposes (Clark et al., 2006). The heart rate method may be used reliably, however, if temperature effects and the effects of feeding are known and corrected for (Butler et al., 2002; Clark et al., 2005a; Clark et al., 2006).

The basis of the heart rate method for estimating metabolic rate lies in Fick's convection equation for the circulatory system, which can be written as:

$$\dot{V}_{O_2} = f_H \times V_s \times (O_{2a} - O_{2v}) \quad (1)$$

where  $\dot{V}_{O_2}$  is the rate of oxygen consumption,  $f_H$  is heart rate,  $V_s$  is cardiac stroke volume, and  $(O_{2a} - O_{2v})$  is the oxygen content difference between arterial and venous blood. The product of  $V_s$  and  $(O_{2a} - O_{2v})$  is termed the cardiac  $O_2$  pulse and represents the amount of oxygen taken up by the tissues per heartbeat. If changes in the rate of oxygen consumption are mediated primarily by changes in heart rate (assuming the cardiac  $O_2$  pulse changes in a constant or curvilinear fashion with respect to changing oxygen consumption),  $f_H$  and  $\dot{V}_{O_2}$  will co-vary in a predictable, linear manner.

*Activity:* The relationship between  $f_H$  and  $\dot{V}_{O_2}$  at a given  $T_b$  for any given activity over a range of intensities is generally rectilinear or curvilinear in birds, (Grubb et al., 1983; Bevan et al. 1994; Green et al. 2001; Ward et al. 2002) mammals (Fedak et al., 1988; Williams et al., 1991; Williams et al., 1993), reptiles (Bennett 1972; Gleeson et al. 1980; Frappell et al. 2002; Butler et al. 2002; Clark et al., 2006) and some fish (Armstrong 1986; Lucas 1994; Webber et al., 1998; Clark et al. 2005a). Thus, for most vertebrates, the cardiac  $O_2$  pulse changes systematically during any activity such that a strong relationship between  $f_H$  and  $\dot{V}_{O_2}$  is maintained (Wilson 1974; Gleeson et al. 1980; Grubb et al. 1983; Butler et al. 1992; Clark et al. 2005a,b). However, this has not always been the case when comparing the  $f_H / \dot{V}_{O_2}$  relationship between different activities. For example, barnacle geese and bar-headed geese displayed a different  $f_H / \dot{V}_{O_2}$  relationship (different slopes and intercepts) when walking/running on a treadmill compared with flying in a wind tunnel (Ward *et al.* 2002) and king penguins displayed a different  $f_H / \dot{V}_{O_2}$  relationship when walking/running on a treadmill compared to thermoregulating at low environmental temperatures (Froget et al. 2002). Tegu lizards in the present study, however, retained the same  $f_H / \dot{V}_{O_2}$  relationship between the activity states we have defined as sleep, quiet wake, alert and moving.

*Temperature:* For some species of fish (Claireaux et al. 1995; Clark et al. 2005a), as well as for marine iguanas (Butler et al. 2002) and Rosenberg's goanna (Clark et al., 2003, 2006), increases in temperature caused a right shift in the linear regression that described the relationship between  $f_H$  and  $\dot{V}_{O_2}$ . This was true for animals at rest as well as during exercise and recovery and was also true during feeding in the goanna (Clark et al., 2006). Thus, for



most vertebrates, the cardiac O<sub>2</sub> pulse changes systematically during exercise and proportionally across temperature such that a strong relationship between  $f_H$  and  $\dot{V}_{O_2}$  is maintained (Wilson 1974; Gleeson et al. 1980; Grubb et al. 1983; Butler et al. 1992; Clark et al. 2005a,b). As a consequence, a common equation can be used to relate  $f_H$  and  $\dot{V}_{O_2}$  as long as a factor correcting for body temperature is included (Butler et al, 2002; Clark et al., 2006). This was not necessary in the case of the tegu lizards as the relationship between  $f_H$  and  $\dot{V}_{O_2}$  remained constant for all activity levels across all temperatures. The effects of temperature and activity state were to extend a common relationship along a single curve.

*The correlation between breathing frequency and metabolic rate*

Since the Fick equation can also be described in terms of breathing frequency it seemed reasonable to consider whether  $f_R$  might be as good a proxy for metabolic rate as  $f_H$ . The Fick's convection equation for ventilation can be written as:

$$\dot{V}_{O_2} = f_R \times V_T (F_{IO_2} - F_{EO_2}) \quad (2)$$

where  $\dot{V}_{O_2}$  is the rate of oxygen consumption,  $f_R$  is the breathing frequency,  $V_T$  is the tidal volume, and  $(F_{IO_2} - F_{EO_2})$  is the difference in the fraction of oxygen in inspired and expired air. The product of  $V_T$  and  $(F_{IO_2} - F_{EO_2})$  is termed the ventilatory O<sub>2</sub> pulse and represents the amount of oxygen extracted from inspired air per breath. In this case, if the product of  $V_T$  and  $(F_{IO_2} - F_{EO_2})$  also changes in a constant or curvilinear fashion with respect to changing oxygen consumption, breathing rate could also be used to estimate metabolic rate in the field.

In all cases, however, the correlation coefficients for the regressions of  $f_R$  against  $\dot{V}_{O_2}$  turned out to be lower than those for the regressions of  $f_H$  against  $\dot{V}_{O_2}$ . The differences were not great, however.

Again, the effects of temperature and activity state were to extend a common relationship along a single curve. Similar effects of temperature on ventilation have been shown for the varanid lizard (Wood et al., 1977). While the correlation between  $f_R$  and metabolic rate may not be as tight as the correlation between  $f_H$  and metabolic rate, it may still be a useful alternative method for estimating  $\dot{V}_{O_2}$  when  $f_R$  can be measured but  $f_H$  can not.

#### *Predicting Metabolic Rates from Energy Budgets and Heart Rate/Breathing Rate*

Any regression equation can serve as a predictive tool, but the further the coefficient of determination ( $r^2$ ) is from 1, the less the confidence in the accuracy of that prediction. Most studies in which the heart rate method has been validated against metabolic rate have achieved  $r^2$  values around 0.8 and higher; that is, around 80% or more of the variation in  $\dot{V}_{O_2}$  could be explained by variations in  $f_H$ . In the present study, the best  $r^2$  values obtained were slightly lower, being 0.63 and 0.74 using  $f_R$  and  $f_H$  as predictors of metabolic rate, respectively. For these lizards, the predictive powers of  $f_R$  and  $f_H$  were maximized when the effects of changes in temperature, digestive state and activity were pooled. This reflects the uniform dependence of changes in metabolism, regardless of cause (temperature, digestive state or activity) on changes in  $f_R$  and  $f_H$  for increasing  $O_2$  delivery. In order to determine the extent to which the calibration equations derived in Series 2 could be used in tegu lizards in

general, validation experiments must be performed using a different population of lizards.

This remains to be done.

Finally, it is important to remember that the relationship between heart rate and metabolic rate may not remain constant in different seasons and physiological states.

Amongst the various factors that have been shown to influence the relationship between  $f_H$  and  $\dot{V}_{O_2}$  in various studies are: age, season, breeding state, and physical fitness (Thorarensen et al., 1996; Butler et al., 2000; Hawkins et al., 2000; Green et al., 2001; Froget et al., 2001; Butler et al., 2002; Clark et al., 2003; Fahlman *et al.* 2004). Thus, if either regression equation is to be used to predict metabolic rates of *T. merrianae* in the field throughout the year, they should be used cautiously.

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## Figure Captions:

**Fig 1:** Four, 10 second sample traces of EEG and EMG signals during wake (a and c) and sleep (b and d). While the amplitude of the EMG trace is clearly reduced in sleep, differences in the EEG traces were not always evident (compare a vs b with c vs d)

**Fig 2:** A-D; EEG power spectral density of 16-second epochs during both wake and sleep. A) period of wake with high frequency activity; B) period of sleep with high frequency activity; C) period of wake with low frequency activity; D) period of sleep with low frequency activity

**Fig 3:** Effects of sleep versus wake on A) EEG amplitude, B) EMG amplitude, C) oxygen consumption, D) CO<sub>2</sub> production (all expressed as % change relative to Alert), E) heart rate and F) breathing frequency. \* indicates significantly different from alert. + indicates significantly different from wake

**Fig 4:** Mean values of each variable in Fick's equation for oxygen transport by circulatory convection (rate of oxygen consumption = heart rate x the cardiac O<sub>2</sub> pulse) as a function of temperature and activity state. (See Table 1 for statistics)

**Fig 5:** Mean values of each variable in Fick's equation for oxygen transport by ventilatory convection (rate of oxygen consumption = breathing frequency x tidal volume x O<sub>2</sub> extraction from the lung) as a function of temperature and activity state. (See Table 1 for statistics)

**Fig 6.** Heart rate plotted as a function of mass-specific oxygen consumption colour coded by temperature (A) and activity (B). The best-fit linear regression is shown along with the 95% prediction intervals, the regression equation, and the  $r^2$  value for the regression

**Fig 7.** Breathing Frequency plotted as a function of mass-specific oxygen consumption colour coded by temperature (A) and activity (B). The best-fit linear regression is shown along with the 95% prediction intervals, the regression equation, and the  $r^2$  value for the regression

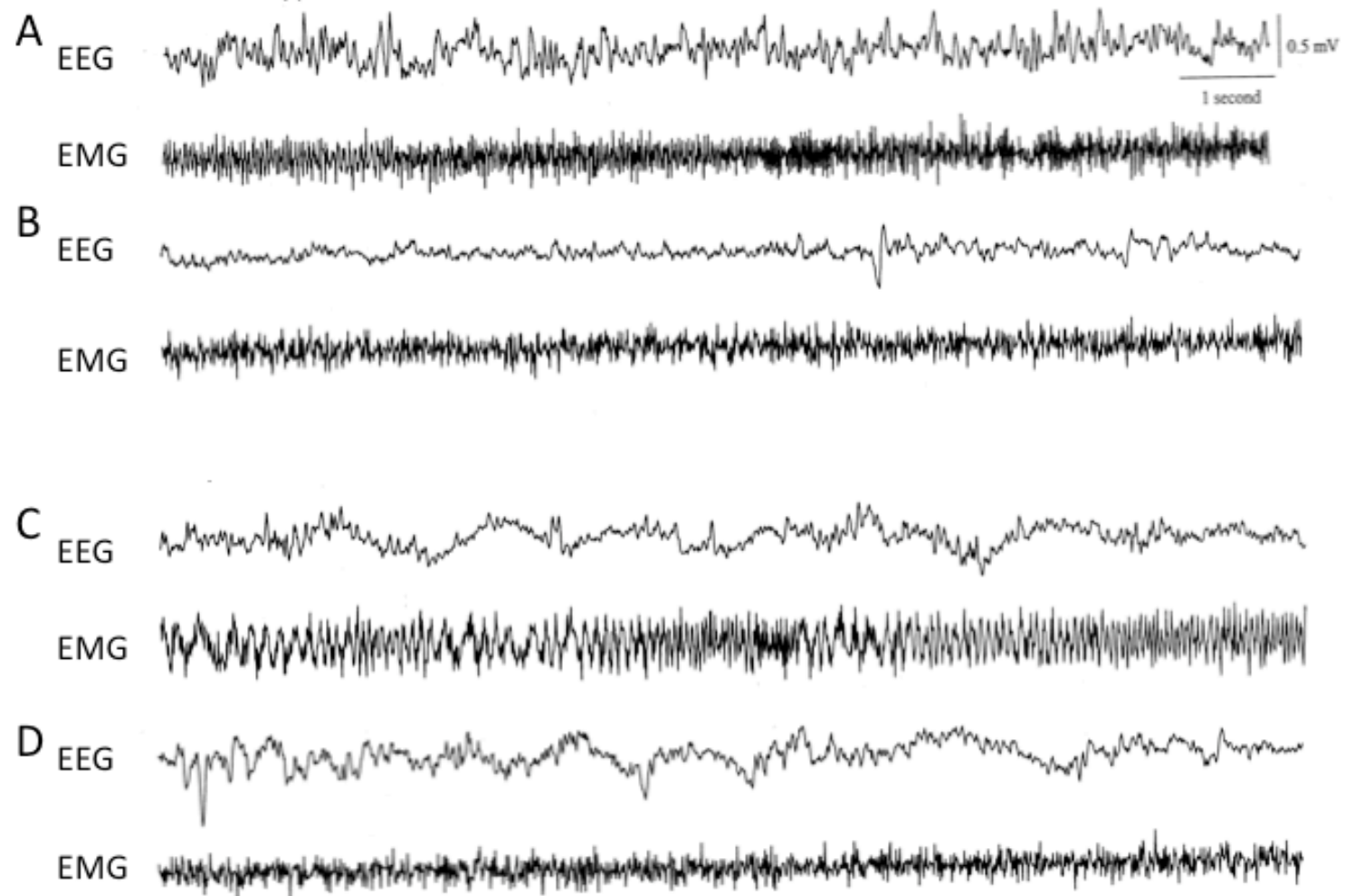


Figure 1

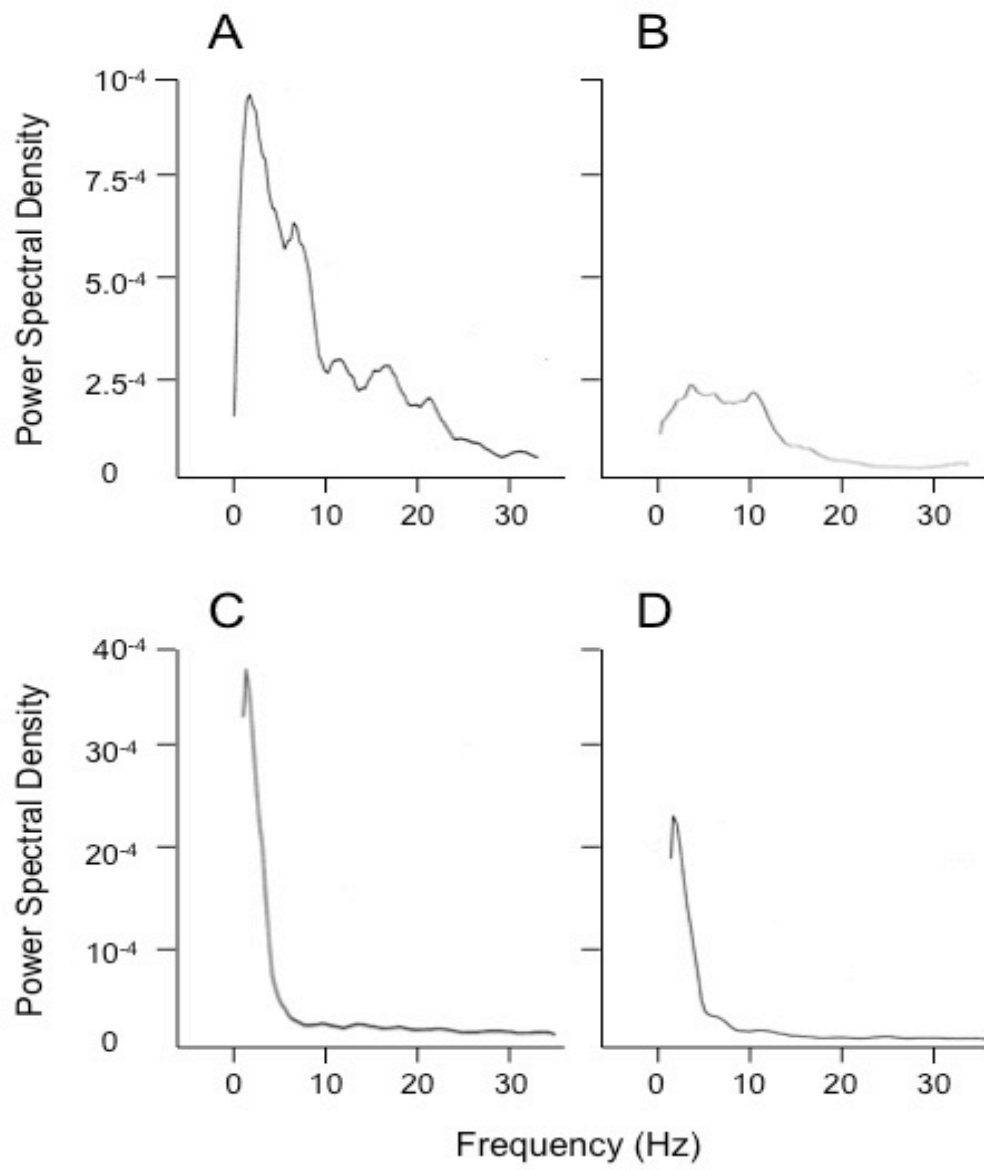


Figure 2

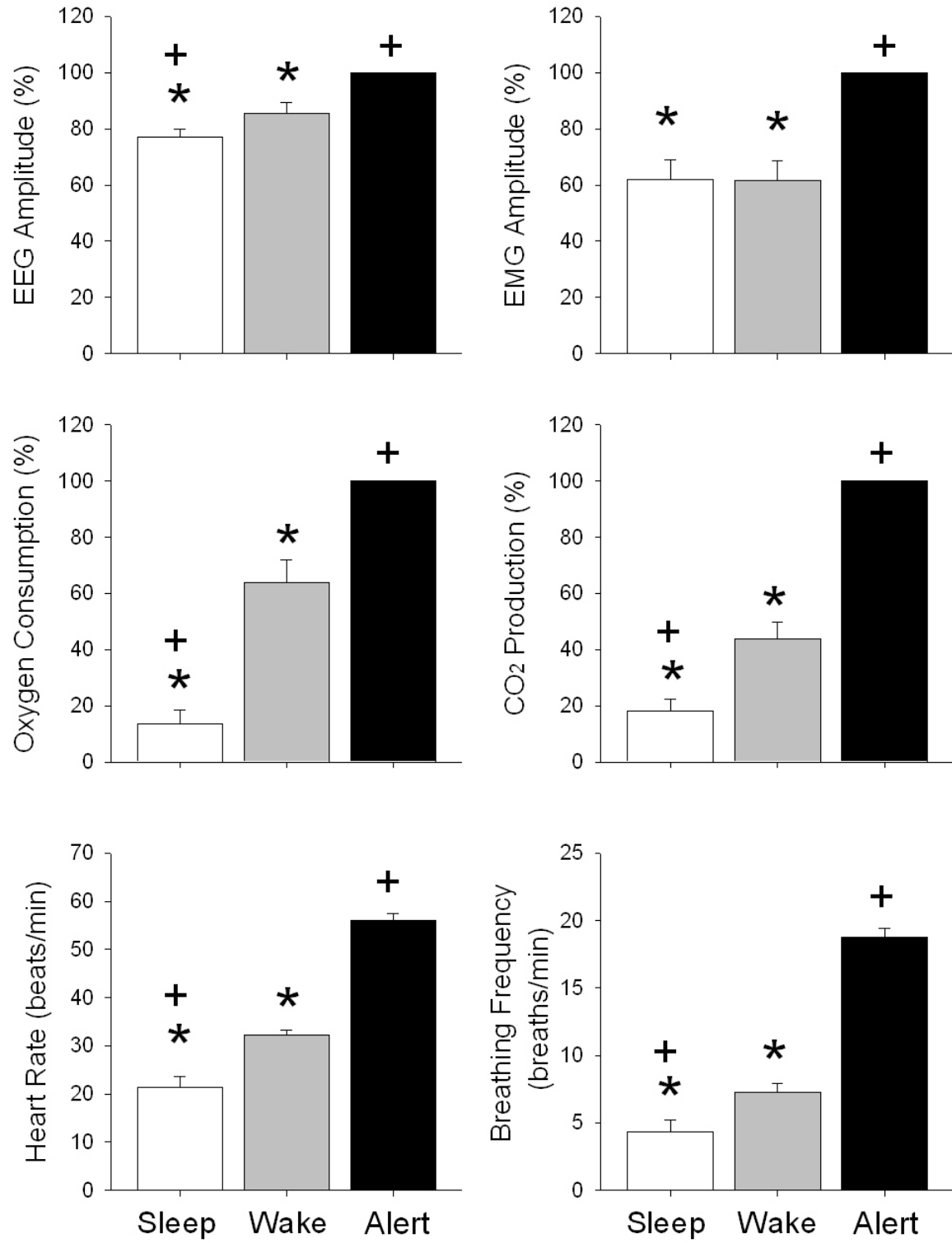
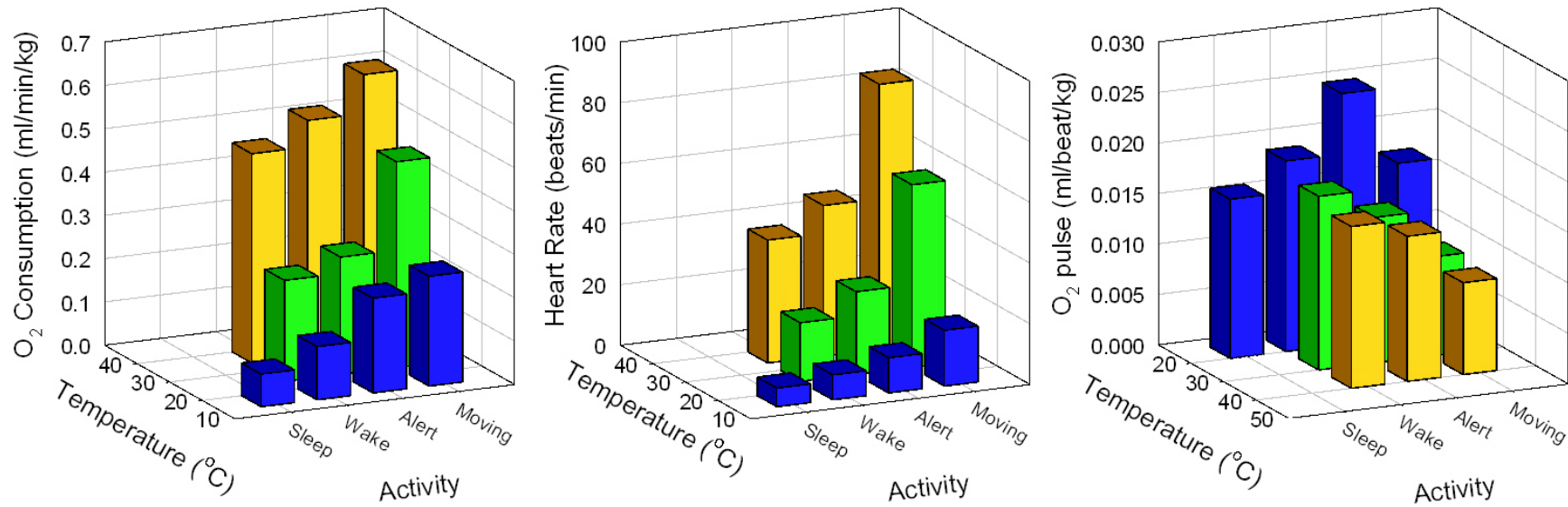


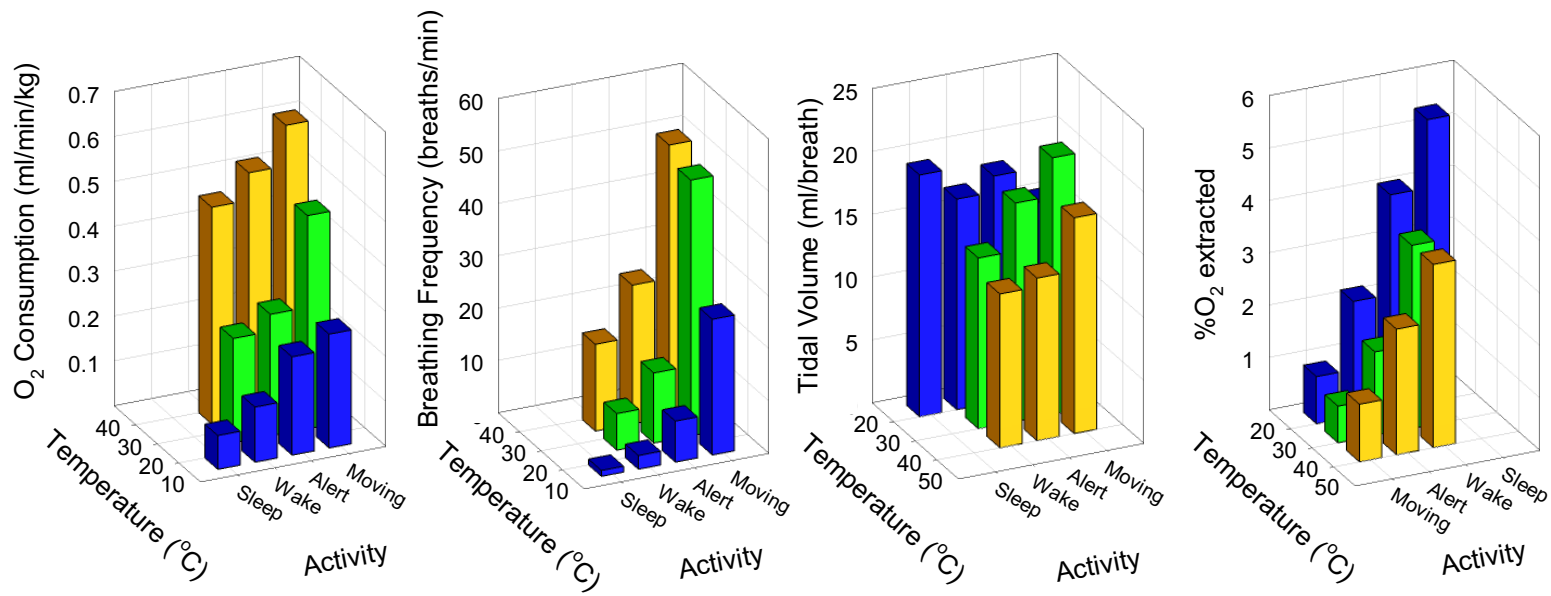
Figure 3





$$\dot{V}O_2 = f_H \times O_2 \text{ pulse}$$

Figure 4



$$\dot{V}_{O_2} = f_R \times V_T \times \%O_2 \text{ extracted}$$

Figure 5

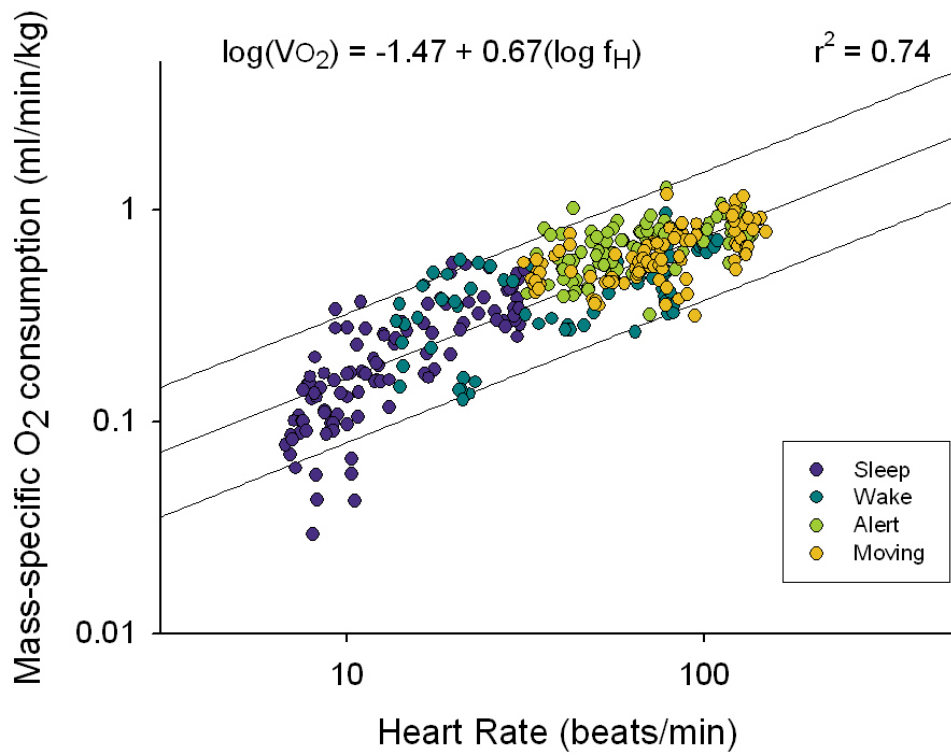
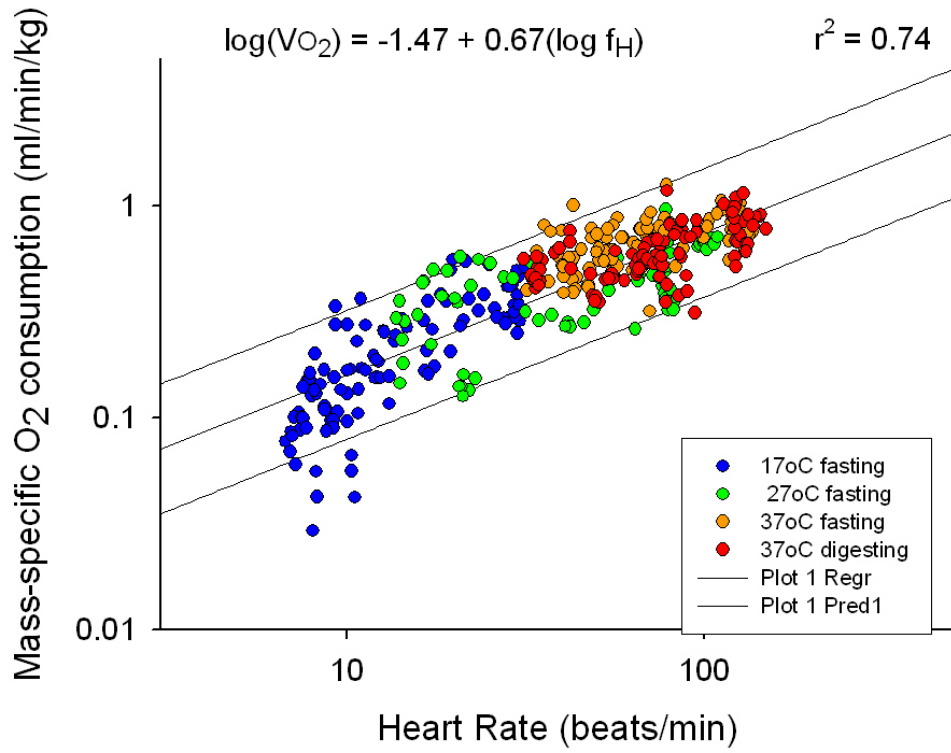


Figure 6

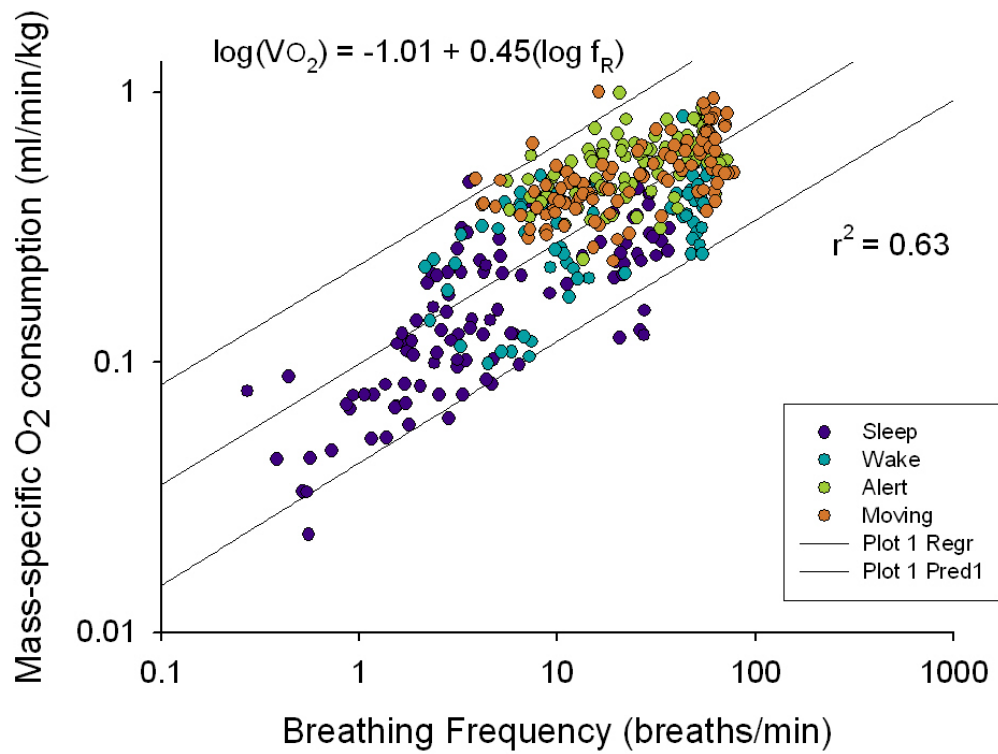
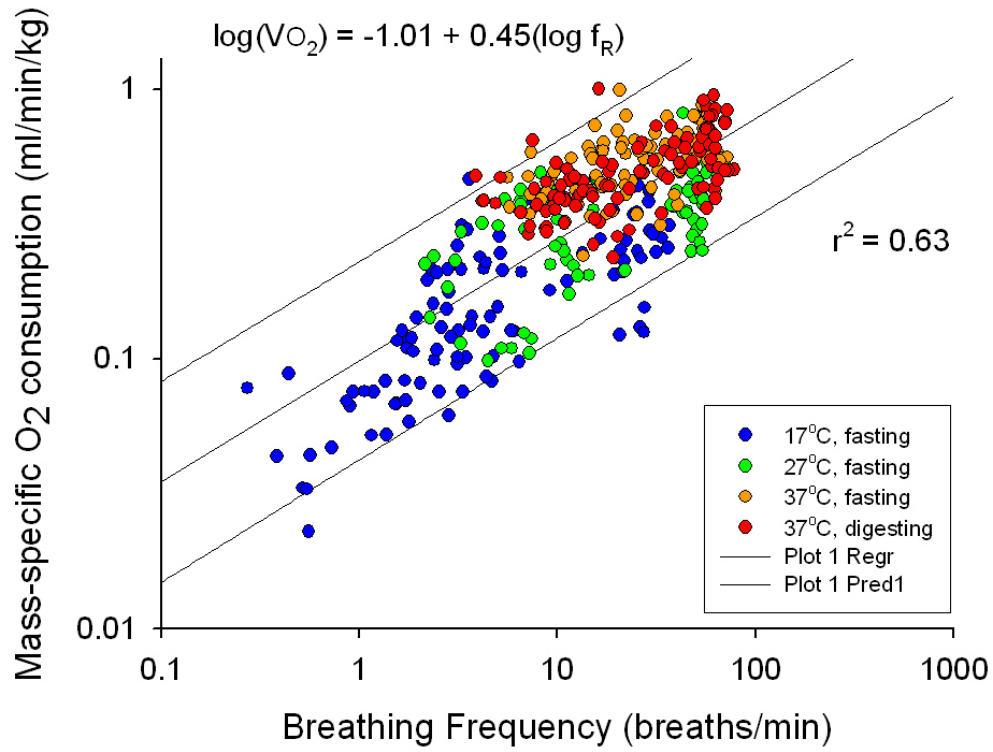


Figure 7

Table 1: Mean values of all variables measured in Series 2. Values for activities that were statistically different from those measured in the wake state are indicated by a \*, and values for temperatures that were statistically different from those measured at 27°C are indicated by a #

Variable	Activity	17°C	27°C	37°C	37°C+Food
<b>O<sub>2</sub> Consumption (ml/min/kg)</b>	Sleep	0.08 ± 0.01*	-	-	-
	Wake	0.12 ± 0.02#	0.23 ± 0.05	0.48 ± 0.03#	0.40 ± 0.03
	Alert	0.22 ± 0.03*##	0.27 ± 0.05*	0.54 ± 0.04*#	0.52 ± 0.06*
	Moving	0.25 ± 0.03*##	0.48 ± 0.08*	0.63 ± 0.06*#	0.66 ± 0.07*
<b>CO<sub>2</sub> Production (ml/min/kg)</b>	Sleep	0.05 ± 0.01*	-	-	-
	Wake	0.10 ± 0.02#	0.20 ± 0.04	0.34 ± 0.04#	0.29 ± 0.03#
	Alert	0.18 ± 0.02*##	0.28 ± 0.05*	0.41 ± 0.03*#	0.40 ± 0.05*#
	Moving	0.28 ± 0.03*##	0.47 ± 0.06*	0.57 ± 0.04*#	0.56 ± 0.05*#
<b>Heart Rate (beats/min)</b>	Sleep	6.2 ± 0.4	-	-	-
	Wake	8.2 ± 1.0#	19.3 ± 4.6	40.5 ± 5.6#	45.3 ± 4.7#
	Alert	11.6 ± 1.8*##	27.3 ± 4.6	49.7 ± 3.6*#	56.2 ± 6.0*#
	Moving	18.4 ± 1.8*##	60.3 ± 3.8*	87.4 ± 5.3*#	89.4 ± 7.1*#
<b>Cardiac O<sub>2</sub> Pulse (ml/beat/kg)</b>	Sleep	0.012 ± 0.002	-	-	-
	Wake	0.015 ± 0.002	0.014 ± 0.004	0.013 ± 0.001	0.010 ± 0.001
	Alert	0.020 ± 0.002*##	0.012 ± 0.003	0.011 ± 0.001	0.010 ± 0.001
	Moving	0.014 ± 0.001#	0.008 ± 0.001*	0.007 ± 0.000*	0.007 ± 0.001*
<b>Tidal Volume (ml)</b>	Sleep	19.2 ± 3.6	-	-	-
	Wake	16.8 ± 2.0	13.6 ± 0.5	12.2 ± 1.4	13.6 ± 1.5
	Alert	18.0 ± 1.6	17.4 ± 4.3	12.9 ± 1.9	12.2 ± 2.5
	Moving	15.3 ± 1.3	20.4 ± 2.3	17.2 ± 1.4	16.0 ± 2.5
<b>Breathing Frequency (breaths/min)</b>	Sleep	1.1 ± 0.1*	-	-	-
	Wake	2.6 ± 0.2#	7.0 ± 1.6	16.6 ± 2.8#	17.6 ± 6.2
	Alert	7.8 ± 1.6*##	13.4 ± 1.1*	26.5 ± 1.8*#	29.6 ± 4.2*
	Moving	25.9 ± 1.1*##	48.9 ± 3.1*	51.9 ± 3.4*	56.5 ± 5.2*
<b>% O<sub>2</sub> Extracted</b>	Sleep	5.4 ± 0.2	-	-	-
	Wake	4.1 ± 0.1	3.5 ± 0.2	3.5 ± 0.1	2.9 ± 0.1
	Alert	2.2 ± 0.1	1.6 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
	Moving	0.9 ± 0.1*	0.7 ± 0.0*	1.1 ± 0.0*	1.1 ± 0.1
<b>Total Ventilation (ml/min/kg)</b>	Sleep	7 ± 1*	-	-	-
	Wake	14 ± 2#	31 ± 8	65 ± 15#	66 ± 16
	Alert	47 ± 11*##	79 ± 25*	106 ± 13*#	104 ± 15
	Moving	130 ± 14*##	327 ± 51*	285 ± 25*	281 ± 38

**Table 2:** Q<sub>10</sub> values of directly measured variables in Series 2.

Variable	Activity	17°C to 27°C	27°C to 37°C	17°C to 37°C
Oxygen Consumption	Sleep	-	-	-
	Quiet	2.3 ± 0.8	2.4 ± 0.8	2.1 ± 0.2
	Alert	1.3 ± 0.1	2.1 ± 0.3	1.6 ± 0.1
	Moving	1.9 ± 0.2	1.4 ± 0.2	1.6 ± 0.1
CO <sub>2</sub> Production	Sleep	-	-	-
	Quiet	2.3 ± 0.4	1.7 ± 0.2	1.9 ± 0.2
	Alert	1.7 ± 0.3	1.6 ± 0.3	1.5 ± 0.1
	Moving	1.8 ± 0.2	1.3 ± 0.2	1.5 ± 0.1
Heart Rate	Sleep	-	-	-
	Quiet	2.0 ± 0.3	2.5 ± 0.5	2.2 ± 0.1
	Alert	2.5 ± 0.6	2.2 ± 0.4	2.2 ± 0.2
	Moving	3.4 ± 0.3	1.5 ± 0.1	2.2 ± 0.1
Breathing Frequency	Sleep	-	-	-
	Quiet	2.9 ± 0.5	2.4 ± 0.6	2.6 ± 0.1
	Alert	2.2 ± 0.6	2.0 ± 0.3	2.0 ± 0.2
	Moving	1.9 ± 0.2	1.1 ± 0.1	1.4 ± 0.1

Table 3: Regression variables and estimation of fit ( $r^2$ ) for mass-specific oxygen consumption versus heart rate data. Simple linear regressions were calculated for pooled untransformed and pooled transformed data. SEE represents the standard error of the estimate. All regressions were significant. (N = 380)

x	y	slope	Intercept	SEE	$r^2$
$f_H$	$s\dot{V}_{O_2}$	$0.007 \pm 0.000$	$0.197 \pm 0.014$	0.148	0.663
$\ln f_H$	$s\dot{V}_{O_2}$	$0.242 \pm 0.008$	$-0.334 \pm 0.029$	0.139	0.704
$\ln f_H$	$\ln s\dot{V}_{O_2}$	$0.669 \pm 0.021$	$-3.184 \pm 0.074$	0.357	0.735

Table 4: Regression variables and estimation of fit ( $r^2$ ) for mass-specific oxygen consumption versus breathing rate data. Simple linear regressions were calculated for pooled untransformed and pooled transformed data. SEE represents the standard error of the estimate. All regressions were significant. (N = 380)

x	y	slope	Intercept	SEE	$r^2$
$f_R$	$s\dot{V}_{O_2}$	$0.008 \pm 0.000$	$0.301 \pm 0.015$	0.190	0.449
$\ln f_R$	$s\dot{V}_{O_2}$	$0.154 \pm 0.007$	$0.086 \pm 0.021$	0.171	0.554
$\ln f_R$	$\ln s\dot{V}_{O_2}$	$0.439 \pm 0.018$	$-2.058 \pm 0.053$	0.431	0.631