

Article

# Variation in Summer and Winter Microclimate in Multi-Chambered Bat Boxes in Eastern Australia: Potential Eco-Physiological Implications for Bats

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**Abstract:** Bat boxes are commonly used as a conservation tool. Detailed knowledge on the influence of box elements on microclimate is lacking, despite eco-physiological implications for bats. Summer and winter box temperature and relative humidity patterns were studied in narrow multi-chambered plywood and wood-cement boxes in eastern Australia. Box exteriors were black or white and plywood boxes comprised vents. Relative humidity was higher in white boxes than black boxes and box colour, construction material, chamber sequence and vents influenced temperatures. Maximum box temperature differences between designs varied by up to 9.0 °C in summer and 8.5 °C in winter. The black plywood box consistently recorded the warmest temperatures. This design comprised a temperature gradient between chambers and within the front chamber (influenced by vent). During the 32-day summer sampling period, the front chamber rarely recorded temperatures over 40.0 °C (postulated upper thermal tolerance limit of bats), while the third and fourth chamber never reached this threshold. At the study site, the tested black boxes are considered most thermally suitable for bats during average summer conditions. However, during temperature extremes black boxes likely become too hot. Wood-cement, a durable material not previously tested in Australia should be considered as an alternative construction material.

**Keywords:** artificial hollow; box humidity; box temperature; roost box; tree cavity-roosting bat

## 1. Introduction

Microhabitat selection can be critical for small-bodied animals [1,2]. One factor that influences microhabitat suitability is microclimate. Temperature affects an animal's energy budget, particularly when they occur outside the animal's thermal neutral zone (TNZ), that is, the zone in which an animal's metabolic rate can be maintained passively [3,4]. In order to minimise metabolic costs, small heterothermic mammals can combat temperature induced metabolic costs through two mechanisms: behavioural adaptation such as seeking microclimates that are favourable, or, using physiological adaptation such as torpor [5–10].

One animal group where microclimate is central to their life cycle is echolocating bats, with many species using tree cavities for roosting [11]. Few studies have been conducted on microclimate roost selection by heterothermic tree-cavity roosting bats. There is some evidence that roost temperature preferences change among seasons and between sexes. For example, studies reported the selection of thermally unstable roost sites and roosts that allowed for passive rewarming [12,13]. In contrast, during the maternity season, energy requirements of females are likely to be high due to milk production, so roost temperatures close to or within the TNZ may be favoured to maintain normothermia and facilitate the growth of young [14–16]. Studies on artificial roosts using bat boxes in the Northern Hemisphere have documented that reproductive female bats, particularly lactating females, selected warm boxes [17–19], although more recent studies documented the use of torpor by pregnant and lactating females and use of thermally labile roosts [10,20,21].

Bat boxes are frequently used for conservation and research purposes [22,23]. However, their use has outpaced the understanding of suitable designs and factors influencing box uptake [22–24]. One factor that is poorly described is the influence of the thermal profile within a box, despite the likely importance of microclimates for tree-cavity roosting bats. Some Northern Hemisphere studies have investigated bat box temperatures [17,19,25–27] but few data are available on bat box thermal profiles in Australia [28–30]. In addition, studies that compared nest boxes to tree hollows showed that boxes differed significantly in temperatures and raised concerns about the suitability of box temperatures, particularly during summer [31,32].

Knowledge of the influence of box design elements on temperature is crucial to provide suitable artificial roosts and to allow desired box microclimates to be attained, such as for maternity roost boxes. This roost type is considered particularly important to support viable local populations, yet records of maternity roosts in boxes are scarce, restricted to only a few species [22]. Box design elements and box placements that may influence box thermal profiles include: multiple large chambers, vents, box size, exterior colour, type of box construction material, thickness of exterior walls, box aspect and extent of shading [17–19,27,30,33,34]. Another potentially important aspect of box microclimate is humidity. Little is known about the significance of humidity in roosts for tree-cavity roosting bats but humidity has been reported to influence evaporative water loss in bats [35]. In addition, humidity has been identified to differ vastly between tree hollows and timber nest boxes [31].

This study compared temperature and relative humidity (RH) of two bat box types during warm (summer) and cool (winter) periods. One type was a multi-chambered plywood box adapted from a North American design [34]. This type contained four fissure-type chambers and vents in an attempt to provide a wide temperature gradient within the box. The other box type was a multi-chambered box with a wood-cement shell. Wood-cement boxes may last several decades [36,37] and are commonly used in Europe [22]. This material has not been widely trialled elsewhere, however, the longevity of these boxes makes wood-cement an attractive option where bat boxes are installed as a long-term conservation measure. The objectives of the study were to investigate the influence of box elements (exterior colour, box construction material, multiple chambers and vents) on box microclimates and to discuss the potential eco-physiological implications of these box elements for bats. Black boxes were hypothesised to provide warmer temperatures to that of white boxes [18,19] and multiple-chambers and vents were expected to influence temperatures within the box [17,34]. The influence of box construction material on box microclimate was uncertain, as was the influence of box colour, multiple-chambers and vents on box humidity.

## 2. Materials and Methods

### 2.1. Study Area and Climate

The study was conducted within a habitat offset area of a coal mine near Muswellbrook in New South Wales, Australia. The vegetation of the study area comprised eucalypt woodland and grassland that had formerly been grazed (see [38] for more details). The climate experienced at the site during the monitoring of the box microclimates was warm during summer 2015 (mean maximum: 29.7 °C; mean minimum: 17.2 °C) and relatively cool during winter 2014 (mean maximum: 17.4 °C; mean minimum: 3.9 °C; data obtained from the mine's on-site weather station at 2 m above ground). The longer-term temperature averages (1991 to 2018) for the region during the summer months is 31.6 °C (maximum) and 16.9 °C (minimum) and 16.6 °C (maximum) and 3.3 °C (minimum) during the winter months (data obtained from a weather station, approximately 35 km away from the study site [39]).

### 2.2. Bat Box Designs

Multi-chambered bat boxes were installed that differed in construction material and design elements. Boxes were made from either plywood or wood-cement, contained entrances measuring 1.5 cm or 2.0 cm and were either painted black or white. Black and white boxes were used in an

attempt to achieve a maximum possible difference in internal temperatures between exterior colour treatments. The external panels of the plywood box were made from 1.9 cm thick marine grade plywood. The plywood box contained four chambers that were divided by 0.9 cm thick plywood panels. This box type contained a  $45 \times 2$  cm horizontal vent across the front panel and a  $2 \times 6$  cm vertical vent on one of the side panels at the location of the fourth chamber (adapted from [34]). Each chamber was open at the bottom. The internal dimensions of the plywood box were: 61 cm (height)  $\times$  45 cm (width) (box depth was made up of the size of the four chambers (either 1.5 cm or 2.0 cm)).

The wood-cement box comprised four wooden chambers enclosed by a 2 cm thick wood-cement shell. The same exterior colours and chamber widths were used as for the plywood box. The height of the wood-cement box was 42 cm and the internal diameter was 15 cm. The lid of the plywood box extended 5 cm beyond the front panel and 2 cm beyond the side and back panels, whereas the wood-cement box did not have a roof overhang (Figure 1). The wood-cement shell was constructed from a mixture of sawdust and cement. The volume ratio of moist sawdust to dry cement was 1:1. The sawdust (obtained from *Eucalyptus oreades* (Blue Mountains ash) chainsaw shavings) was thin and less than 5 mm in length. A sieve was used to exclude larger shavings from the wood-cement mix. The shavings were soaked in cold water for at least 12-h prior to box construction. Calcium chloride was added to the water when mixing the sawdust and cement to accelerate the cement setting process and increase the bonding ability [40]. A mould made from timber was used to shape the wood-cement box.



**Figure 1.** Paired plywood design (**left**) and paired wood-cement design (**right**) installed on poles about 1.5 m above ground.

### 2.3. Field Setup of Boxes

Boxes were installed across four sites in a landscape dominated by grassland that contained isolated trees. The boxes were installed 1.5 m above ground on either hardwood poles or isolated trees. Only microclimate data of boxes installed on poles are reported (Figure 1). Each site comprised two plots. Each plot contained eight bat boxes: paired plywood boxes (one black, one white) installed on a pole and on a tree and paired wood-cement boxes (one black, one white), also installed on a pole and on a tree. Because black boxes aimed to provide warmer box temperatures than white boxes, black boxes were installed to face a north-westerly aspect (afternoon sun) and white boxes faced a south-easterly aspect (morning sun). No other box aspects were tested. Therefore, when box colour is reported and discussed, it is inferred that box aspect would likely have contributed to the difference in microclimate between black and white boxes.

### 2.4. Microclimate Monitoring

Temperature/humidity data loggers (iButton, Dallas TX, USA) (hereafter ‘loggers’) were used to monitor box microclimates in summer and winter. Some loggers recorded both RH and temperature (Hygrochron DS1923) and others only temperature (Thermochron DS1921G). The logger recording interval was one per hour and the logger resolution was 0.5 °C and 0.04% (RH). Loggers were attached

with a short piece of string. One logger was deployed in each box chamber, that is, for the plywood boxes, loggers were installed 10 cm from the top of the box and for the wood-cement box, loggers were installed 5 cm from the top. During a second investigation, an additional logger was installed in each chamber of the black plywood box at a lower position (i.e., 15 cm from the bottom of box) to investigate a potential thermal gradient within chambers of this box design. Each logger faced the same way, that is, the top plane of the loggers faced the back panel of the chamber. Boxes were not closed-off for bats to access the box during the temperature/humidity measurements. Because box use was infrequent and predominantly by solitary bats (unpublished data), it was assumed that bats did not influence logger measurements. Ambient temperatures during the box monitoring were obtained from the mine's on-site weather station.

Temperature and RH data were obtained during the austral summer and early part of autumn (5 February to 8 March 2015) and winter (30 July to 17 August 2015). A subsequent, more detailed investigation was undertaken for the black plywood box only for the warmest day during 18 November 2015 to 17 January 2016 to test the thermal profile within this box (see Table 1 for details). The following variables were collated from the data: 'maximum day' temperature ( $T_{\text{box\_max}}$ ), 'mean warmest day period' temperature (1000–1900 h;  $T_{\text{box\_warmest}}$ ), 'mean warmest day period' RH (1000–1900 h;  $\text{RH}_{\text{box\_warmest}}$ ) and 'mean night' temperature (2000–0700 h;  $T_{\text{box\_night}}$ ). Details and justification of the type of analyses performed are outlined in Table 1 and in the 'statistical analysis' section. Replication of individual chambers per box design in which temperature/RH data were recorded was four, except for winter RH where chamber replication for both black box designs was six. Pairs of boxes installed on poles were selected randomly to obtain temperature and RH data. A preliminary comparison was made between  $T_{\text{box\_max}}$  on the warmest summer day of the same box designs but differing in chamber width (1.5 cm vs 2.0 cm). No significant differences were recorded between boxes comprising different chamber widths ( $F_{1,7} = 0.148$ ,  $P = 0.714$ ; File S1) and chamber widths was not considered further.

**Table 1.** Details and justification of the type of microclimate variables analysed. All four chambers within a box were analysed, except for  $T_{\text{box\_night}}$  and RH where only the front chambers were monitored. RH data were arcsine transformed for statistical analyses. Replication per chamber was four except for winter RH where chamber replication in black boxes was six. Statistical comparisons of chamber temperatures among box designs were restricted between chambers of the same order. For the randomly selected 5-day periods, the variable means over five days were used for the analysis.

Variable	Periods Investigated	Reason
$T_{\text{box\_max}}$ & $T_{\text{box\_warmest}}$ (1000–1900 h)	(i) Warmest summer & winter day of sampling period;	$T_{\text{box\_max}}$ & $T_{\text{box\_warmest}}$ were considered the most relevant variables to investigate box temperature as they are considered to be most influential with regard to eco-physiological implications for bats.  Summer & winter temperature data were collected as these seasons allowed the investigation of box temperatures among & within box designs during both warm & cool ambient temperatures.
	(ii) Coolest winter day of sampling period;	A randomly selected 5-day period during summer & winter was used to investigate the 'average' box microclimate experienced in these seasons.
	(iii) Randomly selected 5-day period during the sampling period (summer & winter).	Investigating temperature among & within box designs during the periods of investigation (i.e., warmest day, coolest day & 5-day period) is of particular relevance to test whether boxes have the potential to cause heat stress (i.e., box temperatures exceeding the upper thermal tolerance limit) in summer [32,41] and/or provide beneficial conditions for bats to passively rewarm in summer & winter [28,42] during both average ambient temperatures & ambient temperature extremes.  The black plywood box recorded the warmest temperature of the box designs. After the initial box temperature monitoring, a detailed investigation was undertaken for this design on the warmest day (20 November 2015) during the subsequent monitoring period. This investigation tested whether a vertical temperature gradient existed within chambers (using loggers in an upper & lower position). Of particular interest was whether the vents in the front & back chambers influenced temperature.

Table 1. Cont.

Variable	Periods Investigated	Reason
Mean night $T_{\text{box\_night}}$	Randomly selected 5-day period (2000–0700 h) during the maternity season	$T_{\text{box\_night}}$ during the maternity season is of interest as heat retention may benefit the development of young if boxes are used for maternity roosting [14,43,44]. An investigation of a 5-day night period was undertaken. However, the differences between night temperatures were minimal among box designs (ranging between 0.2 °C & 0.9 °C) and were considered inconsequential with regard to bat box selection & box night temperature. Therefore, this temperature variable was not modelled & is not considered further.
$RH_{\text{box\_warmest}}$ (1000–1900 h)	Randomly selected 5-day period during the sampling period (summer & winter).	Humidity is of interest as it has the potential to influence evaporative water loss of bats [35]. Humidity has also been identified to differ vastly between tree hollows & timber nest boxes [31]. The warmest day period was used because this period was considered to be most relevant for potential eco-physiological implications for bats.

### 2.5. Thermal Limits

Reported lower TNZs of Australian tree-cavity roosting bats are scarce. Willis et al. [45] reported a lower TNZ threshold for *Vespadelus vulturnus* (little forest bat) of 28 °C and Morris et al. [46] a lower TNZ for *Nyctophilus gouldi* (Gould’s long-eared bat) of 30 °C. Similarly, little information is known about the upper thermal tolerance limit of Australian bats. There is some evidence that exposure to a temperature of >40 °C increases body temperature, resting metabolic rates, thermal conductance and water loss through evaporation [47–49], although some bats have been observed using roosts exceeding 40 °C [49–51]. To discuss the documented box temperatures during summer in relation to thermal limits of bats, a postulated lower TNZ threshold of 30.0 °C and an upper thermal tolerance limit of 40.0 °C were used.

### 2.6. Statistical Analysis

Data were analysed for the variables outlined in Table 1. For the randomly selected 5-day periods, the five day means of  $T_{\text{box\_max}}$ ,  $T_{\text{box\_warmest}}$ , and  $RH_{\text{box\_warmest}}$  were used. The primary samples for the analysed data were sets of box pairs. Linear mixed effects models [52] were employed to separate the random variation among the samples of box pairs from the random variation among the individual boxes. When only one measurement was taken on each box, the variation among the individual boxes comprised the residual variance. Consequently, in models of this type, two random variances were estimated: the variance among box pairs and the residual variance. In many cases, however, multiple measurements were taken on each box. In models on data with this structure, three random variances were estimated: the variance among the box pairs, the variance among the individual boxes in each pair and the residual variance among the measurements on each box. In some cases, multiple measurements were taken on each box on each of a sample of days. A further random effect for the variation among days was fitted in the analyses of such data rather than a fixed effect for day as the mean differences between days were not of interest. Models were fitted using the Mixed procedure in SPSS (Version 25).

A model-reduction procedure was employed to identify the final model for each analysis. In each case, a full-factorial model was initially fitted (including the main effects and all interaction effects up to third order) which was systematically reduced by elimination of non-significant ( $p > 0.05$ ) effects. Non-significant effects were eliminated one at a time starting with those of the highest order of interaction and the highest  $P$ -value. If a third order interaction effect was retained in the final model, its subsidiary two-way effects were also retained and if a two-way effect was retained, its subsidiary main effects were also retained. The fixed and random effects and their test statistics are reported for the final model from each analysis (Supplementary Material File S1). Multiple comparison tests among the levels of factors included in the final models were adjusted for their multiplicity by Sidak’s method [53]. The Sidak correction gives similar, but generally more powerful, results than the better

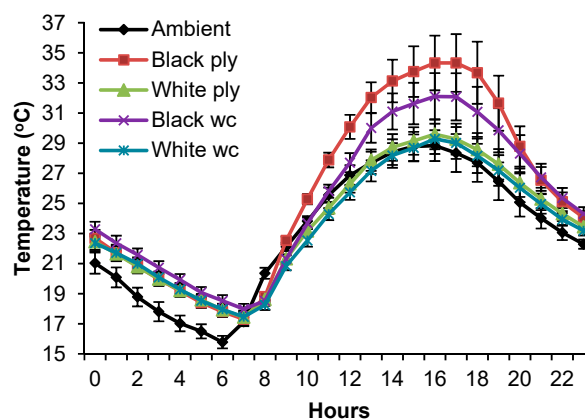
known Bonferroni correction [54]. Apart from presenting the effects and test statistics, the results are reported in terms of the multiple pairwise comparison tests. Multiple pairwise comparison tests were conducted in two families: between box designs and between chambers of the same designs. Records of RH of >70% RH were adjusted to compensate for the reported saturation drift as per the manufacturer's equation [55]. All RH data were arcsine transformed prior to statistical analysis. Given the large extent of statistical analysis output, only a summary of the most pertinent data is provided in the result section to ensure succinctness. References are made to File S1 in which the final models of all the analyses are shown. The study was carried out with approval from Southern Cross University Animal Care and Ethics Committee under permit 14/23.

### 3. Results

#### 3.1. Summer Temperature

##### 3.1.1. Randomly Selected 5-Day Period—Hourly Temperatures

The mean hourly temperature over the 5-day period in summer (16–20 February 2015) showed that the box designs were warmer than ambient during the night. During the day-period, white box temperatures followed ambient temperatures closely, whereas the temperatures of the black boxes were warmer than the white boxes and ambient temperatures (Figure 2).



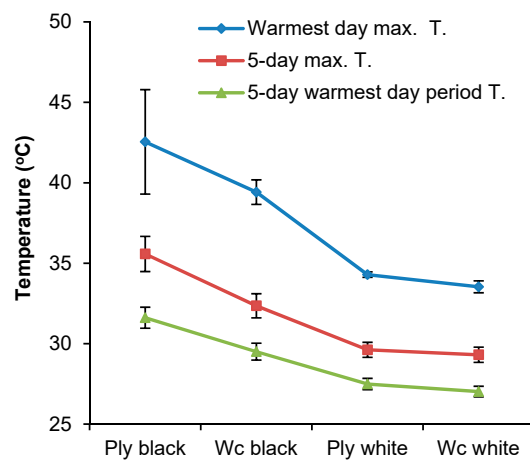
**Figure 2.** Warmest chamber temperature in each box design and ambient temperature over 24-h. Hourly means ( $\pm$ SE) are for the 5-day summer period. ply = plywood; wc = wood-cement.

##### 5-Day Comparisons—between Designs

The 5-day mean maximum day ambient temperature was  $29.3\text{ }^{\circ}\text{C} \pm 1.0$  and  $27.2\text{ }^{\circ}\text{C} \pm 0.3$  for the mean warmest day period. The final model showed that the two-way interaction effect of box design by chamber was significant ( $T_{\text{box\_max}}$ :  $F_{9, 288.00} = 11.060$ ,  $P = <0.001$ ;  $T_{\text{box\_warmest}}$ :  $F_{9, 288.00} = 12.751$ ,  $P = <0.001$ ). The highest  $T_{\text{box\_max}}$  was in the black plywood box ( $T_{\text{box\_max}}$ :  $35.6\text{ }^{\circ}\text{C} \pm 2.0$ ;  $T_{\text{box\_warmest}}$ :  $31.6\text{ }^{\circ}\text{C} \pm 1.3$ ), followed by the black wood-cement box ( $T_{\text{box\_max}}$   $3.2\text{ }^{\circ}\text{C}$  cooler and  $T_{\text{box\_warmest}}$   $2.1\text{ }^{\circ}\text{C}$  cooler) and the two white box designs ( $T_{\text{box\_max}}$   $\sim 6.0\text{ }^{\circ}\text{C}$  and  $T_{\text{box\_warmest}}$   $\sim 4.5\text{ }^{\circ}\text{C}$  cooler than the black plywood box; Figure 3).

The mixed effect models showed that the comparisons of both  $T_{\text{box\_max}}$  and  $T_{\text{box\_warmest}}$  between black (warmer) and white (cooler) designs were significantly different for most chamber comparisons (File S1). The pairwise comparisons between designs of the same chambers showed that the black plywood box comprised the most comparison differences. All chambers of this design were significantly warmer than all chambers of the other three box designs except the fourth (back) chamber of the black wood-cement design. There were no significant differences between the two white box designs between any chambers (File S1). The greatest differences were between the front chambers

of the black plywood and the white wood-cement box (5-day  $T_{\text{box\_max}}$  difference: 6.3 °C;  $df = 19.82$ ,  $P = <0.001$ ; 5-day  $T_{\text{box\_warmest}}$  difference: 4.6 °C;  $df = 15.05$ ,  $P = <0.001$ ; Figure 3; Table 2).



**Figure 3.** Summary of comparisons of mean ( $\pm$ SE) front chamber temperatures for each box design ( $n = 4$ ) during the periods of examination:  $T_{\text{box\_max}}$  on warmest summer day (ambient: 35.4 °C), 5-day  $T_{\text{box\_max}}$  (ambient: 29.3 °C  $\pm$  1.0) and 5-day  $T_{\text{box\_warmest}}$  (ambient: 27.2 °C  $\pm$  0.3). ply = plywood; wc = wood-cement.

**Table 2.** Pairwise comparison of temperatures in front chambers ( $n = 4$ ) between box designs for 5-day  $T_{\text{box\_max}}$ , 5-day  $T_{\text{box\_warmest}}$  and warmest day  $T_{\text{box\_max}}$ . ply = plywood; wc = wood-cement.

Box Design Comparisons		5-Day $T_{\text{box\_max}}$		5-Day $T_{\text{box\_warmest}}$		Warmest Day $T_{\text{box\_max}}$	
		Mean Difference (°C)	<i>P</i>	Mean Difference (°C)	<i>P</i>	Mean Difference (°C)	<i>P</i>
Black ply	White ply	6.0	<0.001	4.1	<0.001	8.3	0.001
	Black wc	3.2	<0.001	2.1	0.004	3.1	0.360
White ply	White wc	6.3	<0.001	4.6	<0.001	9.0	<0.001
	Black wc	-2.7	0.001	-2.0	0.007	-5.1	0.033
Black wc	White wc	0.3	0.997	0.5	0.933	0.8	0.998
	White wc	3.0	<0.001	2.5	0.007	5.9	0.016

### 5-Day Comparisons—Chambers within Designs

The greatest difference between chambers within a box design was between the front and back chamber of the black plywood box (5-day  $T_{\text{box\_max}}$  difference: 3.7 °C;  $df = 288.00$ ,  $P = <0.001$ ;  $T_{\text{box\_warmest}}$  difference: 2.1 °C;  $df = 288.00$ ,  $P = <0.001$ ; Table 3). Comparisons of 5-day  $T_{\text{box\_max}}$  and 5-day  $T_{\text{box\_warmest}}$  between chambers within the same box design showed that the black plywood box comprised the most differences (11 (92%) out of the 12 pairwise comparisons). The black wood-cement box comprised a significant temperature difference among six comparisons (50%), while the white plywood box comprised one and the white wood-cement none.

**Table 3.** Statistically significant ( $P = <0.05$ ) pairwise comparison between chamber temperatures within each box design for 5-day  $T_{\text{box\_max}}$ , 5-day  $T_{\text{box\_warmest}}$  and warmest day  $T_{\text{box\_max}}$  ( $n = 4$  for each chamber). ply = plywood; wc = wood-cement; 1 = front chamber; 4 = back chamber.

Box Design	Chamber Comparisons	5-day $T_{\text{box\_max}}$		5-day $T_{\text{box\_warmest}}$		Warmest day $T_{\text{box\_max}}$	
		Mean Difference (°C)	<i>P</i>	Mean Difference (°C)	<i>P</i>	Mean Difference (°C)	<i>P</i>
Black ply	2	1.0	0.015	-	-	-	-
	1 3	2.8	<0.001	1.3	<0.001	3.6	0.001
	4	3.7	<0.001	2.3	<0.001	4.5	<0.001
	3	1.8	<0.001	1.0	<0.001	-	-
	2 4	2.7	<0.001	1.8	<0.001	2.9	0.011
	3 4	0.9	0.032	0.8	<0.001	-	-

Table 3. Cont.

Box Design	Chamber Comparisons		5-day $T_{\text{box\_max}}$		5-day $T_{\text{box\_warmest}}$		Warmest day $T_{\text{box\_max}}$	
			Mean Difference ( $^{\circ}\text{C}$ )	$P$	Mean Difference ( $^{\circ}\text{C}$ )	$P$	Mean Difference ( $^{\circ}\text{C}$ )	$P$
Black wc	1	2	1.2	0.002	0.7	0.001	-	-
		3	2.0	<0.001	1.2	<0.001	2.5	0.035
		4	1.4	<0.001	0.7	0.001	-	-
White ply	1	3	-	-	0.6	0.008	-	-

### 3.1.2. Maximum Temperature Comparisons on Warmest Day

#### Between Designs

On the warmest summer day during the sampling period (8 February 2015; max. ambient  $35.4^{\circ}\text{C}$ ), the black plywood box recorded the highest  $T_{\text{box\_max}}$ , followed by the black wood-cement box. The two white box designs comprised  $T_{\text{box\_max}}$  slightly below that of the maximum ambient temperature. The greatest temperature difference between ambient and a box design was in the front chamber of the black plywood box ( $7.0^{\circ}\text{C}$ ; Figure 3). The final model showed that the two-way interaction effect of box design by chamber was significant ( $F_{9, 36.00} = 2.631$ ,  $P = 0.019$ ).

The pairwise chamber comparisons of  $T_{\text{box\_max}}$  showed that the black plywood box comprised significantly warmer chambers than the white boxes in chambers one (front), two and three, whereas there were no significant differences between the chambers of this box compared to the chambers of the black wood-cement box. The black wood-cement box comprised a significantly warmer front chamber to that of the front chambers of the two white box designs, while chamber two, three and four (back) were not significantly different between this design and the white box designs. There were no significant differences between the two white box designs between any chambers (File S1). The greatest temperature difference between chambers was within the front chambers of black plywood box and the white wood-cement box ( $9.0^{\circ}\text{C}$ ;  $df = 18.14$ ,  $P = <0.001$ ; Table 2).

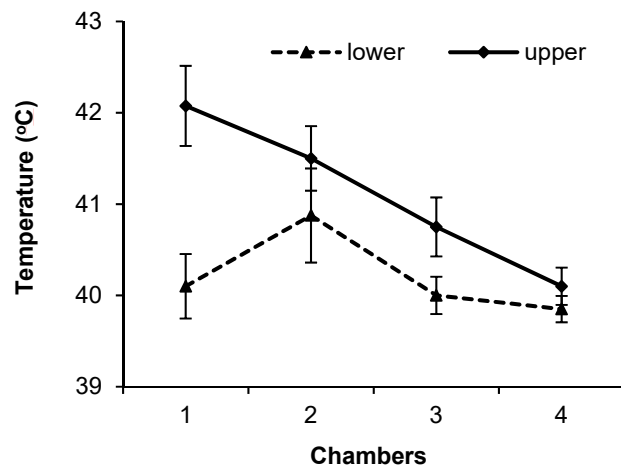
#### Within Designs

The pairwise chamber comparisons of  $T_{\text{box\_max}}$  within the same box design on the warmest summer's day during the sampling period showed that the black plywood box comprised the most significant differences with three of the six pairwise comparisons being significantly different. The black wood-cement boxes comprised one significantly different chamber comparison and none for the two white box designs. The greatest difference between chambers within a box design was between the front and back chamber of the black plywood box (difference:  $4.5^{\circ}\text{C}$ ;  $df = 36.00$ ,  $P = <0.001$ ; Table 3).

### 3.1.3. Detailed Investigation of the Black Plywood Box

The detailed investigation of the black plywood box took place following the ones described above. This investigation examined whether there was a vertical gradient in temperature within chambers of the box design that recorded the warmest temperatures. On the warmest day during this sampling (20 November 2015), the  $T_{\text{box\_max}}$  measured within the box chambers was close to or above the ambient temperature of  $39.5^{\circ}\text{C}$ . There was a marked temperature gradient across the chambers with the rear chamber (chamber 4) being the coolest. The  $T_{\text{box\_max}}$  at the upper chamber position was higher than at the lower position across all chambers. The temperature gradient was less apparent at the lower position presumably due to the influence of the vent in the first chamber (Figure 4). The final model showed that there was a significant difference temperature between the upper ( $42.1^{\circ}\text{C} \pm 0.4$ ) and lower ( $40.1^{\circ}\text{C} \pm 0.4$ ) logger within the front chamber ( $df = 21.00$ ,  $P = <0.001$ ), whereas there were no significant differences within upper and lower logger position in the other three chambers (File S1).

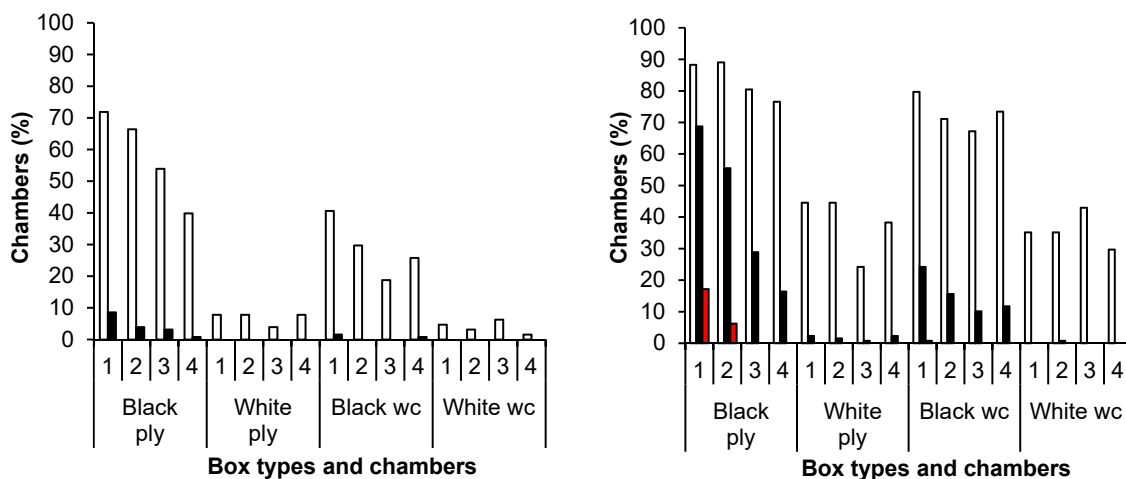




**Figure 4.** Mean ( $\pm$ SE)  $T_{\text{box\_max}}$  recorded in four black plywood boxes on 20 November 2015 when the ambient temperature reached a maximum of 39.5 °C. Loggers were placed in each of the four chambers at an upper and lower position (15 cm and 45 cm from top respectively). 1 = front chamber; 2 = second chamber; 3 = third chamber; 4 = back chamber.

### 3.1.4. Comparison of Box Temperatures Vs Thermal Limit Thresholds

The 32-day summer period in which four replicates per box design were monitored resulted in 128  $T_{\text{box\_max}}$  and 128  $T_{\text{box\_warmest}}$  chamber records. The lower TNZ threshold of local bats was hypothesised to be 30.0 °C. The number of times in which chamber  $T_{\text{box\_warmest}}$  exceeded this threshold was highest for the black plywood box design, followed by the black wood-cement design and the two white box designs (Figure 5). A few chambers of the black plywood boxes exceeded 35 °C during  $T_{\text{box\_warmest}}$ , whereas only one chamber of the black wood-cement boxes exceeded this threshold and none of the chambers of the two white box designs (Figure 5).  $T_{\text{box\_warmest}}$  did not exceed 40.0 °C (hypothesised upper thermal tolerance limit) in any of the box designs (Figure 5). A similar pattern was documented for  $T_{\text{box\_max}}$  over the 32-days monitoring period, although, as would be expected, the frequency of chambers for all designs exceeding the 30 °C and 35 °C thresholds was higher. The only records of chambers exceeding a  $T_{\text{box\_max}}$  of 40 °C during the 32-day monitoring period were in the first (17%) and second (8%) chambers of the black plywood design (Figure 5).

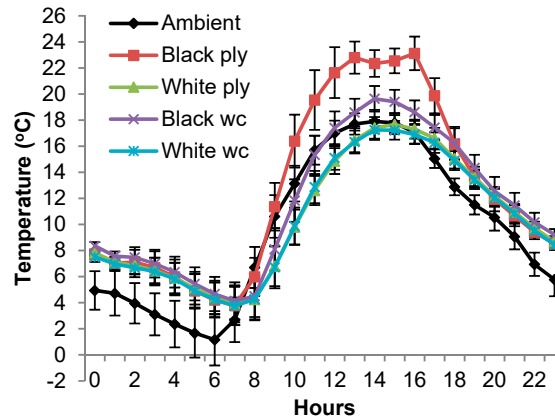


**Figure 5.** Percentage of chambers where chamber temperatures during  $T_{\text{box\_warmest}}$  (left) and  $T_{\text{box\_max}}$  (right) reached >30.0 °C (white bars), >35 °C (black bars) and >40 °C (red bars) during the 32-day monitoring period (5 February–8 March 2015). X-axis: 1 = front chamber; 2 = second chamber; 3 = third chamber; 4 = back chamber; ply = plywood; wc = wood-cement.

### 3.2. Winter Temperature

#### 3.2.1. Randomly Selected 5-Day Period—Hourly Temperatures

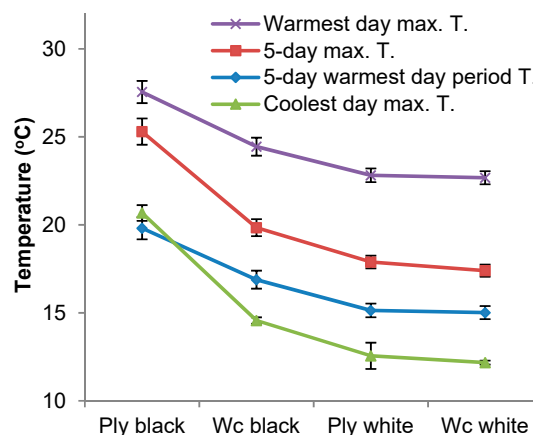
Similar to the summer investigation, the winter mean hourly temperature over the 5-day period (10–14 August 2015) showed that the box designs were warmer than the ambient temperature during the night. During the day, the temperatures of the two white box designs and the black wood-cement box closely resembled ambient temperature, whereas the temperature of the black plywood box was higher than the other box designs and ambient temperature (Figure 6).



**Figure 6.** Warmest chamber mean ( $\pm$ SE) temperature in each box design and ambient temperature over 24-h. Hourly means are for the 5-day winter period. ply = plywood; wc = wood-cement.

#### 5-Day Comparison—between Designs

The 5-day mean maximum day ambient temperature was  $18.3\text{ }^{\circ}\text{C} \pm 0.8$  and  $15.6\text{ }^{\circ}\text{C} \pm 0.4$  for the mean warmest day period. The final model showed that the two-way interaction effect of box design by chamber was significant ( $T_{\text{box\_max}}$ :  $F_{9, 288.000} = 48.070, P = <0.001$ ;  $T_{\text{box\_warmest}}$ :  $F_{9, 288.000} = 27.497, P = <0.001$ ). The highest 5-day box temperatures were in the black plywood box ( $T_{\text{box\_max}}$ :  $25.3\text{ }^{\circ}\text{C} \pm 1.5$ ;  $T_{\text{box\_warmest}}$ :  $19.8\text{ }^{\circ}\text{C} \pm 0.6$ ), followed by the black wood-cement box ( $5.5\text{ }^{\circ}\text{C}$  ( $T_{\text{box\_max}}$ ) and  $2.9\text{ }^{\circ}\text{C}$  ( $T_{\text{box\_warmest}}$ ) cooler) and the two white box designs ( $\sim 7.5\text{ }^{\circ}\text{C}$  ( $T_{\text{box\_max}}$ ) and  $\sim 5.0\text{ }^{\circ}\text{C}$  ( $T_{\text{box\_warmest}}$ ) cooler than the black plywood box; Figure 7).



**Figure 7.** Summary of comparisons of front chamber mean ( $\pm$ se) maximum temperatures for each design ( $n = 4$ ) during the periods of examination:  $T_{\text{box\_max}}$  on warmest winter day (ambient:  $24.0\text{ }^{\circ}\text{C}$ ),  $T_{\text{box\_max}}$  on coolest winter day (ambient:  $12.5\text{ }^{\circ}\text{C}$ ), 5-day  $T_{\text{box\_max}}$  (ambient:  $18.3\text{ }^{\circ}\text{C} \pm 0.8$ ) and 5-day  $T_{\text{box\_warmest}}$  (mean ambient  $15.6\text{ }^{\circ}\text{C} \pm 0.4$ ). ply = plywood; wc = wood-cement.

The mixed effect models showed that the pairwise comparisons of both  $T_{\text{box\_max}}$  and  $T_{\text{box\_warmest}}$  between black and white box designs were significantly different for all comparisons (File S1). As for the summer 5-day investigation, the pairwise comparisons showed that all chambers of the black plywood design were significantly warmer than all chambers of the other three box designs except the fourth (back) chamber of the black wood-cement design. Comparisons between the two white box designs did not result in significant temperature differences between any chambers (File S1). The greatest differences were between the front chambers of the black plywood and the white wood-cement box (5-day  $T_{\text{box\_max}}$  difference: 7.9 °C;  $df = 34.44$ ,  $P = <0.001$ ; 5-day  $T_{\text{box\_warmest}}$  difference: 4.8 °C;  $df = 23.57$ ,  $P = <0.001$ ; Figure 7; Table 4).

**Table 4.** Pairwise comparison of temperature in front chambers between box designs for 5-day  $T_{\text{box\_max}}$ , 5-day  $T_{\text{box\_warmest}}$ ,  $T_{\text{box\_max}}$  warmest day and  $T_{\text{box\_max}}$  coolest day. ply = plywood; wc = wood-cement.

Box Design Comparisons		5-Day $T_{\text{box\_max}}$		5-Day $T_{\text{box\_warmest}}$		$T_{\text{box\_max}}$ warmest day		$T_{\text{box\_max}}$ coolest day	
		Mean Difference (°C)	P	Mean Difference (°C)	P	Mean Difference (°C)	P	Mean Difference (°C)	P
Black ply	White ply	7.4	<0.001	4.7	<0.001	4.7	<0.001	8.1	<0.001
	Black wc	5.5	<0.001	2.9	<0.001	3.1	<0.001	6.1	<0.001
	White wc	7.9	<0.001	4.8	<0.001	4.9	<0.001	8.5	<0.001
White ply	Black wc	−2.0	<0.001	−1.8	<0.001	−1.6	0.005	−2.0	0.002
	White wc	0.5	0.656	0.1	0.997	0.1	1.000	0.4	0.973
Black wc	White wc	2.4	<0.001	1.9	<0.001	1.8	0.002	2.4	<0.001

### 5-Day Comparison—Chambers within Designs

Comparisons between chambers within the same box design showed that the black plywood box comprised the most significant 5-day  $T_{\text{box\_max}}$  and  $T_{\text{box\_warmest}}$  temperature differences, with 10 (83%) of the 12 comparisons being significant. The black wood-cement box comprised four (33%) significantly different chamber comparisons, whereas none of the chambers significantly differed in temperature within the white boxes. The greatest temperature difference between chambers within a box design was between the front and back chamber of the black plywood box (5-day  $T_{\text{box\_max}}$  difference: 5.7 °C;  $df = 288.00$ ,  $P = <0.001$ ;  $T_{\text{box\_warmest}}$  difference: 2.9 °C;  $df = 288.00$ ,  $P = <0.001$ ; Table 5).

**Table 5.** Statistically significant ( $P = <0.05$ ) pairwise comparison between chambers within each box designs for 5-day  $T_{\text{box\_max}}$ , 5-day  $T_{\text{box\_warmest}}$ , warmest day  $T_{\text{box\_max}}$ , coolest day  $T_{\text{box\_max}}$  ( $n = 4$  for each chamber). ply = plywood; wc = wood-cement; 1 = front chamber; 4 = back chamber.

Box Design	Chamber Comparison	5-Day $T_{\text{box\_max}}$		5-Day $T_{\text{box\_warmest}}$		$T_{\text{box\_max}}$ warmest day		$T_{\text{box\_max}}$ coolest day		
		Mean Difference (°C)	P	Mean Difference (°C)	P	Mean Difference (°C)	P	Mean Difference (°C)	P	
Black ply	1	2	2.5	<0.001	1.3	<0.001	1.3	<0.001	2.4	<0.001
		3	5.0	<0.001	2.5	<0.001	3.2	<0.001	5.4	<0.001
		4	5.7	<0.001	2.9	<0.001	3.3	<0.001	6.4	<0.001
	2	3	2.5	<0.001	1.2	<0.001	1.9	<0.001	3.0	<0.001
		4	3.2	<0.001	1.6	<0.001	2.0	<0.001	4.0	<0.001
	Black wc	1	2	1.1	0.001	0.7	0.003	-	-	-
2		4	−0.9	0.003	−0.6	0.014	-	-	-	-

### 3.2.2. Maximum Temperature Comparisons on Warmest and Coolest Day

#### Between Designs

On the warmest day (max. ambient 24.0 °C) and coolest day (max. ambient 12.5 °C) in winter, the chambers of the black plywood box recorded the highest  $T_{\text{box\_max}}$  and were up to 3.5 °C on the warmest and 8.2 °C on the coolest day above maximum ambient temperature. The  $T_{\text{box\_max}}$  of the

black wood-cement box chambers were slightly above maximum ambient temperature, whereas the white box designs were slightly below (Figure 7; File S1).

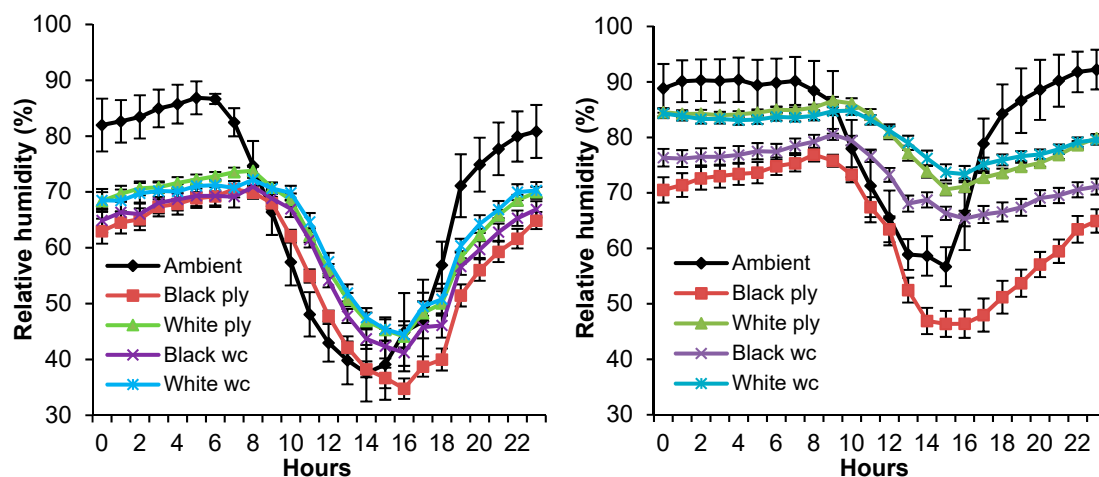
The final model showed that the two-way interaction effect of box design by chamber was significant (warmest day  $T_{\text{box\_max}}$ :  $F_{9, 36.000} = 20.492$ ,  $P = <0.001$ ; coolest day  $T_{\text{box\_max}}$ :  $F_{9, 36.000} = 18.868$ ,  $P = <0.001$ ). The black plywood box was significantly warmer compared to the black wood-cement boxes in six (75%) of the eight chamber comparisons, whereas there were no significant differences between the chambers of the two white box designs (File S1). The greatest temperature difference was between the front chambers of the black plywood box and the white wood-cement box (warmest day  $T_{\text{box\_max}}$  difference:  $4.9\text{ }^{\circ}\text{C}$ ;  $df = 22.64$ ,  $P = <0.001$ ; coolest day;  $T_{\text{box\_max}}$  difference:  $8.5\text{ }^{\circ}\text{C}$ ;  $df = 46.68$ ,  $P = <0.001$ ; Figure 7; Table 4). The mixed effect models for  $T_{\text{box\_max}}$  showed that 11 (69%) pairwise comparisons between the chamber temperatures of black (warmer boxes) and white box designs were significantly different on the warmest day (88% (7) for black plywood; 50% (4) for black wood-cement) and 12 (75%) pairwise comparisons on the coolest day (100% (8) for black plywood; 50% (4) for black wood-cement; File S1).

### Within Designs

On the warmest and coolest winter day, the pairwise comparisons among chambers within the box designs significantly differed only within the black plywood box. The greatest difference between chambers within this design was between the front and back chamber. On the warmest day, the  $T_{\text{box\_max}}$  difference was  $3.3\text{ }^{\circ}\text{C}$  ( $df = 36.00$ ,  $P = <0.001$ ) and  $6.4\text{ }^{\circ}\text{C}$  on the coolest day ( $df = 36.00$ ;  $P = <0.001$ ; Table 5).

### 3.3. Relative Humidity—Summer and Winter

Over the randomly selected 5-day summer and winter sampling periods, ambient RH was higher at night than in boxes. In summer, the black plywood box (front chamber data) comprised a similar RH during the daytime to that of ambient RH, whereas the other three box designs were slightly above ambient RH during most parts of the daytime (Figure 8). In winter, the difference in RH between the white (higher RH) and black (lower RH) box designs (particular the black plywood box) was markedly higher during the daytime (Figure 8).



**Figure 8.** Mean front chamber RH in each box design and ambient RH. **Left:** 5-day summer hourly means ( $\pm$ SE) ( $n = 6$  for each plywood box design;  $n = 4$  for each wood-cement box design). **Right:** 5-day winter hourly means ( $\pm$ SE) ( $n = 4$  for each box design). ply = plywood; wc = wood-cement.

In summer, the ambient RH for the 5-day ‘warmest day period’ was  $50.9\% \pm 2.2$ . The final model showed that the effect of box design was significant for both summer ( $F_{3, 10.208} = 150.831$ ,  $P = <0.001$ ) and winter ( $F_{3, 12.000} = 50.782$ ,  $P = <0.001$ ) during  $\text{RH}_{\text{box\_warmest}}$ . The highest  $\text{RH}_{\text{box\_warmest}}$

was recorded in the white wood-cement box ( $55.8\% \pm 0.8$ ), followed by the white plywood (2.1% lower), the black wood-cement (5.0% lower) and the black plywood (10.1% lower). In winter, the 5-day ‘warmest day period’ ambient RH was  $72.1\% \pm 2.0$ . The highest  $RH_{\text{box\_warmest}}$  was in the white wood-cement box ( $78.1\% \pm 0.4$ ), followed closely by the white plywood (1.2% lower), the black wood-cement (8.7% lower) and the black plywood (22.9% lower). The mixed effect models showed that the pairwise comparisons were significant between the designs during both the summer and the winter sampling periods, except between the two white boxes (Table 6).

**Table 6.** Pairwise comparison of box designs in front chambers for ‘warmest day period’ RH over the 5-day period for summer and winter. ply = plywood; wc = wood-cement.

Box Design Comparisons		Summer RH		Winter RH	
		Mean Difference (%)	P	Mean Difference (%)	P
Black ply	White ply	−8.0	<0.001	−21.7	<0.001
	Black wc	−5.1	<0.001	−14.2	<0.001
White ply	White wc	−10.1	<0.001	−22.9	<0.001
	Black wc	3.0	0.014	7.5	0.010
Black wc	White wc	−2.1	0.086	−1.2	0.989
	White wc	−5.0	<0.001	−8.7	0.003

#### 4. Discussion

The deployment of bat box designs that differ in thermal profiles and the use of boxes that offer a thermal gradient within the box itself is likely an important factor for boxes to be a suitable artificial roost resource for heterothermic bats. Box microclimate is considered particularly important during ambient temperature extremes [32,41,56] and for energy conservation, such as through passive rewarming from torpor [28,57] and through warm roosts for dependent young and lactating females [14,43].

The black plywood box provided the warmest box temperatures of the four designs, followed by the black wood-cement box. Temperatures between the two black box designs were frequently significantly different, particularly for the front chamber comparisons. The two white box designs recorded similar box temperatures among them. Differing exterior colours (black boxes facing afternoon sun and white boxes facing morning sun), box construction materials, multiple chambers (chamber sequence) and chamber vents were shown to influence temperatures between box designs and within box designs, both in summer and winter.

##### 4.1. Box Colour

Box colour consistently influenced  $T_{\text{box\_max}}$  and  $T_{\text{box\_warmest}}$ , with black boxes being warmer than white boxes in both summer and winter. The influence of box colour on temperature is expected to have been amplified by box aspect with black boxes facing the afternoon sun and white boxes facing the morning sun. Previous studies have shown that differing box colours influenced box temperature significantly (Kerth et al. [18] comparing black and white wood-cement boxes; Lourenço and Palmeirim [19] comparing black, green and white plywood boxes; Doty et al. [28] comparing black and white plywood boxes; Griffiths et al. [30] comparing white and green plywood boxes). In contrast, Goldingay [58] found no significant temperature difference between brown and green timber nest boxes. The maximum difference in  $T_{\text{box\_max}}$  of boxes made from the same construction material but differing in exterior colour was  $8.3\text{ }^{\circ}\text{C}$  (summer) and  $8.1\text{ }^{\circ}\text{C}$  (winter) between the plywood boxes and  $3.3\text{ }^{\circ}\text{C}$  (summer) and  $2.4\text{ }^{\circ}\text{C}$  (winter) between the wood-cement boxes. The difference between the plywood boxes in summer is similar to that reported by Lourenço and Palmeirim [19] (mean maximum temperature difference:  $9.2\text{ }^{\circ}\text{C}$  between black and white boxes; Portugal). In contrast, a study carried out in Germany, Kerth et al. [18] reported that during summer, the maximum temperature difference

between sun-exposed white and black wood-cement boxes was up to 20 °C and up to 4.4 °C between shaded boxes during summer.

#### 4.2. Box Construction Materials

Bideguren et al. [56] investigated the internal temperatures of bat box designs made from different construction materials in a Mediterranean climate. In other climates, the investigation of box temperature differences between plywood, a commonly used construction material in Australia and North America [22,59] and wood-cement, a commonly used material in Europe [22], has not been assessed in detail. Rueegger et al. [60] reported that  $T_{\text{box\_max}}$  and  $T_{\text{box\_warmest}}$  of shaded and different coloured nest boxes made from plywood, polyvinyl chloride and wood-cement boxes did not differ significantly during cooler seasons. Studies reported that tree hollows are cooler and have a greater buffer capacity from ambient temperature than timber boxes [31,32] or white and black wood-cement boxes [18].

Wood-cement reduced the effect of black exterior colour for warm box temperature compared to plywood. This resulted in the black wood-cement box adding an additional variation of available box microclimate, recording lower temperatures to that of the black plywood box but higher temperatures to that of the white boxes. The difference during the 5-day  $T_{\text{box\_warmest}}$  period between the black boxes was greater during winter (5.5 °C) compared to summer (3.2 °C). In contrast, box material had little influence on microclimate when painted white.

#### 4.3. Multiple Chambers and Vents

This study investigated potential temperature gradients within box designs, that is, between chambers and within chambers. Providing a temperature range within a box is likely important for bats to select suitable temperatures throughout the day [17,19,50]. Temperature differences were significant between chambers of the black plywood box (maximum difference: 6.4 °C), whereas significant temperature differences between the chambers were sporadic for the black wood-cement box and practically absent for the white box designs (Tables 3 and 5). The detailed investigation of the black plywood box during the warmest day of the sampling period showed that the front chamber of this box comprised a 2.1 °C warmer maximum temperature in the upper section of the chamber to that of the lower section. The localised cooling effect in the lower section of the chamber was likely due to the chamber's vent. A previous study conducted in the Midwest region of the USA also documented temperature differences within boxes [61]. They found that two of their box designs could differ by more than 10 °C between upper and lower portions of the boxes during warm and clear days. Both of these designs used multiple chambers and one design also contained vents. These data show that boxes can be designed to provide a large thermal range within the box and thus, reduce the likelihood of the entire box comprising temperatures above the thermal tolerance limit of bats.

#### 4.4. Suitability of Recorded Box Temperatures for Bats

##### 4.4.1. Maternity Roosts

Given the importance of maternity roosts to sustain viable populations and the low use of bat boxes to rear young by many species, designing bat boxes suitable for maternity roosting is crucial [22]. It is likely that a warm thermal profile of a box is one factor selected by breeding females. The data indicate that at the study area, black painted boxes (facing the afternoon sun) are likely most suitable for maternity roosting during average ambient temperatures. Black boxes, in particular the front chamber of the black plywood box, most frequently provided temperatures within the postulated thermal limits (30.0 to 40.0 °C) and for the longest day period. In addition, the vent for the front chamber and the multiple chambers of the black plywood box resulted in a thermal gradient, providing cooler areas to that of the upper section of the front chamber. In very warm climates, box designs

that facilitate warm roosts, such as the black boxes tested here, are unlikely to be suitable during hot ambient conditions [41,49,56] with dependent young particularly vulnerable to heat stress.

The tested box designs did not allow for bats to access different chambers internally (following [34]). It is unknown if bats migrate to different chambers during the day through the entrances at the bottom of the chambers. This aspect should be investigated to ensure bats are able to make use of the thermal gradient within a multi-chambered box during the day. If access between chambers within the box were to be provided, this may reduce the intended thermal differences among chambers. Extending the external front and side panels by a few centimetres below the bottom edge of the internal chamber panels would offer some protection for bats to switch chambers via the bottom entrances during the day.

#### 4.4.2. Day Roosts Other Than Maternity Roosts

There is evidence that heterothermic bats are well adapted to roosting in conditions where ambient temperatures are below the TNZ [62] and are known to employ torpor extensively to reduce energy costs [28,62,63]. Bats may select roosts that facilitate torpor [64,65]. Such roosts may be thermally labile that allow passive rewarming from sun exposure [12,57,62]. Doty et al. [28] showed that *N. Gouldi* selected warm boxes in winter to passively rewarm from torpor to increase the time spent normothermic and increase the active time at night. Therefore, the black boxes tested, particularly the black plywood box, are likely to provide energy budget benefits for bats that use passive rewarming during cool ambient temperatures.

#### 4.4.3. Thermal Limits and Box Temperature

Little information is available on how well Australian tree-cavity roosting bats cope with high temperature extremes. Bats in hot Australian regions are believed to cope well with warm roost temperatures [49,62]. During the 32-day monitoring period in summer, no chamber of any box design exceeded 40 °C during  $T_{\text{box\_warmest}}$ .  $T_{\text{box\_max}}$  exceeded 40 °C in the front chamber of the black plywood box on 22 (17%) occasions and on eight (6%) occasions in the second chamber but never in the third and back chamber or in the other box designs. In contrast, when the black plywood box was investigated in more detail the following summer,  $T_{\text{box\_max}}$  on the warmest day exceeded 40 °C in the upper section of all chambers and the loggers measuring temperatures at the lower section of the chambers were either just above or just below 40 °C (Figure 4).

It is unclear if short durations of temperatures over 40.0 °C are detrimental to bats [50,51,66] and likely varies between species and location [19,49]. Some bat box studies conducted in warm climates have raised concerns that bat boxes become lethal during hot ambient conditions [41,56], with Hoeh et al. [61] recording box temperatures of up to 61 °C. The data obtained in this study indicates that at the study site, box temperatures experienced in black boxes (with an afternoon sun aspect) installed on poles with a natural groundcover underneath, were suitable for bats on an average summer day (mean maximum: 29.7 °C). However, during spells of very hot ambient temperatures or in climates experiencing warmer ambient temperatures to that at the study site, the availability of box designs buffering from very hot ambient temperatures and providing a thermal gradient are important. This study indicated that: white boxes, box materials that buffer warm ambient temperature (e.g., wood-cement over plywood) and box designs that comprise a temperature gradient within the box (e.g., multiple chambers with vents) can negate hot ambient temperature to some extent. Consideration of these box design elements and identifying other design elements to further improve the buffering capacity from extreme heat [56,67] may be particularly important given the predicted climate warming and increased periods of extreme heat [68].

#### 4.5. Relative Humidity

Humidity has the potential to influence the evaporative water loss of bats [35]. However, it is unclear whether RH is a factor in roost selection with roost humidity likely influenced by the

presence of bats, particularly where bats congregate in colonies [25]. Both box colour and box material influenced RH. Box material was shown to influence RH for black boxes but not for white boxes. The black plywood and black wood-cement box differed significantly during the 5-day  $RH_{\text{box\_warmest}}$  (black plywood 5.1% (summer) and 14.2% (winter) drier than black wood-cement), whereas the white plywood and the white wood-cement box did not differ significantly. In addition, the comparisons showed that white boxes (with a morning sun aspect) provided higher RH conditions than black boxes (with an afternoon sun aspect; maximum difference between the 5-day  $RH_{\text{box\_warmest}}$  in summer: 10.1%; winter: 22.9%). It is likely that the higher RH recorded in the white boxes mimic natural roosts more closely. A previous study on timber nest boxes documented boxes to provide lower mean daily RH (mean 76–78%) to that of tree hollows (mean 90%) [31], indicating that boxes do not closely mimic RH of tree hollows.

## 5. Management Implications

Roost temperatures can influence bat thermoregulation and energy expenditure during both summer and winter. This study showed that box design elements can be used to influence box temperature. Bat thermoregulatory theory and some empirical evidence [18,19,28] support the view that black boxes (facing an afternoon sun aspect) are likely more suitable for passive rewarming during temperatures below the TNZ, as well as for maternity roosting in climates with cool and moderate temperatures, except during periods of ambient temperature extremes. This is also supported by the data obtained in this study. However, there is a lack of detailed knowledge of thermal roost preferences by bats and preferences may differ between sexes, seasons and species.

When devising a bat box program in cool and moderate climates, the use of a variety of box designs that provide differing microclimates and offer an internal thermal gradient should be considered. Deploying paired black (comprising a thermal gradient) and white multi-chambered boxes may be a suitable approach. In very warm climates, however, black boxes should be avoided altogether as they can become ecological traps (see [56] for details). Even in moderately warm climates, such as at the study site, the use of black box designs that provide limited internal thermal gradients should be considered with caution, particularly in light of the predicted increase of days of extreme heat [68]. Furthermore, the placement of boxes should also be considered when deploying bat boxes, such as installing boxes in a variety of aspects and a variety of sun and shade exposed locations [18,69].

This study used wood-cement as a bat box material for the first time in Australia. Black painted wood-cement boxes were shown to diversify box microclimate compared to the other designs tested, being warmer than the two white box designs and cooler than the black plywood design. Wood-cement is likely a suitable alternative construction material to that of plywood in temperate Australia but its use by bats remains to be documented. This material has the potential to reduce box maintenance costs for long-term box programs compared to the commonly used plywood boxes [22,60]. More research is required to test box design elements further, including wood-cement and voluminous-type boxes, across differing climates. Additional artificial hollow provision methods such as mechanical creation of hollows into trees [70,71] and large bat ‘houses’ [34,56] should also be further advanced and tested.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2076-3298/6/2/13/s1>.

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