



## Research

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# Subtropical mouse-tailed bats use geothermally heated caves for winter hibernation

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We report that two species of mouse-tailed bats (*Rhinopoma microphyllum* and *R. cystops*) hibernate for five months during winter in geothermally heated caves with stable high temperature (20°C). While hibernating, these bats do not feed or drink, even on warm nights when other bat species are active. We used thermo-sensitive transmitters to measure the bats' skin temperature in the natural hibernacula and open flow respirometry to measure torpid metabolic rate at different ambient temperatures ( $T_a$ , 16–35°C) and evaporative water loss (EWL) in the laboratory. Bats average skin temperature at the natural hibernacula was  $21.7 \pm 0.8^\circ\text{C}$ , and no arousals were recorded. Both species reached the lowest metabolic rates around natural hibernacula temperatures (20°C, average of  $0.14 \pm 0.01$  and  $0.16 \pm 0.04 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  for *R. microphyllum* and *R. cystops*, respectively) and aroused from torpor when  $T_a$  fell below 16°C. During torpor the bats performed long apnoeas ( $14 \pm 1.6$  and  $16 \pm 1.5$  min, respectively) and had a very low EWL. We hypothesize that the particular diet of these bats is an adaptation to hibernation at high temperatures and that caves featuring high temperature and humidity during winter enable these species to survive this season on the northern edge of their world distribution.

## 1. Introduction

Torpor and hibernation are common physiological adaptations used by mammals to conserve energy. These behaviours have been studied mostly in the context of adaptation to negative energy balance and extended periods of water and food shortage, and have been described in species from desert and tropical habitats. In recent years, different ecological interactions were found to affect the use of torpor and hibernation [1–5]. The Rhinopomatidae (mouse-tailed bats) are a monotypic family of subtropical insectivorous bats inhabiting semi-arid and warm regions in Asia and Africa. Two species, the greater mouse-tailed bat (*Rhinopoma microphyllum*) and the lesser mouse-tailed bat (*Rhinopoma cystops*) are medium-sized insectivorous bats with body masses of approximately 25 g and 12 g, respectively, and are both well adapted to arid environments [6,7]. *Rhinopoma microphyllum* and *R. cystops* inhabit the dry and warm regions of Israel, which is the northern edge of their world distribution [8]. These species have limited tolerance to low ambient temperatures and it has been suggested that they are unable to perform deep torpor or hibernation [6].

We have previously found that during summer, *R. microphyllum* exhibit complete sexual segregation, inhabiting warm and dry caves (28–32°C). We also found that lactating females remain almost normothermic during the day and perform long foraging bouts during the night, while males and non-lactating females use daily torpor during the day and perform short foraging bouts during the night [9,10]. During late summer (August) both species accumulate large amounts of body fat, sometimes reaching 50% of their body mass, a phenomenon typical for hibernators. This increase in body fat is achieved by switching diet preference during summer (mid-July) from coleopterans and heteropterans to mainly fat-rich winged carpenter ants [11]. This fat-rich food

contains a high fraction of saturated and mono-unsaturated fatty acids (SFA and MUFA, respectively), but almost no polyunsaturated fatty acids (PUFA) [12] which are considered important for hibernation success at low temperatures [13–17]. As a result, mouse-tailed bat pre-winter body fat is lacking in PUFAs and is extremely saturated [11,12], which is atypical for hibernators.

During late October, both mouse-tailed bat species move to caves in which they remain throughout the winter [12]. Based on our observations of no foraging activity during the winter months, and the accumulation of large amounts of saturated body fat before winter [11,12], we hypothesized that mouse-tailed bats hibernate during winter at high ambient temperatures. To test these hypotheses, we monitored skin and hibernaculum temperature of free-ranging mouse-tailed bats during winter, and examined the effect of ambient temperature on energy expenditure and water loss under controlled conditions in these two species.

## 2. Material and methods

### (a) Winter hibernacula

A winter colony of both *R. microphyllum* and *R. cystops* was discovered during a field survey on 2003 on a cliff by the Sea of Galilee (32°46' N 35°32' E, 100 m below sea level). The cave is a wet karstic cave in Eocene limestone formed by a phreatic and hypogenic dissolving process in a confined aquifer. The dissolving has created a maze network of mainly horizontal branched tunnels with some halls and typical domes in which the bats hang. The temperature in this cave is relatively high and constant due to geothermic activity (speleological data from the Israeli Cave Research Unit, The Hebrew University, Jerusalem). From 2003 to 2013, we visited this cave three to eight times a year, and counted the bats directly by observation or photography. We also acoustically identified and counted bats emerging from this cave during winter nights, using Pettersson D500 (Pettersson Elektronik, Sweden) and ANABAT bat detectors (Titley Scientific, Australia). During 2003 and 2004, we placed paper sheets on the cave floor beneath the roosting bats for faeces collection.

### (b) Cave and skin temperature

We use thermal sensitive transmitters to record skin temperature ( $T_{sk}$ ) in hibernating mouse-tailed bats. Skin temperature of small mammals (including bats) was validated and is considered to be a good predictor for body temperature, and is used in numerous studies to measure the use of torpor and hibernation in free-ranging small mammals including bats [18–20].

Nine adult *R. cystops* (five females and four males) were captured during daytime (15 January 2012) in their natural hibernacula using a hand net, and body mass was measured using an electronic scales (Micron, China,  $\pm 0.01$  g). To attach the temperature transmitters for skin temperature detection, we removed a patch of hair between the bats' shoulder blades using a depilatory cream (Veet®, Reckitt Benckiser, UK). The area was then washed gently with a wet cotton ball and dried. Then, a calibrated temperature sensitive radio transmitter (BD-2T 0.35 g, Holohil, Canada) was attached between the bat's shoulder blades using medical adhesive (Perma-Type Company, USA). After attaching the transmitter, the bats were immediately returned to the hibernacula.

To cover a large area of the cave, we positioned two RX-900 receivers (Televilt, Sweden) inside the cave, one in the outer section and the other deeper inside the cave maze. We used four dipole antennas (two per receiver) connected to the receivers with 20 m long cables. Voltage to each receiver was supplied by a 12 V car battery (replaced every 5–8 days).

Ambient temperature was recorded every 90 min using four I-Button temperature data loggers (0.5°C resolution, Maxim Integrated, USA) in four different rooms next to each antenna. Relative humidity was measured on December 2004 and again on June 2013, using a mechanical hygrometer (Fischer, Germany).

Of the nine *R. cystops* fitted with temperature sensitive radio transmitters on 15 January 2012, we were able to monitor skin temperatures for only eight. The ninth bat disappeared the day it was tagged and probably moved to another cave on that cliff. On 19 January, one of the receivers stopped working (chewed up by *Hystrix indica* inhabiting this cave), and we lost the signal of three more bats. Five remained within the range of the other receiver throughout the 25 days of transmitter battery life.

### (c) Torpid metabolic rate

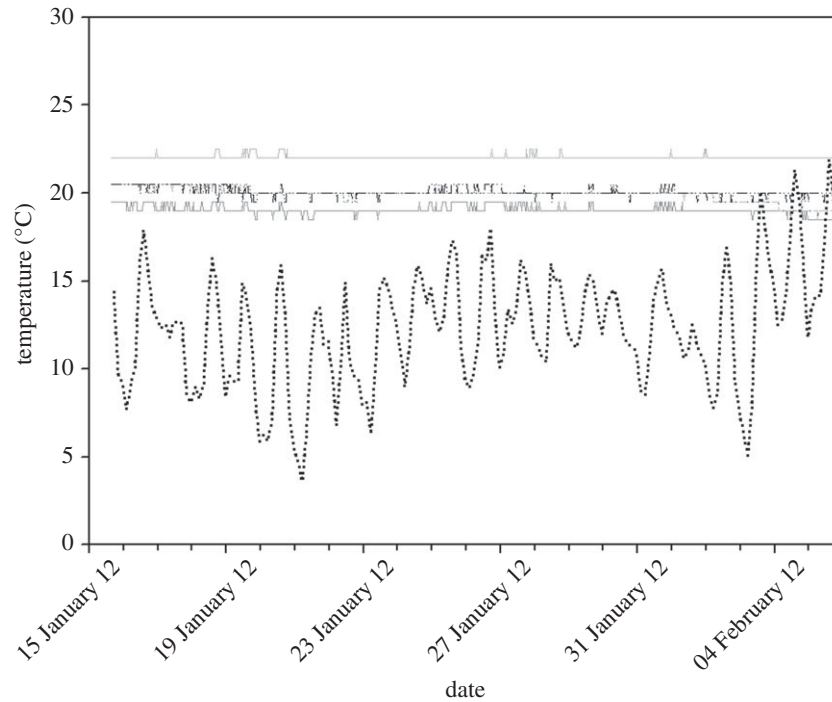
For metabolic measurements 14 adult bats (*R. microphyllum*, five males and four females; *R. cystops*, two males and three females) were captured on 12 February 2012 in their hibernacula as described previously. The bats were brought to the laboratory, marked individually with bat rings (Porzana, UK, for *R. microphyllum*—4.2 mm, for *R. cystops*—2.6 mm) and placed in a custom-made wooden cage in a temperature-controlled room at 20°C, which resembles the ambient temperature of the bats' natural hibernacula. An animal was considered in torpor when its metabolic rate decreased under  $0.7 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  [21] and/or was performing intermittent breathing patterns. TMR of the bats was measured using an open-flow respirometry system. Bats (all fasted at least 2 days in the laboratory and probably much longer in their hibernacula) were weighed using electronic scales (Micron, China,  $\pm 0.01$  g) and introduced individually into a metabolic chamber (Perspex chambers; 250 ml for *R. microphyllum* and 210 ml for *R. cystops*).  $\text{O}_2$  consumption and  $\text{CO}_2$  production were measured by gas analyser (FoxBox, Sable Systems Int., USA). Dry air passed through the metabolic chamber at a constant flow of  $100 \text{ ml min}^{-1}$ . Data were collected at 5-s intervals using a PC and ADAMview software (Advantech, USA). After placing a bat in the chamber, the chamber was immersed in a temperature-controlled water bath at 20°C, reflecting natural hibernacula temperature.

To detect the influence of ambient temperature on TMR the metabolic rate of each bat was measured at various temperatures on 2 consecutive days, each starting with 120 min of acclimation to 20°C. On day 1, TMR was recorded at 19°C, 18°C, 17°C and 16°C for 120 min each. We did not reduce  $T_a$  below 16°C as at this temperature the bats started to get stressed and as this temperature was reported as lethal to this species [6]. On the second day, TMR was recorded at 20°C, 25°C, 30°C and 35°C (33°C is the highest temperature we recorded in the summer roosts [9]) for 120 min each. The experiment was stopped when a bat aroused from torpor, i.e. no apnoea cycles were detectable and  $\text{CO}_2$  levels in the chamber rose above 0.7%.

For data analysis alone the last 30 min of each temperature were used.  $\text{VO}_2 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  was calculated according to Withers [22]. For comparisons of TMR, we used the average value of  $\text{VO}_2$  for the last 30 min of measurement at each temperature.

During November and February 2013, 10 *R. cystops* were measured for TMR as described above at 20°C. At the end of each measurement  $T_{sk}$  was taken from a hairless area of the dorsal side, by an infra-red thermometer GM300 (Benetech, China). We avoided invasive procedures as these are protected species in Israel.

At the end of the 2-day experiment, we prepared the bats for release back to nature: bats were hand-fed daily with a syringe for two weeks with 1 ml of squeezed darkling beetle larvae (*Zophobas morio*) enriched with high nutrient food for pets (Recovery, Royal Canin). The bats were fed in order to increase their weight which, we assumed, will increase their chance for survival in nature. The bats were returned to the hibernacula in late March. Two of the bats used in the experiments were recaptured in good



**Figure 1.** Ambient temperature outside the cave (dashed black line) and temperature at four different rooms in the hibernacula cave (grey lines) during the study. The two middle rooms are almost identical in their temperature. The temperature in this cave is higher than that outside the cave owing to geothermic activity.

shape 1 year later. We assume that no harm was caused to the bats from the captivity period.

#### (d) Evaporative water loss

For EWL measurements an additional 10 adult bats (*R. microphyllum*, three males and two females; *R. cystops* three males and two females) were captured in February 2013 in their winter hibernacula as described before. The bats were placed in individual Perspex metabolic chambers (250 ml for both species) with constant air flow of  $100 \text{ ml min}^{-1}$  of a  $\text{CO}_2$  free, dry air from a cylinder. Air supply to the animals was controlled by an eight-channel gas controller and monitor (Flow bar-8, Sable Systems Int.). The air flowed to an eight-channel gas multiplexer (V5, Sable Systems Int.) that selected channels sequentially for sampling. Air samples were passed through a water vapour analyser (RH-300, Sable Systems Int.). To make sure the bats were hibernating during the measurements, we also measured  $\text{O}_2$  and  $\text{CO}_2$  levels in the air sample: the air coming out from the RH-300 was dried with a drierite (W. A. Hammond, USA) column and passed through an  $\text{O}_2/\text{CO}_2$  analyser (FoxBox, Sable Systems Int.). Data regarding water vapour,  $\text{O}_2$  and  $\text{CO}_2$  were collected every 5 s using Expedata software (Sable Systems Int.). EWL was calculated using Lighton's 10.11 equation [23]. The bats were kept unfed during the 2 days of measurements in the laboratory and then returned to their hibernacula.

### 3. Results

In the winters of 2003–2013, we counted 200–500 individuals of *R. cystops* and 50–500 of *R. microphyllum* in the observed winter roost. *Rhinopoma microphyllum* was often found in clusters while *R. cystops* always maintained a distance of forearm and tail from each other. During all our visits, the bats' eyes were opened and they were able to swiftly fly (within seconds) in response to disturbance. Bats were making audible calls, which were detectable before entering the cave during both day and night. In contrast to the summer roosts, in the hibernacula no fresh faeces were observed on the cave floor or on the collection paper sheets positioned

under the clusters of bats at any point of their stay there. However, much fresh urine was absorbed onto these sheets.

Six other species of insectivorous bats were identified visually and acoustically in the same cave during winter: *Rhinolophus ferumequinum*, *R. blassi*, *R. hipposideros*, *Miniopterus schreibersii*, *Taphozous perforatus* and *Myotis capaccini*. Unlike all of these latter species, which were observed emerging from the cave to feed on winter nights and whose faeces were found under their roosting spots, *R. cystops* and *R. microphyllum* remained inside the cave even on relatively warm nights (greater than or equal to  $20^\circ\text{C}$ ), when many aerial insects were active.

During the study we found another five caves with winter colonies of *Rhinopoma* in Israel, four of them along the Syrian–African rift valley and all of which are warm and humid. This area is geologically active and contains many hot springs and underground warm-water cisterns.

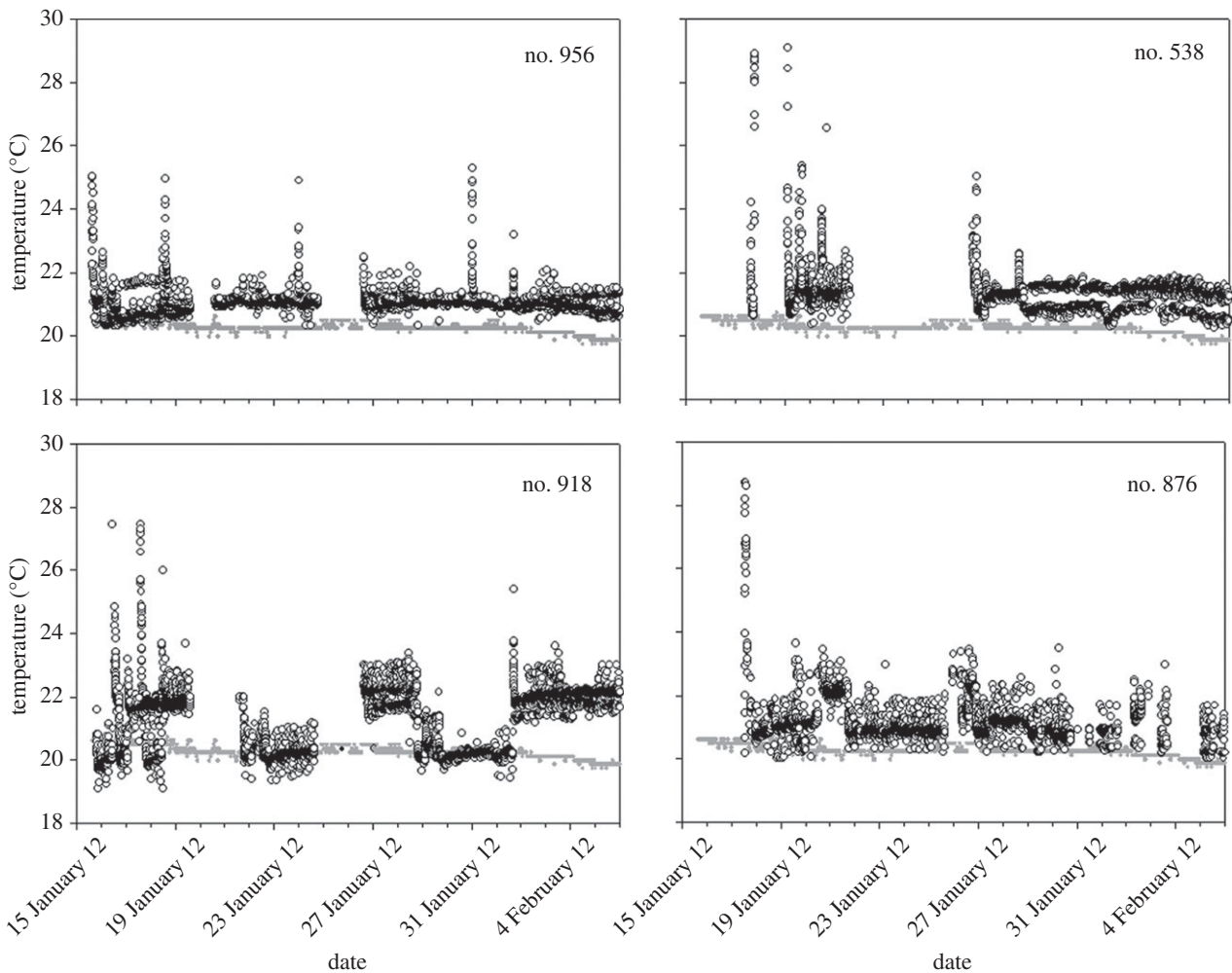
#### (a) Cave and skin temperature

While ambient temperatures outside the cave fluctuated considerably during winter from  $4$  to  $22^\circ\text{C}$  (figure 1), the temperature inside the cave remained relatively constant at about  $19^\circ\text{C}$  in the outer parts and at about  $22^\circ\text{C}$  in the inner parts of the cave (figure 1). Relative humidity in the cave was approximately 100%.

$T_{\text{sk}}$  of all bats recorded was  $1$ – $3^\circ\text{C}$  above average cave temperature (table 1). All bats exhibited sporadically short events of higher  $T_{\text{sk}}$ . These events were more common during the first days after transmitter attachment and always lasted less than 40 min (figure 2).

#### (b) Torpid metabolic rate

After the mouse-tailed bats were introduced into the metabolic chamber at  $20^\circ\text{C}$  they reduced their metabolic rate within 2 to 25 min and entered torpor.  $T_{\text{sk}}$  of torpid bats at  $20^\circ\text{C}$  was  $22.3 \pm 1.7$ . The torpid bats performed typical cycles of apnoea followed by a short ventilation period. These cycles were temperature-dependent. Both species



**Figure 2.** Skin temperature ( $T_{sk}$ ) of four *Rhinopoma cystops* during January–February 2013 (black circles). Grey line, cave temperature (average of four sensors).

**Table 1.** Forearm length, body mass, monitoring period and skin temperature of nine hibernating *Rhinopoma cystops* during winter 2012.

no.	sex	start	end	mass (g)	forearm (mm)	average $T_{sk}$ ( $^{\circ}$ C)	min $T_{sk}$ ( $^{\circ}$ C)	max $T_{sk}$ ( $^{\circ}$ C)*
339	F	15 Jan	19 Jan	11.20	55.3	22.02	20.54	24.96
379	F	15 Jan	19 Jan	11.60	55.8	21.2	20.35	23.5
499	F	15 Jan	19 Jan	11.90	57.9	22.57	19.5	23.73
538	M	15 Jan	6 Feb	10.90	57.7	21.21	19.51	29.09
738	M	15 Jan	31 Jan	14.90	59	23.18	19.78	25.48
877	F	15 Jan	6 Feb	10.40	55.2	21.22	19.49	23.6
918	F	15 Jan	6 Feb	11.70	56.9	21.11	19.09	27.4
938	M	15 Jan	15 Jan	15.20	60.8	—	—	—
957	M	15 Jan	6 Feb	13.50	59.5	21.03	19.41	25.2

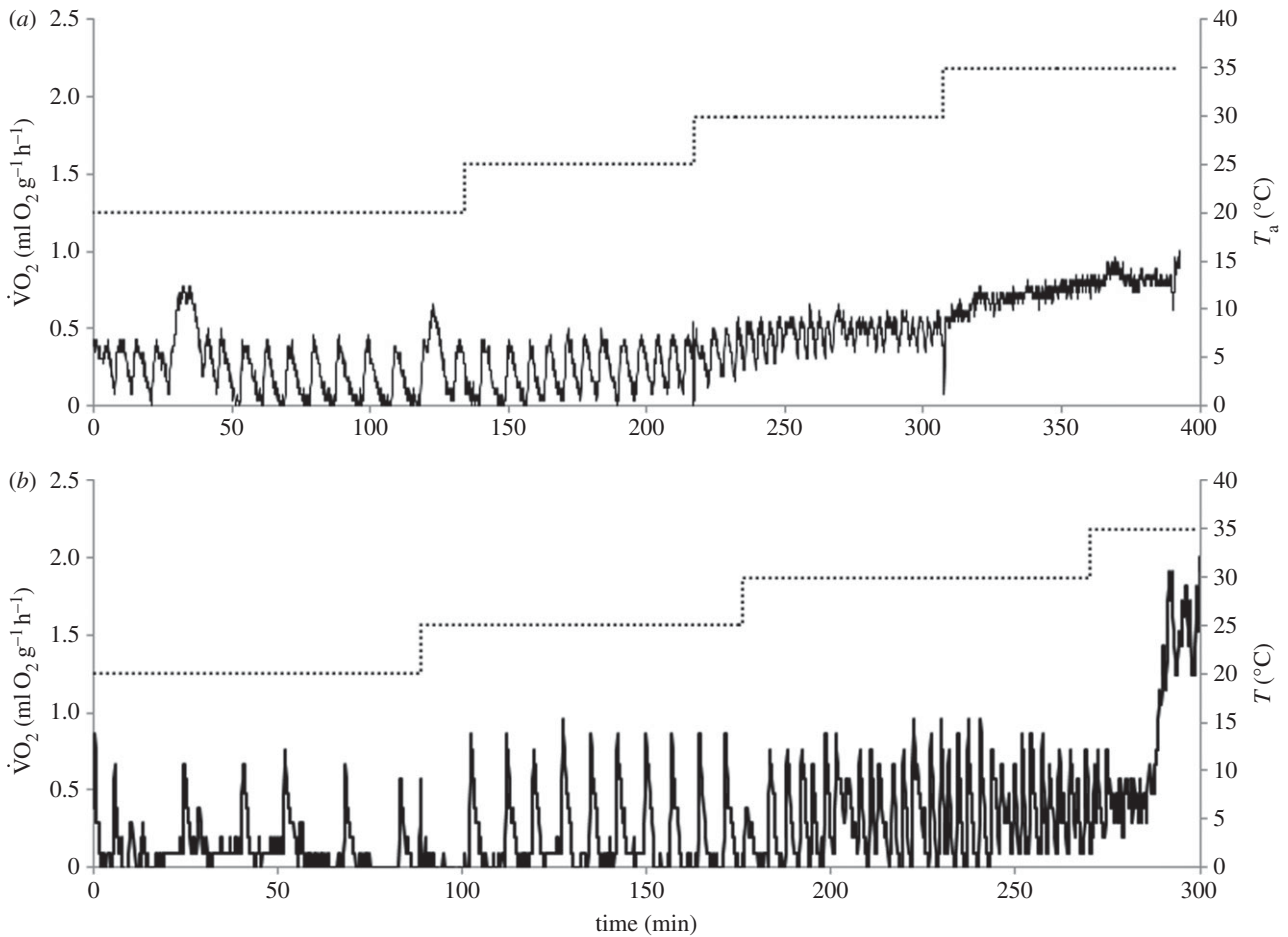
showed the longest cycles around natural hibernacula temperature of  $20^{\circ}$ C with an average of  $14 \pm 1.6$  min for *R. cystops* and  $16 \pm 1.5$  min for *R. microphyllum* (figure 3), and with a maximum cycle length of 28 min.

The two species differed significantly in their torpid metabolic rates (TMRs) at different temperatures (two-way repeated measure ANOVA  $F = 7.9$ ,  $p < 0.001$ ). *R. cystops* had significantly lower TMRs at the lowest measured temperature of  $16^{\circ}$ C (figure 4, *post hoc* with Holm–Sidak method: difference of means = 1.59,  $t = 7.1$ ,  $p < 0.001$ ), and *R. microphyllum* had significantly lower TMRs at the highest measured temperature

of  $35^{\circ}$ C (figure 4, *post hoc* Holm–Sidak method: difference of means = 0.345,  $t = 2$ ,  $p = 0.04$ ).

Average TMR at  $20^{\circ}$ C was  $0.14 \pm 0.01$  and  $0.16 \pm 0.04$   $\text{ml O}_2 \text{g}^{-1} \text{h}^{-1}$  for *R. microphyllum* and *R. cystops*, respectively (figure 4b). In *R. microphyllum*, TMR increased significantly at  $16^{\circ}$ C, while in *R. cystops* it increased significantly at  $35^{\circ}$ C. Six out of eight *R. microphyllum* started arousing from torpor when the ambient temperature was lower than  $17^{\circ}$ C, and only two remained torpid. However, TMR of these two individuals was significantly higher than TMR of *R. cystops* at the same temperature (*post hoc* with Holm–Sidak method:





**Figure 3.** An example of oxygen consumption  $\dot{V}O_2$  ml  $O_2$   $g^{-1}$   $h^{-1}$  of *Rhinopoma microphyllum* (a) and *Rhinopoma cystops* (b) in the laboratory at different ambient temperatures (20–35°C at 5°C intervals, 120 min at each temperature). Dashed line, ambient temperature, solid line, oxygen consumption. Peaks represent ventilations between apnoeas.

difference of means = 1.59  $t = 7.1$ ,  $p < 0.001$ ). When ambient temperature was raised above 20°C, while TMR of both species increased, at these temperatures that of *R. cystops* was significantly higher than that of *R. microphyllum*, and reached normothermic values at 35°C of about  $0.91 \pm 0.2$  ml/ $O_2$  h (figure 4).

We found significantly lower TMR in *R. microphyllum* at 20°C in the 2013 group (see below, EWL experiment) compared with the 2012 group at the same time of the year ( $0.14 \pm 0.01$  versus  $0.087 \pm 0.01$  ml/ $O_2$   $g^{-1}$   $h^{-1}$ ,  $t$ -test  $t = -5.19$ ,  $p < 0.001$ ). This may be related to the fact that the first group was kept in the laboratory for a few days before measurements in low humidity and constant noise from the climate chamber compressor, which might stress the bats. No significant difference between the years was found in *R. cystops* ( $t$ -test,  $p = 0.8$ ).  $Q_{10}$  values for the torpid mouse-tailed bats were higher than 2 in both species (table 2).

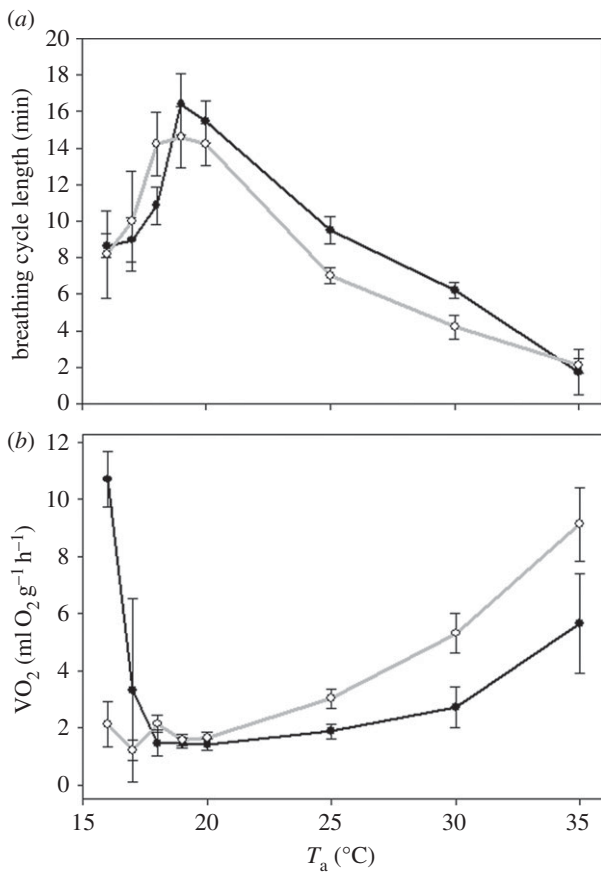
### (c) Evaporative water loss

Total EWL at hibernacula temperature (20°C) was  $0.29 \pm 0.05$  mg  $H_2O$ (h g) $^{-1}$  for *R. microphyllum* and  $0.38 \pm 0.1$  mg  $H_2O$ (h g) $^{-1}$  for *R. cystops*. There was no significant difference in total EWL between the species ( $t$ -test  $t = -1.5$ ,  $p = 0.16$ ). We suggest that the main channel for EWL in mouse-tailed bats is cutaneous, as most of the time the bats were in apnoea, with their mouth and nostrils shut.

## 4. Discussion

Our results show that both *R. cystops* and *R. microphyllum* hibernate during winter. During hibernation, the bats showed intermitted breathing, with bouts of ventilation and apnoea, as reported in several other hibernating rodents, bats and marsupial species [24]. During the ventilation and apnoea, uptake and clearing of  $O_2$  and  $CO_2$  was rapid and greatly reduced respectively, and we assume that as reported in other species, blood oxygen and  $CO_2$  tension change accordingly [24]. The fact that apnoea cycles length changed with temperatures suggests that breathing bouts are correlated with metabolic demands, and most probably with blood gas levels.

Even though these two species have the option to hibernate in cold caves nearby, they prefer to hibernate in a geothermally heated cave with warm and a stable ambient temperature of 19–23°C. This behaviour suggests that this  $T_a$  is of advantage for the mouse-tailed bats during their winter hibernation, which is indeed supported by our findings that lowest TMR, and hence highest energy conservation, and longest cycles of apnoea are achieved around 20°C. It is possible that the existence of these geothermally heated caves enables mouse-tailed bats to extend their distribution northwards along the Syrian–African rift valley. In contrast to the outmoded assumption that hibernation is associated with low temperatures, we present an example of a bat that is not only able to hibernate at high temperatures, but prefers to hibernate at high ambient temperature and is able to maintain low



**Figure 4.** Average ( $\pm$ s.e.) of (a) breathing cycle length (min) and (b) metabolic rates (oxygen consumption  $VO_2$   $ml O_2 g^{-1} h^{-1}$  of *R. microphyllum* (close symbols, black line) and *Rhinopoma cystops* (open symbols, grey line) at different temperatures: 20–35°C at 5°C intervals, 120 min at each temperature. For calculations, the last 30 min at each temperature were used. Asterisks (\*) indicates significant difference from the other species, (\*\*) indicates significant difference from 20°C in the same species,  $p < 0.05$ . Two-way repeated measure ANOVA with *post hoc* Holm–Sidak method.

TMRs at temperatures of, and above, 30°C, while arousing at temperatures below 16°C.

Although it is well established that mammals and birds can reduce their metabolic rates by the use of torpor and hibernation, the mechanism underlying this reduction is still controversial. It was argued that reduction of energy metabolism during hibernation cannot be explained by  $Q_{10}$  effect alone (a measure of the rate of change of a biochemical system as a consequence of changing the temperature by 10°C), since  $Q_{10}$  value of biochemical systems is typically 2, while the  $Q_{10}$  values measured for daily heterotherms and hibernating mammals in temperatures range of 20–30°C are usually higher than 2 (average of 4.11 and 2.24 in hibernators and daily heterotherms, respectively [25]), which implies that animals use metabolic inhibition to maximize energy savings [23]. The  $Q_{10}$  values in the mouse-tailed bats were between 2.5 and 4 in the temperature range measured (table 2) which is close to the ones measured in real hibernators, and suggests that the bats used metabolic inhibition.

Even though both *R. cystops* and *R. microphyllum* hibernate at high temperatures compared with most other hibernating mammals, their TMR at their natural hibernacula temperature is comparable with the TMR of other small (less than 150 g) hibernators at the same temperature range (figure 5). We found *R. cystops* to be more tolerant to low temperatures than

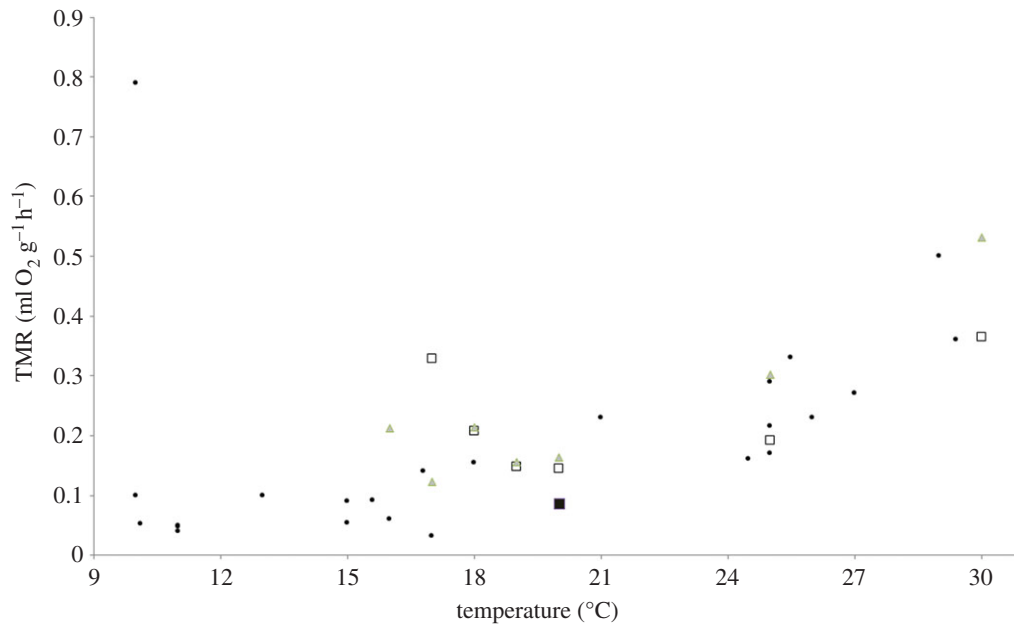
**Table 2.**  $Q_{10}$  values ( $\pm$ s.d.) of *Rhinopoma microphyllum* and *Rhinopoma cystops*.

temperature range	<i>R. microphyllum</i>	<i>R. cystops</i>
$Q_{10}$ 20–30°C	$2.5 \pm 0.5$	$3.5 \pm 1.3$
$Q_{10}$ 25–35°C	$4.0 \pm 0.8$	$3.1 \pm 1.2$

*R. microphyllum*, which may explain its wider distribution in Israel: during summer it is found at higher altitudes and in larger areas of the Mediterranean region [26]. Kulzer [6] reported that mouse-tailed bats die at ambient temperature of 15°C, and that when their  $T_b$  is below 19°C, their re-warming rate is significantly low in comparison with other bat families. Based on our previous work on summer torpor and activity  $T_{sk}$  [9] and on the present observations on hibernation  $T_{sk}$  in mouse-tailed bats, we suggest that mouse-tailed bats defend torpor body temperature of about 20°C, and start thermoregulating if  $T_a$  drops below 19°C. This finding is unusual for hibernators as for most hibernators, the minimum  $T_b$  is limited by the freezing point of body fluids (approx. 0°C), which prevents a further drop of  $T_b$  [27]. For the mouse-tailed bats, using geothermally heated caves and keeping body temperature at this relatively high level will have adaptive significance, since it will significantly reduce the energetic cost of arousals, and therefore, the total cost of hibernation.

We suggest that mouse-tailed bats started arousing and increased TMR at 16°C owing to their body fatty acid composition. We previously found that towards the end of summer, during the pre-hibernation period, mouse-tailed bats feed mainly on queens of carpenter ants [11]. These queen ants are an extremely poor source of PUFA (0.5%) and rich in SFA (approx. 44%) and MUFA (approx. 55.5%) [12]. We also found that this extremely saturated lipid profile is reflected in the mouse-tailed bats' pre-hibernation tissue, which is, to the best of our knowledge, the most saturated ever recorded in a mammal [12] and differs from the typical PUFA-rich food preference and body composition of other hibernating mammals [17]. High levels of dietary PUFAs in pre-hibernating mammals are known to positively affect torpor and hibernation, for example, by increasing depth and duration of torpor bouts [14,15,28], but the function of these fatty acids during hibernation is still unknown [29,30]. Since mouse-tailed bats fat is saturated, unlike other hibernators, it should restrict their ability to hibernate at low temperatures.

During hibernation, hibernating mammals are motionless and their eyes are closed, while mouse-tailed bats hibernate with their eyes open, instantly react to stimuli, and swiftly become active after being disturbed, even when their body temperature is still far below normothermy (approx. 37°C). This can be explained by the relatively high  $T_b$  of mouse-tailed bats during hibernation. We observed mouse-tailed bats actively flying when their body temperature was still low (approx. 23°C) and argue that they can perform many basic functions at relatively low  $T_b$  (see also [9]). Quantitative data on locomotor performance at low  $T_b$  in torpid marsupials suggest that they can run at  $T_b$  far below 20°C [31]. The ability to react fast to threat may be especially important for a small mammal hibernating at a cave entrance in a subtropical winter. Unlike winters in temperate regions, in a subtropical climate other mammals, reptiles and birds of



**Figure 5.** Body temperature and metabolic rate in hibernating mammals in the temperature range of 10–30°C (black filled circles, data from [21]). Open squares, ambient temperature and TMR measured in *Rhinopoma microphyllum* on 2012, black square, on 2013. Grey triangles, ambient temperatures and TMR measured in *Rhinopoma cystops* on 2012. (Online version in colour.)

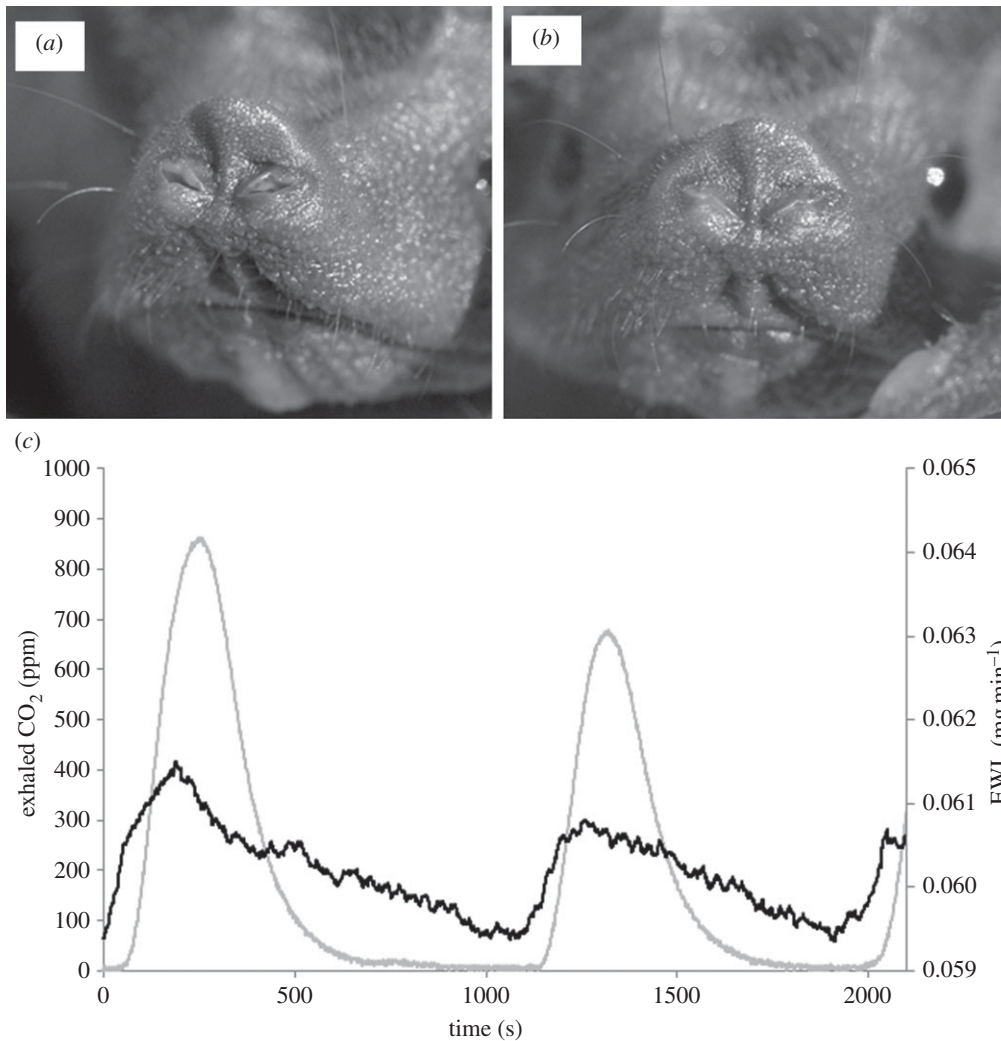
prey are present and active during the winter and might be a threat to the hibernating bats. Therefore, vocal communication between individuals like we heard when entering the hibernacula and a fast response may be crucial for survival and adds another adaptive significance of hibernation at high temperatures.

Why do these bats hibernate during the Israeli temperate winter? This is especially interesting as other bat species inhabiting the same cave remain active and forage. A possible explanation is that for a subtropical bat at the northern edge of its distribution, the Mediterranean winter is too cold to be active and to survive so they hibernate in geothermally heated warm protected caves. However, another subtropical bat, *T. perforatus*, inhabits the same cave during winter but remains active. This may suggest that other factors influence the use of torpor in these species. One possibility is that in contrast to the active bat species, the mouse-tailed bats in northern Israel are diet-specialists. During early summer they consume mainly coleopterans and heteropterans, which are scarce during winter, and from July they feed almost solely on winged ants, which are absent during the winter months [11]. Other factors, like predation risk, were suggested as possible contributors to the use of torpor and hibernation [1], but these remain to be studied.

We did not record any periodic arousals during hibernation in any of the mouse-tailed bats in their natural hibernacula. We expected to find more than few such arousals during the 25 days of  $T_{sk}$  recording. In other temperate zone bats hibernating at ambient temperature around 15°C and above, arousals appear almost every day [32–35]. One exception is the large (60 g) subtropical insectivore bat *Hipposideros terasensis*, hibernating at relatively warm hibernacula around 20°C. In this bat, torpor bouts lasted from 1.6 to 19 days, and arousals and foraging bouts are observed throughout winter (70% of torpor bouts were shorter than 10 days) [36]. We monitored mouse-tailed bats  $T_{sk}$  for 25 days and did not record a single arousal in any of the eight bats recorded. Nevertheless, we did record sporadic increases of 3–8°C in body temperature up to 25–29°C for a very short

duration (20–60 min, figure 2 and table 1). Based on these findings, we suggest that mouse-tailed bats do not perform arousals, or that they perform unusual, extremely long torpor bouts for hibernation in high ambient temperatures. Except for the energy saving, reducing arousal frequency can reduce possible oxidative stress or other damage to the brain and other tissues due to hypoxia during arousals (reviewed by [37,38]).

In contrast to other hibernating bats [32,36,39] mouse-tailed bats remain in their hibernacula all winter long and avoid foraging or drinking. During our study the mouse-tailed bats were never observed drinking water, neither during warm winter nights above 20°C nor during the summer, suggesting that they are very efficient at conserving water. It was previously suggested that hibernating mammals lose water through EWL until a specific threshold at which they must arouse to drink [40–42]. According to this hypothesis, if mouse-tailed bats are very efficient in reducing water loss during hibernation, this might significantly contribute to reducing arousals during hibernation or even to avoiding them. Indeed, mouse-tailed bats possess a very low density of capillaries in their skin, which reduces EWL [43]. Accordingly, we found that the total EWL of mouse-tailed bats is exceptionally low, even under the zero relative humidity conditions we used in the laboratory. As cave relative humidity is close to 100%, the values of EWL in wild hibernating mouse-tailed bats should be even lower and close to zero. Even though most of the water loss is through the skin, we suggest that during apnoea in which nostrils are closed, pulmonary water loss should be greatly reduced. It was previously suggested in other bat species that during bat apnoea the glottis is closed and prevents water loss, but there is contradicting evidence to this theory [41,44,45]. Mouse-tailed bats have distinctive valves in their nostrils (figure 6). During apnoea these valves are shut and only flutter (open and shut rhythmically with diaphragm movement) during the ventilation cycles between the apnoeas. This results in greater total EWL than during apnoea (figure 6). We suggest that in mouse-tailed bats the nostril valves function analogically with the spiraculum of the insects' trachea [46] and



**Figure 6.** *Rhinopoma microphyllum* nostrils. (a) Valves are open and (b) valves are shut. (c) Total evaporative water loss (black line) and CO<sub>2</sub> exhaled (grey line) during hibernation. There is a clear effect of apnoea and ventilation (as indicated by CO<sub>2</sub> exhalation) on pulmonary water loss.

contribute to reducing pulmonary water loss. We observed these nostril valves in another two arid zone bat species; *Taphozous nudiventris* and *T. preforatus*. In Israel, these two species occupy the same habitats as mouse-tailed bats, and we suggest this might be a common mechanism for reducing water loss in arid zone bats.

In summary, both species of mouse-tailed bats enter a very efficient hibernation, which they perform in geothermally heated, stable microclimate of warm hibernacula, maintaining their body temperature above 20°C. Unlike other hibernators, we observed no periodic arousals or periods of normothermy during hibernation, and even though it is possible that the recording period was too short to record such arousals, we suggest that their ability to function at low  $T_b$ , combined with their efficient water economy reduces the need for periodic arousals in these subtropical hibernating species. Their pre-hibernation diet in Israel, and as a result their body fatty acid composition, may contribute to their hibernation

success, but at the same time we hypothesize it forces them to maintain their body temperature above 16°C. Hibernating in the moderate Israeli winter raises questions regarding the selective forces and adaptive significance of using hibernation in this genus, and remains to be studied. The finding that mouse-tailed bats use specific caves with constant high temperature and humidity during winter, enabling these species to survive on the northern edge of their distribution, highlights the importance of protecting such caves for the conservation of these species.

**Ethics statement.** All procedures were carried out under permit no. 2009/31867 from the Israel Nature Reserves and Park Authority (NPA) and the Ethics Committee license no. L-09-002.

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