



Flood-induced multiday torpor in golden spiny mice (*Acomys russatus*)

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Abstract. Mammalian and avian torpor is widely viewed as an adaptation for survival of cold winters. However, in recent years it has been established that torpor can also be expressed in summer and that the functions of torpor are manifold, including survival of adverse environmental events such as fires, storms, heat waves and droughts. Here we provide the first evidence on (1) torpor induction via an accidental flooding event in mammals (in captivity) and (2) expression of multiday torpor by spiny mice, lasting >7 times as long as usually observed for this desert rodent. Our data suggest yet another function of mammalian torpor, as a response to flood, in addition to many other adverse environmental events, and not just in response to cold.

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Introduction

Mammalian and avian torpor is characterised by pronounced temporal reductions in metabolism, body temperature (T_b) and other physiological functions and is especially effective for energy conservation when ambient temperatures (T_a) are low (Boyer and Barnes 1999; Ruf and Geiser 2015). Therefore, it is not surprising that torpor expression for energy conservation in winter is used by many species. However, in recent years it has been established that torpor can also be expressed in summer and in the tropics, and that the functions of torpor are manifold, including facilitation of reproduction, reduction of inter- and intraspecific competition, and survival of adverse environmental events such as fires, storms, heat waves and droughts (Morrow and Nicol 2009; Levy *et al.* 2011; McKechnie and Mzilikazi 2011; Kronfeld-Schor and Dayan 2013; Stawski *et al.* 2015; Dausmann and Warnecke 2016; Nowack *et al.* 2017). Flooding is another potential challenge especially for small mammals because it can result in wet fur, which substantially reduces its insulative properties (Withers *et al.* 2016) and thus increases heat loss and energy expenditure (Dawson and Fanning 1981). This is further exacerbated by the reduced ability or opportunities for foraging and often reduced food availability during or after floods that could, however, be compensated for by the use of torpor, as is the case after fire (Stawski *et al.* 2015). However, data that show direct evidence on torpor use in relation to flood events are currently restricted to a single observation on a captive bird, the dusky woodswallow (*Artamus cyanopterus*). After immersion into water, apparently due to disturbance, this

passerine expressed much deeper torpor than in air and did survive (Maddocks and Geiser 2007). Torpor in mud as a consequence of flooding has also been proposed as the avenue for winter survival by birds by Aristotle in ancient Greece (Lincoln and Peterson 1979), before it was recognised that they can migrate.

One species that is potentially exposed to floods is the golden spiny mouse (*Acomys russatus*), a small diurnal rodent that is distributed over extreme arid, rocky regions in the Middle East. In most of its habitat the average annual rainfall is below 100 mm year⁻¹. However, rain that falls far away, or highly localised storms that develop suddenly, can cause unexpected flash floods through the dry wadi beds that are the preferred habitat of *A. russatus* (Shargal *et al.* 2000). These floods are unpredictable but frequent, and appear very fast, especially around the Syrian African Rift Valley, since rain that falls in Jerusalem and the Hebron mountains ~1000 m above sea level flows to 400 m below sea level over only a short distance (~1400 m in less than 50 km). However, it is still unknown whether and how species living in these wadi beds survive such floods. In nature, *A. russatus* have been described entering short bouts of torpor lasting for 7.5 h, on average, with a reduction of T_b to ~25°C (Levy *et al.* 2011, 2012b), and expressing torpor also when they are fat (Gutman *et al.* 2006; Levy *et al.* 2011, 2012b). The latter is mainly a characteristic of seasonal hibernators that store fat for winter and can use multiday torpor bouts rather than daily torpor (Boyer and Barnes 1999; but see Vuarin *et al.* 2013).

We report an observation of T_b fluctuations during an accidental flooding event in captive *A. russatus* in comparison with individuals that were exposed to the same environmental conditions, but without exposure to water. We were especially interested to examine whether flood exposure resulted in deeper and longer torpor in this species and, considering its ability to maintain large fat stores (Gutman *et al.* 2006), whether it was capable of expressing multiday bouts of torpor.

Methods

Adult male *A. russatus* ($n = 17$, average body mass 62 g) were obtained from the breeding colony at the zoological research garden at Tel Aviv University. Mice were individually housed in plastic cages (33 × 18 × 13 cm) and kept outside under a roof under natural temperature and light conditions, provided with a shade. Food and water were provided *ad libitum*. Data were collected in September and October 2015 when sunrise and sunset were around 0530 and 1730 hours, respectively.

Before measurements, mice were implanted with data loggers (Thermochron iButton, DS1922L, Maxim Integrated Products Inc., Sunnyvale, CA, USA) in the abdominal cavity and, after a two-week recovery period, T_b was sampled every 10 min at a resolution of 0.06°C. The iButtons weighed ~5% of each animal's body mass. For data logger implantation, mice were anaesthetised with isoflurane in medical-grade oxygen using an anaesthetic machine (Ohmeda, 1.5% vol., 1 L min⁻¹) and implanted with an iButton in the abdominal cavity. Both the abdominal wall and the skin were sutured with absorbable surgical sutures, with a cutting needle (5–0 Dexon), and the incision was treated with topical antibiotic (silver sulfadiazine 1%; Silverol Cream). Prophylactic antibiotics (Baytril 5%, 24 mg kg⁻¹) and artificial tear ointment (to prevent desiccation) were administered preoperatively. Removal of data loggers was conducted as described above on 18 October 2015.

Actograms were generated using CTools 7.0 by Daan van der Veen. T_a was measured next to the cages every 10 min using the same data logger model/resolution (iButton, DS1922L) as described above.

Animals were checked every second day to not unduly interfere with the T_b -rhythm experiments. On 7 October heavy rain began early in the morning. Over the night, 40 mm fell and T_a fell to 22.7°C (records from the Israel meteorological service and iButton respectively). When animals were checked on 9 October at 0930 hours (on 8 October the University was closed because of Sukkot holidays) animal #22 was cold but alive in its cage with wet bedding material with moist rather than wet fur. The animal was removed from the cage using a small plastic jar lined with a paper towel. Wet bedding in the cage was exchanged with dry bedding and the animal was dried and put back into the cage within 20 min. All of this was conducted under the prevailing thermal conditions and the animal was not held by hand or partially rewarmed in any other way. Animal #21 was found floating dead in its cage in a water level of 6–8 cm. The flooding of the two cages was caused by a leak directly above the cages; all other cages ($n = 15$) were dry.

All procedures were conducted in accordance with the National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011), and

approved by Tel Aviv University Animal Ethics Committee. The flooding of the cages was not part of the planned experiment, and resulted from damage to the shading roof of the cages.

Results and discussion

During the experimental period T_a ranged from 22.8 to 29.6°C with a mean T_a of $26.5 \pm 1.6^\circ\text{C}$. During this period food was offered *ad libitum* and none of the animals entered torpor (Fig. 1): mean T_b was $35.9 \pm 0.3^\circ\text{C}$ (mean \pm s.d.; $n = 15$, dry animals) and fluctuated between mean minima of $33.0 \pm 0.7^\circ\text{C}$ (individual minimum 31.6°C) and mean maxima of $38.4 \pm 0.2^\circ\text{C}$ (individual maximum 38.7°C).

On the night during the rain event when T_a fell below 24°C, two individuals substantially reduced T_b after their bedding material was soaked with water (#22: Fig. 1) or the cage was flooded (#21, not shown). During that night T_b fell from ~38°C after 0410 hours in both individuals. In #22, T_b fell below 30°C within 40 min and after torpor entry the animal maintained a large T_b – T_a differential of >3°C for most of the torpor bout, but briefly before arousal was initiated by removing it from the cage, its T_b fell to a minimum of 24.0°C. The maximum cooling rate of #22 was 0.25°C min⁻¹ over 30 min. After wet bedding material had been replaced and #22 had been dried and placed back into its cage, arousal from torpor was slow and the T_b increased from 24.0 to 30°C over 350 min, but T_a did not exceed T_b during any part of the arousal process. The torpor bout duration was 57 h or 2.4 days. In #21, T_b fell within 30 min from ~38°C to below T_b 25°C. The minimum T_b recorded was 23.7°C, which was near T_a (T_b – T_a <1°C), and this T_b was reached within 290 min. The T_b of #21 fluctuated somewhat with T_a . However, unlike #22, #21 was dead when found in the cage.

Under the experimental conditions, outdoors and singly housed, all spiny mice had higher T_b s during the night. In contrast, after the flooding, when #22 had rewarmed from the multiday torpor, it switched to diurnal activity with T_b maxima during the day (Fig. 2).

We provide the first evidence on (1) torpor induction via a flooding event in mammals and (2) expression of multiday torpor by spiny mice, lasting >7 times as long as usually observed for the species. Our data suggest yet another function of mammalian torpor, in addition to many others, in relation to adverse environmental events such as fires, storms or heat waves and not just in response to cold winters (Nowack *et al.* 2017) and support the interpretation that water immersion-induced torpor observed previously in woodswallows was an adaptive event (Maddocks and Geiser 2007).

The obvious question that arises from our observation is whether the T_b reduction observed after the rain event in the spiny mouse was a controlled torpor entry or an induction of uncontrolled hypothermia due to excessive heat loss (Wang and Peter 1975; Geiser *et al.* 2014). We argue that it was an induction of a controlled torpor bout for several reasons using the rationale outlined in Geiser *et al.* (2014):

- (1) The initial reduction of T_b of #22 was fast at 0.25°C min⁻¹, about twice that of *A. russatus* (~54 g) in air (Levy *et al.* 2011) and that observed for the smaller (~40 g) poorwill (*Phalaenopterus nuttallii*) at 0.15°C min⁻¹ (Lasiewski and Lasiewski 1967), suggesting that they did not try to defend a

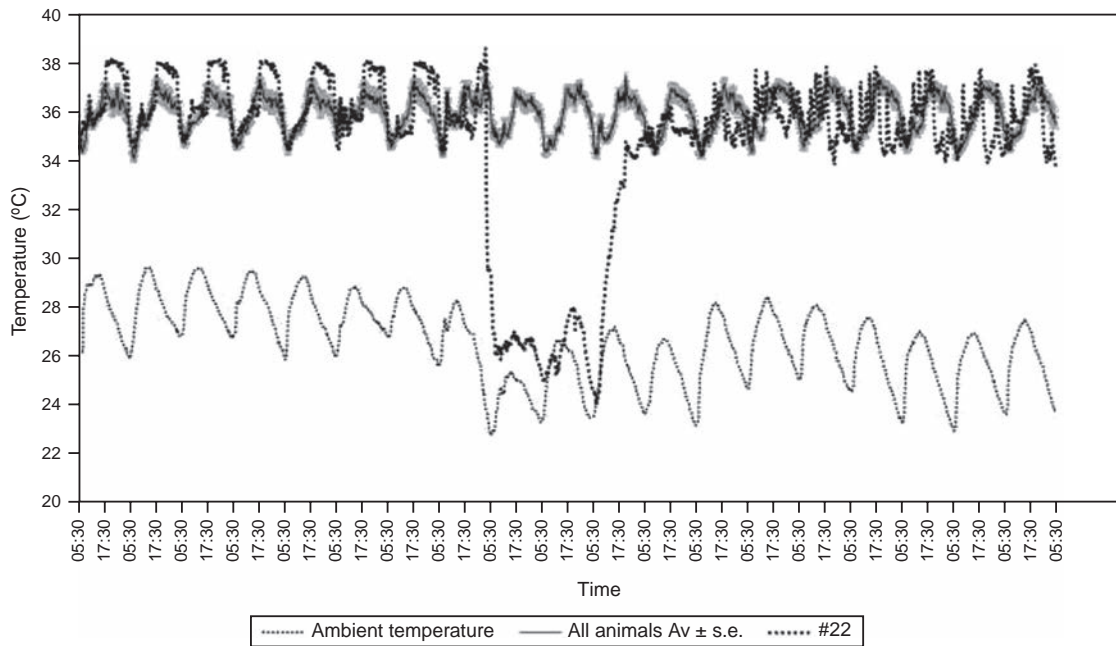


Fig. 1. Mean ± s.e. (grey shading) body temperature of 15 golden spiny mice in dry cages, and #22 using multiday torpor in a wet cage, kept outdoors in individual cages, and the same ambient temperature.

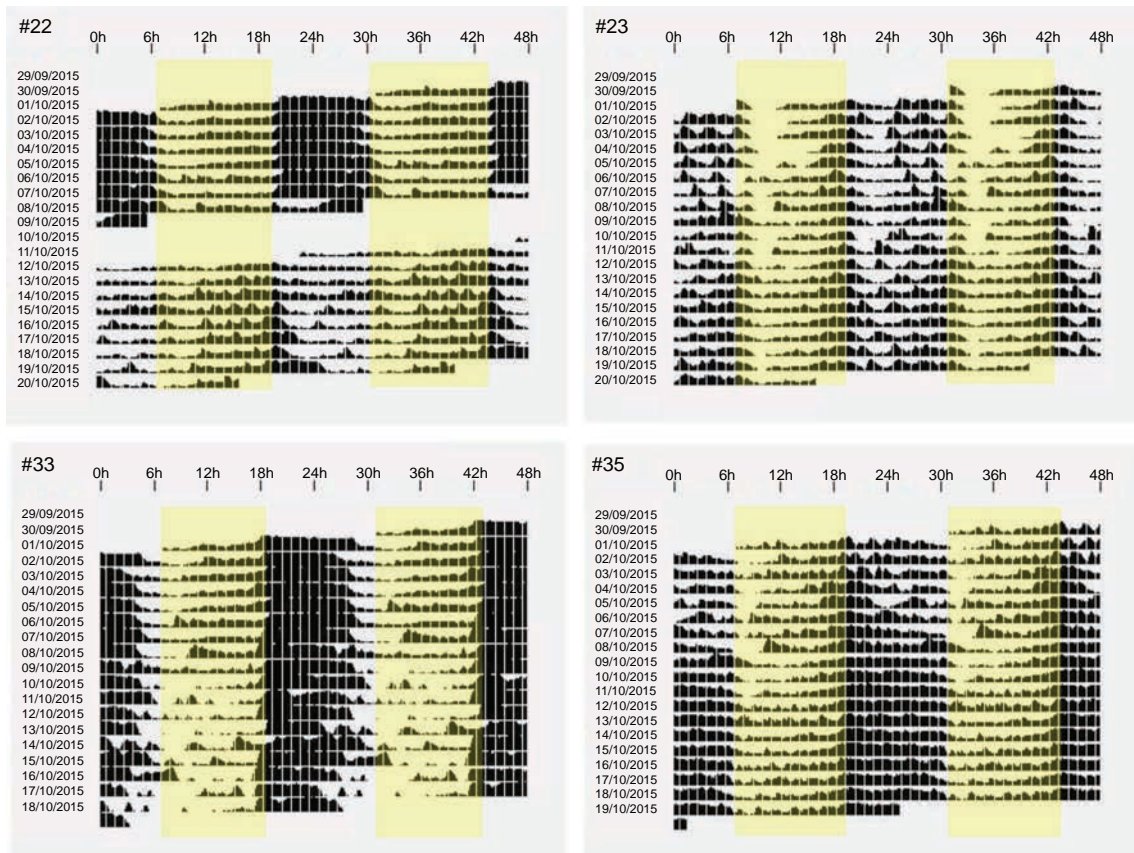


Fig. 2. Double daily plots of body temperatures of #22, which used multiday torpor, and #23, 33 and 35 for comparison. The T_b range for each day (y-axis) is 34 to 38°C; the x-axis shows clock time (the yellow background indicates daytime). Each row represents two consecutive days. In #22, the multiday torpor bout is depicted as missing data (7 to 9 October) since T_b were too low to be registered on the T_b-range shown. Also note the postflood change in T_b rhythm in #22, unlike in the other individuals.

high T_b . In contrast, the maximum cooling rate during induction into hypothermia by mild cold exposure ($\sim T_a$ 16°C) in baby rats at 26 g body mass was only 0.045°C min⁻¹, because the animals attempted to maintain a high T_b but failed to do so because of insufficient heat production (Geiser *et al.* 2014). In adult rats exposed to a helium–oxygen (HELOX) atmosphere, which increases heat loss and thermal conductance by ~ 2 -fold in comparison to air (Thomas *et al.* 1998), and $T_a = -10^\circ\text{C}$ (Wang and Peter 1975), hypothermia was induced with a slow reduction in T_b . The cooling rate in these rats was only 0.11°C min⁻¹ despite the fact that HELOX exposure at $T_a = -10^\circ\text{C}$ is roughly equivalent to a T_a of -30°C (Holloway and Geiser 2001), requiring an increase in metabolism that exceeds the maximum heat production in rats (Wang and Peter 1975). In contrast, exposure to water at $T_a = 20$ – 25°C , similar to the T_a that *A. russatus* experienced here, increased metabolism by only ~ 2 -fold or less in water rats (Dawson and Fanning 1981). These differences suggest that the spiny mouse was ‘willing’ to reduce its T_b and did enter a bout of torpor for energy conservation to increase its chance of survival during the flood event.

- (2) The T_b during torpor in #22, unlike for #21, was maintained $\sim 3^\circ\text{C}$ above T_a apparently via thermoregulatory heat production (Boyer and Barnes 1999; Ruf and Geiser 2015). This suggests that the reduction of T_b not only at torpor entry but also during the torpor bout was a regulated physiological process. $T_b - T_a$ differentials of 2– 3°C are commonly maintained during torpor in daily heterotherms.
- (3) As T_a was below T_b during the rewarming process and no other external heat was supplied, rewarming from torpor in the spiny mouse, although it was slow, was entirely due to endogenous heat production.
- (4) The animal survived the >2 -day torpor bout, which shows that it is capable of more than just daily torpor. In contrast, although #21 also cooled rapidly after the rain event, its T_b was almost identical to that of T_a and because of its demise was unable to rewarm.

We also found that after the multiday torpor, #22 switched its T_b pattern and its T_b was higher during the day than during the night, in contrast to all other individuals under the same conditions. Energy expenditure for thermoregulation depends on T_a and the thermo-neutral zone of the species (a range of T_a s in which the animal is not investing energy in thermoregulation). The thermo-neutral zone of golden spiny mice is $\sim 31^\circ\text{C}$ (Shkolnik and Borut 1969). Since being active during the day or during the night exposes the animal to very different T_a s, choosing to be active at the phase in which T_a is within or closer to the species’ thermo-neutral zone will result in lower energy expenditure for thermoregulation. Spiny mice are well known for their ability to switch their activity and T_b rhythms between nocturnal and diurnal (Levy *et al.* 2007, 2012b), also in response to T_a : in the high mountains of the Sinai desert *A. russatus* are diurnally active, especially in winter, preferring the higher T_a s during the day than during the cold nights (Haim and Borut 1975), and at the Judean desert, energy expenditure during foraging was found to be always lower during the day than during the night (Levy *et al.* 2012a). It is possible that the switch by #22 to the warmer part of the diel cycle resulted also from

thermoregulatory considerations and reduced its energy expenditure.

Conflicts of interest

The authors declare no conflicts of interest.

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