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4 **Effect of roost choice on winter torpor patterns of a free-ranging insectivorous bat**

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17 **Running head:** Roost choice and torpor in a microbat

18

19 **Abstract**

20 Gould's wattled bat (*Chalinolobus gouldii*) is one of only three native Australian mammals
21 with an Australia-wide distribution. However, there are currently no data available on the
22 thermal physiology of free-ranging *C. gouldii*. Therefore, we aimed to quantify the effect of
23 roost choice on daily skin temperature fluctuations during winter in *C. gouldii* living in an
24 agricultural landscape in a temperate region. Ambient conditions consisted of long periods
25 below 0°C and snow. Some individuals roosted high in dead branches whereas another

26 individual roosted in a large cavity located low in a live tree. Torpor was employed on every
27 day of the study period by all bats, with bouts lasting for over five days. The skin temperature
28 of individuals in the dead branches tracked ambient temperature and they displayed skin
29 temperatures below 3°C (lowest recorded -0.2°C) on 67% of bat days. In contrast, the
30 individual in the tree cavity maintained a larger skin-ambient temperature differential likely
31 influenced by the internal cavity temperature. Our study presents the lowest skin temperature
32 recorded for a free-ranging Australian microbat and reveals that roost choice affects the
33 thermal physiology of *C. gouldii* ensuring survival during periods of cold weather and limited
34 food supply.

35

36 **Introduction**

37 Although interest in the biology of free-ranging bats and other mammals has increased
38 recently, information relating to their physiological ecology remains scarce. Importantly,
39 research concerning the ecology, behaviour and physiology of animals has been identified as
40 being crucial to conservation practices with different species requiring specific habitat
41 characteristics (Cristine *et al.* 2014). As bats encompass one-fifth of all mammalian species,
42 understanding the biology of the group is vital. The significance of bats to human interests, in
43 particular agriculture, is becoming increasingly evident (Kunz *et al.* 2011). In particular, bats
44 consume large amounts of pest insects nightly, reducing the impact of these pests on crops
45 and highlighting the importance of conserving bats within these landscapes (Cleveland *et al.*
46 2006; Kalka *et al.* 2008; Kunz *et al.* 2011).

47

48 Bats are particularly sensitive to changes in weather as most are small, weighing less than
49 25g, and with their large wing membranes bats have an especially high surface area to
50 volume ratio. In addition, many of the specialisations that are characteristic of bats, such as

51 flight and echolocation, are energetically expensive (Fenton *et al.* 1998; Neuweiler 2000).
52 The majority of bats feed on insects, the abundance of which is highly correlated to climatic
53 conditions and decrease in number when ambient temperatures (T_a) are low (Richards 1989;
54 Pavey and Burwell 2004; Stawski 2012). Therefore, physiological strategies for obtaining
55 and/or conserving energy are of vital importance to bats. To overcome energetic bottlenecks
56 and periods of inclement conditions, many bats employ torpor to save energy by reducing
57 body temperature (T_b), metabolic rate and heart rate (Turbill and Geiser 2008; Stawski *et al.*
58 2014; Ruf and Geiser 2015). Although torpor can be employed for a range of reasons (Geiser
59 and Brigham 2012), it is particularly useful during winter in temperate regions when T_a
60 approaches or surpasses freezing, and food abundance is drastically reduced (Turbill and
61 Geiser 2008).

62

63 Bats roost in a wide variety of locations, such as caves, rock crevices, man-made structures,
64 tree hollows or under bark (Brigham *et al.* 1997; Lumsden *et al.* 2002a; Law and Chidel
65 2007; Sagot and Chaverri 2015). All of these roosts provide varying thermal properties with
66 regard to variation in daily T_a , ranging from very stable to widely fluctuating (Law and
67 Chidel 2007; Coombs *et al.* 2010; Doucette *et al.* 2011; Clement and Castleberry 2013; Otto
68 *et al.* 2016). It has been well documented that many bats will select daytime roosting
69 locations based on their energetic needs (Lausen and Barclay 2003; Lourenço and Palmeirim
70 2004; Willis and Brigham 2005; Stawski *et al.* 2008; Otto *et al.* 2016). For example, cool
71 roosts with little daily fluctuation in T_a , such as caves or buildings, are beneficial for
72 prolonged torpor use during periods of low resource availability and ensure that bats can
73 avoid exposure to below freezing T_a (Stawski *et al.* 2008; Doucette *et al.* 2011; Jonasson and
74 Willis 2012; Otto *et al.* 2016). Conversely, roosts with large daily temperature fluctuations,
75 such as under tree-bark, promote deep torpor in the early morning to save energy, but allow

76 for passive or partially passive arousals in the afternoon with warming T_a (Turbill 2006a;
77 Stawski *et al.* 2008; Doucette *et al.* 2011; Otto *et al.* 2016). Passive rewarming is known to
78 significantly decrease the costs associated with active arousals (Geiser *et al.* 2004; Currie *et*
79 *al.* 2015) and selection of thermally labile roosts would therefore be advantageous during
80 times when foraging in the evenings could provide a significantly positive energetic return.
81 As the energetic requirements of individuals fluctuate throughout the year with response to
82 climate, food availability and reproductive status (Turbill 2006b; Stawski *et al.* 2014), bats
83 often move between roosting habitats throughout the year. Consequently, bats require a
84 variety of roosting locations within their habitat in order to effectively balance their energy
85 budgets.

86

87 We investigated the thermal biology and torpor patterns of free-ranging Gould's wattled bats
88 (*Chalinolobus gouldii*, 10-20g) in relation to roost choice during mid-winter in a temperate
89 area to establish the physiological capabilities of this species. Further, as natural habitats are
90 decreasing due to expanding urban and agricultural areas, reducing the potential roosting
91 choices for bats, we also aimed to understand how bats cope physiologically within an
92 agricultural landscape and limited roost choices. It is surprising that limited data are available
93 on this species given its Australia-wide distribution in a large variety of habitats and climates.
94 Data are available on roosting and foraging ecology in autumn and spring and demonstrates
95 that they typically roost in tree hollows and buildings and can move large distances between
96 roosts and foraging areas (Lumsden *et al.* 2002a, b). However, very little is known about the
97 thermal physiology of *C. gouldii* and these data have only been collected under laboratory
98 conditions, which have shown that they do indeed use torpor (Kulzer 1970; Hosken and
99 Withers 1997). Moreover, data on winter roosting habits and torpor patterns in free-ranging
100 bats are scarce, particularly for Australian species that are thought to generally experience

101 mild winters. As *C. gouldii* are common across the Australian continent they are regularly
102 exposed to low temperatures in the higher elevations. Prolonged torpor during winter in the
103 wild has been documented for its congener *C. morio* (Turbill 2006b), and therefore we
104 hypothesised that during winter *C. gouldii* would regularly employ long bouts of torpor
105 interspersed with short normothermic periods on warmer evenings associated with the
106 potential to feed. Further, as previous studies have shown that roost choice affects bat thermal
107 physiology (Turbill 2006a; Stawski *et al.* 2008; Doucette *et al.* 2011), we also predicted that
108 roost choice would influence daily skin temperature (T_{skin}) fluctuations.

109

110 **Materials and Methods**

111 Our field study was conducted on a grazing agricultural property (30°30'39.2904"S,
112 151°32'57.1488"E) near Armidale, Australia, during the austral mid-winter in July 2015 with
113 a long-term mean minimum T_a of -0.4°C and mean maximum T_a of 12.9°C. *Chalinolobus*
114 *gouldii* were originally removed from the roof of a building by hand and subsequently
115 transferred to the University of England. All bats were held in outdoor aviaries over a two-
116 month period for another project (Currie *et al.*, in preparation). Upon completion of this
117 research five bats were fitted with temperature-sensitive transmitters (~0.5 g, LB-2NT,
118 Holohil Systems, Inc., Carp, Ontario, Canada) by removing a small patch of fur from
119 between the shoulder blades and attaching the transmitter using a latex adhesive (B-520;
120 Factor2; Lakeside, Arizona). These transmitters are known to have no effect on the body
121 condition of bats (Jonasson and Willis 2012). Before attachment all transmitters were
122 calibrated in a water bath against a precision thermometer to the nearest 0.1°C between
123 temperatures of 1.5 to 41°C. Cubic polynomial equations were then fitted ($R^2 > 0.99$, $p <$
124 0.0001) in order to convert the pulse interval obtained from the transmitters into skin
125 temperature (T_{skin}).

126

127 Bats were released after dusk and were radio-tracked with receivers (ICOM IC-R10, Icom
128 Inc. Osaka, Japan) and antennae (Yagi, Sirtrack® Limited, Hawkes Bay, New Zealand) to
129 their roost locations the following morning. Once located, a multi-channel custom-made data
130 logger (slightly modified version of that described in Körtner and Geiser 2000) and an
131 antenna were placed within reception range of each bat. These loggers recorded the pulse
132 interval of each transmitter every 10 minutes and the data were downloaded twice a week.
133 Additionally, an iButton data logger ($\pm 0.5^{\circ}\text{C}$, iButton thermochron DS1921G, Maxim
134 Integrated Products, Inc., Sunnyvale, California, USA) was suspended from a tree in an
135 inverted styrofoam cup to record ambient temperatures (T_a) every 10 minutes throughout the
136 study. We attempted to place an iButton in a tree cavity where one of the bats was located,
137 however, we were unable to get it in deep enough to eliminate the influence of outside T_a .
138 The heights of each of the roosts were recorded, the diameter at breast height (DBH), height
139 of the roost trees and foliage.

140

141 The first individual was released ~6 km from the original capture location and, interestingly,
142 this female was found the following day roosting in a tree ~20 m from the building where she
143 was originally captured two months prior. Therefore, the remaining individuals were released
144 at the original capture location due to the evident strong home roost fidelity, and were indeed
145 subsequently found roosting in nearby trees. The agricultural property consisted of cleared
146 grazing land and small pockets of live and dead eucalypt trees, with ~1 km of open space
147 between these pockets. The trees in these remnants were not clustered and widely spaced
148 apart with little vegetation on the ground except for grazing grass.

149

150 Data could only be obtained from three of the five individuals, as the remaining two
151 individuals were not found after release, possibly due to transmitter failure. Therefore,
152 continuous data were collected for a total of 17 bat days (2448 data points) from three
153 individuals: female #1 = 6 days (15.8 g), female #2 = 8 days (13.5 g) and male #1 = 3 days
154 (15.0 g). We defined torpor bouts as periods of longer than 30 minutes when T_{skin} was below
155 28°C . Importantly, while T_{a} does influence the transmitters and often creates a large
156 differential between T_{b} and T_{skin} during activity and normothermic rest periods, this
157 differential is $<2^{\circ}\text{C}$ during torpor, which is the main focus of the current study (Barclay et al.
158 1996; Willis and Brigham 2003). Data were analysed in R (R version 3.0.1, The R
159 Foundation for Statistical Computing, 2013) and significance was assumed if $p < 0.05$.
160 Means are presented ± 1 standard deviation. To test for a difference in daily minimum T_{a} on
161 days when bats remained torpid versus days when bats aroused, a Welch Two Sample t-test
162 was performed. A one-way analysis of variance was undertaken to test for differences among
163 individuals for T_{b} variables, followed by a post-hoc Tukey test to verify which individuals
164 differed from each other. A linear mixed effects model (packages ‘lme4’ and ‘MuMIn’) was
165 fitted to determine the relationship between the measured variables (daily minimum T_{b} and
166 torpor bout duration) and daily minimum T_{a} ; individuals were accounted for as a random
167 factor.

168

169 All procedures were approved by the University of New England Animal Ethics Committee
170 (AEC15-024) and New South Wales National Parks and Wildlife Service (SL100791).

171

172 **Results**

173 Over the study period weather conditions consisted of days with frost and snow and a mean
174 daily T_{a} of $5.3 \pm 1.9^{\circ}\text{C}$ ($n = 12$, range: 1.8 to 7.7°C). The T_{a} fluctuated from a mean minimum

175 T_a of $0.5 \pm 2.1^\circ\text{C}$ ($n = 12$, range: -3.0 to 3.5°C) to a mean maximum T_a of $11.2 \pm 1.9^\circ\text{C}$ ($n =$
176 12 , range: 7.0 to 14°C), with a mean daily T_a range of $10.7 \pm 2.3^\circ\text{C}$ ($n = 12$, range: 7.0 to
177 14.5°C). Temperatures fell below 0°C on 42% of the study days.

178

179 All roost trees were *Eucalyptus* spp. ranging from 9m up to 12m in height. Female #1 and
180 male #1 roosted separately in high branches of dead trees with no foliage throughout the
181 entire recording period (female #1: branch height = 9 m, branch diameter = ~ 0.1 m, DBH =
182 0.9 m; male 1: branch height = 6 m, branch diameter = ~ 0.2 m, DBH = 1.3 m. Conversely,
183 female #2 roosted low (0.7m from the ground) in a large cavity (diameter = 1.6m) inside a
184 living tree (DBH = 1.6 m) with thick crown foliage. Both females were found roosting in a
185 large stand of remnant trees (>30 trees) near the main homestead, which was much larger
186 than other stands of trees in the area. On the other hand, the male roosted in a tree within a
187 very small remnant, ~ 1 km from the females, that consisted of <10 trees.

188

189 Due to the small sample size we could not accurately test for a roost effect. However, it is
190 likely that roost choice influenced daily patterns of torpor T_{skin} as, for all T_{skin} variables
191 measured, female #2 was significantly higher than female #1 and male #1 (results from a
192 post-hoc Tukey test, female #1 and male #1 did not differ for any of the variables; Fig. 1).
193 Importantly, throughout this cold winter period all individuals employed torpor on every day
194 data were collected, regardless of roost choice, with torpor bout durations ranging from 20.8
195 to 126.5 h (Fig. 1). Daily mean torpor T_{skin} was significantly higher for female #2 ($13.2 \pm$
196 1.3°C , $n = 1$, $N = 8$) when compared to the other individuals ($6.4 \pm 1.3^\circ\text{C}$, $n = 2$, $N = 9$; $p <$
197 0.0001 , $f_{2,14} = 54.4$). This was also the case for both the daily minimum and maximum T_{skin}
198 during torpor (min: $p < 0.0001$, $f_{2,14} = 94.8$; max: $p = 0.002$, $f_{2,8} = 15.6$). In both cases female
199 #2 had a torpor T_{skin} at least 5°C greater than the other individuals. Further, the $T_{\text{skin}} - T_a$

200 differential during torpor bouts was significantly larger ($p < 0.0001$, $f_{2,1680} = 766.2$) for female
201 #2 (5.8 ± 1.1 , $n = 1$, $N = 3$) in comparison to both female #1 and male #1 (0.5 ± 0.001 , $n = 2$,
202 $N = 2$). Interestingly, the absolute lowest T_{skin} recorded throughout the study was -0.2°C for
203 female #1, and in fact the T_{skin} of female #1 and male #2 combined fell below 3°C during
204 torpor on 67% of bat days. The corresponding T_{a} at this time was -0.5°C , therefore the $T_{\text{skin}} -$
205 T_{a} differential was 0.3°C and, based on T_{b} values obtained for *C. gouldii* in the laboratory
206 (Currie et al., in preparation, measured at T_{a} down to -2.0°C), T_{b} was $\sim 1^{\circ}\text{C}$.

207

208 For all individuals, the expression of torpor was influenced by ambient conditions, with
209 torpor bout duration increasing with decreasing daily minimum T_{a} ($p = 0.015$, $f_{1,3} = 25.6$, R^2
210 $= 0.8$; Fig. 2). Further, daily minimum T_{a} on days individuals remained torpid and expressed
211 multiday torpor bouts ($-0.4 \pm 1.8^{\circ}\text{C}$, $n = 3$, $N = 11$) was significantly lower in comparison to
212 days when bats aroused ($2.4 \pm 0.7^{\circ}\text{C}$, $n = 3$, $N = 6$; $p = 0.0003$, $t = -4.7$, $df = 14.0$). However,
213 while for female #1 and male #1 daily mean torpor T_{skin} significantly decreased with daily
214 minimum T_{a} ($p < 0.0001$, $f_{1,7} = 91.7$, $R^2 = 0.9$; Fig. 3), no significant relationship was found
215 for female #2 ($p = 0.252$, $f_{1,6} = 1.6$, $R^2 = 0.2$; Fig. 3).

216

217 **Discussion**

218 Our study is the first to document the thermal physiology of free-ranging *C. gouldii*. In
219 particular, we provide important new information on the effect of roost choice on T_{skin} during
220 mid-winter in a temperate agricultural area. Torpor was employed by all individuals on every
221 day of the study period and we recorded the lowest T_{skin} for an Australian microbat. This
222 result is perhaps not unexpected as a previous laboratory study found that at a T_{a} of 4°C *C.*
223 *gouldii* displayed a corresponding T_{b} of 5.3°C (Hosken and Withers 1997). While this
224 previous study did not test the bats at T_{a} lower than 4°C , our current study on free-ranging

225 bats suggests that this southern hemisphere hibernating bat is able to remain torpid at very
226 low T_a and also withstand T_{skin} at least down to -0.2°C ($T_b \sim 1^{\circ}\text{C}$), which is similar to northern
227 hemisphere hibernators (Ruf and Geiser 2015). Moreover, two of the individuals in this study
228 had T_{skin} during torpor that was $\leq 3^{\circ}\text{C}$ on 67% of bat days, further emphasising their ability to
229 withstand low T_b over extended periods.

230

231 Similar to other hibernating bats (Turbill and Geiser 2008; Stawski *et al.* 2014), torpor bouts
232 lasted from just less than 24h to over five days and torpor duration significantly increased
233 with decreasing T_a . On the days that bats did arouse the daily minimum T_a was significantly
234 higher in comparison to days bats remained torpid. The energetic costs of remaining
235 normothermic and foraging would be extremely high on cold days (Hosken and Withers
236 1997), therefore remaining torpid would become more beneficial. Importantly, torpor and
237 arousals can be facilitated by roost choice, with cool stable roosts promoting torpor and
238 roosts with fluctuating daily temperatures enabling passive arousals to save energy (Stawski
239 *et al.* 2008; Doucette *et al.* 2011; Currie *et al.* 2015). This is important in terms of food
240 availability, as insect activity is reduced on colder days, suggesting that energetic returns
241 would be negligible (Richards 1989; Pavey and Burwell 2004; Stawski 2012).

242

243 While all individuals roosted in trees throughout the study period, two roosted in high
244 branches of dead trees, similar to previous studies on *C. gouldii* (Lumsden *et al.* 2002b),
245 while the remaining individual roosted low to the ground in the cavity of a live tree.
246 Interestingly, the depth of torpor bouts was deeper for individuals roosting in dead trees in
247 comparison to the individual that roosted in a live tree. Therefore, even though the
248 relationship between torpor bout duration and T_a was similar for all individuals, the daily T_{skin}
249 fluctuations differed, likely in response to roost microclimate. The T_{skin} of individuals that

250 roosted in the high branches of dead trees closely followed T_a during the whole study and
251 only deviated when they aroused. This was likely facilitated by the small diameter of the
252 roosts and also that these individuals were probably roosting solitarily. Indeed, a previous
253 study revealed that the microbat *Plecotus auritus* displays lower T_{skin} during torpor and roosts
254 in thinner trees that are thermally unstable with low minimum T_a in comparison to two other
255 species (Otto *et al.* 2016).

256

257 On the other hand, the individual that roosted low in the live tree cavity experienced daily
258 T_{skin} fluctuations that were much higher than the daily fluctuations in T_a . As the diameter of
259 this tree was 8-fold larger than that of the branches it was likely buffered and therefore
260 warmer than outside conditions. A previous study revealed that live trees are warmer than
261 dead trees during winter and also lose heat more slowly; likely due to the fluids in live trees
262 and the heat these fluids retain (McComb and Noble 1981; Coombs *et al.* 2010). It is also
263 possible that the individual in the tree cavity was roosting with other individuals as *C. gouldii*
264 are known to roost in colonies of up to 20 individuals (Lumsden and Bennett 2000), which
265 would elevate the temperature within the roost and hence influence T_{skin} . Clustering
266 behaviour and social thermoregulation not only saves energy during normothermic periods,
267 but it also reduces evaporative water loss during torpor and the energetic costs of arousals
268 (Willis and Brigham 2007; Boratyński *et al.* 2012). Further, the individual that was roosting
269 in the live tree cavity was ~2g lighter than the other two individuals, suggesting that this bat
270 was more energetically constrained. Therefore, it would be beneficial for this lighter bat to
271 roost in a warmer environment to help facilitate arousals to feed and gain weight, whereas the
272 individuals in better body condition may choose to remain torpid in a cooler roost as they do
273 not need to feed and can therefore reduce exposure to predators (Stawski and Geiser 2010).

274

275 Our study has revealed that appropriate roosting options for bats are vital for certain life
276 history strategies, such as torpor use to save energy during cold weather. In addition to *C.*
277 *gouldii* from the current study, it is known that many tree-roosting bats prefer roosting in
278 large trees (Brigham *et al.* 1997; Law and Anderson 2000; Lumsden *et al.* 2002b) and that
279 thermal physiology is indeed affected by roost choice (Stawski *et al.* 2008; Doucette *et al.*
280 2011; Otto *et al.* 2016), which emphasises the importance of maintaining old-growth stands
281 of native vegetation in agricultural areas to encourage bat biodiversity. This is also vital as
282 many bats show strong habitat and roost fidelity (Lumsden *et al.* 2002a; Lučan *et al.* 2009).
283 Indeed, in our current study one individual that was released following two months in
284 captivity at a significant distance from her initial capture location, was found roosting in a
285 tree close to her original building roost the following morning. Importantly, these data
286 suggest that relocating bats is not a viable practice. Further, our research has provided new
287 data on free-ranging *C. gouldii* that have revealed that the thermal physiology of this southern
288 hemisphere hibernating bat is similar to that of northern hemisphere hibernating bats (Stawski
289 *et al.* 2014). This highlights the importance of studying the physiological ecology of southern
290 hemisphere bats, for which data are lacking, to determine the similarities and differences
291 among bats worldwide. It would be interesting to obtain a larger data set for the thermal
292 physiology of free-ranging *C. gouldii* in other parts of its range and also in undisturbed
293 habitats to determine if this species shows long-term adaptations to specific habitats or shows
294 phenotypic plasticity throughout its range. While the results of studies on the ecosystem
295 services provided by bats will likely be beneficial in encouraging agricultural practices that
296 maintain biodiversity (Kunz *et al.* 2011), developing these management practices will rely on
297 the information ascertained by studies such as ours, that provide vital details regarding
298 roosting ecology and its strong relationship to thermal physiology.

299

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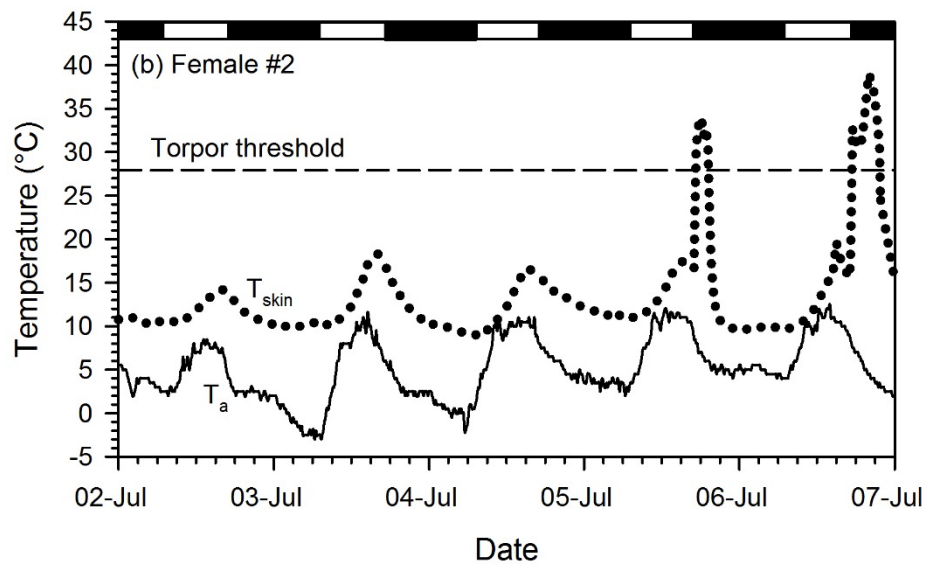
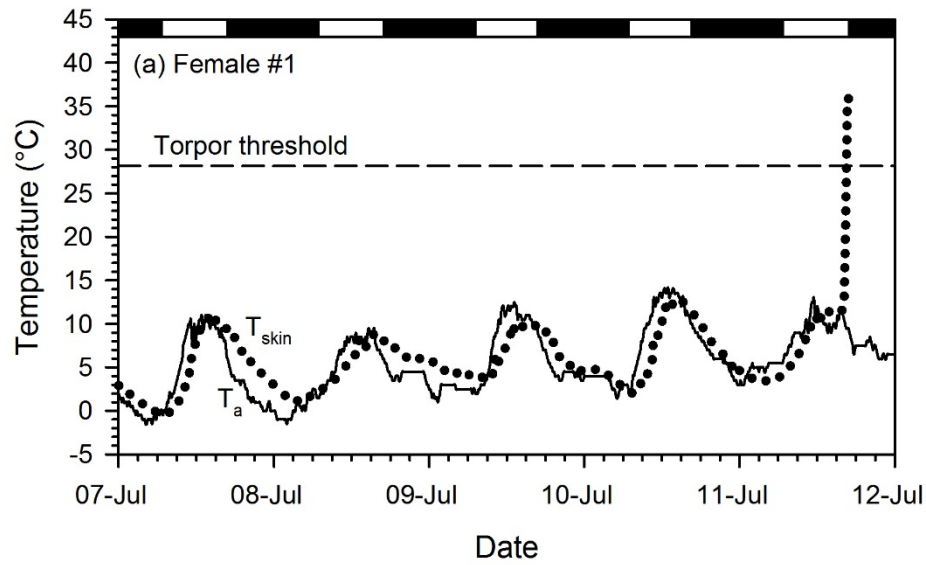
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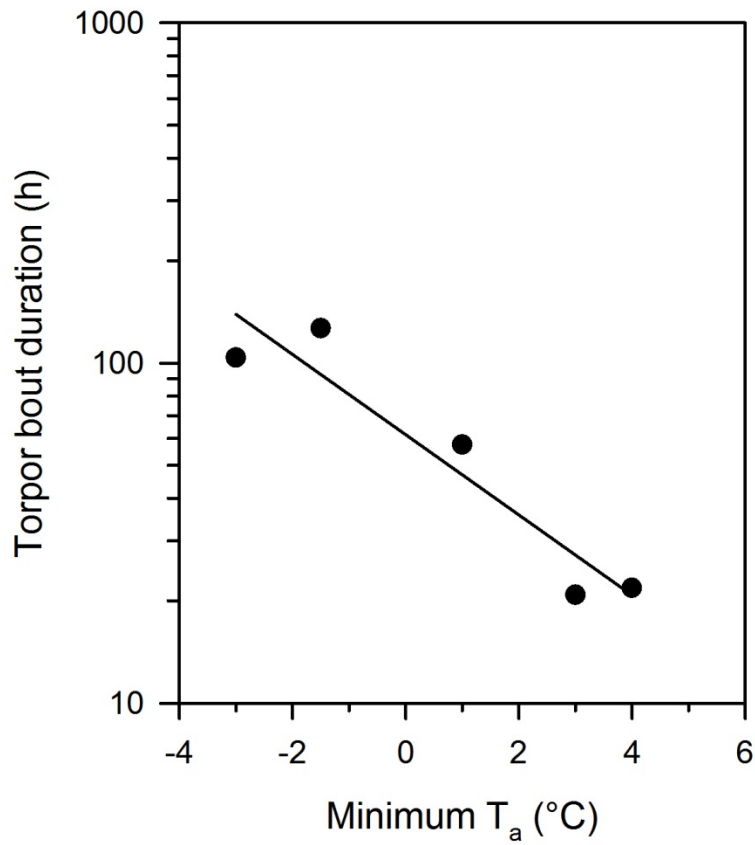
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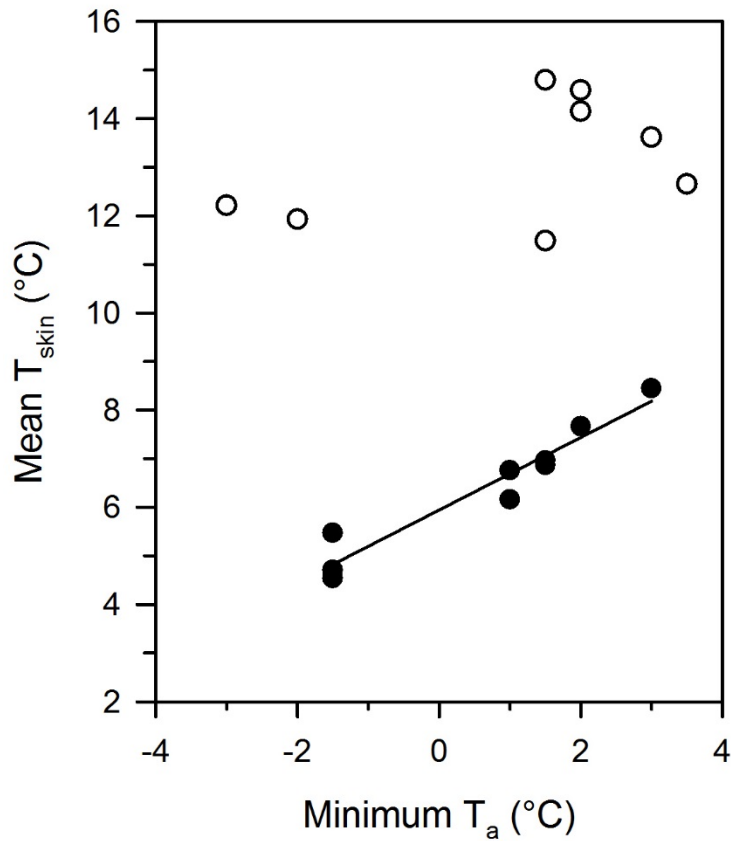
436 **Fig. 1.** Skin temperature (T_{skin} ; dotted line) and ambient temperature (T_a ; solid line) traces
 437 over five days of the study period for (a) female #1 and (b) female #2. The dashed line
 438 represents the torpor threshold and the black and white bars at the top of the graphs represent
 439 night and day, respectively.



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442 **Fig. 2.** For all individuals, as daily minimum ambient temperature (T_a) decreased, torpor bout
443 duration increased ($p = 0.015, f_{1,3} = 25.6, R^2 = 0.8, y = -15.1x + 76.2$)



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445

446 **Fig. 3.** Daily mean torpor skin temperature (T_{skin}) significantly increased with daily minimum
 447 ambient temperature (T_a) for female #1 and male #1 that roosted in high branches of dead
 448 trees (solid circles with regression line: $p < 0.0001$, $f_{1,7} = 91.7$, $R^2 = 0.9$, $y = 0.7x + 5.9$).
 449 However, no significant relationship was found for female #2 that roosted in a low large
 450 cavity in a live tree (open circles: $p = 0.252$, $f_{1,6} = 1.6$, $R^2 = 0.2$).