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Comparing the thermal suitability of nest-boxes and tree-hollows for the conservation-management of arboreal marsupials

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Abstract

Hundreds of species rely on tree-hollows for shelter and breeding, however land-clearing has reduced their availability worldwide. While nest-boxes are deployed extensively in hollow-deficient habitats, their thermal value for arboreal marsupials compared to tree-hollows is unclear, particularly in temperate environments. We analysed thermal regimes in nest-box and tree-hollow pairs during summer and winter environmental conditions. Using a biophysical model, we quantified the relative suitability of den-sites for several marsupial species, estimating the impact of microclimates (and ambient conditions) on predicted heat-production and heat-loss. Nest-box temperatures were strongly influenced by ambient temperatures and solar radiation, whereas tree-hollows buffered external temperature fluctuations. On average, nest-boxes reached maximum temperatures 8°C higher than tree-hollows in summer, and 3°C higher in winter, with maximum temperatures of 52°C recorded in nest-boxes, compared to 38°C in tree-hollows. During summer, estimated heat-loss required by marsupials was 1.5-2.4 times higher in nest-boxes than tree-hollows. Conversely, predicted winter heat-production requirements were slightly lower in nest-boxes (0.95-0.97 of hollow requirements). Our study emphasises the importance of retaining tree-hollows as thermal refuges for hollow-dependent marsupials in temperate zones to reduce thermoregulatory costs during heat-events. Current nest-box designs are likely of limited value during high temperatures and solar radiation loads if they consistently reach temperatures exceeding species upper critical temperatures, however may provide suitable microclimates during winter. With increasing and more prolonged heat-events predicted under climate change, future conservation-management programs should focus on improving nest-box thermal properties to enhance suitability for wildlife.

1. Introduction

Tree-hollows form essential habitat for hundreds of species worldwide, particularly birds and mammals (Scott et al. 1980; Goldingay 2009, 2011). For many vertebrates, tree-hollows are critical for shelter, breeding and predator protection (Gibbons & Lindenmayer 2002; Wesołowski 2002), however, widespread land-clearing has dramatically reduced their availability (Eyre et al. 2010). Hollows large enough for arboreal mammals can take at least 100 years to develop (Wormington & Lamb 1999; Gibbons et al. 2000). Ongoing habitat loss combined with time-lags in hollow-development, will likely maintain the deficit of tree-hollows (Gibbons et al. 2008; Vesk et al. 2008). This is of major concern for hollow-dependent species, as den availability and quality can impact survival, growth and reproduction (Dawson et al. 2005; Catry et al. 2011).

To compensate for tree-hollow loss, nest-boxes have been deployed by wildlife managers, individuals and community groups to support a range of hollow-dependent species (Beyer & Goldingay 2006; Goldingay & Stevens 2009; British Trust for Ornithology). Nest-boxes can be highly valuable for conservation (Durant et al. 2009; Goldingay et al. 2015). However, low occupancy (Lindenmayer et al. 2009) and suboptimal cavity temperatures (Catry et al. 2011) can limit their value. While there has been considerable research into how nest-box design and placement influence occupancy, surprisingly few data are available on thermal suitability of nest-boxes, despite thermal properties likely being a key direct driver of their value for wildlife (Sedgeley 2001).

For endotherms, inappropriate den temperatures almost certainly have acute and long-term impacts, influencing survival during extreme conditions, and increasing costs associated with thermoregulation. Endotherms have an optimal range of environmental temperatures (thermo-neutral zone: TNZ), within which thermoregulatory costs are minimal (Lovegrove et al. 1991). Below their TNZ, metabolic heat-production (thus energy costs) increase, while above the TNZ, water costs rise because evaporative heat-loss is used to avoid overheating (Dawson 1969).

Hollow-dependent species can minimize thermoregulatory costs by selecting dens providing temperatures closest to their TNZ.

Den microclimates influence breeding success and survival. Due to high thermoregulatory costs, animals experiencing unfavorable microclimates are likely to invest fewer resources in growth and reproduction (Garcia-Navas et al. 2008). Tree swallow (*Tachycineta bicolor*) chicks in cooler nests have lower survival, slower growth rates, and smaller body size than those in warmer nests (Dawson et al. 2005). However, higher temperatures are not always beneficial: extreme den temperatures during a heat-wave led to 22% juvenile mortality in a lesser kestrel (*Falco naumanni*) population, with heat-related deaths occurring over two days when ambient temperatures exceeded 39°C (Catry et al. 2011). Some evidence suggests that arboreal marsupials may avoid dens experiencing temperature extremes (Isaac et al. 2008a; Goldingay 2015), which is almost certainly related to factors discussed above.

Despite the potentially high fitness consequences of denning in thermally sub-optimal microclimates, the thermal suitability of nest-boxes across seasons has not been comprehensively studied across the range of environments where they are deployed. The few previous studies suggest that thermal properties of empty nest-boxes and tree-hollows differ significantly, with tree-hollows buffering extremes in daily temperature fluctuations more than nest-boxes (McComb & Noble 1981; Isaac et al. 2008b). However, Isaac et al. (2008b) only compared nest-box microclimates to tree-hollows during summer in a tropical climate, and McComb and Noble (1981) only compared microclimates in a few pairs of nest-boxes and tree-hollows across seasons in a humid subtropical climate. Minimal research has examined the thermal suitability of nest-boxes in temperate Australia, a region that experiences a wide temperature range, has undergone extensive habitat loss, and has had many nest-boxes installed (Lindenmayer et al. 2003; Harper et al. 2005).

Artificial and natural dens differ in structure, which is likely to drive differences in their thermal properties. Characteristics that influence nest-box temperatures include their insulative properties (relating to wall thickness and construction materials), orientation, and level of solar exposure (García-Navas et al. 2008; Charter et al. 2010; Goldingay 2015). Temperatures in tree-hollows are also influenced by their structure (including wall thickness, cavity size and entrance area), in addition to tree health (Paclik & Weidinger 2007; Coombs et al. 2010). Such differences in tree-hollow and nest-box properties may create disparities in their suitability for wildlife under different environmental conditions, between seasons or times of day (Vel'ky et al. 2010). To maximise the success of nest-boxes for conservation it is essential to understand the drivers of variation in cavity temperatures.

While nest-box temperatures are likely to differ from those in tree-hollows, it is important to determine whether these translate to biologically meaningful differences in fitness for species using them. Few studies have examined the fitness consequences of denning in nest-boxes, and these focused predominantly on reproductive success in birds (e.g. Dawson et al. 2005; Charter et al. 2010), with little information about arboreal hollow-dependent mammals. Overall, studies of den microclimates rarely relate differences in temperature to eco-physiological consequences for species (although see Willis & Brigham 2005, 2007), important information for predicting and testing drivers of fitness.

We investigated how daily fluctuations in thermal microclimates differed between nest-boxes and tree-hollows across seasons in a temperate environment. We also examined factors influencing daytime den temperatures. We determined the relative thermal suitability of nest-boxes for four hollow-dependent marsupial species across seasons by estimating the energy and water costs of denning in nest-boxes, tree-hollows, or outside in a sheltered position, using a biophysical model that predicts how morphology, physiology and behaviour interact with the environment to determine animals' metabolic rate and rate of evaporative heat-loss (Porter & Kearney 2009). Our research will inform management decisions regarding nest-box design and installation, with a particular focus on understanding daily fluctuations in cavity temperature during extreme conditions.

2. Materials and methods

2.1 STUDY AREA AND SPECIES

We conducted this research in the Strathbogie Ranges, (36°79' S, 145°80' E) Victoria, Australia (Figure S1). The area has an average altitude of c. 570 m above sea level (a.s.l) and high annual rainfall (c. 1000 mm). It experiences considerable thermal variation, with temperatures exceeding 40°C during summer and falling below 0°C during winter. Temperatures range from a mean monthly maximum of 27.4°C in February to a minimum of 1.7°C in July (Bureau of Meteorology).

This region has experienced substantial habitat loss and fragmentation (Martin & Handasyde 2007), but retained some eucalypt-dominated open sclerophyll forest, which provides habitat for arboreal marsupials that rest in tree-hollows during the day. These include sugar gliders (*Petaurus breviceps*), common ringtail possums *(Pseudocheirus peregrinus,* henceforth common ringtail), greater gliders *(Petauroides volans),* common brushtail possums *(Trichosurus vulpecula,* common brushtail), and mountain brushtail possums *(Trichosurus cunninghami,* mountain brushtail) (Downes et al. 1997). Greater gliders and brushtail possums use a suite of tree-hollows solitarily (Lindenmayer et al. 2004; Martin 2005; Harper 2006), while common ringtails typically rest in small groups in dreys or tree-hollows (Pahl 1987). Nest-box use has been recorded for common ringtails, both brushtail *spp.* (Lindenmayer et al. 2003; Harper et al. 2005) and sugar gliders (Menkhorst 1984; Goldingay et al. 2015).

2.2 COMPARISON OF DEN THERMAL MICROCLIMATES

We compared daytime thermal microclimates of nest-boxes, tree-hollows, and ambient conditions during two summer periods (27/11/2014 to 3/01/2015 and 2-27/02/2015 between 06:00-20:30h) and winter periods (29/06/2014 to 2/08/2014 and 7/06/2015 to 5/07/2015 between 07:30-17:30h). Sampling periods within each season had similar environmental conditions (Figure S2).

We selected 41 tree-hollows spread over 150 km^2 (Figure S1), ranging from 481-674 m a.s.l. We considered tree-hollows suitable if the entrance and cavity were large enough to accommodate common ringtails (Beyer & Goldingay 2006), less than 5.5 m high (for safety), and in live trees. Dead trees were excluded as their thermal properties may differ (Wiebe 2001; Paclik & Weidinger 2007). We used hollows in *Eucalyptus* trees with entrances opening on the trunk (n=20) or tree-base (n=21) to reflect the natural range used by wildlife (K. Handasyde unpublished, based on radio-tracking data for brushtail *spp.*). We measured the entrance orientation (measured as °, converted into cardinal direction: north, n=13; east, n=13; south, n=7; west, n=8), entrance height above ground (to the nearest 5 mm), and DBH (mean ± sd: 1.3 ± 0.5 m). Hollows varied in structure: entrance area range 38.48-30210 cm^2; cavity depth range 18-140 cm; and cavity volume range 0.002-5.655 m^3.

We installed 40 plywood nest-boxes (300x370x475 mm, 17 mm thick) between February and June 2014. One nest-box was relocated after the first two sampling periods (it was too dangerous to access) and paired with a different base-hollow for the remaining periods. Nest-boxes were painted dark-green, consistent with common practice. Each nest-box was mounted on a tree within 17m (mean ± sd, 8.1 ± 2.7 m) of a tree-hollow, with the entrance at the same height and orientation, and similar canopy cover (mean difference ± sd, 5.8 ± 4.1%; t_{39}=1.36, *P*=0.18). We calculated canopy openness above each den by analyzing hemispherical photos, taken with a fisheye len (Sigma 8mm 6.3, Japan) attached to a full frame camera (Canon 5D MkII, Japan), using Gap Light Analyzer (Version 2.0) (Beckschäfer et al. 2013). We initially covered nest-box entrances with wire-mesh to exclude wildlife (02/2014 to 3/01/2015), then uncovered entrances to allow access (from 4/01/2015). After nest-box entrances were uncovered, we checked each den (nest-boxes and tree-hollows) for occupants using a camera (Nikon Coolpix P310, Japan) on a pole (summer: 15 days; winter: 20 days). Animals were not further disturbed after determining occupation status.

Using thermal data loggers (Thermochron iButton; Alfa-Tek, Bayswater, Australia) mounted in plastic mesh or holders, we recorded cavity temperatures (°C; ± 0.5) simultaneously in each nest-box and tree-hollow, as well as ambient temperture (T_a), at 30-minute intervals during all sampling periods. iButtons were positioned on the back wall of each nest-box suspended by string 2 cm below the entrance (25.5 cm above cavity base and 22 cm from lid), with the temperature recording side facing into the cavity. This central location aimed to minimise the impact of sun and wind exposure and reduce the chance of animals sitting on the logger, while sampling the temperature adjacent to the upper half of the body of larger species (brushtail possums); logger position may have a minor effect on the recorded temperatures due to temperature gradients in nest-boxes (Goldingay 2015). We secured iButtons in each tree-hollow away from the entrance and near where a marsupial might rest; the exact position varied with tree-hollow structure. To record T_a, iButtons were placed in permanent shade behind each nest-box, facing away from adjacent surfaces.

2.3 STATISTICAL ANALYSIS

Occupied dens (3/70 den checks), and those deemed likely to be occupied during the non-monitoring period, indicated by atypical jumps in temperature (over 10°C), were examined separately. We also excluded records when iButtons were ejected from dens or faulty (seven during 29/06/2104 to 2/08/2014; one during 27/11/2014 to 3/01/2015).

Daily maximum (T_{denMAX}), mean ($T_{denMEAN}$) and minimum (T_{denMIN}) cavity temperatures, and maximum hourly difference between den temperature and T_a during daylight hours were calculated for each nest-box ($T_{boxDIFF}$) and tree-hollow ($T_{holDIFF}$). To examine the effect of den-type, weather and den characteristics on cavity temperatures across seasons, we fitted linear mixed-effects models with the package '*nlme*' (Pinheiro et al. 2015) using the software 'R' (R Core Team 2014). We included den-type within site as a random effect to account for repeated measures in each den and spatial correlations, and fit a corARMA correlation structure, assuming correlation across days for each den. For models with only categorical predictors, we fit a varIdent variance structure (Zuur et al. 2009). For models with continuous predictors, a variance structure was fitted based on Akaike's Information Criteria (AIC), as multiple structures were appropriate (Zuur et al. 2009). Where residuals plots indicated deviations from homoscedasticity or normality, variables were log or square-root transformed.

We constructed models for each response variable to determine the effect of den-type on cavity temperatures across seasons. We tested the effect of T_a and solar exposure (proportion canopy openness x total daily solar radiation over 24 hours, kWh m^2) on den temperatures (T_{denMAX} and $T_{denMEAN}$) in summer and winter. Because minimum temperatures predominantly occurred very early in the morning, and were thus more likely influenced by exposure to cold sky rather than solar radiation, we included canopy openness rather than solar exposure as a predictor for T_{denMIN}. We also analysed the relationship between den and site characteristics on both $T_{boxDIFF}$ and $T_{holDIFF}$ during summer and winter. Models included site openness (%), cardinal direction (aspect), den height, tree DBH, and hollow type (for $T_{holDIFF}$ only).

Continuous predictor variables were standardised (mean subtracted, then divided by the standard deviation) to allow each model to be fitted without the scale of predictors altering their influence on the results (Quinn & Keough 2002). We selected model predictors and interactions between predictors using AIC (Burnham & Anderson 2002), where the best-fitting model for each analysis had the lowest AICc. Full models only included interactions that were deemed biologically relevant; for example, interactions between DBH and aspect were excluded, as this was not considered meaningful.

2.4 MODELING ECO-PHYSIOLOGICAL CONSEQUENCES OF THERMAL MICROCLIMATES

We used the model of Porter & Kearney (2009) to calculate the metabolic rate that would allow an endotherm to maintain core temperature, given the environmental conditions (denning during the day in nest-boxes, tree-hollows, and outside under ambient conditions) and its traits for four marsupial species (see below). We assumed heat-loss was required when the predicted metabolic rate to maintain homeothermy was below the basal metabolic rate (i.e. we calculated how much additional heat must be lost to allow the animal to maintain its basal metabolic rate and core temperature). As panting is a key mechanism of heat-loss for marsupials (Robinson & Morrison 1957), respiratory heat-loss was only included in heat-loss estimates when animals were not actively offloading heat.

To parameterize the model, we estimated morphological characteristics of an average adult female common brushtail, mountain brushtail, greater glider and common ringtail, and the environmental conditions in each denning location (Table S1). Using museum specimens (*n*=9-21 for each species, Museum Victoria collection), we measured body length (mm) to estimate values for posture (ratio of body length:width) when denning, and ventral and dorsal fur depth using vernier calipers (to the nearest mm). We simulated behavioural responses to temperature by gradually altering posture and fur depth to minimize costs; posture changed from curled in a near-perfect sphere (1.001) with fur as the average of dorsal fur depth for cold conditions, to fully uncurled with the average of dorsal and ventral fur depth for high temperatures. For animals resting outside, we assumed a sheltered position, with the modeled wind-speed 50% of wind-speed recorded in the open. We calculated seasonal daytime heat-production and heat-loss costs (MJ) for each den and outside under ambient conditions for each species by summing estimated daily values across summer and winter.

3. **Results**

3.1 COMPARISON OF TEMPERATURES IN NEST-BOXES AND TREE-HOLLOWS

For dens unoccupied by an animal, T_{denMAX} in nest-boxes was 8.0°C higher on average than tree-hollows in summer, 3.0°C higher in winter, and showed greater variation (Figure 1; Table S2). The highest nest-box temperature recorded was 52.1°C in summer and 41.1°C in winter, whereas tree-hollows reached 38.1°C in summer and 20.7°C in winter. $T_{denMEAN}$ was also higher in nest-boxes than tree-hollows: 3.6°C higher in summer and 1.2°C in winter (Figure 1; Table S2). Conversely, across both seasons, daytime T_{denMIN} remained higher in tree-hollows (lowest -3.5°C) than nest-boxes (lowest -5.4°C) (Figure 1; Table S2).

The limited data we were able to collect suggested that occupied dens were warmer than unoccupied dens. During summer, one tree-hollow containing cockatoo chicks for approximately 29 days, was substantially warmer ($T_{denMEAN}$ 27.2°C) when occupied than when unoccupied (18.1°C) under similar ambient conditions. A common brushtail occupied one tree-hollow on two separate days. Under similar ambient conditions, $T_{denMEAN}$ on an occupied day (22.5°C) was considerably warmer than on the previous and following days (18.5°C), when the den was unoccupied. A sugar glider occupying a different tree-hollow had a negligible effect on den temperature. No nest-box use was recorded during the study.

3.2 EFFECT OF WEATHER AND HABITAT CHARACTERISTICS ON DEN TEMPERATURES

The effects of T_a and solar exposure were dependent on den-type: nest-box temperatures responded more strongly to changing environmental conditions than tree-hollows (Figure 2; Table 1). During both seasons, T_{denMAX}, $T_{denMEAN}$ and T_{denMIN} increased substantially more in nest-boxes as T_a increased compared to tree-hollows (Figure 2a, c; Table 1), leading to greater disparity in temperature between den-types at higher T_a. For example, based on the fitted models, during an average summer day (T_a 28°C, daily solar exposure 1.5 kWh m^2), nest-boxes were predicted to be 9°C warmer than tree-hollows (31.4°C versus 22.3°C respectively). Under extreme recorded weather conditions (40°C, 4.1 kWh m^2), the predicted difference between den-types rose to 19.6°C (45.7°C versus 26.1°C). T_{denMAX} and $T_{denMEAN}$ in nest-boxes also increased as exposure to solar radiation increased, but decreased slightly in tree-hollows (Figure 2b, d; Table 1). During summer, T_{denMIN} increased slightly more with increasing T_a at sites with higher canopy openness (Table 1). For $T_{holDIFF}$ and $T_{boxDIFF}$, canopy openness, den aspect, den height, tree DBH and hollow type (for $T_{holDIFF}$ only) had little impact on den temperatures as the null models had the best fit (Table 1).

3.3 PREDICTED ECO-PHYSIOLOGICAL CONSEQUENCES OF DEN TEMPERATURES

Estimates of TNZs from the biophysical model for the four arboreal marsupials were similar to those previously observed (see Table S1). This suggests that the model captures key heat exchange processes for these species.

Den-type (nest-box, tree-hollow, none/outside) had a strong effect on estimated heat-loss required for all species (Table 2; Table S3). During summer, total heat-loss required in nest-boxes was approximately double that required in tree-hollows (e.g. 2.4 and 1.5 times higher for common brushtails and common ringtails, respectively), and 1.3-2.3 times higher compared to resting outside for all species (Table 2; Table S3). During summer, the predicted average daytime heat-loss (calculated as the % basal metabolic heat-production required to be lost via evaporative cooling) for mountain brushtails was higher in nest-boxes (29.5%) than in tree-hollows (10.5%), with mean hourly rates of heat-loss required in nest-boxes up to 5.4 times that required in tree-hollows.

Heat-loss requirements were higher for larger species, with higher predicted heat-loss required for mountain brushtails than common ringtails when resting in nest-boxes during summer (29.5% and 10.3% basal metabolic rate, respectively, Table 2). During winter, predicted heat-loss required was typically slightly lower in nest-boxes, and similar between tree-hollows or resting outside (Table 2; Table S3).

The total predicted daytime heat-production (MJ) required during winter for animals in tree-hollows was 1.04-1.05 times that in nest-boxes, but marginally higher in nest-boxes during summer for most species (Table 2; Table S3). Predicted daytime heat-production (% basal) for common ringtails in winter was lower in nest-boxes (187.7%) than tree-hollows (197.0%), with the highest costs predicted for an animal resting outside (203.4%).

4. **Discussion**

Nest-boxes support a range of hollow-dependent wildlife, particularly birds (Catry et al. 2011) and mammals (Durant et al. 2009), across a wide range of ecosystems globally (Harper et al. 2005; Isaac et al. 2008b; Charter et al. 2010). Despite the widespread use of nest-boxes, their thermal value has received little attention. Our study demonstrates that thermal properties of nest-boxes and tree-hollows differ substantially. We found that nest-boxes can experience highly fluctuating temperatures that are likely to pose risks for wildlife during very hot weather. Our modeling showed that eco-physiological costs of thermoregulation are likely to be considerably higher for arboreal marsupials in nest-boxes compared to tree-hollows during summer, but marginally lower during winter. These findings highlight the importance of retaining tree-hollows as thermal refugia. Although nest-boxes clearly provide valuable habitat for arboreal marsupials under many environmental conditions, we found that during hot weather they provide inadequate protection from extreme daytime temperatures, a critical issue with the predicted increase in heat-waves with climate change (Coumou & Rahmstorf 2012).

4.1. NEST-BOX AND TREE-HOLLOW THERMAL PROFILES

In our study, nest-boxes reached greater daytime temperature extremes than tree-hollows, consistent with the few previous studies examining natural versus artificial den temperatures (McComb & Noble 1981; Isaac et al. 2008b). We found that nest-box microclimates responded more strongly to changes in ambient temperature and solar radiation than tree-hollows, which may largely be attributed to differences in physical structure. Tree-hollows large enough for the possums at our site typically occurred in trees over 1m DBH (Martin 2005). While this can vary among tree species (Wormington & Lamb 1999; Gibbons et al. 2000), wood surrounding tree-hollows will generally be much thicker than nest-box walls. Tree-hollows with thicker walls have greater heat-retaining capacity (Coombs et al. 2010), and slower rates of heat-gain from the external environment due to low thermal inertia (Derby & Gates 1966). Our results are consistent with this: nest-box cavities heated and cooled faster than tree-hollows, indicating that nest-boxes have lower insulative capacity. However, our results are based on empty dens, and the thermal properties of occupied dens may show some differences. Dens provide wildlife with protection from daily temperature fluctuations and extremes (Cooper 1999). Our study indicates that nest-boxes of one commonly used design may have limited capacity to perform this critical function under extreme conditions, with wildlife being exposed to substantially hotter daytime temperatures in nest-boxes than in tree-hollows, even in temperate environments. Retaining large trees and reforestation are therefore vital in regions experiencing large daily and seasonal temperature fluctuations and high average temperatures, because nest-boxes of commonly used designs are unlikely to provide suitable thermal microclimates throughout the whole year.

Along with ambient temperature, solar radiation influenced daytime den temperatures, particularly in nest-boxes. Higher solar exposure (calculated from daily solar radiation and canopy openness) increased daytime temperatures in nest-boxes. Conversely, temperatures in tree-hollows decreased when solar exposure was high, possibly due to higher transpiration rates (water-loss) as radiation heated the leaves (Gates 1964; Mehajan et al. 2008), enhancing water flow through the trunk to heighten cooling (Vines 1968). However, further research is required, as factors governing tree-trunk temperatures, and thus tree-hollow temperatures, are not well understood. We also analysed several physical traits typically used when examining den temperature profiles (e.g. Isaac et al. 2008a), however none showed strong relationships with den temperatures. A few studies have found that orientation affects nest-box temperatures in fields (Adria et al. 2006; Butler et al. 2009), however, in our study, orientation had minimal effect, consistent with Stamp et al. (2002) who also worked in forests. Den height also had no effect on cavity temperatures, possibly because canopy openness (thus solar exposure) did not differ with height above the ground across the range measured here. In contrast with Isaac et al. (2008a) who found that maximum daytime temperatures were lower in hollow-bearing trees with larger DBH, in our study, which focused on comparing nest-boxes with the natural range of tree-hollows available, DBH had minimal impact on den temperatures. Collectively, our results suggest that canopy openness may be more influential than orientation in regulating exposure to solar radiation in forested environments, particularly for nest-boxes. In regions prone to high ambient temperatures, nest-boxes should be installed in sites with high canopy cover to reduce excessive heating from solar exposure.

4.2 MODELED ECO-PHYSIOLOGICAL CONSEQUENCES FOR ARBOREAL MARSUPIALS

Our study revealed seasonal differences in the value of natural and artificial dens for arboreal marsupials due to variation in thermal microclimates. Nest-box use was predicted to substantially increase heat-loss requirements in summer, and therefore the potential for heat-stress and dehydration, but slightly reduce energy requirements in winter. Marsupials predominantly rely on evaporative heat-loss to maintain homeothermy at temperatures above their TNZ (Robinson & Morrison 1957). Water-loss rates can increase substantially at high ambient temperatures (Dawson 1969), and evaporative heat-loss may not be sufficient to maintain homeothermy. In addition, free-water can be limited during the hot, dry conditions typical of temperate Australian summers. Under such conditions, dehydration and heat-stress are more likely for animals in nest-boxes than in tree-hollows, where evaporative heat-loss is predicted to be 1.5-2.4 times higher. Estimates of heat-loss required in our study were based on temperatures in empty dens, however the expected added thermal impact of occupation by an endotherm (Kearney et al. 2011; J. Rowland, unpublished data) would inflate eco-physiological costs over summer. Under high ambient temperatures, animals may avoid nest-boxes reaching high temperatures (Goldingay 2015) and trade-off predator protection to avoiding acute heat-stress if suitable shelter is not available (Havera 1979). Alternatively, wildlife using thermally unsuitable nest-boxes may experience reduced growth and body condition, and high mortality rates, particularly juveniles (Catry et al. 2011). Temperature-related mortality and reduced fitness are important to address in the future because conservation-management programs using nest-boxes often target endangered species (e.g. Leadbeater’s possum: Lindenmayer et al. 2009). Nest-boxes can contribute to species conservation, but may also contribute further to population declines during rare, but increasingly frequent, catastrophic heat events, which can cause substantial mortality (Catry et al. 2011). Investing in improved nest-box designs to buffer extreme temperatures is of high-priority to ensure nest-boxes are of maximum value for wildlife.

During winter, the predicted heat-production costs required for thermoregulation were slightly lower for marsupials in nest-boxes compared to tree-hollows, and highest when resting outside. This is because nest-boxes were typically warmer than tree-hollows during the day, thus animals occupying tree-hollows spent more time exposed to temperatures below their TNZ (98.7-100% versus 90.1-99.8%, respective). Further, animals resting outside are subjected to wind, increasing convective heat-loss, and thus energy costs required to maintain homeothermy (Dawson & Brown 1970). Cooler dens are probably most challenging for smaller mammals and juveniles that are more susceptible to hypothermia due to their increased thermal conductance and/or poor thermoregulatory capabilities (Aschoff 1981; Holloway & Geiser 2000; Porter & Kearney 2009). Higher cumulative energy costs from resting in colder microclimates may also cause progressive decline in body condition when food availability is low (Speakman 1997) or foraging restricted (e.g. during rain: van den Oord et al. 1995). In our study, heat-production costs were calculated for unoccupied dens, thus costs in occupied dens are likely lower than predicted here. Energy costs may be further reduced in occupied well-insulated tree-hollows where more heat is likely to be retained, or if multiple animals den together (e.g. mountain brushtails: Martin 2005; sugar gliders: Durant et al. 2009). A systematic study is required to fully understand all factors driving thermal differences in tree-hollow temperatures.

The relative value of nest-boxes and tree-hollows for particular species' depends on their physiology, morphology and behaviour. In general, larger mammals with thicker fur are more sensitive to high temperatures than smaller species with thinner fur (Robinson & Morrison 1957; Phillips & Heath 1995; Blanckenhorn 2000), with the converse true in cold environments. This is evident in the predicted relative costs (i.e. % basal heat-production or heat-loss required) for mountain brushtails (large, thick fur) compared to common ringtails (smaller, thinner fur) (see Table 2). However, larger animals have higher energy and water reserves, increasing their ability to withstand high physiological costs over short periods (McKechnie & Wolf 2010). Den use patterns also influences the relative value of nest-boxes. While we focused on arboreal marsupials exhibiting diurnal den use, our finding that nest-boxes had lower minimum temperatures than tree-hollows (mean nighttime temperature 8.46°C and 10.96°C respectively) and more closely tracked ambient conditions, suggests that nest-boxes may provide lower thermal quality habitat than tree-hollows for temperate species denning nocturnally, including many birds species (Goldingay & Stevens 2009).

4.3 CONSERVATION IMPLICATIONS FOR HOLLOW-DEPENDENT SPECIES

Our biophysical modeling provided a useful method for estimating the relative costs of denning in nest-boxes versus tree-hollows for endotherms. We showed that nest-boxes and tree-hollows vary in their temperature regimes and thermal value for several arboreal hollow-dependent marsupials across seasons, with the largest disparities occurring when thermoregulatory costs are highest. Our study provides new information about fitness consequences of differing thermal microclimates of natural versus artificial dens. Our results indicate that tree-hollows generally provide better microclimates for endotherms, however nest-boxes are still valuable during milder environmental conditions. While we only assessed one nest-box design, our findings are broadly applicable to nest-boxes used globally for various species. However more research is needed on the thermal properties of artificial hollows, including nest-boxes with different dimensions that target different species (Beyer & Goldingay 2006), variation in construction materials (e.g. timber vs. clay pots, Catry et al. 2011), and those designed to more closely mimic tree-hollow properties (e.g. chainsaw cavities, Hurley & Harris 2014).

The persistence of hollow-dependent species worldwide, under both current and future climates, requires long-term conservation-management that prioritises retaining large, hollow-bearing trees and habitat regeneration. However the increasing global deficiency of tree-hollows

(Gibbons et al. 2008; Vesk et al. 2008) means that nest-boxes will be increasingly important, thus we must ensure these are of the highest-value for wildlife. Clear guidelines for nest-box design and deployment based on scientific evidence of their suitability for target species and prevailing environmental conditions, are critical to inform conservation decisions. We recommend that in environments prone to high temperatures, nest-boxes should be installed in shaded sites to limit high cavity temperatures and adverse impacts on inhabitants. Future efforts should be directed at improving nest-box design and deployment to improve their quality as habitat, especially to buffer against large temperature fluctuations. Actions should include altering surface thermal reflectance (S.R. Griffiths, J.A. Rowland, unpublished data) and increasing the insulative value of nest-boxes, along with installing nest-boxes with differing thermal properties to enable animals to select thermally suitable dens under different environmental conditions.

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Table 1. Parameter estimates for models of effect of den-type and ambient conditions on maximum (T_{denMAX}), mean ($T_{denMEAN}$) and minimum (T_{denMIN}) daytime den temperature, and den and site characteristics on maximum daytime temperature difference ($T_{holDIFF}$ and $T_{boxDIFF}$). Variables included in models for T_{denMAX}, $T_{denMEAN}$ and T_{denMIN} were: Den-type (nest-box, tree-hollow), ambient temperature, and solar exposure or canopy openness above each den. Variables included in full models for $T_{holDIFF}$ and $T_{boxDIFF}$ included: canopy openness, den height, aspect, tree DBH, and hollow type (for $T_{holDIFF}$ only). Coefficients (95% CI) for best fitting models and interactions are presented (lowest AIC_c). Response variables for $T_{boxDIFF}$ and T_{denMAX} were log transformed to meet model assumptions.

Response variable	Predictor variables	**Summer**	**Winter**
T_{denMAX}	(Intercept)	3.39 (3.36, 3.41)	2.35 (2.31, 2.38)
	Den-type (Hollow)	-0.32 (-0.35, -0.28)	-0.30 (-0.35, -0.26)
	T_{aMAX}	0.14 (0.14, 0.15)	0.27 (0.27, 0.28)
	Solar exposure	0.03 (0.03, 0.04)	0.10 (0.09, 0.11)
	Den-type (Hollow): T_{aMAX}	-0.05 (-0.05, -0.04)	-010 (-0.11, -0.09)
	Den-type (Hollow): Solar exposure	-0.05 (-0.05, -0.04)	-0.18 (-0.19, -0.17)
$T_{denMEAN}$	(Intercept)	23.29 (22.98, 23.6)	8.2 (7.9, 8.4)
	Den-type (Hollow)	-3.82 (-4.26, -3.38)	-1.0 (-1.36, -0.69)
	T_{aMEAN}	3.43 (3.39, 3.46)	1.89 (1.84, 1.94)
	Solar exposure	0.86 (0.81, 0.91)	1.15 (1.10, 1.21)
	Den-type (Hollow): T_{aMEAN}	-1.38 (-1.44, -1.32)	-0.54 (-0.61, -0.48)
	Den-type (Hollow): Solar exposure	-1.28 (-1.37, -1.20)	-1.08 (-1.14, -1.01)
T_{denMIN}	(Intercept)	13.84 (13.54, 14.13)	4.95 (4.73, 5.16)
	Den-type (Hollow)	2.54 (2.12, 2.96)	1.00 (0.71, 1.30)
	T_{aMIN}	3.85 (3.79, 3.90)	2.39 (2.32, 2.46)
	Canopy openness	0.19 (-0.02, 0.40)	-
	Den-type (Hollow): T_{aMIN}	-1.26 (-1.45, -1.28)	-0.79 (-0.88, -0.70)
	T_{aMIN}: Canopy openness	0.12 (0.08, 0.16)	
$T_{holDIFF}$	(Intercept)	2.93 (2.53, 3.32)[a]	1.34 (1.13, 1.54)[b]
$T_{boxDIFF}$	(Intercept)	1.45 (1.33, 1.57)[c]	0.98 (0.91, 1.05)

[a] Model $AIC_c < 2$ lower than competing models with either DBH or hollow type

[b] Model $AIC_c < 2$ lower than competing models with either den height or hollow type

[c] Model $AIC_c < 2$ lower than competing models with openness

Table 2. Total predicted daytime heat-loss and heat-production (mean + 95% confidence intervals, MJ) for arboreal marsupials denning in nest-boxes, tree-hollows, or outside under shaded ambient conditions in the Strathbogie Ranges, Victoria, during summer (n = 37 sites; 53 days) and winter (n = 32; 49 days. See Table S3 for statistical analyses.

	Summer			Winter		
Species	**Heat-loss (MJ)**					
	Nest-box	Tree-hollow	Ambient	Nest-box	Tree-hollow	Ambient
Common brushtail	3.53 (3.33, 3.74)	1.50 (1.43, 1.57)	2.58 (2.46, 2.69)	0.98 (0.97, 0.99)	1.04 (1.03, 1.05)	1.04 (1.04, 1.05)
Mountain brushtail	5.54 (5.30, 5.79)	2.65 (2.50, 2.81)	4.27 (4.12, 4.43)	1.24 (1.23, 1.26)	1.30 (1.29, 1.31)	1.30 (1.29, 1.31)
Greater glider	1.94 (1.82, 2.07)	0.85 (0.82, 0.88)	1.40 (1.34, 1.47)	0.62 (0.61, 0.62)	0.66 (0.65, 0.66)	0.65 (0.65, 0.66)
Common ringtail	1.25 (1.17, 1.34)	0.85 (0.83, 0.86)	1.00 (0.97, 1.03)	0.84 (0.83, 0.85)	0.90 (0.89, 0.914)	0.92 (0.908, 0.93)
	Heat-production (MJ)					
	Nest-box	Tree-hollow	Ambient	Nest-box	Tree-hollow	Ambient
Common brushtail	25.04 (25.02, 25.07)	24.93 (24.90, 24.95)	25.27 (25.24, 25.31)	19.17 (19.03, 19.31)	20.08 (19.94, 20.22)	20.37 (20.26, 20.48)
Mountain brushtail	34.18 (34.16, 34.19)	34.16 (34.15, 34.18)	34.28 (34.26, 34.29)	22.69 (22.57, 22.81)	23.51 (23.35, 23.66)	23.75 (23.64, 23.87)
Greater glider	16.52 (16.50, 16.53)	16.37 (16.35, 16.40)	16.62 (16.60, 16.64)	12.69 (12.59, 12.79)	13.32 (13.23, 13.42)	13.40 (13.32, 13.48)
Common ringtail	15.24 (15.16, 15.31)	15.94 (15.72, 16.17)	16.22 (16.11, 16.32)	15.40 (15.27, 15.54)	16.16 (16.05, 16.27)	16.69 (16.60, 16.78)

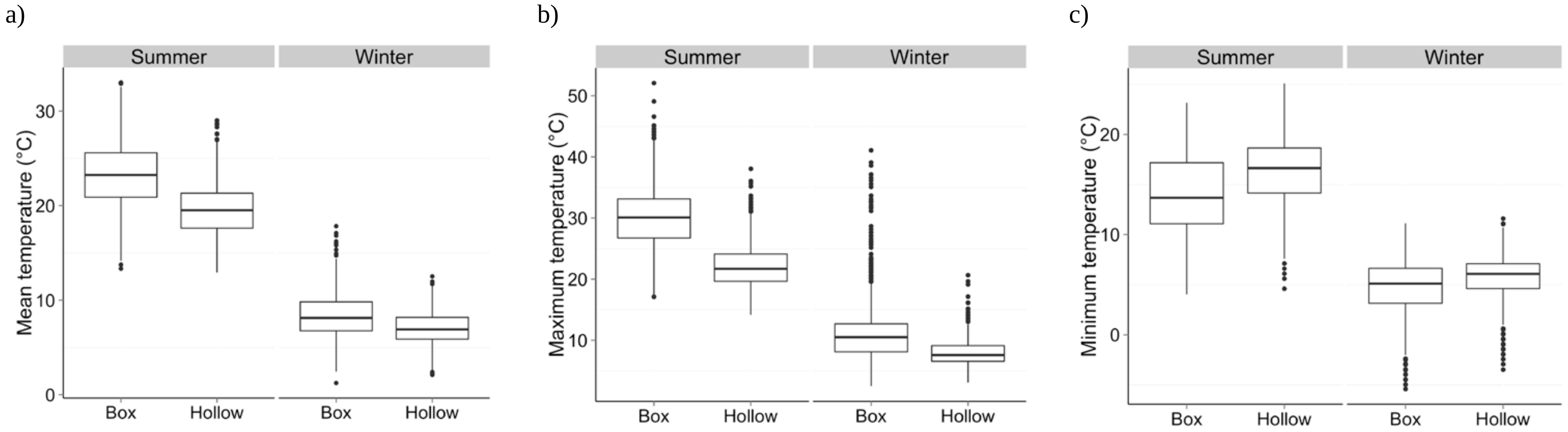

Figure 1. Daily daytime a) mean, b) maximum, and c) minimum temperature within nest-boxes and tree-hollows during summer (n = 65 days) and winter (n = 60 days) sampling periods in the Strathbogie Ranges, Victoria. Nest-boxes had significantly higher T_{denMAX} and $T_{denMEAN}$, and significantly lower T_{denMIN} than tree-hollows during both summer and winter. See Table S2 for statistical analyses.

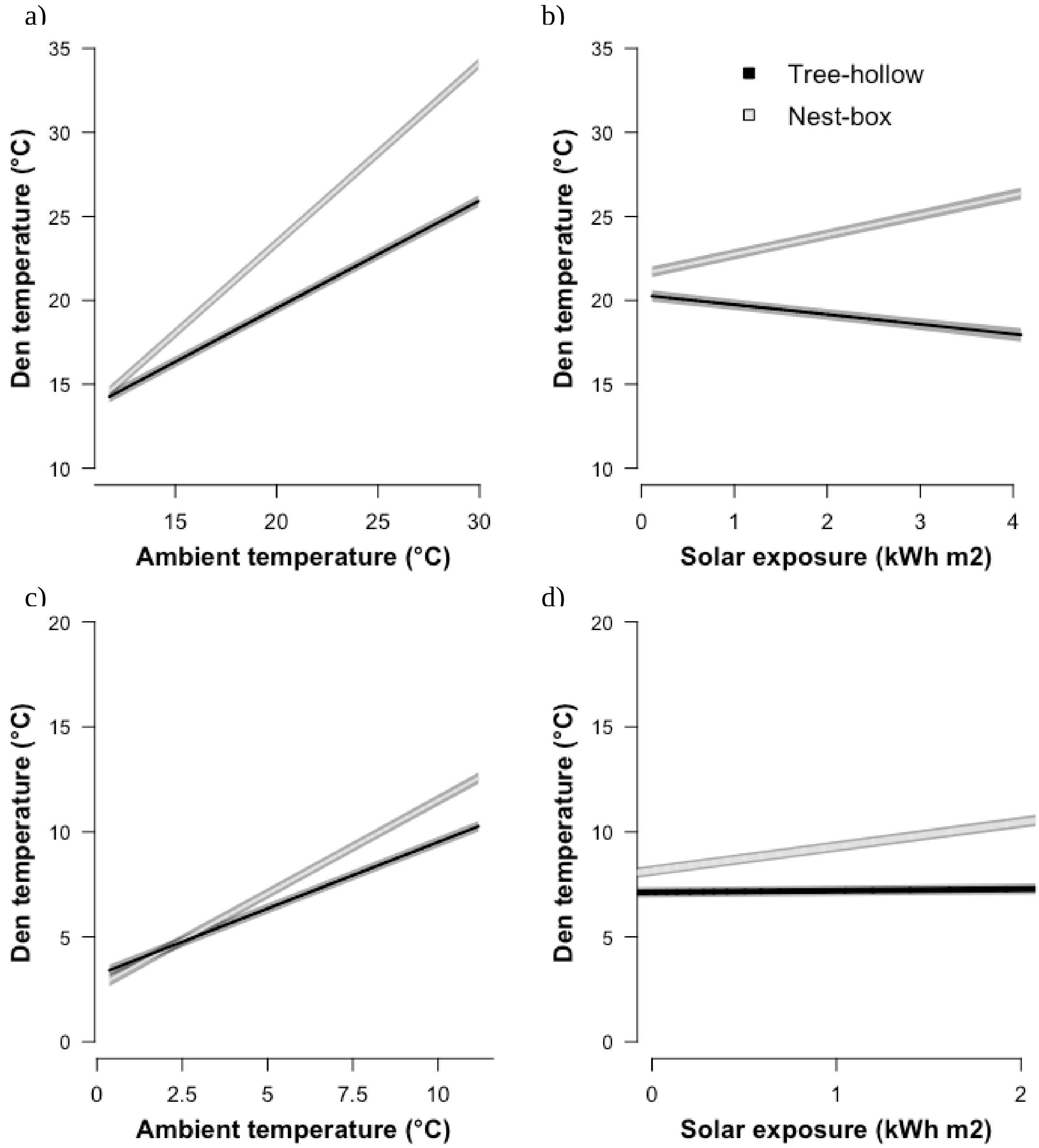

Figure 2. The modeled fixed-effects of ambient temperature (a,c) and solar exposure (b,d) on mean daytime temperatures of tree-hollows and nest-boxes during summer (a, b) and winter (c, d) in the Strathbogie Ranges, Victoria, (holding other variables at the mean value). Grey bars represent 95% confidence intervals. See Table 1 for statistical analyses.

Supplementary material

Table S1 Species trait values and climate data used to model eco-physiological costs of den microclimates on Australian arboreal marsupials, and estimated thermoneutral zones (TNZ) for each species. Measurements of fur depth and body dimensions were taken from female specimens of common brushtail possum (*Trichosurus vulpecula*; n = 21), mountain brushtail possum (*Trichosurus cunninghami*; n = 9), common ringtail possum (*Pseudocheirus peregrinus*; n = 17), and greater glider (*Petauroides volans*; n = 17) held at Museum Victoria.

Variables	**Species parameter estimates**			
	Common brushtail possum	Mountain brushtail possum	Common ringtail possum	Greater glider
Body mass (kg)	Summer: 2.2 Winter: 2.3[a]	3.4[b]	Summer: 0.9 Winter: 1.0[c]	1.25[d]
Posture (length:width)[e]	1.001 – 4	1.001 – 3.5	1.001 – 4	1.001 – 5.5
Fur thermal conductivity, W/m°C[f]	0.04	0.04	0.04	0.04
Fur depth (mm)	17-23	21-28	11-15	18-30
Core temperature (°C)	36.2[g]	37.3[h]	37.4[c]	35.4-39.1[i]

Climate variables

Wind-speed (m/s)	0.01 in nest-box and hollow; otherwise assumed 50% of wind-speed measured in the open at 9 am[j]
Humidity (%)	Relative humidity at 9 am[j]

Thermoneutral zones

	Common brushtail possum	Mountain brushtail possum	Common ringtail possum	Greater glider
Model-estimated average	16.8°C – 24.5°C	14.6°C – 23.7°C	17.2°C – 24.5°C	22.8°C – 28.6°C
Previously observed	15°C – 25°C[k,l]		20°C[i]	20°C – 30°C[m]

[a] Clinchy *et al.* 2004, [b] Martin 2005, [c] Munks & Green 1995, [d] Tyndale-Biscoe & Smith 1969, [e] Posture changed to simulate behavioural responses to changes in temperature, from curled to uncurled posture, [f] Default mammal value, see Porter & Kearney 2009, [g] Dawson & Hulbert 1970, [h] Gemmell & Cepon 1993, [i] Rubsamen *et al.* 1984, [j] Bureau of Meteorology Strathbogie North Station 082043, [k] Dawson 1969, [l] van den Oord et al. 1995, [m] Munks 1990.

Table S2. Parameter estimates for linear mixed models of effect of Den-type (nest-box, tree-hollow) and Season (summer, winter) on maximum, mean and minimum daytime den temperature. Best fitting model presented (lowest AIC_c value). In all cases, competing models had ΔAIC values greater than 2.

Response variables	Predictor variables	Coefficients (95% CI)
T_{denMAX}	Intercept	30.2 (29.6, 30.8)
	Den-type (Hollow)	-8.0 (-8.7, -7.4)
	Season (Winter)	-19.3 (-19.8, -18.8)
	Den-type (Hollow): Season (Winter)	5.1 (4.5, 5.6)
T_{denMEAN}	Intercept	23.2 (22.9, 23.4)
	Den-type (Hollow)	-3.6 (-3.9, -3.3)
	Season (Winter)	-15.0 (-15.3, -14.7)
	Den-type (Hollow): Season (Winter)	2.4 (2.0, 2.8)
T_{denMIN}	Intercept	14.0 (13.6, 14.3)
	Den-type (Hollow)	2.3 (2.0, 2.7)
	Season (Winter)	-9.0 (-9.3, -8.7)
	Den-type (Hollow): Season (Winter)	-1.4 (-1.8, -1.0)

Table S3. Effect of den-type on total predicted daytime heat-loss and heat-production (MJ) for arboreal marsupials denning in nest-boxes, tree-hollows or under ambient conditions (outside) in the Strathbogie Ranges, Victoria, during summer (n = 37 sites; 53 days) and winter (n = 32; 49 days). Data for summer heat-loss were square-root transformed to improve normality. Values are coefficient (95% CI), with bold-type indicating where 95% CI do not overlap zero.

		Heat-loss (MJ)		**Heat-production (MJ)**	
Species	Comparison	Summer	Winter	Summer	Winter
Common brushtail possum (*Trichosurus vulpecula*)	Intercept	**0.05** (0.049, 0.051)	**1.04** (1.03, 1.05)	**25.27** (25.24, 25.30)	**20.37** (20.24, 20.51)
	Den-type (Nest-box)	**0.008** (0.007, 0.01)	**-0.06** (-0.08, -0.05)	**-0.23** (-0.27, -0.19)	**-1.20** (-1.39, -1.01)
	Den-type (Hollow)	**-0.012** (-0.014, -0.01)	-0.005 (-0.02, 0.01)	**-0.35** (-0.39, -0.31)	**-0.29** (-0.48, -0.10)
Mountain brushtail possum (*Trichosurus cunninghami*)	Intercept	**0.065** (0.063, 0.067)	**1.30** (1.29, 1.32)	**34.28** (34.26, 34.29)	**23.75** (23.62, 23.88)
	Den-type (Nest-box)	**0.009** (0.007, 0.01)	**-0.06** (-0.08, -0.04)	**-0.10** (-0.12, -0.08)	**-1.06** (-1.25, -0.88)
	Den-type (Hollow)	**-0.014** (-0.016, -0.012)	-0.002 (-0.02, 0.02)	**-0.11** (-0.13, -0.09)	**-0.25** (-0.43, -0.06)
Greater glider (*Petauroides volans*)	Intercept	**0.037** (0.036, 0.038)	**0.65** (0.65, 0.66)	**16.62** (16.60, 16.64)	**13.40** (13.31, 13.49)
	Den-type (Nest-box)	**0.007** (0.005, 0.008)	**-0.04** (-0.05, -0.03)	**-0.10** (-0.14, -0.07)	**-0.71** (-0.84, -0.58)
	Den-type (Hollow)	**-0.008** (-0.001, -0.007)	0.003 (-0.01, 0.01)	**-0.25** (-0.28, -0.21)	-0.08 (-0.21, 0.05)
Common ringtail possum (*Pseudocheirus peregrinus*)	Intercept	**0.032** (0.03, 0.032)	0.92 (0.91, 0.93)	**16.22** (16.06, 16.37)	**16.69** (16.58, 16.80)
	Den-type (Nest-box)	**0.004** (0.003, 0.005)	**-0.08** (-0.09, -0.07)	**-0.98** (-1.19, -0.76)	**-1.29** (-1.45, -1.13)
	Den-type (Hollow)	**-0.003** (-0.004, -0.001)	**-0.02** (-0.03, -0.01)	**-0.27** (-0.49, -0.06)	**-0.53** (-0.69, -0.37)

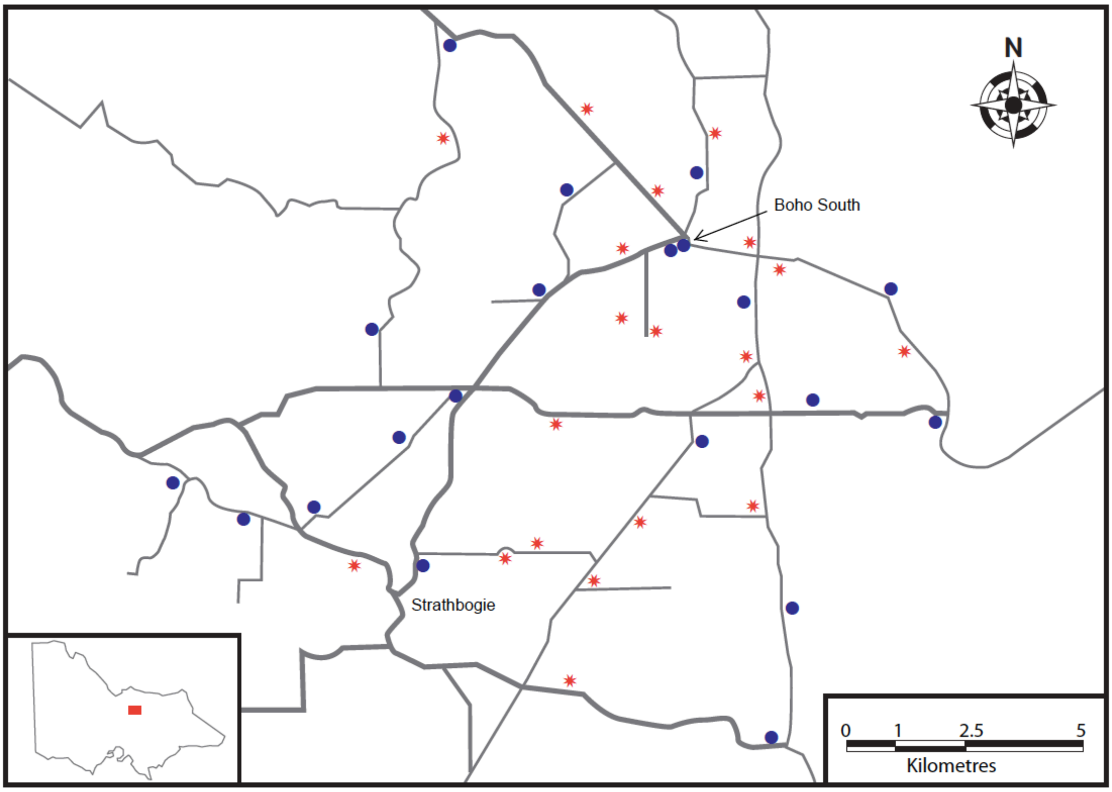

Figure S1. Location of study sites in the Strathbogie Ranges, Victoria, Australia, symbols indicate location of paired nest-boxes and tree-hollows. Tree-hollows either had the entrance located in the trunk (*) or base (•) of the tree. Insert shows Victoria with location of the study site shaded in red.

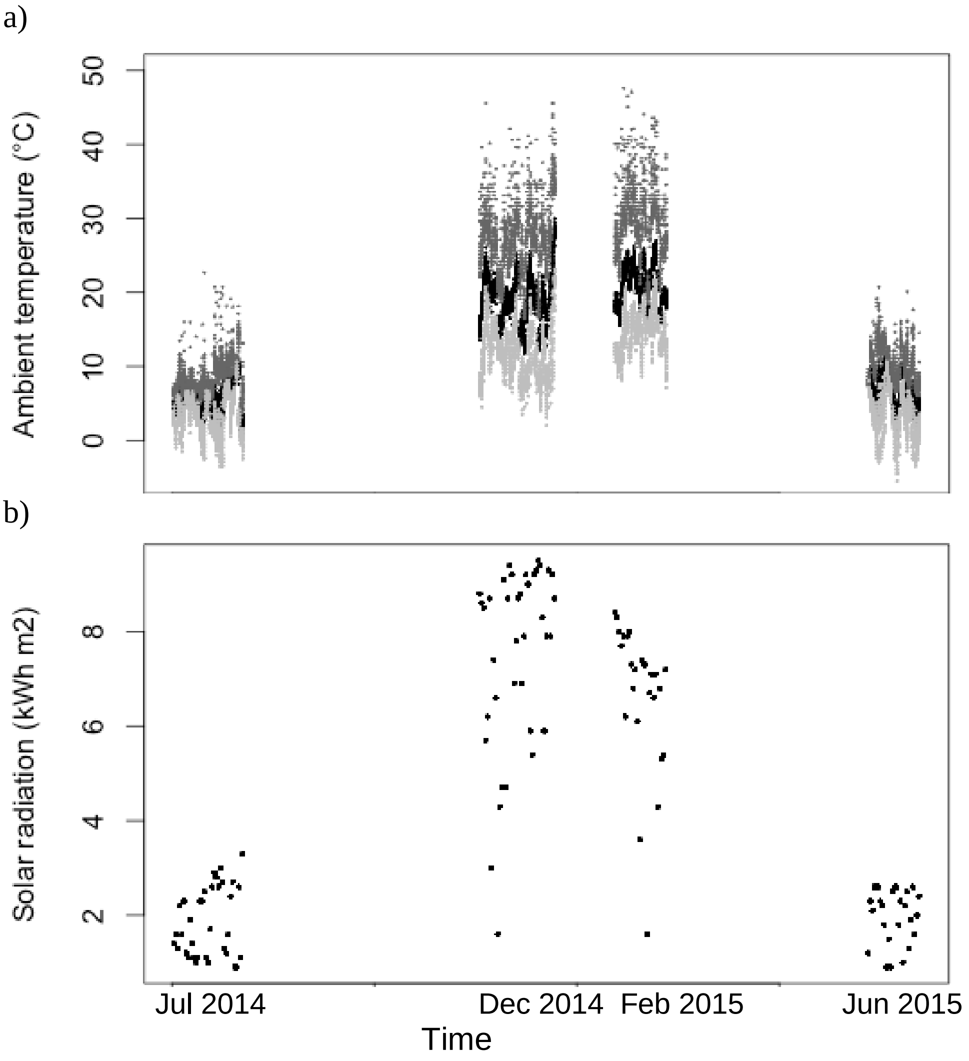

Figure S2. a) Daily maximum (medium-grey), mean (black), and minimum (light-grey) ambient temperature (°C), and b) total daily solar radiation (kWh/m^2) over 24 hours in the Strathbogie Ranges, Victoria, during the sampling periods.

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