

## RESEARCH ARTICLE

# A new cue for torpor induction: charcoal, ash and smoke

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## ABSTRACT

Recent work has shown that the use of torpor for energy conservation increases after forest fires in heterothermic mammals, probably in response to the reduction of food. However, the specific environmental cues for this increased torpor expression remain unknown. It is possible that smoke and the novel substrate of charcoal and ash act as signals for an impending period of starvation requiring torpor. We therefore tested the hypothesis that the combined cues of smoke, a charcoal/ash substrate and food shortage will enhance torpor expression in a small forest-dwelling marsupial, the yellow-footed antechinus (*Antechinus flavipes*), because like other animals that live in fire-prone habitats they must effectively respond to fires to ensure survival. Activity and body temperature patterns of individuals in outdoor aviaries were measured under natural environmental conditions. All individuals were strictly nocturnal, but diurnal activity was observed shortly after smoke exposure. Overall, torpor in females was longer and deeper than that in males. Interestingly, while both males and females increased daily torpor duration during food restriction by >2-fold as anticipated, a combination of food restriction and smoke exposure on a charcoal/ash substrate further increased daily torpor duration by ~2-fold in both sexes. These data show that this combination of cues for torpor induction is stronger than food shortage on its own. Our study provides significant new information on how a small forest-dwelling mammal responds to fire cues during and immediately after a fire and identifies a new, not previously recognised, regulatory mechanism for thermal biology in mammals.

**KEY WORDS:** Activity, Behaviour, Fire, Heterothermy, Mammal, Physiology

## INTRODUCTION

The physiological and behavioural responses of individual mammals to fire have attracted some recent scientific attention (Scesny, 2006; Dickinson et al., 2010; Doherty et al., 2015; Perry and McDaniel, 2015; Doty et al., 2016; Matthews et al., 2016; Nowack et al., 2016a; Stawski et al., 2016). For example, volant bats employed less torpor, characterised by reduced metabolic rate and body temperature ( $T_b$ ), shortly after a wildfire in comparison to 2 years later (Doty et al., 2016). This response is probably because of the ease of foraging in the canopy and an increase in aerial insects after a wildfire. In contrast, for most terrestrial animals, food and water resources will be reduced once a fire has passed through an area (VanTassel et al., 2015; Coleman and Rieske, 2006) and

ground cover and refuges will be scant or completely obliterated (Matthews et al., 2016; Stawski et al., 2016). Consequently, in terrestrial mammals, torpor has been shown to be an important energy-saving mechanism that can increase the chances of post-fire survival (Matthews et al., 2016; Nowack et al., 2016a; Stawski et al., 2016). While energy savings by using torpor can compensate for food shortage, they can potentially also reduce foraging requirements and other above-ground activities. This would be important in a denuded landscape that exposes small mammals to predators, which are often attracted to recently burnt areas (McGregor et al., 2014).

Previous studies on the response of free-ranging terrestrial mammals to fire have suggested that food reduction is likely to be an important, if not the primary cause of the increase in torpor use (Matthews et al., 2016; Nowack et al., 2016a; Stawski et al., 2016). A decrease in food abundance is indeed a well-known trigger for torpor induction in many heterothermic species, and other environmental cues, such as low ambient temperature ( $T_a$ ) and a change in photoperiod, are often precursors to a reduction in food availability (Ruf et al., 1991; Song and Geiser, 1997; Stawski and Geiser, 2010; Levy et al., 2012; Williams et al., 2014; Vuarin et al., 2015). However, in a post-fire landscape, other environmental signals may be used as cues for torpor expression and the most obvious of these are smoke, charcoal and ash.

Indeed, a small marsupial (fat-tailed dunnart, *Sminthopsis crassicaudata*) from the Australian arid zone was shown to respond to smoke and arouse from torpor (Stawski et al., 2015). However, as far as torpor expression is concerned, food availability remained the main driver and, in fact, smoke and a charcoal and ash substrate reduced torpor use somewhat (Stawski et al., 2015). Nevertheless, dunnarts live in a variety of open habitats, such as grasslands and claypans (Morton, 1978; Warnecke et al., 2012; Morton and Dickman, 2013), whereas many other of Australia's small mammals live in forests along the east coast where regular and extensive wildfires can be devastating. Arguably, fire constitutes the most severe threat for species living in such forest and woodland habitats and one of these is the yellow-footed antechinus (*Antechinus flavipes*) (Kelly, 2006; Crowther, 2013). Antechinus are semi-arboreal, with nests often located in rock fissures, underground burrows or high in trees, and forage predominantly on arthropods on the ground and also in trees (Dickman, 1980; Marchesan and Carthew, 2004; Lada et al., 2007). While antechinus can use torpor, they usually do not employ torpor every day and often only in response to adverse conditions, and generally females are more likely to use torpor than males (Geiser, 1988; Rojas et al., 2014). Conversely, both male and female arid-zone dunnarts use torpor frequently throughout the year and even express spontaneous torpor (food *ad libitum*) under laboratory conditions (Warnecke et al., 2008; Stawski et al., 2015). These differences in habitats and torpor use between dunnarts and antechinus may result in varying responses to fire cues.

The aim of our study was to provide quantitative data on how a combination of food restriction, smoke and a charcoal/ash substrate,

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as experienced in a forest during and after a fire, affect activity and torpor expression. Therefore, yellow-footed antechinus were investigated under semi-natural conditions to determine which cue or which combination of cues elicits a behavioural or physiological response. Yellow-footed antechinus are known to survive forest fires *in situ* and the survivors were found nesting in crevices in large rocky outcrops after a devastating wildfire (Matthews et al., 2016). Importantly, yellow-footed antechinus employed more torpor in this denuded landscape than in a laboratory study (Geiser, 1988) and also in free-ranging individuals (Rojas et al., 2014). Similar field observations with an increase in torpor use were also made for brown antechinus (*Antechinus stuartii*) and echidnas (*Tachyglossus aculeatus*) after management burns (Nowack et al., 2016a; Stawski et al., 2016). We therefore hypothesised that yellow-footed antechinus will increase torpor use in response to a decrease in food availability, but that smoke and a charcoal/ash substrate will further enhance torpor expression.

## MATERIALS AND METHODS

Eight yellow-footed antechinus, *Antechinus flavipes* (Waterhouse 1838) (three males, five females), were originally captured in the wild from Aberaldie Nature Reserve (31°04'24"S, 151°25'34"E), a eucalypt woodland, using box aluminium traps (Type A; Elliott Scientific Equipment, Upwey, Australia) baited with a peanut butter, honey and oats mixture. Polyester fibre was provided for bedding material and each trap was covered in a plastic bag to prevent moisture collecting in the traps. Once captured, antechinus were transported to the University of New England and were housed in individual mesh cages (2.0×0.6×0.6 m) in outdoor aviaries and provided with three wooden nest boxes with shredded paper for bedding material, each located at different heights (bottom 0.1 m, middle 1.0 m, top 1.8 m above ground), bark chips on the floor, climbing sticks and running wheels. Water was provided *ad libitum* throughout the study period and food (a mixture of canned cat food and dry cat food soaked in water, supplemented with a vitamin powder) was provided *ad libitum* on non-experimental days. Twice a week throughout the study period, food was restricted to 50% of the normal daily food intake, which was determined prior to the experiment by weighing the amount of food each animal consumed when given food *ad libitum*. Experimenters removed leftover food and placed new food in each cage around 14:00 h daily; this process only took ~5 min. As animals were located outdoors, they experienced natural photoperiod and ambient conditions throughout the study period.  $T_a$  was recorded at 10 min intervals using iButton data loggers ( $\pm 0.5^\circ\text{C}$ , iButton thermochron DS1921G, Maxim Integrated Products, Inc., Sunnyvale, CA, USA). Body mass (g) of all individuals was recorded at the start and end of the experiment and nest choice was recorded daily during feeding time using the transmitter signal (see below) without disturbing the animals.

Throughout the study period during the austral winter (30 June to 24 July 2014), antechinus were exposed to control and experimental conditions (see Table 1 for the experimental protocol). In addition to control conditions (food and water provided *ad libitum*), animals were subjected to four different one-day experimental treatments: (1) food restriction only: this treatment was repeated three times (see Table 1) and was 50% of the normal daily food intake; (2) smoke+food restriction: the introduction of smoke and food restriction; (3) smoke+sawdust+food restriction: the introduction of smoke along with sawdust (a novel substrate) and food restriction; and (4) smoke+charcoal/ash+food restriction: the introduction of smoke along with a charcoal/ash substrate (two fire variables) and food restriction.

**Table 1. Experimental protocol**

Day	Treatment
0–2	Control
3	Smoke+food restriction
4–5	Control
6	Food restriction
7–9	Control
10	Smoke+sawdust+food restriction
11–12	Control
13	Food restriction
14–16	Control
17	Smoke+charcoal+food restriction
18–19	Control
20	Food restriction
21–24	Control

Food restriction is 50% of the daily food intake.

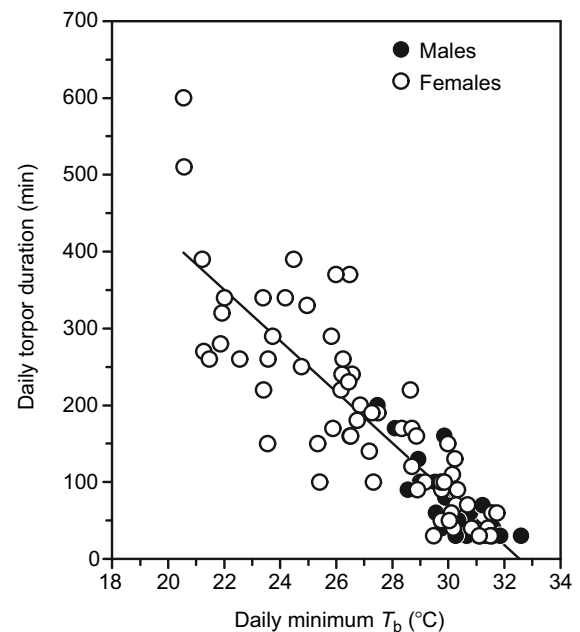
Food restriction was followed by at least 2 days of food *ad libitum*. This experimental protocol was designed to take into account the restricted battery life of the temperature-sensitive radio-transmitters used to record  $T_b$  (see below for more details) to ensure all the necessary data were obtained during this time period. Furthermore, the order of the experimental treatments ensured that animals had time to recover and experience control conditions in between each treatment. Smoke was produced by creating a fire in a large metal bin just outside the outdoor aviary burning *Eucalyptus* leaves and twigs. For all three smoke treatments, the fire was lit at 14:00 h and after 30 min the fire was doused in water and sand. The abundance of smoke particles in the air just outside the aviary was measured using a smoke spot tester kit (Testo 308, Professional Equipment, Janesville, WI, USA) that uses a scale ranging from 0 (clean air) to 6 (very thick smoke). For the first 5 min of the smoke treatment, the smoke reading was ~1 (light smoke) and this gradually increased to a maximum reading of ~5 (thick smoke) within 10 min of lighting the fire, ensuring that all individuals experienced thick smoke for 20 min during the treatment. Before the second fire event, 50 g of sawdust was scattered on the floor of each cage to introduce a novel substrate in conjunction with smoke to test whether any potential responses to charcoal/ash in the subsequent treatment were specifically to charcoal/ash or generally to a novel substrate. For the subsequent fire event, 50 g of charcoal and ash was used as the substrate for each cage to mimic post-fire conditions. Both of these novel substrates were left in the enclosures from shortly prior to smoke exposure (14:00 h) until 14:00 h the following day.

We recorded the activity of six individuals and the  $T_b$  patterns of all eight individuals. To continuously measure activity, a custom-made logger was used to record activity events from one passive infrared sensor per cage for six of the cages (only six sensors were available); data were summed over 10 min. Sensors were attached to the lower part of six of the cages to pick up any animal movement close to the feeding dishes and running wheels. Black plastic sheets were attached to all sides of the lower portion of the cages to ensure the sensors only recorded movement from the target individual and also to prevent triggering of the sensors by the movement of the experimenters lighting the fire. Further, we tested the activity sensors without animals in the cages and lit a fire to produce smoke to ensure the sensors did not react to smoke, which they did not. To account for movement created by the experimenters as they replaced food and water, these short time periods (~5 min) were noted and the data removed from analyses. The activity logger failed during the first week of the experiment; therefore, only activity data after

day 8 of the study period are available (refer to Table 1 for protocol). The  $T_b$  of all antechinus was recorded every 10 min by a remote receiver/data logger (Körtner and Geiser, 2000) and an antenna that picked up the signals from implanted temperature-sensitive radio-transmitters (1.7–2.1 g, Sirtrack, Havelock North, New Zealand). All transmitters were initially calibrated in a water bath in a temperature range of 14–41°C to the nearest 0.1°C and coated with inert wax (Paraffin/Elvax, Mini Mitter, Respironics Inc., OR, USA). Waxed transmitters weighed <10% of body mass as recommended for small terrestrial mammals (Rojas et al., 2010) and were implanted into the intraperitoneal cavity of each individual as described in Stawski et al. (2015). Following this surgical procedure, all animals were allowed to recover for 1 week before the experiment began.

The start and end times for each activity bout that lasted for longer than 30 min were recorded. To define torpor bouts, we calculated the torpor  $T_b$  thresholds ( $T_{b,onset}$ ) from eqn 4 in Willis (2007), which were 32.5°C for females and 33.0°C for males. As maximum  $T_b$  for antechinus during this study was ~40°C, a drop of >7°C was deemed appropriate for defining torpor and, importantly, energetically significant decreases in  $T_b$  may have been overlooked if the widely used threshold of 30°C had been used (Barclay et al., 2001; Brigham et al., 2011). Torpor bout duration was calculated as the period for which  $T_b$  fell below the  $T_{b,onset}$  for intervals of longer than 30 min. All torpor bouts employed during each day were combined for analyses and termed ‘daily torpor duration’.

All statistical analyses were performed in R (R version 3.0.1, The R Foundation for Statistical Computing 2013). To determine whether total nightly activity duration, daily torpor duration and daily minimum  $T_b$  differed between the sexes for each treatment group, analyses of covariance (function ‘aov’) were used with daily minimum  $T_a$  and the initial body mass as covariates for both variables and daily minimum  $T_b$  as an additional covariate for daily torpor duration. Total nightly activity duration was the same for males and females and was therefore analysed together. In contrast, as daily torpor duration and daily minimum  $T_b$  differed between sexes for all of the treatment groups ( $P \leq 0.04$ ), the effect of each treatment on these variables was analysed separately for the two sexes. Linear mixed effects models were then fitted (package ‘nlme’) to determine whether treatments (control, food restriction only, smoke+food restriction, smoke+sawdust+food restriction, smoke+charcoal/ash+food restriction) had an effect on the measured variables (total nightly activity duration, daily torpor duration, daily minimum  $T_b$ ). As daily torpor duration significantly increased with decreasing daily minimum  $T_b$  in both sexes and all treatment groups ( $n=8$  individuals,  $F_{1,93}=301.4$ ,  $P<0.0001$ ,  $R^2=0.7$ ; Fig. 1), daily minimum  $T_b$  was included as a covariate. In addition, daily minimum  $T_a$  and initial body mass were included as covariates for all variables. To account for repeated measures, individuals were included as a random effect. This approach allowed us to compare control and treatment days for each individual. If there was a significant effect of treatment, a *post hoc* Tukey test (package ‘multcomp’) was performed to determine which treatment groups differed. The proportional increase in daily torpor duration between the food restriction only and the smoke+charcoal/ash+food restriction treatment was similar for the two sexes and therefore this variable was tested for males and females combined. To determine the relationship between activity bout duration/daily minimum  $T_b$  and daily torpor duration, a linear mixed effects model (packages ‘lme4’ and ‘MuMIn’) was fitted and again initial body mass was included as a covariate and individuals were included as a



**Fig. 1. The relationship between daily minimum body temperature ( $T_b$ ) and daily torpor duration for all individuals ( $n=8$ ) and treatments.** Linear mixed effects model:  $F_{1,93}=301.4$ ,  $P<0.0001$ ,  $R^2=0.7$ ,  $y=-33.214x+1081$ . As the slope of this relationship did not differ between males and females ( $P=0.928$ ,  $F_{1,91}=0.01$ ), they were regressed together.

random factor. As the slope of this relationship did not differ among individuals or sexes, all study animals were pooled together for this test. A Rayleigh test was used to determine the average timing of the start and end of activity bouts and a Watson–Williams  $F$ -test was performed to determine any differences between treatment groups (package ‘circular’). Significance was assumed at  $P<0.05$  and means are shown as the mean of the mean of each individual  $\pm 1$  s.d., with  $n$ =number of individuals/days and  $N$ =number of observations.

Permits to conduct this study were granted by the University of New England Animal Ethics Committee and the New South Wales National Parks and Wildlife Service.

## RESULTS

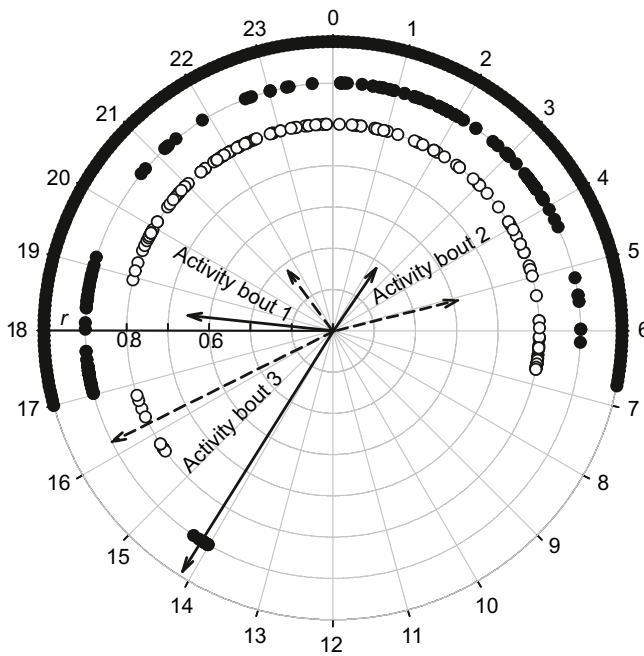
Antechinus experienced natural weather conditions with  $T_a$  fluctuating on average by  $13.7 \pm 0.8^\circ\text{C}$  ( $n=25$ ; range: 6.0–20.0°C) each day, with a daily mean minimum  $T_a$  of  $2.4 \pm 0.4^\circ\text{C}$  ( $n=25$ ; range:  $-0.5$  to  $6.5^\circ\text{C}$ ) and a daily mean maximum  $T_a$  of  $16.1 \pm 0.7^\circ\text{C}$  ( $n=25$ ; range: 7.0–21.5°C). Throughout the study period, mean daily  $T_a$  was  $6.7 \pm 0.4^\circ\text{C}$  ( $n=25$ ; range: 3.2–9.7°C). None of these  $T_a$  variables differed among treatment regimes ( $P \geq 0.123$ ).

Males were significantly heavier than females ( $F_{1,13}=39.7$ ,  $P<0.0001$ ) and body mass did not change over the course of the study for either sex (females:  $F_{1,8}=0.2$ ,  $P=0.696$ , start  $29.9 \pm 1.4$  g, end  $30.8 \pm 1.7$  g; males:  $F_{1,4}=0.1$ ,  $P=0.834$ , start  $47.7 \pm 3.9$  g, end  $49.3 \pm 6.0$  g).

Nest choice was the same for both sexes ( $F_{1,17}=0.2$ ,  $P=0.629$ ) and during all treatments antechinus showed a significant predilection in nest choice ( $F_{2,17}=4.4$ ,  $P=0.029$ ), preferring the middle ( $52.7 \pm 14.0\%$ ,  $n=8$ ,  $N=60$ ) and highest ( $47.0 \pm 14.1\%$ ,  $n=8$ ,  $N=74$ ) nest boxes in comparison to the nest box on the ground ( $1.3 \pm 0.8\%$ ,  $n=2$ ,  $N=2$ ).

Throughout the study, all antechinus maintained nocturnality and activity records were clustered around two main time periods during the night (activity bout 1 and 2; Fig. 2). The duration of total nightly





**Fig. 2.** Circular distribution of the time of day (24 h) for the start (black circles) and end (white circles) of activity for all individuals ( $n=8$ ). Vectors of the means derived from Rayleigh's test ( $P<0.0001$  for all) for the start (solid arrows) and end (dashed arrows) of all activity bouts. The radial axis is a proportional scale (i.e. 0–1) as vector length ( $r$ ) is a measure of the dispersion from the mean, i.e. a longer arrow indicates stronger clustering. Night is indicated by the thick black solid line on the outer circle.

activity differed neither between the sexes nor among the treatments and lasted on average for  $484.7\pm 82.6$  min ( $n=6$ ,  $N=78$ ; Table 2). The timing of these two activity periods was also similar for the different sexes and treatment regimes (Watsons–Williams  $F$ -test:  $P=0.123$  to  $0.927$ ). The first activity period commenced on average at  $18:20\pm 00:36$  h ( $n=6$ ,  $N=95$ ; Rayleigh test:  $z=1.6$ ,  $P<0.0001$ ) and ended approximately 200 min later at  $21:38\pm 00:20$  h ( $n=6$ ,  $N=83$ ; Rayleigh test:  $z=0.1$ ,  $P<0.0001$ ; Fig. 2). Activity then resumed for bout 2 at  $02:24\pm 00:23$  h ( $n=6$ ,  $N=97$ ; Rayleigh test:  $z=4.3$ ,  $P<0.0001$ ) and ended for the daytime rest period after about 150 min at  $05:00\pm 00:23$  h ( $n=6$ ,  $N=112$ ; Rayleigh test:  $z=1.8$ ,  $P<0.0001$ ; Fig. 2). Consistently for all antechinus, on days with smoke exposure, a third additional activity period (activity bout 3; Fig. 2) started shortly after the introduction of smoke at  $14:02\pm 00:05$  h ( $n=6$ ,  $N=12$ ; Rayleigh test:  $z=3.1$ ,  $P<0.0001$ ) and lasted for approximately 130 min, ending at  $16:14\pm 00:31$  h ( $n=6$ ,  $N=12$ ; Rayleigh test:  $z=0.7$ ,  $P<0.0001$ ; Fig. 2).

Unlike for activity, the expression of daily torpor was strongly affected by treatment and therefore the length of total nightly activity was not related to daily torpor duration ( $P=0.700$ ). For all individuals during all treatments, torpor bouts predominantly occurred during the daytime, with 88% of torpor entries ( $n=8$ ,  $N=169$ ) and 89% of arousals from torpor ( $n=8$ ,  $N=169$ ) occurring between sunrise and sunset. Daily torpor duration for all treatments was shorter for male ( $n=3$ ) than for female ( $n=5$ ) antechinus, with a 10-fold difference under control conditions and ~2- to 3-fold difference under all other experimental conditions (daily torpor duration:  $P\leq 0.04$ ; Figs 3 and 4).

Consequently, for males the treatment conditions resulted in a more substantial increase in daily torpor duration (Table 2), with a 6.5-fold increase when food was restricted only (daily torpor duration:  $P<0.0001$ ,  $z=4.4$ ) and also in conjunction with smoke and

sawdust (daily torpor duration:  $P<0.0001$ ,  $z=3.9$ ; Fig. 4). The effect was even larger (14.3-fold) when charcoal/ash was added to the treatment (daily torpor duration:  $P<0.0001$ ,  $z=8.4$ ; Figs 3 and 4). Overall, the lengthening of torpor duration by food restriction was accompanied by a significant  $\sim 2.5^\circ\text{C}$  drop in daily minimum  $T_b$  ( $n=3$ ;  $P<0.0001$ ,  $z=-4.7$  to  $-2.7$ ; Tables 2 and 3), regardless of smoke exposure or substrate change.

For females, because of the more pronounced baseline torpor expression under control conditions, changes in daily torpor duration were smaller but also significant (Table 2), with a ~2.2-fold increase in daily torpor duration when food was restricted only (daily torpor duration:  $P<0.0001$ ,  $z=5.9$ ) and also in combination with smoke and sawdust (daily torpor duration:  $P<0.0001$ ,  $z=4.4$ ). Again, a charcoal/ash substrate augmented the effect of food reduction to a 3.5-fold increase (daily torpor duration:  $P<0.0001$ ,  $z=7.9$ ; Figs 3 and 4). Interestingly, this significant  $2.0\pm 0.5$ -fold ( $n=7$ ) enhancement in daily torpor duration by the addition of the charcoal/ash substrate over food restriction alone was the same in males and females (proportional increase in daily torpor duration:  $F_{1,5}=1.5$ ,  $P=0.189$ ). The overall longer torpor bouts in females were associated with lower daily  $T_b$  minima and these significantly decreased from control conditions by  $\sim 3.5^\circ\text{C}$  when food was restricted only (daily minimum  $T_b$ :  $P<0.0001$ ,  $z=-6.8$ ) and also in conjunction with smoke and sawdust (daily minimum  $T_b$ :  $P<0.0001$ ,  $z=-3.1$ ). An even more pronounced  $5.4^\circ\text{C}$  reduction in daily  $T_b$  minima was observed after the addition of the charcoal/ash substrate (daily minimum  $T_b$ :  $P<0.0001$ ,  $z=-7.8$ ; Tables 2 and 3).

Importantly, the effect of the three interspersed food restriction only treatments (on days 6, 13 and 20) on torpor bout duration remained consistent (males:  $F_{2,4}=0.6$ ,  $P=0.589$ ; females:  $F_{2,6}=0.1$ ,  $P=0.916$ ). Therefore, the increase in torpor bout duration in response to the addition of a charcoal/ash substrate for both males and females was not an accumulative effect of repeated food restriction treatments.

## DISCUSSION

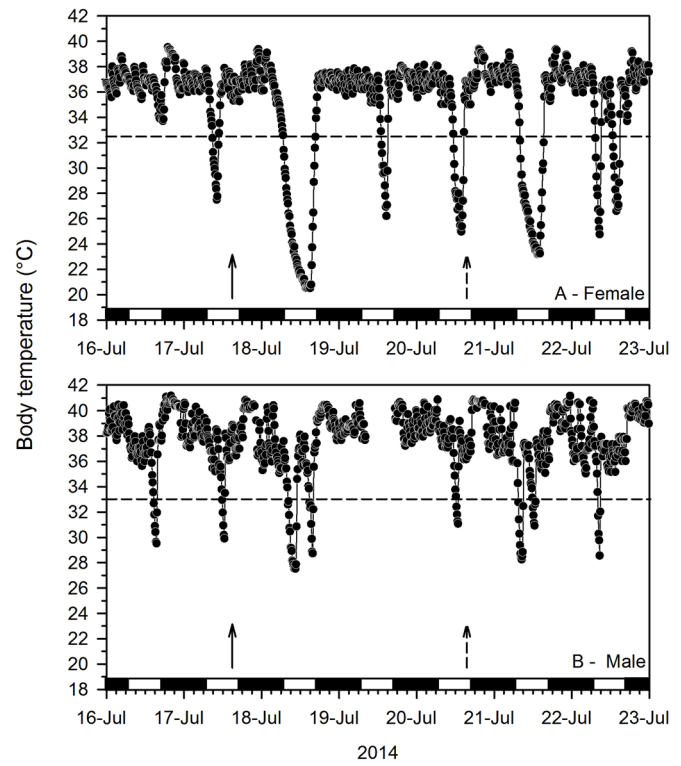
Our study provides compelling evidence that a charcoal and ash substrate enhances torpor expression in a forest-dwelling small terrestrial mammal. Previous work has revealed that free-ranging yellow-footed antechinus can survive even a severe wildfire *in situ* and several months after the fire were able to deal with the depletion of resources and vegetative cover by extensive torpor use (Matthews et al., 2016). However, the environmental cues that triggered this response remained obscure and the present study aimed to close this knowledge gap.

The instant alarm response to smoke exposure was very obvious, with all antechinus being active for several hours after the introduction of the stimulus. Importantly, all antechinus responded while the smoke produced by the fire was still light, suggesting that in the wild, antechinus would probably detect an approaching fire front quite early. Further, the third activity bout in the afternoon (in addition to two activity bouts at night) started in response to smoke at a time when the animals would not have known whether they would be fed or not; therefore, this emergency response was not food related. The intrusion of smoke into a nest site would indicate the vulnerability of this particular site to fire and heat (Dickinson et al., 2010; Perry and McDaniel, 2015), probably causing animals to retreat into a more secure shelter. In our experimental setup, such secure sites like underground burrows or rocky crevices (Howard et al., 1959) were not provided, perhaps explaining the protracted length of the activity bout following smoke exposure. This emergency response to smoke is not unique to antechinus and has

**Table 2. Statistical results from the linear mixed effects models for total nightly activity duration, total daily torpor duration and daily minimum body temperature**

Variable	Sex		Food restriction		Smoke+food restriction		Smoke+sawdust +food restriction		Smoke+charcoal +food restriction		Daily minimum $T_a$		Daily minimum $T_b$		Body mass		Sex	
	t	P	t	P	t	P	t	P	t	P	t	P	t	P	t	P	t	P
Nightly activity duration	1.0	0.317	NA	NA	2.4	0.217	-1.1	0.272	0.1	0.940	NA	NA	-1.2	0.314	0.2	0.828		
Daily torpor duration	4.4	<0.0001	3.9	0.0002	3.9	0.0002	8.4	<0.0001	0.6	0.545	-6.4	<0.0001	1.1	0.474	NA	NA		
Daily minimum $T_b$	5.9	<0.0001	4.4	<0.0001	5.9	<0.0001	7.9	<0.0001	0.2	0.810	-8.6	<0.0001	0.6	0.598	NA	NA		
	-4.7	<0.0001	-2.7	0.009	-3.9	0.0002	-4.5	<0.0001	1.6	0.122	NA	NA	-0.2	0.881	NA	NA		
	-6.8	<0.0001	-4.2	<0.0001	-3.1	0.003	-7.8	<0.0001	2.3	0.025	NA	NA	-0.6	0.615	NA	NA		

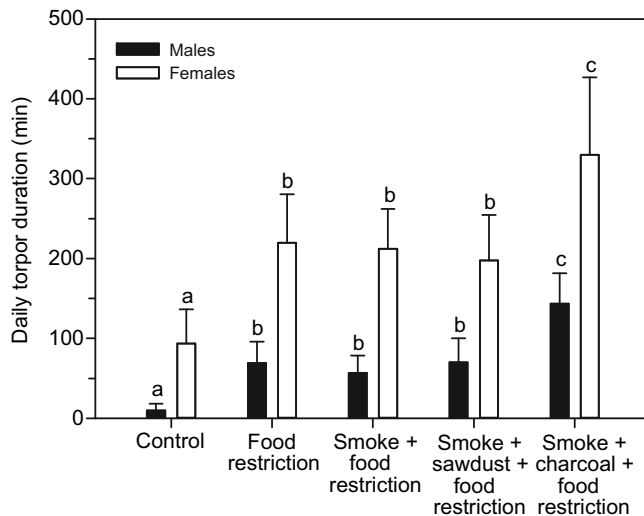
The t- and P-values are shown for each of the treatments (food restriction only, smoke+food restriction, smoke+sawdust+food restriction, smoke+charcoal/ash+food restriction) and all of the covariates [daily minimum ambient temperature ( $T_a$ ), daily minimum body temperature ( $T_b$ ), body mass, sex]. If a treatment was not tested or a covariate was not applicable to the model, 'NA' is shown. F, female; M, male.



**Fig. 3. Body temperature traces of antechinus over 1 week during the study period.** (A) Female antechinus; (B) male antechinus. The dashed lines represent the torpor threshold, the solid arrows show when the smoke +charcoal/ash+food restriction treatment was implemented for 1 day, the dashed arrows show when food was restricted for 1 day, and the black and white bars at the bottom of the graphs denote night and day, respectively.

also been shown for two other small marsupials, the fat-tailed dunnart and the eastern pygmy possum (Stawski et al., 2015; Nowack et al., 2016b). Interestingly, in these two species, smoke was sensed even by torpid individuals and induced rapid arousal, whereas none of the antechinus were still torpid when smoke was introduced in the present study. Arousal from torpor in response to smoke has also been recorded in bats (Scesny, 2006) and it is possible that fire cues other than smoke may influence activity and arousal from torpor, such as an increase in  $T_a$  or changes in humidity (Dickinson et al., 2010; Perry and McDaniel, 2015). Regardless, it is likely that smoke is perceived as a warning signal and will elicit an escape response in both torpid and normothermic small mammals, therefore strengthening the chances of surviving an approaching fire front provided a secure shelter can be found in time.

In contrast, smoke exposure did not seem to entail long-term effects in the antechinus, as activity during the subsequent night followed normal patterns and torpor expression did not increase beyond that caused by food restriction alone. Again these results match those seen in fat-tailed dunnarts (Stawski et al., 2015). Hence, smoke can trigger a vital escape response, but appears to do little to prepare these animals for the challenging conditions during the aftermath of a fire. This cue is apparently provided by what is left after a fire has passed; namely, charcoal and ash. While food restriction appears to be a strong trigger for torpor in antechinus and other mammals, when smoke exposure and food restriction were augmented with a charcoal and ash substrate, torpor use in antechinus increased significantly further, suggesting that this combination provides a stronger cue for animals to conserve energy.



**Fig. 4. Mean daily torpor duration of antechinus in the study conditions.** Daily torpor duration (means $\pm$ s.d.) differed significantly between males ( $n=3$ ) and females ( $n=5$ ) in each treatment ( $P<0.04$ ). Therefore, linear mixed effects models were performed separately for the two sexes and a significant difference was found among the treatments for both ( $P<0.0001$ ). Different letters indicate a significant difference between treatments (*post hoc* Tukey test) for each sex separately.

In this regard, the present study differs from the similar study conducted on dunnarts, in which a charcoal and ash substrate did not enhance torpor expression when food was withheld (Stawski et al., 2015). However, wild dunnarts use torpor almost every day during winter and spontaneous torpor (room temperature and food provided *ad libitum*) under laboratory conditions is also seen regularly (Geiser and Baudinette, 1987; Warnecke et al., 2008). Therefore, in dunnarts it is likely that the torpor-inducing stimuli of low  $T_a$  and food restriction might already elicit a maximal torpor response to save energy and further augmentation is not possible. Yellow-footed antechinus, in contrast, do not employ torpor as often as dunnarts and, importantly, do not always do so in response to food restriction (Geiser, 1988; Rojas et al., 2014). Therefore, the response of antechinus to a charcoal/ash substrate by increasing torpor use suggests that they might adjust thermal biology on a finer scale and incorporate more subtle cues than just food restriction alone. As a result, antechinus may be influenced more by additional

**Table 3. Mean daily minimum  $T_b$  for female and male antechinus in each treatment**

Treatment	Sex	Minimum $T_b$ ( $^{\circ}$ C)	Sex difference ( $P$ -value)
Control	F	29.8 $\pm$ 1.3 <sup>a</sup>	<0.0001
	M	32.5 $\pm$ 0.6 <sup>a</sup>	
Food restriction	F	26.2 $\pm$ 1.4 <sup>b</sup>	0.005
	M	30.4 $\pm$ 1.0 <sup>b</sup>	
Smoke+food restriction	F	26.9 $\pm$ 1.2 <sup>b</sup>	0.029
	M	30.6 $\pm$ 1.1 <sup>b</sup>	
Smoke+sawdust+food restriction	F	27.1 $\pm$ 1.9 <sup>b</sup>	0.046
	M	30.1 $\pm$ 0.8 <sup>b</sup>	
Smoke+charcoal+food restriction	F	24.4 $\pm$ 1.5 <sup>c</sup>	0.040
	M	29.5 $\pm$ 1.1 <sup>b</sup>	

Linear mixed effects models were performed separately for the two sexes (F, female,  $n=5$ ; M, male,  $n=3$ ) and a significant difference was found among the treatments for both ( $P<0.0001$ ). Different letters indicate a significant difference between means ( $\pm$ s.d.) for the different treatments, for each sex separately.

environmental cues and could perceive charcoal and ash as a long-term challenge (Matthews et al., 2016). Further, dunnarts are smaller with less body energy reserves and also occur in relatively open habitats in the arid zone (Morton, 1978; Warnecke et al., 2012; Morton and Dickman, 2013), suggesting they would be accustomed to foraging in the open with a high risk of predation and a fire would not change this substantially. Arid-zone dunnarts also show little site fidelity (Dickman et al., 2001) and the usually patchy grassland fires might simply result in a home range shift. Conversely, antechinus prefer more cluttered and complex habitats in forests (Stokes et al., 2004; Kelly, 2006; Crowther, 2013), remain in their familiar home range even after fire (Stawski et al., 2016) and would therefore experience a much greater increase in post-fire predation pressure when vegetation cover is reduced to charcoal and ash.

By employing energy-saving torpor, animals are able to reduce foraging activity, suggesting that torpor and activity are intrinsically linked (Ruf et al., 1991). For example, free-ranging brown antechinus displayed both a reduction in activity and an increase in torpor use after a management burn (Stawski et al., 2016). In contrast, we could not detect such a link in the present data set. With the exception of the emergency response to smoke, activity remained constant throughout all treatments. This is partly explained by the temporal separation of torpor during daytime and nocturnal activity in captive animals. Furthermore, the link between torpor and activity applies specifically to foraging and this is an easy task if ready-made food is provided in a dish. Hence, the locomotor activity recorded in our experiment may have included only a small part of foraging activity. The extensive daytime activity seen in wild yellow-footed antechinus, which are presumed to be the most diurnal of the antechinus species (Kelly, 2006; Matthews et al., 2016), as well as in brown antechinus (Stawski et al., 2016), is likely to reflect the true foraging effort under natural conditions. The animals in our study had no reason to forage during the day as they were fed in the late afternoon.

Torpor appears to be an important survival strategy for many small mammals in response to catastrophic events (Lovegrove et al., 2014; Nowack et al., 2015, 2016a; Stawski et al., 2016), but how animals employ torpor immediately or in the short to long term after a fire varies among species (Doty et al., 2016). Importantly, our study identifies a charcoal and ash substrate as a new cue that signals antechinus to enhance torpor use to save energy. In rats and mice, exposure to particulate matter, such as ash, results in reduced heart rate or heart rate variability via vagal regulation and also causes lung inflammation (Swoap et al., 2008; Pham et al., 2009; Farraj et al., 2011). Additionally, as  $T_b$  regulation involves the hypothalamus (Heller et al., 1977; Seebacher, 2009), a fast adjustment to the thermal physiology of the animal can be made, as the presence of these products of fire will herald a potential reduction in food availability. The additional acute cue of food restriction will further amplify this response, resulting in enhanced torpor expression in a species that does not enter torpor every day (Geiser, 1988; Rojas et al., 2014). Such a response is particularly pertinent in the forest habitat where antechinus are found (Kelly, 2006; Crowther, 2013) and where a charcoal and ash substrate will be present in large amounts after a fire, accompanied by a reduction in food resources and ground cover and increasing predation pressure (Stawski et al., 2016). Importantly, as the body mass of antechinus before and after the current experiment did not differ, the animals did not lose significant amounts of mass after food restriction and would have replaced any lost body mass the following day when food was provided. Therefore, by employing more torpor in response to a combination of food restriction and a charcoal and ash substrate, antechinus will increase their chance of survival after a fire by saving energy and reducing



exposure to predators. More studies such as ours would be beneficial to further reveal the relationship between fire cues and activity and torpor, perhaps in conjunction with different food regimes. Regardless, the emergency response to smoke and the longer term response to charcoal and ash could explain the survival of antechinus during and immediately after a fire.

#### Acknowledgements

For help with animal maintenance we would like to thank Daniella Rojas and Chris Wacker.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

C.S. designed the study, conducted the experiments and analysed the data. F.G. provided logistical support. All authors wrote the manuscript.

#### Funding

This research was funded by a University of New England Postdoctoral Research Fellowship and an Australian Research Council Discovery Early Career Researcher Award to C.S., the German Academic Exchange Service (Deutscher Akademischer Austauschdienst) and the A.F.W. Schimper-Stiftung für Ökologische Forschungen to J.N. and the Australian Research Council to F.G.

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