Thermoregulation by captive and free-ranging Egyptian rousette bats (*Rousettus aegyptiacus*) in South Africa

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Running Head: Thermoregulation by Egyptian rousette bats.

Many bats, including some small-bodied tropical and sub-tropical Pteropodidae, use torpor to offset energetic constraints. We tested the hypothesis that medium sized (110-160 g) cave-roosting Egyptian rousette bats (*Rousettus aegyptiacus)* at the southern extent of their range are able to employ torpor. We measured daytime body temperatures (T_b) of nine wild individuals using implanted temperature-sensitive radio-transmitters. The bats roosted in a cave on Table Mountain, Cape Town, South Africa (a typically cool and wet area). Daily mean cave temperature (T_c) ranged between 7 and 12 °C (mean 9.3 °C). All wild individuals exhibited a circadian cycle in T_b, with an average of 37.7 °C upon return from foraging, decreasing to 35.5 °C by mid-day. Before emergence for feeding, T_b increased to about 37 °C. No individual allowed T_b to drop below 34 °C indicating (assuming a threshold temperature of 30 °C) that individuals in this population do not use torpor. Bats were active throughout the day within the roost and metabolic heat production may have contributed to the maintenance of rest phase T_b. Ten individuals implanted with temperature sensitive passive integrated transponder tags were held in captivity at temperatures of 25-30 °C and subjected to food deprivation for 2-3 days. The lowest T_b recorded for any captive individual was 33.4 °C despite losing an average of 10% of their initial body mass. Despite exposure to cool T_a in the cave, and often cold, wet and windy conditions whilst foraging, or substantial food restriction in captive bats, *R. aegyptiacus* did not resort to using torpor.

Torpor is a temporary state of heterothermy characterized by reduced metabolic rate (MR) and other physiological variables (i.e. heart rate, respiration rate, body temperature (T_b); Geiser 2004; Ruf and Geiser 2014;), which results in a pronounced reduction in daily energy expenditure (Geiser 1994, 2004). The high MR typical of endothermic mammals requires high energy intake to maintain and thus endothermic homeothermy can become prohibitively expensive when food abundance decreases and/or ambient temperature (T_a) is low. Many small mammals respond to elevated energy demands and/or reduced energy supply by using torpor, a physiological mechanism that reduces these costs.

Body size is an important determinant of the energetic costs of maintaining normal elevated T_b, usually 37-40 °C in endothermic animals (for review see McNab 2002). Small endotherms have higher mass-specific MRs than larger ones to compensate for the higher rate of mass-specific heat loss associated with their proportionately larger surface area (McNab 2002). They are also constrained by body size in how much energy can be stored (usually in the form of fat). Smaller endotherms are thus more likely to face energetic shortfalls, and have been more commonly reported to employ torpor (Ruf and Geiser 2014).

Torpor is used by various bats to offset energetic constraints, but most investigations have focused on insectivorous species with body mass < 40 g (Stawski et al. 2014). Fruit- and nectar-feeding bats might also be expected to face seasonal energy shortages when these foods are in short supply. Members of the super-family Pteropodidae feed primarily on fruit and nectar, and belong to a relatively old lineage of bats (Teeling et al. 2005; Eick et al. 2005). There is growing evidence that torpor is a plesiomorphic trait widespread in basal lineages (Geiser 2008), especially among species with smaller body sizes. Laboratory studies have established that several small (16-18 g) pteropodids express daily torpor with T_b falling as low as 17.2 °C,

especially when food is restricted (Bartels et al. 1998; Geiser et al. 2001; McNab and Bonnacorso 2001; reviewed by Stawski et al. 2014). In contrast, most medium sized tropical and semi-tropical pteropodids (50-200 g) appear not to use torpor (Bartholomew et al. 1970; McNab and Bonnacorso 2001; Riek et al. 2010; Downs et al. 2012; but see McNab 1989), suggesting that the energetic demands of medium-sized bats in the tropics may not be stringent enough to warrant the use of torpor. Fruit-eating bats exploit a food source that is rich in energy and its availability may be more predictable than that of insect prey. Most are also larger than insectivorous bats and may be able to store sufficient energy in the form of fat reserves to avoid using torpor. Furthermore, most fruit-eating bats are restricted to the tropics or subtropics where seasonal fluctuations in food availability are often less pronounced than in temperate latitudes, although some species migrate in response to spatial and/or temporal variation (e.g. Thomas 1983).

The Egyptian rousette bat (*Rousettus aegyptiacus*) is a widespread fruit-eating bat distributed from the Middle East, and Pakistan, through sub-Saharan Africa, and has a geographic range that extends into the temperate regions at the southern tip of Africa (Monadjem et al. 2010). Here the wet winters typical of the Cape Floral Kingdom (Manning 2007), may prevent effective foraging during inclement weather and produce conditions favoring the use of torpor. *Rousettus aegyptiacus* is a medium sized flying fox (mass 110-160 g) and its ability to accrue sufficient fat deposits to use during such times may be limited. Thus it is a pteropodid that despite its relatively large size occurs in an environment in which torpor may have considerable adaptive value.

Previous studies on captive *R. aegyptiacus* indicate that individuals maintain a normothermic T_b under various T_a conditions (Kulzer 1963; Noll 1979). Korine et al. (2004)

reported that the resting metabolic rate of Egyptian rousette bats decreased significantly (~19 %) during late pregnancy. These authors argued that this metabolic depression is a compensatory mechanism for the high energy requirements of reproduction (Speakman 2008). Korine et al. (2004) concluded that reproductive females likely use more than one strategy to cope with the high energetic demands and that they may shift strategies according to food availability and reproductive phase, but they found no direct evidence of torpor use. Instead, in their study area in Israel, *R. aegyptiacus aegyptiacus* has adaptations that allow maintenance of T_b at <u>high</u> T_a, despite restricted access to water (Korine and Arad 1993). The large geographic and environmental range of *R. aegyptiacus*, the geographically isolated populations of its sub-species (*R. aegyptiacus leachii* in southern and eastern Africa, including our study area; Kwiecinski and Griffiths 1999; Happold and Happold 2013), and the fact that in other bat species, different populations exhibit different thermoregulatory patterns (e.g. McNab and Bonnacorso 2001; Dunbar and Brigham 2010), led us to hypothesize that in populations of *R. aegyptiacus* in the southern extent of its range, torpor has evolved. Our purpose was thus to test the prediction that free-ranging, cave-roosting *R. aegyptiacus* at the southern extent of its range, employs torpor during winter when thermoregulatory costs are high, despite the species' relatively large body mass compared to that of other fruit-eating bats shown to use torpor. We measured daytime (i.e. rest-phase) T_b using implanted temperature-sensitive radio-transmitters in wild, free-ranging individuals near Cape Town, South Africa, the most southerly location for the species, and employed the commonly used - albeit arbitrary - T_b threshold for torpor of 30 °C (Brigham et al. 2011). Our winter study period encompassed the period of mating and early pregnancy.

We combined our field data with a subsequent laboratory study designed to address whether *R. aegypticus* in southern populations possesses the physiological capacity for

reductions in T_b greater than those observed in free-ranging individuals. The expression of heterothermy by endotherms is generally greater under natural compared to artificial environments (Geiser et al. 2000). However, restricted feeding is an important cue for heterothermy (Genin and Perret 2003; Canale et al. 2012), and studies involving captive individuals whose food intake can be manipulated experimentally remain instructive for evaluating whether or not a particular species or sub-species is capable of torpor.

MATERIALS AND METHODS

Field study

Our study was conducted near Cape Town, South Africa (33°55'S, 18°22'E), from May-September (austral winter) 2011. The bats roosted in a cave near the top of the western side of Table Mountain (elevation ~ 720 m asl). Mean daily cave temperature ranged from 7 to12 °C over the duration of the study, which is well below the species' thermal neutral zone (31-36 °C; Korine and Arad 1993) and should result in high thermoregulatory costs. We placed two iButton data loggers (Maxim Integrated DS1921, San Jose, CA, USA) on rock ledges in the front chamber to record cave temperature (T_c; $\pm$ 0.5 °C) every two hours. For comparison, we also obtained T_a data from the Cape Town airport (elevation ~ 40 m), 22.8 km from the cave. The region has a coastal climate with cool wet winters. Mean annual rainfall varies from 1000 to 2000 mm and mean annual temperature ranges from approximately 22 °C in the city center to 16 °C on the top of Table Mountain (Cowling et al. 1996).

We captured *R. aegyptiacus* in mist nets at dusk as they emerged from the cave. The cave is used year-round as a diurnal roost by a mixed group of approximately 300 males and females. We determined the sex of individuals through examination of genitalia and confirmed all were

adults by the presence of cartilaginous epiphyseal plates in their finger bones detected by trans-illuminating the bat's wings (Anthony 1988). Males had enlarged testes but it was too early in the reproductive season to determine whether females were pregnant.

To collect data on body temperature, a veterinarian intraperitoneally implanted BD-2TH temperature sensitive radio-transmitters (Holohil Systems Inc., Carp, ON, Canada) into 12 bats (7 males and 5 females) under oxygen/isoflurane anesthesia. Before implanting transmitters, we calibrated them in a water bath at 10 °C intervals from 0 to 40 °C and compared pulse rate and temperatures to the manufacturer's calibration curves to ensure accuracy. Transmitter mass was <3 g (~2 % of body mass). Bats were kept individually in cloth bags for 36 hours post surgery before being released into the cave on 30 May 2011. Post surgery, captive bats were offered unlimited amounts of banana, apple, and guava nectar every 8 hours.   All protocols involving wild bats were approved by the University of Cape Town's Animal Ethics Committee (No. 2010\V3\DJ), and the work was carried out under permit from South African National Parks.

Once we released the tagged bats, we deployed a Lotek SRX 400 data-logger/scanner receiver (Lotek Wireless Inc., Newmarket, ON, Canada) within the cave. This device scanned for transmitter signals every 10 min and stored transmitter pulse-rate data for each individual in range. Two chambers in the cave were used by the bats, although only individuals roosting in the front chamber (used by the majority of bats) were typically detected by the receiver. To minimize disturbance to the colony we did not position the data logger deeper into the cave as we had to regularly change batteries and download data.

On two days during the field study, we used an infrared night vision video recorder (Bell & Howell model DNV16HDZ, New York, NY, USA) in the front chamber to record activity

(both vocal and behavioral) of the bats. It was not possible from these recordings to identify individuals.

Laboratory study

We investigated thermoregulatory responses to restricted feeding in ten *R. aegyptiacus* during November 2015. The bats, from the same subspecies as the wild individuals (*R. a. leachii*), were caught at a large maternity roost near Ga-Mafefe (24°12'N; 30°36'E) in the Limpopo Province of South Africa, and were housed in the Animal Facility, Centre for Emerging and Zoonotic Diseases, National Institute for Communicable Diseases, National Health Laboratory Services, Sandringham, Gauteng, South Africa. They were in captivity for approximately 12 months (for other research) before the experiments described here took place. All protocols involving captive bats were approved by the University of Pretoria's Animal Ethics Committee (EC056-15) and the National Health Laboratory Services Animal Ethics Committee (136-12, 146-15).

We measured T_b using temperature-sensitive passive integrated transponder (PIT) tags (sensitive down to 25 ^{o}C; BioMark, Boise, ID, USA) injected subcutaneously between the scapulae of each bat. Signals from the PIT tags were detected and recorded every 15 seconds for 48-72 hours using two PIT tag readers and portable transceiver systems (model FS2001, Destron Fearing, St. Paul, MN, USA), linked to a personal computer. We used PIT tags from the same batch as those used previously by Whitfield et al. (2015), who found a mean difference between actual and measured temperature of 0.02 ± 0.09 °C. The temperature and humidity of the Animal facility were monitored by HAXO-8 Temperature and Humidity LogTags (LogTag®, Auckland, New Zealand).

During measurements, bats were individually housed in custom-built cylindrical cages (diameter 350 mm, depth 370 mm) to facilitate continuous reception of signals from the PIT tags. These cages where large enough to allow crawling, wing stretching and flapping. The daily temperature in the facility never went below 24.7 °C and never exceeded 31.0 with humidity ranging between 35 and 65 % on a daily basis. The animals received natural light from sunrise to sunset with a maximum of 4 hours of artificial light (not direct) during the course of the day. Body-mass loss was induced by reducing the available food mass to 40 % of the bats' initial body mass (e.g. 40 g per day for a 100 g bat) for each of 2-3 successive days. This resulted in a 3-11% loss of body mass per day.

RESULTS

Field study

Both temperature loggers in the cave indicated that the mean daily T_c was relatively stable from 14 May - 21 September, ranging between 7.0 and 12.3 °C. As the bats roosted against the rock walls and ceiling of the cave, our T_c measurements were reasonable approximations of the environmental temperatures experienced by the bats. T_c was considerably lower (due to the elevation difference) and more stable than mean daily T_a measured at the Cape Town Airport, which ranged from ~9-17 °C (Fig. 1).

Of the 12 bats implanted with radio-transmitters, the signals from 11 were detected by the receiver after the bats' release, indicating that they returned to the cave. Eight individuals provided at least 6 days of data each (5 males and 3 females). In all instances (n = 288 bat-nights), bats which roosted in the cave during the day, left at night (usually between 2000 and 2100 hr, although sometimes as late as 2330 hr), presumably to forage. In total, we collected
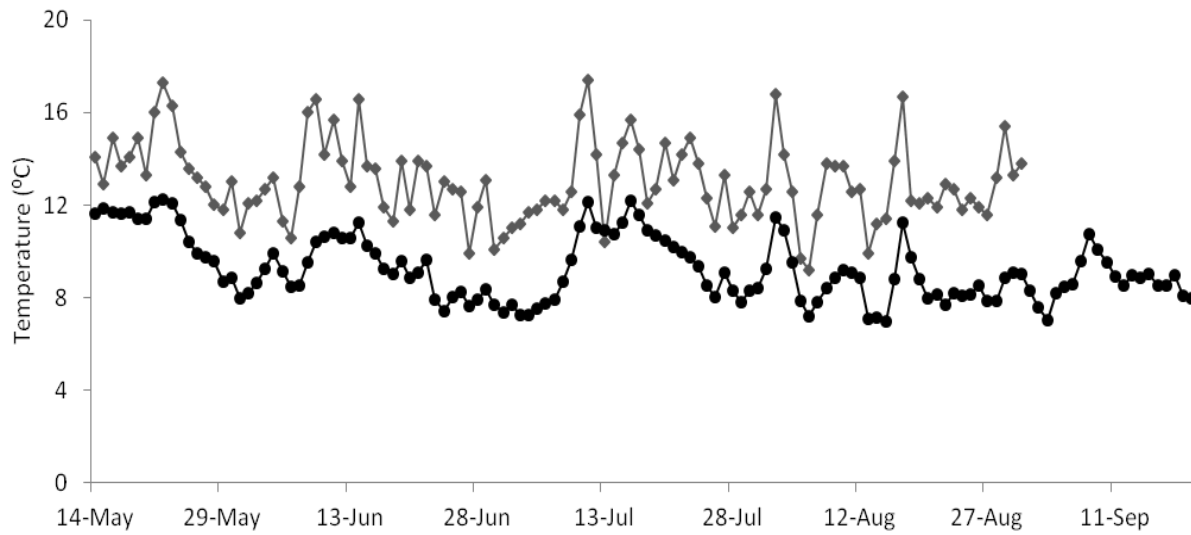
Figure 1: Mean daily temperature (°C) of rock ledges within the roost cave of Egyptian rousette bats (*R. aegypticus*) near Cape Town South Africa (black line) compared to ambient air temperature (°C) recorded at the Cape Town International airport (23 km from the roost) (grey line).

209 complete days of data (where an individual was in range of the receiver from dawn until dusk) and an additional 103 partial days of data (signals detected for part of a given day but sufficient to determine entry, exit, minimum and maximum temperatures). We presume these latter bats either switched between chambers or moved to a location in the front chamber which was not detectable by the receiver. Of the 209 complete days of data, 91 came from one male, and of the 103 partial days, 64 came from one female.

All bats arrived at the cave in the morning with high T_b (mean $\pm$ SD T_b for 8 individuals was 38.2°$\pm$1.2), presumably after flying up to the top of the mountain from foraging areas lower on the slopes (Barclay and Jacobs 2011). On average, bats cooled by 2.4° C degrees (to a mean of 35.8 °$\pm$1.1) during the day and then warmed by 1.6° C degrees before departing with a mean T_b of 37.4°$\pm$1.0 (Table 1).

The lowest T_b recorded for any wild bat was 32.0° C. For the period 8-17 June 2011, one male had a minimum daily T_b ranging from 32.0-33.8° C. We recorded no minimum T_b for any other individual, male or female, that fell below 34.0° C, and for 7 of the 8 bats, mean minimum T_b values were $\geq$ 35.5° C (Table 1). Minimum T_b typically occurred between 1500 and 1700 hr. T_b did fluctuate by several degrees throughout the day for all bats (Fig. 2). These fluctuations are unlikely to be sampling errors as they lasted for longer than a single record. Periodic flights within the cave (see below) may have warmed bats slightly.

The video recordings showed that bats were awake and active all day. We made recordings on 19 and 26 July 2011. Each day, we recorded the group of bats in the front chamber of the cave for 9 intervals of 50 mins each beginning at 0900 and concluding at approximately 1600. During each interval we counted the total number of flights initiated by any of the 80-100 bats in view. The mean number of flights per 50 min was 192.4 (range 113-319),

ID	Sex	n	Enter	Exit	Minimum	Maximum
080	m	6	37.9+/-1.3	37.5+/-0.7	36.2+/-0.3	38.4+/-1.0
101	m	93	37.9+/-0.8	37.1+/-0.5	35.5+/-0.5	38.2+/-0.6
198	m	19	36.7+/-2.0	36.6+/-1.7	34.8+/-2.3	37.4+/-1.9
469	m	24	39.2+/-0.6	38.4+/-1.2	36.4+/-0.8	39.4+/-0.5
712	m	13	38.1+/-0.6	37.7+/-0.6	35.5+/-0.2	38.4+/-0.7
034	f	19	38.9+/-1.2	38.7+/-0.8	37.0+/-0.7	39.4+/-1.0
059	f	35	38.2+/-1.4	37.8+/-1.0	36.6+/-0.8	39.1+/-1.9
174	f	101	38.3+/-1.1	37.3+/-0.8	35.6+/-0.8	38.7+/-0.9

Table 1: Mean (+/- *SD)* body temperatures (^oC) of individual free-ranging male (m) and female (f) *Rousettus aegyptiacus* roosting in a cave in Cape Town, South Africa. Values are given for body temperature when the bat entered the cave after foraging (Enter), left near sunset to forage (Exit), and the minimum and maximum daily body temperatures. n is number of days of data.
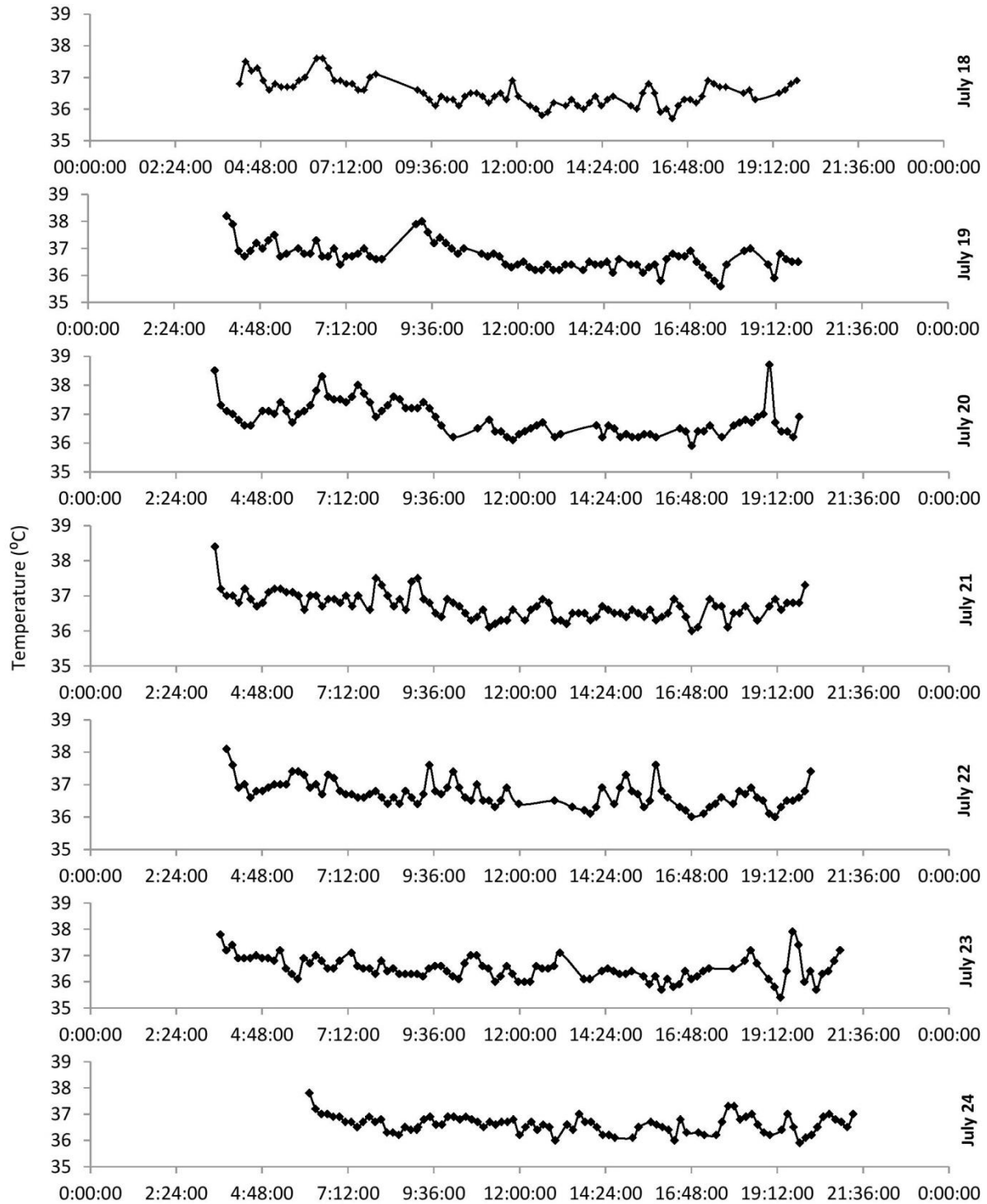
Figure 2: Core body temperatures (oC) for a free-ranging male Egyptian rousette bats (*Rousettus aegyptiacus*) (M101) while roosting in a cave in Cape Town, South Africa from 18 to 24 July 2011. Temperature was recorded every 10 minutes except when the bat was out of range of the receiver (e.g. top panel ~0800 to 0900h).

or approximately 2 per interval per bat. There was no evident pattern of increase or decrease in flights with time of day but the small sample precludes statistical analysis. The bats also vocalized virtually non-stop. There were numerous interactions between individuals that appeared to be agonistic. In many instances after such interactions, some bats took flight. These flights appeared to be voluntary and not forced by interactions between animals or from losing a foothold on the cave ceiling. There was no evidence of increased clustering or change in group size in the front chamber before bats left to feed at night.

Laboratory study

The restricted feeding protocol resulted in a mean ± SD body-mass loss of 10.7 ± 3.5 % (n = 10) compared to initial mass, with a maximum of 16.4 % in one individual. Mean minimum T_b associated with restricted feeding was 34.4 ± 0.6 °C, and the lowest single T_b value for any individual was 33.4 °C. Minimum T_b was not significantly related to body mass loss (Pearson Product Moment Correlation Coefficient = -0.176, $P = 0.63$). There was no obvious daily cycle in T_b.

DISCUSSION

Despite cool temperatures in the roost cave, below the thermal neutral zone of *R. aegyptiacus*, and occasional cool, wet weather in the winter in Cape Town, our data indicate that torpor (using the 30 °C threshold; Brigham et al. 2011), was not used by free-ranging Egyptian rousette bats of either sex during our study period. Most individuals we tagged did undergo a predictable daily reduction in T_b (approximately 2.5 °C) consistent with that found for captive *R. aegyptiacus* acclimated to either 30 or 15 °C (Noll 1979), and another southern African pteropodid

(*Epomophorus wahlbergi*; Downs et al. 2012). We conclude that torpor is not commonly (if ever) employed by this species. However, whether termed torpor or not, the daily cycle of body temperature likely did save bats some energy as even small drops in T_b result in considerable energy savings (Studier 1981).

Our measurements of T_b were similar to those for captive conspecifics in Israel (Korine et al. 2004). Korine et al. (2004) reported that both captive and free-ranging *R. aegyptiacus* in Israel responded to increased energetic costs during reproduction by increasing food intake. In our study, radio-tagged *R. aegyptiacus* always left the cave at night, regardless of weather conditions, and did not return until just before dawn. As found previously at our study site (Barclay and Jacobs 2011), bats forage during these nocturnal hours and their consistent departures indicate that food was available throughout our study. For some captive *R. aegyptiacus* acclimated to either 15 or 30 °C, both resting metabolic rate and heart rate approximately doubled when animals were exposed to temperatures decreasing from 30 to 10 °C (Noll 1979). This suggests that, compared to a warmer cave with temperatures within the thermoneutral zone of *R. aegyptiacus*, the free ranging bats at our study site may spend twice as much energy during the day. Even so, torpor was not employed.

Our video data are also consistent with the idea that torpor was not used, as both vocal and behavioral activity continued inside the cave all day. Bats may have flown on purpose to generate heat and thus maintain a relatively constant T_b during the day, but the video recordings do not allow us to confirm this. The apparently agonistic interactions between individuals would be consistent with male territorial defense during the breeding season. The need for territory defense would likely increase the benefit for males to remain active and not use torpor unless they faced energetic emergencies.

The increase in T_b we recorded for free-ranging bats just before emergence in the evening could suggest that bats were flying in the cave to re-warm from the slightly decreased T_b during the day, or clustered to increase temperature before departing to feed at night. However, there was no obvious increase in flight or vocal activity towards emergence (it was consistent all day) and no evidence that the cluster size in the front room changed throughout the day. The fact that our measure of diurnal T_b was so similar to that of Noll (1979) for captive bats, suggests that the temperature fluctuations we found were simply part of a circadian rhythm.

The laboratory data for individuals experiencing restricted feeding are consistent with the absence of torpor expression by free-ranging individuals, and moreover are consistent with the notion that this species does not possess the physiological capacity for torpor. Despite body mass loss of up to ~16 % of *ad libitum* feeding body mass, none of the captive bats decreased T_b below 33.4 °C, a value only slightly above the minimum of 32.0 °C observed in wild bats in the field study. These laboratory data hence support the view that medium sized pteropodids are generally not heterothermic and that the physiological capacity for torpor may have been lost in larger members of this family (Stawski et al. 2014).

In summary, in winter, *R. aegyptiacus* near Cape Town, South Africa roosted each day in a relatively cold cave, but maintained elevated T_b close to typical normal eutherian temperatures throughout the day. The small daily fluctuations in T_b are not consistent with torpor but could potentially reflect a small depression in metabolic rate (Studier 1981). Given that other pteropodids can use torpor (e.g. Bartels et al. 1998; Riek et al. 2010), the question is why *R. aegyptiacus* does not use it to save energy. Reproduction may constrain its use. Males may not be able to afford to go into torpor because they are undergoing spermatogenesis and defending mating territories (Monadjem et al. 2010), and females may be early in gestation or lactation

which increases the cost of using torpor because it slows fetal development (although reproductive females in some species still use torpor; e.g. Hamilton and Barclay 1994; Dzal and Brigham 2013; Rintoul and Brigham 2014). Another potential reason is that the net benefit of using torpor for energy conservation is relatively low given that individuals seem to have ample access to high energy food and thus no energetic constraints. Thomas (1984) postulated that many fruit-eating pteropodids may actually have to over ingest energy to meet certain nutrient (e.g. protein) requirements. Thus, remaining active all day and using energy to maintain elevated body temperatures, despite cool cave temperatures, may be a means of coping with an energy surplus. However, reducing foraging time and thus potential exposure to predators would likely favor torpor use. The existence of torpor, or its loss in some species, is therefore likely a result of complex interactions among body size, diet, environment, and the availability of other strategies to obtain a positive energy balance.

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