Heart rate as a predictor of metabolic rate in heterothermic bats

Shannon E. Currie, Gerhard Körtner, Fritz Geiser

Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale NSW 2351, Australia

Abstract

While heart rate ($f_H$) has been used as an indicator of energy expenditure, quantitative data showing the relationship between these variables are only available for normothermic animals. To determine whether $f_H$ also predicts oxygen consumption ($V_O^2$) during torpor we simultaneously measured $V_O^2$, $f_H$ and subcutaneous body temperature ($T_{sub}$) of a hibernator, Gould’s long-eared bats ($Nyctophilus gouldi$, 9 g, n=18), at ambient temperatures ($T_a$) between 0 and 25°C. At rest, $f_H$ of normothermic resting bats was negatively correlated with $T_a$, with maximum $f_H$ of 803 bpm ($T_a=5^\circ C$). During torpor the relationship between $f_H$ and $T_a$ was curvilinear, and at low $T_{sub}$ (~6°C) $f_H$ fell to a minimum average of 8 bpm. The minimum average values for both $V_O^2$ and $f_H$ in torpor reported here were among the lowest recorded for bats. The relationship between $f_H$ and $V_O^2$ was significant for both resting ($r^2=0.64$, $p<0.001$) and torpid bats ($r^2=0.84$, $p<0.001$), with no overlap between the two states. These variables were also significantly correlated ($r^2=0.44$, $p<0.001$) for entire torpor bouts. Moreover, estimates of $V_O^2$ from $f_H$ did not differ significantly from measured values during the different physiological states. Our study is the first to investigate the accuracy of $f_H$ as a predictor of $V_O^2$ during torpor and indicates the reliability of this method as a potential measure of energy expenditure in the field. Nevertheless, $f_H$ should only be used to predict $V_O^2$ within the range of activities for which robust correlations have been established.

Introduction

Energy is essential for all life processes and therefore its appropriate use and acquisition are crucial for animals. Measurements of energy expenditure of free-ranging animals is of particular interest to ecological and evolutionary physiologists as they provide an understanding of how animals budget their energy. Use of energy in captive animals is often quantified as basal metabolic rate (BMR) in resting animals under thermo-neutral conditions, and it is often assumed to be proportional to field metabolic rates (FMR). However such extrapolations can misrepresent energy demands of individuals in the wild (Nagy, 1987; Koteja, 1991). Ambient temperature ($T_a$), activity, reproductive status,
resource availability, and predator avoidance are just a few of the challenges faced by animals in their natural habitats that require appropriate adjustments of energy expenditure which are not likely represented by extrapolations from BMR. This is particularly evident in a species whose BMRs are lower than expected based on size, and have a FMR that is much higher than predicted (Geiser and Coburn, 1999).

Currently, there are two widely used quantitative approaches for direct measurement of FMR that overcome these problems -- the doubly labelled water (DLW) and heart rate ($f_H$) methods (Nagy, 1987; Speakman, 2000; McCarron et al., 2001). The DLW method quantifies CO$_2$ production over time by measuring the proportional washout of isotopically labelled oxygen and hydrogen from body water. It is generally used as a measure of daily energy expenditure (DEE) in the field and has been the most widely applied approach for measuring FMR. The high metabolic turnover and elusive nature of many small animals means that DLW measurements are often restricted to short time periods reducing the robustness of the technique. Moreover, free-ranging small animals show enormous differences in energy expenditure between activity and rest, the differences of which cannot be made apparent from average DEE.

An extreme example of temporal fluctuations in energy expenditure and body temperature ($T_b$) is torpor which is characterised by a controlled reduction in metabolism, often to up to 1-10% of BMR (Geiser, 2004). Bouts of torpor can last for short periods of time <24h in daily heterotherms or up to days or weeks in hibernators (Geiser and Ruf, 1995). Although DLW provides a valid measure of energy expenditure over time, this approach cannot reveal the pronounced short-term physiological changes, and metabolic savings, associated with heterothermy. For example, relative low FMR in insectivorous bats for their size (Nagy et al., 1999) only suggests that these bats may have used torpor during the period of sampling but could not provide any further information regarding torpor use in the energy budgets of these animals.

The heart rate method, on the other hand, relies on the intrinsic relationship between oxygen consumption (VO$_2$) and $f_H$, and can provide instantaneous and continuous measurements of metabolism over extended periods (~1yr; McPhee et al., 2003). This permits comparisons of energy expenditure across various activities and life stages and provides a more specific understanding of the various components of an animal’s cost of living. Several validation studies comparing both DLW and $f_H$ to standard respirometry show that $f_H$ is as accurate a predictor of metabolic rate as DLW in homeothermic birds and mammals (Bevan et al., 1994; McCarron et al., 2001; Butler et al., 2004).

As the relationship between $f_H$ and VO$_2$ changes with exercise it has been important to validate the method for a range of activities. Statistically significant relationships between $f_H$ and VO$_2$ have been demonstrated for normothermic animals whilst walking (Bevan et al., 1994), diving (Bevan and
Butler, 1992), flying (Weimerskirch et al., 2000; Ward et al., 2002) and swimming (Nolet et al., 1992; McPhee et al., 2003); with \( f_{\text{H}} \) providing a more precise estimate of \( \dot{V}O_2 \) than DLW in some cases. Unfortunately, current data are mainly limited to large homeothermic mammals and birds. However, in recent years technological advancements have led to the development of small, lightweight devices for \( f_{\text{H}} \) telemetry, making measurements of heart rate in small animals feasible (Dechmann et al., 2011).

Considering the dramatic changes in \( \dot{V}O_2 \) between rest and torpor, and the fact that >1/2 of all mammalian orders contain heterothermic species (Geiser, 2013), knowledge about whether the same relationship between \( \dot{V}O_2 \) and \( f_{\text{H}} \) applies is highly desirable. It is known the heart rate decreases with metabolic rate during torpor, but whether it can be used to estimate energy expenditure has not been established. We therefore aimed to determine the accuracy of \( f_{\text{H}} \) as a measure of \( \dot{V}O_2 \) in long-eared bats (\textit{Nyctophilus gouldi}) during normothermia and torpor as a function of \( T_b \) and \( T_a \). This insectivorous bat hibernates in temperate areas of Australia and spends a large proportion of its life in a state of torpor (Turbill and Geiser, 2008), but there are no data on FMR for the species. Additionally, we investigated the precision of the \( f_{\text{H}} \) method during entire torpor bouts, incorporating the transitional periods of torpor entry and arousal. Detailed knowledge of the relationship between \( \dot{V}O_2 \) and \( f_{\text{H}} \) is particularly important for the study of bats because their metabolism changes substantially between activity, rest, and especially during torpor, which may be used throughout the year.

**Results**

All bats entered torpor overnight or in the early morning and, following a partial arousal associated with ECG lead attachment, \( \dot{V}O_2 \) and \( f_{\text{H}} \) fell concurrently and reached steady-state minima when \( T_{\text{sub}} \) was declining and within ~2°C of minimum \( T_{\text{sub}} \) (Fig. 1). Disturbance did not affect minimum \( \dot{V}O_2 \) values (paired t-test; \( t=2.0478, \text{df}=9, p>0.05 \)). At \( T_a \) below 20°C bats remained torpid until shortly after lights off when \( \dot{V}O_2, f_{\text{H}} \) and \( T_{\text{sub}} \) increased beginning with an increase in \( \dot{V}O_2 \) and \( f_{\text{H}} \) associated with evening arousal.

Mean resting \( f_{\text{H}} \) of normothermic bats was a linear function of \( T_s \) (\( r^2 = 0.82 \)) and increased with decreasing temperature from 228 to 706 bpm at \( T_s \) between 25 and 2°C (Fig. 2). The corresponding mean resting \( \dot{V}O_2 \) ranged from 1.27 to 11.18 ml g\(^{-1}\) h\(^{-1}\) (not shown). Normothermic resting \( T_{\text{sub}} \) was not affected by \( T_s \) and the mean was 34.5±1.0°C (\( n=8 \)). The maximum \( f_{\text{H}} \) recorded was 803 bpm at a \( T_s \) of 5°C. During torpor, \( f_{\text{H}} \) was reduced curvilinearly with \( T_s \) to values as low as 3.5% of resting heart rates at the same \( T_s \), while \( \dot{V}O_2 \) was reduced to ~1% resting \( \dot{V}O_2 \) (Figs 3 & 4). The maximum average resting \( f_{\text{H}} \) was ~90-fold higher than the minimum average \( f_{\text{H}} \) in torpor.
At Ta between 1 and 25°C the mean f_H over 30 mins of torpid bats ranged from 8 to 144 bpm with correspondent VO_2 from 0.02 to 0.46 ml g^{-1} h^{-1}. Even at Ta of 25°C average f_H during torpor was only 35% of normothermic bats. Heart rate during torpor was a curvilinear function of T_{sub} when plotted on a linear scale, with a Q_{10} of 2.0 (Fig. 3). Average f_H of normothermic bats ranged from 1.3-fold to 4-fold the values predicted by the extrapolated curve of torpid bats against T_{sub} (Fig. 3). The minimum f_H recorded over 1 min was 5 bpm at Ta = 0°C.

Log_{10} transformation resulted in a linear function for both VO_2 and f_H against T_{sub} during torpor (Fig. 4). The Q_{10} of VO_2 for torpid bats (2.5) was similar to f_H (2.0), resulting in two near parallel curves. The slopes of log_{10} transformed f_H and VO_2 against T_{sub} during torpor did not differ significantly (ANCOVA; p>0.05) (Fig. 4). As average minimum f_H increased from 15 bpm at T_{sub} 5.5°C to 113 bpm at T_{sub} 26°C average minimum VO_2 increased from 0.04 to 0.40 ml g^{-1} h^{-1}. Consequently, an increase in f_H by approximately 100 bpm resulted in a 10-fold increase in VO_2.

The VO_2 and f_H were strongly correlated at rest and during torpor (Fig. 5). However extrapolation of the line derived from bats during torpor fell below values for normothermic bats and the slopes differed enormously (ANCOVA; p<0.01) (Fig. 5). There was no overlap in recorded averages, with none of the normothermic points falling on the torpor regression and vice-versa. The relationship between VO_2 and f_H in resting normothermic bats is described by the equation:

\[ \dot{V}O_2 = 0.02(f_H) - 3.458, \quad (r^2=0.64, \ p<0.001) \]

where f_H is in bpm and VO_2 is measured in ml g^{-1}h^{-1}. For each individual, estimates of resting VO_2 did not differ significantly from direct measurements at the same f_H (paired t-test; t=0.33, df=9, p=0.749). This was calculated by sequentially removing data from one individual from equation 1 and recalculating the regression using data from the remaining bats. During torpor the relationship between VO_2 and f_H was described by the equation:

\[ \dot{V}O_2 = 0.004(f_H) - 0.013, \quad (r^2=0.84, \ p<0.001). \]

Discussion

Our study is the first to provide continuous quantitative data on heart rate, metabolic rate and T_{sub} simultaneously and as a function of T_a for a microbat. We demonstrate a strong positive correlation...
between metabolism and cardiac function at rest and during torpor. The data suggest that \( f_H \) can be used to reliably quantify energy expenditure of bats, at least during torpor and rest, in the wild.

Bats showed a strong proclivity to enter torpor in captivity and despite disturbance associated with heart rate measurements, exhibited similar temporal patterns of torpor use described for the same species in previous studies (Geiser and Brigham, 2000). In addition, our results showed that the relationship between \( \dot{V}O_2, f_H \) and \( T_{sub} \) as bats entered torpor progressed in a pattern qualitatively similar to other hibernators and daily heterotherms (Lyman, 1958; Swoap and Gutilla, 2009). The minimum average values for both \( \dot{V}O_2 \) and \( f_H \) in torpor reported here were amongst the lowest recorded for bats. At \( T_a \) of 9-11°C minimum \( \dot{V}O_2 \) of torpid bats was not significantly different from previous data for this species (Geiser and Brigham, 2000; two-tailed t-test, \( t=0.778, \text{df}=21, \text{p}>0.05 \)). Minimum mean \( f_H \) (8bpm) in particular was well below values reported for unrestrained northern hemisphere bats of a similar mass (40bpm; Kulzer, 1965). Moreover, the absolute minimum of 5 bpm was similar to that measured in much larger hibernators such as woodchucks (\( Marmota monax \) 3-5kg; Lyman, 1958) and dormice (\( Glis glis \) ~150g; Elvert and Heldmaier, 2005).

Interestingly, reported \( f_H \) values of ~40bpm in other studies were similar to those of thermo-regulating individuals (bats that maintained a \( T_{sub}-T_a \) differential >2°C when in torpor) at the same \( T_a \) in our study (not shown). This suggests that bats in previous investigations were not thermo-conforming and were not in steady-state torpor. It also indicates the need for simultaneous measurements of other physiological variables such as \( T_b \) to enhance the reliability of \( f_H \) data in torpor.

The maintenance of higher \( T_b-T_a \) differentials in thermo-regulating torpid bats will reduce the energy savings associated with torpor when compared to animals who are thermo-conforming at the same \( T_a \) because a higher differential results in higher heat loss that needs to be compensated for. However small this difference may be, extended periods of time spent thermo-regulating during torpor will increase energy demands. In free-living bats, increased energy expenditure associated with disturbance, including that caused by pathogens, has been suggested to deplete energy stores required for survival of the hibernation season, increasing mortality (Speakman et al., 1991; Thomas, 1995; Warnecke et al., 2013). Therefore precise and detailed measurements of \( f_H \) for bats in different physiological states are required if the \( f_H \) method is to be used to quantify energy expenditure in the wild. To investigate this in free-ranging animals measurements of temperature are required, both of the individual and their surroundings, to enable better interpretation of data and provide information regarding \( T_b-T_a \) differentials. It also has the potential to provide instant information remotely regarding natural disturbances of bats during torpor (i.e. perceived predation risks etc) and how often thermo-regulatory heat production is used in free-living animals.
We show that during steady-state torpor and at rest the relationships between $f_{H}$ and $\dot{V}O_2$ are strongly linear. However, this same relationship may not be maintained during more dynamic periods of torpor entry and arousal. During entry into torpor peripheral blood flow is restricted and $T_b$ declines associated with the change in $T_b$ set point (Lyman et al., 1982). Although there is little change in blood pressure, associated with increased viscosity of cold blood, heart rate and metabolism are actively suppressed as demonstrated by high $Q_{10}$ (Milsom et al., 1999; Geiser, 2004). As animals arouse from torpor, there is an enormous increase in $\dot{V}O_2$ and $f_{H}$ required for increasing $T_b$. Associated with this is a decrease in blood viscosity and reperfusion of the peripheries and organs which could alter the relationship between $f_{H}$ and $\dot{V}O_2$. We therefore investigated whether or not a strong linear correlation remains between $\dot{V}O_2$ and $f_{H}$ when averaged across a complete torpor bout (i.e. from peak values after partial arousal before torpor, to peak values following final arousal; refer to arrows in Fig. 1). Our results show that the relationship between $\dot{V}O_2$ and $f_{H}$ was still significant with the inclusion of torpor entry and arousal ($r^2=0.44$, $p<0.001$), regardless of time spent in torpor or whether $T_a$ remained constant throughout a torpor bout (Fig. 6). This signifies the precision of $f_{H}$ as a predictor of $\dot{V}O_2$, not only during steady-state conditions but throughout the transition between physiological states.

Nevertheless, $f_{H}$ should only be used to predict $\dot{V}O_2$ within the range of activities for which robust correlations have been established (Nolet et al., 1992). This is of particular importance when studying heterothermic animals as we have demonstrated a distinct difference between resting and torpor regressions; with no overlap between the two states. At rest both $f_{H}$ and $\dot{V}O_2$ were related to $T_a$ in a linear fashion but this became curvilinear when animals were in torpor. Not surprisingly, none of the values for torpor fell near the line derived from $f_{H}$ against $\dot{V}O_2$ in normothermic resting bats. Our results support the findings of Song et al. (1997) and demonstrate that torpor is not just an extrapolated reduction of $f_{H}$ and $\dot{V}O_2$ as a function of temperature differentials. Moreover, extrapolation from the regression of torpid bats underestimated resting $\dot{V}O_2$ by as much as 75%, emphasizing the importance of determining correlations for different physiological states.

Essential to any study of energy expenditure in bats is an understanding of the physiological mechanisms and costs associated with flight. Flight is the most energetically expensive form of locomotion (Schmidt-Nielsen, 1972) and energy expenditure in small (5g) flying bats has been shown to be $>16$ times higher than at rest (Voigt and Lewanzik, 2012). Strong correlations between $f_{H}$ and $\dot{V}O_2$ during flight have been reported for geese flying in a wind tunnel and this relationship differed significantly from that of walking geese, with no overlap between exercises (Ward et al., 2002). This illustrates that extrapolations from resting values may be grossly inaccurate for flying bats. In phyllostomid bats heart rate doubled at the onset of flight, while oxygen consumption increased 4-fold.
and both $f_{HI}$ and VO$_2$ returned to resting levels within 30 seconds of landing (Thomas and Suthers, 1972), further indicating a need for calibration over fine time scales. As flight is essential for survival of all bats and constitutes the highest energetic demands on individuals, determination of correlations between $f_{HI}$ and VO$_2$ during flight for bat species is essential before this method can be used to quantify energy expenditure in the field.

The DLW method can only provide average energy expenditure over time with relatively low values in bats suggestive of torpor use (Nagy et al., 1999). Speakman and Racey (1988) showed that a 5-fold range of average energy metabolism could be generated using this method, when bats employed torpor to differing degrees. Here we show that regardless of torpor bout length there is a strong correlation between $f_{HI}$ and VO$_2$ across a complete torpor bout and at rest, consistent with the potential for the $f_{HI}$ method to reliably measure field energy expenditure. Although it has been suggested in the past that the $f_{HI}$ method becomes prohibitively expensive when applied to animals smaller than 1kg (Butler et al., 2004), miniaturized heart rate transmitters are becoming more readily available and can be used on animals as small as 10g (Dechmann et al., 2011). Our study highlights the need for validation of this method for small heterothermic animals as torpor plays an important role in energy budgets for these animals and extrapolations from resting values are grossly inaccurate. This may also warrant the development of a torpor ‘cut off method’ for heart rate similar to those used for T$_b$ or metabolic rate in most studies of heterothermy in free-living mammals and birds.

Material and Methods

We used open-flow respirometry, electrocardiograms (ECG) and temperature-sensitive passive integrated transponders to measure the relationship between metabolic rate and heart rate of long-eared bats (Nyctophilus gouldi) during torpor at a range of T$_a$ (0-25°C). Measurements were conducted on a total $n=9$ female and $n=9$ male N. gouldi (mass at capture: 10.5±1.5 g) from May-July 2011 and March-July 2012 (Autumn/Winter). Bats were captured in mist nests at Imbota Nature Reserve and Newholme Field Station near Armidale, New South Wales (30°35'S, 151°44'E). Both field sites are temperate open woodland areas at approximately 1000 m elevation. Captured animals were transferred to the University of New England and kept in captivity for a maximum period of seven months. Bats were kept in large outdoor flight cages with maximum 8 animals per cage and provided mealworms and water ad libitum. Twice weekly mealworms were dusted with a supplement of Wombaroo™ Insectivore Rearing Mix. Additional food of moths and other flying insects were attracted into cages by a UV light. Bats remained within 1g of their body mass at the time of capture while in captivity.
This study was conducted under a scientific license provided by the NSW Parks and Wildlife Authority (SL100084) and with Animal Ethics approval from the University of New England (AEC11-016).

Transponder Implantation

Subcutaneous body temperature (T_{sub}) was measured using temperature-sensitive transponders (IPTT-300 Bio Medic Data Systems Implantable Programmable Temperature Transponder, Delaware, 0.13 g, 14 mm x 2 mm) implanted interscapularly. For small mammals T_{sub} is closely related to T_{b}, particularly during torpor when T_{b}-T_{a} differentials are often 1°C or less (Wacker et al., 2012). Transponders were calibrated over a range of 5 to 40°C to the nearest 0.1°C against a precision reference thermometer in a water bath prior to use.

Bats were given a minimum of 3 days to acclimate to captivity and ensure stable body mass before transponder implantation. Transponders were implanted under general Isoflurane/oxygen anaesthesia. The skin was sterilized with 70% alcohol before a small (~3 mm) incision was made in the skin just below the shoulder blades for transponder insertion. The insertion site was closed with a single suture (chronic gut, Ethicon, Somerville USA) and the entire process was complete within fifteen minutes. Bats were given 24h to recover in a warm room before being returned to outdoor flight cages.

Respirometry

Bats were placed in respirometry chambers in the early evening and metabolic rate, measured as oxygen consumption (V_{O2}), was monitored overnight and throughout the following day(s) to allow animals to undergo their usual daily thermal cycle. Bats were weighed (±0.1 g) immediately prior to measurement and were removed from the chamber following arousal from torpor on subsequent days and reweighed. A linear rate of mass loss was assumed over each day to calculate mass-specific V_{O2} values.

Respirometry chambers were made from modified polycarbonate enclosures with clear lids (0.26, 0.40, or 0.53 L), lined with a small patch of hessian from which the bats could roost. Chambers were placed inside a temperature-controlled cabinet. Chamber size was randomized between measurements and the values obtained were not affected by chamber volume (ANOVA; p>0.05). The T_{a} (±0.1°C) was recorded using a calibrated thermocouple placed 5 mm within the chamber and read using a digital thermometer. Air flow (165-230 ml min^{-1}) was adjusted based on chamber size to ensure that 99% equilibrium was reached within <11 minutes, controlled with rotameters and measured with mass flowmeters (Omega FMA-5606; Stamford, CT, USA).
Oxygen concentration was measured in a constant temperature room using either Sable Systems FC-1B Oxygen Analyser or FOX Field Oxygen Analyser (Version 1.01, FXO301-01R). Measurements were taken from the chamber every minute for 15 minutes and then switched to outside air for reference readings (3 min) using solenoid valves. Outputs of the digital thermocouple thermometer, flowmeter and oxygen analyser were recorded using custom data-acquisition software (G.K.) onto a personal computer. The VO₂ was calculated using standardised gas volumes and Eq. 3a of Withers (1977). A respiratory quotient of 0.85 was assumed throughout.

Tsub was read from each animal with a DAS-7006/7R/S Handheld Reader (Bio Medic Data Systems) which was connected to a personal computer and programmed to take readings every minute, concurrent with respirometry measurements. Tb was measured to the nearest 0.1°C by inserting a fine calibrated thermocouple probe 1.5-2 cm rectally. Rectal Tbs were taken within 30 seconds of removal from respirometry chambers and compared to simultaneous readings of Tsub, with Tsub ≤ 1.5°C of Tb when animals were in torpor. Transponder function varied and on occasion transponders temporarily stopped working when the Tsub of animals in torpor fell below 7°C. In these cases Tsub was estimated to be 0.5°C above Ta, as this was the average differential for animals with similar VO₂ whose transponders continued to work at low Ta.

Electrocardiograms

Measurements of fH were recorded using the methods of Zosky (2001). Individuals were placed in respirometry chambers in the evening and left until the following morning, when VO₂ reached steady state values, ECG wires (Lead I arrangement) were attached to adhesive electrodes on the bat’s forearm just after lights on. This resulted in a partial arousal from torpor in most cases, however, VO₂ soon returned to similar or lower values than prior to the disturbance and did not differ significantly (paired t-test; t=2.0478, df=9, p>0.05). The data were therefore considered representative of steady-state torpor.

Electrodes were fashioned from Kendall Care Resting ECG Electrodes (Tyco Healthcare Group) cut into strips of appropriate length and width to fit the forearm of the bat. Lead wires were made from modified Kittycat™ Paediatric Monitoring Electrodes (Tyco Healthcare Group) fitted with customised clips at one end. ECGs were measured using either a FE132 BioAmp or ML135 Dual BioAmp (ADInstruments) connected to a Powerlab 4/35 Data Acquisition System and recorded with LabChart Pro v7.3 software. Electrocardiograms were analysed to calculate instantaneous fH, which was averaged per second using LabChart Pro v7.3 and exported to Microsoft Excel for further analysis.

Statistical Analyses
For the purpose of our study, only data for normothermic resting and thermo-conforming animals in steady-state torpor were used for regression analyses (bats that maintained a high $T_{sub} - T_a$ differential when in torpor were considered to be thermo-regulating, and were excluded from analyses). Average minimum values of $V\dot{O}_2$, $f_H$, and $T_{sub}$ during torpor were taken from times when all variables were lowest for at least 30 min. At $T_a$ below 10°C periods of apnoea were generally longer than 30 min (S.E. Currie, unpublished), and in such cases the sampling time was extended to 45 min to include representative periods of breathing to be able to estimate metabolic rate indirectly. Average $V\dot{O}_2$ and $f_H$ during torpor were calculated for thermo-conforming animals that entered torpor for <24h exposed to constant $T_a$. Averages were taken from peak values following partial arousals in the morning, to the peak following arousal from torpor in the afternoon or following lights out (for example; Fig. 1- time between the arrows). On occasion torpor lasted for >24h or bats were exposed to more than one $T_a$ during a torpor bout. Animals were exposed to a maximum of 3 different $T_a$s for ≥1.5hrs each. The average $V\dot{O}_2$ under these conditions fell within the range of values for animals exposed to only one temperature, and therefore all data were pooled for analysis.

The Q_{10} for $V\dot{O}_2$ of thermo-conforming torpid bats was calculated using the following equation: $Q_{10} = (V\dot{O}_21/ V\dot{O}_22)^{10/(T_b1 - T_b2)}$. Values for resting $V\dot{O}_2$, $f_H$, and $T_{sub}$ were taken from the period following arousal. Due to impedance of the ECG associated with bat movement and/or individuals’ intolerance of the electrodes, resting values could only be averaged over a 5-min period. Furthermore, following arousal from torpor bats often moved out of range of the transponder scanner, which was ~ 5cm, and therefore $T_{sub}$ was occasionally unavailable. This resulted in more $f_H$ values of resting normothermic bats against $T_a$ than $T_{sub}$.

Statistical analyses were performed using R v2.15.2. Standardized major axis regressions were performed using the smatr package (Warton et al., 2012) and we accounted for pseudo-replication by using the degrees of freedom as for mixed effect linear modelling that are adjusted for repeated measures. Two sample t-tests were used to compare mean $V\dot{O}_2$ before and after disturbance associated with ECG lead attachment, and predicted $V\dot{O}_2$ values with measured values. Analysis of covariance (ANCOVA) was used to compare slopes of regression equations. Means are reported ± SD for the number of individuals ($n$).

Acknowledgements

The authors thank Daniella Rojas, Artiom Bondarenco and Chris Wacker for their tireless help with animal care and experimentation, and Kodie Noy, Danielle Sisson and Kathryn Lambert for their help in the field.

Funding
Financial support for this study was received from the University of New England and the Australian Research Council.

**Author Contributions**

All authors contributed to writing the paper and devising the study; S.E.C collected and cared for the bats, carried out the experimental protocol and analysed the data. G.K. and F.G were also involved in data analysis and initial design of the experimental protocol.

**Competing Interests**

No competing interests declared.
Fig. 1. Representative example of heart rate ($f_h$; dotted line), oxygen consumption ($\dot{V}O_2$; solid line), and subcutaneous temperature ($T_{sub}$; filled circles- missing data are from the bat moving out of range of the scanner) of *N. gouldi* at ambient temperature of 10°C; the dark bar on the horizontal axis represents scotophase. The animal entered torpor in the early morning prior to lights on, exhibited a partial arousal associated with ECG lead attachment (indicated by solid arrow) and then proceeded to re-enter and remain in torpor until spontaneously arousing when the lights went off in the evening (indicated by dashed arrow).

Fig. 2. Heart rate of normothermic resting (open circles) and torpid (filled circles) *N. gouldi* as a function of ambient temperature. Each point represents an individual measurement taken from 18 individuals in total. Heart rate in resting normothermic bats increased linearly with decreasing $T_a$: $f_h$(bpm) = 664.8 – 12.68$T_a$(°C), $r^2$=0.82, p<0.01.

Fig. 3. Heart rate of normothermic resting (open circles) and torpid (filled circles) *N. gouldi* as a function of subcutaneous temperature. Heart rate increased in a curvilinear pattern with increasing subcutaneous temperature, however the extrapolated curve for data on heart rate in torpid bats fell below values obtained for normothermic resting individuals.

Fig. 4. Average heart rate ($f_h$; filled circles) and oxygen consumption ($\dot{V}O_2$; open circles) of torpid bats plotted on a logarithmic scale against subcutaneous body temperature ($T_{sub}$). Linear regressions for $\log_{10}f_h = 0.03(x) + 0.98$ (solid line); and $\log_{10}\dot{V}O_2 = 0.04(x) - 1.62$ (dashed line).

Fig. 5. Oxygen consumption (ml g$^{-1}$h$^{-1}$) as a function of heart rate (bpm) in normothermic resting (open circles, $n = 34$) and torpid (filled circles, $n = 74$) *N. gouldi* at ambient temperatures between 1 and 25°C. Dashed line represents the regression equation for resting individuals ($\dot{V}O_2 = 0.02(f_h) - 3.458$, $r^2$=0.46, p<0.001) and the solid line represent the regression equation for torpid individuals ($\dot{V}O_2 = 0.004(f_h) - 0.013$, $r^2$=0.84, p<0.001).

Fig. 6. Oxygen consumption (ml g$^{-1}$h$^{-1}$) as a function of heart rate (bpm) averaged for an entire torpor bout (including entry after partial arousal and final arousal) for *N. gouldi* at ambient temperatures between 1 and 25°C, each point represents one bout. The regression equation was; $\dot{V}O_2 = 0.008(f_h) + 0.163$, $r^2$=0.44, p<0.001.
References


McCarron, H. C. K., Buffenstein, R., Fanning, F. D. and Dawson, T. J. (2001). Free-ranging heart rate, body temperature and energy metabolism in eastern grey kangaroos (Macropus giganteus) and red kangaroos (Macropus rufus) in the arid regions of south east Australia. J. Comp. Physiol. (B) 171, 401-411.


