**Thermoregulation under semi-natural conditions in two species of African
barbets (Piciformes: Lybiidae).**

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Abstract

Torpor has been documented in a number of avian orders, but our understanding of the phylogenetic distribution of this phenomenon is far from complete, and several groups have yet to be investigated. We examined patterns of rest- and active-phase thermoregulation in Acacia Pied Barbets (*Lybius leucomelas*) and Crested Barbets (*Trachyphonus vaillantii*) held in outdoor aviaries, using surgically-implanted miniature data loggers to record body temperature (T_b). Both species exhibited large circadian rhythms of T_b , with maximum active-phase and minimum rest-phase T_b (pT_{bmin}) values of 42.3 ± 0.1 °C and 37.7 ± 0.6 °C respectively in Acacia Pied Barbets ($n = 3$) and 42.5 ± 0.4 °C and 37.0 ± 0.6 °C respectively in Crested Barbets ($n = 5$). Neither species exhibited any indication of torpor, despite being subjected to a food restriction treatment that is known to elicit torpor in other taxa. Instead, restricted feeding resulted in small but significant increases in the amplitude of circadian T_b rhythms, with pT_{bmin} values 0.3 - 0.6 °C lower than during ad libitum food availability. The responses of barbets maintained in aviaries may not, however, be representative of those of free-ranging individuals. Body temperature traces we obtained from Acacia Pied Barbets in a field laboratory in the Kalahari Desert reveal reductions in T_b to below 32 °C during the rest-phase, suggesting that in natural habitats, this species may routinely exhibit facultative hypothermia. The amplitudes of circadian T_b rhythms we observed under semi-natural conditions in both species are significantly greater than expected on the basis of an allometric equation based largely on northern Hemisphere data, but are similar to those observed in other southern African species.

Introduction

Many birds and mammals use facultative, reversible reductions in metabolic rate and body temperature (T_b) to avoid mismatches between energy supply and demand (Lyman et al. 1982, Dawson and Whittow 2000). Avian facultative hypothermic responses involve increases in the amplitude of circadian T_b rhythms, and are often categorized as either daily torpor, which may involve T_b reductions of more than 30°C below rest-phase normothermic values, or shallow rest-phase hypothermia, comprising more moderate reductions (Prinzinger et al. 1991, Reinertsen 1996). However, the biological reality of this distinction remains unclear (McKechnie and Lovegrove 2002).

In the last decade, torpor has been reported for the first times in two avian orders, namely the Coraciiformes (Merola-Zwartjes and Ligon 2000, Cooper et al. 2008,) and Columbiformes (Schleucher 2001). Nevertheless, our understanding of the phylogenetic distribution of this phenomenon remains far from complete, and there are numerous orders in which the occurrence of facultative hypothermic responses has not been investigated. Inferring the evolutionary history of avian torpor is complicated by the large body masses (M_b) of several phylogenetically ancient orders (Geiser 1998), and arguably the most interesting taxa in this respect are those that include species with small M_b but which diverged relatively early in avian evolutionary history, namely the Piciformes, Galbuliformes, Bucerotiformes, Upupiformes and Trogoniformes (Sibley and Ahlquist 1990, but see also Hackett et al. 2008).

The African barbets (Piciformes: Lybiidae) include a number of species for whom torpor could conceivably confer selective advantages. In addition to relatively low M_b (< 100 g), many African barbets are largely frugivorous and occur in arid habitats, two factors thought to favour the capacity for torpor (Geiser 2004,

McKechnie and Lovegrove 2002). In this study, we investigated whether two species, the Acacia Pied Barbet (*Lybius leucomelas*) and the Crested Barbet (*Trachyphonus vaillantii*), use torpor during winter when housed under semi-natural conditions. We also report field data from two Acacia Pied Barbets that reveal deeper reductions in T_b than we observed in captive birds.

Materials and methods

Capture and housing

Three Acacia Pied Barbets and five Crested Barbets with mean $\pm$ SD M_b of 35.3 ± 1.6 g and 72.8 ± 8.1 g respectively were trapped during June 2008 using mist nets or spring traps baited with fruit. The Acacia Pied Barbets and three of the Crested Barbets were caught near Mafikeng, South Africa ($25^\circ 45'$ S $25^\circ 48'$ E) and the remaining two Crested Barbets were caught in Pretoria, South Africa ($25^\circ 55'$ S $28^\circ 15'$ E). The birds were housed in outdoor aviaries (5 m long, 1.8 m wide, 1.8 m high) at the University of Pretoria Experimental Farm, where they were provided with an ad libitum diet of fruit (pawpaws, apples, and oranges) and softbill/mynah pellets (Avi-Products, Linkhills, KZN, South Africa), as well as an ad libitum supply of water. The birds experienced natural cycles of air temperature and photoperiod, and roosted in wooden nest boxes.

Measurement of air and body temperature

Air temperature (T_a) was measured and recorded using a Thermochron iButton (model DS1922L, Dallas Semiconductor, Sunnyvale CA, U.S.A.), placed in a shaded site under the ceiling of the aviaries. Body temperature (T_b) was measured and

recorded using iBBats (model 1922L, Alphaspace Mont St-Hilaire, Canada), with a mean mass of 1.7 g. The iBBats were calibrated against a mercury thermometer with accuracy traceable to the US National Bureau of Standards in a circulating water bath over the temperature range of 25 – 45°C before implantation, as well as after removal in order to correct for any drift during the course of the study. The iButton used for T_a measurements was similarly calibrated. After being coated with surgical wax (Elvax, Mini Mitter, Bend, OR, U.S.A.), the iBBats were implanted intraperitoneally under inhalation anaesthetic (Isoflurane, Abbott Laboratories, North Chicago, IL, U.S.A.) by a veterinarian at the Onderstepoort Bird and Exotic Animals Clinic. Implantation took place on 4 July 2008, but only data from 9 July onwards were included in the analyses. The iBBats recorded T_b every 15 minutes.

Restricted feeding treatment

On six occasions during the study period (July/August 2008), food was removed from the aviaries at approximately 09:00 and the birds did not have access to food until the following morning, a similar protocol to that previously used to induce facultative hypothermic responses in mousebirds (McKechnie and Lovegrove 2001a) and passerines (McKechnie and Lovegrove 2003).

Field data collection

In July and August 2007, we obtained overnight traces of T_b in two Acacia Pied Barbets (M_b = 27 and 31 g respectively) in a field laboratory in the Molopo Nature Reserve (25° 47' S, 22° 56' E) in the Kalahari Desert [see Smit and McKechnie 2010 for a full description of this site]. The two barbets were trapped using spring traps within one hour before sunset and placed in 1.7 L plastic respirometry chambers

shortly after sunset on the same day. No food was provided. The respirometry chambers were immediately placed into a darkened environmental chamber constructed from a 67 L coolbox plumbed with copper tubing, through which water was pumped by a temperature-controlled circulator (model ME, Julabo Labortechnik, Seelbach, Germany). During the measurements described here, the T_a within the respirometry chambers varied between 5 and 20 °C, as part of a ramped profile we used for measurements of respiratory gas exchange. The birds spent the night in the chambers, and their T_b s were recorded every 15 s using lubricated fine-gauge Teflon-coated Cu-Cn thermocouples (IT-18, Physitemp, Clifton NJ) inserted into the birds' cloacae to a depth at which a slight withdrawal did not result in a change in the temperature reading (~ 2 cm). Each thermocouple was secured by attaching the wire to the tail feathers immediately behind the cloaca, using adhesive tape and a light wire paper clip. Data from the thermocouples were acquired using a TC-1000 thermocouple meter (Sable Systems, Las Vegas, NV, USA), and recorded using a UI-2 Universal Interface and Expedata software (Sable Systems, Las Vegas, NV, USA). In both cases, the thermocouples remained securely attached until the birds were removed from the chambers around sunrise the following morning.

Data analyses

Minimum rest-phase T_b (ρT_{bmin}) and maximum active-phase T_b (αT_{bmax}) were calculated as the mean of the lowest and highest five data points within each 24-hr period, respectively (i.e., the lowest and highest 1-hr mean values). All values are presented as mean $\pm$ SD. Variables were compared within species using repeated-measures analysis of variance (RM-ANOVA). All statistical procedures were carried out using Statistica version 8.0 (Statsoft, Tulsa, OK).

Results

Ad libitum feeding

Both species exhibited distinct circadian cycles of T_b (Figure 1). Neither species defended an obvious setpoint during the rest-phase; instead, T_b decreased sharply after sunset, and then decreased more slowly, at $0.23 \pm 0.04 \text{ } ^\circ\text{C h}^{-1}$ to $\rho T_{b\min} = 37.7 \pm 0.6 \text{ } ^\circ\text{C}$ ($n=3$ individuals, 93 bird-days) in Acacia Pied Barbets and at $0.36 \pm 0.04 \text{ } ^\circ\text{C h}^{-1}$ to $\rho T_{b\min} = 37.0 \pm 0.6 \text{ } ^\circ\text{C}$ ($n=5$ individuals, 130 bird-days) in Crested Barbets, until around midnight, after which T_b gradually increased again until before sunrise (Figure 1). The amplitude of circadian T_b cycles (R_T) was $4.6 \pm 0.7 \text{ } ^\circ\text{C}$ ($n = 3, 93$) in Acacia Pied Barbets and $5.5 \pm 0.4 \text{ } ^\circ\text{C}$ ($n = 5, 130$) in Crested Barbets. Neither $\rho T_{b\min}$ nor the rate of cooling during the first part of the rest phase was significantly correlated with T_a in either species.

The mean active-phase T_b of Acacia Pied Barbets was $41.9 \pm 0.1 \text{ } ^\circ\text{C}$ ($n = 3, 93$), and in Crested Barbets was $42.1 \pm 0.4 \text{ } ^\circ\text{C}$ ($n = 5, 130$). In both species, T_b was less labile during the active phase compared to the rest-phase (Figure 1), and daily maximum T_b was $42.3 \pm 0.1 \text{ } ^\circ\text{C}$ ($n = 3, 93$) and $42.5 \pm 0.4 \text{ } ^\circ\text{C}$ ($n = 5, 130$) for Acacia Pied and Crested Barbets respectively.

Restricted feeding

Both species responded to food deprivation by exhibiting small decreases in $\rho T_{b\min}$ (Figure 1), and at no stage did either species exhibit the more pronounced T_b reductions typical of torpor. In Acacia Pied Barbets, $\rho T_{b\min}$ during restricted feeding was $37.3 \pm 0.6 \text{ } ^\circ\text{C}$, significantly lower than during *ad libitum* feeding (RM-ANOVA,

$F_{1,107} = 23.30$, $P < 0.005$). Similarly, R_T during restricted feeding was 5.2 ± 0.7 °C, significantly greater than during *ad libitum* feeding (RM-ANOVA, $F_{1,107} = 35.82$, $P < 0.005$). Neither the mean nor maximum αT_{bmax} varied significantly with feeding treatment. In Crested Barbets, ρT_{bmin} decreased significantly to 36.4 ± 0.8 °C (RM-ANOVA, $F_{1,149} = 48.20$, $P < 0.005$), and R_T increased significantly to 6.1 ± 0.7 °C (RM-ANOVA, $F_{1,149} = 8.20$, $P = 0.0048$).

Field measurements

The two wild-caught barbets exhibited large nocturnal reductions in ρT_b , to respective minima of 31.3 °C and 31.6 °C at approximately 03:00 (Figure 2). Thereafter, ρT_b gradually increased back towards normothermic levels, and was > 39 °C in both individuals when they were removed from the chambers. The average rewarming rate for the two birds was 0.028 °C min^{-1} , much lower than the value of 0.33 °C min^{-1} predicted for a 29-g bird (average M_b for the two individuals) (McKechnie and Wolf 2004). The patterns of ρT_b were similar in the two individuals despite the fact that they were exposed to completely different T_a profiles (Figure 2).

Discussion

Neither species of barbet exhibited any evidence of torpor during *ad libitum* or restricted feeding when held in outdoor aviaries. Instead, metabolic responses to food deprivation involved very shallow reductions in ρT_{bmin} below normothermic rest-phase levels. Many bird species, particularly passerines, respond to restricted feeding and/or low environmental temperatures by reducing ρT_{bmin} a few degrees below normothermic values (e.g., Shapiro and Weathers 1981, Reinertsen and Haftorn 1986, Waite 1991, Schleucher 2001, McKechnie and Lovegrove 2003). These responses are

often referred to as rest-phase hypothermia (Reinertsen 1996) or controlled rest-phase hypothermia (Prinzinger et al. 1991), but it remains unclear whether they represent a physiologically distinct phenomenon from torpor, with the latter characterised by greater reductions in ρT_{bmin} and reduced responsiveness to external stimuli (McKechnie and Lovegrove 2002). The evolutionary and ecological factors that determine whether or not a particular avian taxon exhibits torpor remain unclear. Although phylogenetic position, M_b , and diet are thought to be related to torpor use (McNab 1988, Geiser 1998, McKechnie and Lovegrove 2002), factors such as predation risk may also be important.

The limited data we obtained from two Acacia Pied Barbets in a field laboratory, however, reveal that this species is in fact capable of more pronounced reductions in ρT_{bmin} , with both individuals exhibiting ρT_{bmin} values below 32 °C. These data, although limited to a single ρT_b trace from each of two individuals, suggest that the responses to food deprivation we observed in barbets maintained in captivity should be viewed only as responses to restricted feeding under semi-natural conditions, and are probably not representative of winter patterns of thermoregulation in free-ranging barbets. The two wild barbets were caught shortly before sunset, and had probably been feeding throughout the day. Geiser et al. (2000) showed that torpor is generally much more pronounced in free-ranging populations compared with captive individuals, and our observations for Acacia Pied Barbets are consistent with these authors' findings.

Captive individuals of both barbet species exhibited circadian rhythms of T_b , as is typical for birds, but the amplitudes of the barbets' circadian rhythms were considerably larger than expected on the basis of M_b . In Acacia Pied Barbets, the R_T value of 4.6 °C observed during ad libitum feeding is equivalent to 159 % of the R_T

predicted by Aschoff (1982), with a corresponding value for Crested Barbets of 248 %. Neither species of barbet defended an obvious T_b setpoint during the rest-phase (Figure 1). The patterns of gradual decreases in ρT_b towards a minimum value, followed by gradual increases, are qualitatively similar to the rest-phase thermoregulatory patterns in Laughing Kookaburras (Figure 2 in Cooper et al. 2008).

The larger-than-predicted circadian rhythms of T_b we observed in Acacia Pied Barbets and Crested Barbets are not, however, atypical compared to those of other southern African non-passerines. Values of R_T are available for seven such species, and with only one exception they all lie outside the conventional upper 95% prediction interval for Aschoff's (1982) non-passerine data (Figure 3). Aschoff's analysis was based largely on data from species inhabiting temperate latitudes in the northern Hemisphere, but the data currently available for southern African species suggest that it does not adequately predict the amplitudes of circadian T_b rhythms for species from tropical and sub-tropical regions. Most studies of circadian rhythms in avian and mammalian T_b have focused on the scaling of R_T , (e.g., Aschoff 1982; Mortola and Lanthier 2004), and the adaptive significance of inter- and intraspecific variation in this parameter has received less attention. Lovegrove and Heldmaier (1994) proposed that variation in R_T is correlated with daily fluctuations in environmental temperature, and/or environmental factors favouring energy conservation. For instance, greater R_T -values arising from lower than expected normothermic ρT_{bmin} values are thought to be indicative of selection for energy conservation via reduced rest-phase metabolic rates (Lovegrove and Heldmaier 1994). Whereas environmental correlates of avian R_T have yet to be investigated in a comparative analysis, it is tempting to speculate that the differences between southern African species and those included in Aschoff's (1982) analysis may reflect broad-

scale latitudinal variation in climatic and/or biotic factors. For instance, the magnitude and direction of avian seasonal metabolic adjustments are correlated with latitude, a finding that has been interpreted in the context of latitudinal variation in the need for cold tolerance *vs.* energy conservation (Smit and McKechnie in press). It may thus be that the large R_T values of southern African non-passerines reflect selection for reduced energy demands, possibly associated with factors such as winter decreases in food availability (Smit and McKechnie in press) and/or factors such as the precipitation unpredictability associated with the El Niño Southern Oscillation (Lovegrove 2000).

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Figure legends

Figure 1. Representative traces of body temperature during the period 14-27 July 2008 in one Acacia Pied Barbet (*Lybius leucomelas*) and two Crested Barbets (*Trachyphnous vaillantii*) maintained in outdoor aviaries. Days on which food was withheld from 09:00 onwards are indicated with asterices. The top graph shows air temperature in the aviaries, and the periods between the end and start of twilight as black bars.

Figure 2. Body temperature in two Acacia Pied Barbets (*Lybius leucomelas*) held overnight in darkened metabolism chambers in a field laboratory in Molopo Nature Reserve, Northwest Province, South Africa. The upper graph in each panel shows air temperature (T_a), which varied between 5 and 20 °C as part of a ramped T_a profile used for measurements of gas exchange. Data for the two individuals were collected on 4 July 2007 (left panel) and 10 August 2007 (right panel).

Figure 3. The amplitudes of circadian rhythms of body temperature (R_T) in southern African non-passerines (crosses) are considerably larger than those of the non-passerine species used by Aschoff (1982) (solid circles). The solid line is a conventional linear regression ($\log_{10}R_T = -0.370\log_{10}M_b + 1.044$) and the dashed lines are conventional (i.e., non-phylogenetically-corrected) 95 % prediction intervals. In all but one case, the R_T values for southern African non-passerines fall above the upper 95% prediction interval. Data are shown for the following southern African species: *Elanus caeruleus* (McKechnie and Lovegrove 1999), *Phoeniculus purpureus* (Boix-Hinzen and Lovegrove 1998), *Colius colius* (McKechnie and Lovegrove

2001b), *Falco rupicolis* (Bush et al. 2008), *Ptilopsis grantii* (Smit et al. 2008), *Lybius leucomelas* (present study) and *Trachyphnous vaillantii* (present study).

Figure 1.

Figure 2.

Figure 3.