Can bats can 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, ~10g), 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 low 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 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 ~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 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 rewarming 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 greater than 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 rewarming 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 vespertilionid bat, Gould's long-eared bat (*Nyctophilus gouldi*), as a function of T_a. *Nyctophilus gouldi* is a common and small (~ 10g) 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 by increasing RR, 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 ± 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 (80x55x120 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 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, Fig. 1b). 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^{-1}) 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 minimise 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 11x 22cm 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 ≥ 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 ~ 465 ml 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 ± 0.5°C, 16.9 ± 1.3°C and 21.4 ± 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, ~ 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 (~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., ~ 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 (eupnoeic) 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 ≥ 30s as individuals exhibited apnoeas ≥ 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_{start}), this excludes the time lapsed between exposure and response time. Peak respiratory rate (RR_{peak}) 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_{sub}) values taken from 9 *N. gouldi* during the rewarming process (S.E. Currie, unpublished) to determine T_{sub} that corresponds with RR_{peak} 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_{sub} threshold to ≥ 28°C to account for this difference. In rewarming *N. gouldi* at $T_{sub} \geq 28$°C RR_{peak} averaged 375 ± 69 breaths min^{-1} when T_a was ≤ 15°C (S.E. Currie unpublished). Therefore we considered an RR_{peak} greater than 375 breaths min^{-1} to be indicative of reaching normothermia at these T_a. Similarly, at 20°C rewarming *N. gouldi* reached an average RR_{peak} of 324 ± 57 breaths min^{-1} when T_{sub} was ≥ 28°C (S.E. Currie unpublished), suggesting that an RR_{peak} greater than 324 breaths 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 ≥ 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 ± 11 bpm for T_{sub} 10.6 ± 0.3°C, 32 ± 13 bpm for T_{sub} 16.0 ± 0.9°C, and 46 ± 11 bpm for T_{sub} 20.9 ± 0.4°C, where HR was determined using electrocardiograms, and T_{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 length 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 ± 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 ± 372 s at T_b 12.0 ± 0.0°C and T_a 11.4 ± 0.1°C (n = 2, N = 2), 147 ± 80 s at T_b 18.0 ± 0.0°C and T_a 17.6°C ± 0.1°C (n = 2, N = 2), and 89 ± 70 s at T_b 21.5 ± 1.5°C and T_a 21.0 ± 0.7°C (n = 2, N = 2). The corresponding apnoeic HR was 21 ± 1 bpm at T_a 11.4 ± 0.1°C (n = 2, N = 2), 38 ± 6 bpm at T_a 17.6°C ± 0.1°C (n = 2, N = 2), and 47 ± 7 bpm at T_a 21.0 ± 0.7°C (n = 2, N = 2). Average duration of eupnoeic periods during torpor was 37 ± 6 s at T_a 11.4 ± 0.1°C (n = 2, N = 2), 26 ± 23 s at T_a 17.6°C ± 0.1°C (n = 2, N = 2), and 36 ± 5 s at T_a 21.0 ± 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 ± 14 breaths min^{-1} at T_a 21.3 ± 1.2°C (n = 4, N = 4), 162 ± 34 breaths min^{-1} at T_a 16.9 ± 1.3°C (n = 5, N = 5), and 103 ± 14 breaths min^{-1} at T_a 11.8 ± 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 ± 96 breaths min^{-1} at 11.9 ± 0.4°C (n=5, N=5), 333 ± 54 breaths min^{-1} at 17.1 ± 1.2°C (n=5, N=5), and 309 ± 39 breaths min^{-1} at 21.5 ± 0.9°C (n=5, N=5). Only one bat at $T_a \leq 15$°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 ± 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 ≤ 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 ± 14 bpm at T_a 12.0 ± 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 ± 7 bpm at T_a 17.1 ± 1.3°C (n = 5, N = 5) and 47 ± 5 bpm at T_a 21.2 ± 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 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 ± 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 ~ 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 point of flight and escape fire.

Along with smoke, increased CO and CO_2 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_2 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 ± 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 ± 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 $T_a < 11$°C may not illicit a response, and indeed, bats at $T_a < 15$°C took much longer to rewarm from torpor at low T_a and at times 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 $T_a < 10°C$, 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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Figures

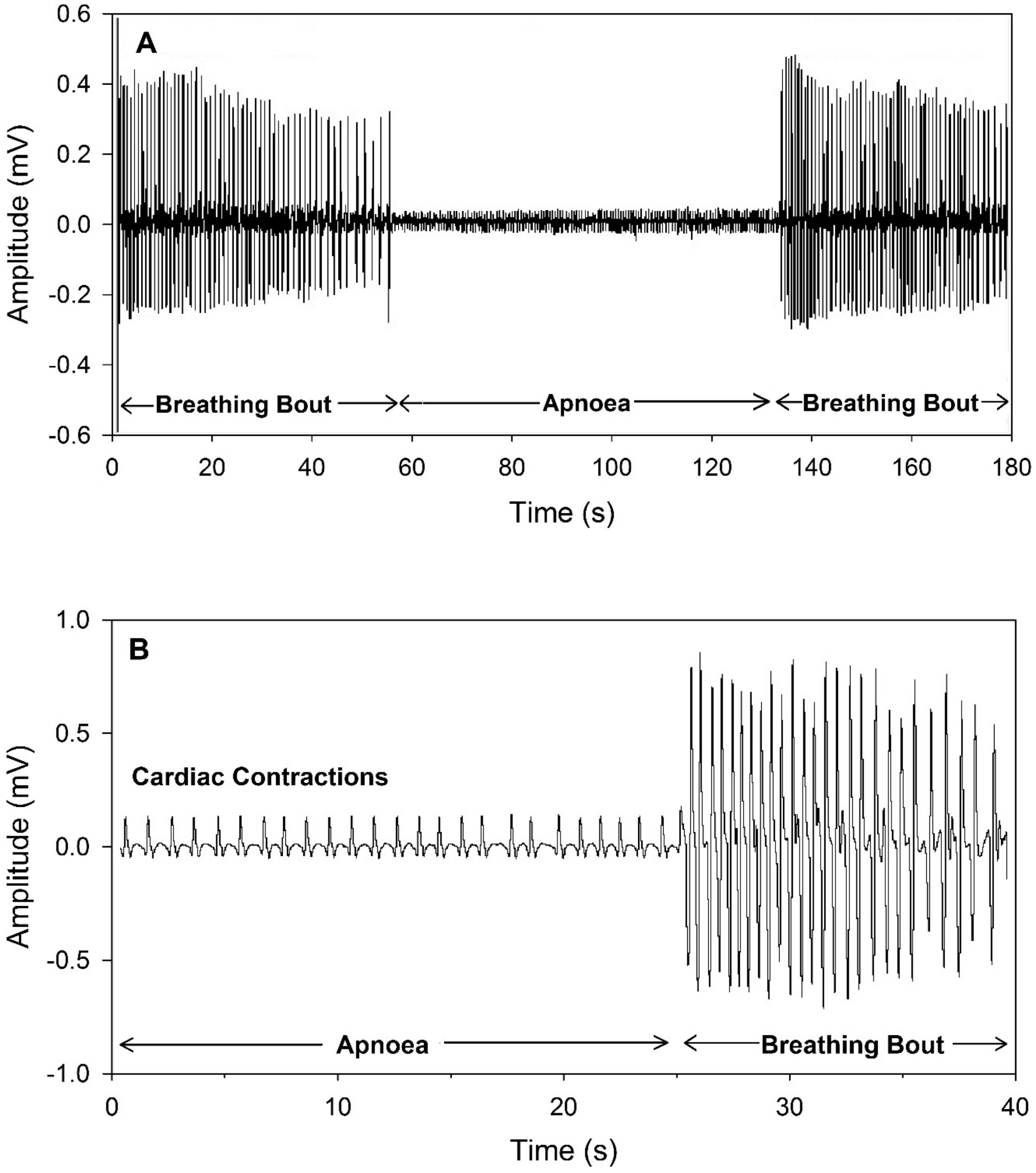

Figure 1. (**a**) Eupnoeic and apnoeic bouts of respiration of an individual male bat at 20°C prior to smoke exposure. (**b**) Example of detectable cardiac contractions (0 to 25 s) during an apnoea of an individual male bat at 17.5°C (HR ~ 55 bpm; RR ~ 75 breaths min^{-}).

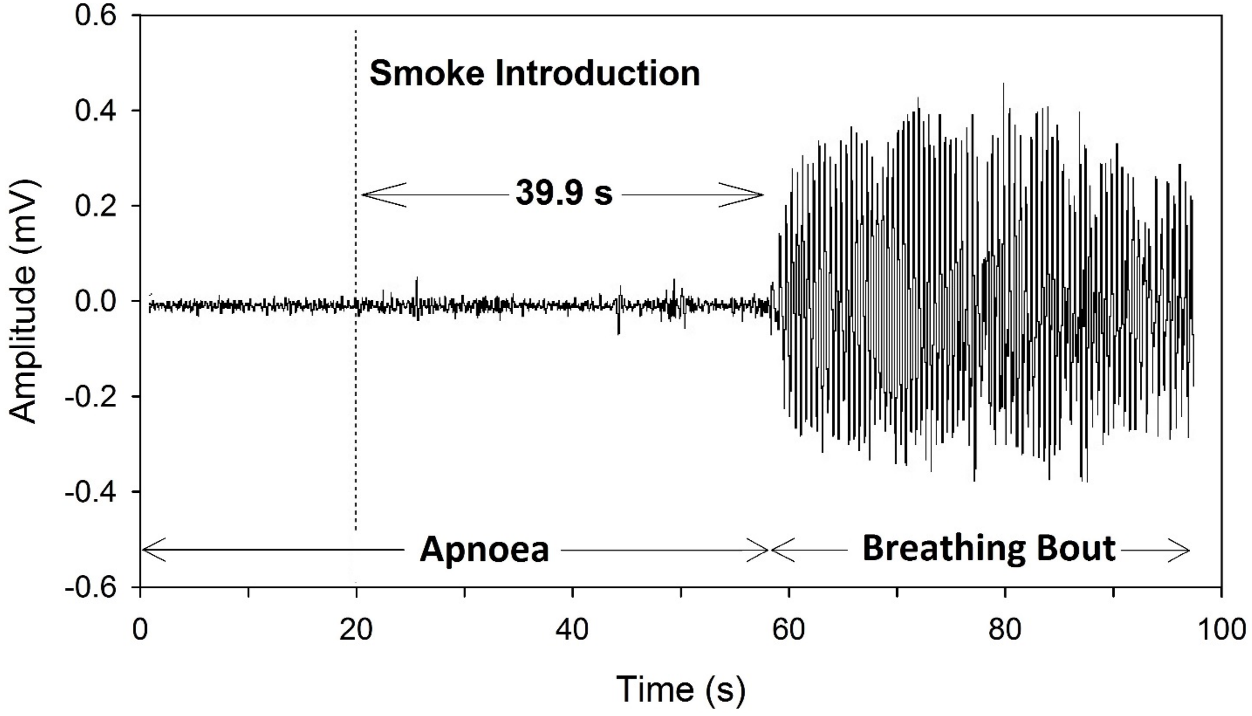

Figure 2. Example of RR in response to smoke. The introduction of smoke (dashed line) to a male bat at T_a 11.8°C resulted in a response after 39.9 s as seen by an increase in RR (RR_{start}). Prior to smoke exposure, the bat was apnoeic.

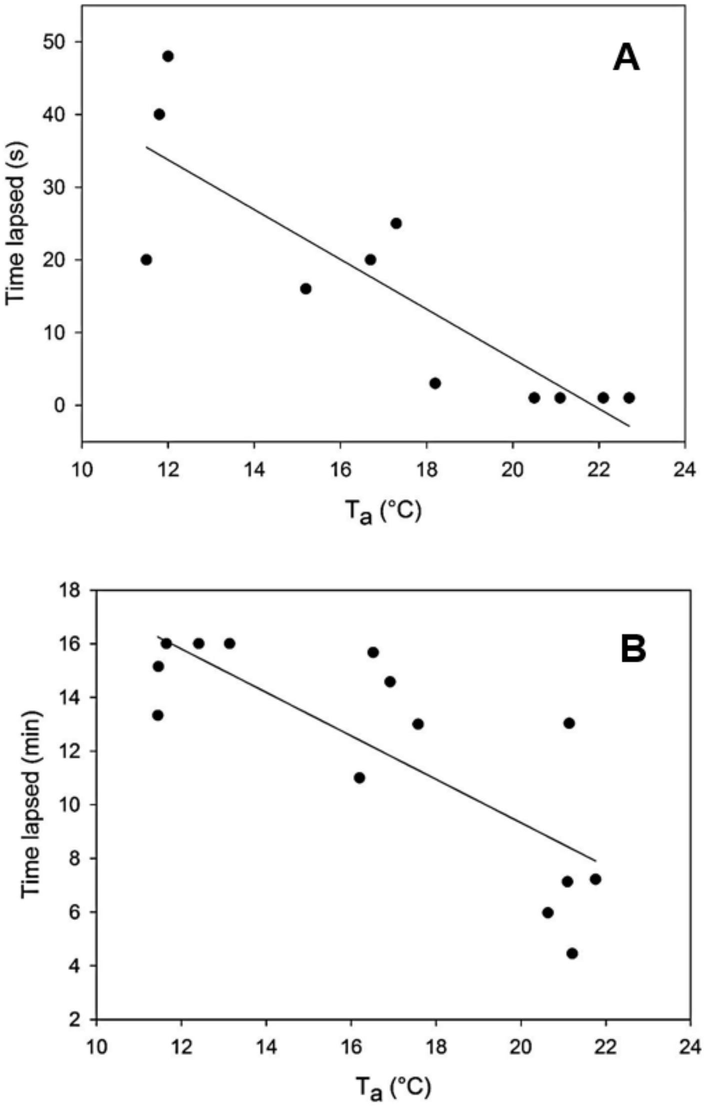

Figure 3. (a) The time lapsed until bats in steady-state torpor sensed smoke was negatively related to T_a and is described by the following equation: Time lapsed (s) = 74.9 – 3.4 * T_a (°C) ($r^2 = 0.73$, $P = 0.0041$), and **(b)** the time taken by bats to reach RR_{peak} (the highest RR in a one-minute 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) ($r^2 = 0.63$, $P = 0.002$).

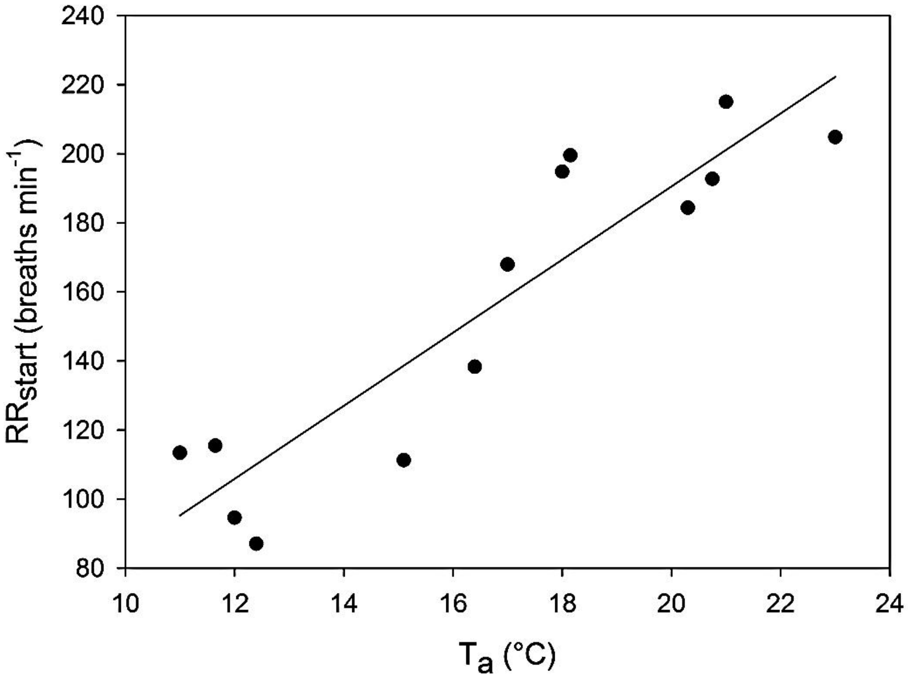

Figure 4. The RR_{start} (the RR within the first minute of post-smoke respiratory response) was positively related to T_a and is described by the following equation: RR_{start} (breaths min^{-1}) = - 21.8 + 10.6 * T_a (°C) ($r^2 = 0.85$, $P < 0.001$).

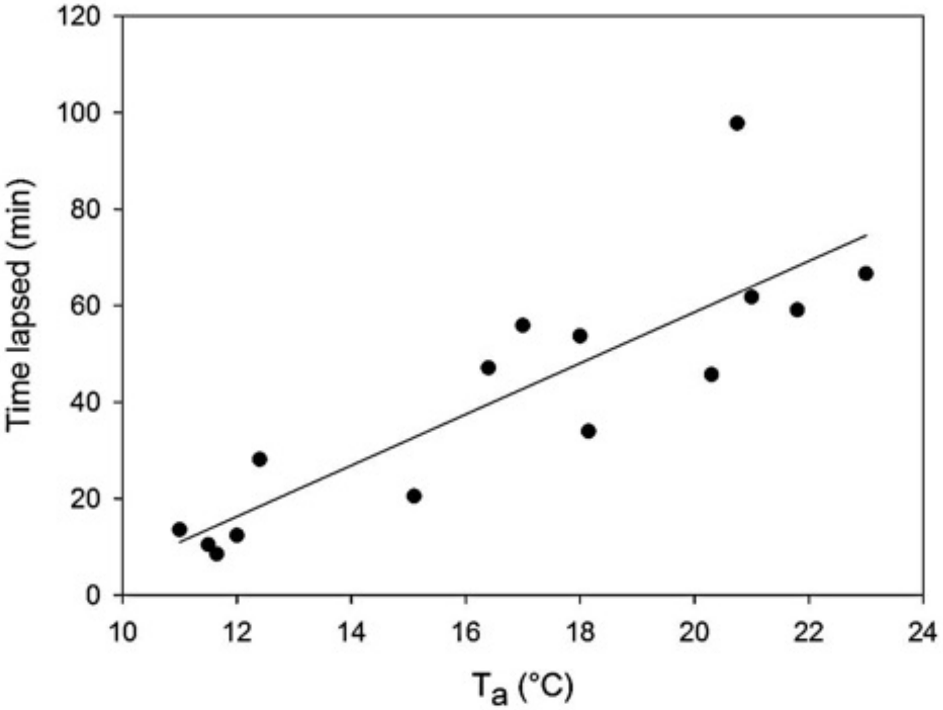

Figure 5. The time lapsed until the first discernible apnoea from the cessation of smoke exposure was positively related to T_a and is described by the following equation: Time lapsed (min) = -47.4 + 5.3 * T_a (°C) ($r^2 = 0.80$, $P < 0.001$).

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).

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