An experimental test of effects of ambient temperature and roost quality on aggregation by little brown bats (*Myotis lucifugus*)

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ABSTRACT

Environmental factors, such as ambient temperature (T_a) or roost/nest quality, can influence social behaviour of small-bodied endotherms because individuals may aggregate for social thermoregulation when T_a is low or select the warmest possible sites for roosting. Female temperate bats form maternity colonies in spring to communally raise pups and exploit social thermoregulation. They also select roosts with warm microclimates because low roost temperature (T_{roost}) delays juvenile development. We studied captive female little brown bats (*Myotis lucifugus*) to test the hypothesis that variation in T_a and T_{roost} influence social group size. First, we predicted that female bats would preferentially select artificially heated roosts over unheated roosts. Second, we predicted that, as T_a decreased, group size would increase because bats would rely more heavily on social thermoregulation. Third, we predicted that experimentally increasing T_{roost} (i.e., roost quality) above T_a would result in larger group sizes due to greater aggregation in high quality roosts. We captured 34 females from a maternity colony and housed them in a flight-tent provisioned with four bat boxes. Each box was outfitted with a heating pad and thermostat. Over the course of eight-days we heated each roost box in sequence to near thermoneutral T_{roost} for two days. Bats preferentially selected heated roosts over unheated roosts but, contrary to our prediction, group size decreased when T_{roost} was much greater than T_a (i.e., when the benefits of a warm roost should have been highest). Our results suggest that social thermoregulation and the availability of warm roosts influence aggregation in bats and have implications for the potential of summer habitat protection and enhancement to help bat populations in the face of threats like white-nose syndrome.

1. Introduction

Virtually all animals engage in social interactions with conspecifics at some point in their lives (e.g., for mating, cooperation, or competition: Alexander, 1974; Silk, 2007) and sociality can have fitness consequences (Kappeler et al., 2013). Social aggregation is often driven by environmental factors that influence the costs and benefits of interactions among individuals. For instance, European badgers (*Meles meles*) are typically solitary but occasionally aggregate in social groups when food resources are scarce (Johnson et al., 2000). Variation in habitat quality can influence social structure (Webber and Vander Wal, 2018), particularly if the costs of competition for high quality habitat increases to a point where individuals disperse and social structure deteriorates. Alternatively, when high quality habitat is limited, individuals may aggregate out of necessity (Silk, 2007).

Thermal characteristics of habitats, such as ambient temperature (T_a) and nest or roost microclimate, can have strong influence on social aggregation. In temperate regions, where T_a fluctuates widely and can

drop below freezing for much of the year, many animals actively associate and exploit social thermoregulation during periods of low T_a or resource scarcity to reduce energy expenditure (Edelman and Koprowski, 2007; Mckechnie and Lovegrove, 2001; Ostner, 2002). Many small-bodied endotherms also select habitats for nesting, denning, or roosting that decrease thermoregulatory costs associated with low T_a (e.g., subterranean burrows: Begall et al., 2007; sites with high levels of radiant heat: Warnecke and Geiser, 2010; sites that reduce convective heat loss: Willis and Brigham, 2005). Thus, individuals may interact due to either passive aggregation in relatively warm, favourable den or nest sites, or active aggregation for social thermoregulation.

Temperate bats are small-bodied endotherms that preferentially select roosting sites with warm microclimates during the active season to decrease thermoregulatory costs (Kerth et al., 2001b; Vonhof and Barclay, 1996; Willis and Brigham, 2005). Roost selection may vary throughout the active season and differs for male and female bats. In spring and early summer, females of many temperate species form maternity colonies to give birth and rear pups (Kunz and Lumsden,

2003). Bats at maternity colonies display fission-fusion dynamics, where individuals temporarily split into sub-groups before subsequently merging again (e.g., Garroway and Broders, 2007; Kerth, 2008; Kerth and Konig, 1999; Willis and Brigham, 2004). For colonies living in tree hollows, females typically change roosts every few days but not all members of the group move together, resulting in variable group size and composition (e.g., Patriquin et al., 2010). Spatial variation in the availability of thermally suitable roosts could be an ecological constraint driving social relationships and stability for bats living in fission-fusion societies (Kerth, 2008; Patriquin et al., 2016; Patriquin and Ratcliffe, 2016).

Understanding roost selection decisions of North American bats is especially important for wildlife conservation in light of threats like habitat loss and white-nose syndrome (WNS). In general, forest-dwelling bats roost in trees that are taller, have larger diameters, and more open canopies relative to random trees (Kalcounis-Ruppell et al., 2005) and bats appear to select roosts based on microclimate within roost cavities (Boyles, 2007; Kerth et al., 2001b; Sedgeley, 2001). Females are thought to select roosts with warm microclimates because exposure to low T_a can induce torpor (e.g., Dzal and Brigham, 2013; Solick and Barclay, 2006) and delay juvenile development (Racey and Swift, 1981). While most temperate bats roost in trees for at least part of their annual-cycle, many species also roost in anthropogenic structures (e.g., buildings, bat-boxes; Lausen and Barclay, 2006). The main advantage of anthropogenic structures over trees appears to be that buildings and bat-boxes studied to date generally have warmer, more stable, microclimates (Kerth et al., 2001b; Lausen and Barclay, 2006). Thus, the available evidence suggests that bats should be most strongly motivated to select the warmest roosts and aggregate in the largest groups for huddling when T_a falls (Speakman and Thomas, 2003).

A number of studies have examined correlations between use of roosts by bats and roost temperature (T_{roost}: Johnson and Lacki, 2014; Sedgeley, 2001; Willis and Brigham, 2007) but few have manipulated roost temperature experimentally to quantify causal effects (although see Kerth et al., 2001b). We experimentally adjusted T_{roost} for a captive colony of female little brown bats (*Myotis lucifugus*) housed in a flight enclosure to test the hypothesis that ambient conditions and habitat quality affect aggregation by bats. We evaluated three predictions: 1) That female bats would preferentially select artificially heated roosts over unheated roosts as found for captive, post-hibernating male bats in the laboratory (Wilcox and Willis, 2016); 2) That roosting group sizes would be negatively correlated with T_a and, especially, the difference between T_{roost} and T_a, because individuals would rely more heavily on social thermoregulation in warm roosts during cold conditions; and 3) Positive effects of roost occupation by bats, and numbers of bats in a given roost, on T_{roost} as observed for free-ranging big brown bats (*Eptesicus fuscus*) roosting in cavities in trembling aspen trees (*Populus tremuloides*) (Willis and Brigham, 2007).

2. Methods

All procedures were approved by the University of Winnipeg Animal Care Committee, conducted in compliance with guidelines of the Canadian Council on Animal Care and approved under Manitoba Conservation Wildlife Scientific Permit number WB16368. Although all sites were negative for *Pseudogymnoascus destructans*, the fungal pathogen that causes WNS in bats, we followed U.S. Fish and Wildlife Service and Canadian Wildlife Health Cooperative (CWHC) guidelines for decontamination by researchers (Canadian Wildlife Health Cooperative, 2015; United States Fish and Wildlife Service, 2016).

2.1. Study site and subjects

We captured 40 adult female little brown bats (*Myotis lucifugus*) on 5 June 2014, from a colony of ~ 250 individuals living in two bat boxes at a summer cottage near Nutimik Lake, Manitoba (50.14°N, 95.69°W).

We captured bats using bucket traps attached to the bottom of each bat box (i.e., a tube with ~ 75 cm diameter consisting of 50 cm of plastic sheeting attached to an additional 50 cm of mesh screening with a blind bottom). Bats entered the trap at dusk by either flying or sliding along the plastic part of the trap and were held in the blind mesh bottom for approximately 30 min until all bats were caught. To minimise variation in roost selection and social thermoregulation resulting from bats being in different stages of reproduction during our experiment (i.e., early- vs. late-pregnancy, pregnancy vs. lactation), we immediately released detectably pregnant individuals after gently palpating the abdomen. No bats were lactating and we did not capture any pups. Although bats used in our experiment (see below) were not palpably pregnant, most if not all were likely in the early stages of pregnancy and we assumed these individuals had similar social tendencies and energetic requirements as pregnant bats because we captured them from a maternity colony alongside females that were in the early stages of detectable pregnancy. To allow for permanent identification, within 30 min of capture, we implanted a uniquely coded passive transponder (PIT tag, Trovan Ltd. ID 100-01, Douglas, UK) subcutaneously between the scapulae for each bat used in our experiment.

2.2. Transport and housing

Bats were transported from the capture site ~ 80 km to the Sandilands Forest Discovery Centre, Manitoba, Canada. During transport, they were suspended in cloth bags in groups of three or four inside a ventilated picnic cooler to dampen noise during transport. Upon arrival at the Forest Centre bats were transferred to a nylon mesh flight tent (2.75 m × 2.75 m × 2.75 m) with a shaded roof but which was otherwise open to ambient conditions. The flight tent was outfitted with four roost boxes constructed from cleanable 'vinyl plywood' each with a volume of 3000 cm^3 (height: 20 cm, length: 30 cm, width: 5 cm). Each roost box was mounted on a 1.5 m stand.

Bats were only disturbed in the flight tent each night at dusk (~ 21:30) for approximately 30 min when they were weighed to the nearest 0.01 g, and food and water were replenished. Throughout captivity bats were provided water and mealworms (larval *Tenebrio molitor*) *ad libitum* on a 75 cm by 50 cm folding table placed in the centre of the flight tent. Bats were trained to eat mealworms by hand during the 5 days prior to the start of our experiment (see below). All bats that were unable to eat meal worms independently during the 5-day training period were released at the capture site (n = 6 bats were released, while n = 34 bats were used in subsequent experiments). Mealworms were gut-loaded with beta carotene multivitamins (Herptivite, California, USA) and nutrient supplements (Repashy Superfoods, Oceanside, California, USA).

2.3. Quantifying group size

Each night at dusk (~ 21:30) when we entered the flight tent to weigh bats, we recorded the number and identities of bats roosting in each box. Occasionally, bats did not roost in a box but, instead, hung on the mesh lining of the flight tent (0.5% of 510 overall bat-days). We did not consider these bat-nights in our analyses because we were interested in group sizes within available roost boxes and because most bats roosted in bat boxes.

2.4. Assessing effects of roost quality

Each bat box was outfitted with a reptile heating mat (160 cm^2, HMA-4, All Living Things™) which allowed us to manipulate T_{roost}. Temperature and humidity data loggers (HOBO Micro Station – H21-002, Onset Computer Corporation, Cape Cod, Massachusetts, USA) were positioned in the centre of each roost box, while an additional shaded data logger was placed adjacent to the flight tent to monitor T_a outside roosts. Roost boxes were not insulated and T_{roost} of heated

boxes fluctuated with T_a so the difference between heated and unheated T_{roost} was relatively small. However, based on values of metabolic rate (MR) calculated using the equation that Jonasson and Willis (2012) derived from Studier (1981) (i.e., MR ($mlO_2 \, g^{-1} \, h^{-1}$) = $0.193(T_b–T_a)^2$ + 0.268), the difference in T_{roost} between heated vs. unheated roosts would have been energetically significant (see Results). We used the sunrise $T_{roost}–T_a$ differential (hereafter, $T_{roost}–T_a$) as an index of 'roost quality' and we assumed that higher values of $T_{roost}–T_a$ reflected higher quality roosts, regardless of whether the roost was heated or not. We used values taken at sunrise because 80% of bats selected roosts within 60 min of sunrise. Therefore, values at sunrise should reflect a criterion used by bats to select roosts. We used both the $T_{roost}–T_a$ differential as well as absolute values of T_a at sunrise as predictor variables to tease apart effects of T_a and T_{roost} on roost selection and aggregation.

We housed bats in the flight tent for 15 nights. For the first five nights, and the last two nights of the experiment we did not manipulate T_{roost} to provide a period during which we could assess roost selection decisions of the bats in the absence of artificial heating. We pooled data from these seven nights (hereafter the "unheated period") for our analyses. We manipulated T_{roost} starting on night six for eight nights to assess how microclimates might influence aggregation and social thermoregulation (hereafter the "heated period"). During the heated period, we first activated the heating mat in one randomly selected roost box for 48 h beginning at ∼ 22:00 (after bats had emerged from roosts for the night), then deactivated the heater in this box and heated a second box for the next 48 h, followed by the third and fourth boxes, each for an additional 48 h. Thus, we heated each roost box in sequence for two days at a time providing artificial heating in one of four available boxes each night. This pattern of heating allowed us to experimentally assess the influence of T_{roost} on roost selection and aggregation.

2.5. Statistical analysis

All statistical analyses were conducted in R (R Core Team, 2017). We used a linear regression to examine the relationship between sunrise $T_{roost}–T_a$ and maximum $T_{roost}–T_a$ to determine whether microclimate in the early morning when bats were presumably selecting roosts predicted daily maximum T_{roost} during the day. We also used an ANOVA to examine the relationship between $T_{roost}–T_a$ and whether, or not, a given box was heated (see below). We then used a linear mixed model ('lmer' function: Bates et al., 2015) to assess the role of ambient conditions and roost microclimate on daily roosting group size. We log-transformed roosting group size prior to analysis to meet assumptions for parametric analyses. We used the $T_{roost}–T_a$ differential for each box on each day, and whether or not a given box was heated or not (roost heating), as predictors of log-transformed day-roosting group size. We also included T_a at sunrise as a predictor of log-transformed day-roosting group size. The 'roost heating' predictor variable was comprised of three levels: 1) heated roost during the heated period; 2) unheated roost during the heated period; and 3) unheated roost during the unheated period prior to activating any of the heated boxes. We included the identity of each individual roost box (i.e., roost 1, 2, 3, or 4) as a random effect in the model to control for potential preferences by bats for one box or another, independent of microclimate. We also tested for correlations between maximum values of $T_{roost}–T_a$ (measured during the day, as opposed to at sunrise, see below) and log-transformed day-roosting group size using linear regressions to determine if the number of bats in a given roost affected T_{roost}. Significance was assessed at α = 0.05 and all results are presented as mean ± standard deviation unless otherwise specified.

2.6. Lagged association rate

To assess temporal patterns in bat associations we calculated the lagged association rate (LAR), which measures the average probability
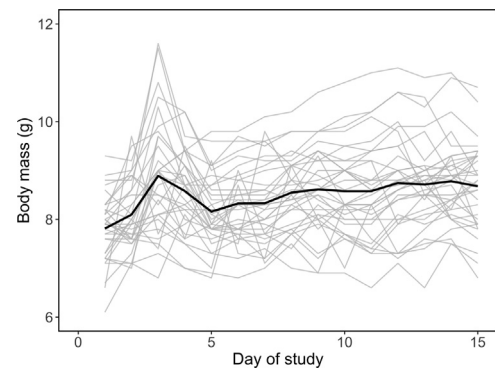

Fig. 1. Temporal change in body mass (g) for little brown bats (N = 34) during captivity. Thin grey lines represent changes in body mass for each individual bat throughout captivity, while the thick black line represents the average body mass on each day.

that pairs of bats roosting together on a given day were still roosting together on subsequent days (Whitehead, 2008). We used a jackknifing technique to compare observed LAR values to a null association rate (Farine, 2013), which is calculated by iteratively removing a single individual and re-calculating the LAR without that individual (Whitehead, 2008).

3. Results

Body mass fluctuated for individuals during captivity, but we found no effect of time in captivity on body mass (Fig. 1). At capture, female little brown bats weighed 8.75 ± 0.8 g and they maintained consistent body mass until release when they weighed, on average, 8.67 ± 0.9 g (Fig. 1).

During our 15-day study period, T_a ranged from a nightly minimum of − 1.0 °C to a daily maximum of 36.0 °C Table 1; Fig. 2). During the unheated period, T_{roost} ranged from a minimum nightly low of 1.7 °C to a daily maximum of 40.3 °C across all boxes. During the heated box period, T_{roost} in unheated boxes ranged from a nightly minimum of 2.9 °C to a daily maximum of 39.3 °C ($T_{roost}–T_a$ differential: − 8.7 to 15.0 °C) and T_{roost} for heated boxes ranged from a nightly minimum of 6.0 °C to a daily maximum of 32.4 °C ($T_{roost}–T_a$ differential: – 2 to 12.1 °C). The high maximum T_{roost} we observed for unheated boxes was driven by a single box that was very warm for two days (for details see Fig. 3). The flight tent was shaded but this higher maximum likely

Table 1

Summary of weather variables for the 15 day study period. Experimental period refers to whether or not roost boxes were heated; $T_{a\,max}$ refers to maximum daily temperature; $T_{a\,min}$ refers to minimum nightly temperature; $T_{a\,sunrise}$ refers to daily temperature at sunrise (∼ 4:20).

| Date	Experimental period	$T_{a\,max}$ (°C)	$T_{a\,min}$ (°C)	$T_{a\,sunrise}$ (°C)	Proportion of bats in heated roost
June 7	No	28.5	− 1.0	0	–
June 8	No	30.0	0	0.5	–
June 9	No	31.0	6.0	8.0	–
June 10	No	36	8.5	11.0	–
June 11	No	26.5	9.0	11.0	–
June 12	Yes	27.0	5.0	8.0	0.00
June 13	Yes	29.5	− 0.5	0.5	0.06
June 14	Yes	20.0	10.0	10.0	0.35
June 15	Yes	12.0	10.5	10.5	0.36
June 16	Yes	31.0	8.5	9.0	0.50
June 17	Yes	30.5	3.5	4.0	0.88
June 18	Yes	33.5	8.0	9.5	0.50
June 19	Yes	23.0	14.0	14.5	0.18
June 20	No	33.0	9.5	10.5	–
June 21	No	40.5	8.5	11.5	–
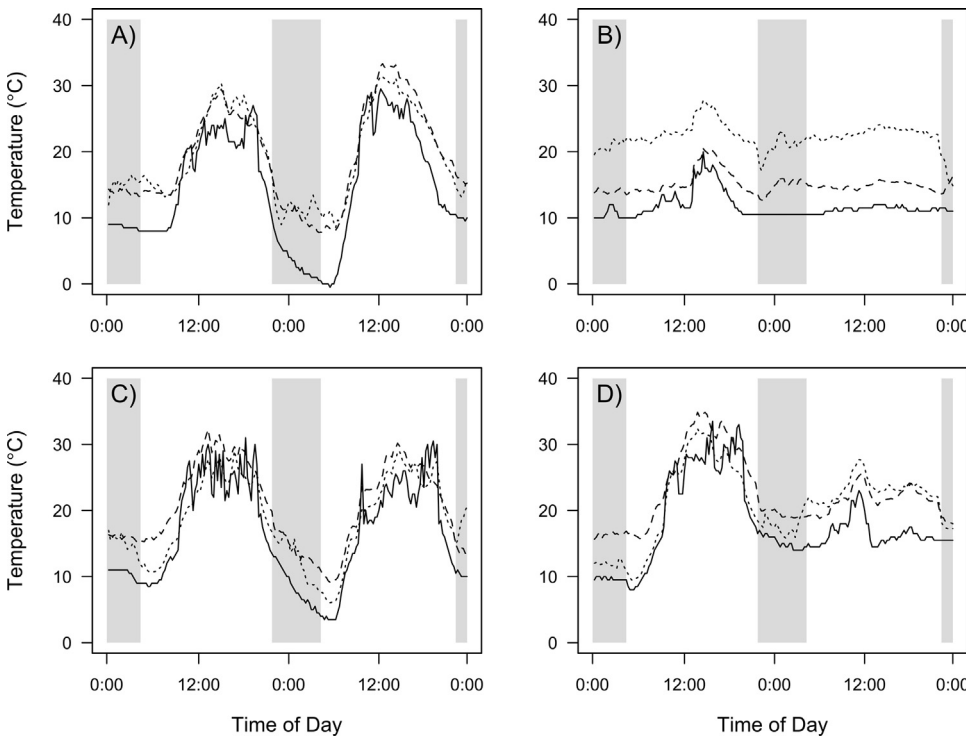
Fig. 2. Comparison of ambient temperature (°C) (solid black lines) to heated roost temperature (°C) (thin dashed lines) and mean temperature inside three unheated roosts (thick dashed lines) for four 48-h heated roost trials on A) June 12–13: box 1 heated; B) June 14–15: box 2 heated; C) June 16–17: box 3 heated; and D) June 18–19: box 4 heated. Grey shaded sections represent the scotophase (21:30 to 04:30 at our high latitude study site).
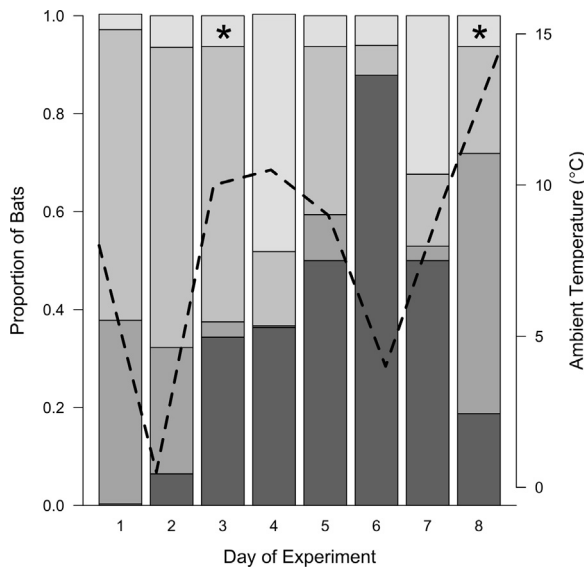

Fig. 3. Proportion of captive little brown bats (*Myotis lucifugus*) roosting in heated (dark grey bars) or one of three unheated roosts (each represented by a lighter shade of grey) during the eight-day period when one roost was heated per day. Right-hand axis and dashed line represents the minimum ambient temperature on each night of the experiment. Asterisks denote unheated boxes that were unusually hot on two days, likely due to sun exposure. We excluded data from these boxes on these days for our calculations of some summary statistics (see Section 3).

reflects sun exposure for that particular box. Excluding that box on those two days, the maximum T_{roost} in unheated boxes during the heated period was 33.7 °C. On average, heated boxes had a consistently higher $T_{roost}–T_a$ differential at sunrise (6.9 ± 4.0 °C) (ANOVA: $F_{2,57}$ = 4.3, p = 0.01) compared to unheated boxes during the heated box period (5.7 ± 3.9 °C) (Tukey's adjusted p-value = 0.03). Although this difference seems relatively small, heated roosts would have saved bats a considerable amount of energy. For example, a bat staying

normothermic at the minimum T_{roost} for heated roosts (i.e., 6 °C) would have benefited from a > 10% reduction in metabolic rate compared to remaining normothermic at the minimum temperature for un-heated roost (i.e., 2.9 °C).

We obtained 15 days of roost selection data from bats with four available roosts per day (i.e., N = 60 roost days). Consistent with our first prediction, during the 8-day heating period mean group size was 11.6 ± 9.3 bats per day in heated roosts (range: 0–29 bats, n = 8 heated roost days) versus only 6.9 ± 4.0 bats (range: 0–20 bats, n = 24 unheated roost days) bats per day in unheated roosts. During the pooled 5 + 2-day unheated period (n = 28 unheated roost days) group size was 7.3 ± 4.0 (range: 1–18) bats per day per roost. We found a positive relationship between sunrise $T_{roost}–T_a$ and maximum daily $T_{roost}–T_a$ (r^2 = 0.20, p = 0.0003; Fig. 4) indicating that T_{roost} at the time when bats were selecting roosts was a reliable predictor of roost microclimate later in the day).

Group size varied depending on whether a given roost was heated (Fig. 3). Consistent with our second prediction, on average during the heated period, heated roosts had larger group sizes than unheated roosts and there was no difference in group sizes recorded in unheated
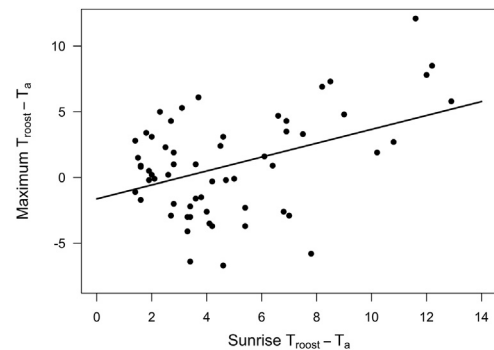

Fig. 4. Positive relationship between maximum $T_{roost}–T_a$ and sunrise $T_{roost}–T_a$ for 60 roost days (15 days with 4 available roosts per day) indicating that microclimate conditions at sunrise predict maximum microclimate conditions during the day.

Table 2

Summary of linear mixed effects model assessing effects of ambient conditions and roost temperature on group size for 34 little brown bats over 15 days in captivity (7 days with no heated roost treatment and 8 days when one roost was heated). The model included the identification of each roost box (i.e., 1, 2, 3, or 4) as a random factor.

Fixed effects	Slope ± S.E.	z-value	p-value
Intercept	2.58 ± 0.42	6.1	**< 0.001**
T_{roost}–T_a	− 0.07 ± 0.03	− 2.4	**0.01**
Sunrise T_a	0.007 ± 0.02	0.36	0.71
Heated roost use			
– Unheated roost use (during heating period)[a]	− 0.57 ± 0.28	− 2.0	**0.04**
– Unheated period[a]	− 0.39 ± 0.29	− 1.3	0.18
Random effects	**Variance ± S.D.**		
Box	0.186 ± 0.43		
Residual	0.469 ± 0.68		

[a] Compared to number of bats roosting in heated roosts during the experimental period.

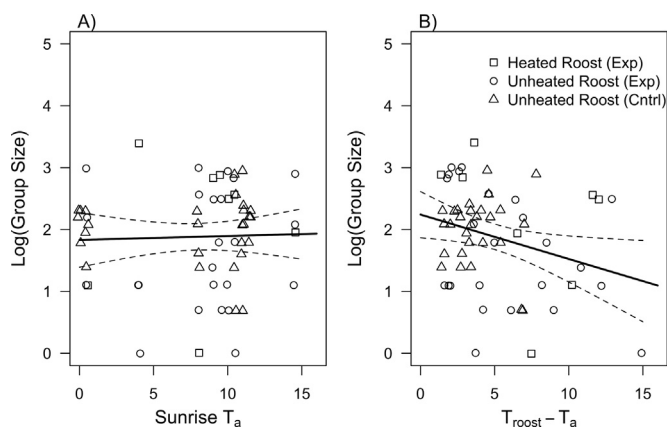**Fig. 5.** Relationship between log-transformed group size (i.e., number of bats roosting in a roost) and A) Sunrise T_a and B) T_{roost}–T_a at sunrise for 34 little brown bats (Myotis lucifugus). Data points represent number of bats roosting in each roost (n = 4 available roosts per day) on each day (n = 15 days) during each stage of the experimental period (N = 60 roost-days). Trend line represents linear regression between log-transformed group size and T_{roost}–T_a for all roost-days combined (see Table 2 for full model presentation). Open squares represent heated roosts during the heating period; open circles represent unheated roost during the heating period; open triangles represent all roosts during the unheated control period.

roosts between the heated and unheated periods (Table 2). However, we found a negative relationship between T_{roost}–T_a and group size (Fig. 5). When T_{roost} approached T_a, bats aggregated into larger groups, possibly to reduce thermal conductance and energy expenditure but, in contrast to our prediction, when T_{roost} was higher than T_a, bats divided into smaller groups and spread out among all roosts (Fig. 3). We did not detect an effect of sunrise T_a on group size. In contrast to our third prediction, we found no evidence of an influence of occupation by the bats on microclimate in either heated or unheated boxes; maximum T_{roost}–T_a during the day was not correlated with group size (heated roosts during the heated period: r^2 = 0.001, $F_{1,6}$ = 0.05, p = 0.83; unheated roosts during the heated period: r^2 = 0.03, $F_{1,22}$ = 0.9, p = 0.34; and all roosts during the unheated period: r^2 = 0.002, $F_{1,26}$ = 0.05, p = 0.83).

We did not find strong evidence of preferential roosting associations among pairs of bats across the timescale of our experiment. Ninety-eight percent of all possible dyads roosted together on at least one night over the course of the experiment. The LAR was relatively stable until day 6, at which point it began to drop but neither observed nor jack-knifed LAR values dropped below an association rate of 0.3 (Fig. 6).

This indicates that, on average, there was at least a 30% chance that any given pair of bats roosted together for all 8 days of our experiment (Fig. 6).

4. Discussion

We found mixed support for our hypothesis that ambient conditions and habitat quality affect social aggregation in bats. In general, bats that aggregated in larger groups in heated roosts were more likely to roost in large groups when T_{roost} was low relative to T_a, presumably to take advantage of social thermoregulation. We found evidence of a preference for heated roosts with warm microclimates, while we also found that social aggregation was greatest on days when the difference between T_{roost} and T_a was lowest. Taken together, our results support the hypothesis that individuals aggregate in larger groups, and higher quality roosts, to decrease the thermoregulatory costs associated with low T_a.

We found that bats roosted in the largest groups when the differential between T_{roost} and T_a was relatively small, i.e., when the thermoregulatory benefit of T_{roost} on its own would have been relatively low (Fig. 4). Thus, variation in ambient conditions affected variation in aggregation. This result was consistent for both artificially heated roosts during the heating period and all roosts during the unheated period, suggesting that bats flexibly adjust group size based on the thermal properties of available roosts. Flexibility in the propensity to aggregate in large social groups could be an important energy saving mechanism for bats at the individual and the colony-level, especially if social thermoregulation decreases energy expenditure when T_{roost} is low relative to T_a. Willis and Brigham (2007) found that estimated energy expenditure of free-ranging big brown bats (*Eptesicus fuscus*) was negatively correlated with group size because of the warming effect of larger numbers of bats, suggesting that social aggregation is a mechanism for energy conservation in bats. Similarly, metabolic rate for Bechstein's bats (*Myotis bechsteinii*) decreased as group size increased during the reproductive period (Pretzlaff et al., 2010). This pattern has also been observed for other taxa. At lower T_a, Abert's squirrels (*Sciuris aberti*) nested communally (Edelman and Koprowski, 2007) and more social vervet monkeys (*Chlorocebus pygerythrus*) had lower thermoregulatory costs during winter (McFarland et al., 2015). While these examples highlight the role of ambient conditions, individual differences in social behaviour could also affect grouping patterns of bats (e.g., Menzies et al., 2013; Webber et al., 2015). Specifically, some bats may prefer to roost in large groups, while others may prefer to roost in smaller groups, which could explain some of the variation we observed in group size. Alternatively, torpor-use could also affect group size. We were not able to quantify torpor expression in our experiment but we predict that bats roosting alone or in small groups would be more likely to use torpor (e.g., Pretzlaff et al., 2010).

Bats aggregated in larger groups when the T_{roost}–T_a differential was low but we did not detect a direct effect of occupation by bats, or variation in group size, on T_{roost}. Given that our boxes were not insulated, the boxes themselves likely had high rates of heat loss. Combined with the fact that group sizes were relatively small compared to those of little brown bats in natural roosts, this could have limited the potential of metabolic heat production by the bats to elevate T_{roost}. However, even if the presence of bats did not directly affect T_{roost}, social aggregation at low T_{roost}–T_a differentials could still provide thermoregulatory benefits. Larger groups of clustering bats could reduce per capita thermal conductance and heat loss by reducing the overall surface to volume ratio for the group, thus decreasing minimum energetic requirements for each group member (Gilbert et al., 2010). For example, in the lesser bulldog bat (*Noctilio albiventris*), both thermal conductance and metabolic rate decreased as group size increased (Roverud and Chappell, 1991). Even in the absence of a direct effect on T_{roost} of roost occupation by large numbers of bats, social aggregation could still facilitate energetic benefits via reduced thermal conductance
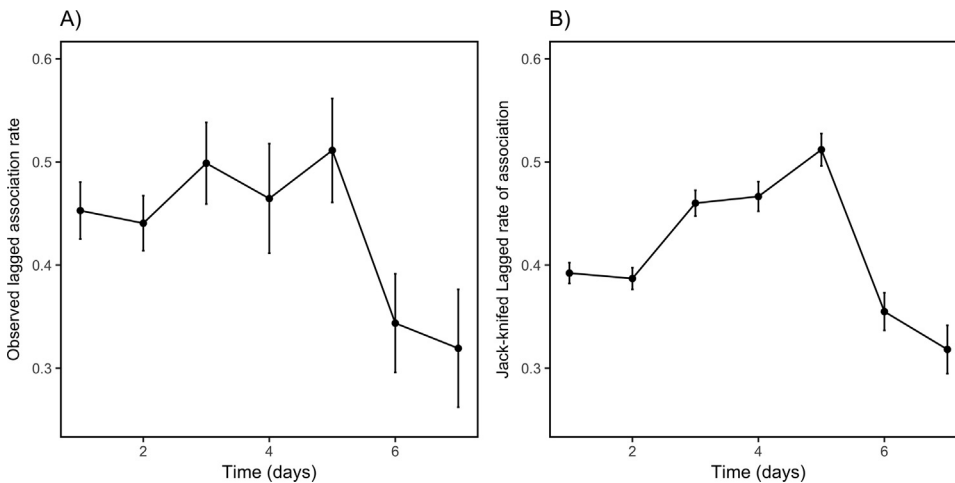
A)

B)

Fig. 6. A) Observed lagged association rate (LAR), calculated as the probability that any pair of bats roosted together on a given day, are still found roosting together on subsequent days, dots represent mean LAR and error bars represent the standard error of all association rates calculated on each day. B) Jack-knifed LAR where error bars represent standard error around the mean jack-knifed LAR.

and energy expenditure during periods of decreased resource availability.

We also observed marginal differences in group size as a function of our metric of roost quality, the T_{roost}–T_a differential (Fig. 4), although there was no effect of absolute T_a on group size, likely due to the smaller sample size for this analysis (n = 15 nights vs. 60 roost-days for the T_{roost}–T_a differential analysis). Group size was slightly, but consistently, higher in heated roosts compared to unheated roosts, suggesting a preference by bats for warmer roosts. Group sizes in heated roosts were small for the first two days of the experiment (Fig. 4), which suggests that bats may have taken a few days to investigate the available roosts and discover which had the warmest microclimates. After these first two days, however, groups were larger when T_{roost} was equal to or only slightly higher than T_a (Fig. 4). These results suggest that bats may reduce energy expenditure by using social thermoregulation when roost microclimate is relatively low and unlikely to provide thermoregulatory benefits. Alternatively, bats that did not roost in large groups when the T_{roost}–T_a differential was small may have used torpor to conserve energy, although we concede we have no data on torpor expression. For free-ranging temperate bat colonies, roosts in buildings appear to be warmer than those in trees (Lausen and Barclay, 2006). This could explain higher torpor use for lactating female big brown bats in trees compared to buildings (Rintoul and Brigham, 2014). Similarly, in barbastelle bats (*Barbastella barbastellus*), although all bats used torpor, torpor depth was shallower for bats roosting in large groups (Russo et al., 2017). Our results, suggest that, if given a choice, female bats will roost communally in warm microclimates. To further evaluate torpor as a predictor for social group size in bats we suggest that, in addition to manipulating T_{roost} as an index of roost quality, future studies also monitor body temperature to determine how individual energetics influences social aggregation.

In the context of conservation and management of endangered bat populations, our results provide experimental support for the assumption that roost microclimate is an important component of habitat quality (Wilcox and Willis, 2016). Our results are particularly important for bat species, like little brown bats, affected by WNS, a recently emerged fungal disease causing staggering mortality of several species of hibernating bats including little brown bats (Frick et al., 2010; Warnecke et al., 2012). Specifically, for bats that survive the winter with WNS, the ability for pregnant females to take advantage of social thermoregulation at maternity colonies may be severely limited, which could influence population demography if females abort pregnancy and abandon reproduction. This also suggests that WNS survivors may have fewer opportunities to exploit social information transfer about high quality roosts (e.g., Kerth et al., 2001a; Kerth and Reckardt, 2003). In a recent experiment, captive little brown bats recovering from WNS in the spring showed a stronger preference for artificially heated

roosts similar to the roosts we used, compared to control bats that were not infected (Wilcox and Willis, 2016). This was presumably because these roosts reduced energy expenditure and/or facilitated healing from the skin damage caused by the WNS fungus (Wilcox and Willis, 2016). In a scenario where a large proportion of female bats do not survive WNS during hibernation (Frick et al., 2010), social thermoregulation at maternity colonies may not be possible which means protection of forest patches with known maternity roosts (i.e., those with the high quality microclimates) should be an urgent conservation priority. For species like little brown bats that regularly rely on bat houses, deployment of artificially heated bat houses, like our experimental roosts, close to existing colonies where bats are likely to find them, could be another management option, as long as heated roosts do not cause bats to aggregate in ways that might increase the transmission of the fungal pathogen that causes WNS or other pathogens or parasites of bats (Webber et al., 2016).

Most of our understanding of roost selection in bats is based on structural characteristics of roosts that are thought to influence microclimate but very few studies have actually measured the microclimates of preferred roosts (Boyles, 2007; Johnson and Lacki, 2014; Olson and Barclay, 2013; Sedgeley, 2001; Willis and Brigham, 2007). Our study is among the first (but see Kerth et al., 2001b) to experimentally manipulate roost microclimate while also controlling for the potential influence of structural characteristics on roost selection by using identical roost boxes. Thus, our results reflect how variation in T_a influences roost selection and group size based solely on microclimate. Our experimental data may also have implications for our understanding of the evolution of sociality in endotherms. Thermoregulatory benefits associated with close social association for small-bodied endotherms include an increased survival rate for adults, particularly during critical periods such as pregnancy and lactation, as well as juveniles. For species with altricial neonates, social thermoregulation can reduce heat loss and increase growth rate and the probability of survival to adolescence (Gilbert et al., 2010). The evolutionary implications of social thermoregulation, therefore, appear directly linked to an animal's ability to optimize its energy budget to cope with a range of environmental conditions.

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