



## Can bats sense smoke during deep torpor?

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### ABSTRACT

While torpor is a beneficial energy-saving strategy, it may incur costs if an animal is unable to respond appropriately to external stimuli, which is particularly true when it is necessary to escape from threats such as fire. We aimed to determine whether torpid bats, which are potentially threatened because they must fly to escape, can sense smoke and whether respiration rate (RR), heart rate (HR) and reaction time of torpid bats prior to and following smoke introduction is temperature-dependent. To test this we quantified RR and HR of captive Australian tree-roosting bats, *Nyctophilus gouldi* ( $n = 5$ ,  $\sim 10$  g), in steady-state torpor in response to short-term exposure to smoke from *Eucalyptus* spp. leaves between ambient temperatures ( $T_a$ ) of 11 and 23 °C. Bats at lower  $T_a$  took significantly longer (28-fold) to respond to smoke, indicated by a cessation of episodic breathing and a rapid increase in RR. Bats at lower  $T_a$  returned to torpor more swiftly following smoke exposure than bats at higher  $T_a$ . Interestingly, bats at  $T_a < 15$  °C never returned to thermoconforming steady-state torpor prior to the end of the experimental day, whereas all bats at  $T_a \geq 15$  °C did, as indicated by apnoeic HR. This shows that although bats at lower  $T_a$  took longer to respond, they appear to maintain vigilance and prevent deep torpor after the first smoke exposure, likely to enable fast escape. Our study reveals that bats can respond to smoke stimuli while in deep torpor. These results are particularly vital within the framework of fire management conducted at  $T_a < 15$  °C, as most management burns are undertaken during winter when bats will likely respond more slowly to fire cues such as smoke, delaying the time to escape from the fire.

### 1. Introduction

Although mammalian torpor can substantially reduce metabolic rate (MR) and body temperature ( $T_b$ ) for energy conservation [1], its drawbacks include compromised sensory and locomotor capabilities [2,3]. Reduced responsiveness at low  $T_b$  decreases the ability of torpid endotherms to respond quickly to environmental stimuli. Many hibernators, such as insectivorous bats, reduce  $T_b$  to near or below 10 °C [1,4,5]. Therefore, responding to a disturbance during a torpid state by rewarming from low ambient temperature ( $T_a$ ) is not only energetically expensive [6], but also requires more time than at warmer  $T_a$  [7,8]. The time needed for a torpid animal to respond to an environmental disturbance, such as smoke, from low  $T_b$  is critical and could determine whether or not that animal is able to escape and survive a fire.

Only a few studies have attempted to determine which types of nontactile disturbances can induce arousal from torpor, and in bats these are generally limited to human interaction, light, sound and conspecific disturbance, rather than environmental events [3,9,10,11]. Research linking physiological coping mechanisms such as torpor to

ecological interactions and/or disturbance remains scant [12] and this is especially true for responses to fire. To our knowledge, only two studies on the effects of fire-associated stimuli on heterotherms have been published. The first showing that torpid fat-tailed dunnarts (*Sminthopsis crassicaudata*) respond to smoke and ash in their environment by arousing from shallow torpor ( $T_b \sim 19$  °C) and subsequently increasing activity and decreasing torpor use [13]. The second study detailed that the arboreal pygmy possum *Cercartetus nanus*, a marsupial hibernator, reacted more slowly in terms of locomotor performance and responsiveness to smoke exposure at  $T_b < 13$  °C [14]. In contrast, bats must be able to fly if they are to escape, and many insectivorous bats are deep hibernators, capable of withstanding  $T_b$  during torpor  $< 5$  °C [5]. Australian bats often roost and hibernate in trees [15,16,17,18,19], where they are prone to exposure to fire. To achieve flight, bats in deep torpor need to raise  $T_b$  substantially further during the rewarming process and therefore are more threatened by fire than species that only need to climb at low  $T_b$ . Bats in North America have been observed attempting to crawl or fly from leaf litter or flushing tree roosts during prescribed burns [20,21,22,23]. However, these studies did not assess

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$T_b$  and depth of torpor prior to smoke exposure or response time to fire stimuli.

In order to better understand how nontactile stimuli affect re-warming from torpor, it is important to quantify the initial response time of bats. During steady-state torpor, heart rate (HR), MR and  $T_b$  of bats are reduced to low levels [24,25]. The breathing pattern of most insectivorous bats becomes arrhythmic and is characterized by periodic extended apnoeas, at times > 1 h, dependent on  $T_a$  [26,27]. When bats arouse from torpor to normothermia, episodic breathing ceases and HR, MR, and respiration rate (RR) increase rapidly followed by an increase in  $T_b$  [24,28,29,30,31]. The HR, MR and RR peak mid to late arousal, usually followed by a decrease in rates as normothermic  $T_b$  is reached [8,30,32].

Although  $T_b$  is a reasonable measure to determine whether or not animals respond to disturbance,  $T_b$  only can increase after MR and respiratory rate (RR) have been raised during arousal [28]. In addition, during re-warming regional temperature differences often occur especially across the body surface [33,34]. Therefore measuring  $T_b$  in torpid animals via skin surface temperature, as is often done in small bats [35,36,37,38], may introduce further delays in assessing response time. As RR falls prior to  $T_b$  when entering torpor and increases prior to  $T_b$  when arousing from torpor [24,31], RR is likely a more accurate indicator of stimulus detection and response than  $T_b$ .

Therefore, to gain a better understanding of whether and how hibernating bats are able to respond to smoke while in deep torpor, we quantified the RR and HR of a vesperilionid bat, Gould's long-eared bat (*Nyctophilus gouldi*), as a function of  $T_a$ . *Nyctophilus gouldi* is a common and small (~10 g) insectivorous bat that roosts in fissures, hollows, and under the bark of trees [17,39,40]. This species hibernates in south-eastern Australia and uses torpor throughout the year, even during summer when conditions are mild [35,41]. Thus, *N. gouldi* are a suitable study species as they employ torpor bouts for up to two weeks during winter [19] and can decrease  $T_b$  as low as 2 °C [41]. Because *N. gouldi* roost in forests [16,17,35], they are also susceptible to wild and management fires. We hypothesized that *N. gouldi* at low  $T_a$  would 1) take longer to respond to smoke, 2) take longer to demonstrate the peak RR after the beginning of smoke exposure, and 3) return to torpor more quickly following the cessation of smoke exposure than bats at high  $T_a$ .

## 2. Methods

### 2.1. Animals

The RR and response to smoke exposure were quantified at the University of New England in Armidale (30°30'S 151°39'E) in NSW Australia, a cool-temperate area surrounded by open eucalypt forest and grazing land, during the Austral winter (June–July 2015). Bats ( $n = 5$  males, body mass =  $10.0 \pm 0.7$  g) were captured in nearby forest using harp traps (© Faunatech Austbat, Australia) and mist nets (© Ecotone, Poland). They were housed together in a large outdoor flight cage with hessian sacks for roosting. Bats were offered mealworms and water ad libitum on all non-experimental days. To provide a diet of appropriate composition, three times a week mealworms were supplemented with approximately 1 g of Wombaroo Insectivore Rearing Mix. Bats were allowed to acclimate to captivity for at least one month prior to the experiment and were kept in captivity for a total of three months. Bats were released at the end of the experiment at the site of capture.

### 2.2. Experimental setup

Bats were placed inside a modified polycarbonate chamber with a clear lid (80 × 55 × 120 mm) inside a temperature-controlled cabinet. The chamber was fitted with a 2300–3300 Hz, 35 mm piezoelectric transducer (model 7BB-35-3, © Murata Manufacturing Co., Kyoto, Japan) covered with a small piece of hessian to ensure bats roosted with

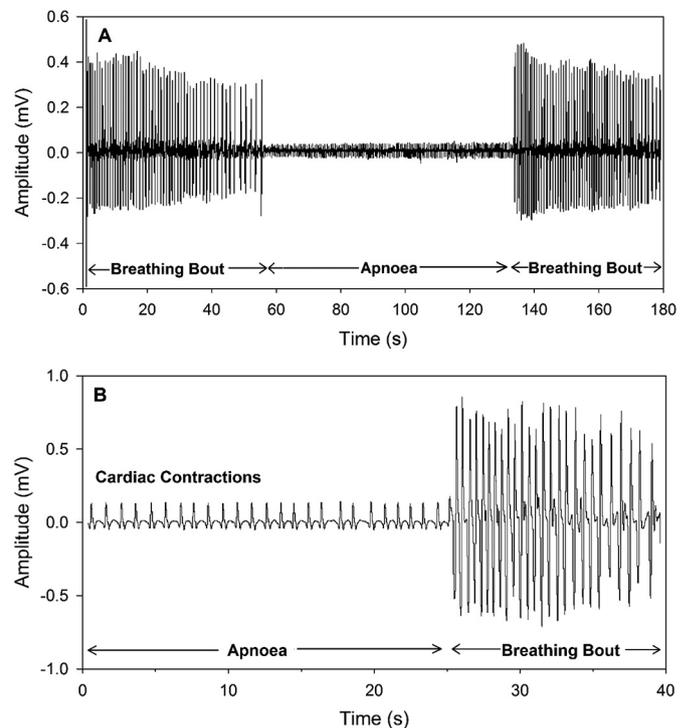


Fig. 1. (a) Eupnoeic and apnoeic bouts of respiration of an individual male bat at 20 °C prior to smoke exposure. (b) Example of detectable cardiac contractions (0 to 25 s) during an apnoea of an individual male bat at 17.5 °C (HR ~ 55 bpm; RR ~ 75 breaths min<sup>-1</sup>).

their chest touching the transducer. Piezoelectric transducers were connected to a PowerLab Data Acquisition System (model 4/35, © A.D. Instruments, Dunedin, NZ) and data were recorded using LabChart Pro software (v7.3, A.D. Instruments, Dunedin, NZ). Piezoelectric transducers are extremely sensitive to pressure and were not only capable of detecting the breathing pattern and movement of bats within the chamber, but also cardiac contractions during periods of apnoea during torpor (Fig. 1a, b). Therefore, it was possible to assess the HR of torpid individuals during apnoeic periods and this was used as a supplementary measure of torpor depth. Previous work on *N. gouldi* have shown that during steady-state torpor HR falls to ~3.5% of resting HR, and that resting HR can be predicted with the following equation: HR (bpm) =  $664.8 - 12.7 * T_a$  (°C), indicating torpor HR below those levels [8].

The  $T_a$  of the chamber was measured using a calibrated thermocouple placed ~3 mm into the chamber and read to the nearest 0.1 °C using a data logger (University of New England E.S.U.), and downloaded to a laptop computer after the cessation of each experiment. Air was pulled from outside through the chamber using an air pump, and air flow was adjusted (~465 ml min<sup>-1</sup>) with a mass flowmeter (© Omega FMA-5606; Stamford, CT, USA). The artificial photoperiod was adjusted to time of year for local conditions. Individuals were monitored visually using a night vision web camera.

To confirm that bats were in steady-state torpor,  $T_b$  ( $n = 4$ ,  $N = 6$ ) was measured in the morning (approximately 09:00 h) on baseline measurement days—those days on which bats were not exposed to smoke. The  $T_b$  was measured using a calibrated thermocouple read by a digital thermometer (© Omega HH-25TC, Stamford, CT, USA) inserted ~1 cm into the rectum. To minimize the effect of handling on  $T_b$ , all measurements were gathered ≤ 1 min of opening the chamber door by timing the process using a stopwatch. Average apnoeic duration during torpor was measured from the period 30 min before  $T_b$  measurements were taken to ensure that bats had sufficient time to reach steady-state torpor. Average HR during apnoeas was determined over 10 min during the corresponding period, and considered the representative HR of

torpid bats. Because the apnoeas were not observed when bats were normothermic and absolute minimum apnoea duration at  $T_b$  12.0 °C and  $T_a$  11.2 °C was 30 s, we were confident that an average apnoea duration of > 30 s was indicative of torpor at all  $T_a$ . The  $T_b$  of bats was not measured during the experiment itself, because handling of bats during the experiment to obtain rectal measurements would have significantly interfered with results.

Smoke was produced by burning a 50 g mixture of dry and fresh *Eucalyptus* spp. leaves that were collected on campus and burnt outdoors in a fireproof container. After ample smoke had been produced, a lid was placed on the container and smoke was transferred into an 11 × 22 cm heat-resistant bag through an exhaust valve using a hand pump. Smoke density was assessed using a smoke meter (Testo 308, Testo AG, Lenzkirch, Germany) which evaluates smoke particle density on a scale of 0 (clean air) to a saturated maximum of values > 6 (thick smoke). To normalize smoke density throughout the experiment, smoke was only transferred through the chamber if initial assessment of smoke in the bag read  $\geq 6$ , indicating thick smoke similar to a wildfire. The bag was attached to an inflow tube leading to the animal chamber, and smoke was drawn from the bag and through the animal chamber by an air pump. To minimize potential damage to the air pump a filter was placed in the airflow following the animal chamber prior to the pump inlet. At a flow rate of  $\sim 465$  ml  $\text{min}^{-1}$ , the delay of smoke from the bag to the chamber was < 1 s. Animals were exposed to smoke for a maximum of 10 min, an arbitrary time we considered to be ample for response, yet safe. Response time of the bat generally occurred prior to the maximum exposure time. However, we decided to ensure the wellbeing of all animals by ceasing smoke exposure as soon as a strong visible reaction (moving completely off the piezo-transducer and attempting to escape the chamber) was observed via the web camera.

### 2.3. Experimental protocol

Approximately one hour prior to sunset, bats were placed in the experimental chambers and exposed to a constant  $T_a$  between 11 and 23 °C (averages  $11.7 \pm 0.5$  °C,  $16.9 \pm 1.3$  °C and  $21.4 \pm 1.1$  °C) during exposure. Individuals were exposed to two experimental protocols at each temperature; 1) a baseline study where torpid individuals were not exposed to smoke and 2) smoke exposure during torpor. No bat was introduced to the chamber on consecutive days, with a minimum of four days in between each experiment per individual. On days when no smoke was drawn through the chamber (baseline) an external stimulus was presented by opening and closing the door to the experimental room at approximately the same time smoke introduction occurred on experimental days. This was to ensure that, on the days where smoke was introduced, data were not confounded by the noise associated with monitoring bats and torpor bouts were therefore comparable. During baseline studies bats showed no signs of response to the stimulus, either through increased RR or movement, thus we deemed the presence of smoke itself to be the factor initiating arousal.

Smoke was introduced to the chamber at approximately 09:00 h,  $\sim 17$  h after the animals were placed in the chamber, and bats were monitored for the entirety of the exposure both visually on the web camera and by monitoring RR on LabChart. Previous studies indicate that *N. Gouldi* enter torpor prior to sunrise (or lights on) and at mild  $T_a$  ( $\sim 20$  °C) will actively rewarm around mid-day [35,38]. As such, 09:00 h was designated an appropriate time to ensure bats were torpid at all  $T_a$ . If a bat showed visual discomfort by attempting to escape the chamber (moving completely off the piezo-transducer, ceasing to hang on the hessian layer, attempting to find a way out of the chamber by moving into corners or into the inlet/outlet), the time of escape attempt was noted and smoke exposure ceased. The bat was removed from the chamber approximately 2 h prior to sunset (i.e.,  $\sim 15:00$  h), offered mealworms and water and returned to the flight cage. No animals showed any prolonged negative response to the brief smoke exposure, continued to feed regularly and maintained weight, therefore we are

confident that animals were not adversely affected. This study was approved by the UNE animal ethics committee (AEC13-150).

### 2.4. Statistical analysis

To ensure that bats were in a similar state of torpor prior to the time of smoke introduction, the duration of apnoeic and respiratory (eu-pnoeic) periods were compared in the hour prior to smoke, on both the baseline and experimental days and at each  $T_a$ . A bat was considered to be in steady-state torpor if apnoeas lasted  $\geq 30$  s as individuals exhibited apnoeas  $\geq 30$  s at all  $T_a$  and the measured  $T_b$  of bats during these conditions was within 1 °C of  $T_a$ .

The behavioural response of bats to smoke exposure was determined using two measures: 1) visually determined response via the web camera (escape behaviour), resulting in complete movement off the piezo-transducer, and 2) rapid and erratic waveforms on piezoelectric recordings that resembled muscle contractions and showed a clear deflection from the respiratory movements.

The RR was analysed from the point of smoke exposure to the first apnoeic period, excluding periods of movement. The RR was averaged over 1-min periods from 1-s averages of breath to breath measurements. We calculated the response time to smoke as the recorded time from smoke exposure to the beginning of respiration. As all bats were apnoeic prior to introduction of smoke, the RR within the first minute of post-smoke respiratory response is reported as the starting respiratory rate (RR<sub>start</sub>), this excludes the time lapsed between exposure and response time. Peak respiratory rate (RR<sub>peak</sub>) was described as the highest RR in a one-minute period after the beginning of smoke exposure.

To assess if bats fully or partially aroused in response to smoke, we used RR and subcutaneous temperature ( $T_{\text{sub}}$ ) values taken from 9 *N. Gouldi* during the rewarming process (S.E. Currie, unpublished) to determine  $T_{\text{sub}}$  that corresponds with RR<sub>peak</sub> values in this study. Torpor entry has been defined as a drop in  $T_b$  below 30 °C [42], however because  $T_b$  lags behind RR, HR and MR during the arousal process [8,30], we reduced the normothermic  $T_{\text{sub}}$  threshold to  $\geq 28$  °C to account for this difference. In rewarming *N. Gouldi* at  $T_{\text{sub}} \geq 28$  °C RR<sub>peak</sub> averaged  $375 \pm 69$  breaths  $\text{min}^{-1}$  when  $T_a$  was  $\leq 15$  °C (S.E. Currie unpublished). Therefore we considered an RR<sub>peak</sub> > 375 breaths  $\text{min}^{-1}$  to be indicative of reaching normothermia at these  $T_a$ . Similarly, at 20 °C rewarming *N. Gouldi* reached an average RR<sub>peak</sub> of  $324 \pm 57$  breaths  $\text{min}^{-1}$  when  $T_{\text{sub}}$  was  $\geq 28$  °C (S.E. Currie unpublished), suggesting that an RR<sub>peak</sub> > 324 breaths  $\text{min}^{-1}$  to be a representative threshold for normothermia. Following smoke exposure all animals returned to torpor, which was indicated by a return to episodic breathing. The first post-smoke apnoeic period was defined as that when an apnoea lasted  $\geq 10$  s. This distinguishable apnoeic period was used to determine the time lapsed between cessation of smoke exposure and re-entry into torpor. A bat was also considered in thermoconforming steady-state torpor if minimum discernible apnoeic HR fell to or below previously reported HR values for thermoconforming torpid *N. Gouldi* from Currie et al. [25],  $27 \pm 11$  bpm for  $T_{\text{sub}}$   $10.6 \pm 0.3$  °C,  $32 \pm 13$  bpm for  $T_{\text{sub}}$   $16.0 \pm 0.9$  °C, and  $46 \pm 11$  bpm for  $T_{\text{sub}}$   $20.9 \pm 0.4$  °C, where HR was determined using electrocardiograms, and  $T_{\text{sub}}$  was within 1 °C of  $T_b/T_a$ .

All statistical analyses were conducted using R (v. 3.4.1) and SPSS (v. 22). A paired *t*-test was used to determine whether RR and duration of apnoeas differed significantly in the hour prior to smoke exposure between baseline and experimental days. Linear mixed effects models (package nlme) [43] were fitted to assess the relationship between  $T_a$  and the measured variables, with animal included as a random factor. These variables include: a) Time until first respiratory response to smoke exposure, b) Time until first movement, c) RR within the first minute of smoke exposure, d) Peak RR, e) Time until peak RR, f) Time until first apnoea from cessation of smoke exposure, and g) Time until thermoconforming steady-state torpor from cessation of smoke exposure.

The  $T_a$  was averaged over the period during which the given variable occurred for each individual (e.g.; the  $T_a$  for time until first movement was averaged over the time from smoke exposure to the first movement, while the  $T_a$  for time until the first apnoea was averaged from the cessation of smoke exposure to the time of the first apnoea). Means are reported  $\pm$  1 s.d. for the number of individuals 'n'; the number of measurements is reported as 'N'.

### 3. Results

#### 3.1. Baseline torpor physiology

All bats entered torpor and thermoconformed during baseline experiments and were considered thermoconforming as  $T_b$  fell within 1 °C of the  $T_a$  at ~09:00 h. During torpor average apnoeic periods were  $417 \pm 372$  s at  $T_b$   $12.0 \pm 0.0$  °C and  $T_a$   $11.4 \pm 0.1$  °C (n = 2, N = 2),  $147 \pm 80$  s at  $T_b$   $18.0 \pm 0.0$  °C and  $T_a$   $17.6 \pm 0.1$  °C (n = 2, N = 2), and  $89 \pm 70$  s at  $T_b$   $21.5 \pm 1.5$  °C and  $T_a$   $21.0 \pm 0.7$  °C (n = 2, N = 2). The corresponding apnoeic HR was  $21 \pm 1$  bpm at  $T_a$   $11.4 \pm 0.1$  °C (n = 2, N = 2),  $38 \pm 6$  bpm at  $T_a$   $17.6 \pm 0.1$  °C (n = 2, N = 2), and  $47 \pm 7$  bpm at  $T_a$   $21.0 \pm 0.7$  °C (n = 2, N = 2). Average duration of eupnoeic periods during torpor was  $37 \pm 6$  s at  $T_a$   $11.4 \pm 0.1$  °C (n = 2, N = 2),  $26 \pm 23$  s at  $T_a$   $17.6 \pm 0.1$  °C (n = 2, N = 2), and  $36 \pm 5$  s at  $T_a$   $21.0 \pm 0.7$  °C (n = 2, N = 2).

#### 3.2. Smoke exposure

At all  $T_a$  tested, bats entered torpor as indicated by an episodic breathing pattern. Individuals were considered in steady-state torpor prior to smoke exposure on experimental days as the duration of apnoeic and eupnoeic periods were not significantly different in the hour prior to smoke exposure between the two treatments (Apnoea: df = 14, t = 0.70, P = 0.495; Eupnoea: df = 14, t = 1.24, P = 0.234). On experimental days, in the hour prior to smoke exposure average apnoeic periods during torpor ranged from an absolute minimum of 69 s at  $T_a$   $22.9$  °C to an absolute maximum of 1567 s at  $T_a$   $11.8$  °C. The relationship between apnoea duration and  $T_a$  was negative and significant (df = 14,  $r^2 = 0.59$ , P = 0.0152), described by the following equation: Apnoea (s) =  $1321.5 - 54.6 * T_a$  (°C). However, eupnoea duration varied widely, with an absolute minimum of 9 s at  $18.2$  °C to an absolute maximum of 79 s at  $22.9$  °C. Thus, the relationship between eupnoea duration and  $T_a$  was not significant (df = 14,  $r^2 < 0.01$ , P = 0.963).

All bats at all  $T_a$  responded to smoke exposure by increasing RR (see Fig. 2 for an example). Bats responded to smoke more quickly at higher  $T_a$  (Table 1). Interestingly, bats at  $T_a < 15$  °C responded over a more variable range (20 s to 48 s). The relationship between response time to smoke exposure and  $T_a$  was negative and significant (df = 10,  $r^2 = 0.73$ , P < 0.0041) (Fig. 3a). All bats continued to rewarm even after the cessation of smoke exposure, and thus all bats reached their  $RR_{peak}$  after smoke exposure stopped. Bats took longer to reach  $RR_{peak}$  at lower  $T_a$  (Table 1). The relationship between the time taken to reach  $RR_{peak}$  and  $T_a$  was negative and significant (df = 13,  $r^2 = 0.63$ , P = 0.002) (Fig. 3b).

The  $RR_{start}$  caused by the initial smoke exposure, was greater at higher  $T_a$ , with an average of  $199 \pm 14$  breaths  $min^{-1}$  at  $T_a$   $21.3 \pm 1.2$  °C (n = 4, N = 4),  $162 \pm 34$  breaths  $min^{-1}$  at  $T_a$   $16.9 \pm 1.3$  °C (n = 5, N = 5), and  $103 \pm 14$  breaths  $min^{-1}$  at  $T_a$   $11.8 \pm 0.6$  °C (n = 4, N = 4). The  $RR_{start}$  was significantly positively correlated with  $T_a$  (df = 12,  $r^2 = 0.85$ , P < 0.001) (Fig. 4).

The  $RR_{peak}$  was not related to  $T_a$  (df = 14,  $r^2 = 0.41$ , P = 0.521) expressing an average of  $286 \pm 96$  breaths  $min^{-1}$  at  $11.9 \pm 0.4$  °C (n = 5, N = 5),  $333 \pm 54$  breaths  $min^{-1}$  at  $17.1 \pm 1.2$  °C (n = 5, N = 5), and  $309 \pm 39$  breaths  $min^{-1}$  at  $21.5 \pm 0.9$  °C (n = 5, N = 5). Only one bat at  $T_a < 15$  °C reached an  $RR_{peak}$  greater than our calculated threshold for normothermia, expressing an  $RR_{peak}$  of

$392$  breaths  $min^{-1}$  at  $T_a$   $12.5$  °C, and was observed to visibly shiver following smoke exposure. However, at  $T_a > 15$  °C, average  $RR_{peak}$  values were similar to our threshold for normothermia with an average of  $326 \pm 45$  breaths  $min^{-1}$ .

#### 3.3. Movement

None of the bats at  $T_a < 15$  °C demonstrated escape behaviour or even minor head movements in response to smoke (although, as previously noted, one bat did shiver), and were thus exposed to smoke for the full 10 min. At  $T_a \geq 15$  °C, only two bats were exposed to smoke for the full 10 min (at  $T_a$   $17.0$  and  $18.1$  °C) and all bats expressed escape behaviour. At  $T_a \geq 20$  °C, all bats quickly responded to smoke via visual expression of escape behaviour and thus all individuals were exposed to smoke for  $\leq 5$  min, with the minimum exposure period being 2 min.

Similarly, the time lapsed until movement in response to smoke exposure, as indicated on the piezo-transducer, was greater at lower  $T_a$  (Table 1). However, the time until first movement was widely variable even at the same  $T_a$ , ranging from an absolute minimum of 1.0 min at  $21.8$  °C to an absolute maximum of 15.5 min at  $13.1$  °C and occurred after smoke exposure had ceased. Nonetheless, the time lapsed until the first discernible movement was negatively correlated with  $T_a$  (df = 13,  $r^2 = 0.48$ , P = 0.01), described by the following equation: Time lapsed (min) =  $18.6 - 0.8 * T_a$  (°C).

#### 3.4. Post-exposure apnoea expression and heart rate

After smoke exposure ceased, bats took less time at low  $T_a$  to return to apnoeic torpor values than at high  $T_a$ , indicated by the time lapsed until the first apnoea > 30 s (Table 1). The time lapsed until the first apnoea from the cessation of smoke exposure ranged from an absolute minimum of 8.5 min at  $11.7$  °C to an absolute maximum of 97.8 min at  $20.8$  °C, and showed a positive linear correlation with  $T_a$  (df = 14,  $r^2 = 0.80$ , P < 0.001) (Fig. 5).

While bats at lower temperatures took less time to return to apnoeic torpor values, none of the bats at  $T_a < 15$  °C returned to HR values consistent with thermoconforming steady-state torpor prior to being removed from the chamber at the cessation of the experiment. The minimum apnoeic HR post smoke exposure at  $T_a < 15$  °C ranged from 52 bpm at  $T_a$   $12.4$  °C to 81 bpm at  $T_a$   $11.7$  °C, at an average of  $65 \pm 14$  bpm at  $T_a$   $12.0 \pm 0.5$  °C (n = 4, N = 4). Interestingly, all bats at  $T_a \geq 15$  °C returned to HR values consistent with thermoconforming steady-state torpor after smoke exposure, although they took more time to express their first apnoea. Bats had a minimum discernible steady-state HR of  $39 \pm 7$  bpm at  $T_a$   $17.1 \pm 1.3$  °C (n = 5, N = 5) and  $47 \pm 5$  bpm at  $T_a$   $21.2 \pm 0.8$  °C (n = 5, N = 5). The amount of time lapsed until bats reached minimum steady-state HR values expressed a trend of decreasing duration with increasing  $T_a$  and ranged from 126 min at  $23$  °C to 256 min at  $15.1$  °C. The relationship between time until HR values were consistent with thermoconforming steady-state torpor and  $T_a$  was negatively correlated and significant at  $T_a \geq 15$  °C (df = 9,  $r^2 = 0.71$ , P = 0.0119).

### 4. Discussion

#### 4.1. General discussion

Our study is the first to quantify the response of HR and RR of a hibernating bat to fire cues during torpor as a function of  $T_a$ . The data show that torpid bats respond to smoke at  $T_a$  between 11 and 23 °C, however the response time was longer at lower  $T_a$ . Hence, bats are able to detect smoke in their environment and appropriately respond by increasing RR and initiating arousal from torpor at low  $T_a$ , but require more time to reach their  $RR_{peak}$  compared to bats at higher  $T_a$ . Further, bats at low  $T_a$  entered torpor more quickly following cessation of smoke

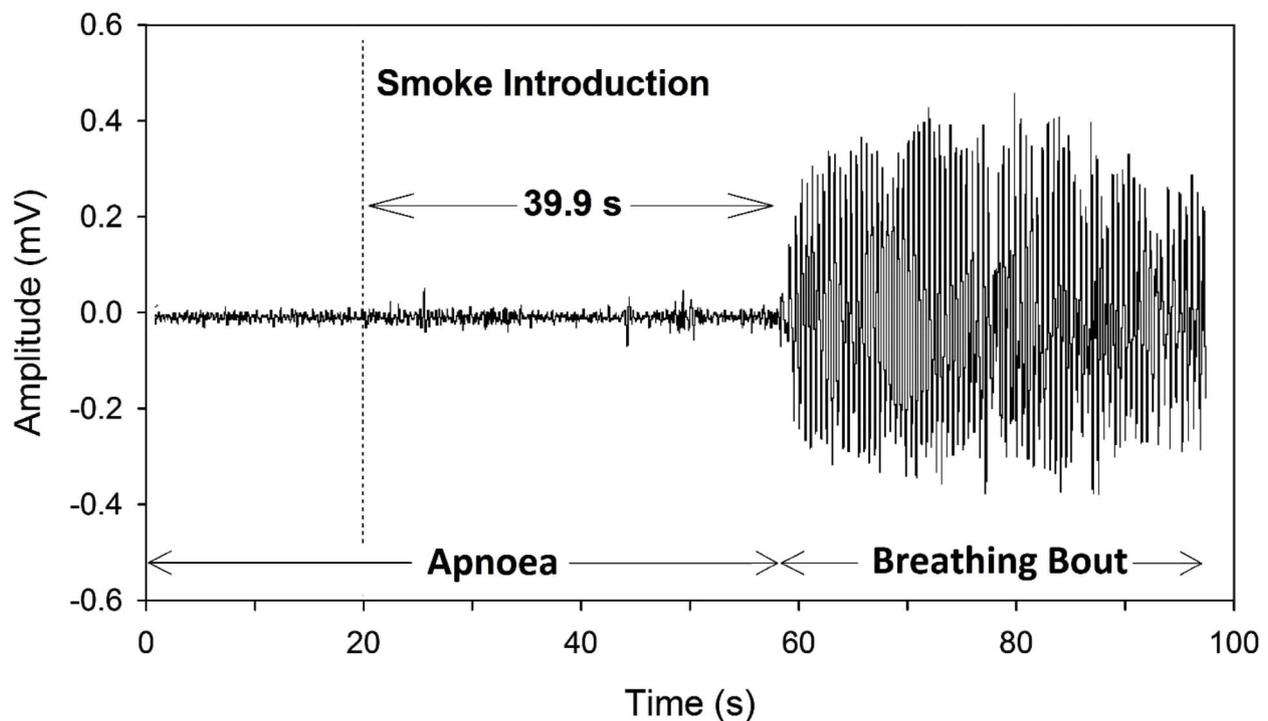


Fig. 2. Example of RR in response to smoke. The introduction of smoke (dashed line) to a male bat at  $T_a$  11.8 °C resulted in a response after 39.9 s as seen by an increase in RR ( $RR_{start}$ ). Prior to smoke exposure, the bat was apnoeic.

exposure, but did not achieve deep, steady-state torpor prior to the end of the experimental day, unlike bats kept at  $T_a \geq 15$  °C.

While our results reveal that bats can respond to smoke, we found that the comparatively lower average  $RR_{peak}$  at  $11.9 \pm 0.4$  °C suggests that most individuals (4 out of 5 bats) did not completely rewarm in response to smoke. Bats also took longer to sense smoke and reach  $RR_{peak}$  at lower  $T_a$ , which is unsurprising because hibernators take longer to rewarm at colder temperatures [7,8,44,45]. However, as these bats did start the arousal process in response to smoke exposure it is likely that continued smoke exposure (> 10 min) may illicit complete arousal. Although two bats at  $T_a \geq 15$  °C only exhibited partial arousal and did not reach  $RR_{peak}$  indicative of normothermic  $T_b$ , they still displayed escape behaviour. It is known that some heterothermic mammals can move during torpor [2,46]. In addition, some bats have shown the capability for flight activity at a low  $T_{skin}$  of 29 °C, using flight to complete the rewarming process [47]. Therefore, these two bats could have displayed escape behaviour even without achieving a normothermic  $T_b$ , hence a lower  $RR_{peak}$ . Because measured  $T_b$  of torpid bats during baseline studies were close to that of the  $T_a$  in the chamber, it is likely that  $T_b$  prior to smoke exposure was the same. We were unable to measure  $T_b$  after smoke exposure to confirm normothermia was achieved, as avoiding contact with bats during the experiment was essential to reduce human interaction and the introduction of other external variables (such as light and sound) which may have influenced torpor re-entry times and/or the level to which bats rewarmed. However, future studies may be able to use remote measures of  $T_b$  to also

assess the  $T_b$  during torpor.

Interestingly, after responding to smoke exposure at low  $T_a$ , bats returned to torpor more quickly than at higher  $T_a$ . However, all bats at  $T_a > 15$  °C achieved the average minimum HR consistent with thermoconforming steady-state torpor prior to the cessation of the experimental day, whereas all bats at  $T_a < 15$  °C did not. This is likely related to the longer duration of arousal time at low  $T_a$  and a trade-off between reducing energy expenditure while maintaining vigilance. *Nyctophilus gouldi* can lower  $T_b$  to  $\sim 2$  °C during torpor [41], however were only exposed to  $T_a > 11$  °C in our study. Because rewarming to normothermia from steady-state torpor at higher  $T_b$  would take less time, it would be more energetically “risky” for bats at low  $T_a$  to re-enter thermoconforming steady-state torpor only to face repeated smoke exposure and, again, arouse from a low  $T_b$ . It has also been suggested that other hibernators may not thermoconform when stressed to ensure that they are poised for arousal [48] and that *Nyctophilus* spp. disturbed or handled during the day do not reach steady-state minimum MR during torpor [41]. Additionally, the proportional cost of arousal from a low  $T_a$  is reduced when animals are thermoregulating during torpor compared to when they are thermoconforming [45,49], therefore it may have been energetically advantageous to thermoregulate at low  $T_a$  in the case of repeated arousals. Nonetheless, bats at  $T_a < 15$  °C may have accounted for the higher cost of thermoregulating at low  $T_a$  by entering torpor more quickly than bats at  $T_a \geq 15$  °C, as shown by apnoea duration. Bats exposed to smoke at higher  $T_a$  may therefore be at a further advantage and afforded sufficient time to rewarm to the

Table 1

Comparison of the time taken for bats to respond to smoke (as shown by an increase in respiratory rate), express the first discernible movement (as shown by rapid and erratic waveforms on piezoelectric recordings), and reach peak respiratory rate ( $RR_{peak}$ ) following smoke exposure, and time taken for bats to express the first apnoea from cessation of smoke exposure at three ambient temperatures ( $T_a$ ). For all variables,  $n = N$ .

$T_a$ (°C)	Time to response (s)	N	Time to first movement (min)	N	Time to $RR_{peak}$ (min)	N	Time to first apnoea following cessation of smoke exposure (min)	N
11.9 ± 0.5	36 ± 14	3	9.2 ± 5.3	4	15.3 ± 1.2	5	14.6 ± 7.8	5
17.0 ± 1.0	16 ± 9	4	4.0 ± 3.1	5	13.6 ± 2.0	4	42.2 ± 14.9	5
21.4 ± 0.8	1 ± 0	4	1.7 ± 1.5	5	7.6 ± 3.3	5	66.2 ± 19.3	5

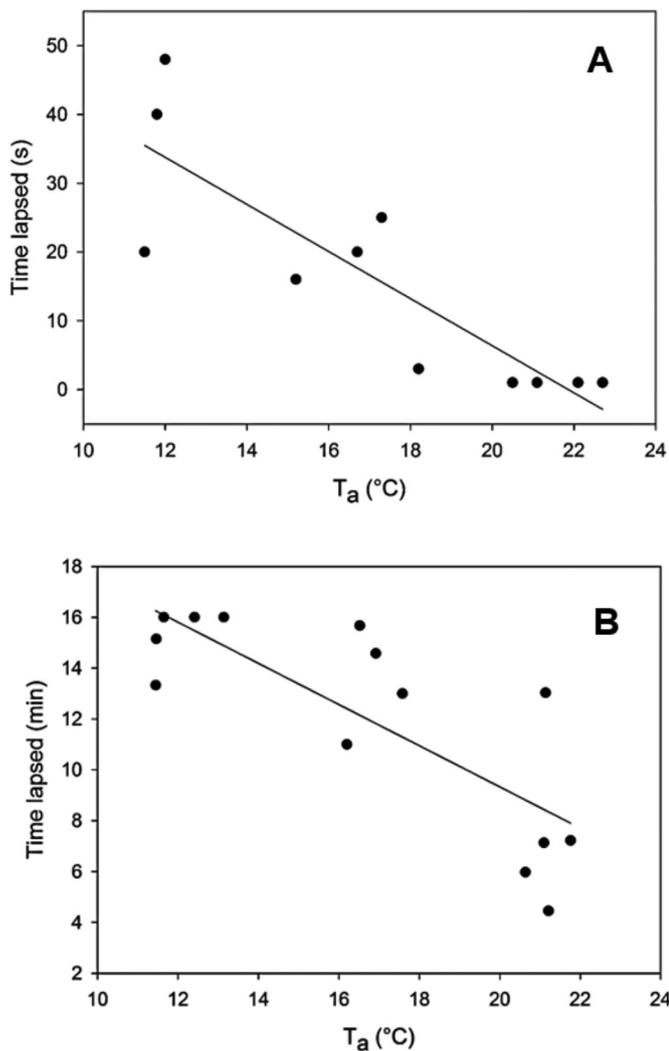


Fig. 3. (a) The time lapsed until bats in steady-state torpor sensed smoke was negatively related to  $T_a$  and is described by the following equation: Time lapsed (s) =  $74.9 - 3.4 * T_a$  (°C) ( $r^2 = 0.73$ ,  $P = 0.0041$ ), and (b) the time taken by bats to reach  $RR_{peak}$  (the highest RR in a one-minute period after the beginning of smoke exposure) showed a significant negative response to  $T_a$  and is described by the following equation: Time lapsed (min) =  $25.5 - 0.8 * T_a$  (°C) ( $r^2 = 0.63$ ,  $P = 0.002$ ).

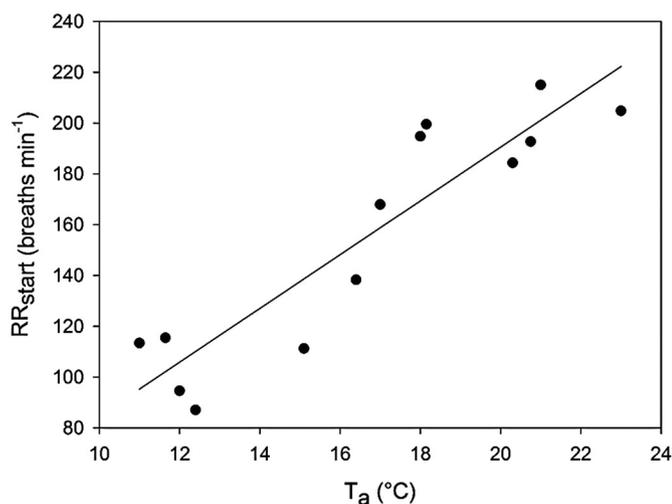


Fig. 4. The  $RR_{start}$  (the RR within the first minute of post-smoke respiratory response) was positively related to  $T_a$  and is described by the following equation:  $RR_{start}$  (breaths  $min^{-1}$ ) =  $-21.8 + 10.6 * T_a$  (°C) ( $r^2 = 0.85$ ,  $P < 0.001$ ).

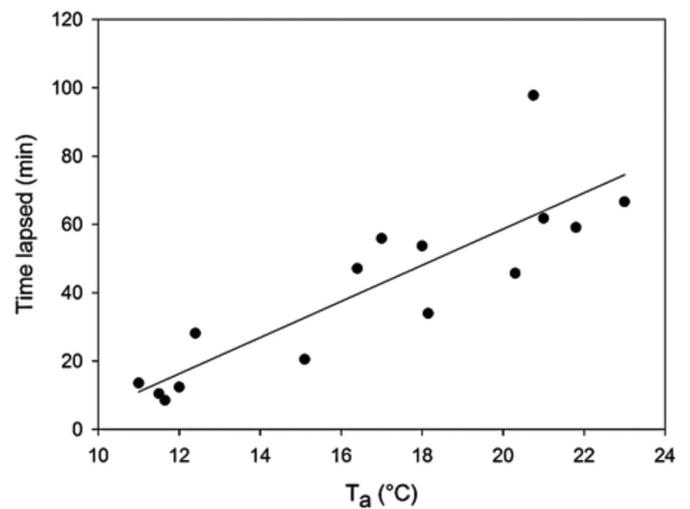


Fig. 5. The time lapsed until the first discernible apnoea from the cessation of smoke exposure was positively related to  $T_a$  and is described by the following equation: Time lapsed (min) =  $-47.4 + 5.3 * T_a$  (°C) ( $r^2 = 0.80$ ,  $P < 0.001$ ).

point of flight and escape fire.

Along with smoke, increased CO and CO<sub>2</sub> content of air during smoke exposure can alter respiratory patterns and can cause a gradual increase in RR and cessation of apnoeas. Previous studies have shown that in response to CO and CO<sub>2</sub> big brown bats (*Eptesicus fuscus*) increase RR 14-fold while torpid at 5 °C, while only increasing RR just over 2-fold at 30 °C [50,51]. Further, when exposed to hypercapnic and hypoxic air, *E. fuscus* did not arouse from torpor and only altered breathing patterns [51], while bats in our study initiated arousal from torpor. Moreover, almost all bats in our study responded to smoke exposure via cessation of apnoeas within 1 min and sustained an increased RR even after smoke was removed from the chamber and outside air replaced smoke. This demonstrates that exposure to smoke as a nontactile stimulant triggered arousal itself. Therefore, we suggest that the increased RR was largely due to other cues introduced by smoke rather than just hypercapnic and hypoxic air, because bats were arousing from torpor. Additionally, smoke is made up of more than hypercapnic and hypoxic air; the olfactory cues and increased particulate matter accompanying smoke exposure also may have stimulated reaction and rewarming.

Our data demonstrate the ability for bats to sense and actively respond to smoke at cool  $T_a$  via an increased RR, however it is essential that further testing is done to understand how this relationship changes near 0 °C. There is very little information on the ability of hibernating bats to respond to nontactile sensory cues at low  $T_a$ . Previous studies have described bats in North America flushing roosts or on the ground, attempting to escape from prescribed fire in winter [20,21]. Other work demonstrates varying responses of torpid bats to other nontactile stimuli, such as light, sound and human presence [3,9,52]. Our study demonstrates that another type of nontactile stimulus, smoke, can stimulate response and rewarming in bats. Even during sleep, another physiological state of inactivity, animals are capable of responding to olfactory cues [53]. Although our study serves as an adequate proxy for response prediction during the wildfire season in the New England region when  $T_a$  is warm (average  $T_a$  at 09:00  $17.5 \pm 1.2$  °C; Australian Bureau of Meteorology, <http://bom.gov.au>, Armidale Airport AWS weather station), management burns are often conducted in May and June when  $T_a$  is lower (average  $T_a$  at 09:00  $8.1 \pm 2.1$  °C). To understand wildlife response to management burns, it is important to know how and if torpid mammals are able to respond and escape when they occur.

In addition to  $T_a$ , it is unclear how smoke levels/particle density are related to sensory cues. In our study, bats were exposed to thick smoke

with a high particle density; management fires, however, are often smaller in scope and conducted at low  $T_a$ , thus the smoke levels of these fires would presumably be much lighter, especially if bats are roosting at elevated heights in trees. The intensity of management fires widely vary and are dependent on fuel load and type as well as soil moisture,  $T_a$ , spread and flame length [54]. Therefore, how insectivorous bats immediately respond to varying levels of smoke remains unknown. For example, a light prescribed fire at  $T_a < 11^\circ\text{C}$  may not illicit a response, and indeed, bats at  $T_a < 15^\circ\text{C}$  took much longer to rewarm from torpor at low  $T_a$  and did not visually demonstrate escape behaviour. Thus it can be inferred that at lower  $T_a$  and light smoke levels, bats may not adequately respond to smoke exposure and are at a much higher risk for acute respiratory failure or inability to escape and sustain burn injury. Aside from smoke levels, the likelihood of injury due to heat from fires decreases with roost height and wind [55]. It is consequently essential to understand how other factors affect heterotherm response time, such as roost ventilation and ambient wind conditions.

#### 4.2. Conclusions

In conclusion, our data show that although bats in steady-state torpor can sense nontactile smoke cues, those in deeper torpor take longer to respond and rewarm. Management fires are often conducted prior to or following winter at cool  $T_a$  when bats are likely to be in deep torpor, therefore bats may be at a greater risk for injury or mortality due to their inability to react quickly. More research is needed to understand how bats respond to  $T_a < 10^\circ\text{C}$ , as sensory and locomotor capabilities are likely even further compromised at lower  $T_a$ . We therefore recommend that particular caution is taken to ensure management fires are conducted at  $T_a$  which would allow for ample rewarming time, permitting escape.

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