Similar hibernation physiology in bats across broad geographic ranges

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Abbreviations

TMR (torpid metabolic rate), EWL (evaporative water loss), WVP (water vapor pressure), $\dot{V}CO_2$ (rate of carbon dioxide production)

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Abstract

Species with broad geographic ranges may experience varied environmental conditions throughout their range leading to local adaptation. Variation among populations reflects potential adaptability or plasticity, with implications for populations impacted by disease, climate change, and other anthropogenic influences. However, behaviour may counteract divergent selection among populations. We studied intraspecific variation in hibernation physiology of *Myotis lucifugus* (little brown myotis) and *Corynorhinus townsendii* (Townsend's big-eared bat), two species of bats with large geographic ranges. We studied *M. lucifugus* at three hibernacula which spanned a latitudinal gradient of 1,500 km, and *C. townsendii* from 6 hibernacula spread across 1,200 km latitude and 1,200 km longitude. We found no difference in torpid metabolic rate among populations of either species, nor was there a difference in the effect of ambient temperature among sites. Evaporative water loss was similar among populations of both species, with the exception of one *C. townsendii* pairwise site difference and one *M. lucifugus* site that differed from the others. We suggest the general lack of geographic variation is a consequence of behavioural microhabitat selection. As volant animals, bats can travel relatively long distances in search of preferred microclimates for hibernation. Despite dramatic macroclimate differences among populations, hibernating bats are able to find preferred microclimate conditions within their range, resulting in similar selection pressures among populations spread across wide geographic ranges.

Keywords: Hibernation, torpid metabolic rate, evaporative water loss, local adaptation, *Myotis lucifugus*, *Corynorhinus townsendii*

Introduction

Studies seeking to understand broad scale ecological and evolutionary processes often consider variation in phenotypic traits among geographically isolated populations (Garland Jr and Adolph 1991; Kawecki and Ebert 2004). Organisms with broad geographic ranges may have to contend with dramatic differences in climate and environmental conditions throughout their range and, thus, exhibit different strategies to cope with this climatic and environmental variability. Some species may adopt a generalist strategy, but specialist-generalist tradeoffs suggest there may be selection for alternate strategies (Kawecki and Ebert 2004). Species with large geographic ranges may instead specialize on a relatively narrow range of environmental conditions (e.g., microclimate specialists), or may migrate long distances seasonally to remain in favorable conditions year-round. Among more sedentary species, intraspecific variation may result from phenotypic plasticity, where different phenotypes arise without underlying genetic change, especially in highly variable environments. Alternatively, populations of widespread species may adapt to local conditions that may be relatively consistent among years, but vary dramatically across the species range.

Although clearly an important topic, many studies do not consider intraspecific variation (or implicitly assume that local adaptation is not a major factor) due to the financial and logistic challenges associated with collecting data from multiple widespread populations. However, intraspecific variation has great evolutionary significance (Gould and Johnston 1972). Varying environments impose different selection pressures among populations, selecting for traits linked to increased fitness in these environments. Challenges posed by the environment can constrain the distribution of the species (e.g., Humphries et al. 2002), and populations that occur at range margins may be more likely to be locally adapted, as local conditions may otherwise prevent the occurrence of the species. Ultimately, resulting differences among populations reflect potential adaptability of the species with broad implications

ranging from coping with climate change and other anthropogenic influences to speciation (Doebeli and Dieckmann 2003; Otto 2018).

From an energetic perspective, local adaptations and specialization will be most pronounced under situations of extreme energetic challenges. For many temperate species, the limitation of food availability and increased thermoregulatory demands of winter represent a combined energetic challenge. Hibernating bats are an excellent study system to address questions of local adaptation in broadly distributed populations. Many species of bats have very broad geographic distributions, often with ranges that cover large parts of entire continents. Therefore, populations of these widely distributed species will experience a wide range of environmental conditions due to latitudinal gradients, the influence of mountain ranges, or the influence of large bodies of water, in some cases resulting in populations persisting in different biomes. Bats are also interesting to consider as they are highly mobile species. Some species of bats migrate latitudinally to seek out favorable conditions year-round, but many more species instead migrate relatively short distances (< 500 km) to seek out appropriate habitat for winter hibernation (Fleming and Eby 2003). For those species that do not overwinter at great distances from their summer breeding grounds, winter poses an extreme energetic challenge. Indeed, hibernation represents the highest period of mortality for many species (Davis 1966; Frick et al. 2010; Lentini et al. 2015). Many species of bats are comprised of populations that hibernate across broad geographic ranges, but little is known about how hibernation energetics vary among populations, or the extent to which local adaptation results in regional phenotypic variation.

The objective of our study was to understand how hibernation physiology varies among populations of species with broad distributions. We studied this question by comparing the physiology of two hibernating bat species across large geographic ranges. We hypothesized that variation in environmental conditions across populations could affect hibernation strategies. We made two alternate predictions. If widely distributed species are locally adapted to regional environmental

conditions, we predicted we would observe physiological differences among bats from different sites. Alternatively, if populations from throughout the species range seek out and select similar hibernacula then we predicted we would not observe differences across our study sites.

Materials and Methods

Study Species and Study Sites– We included two species of hibernating bats in our study. *Corynorhinus townsendii* (Townsend's big-eared bat) is a small (approximately 5 – 13 g body mass) insectivorous bat (family Vespertilionidae). The distribution of *C. townsendii* spans across western North America, extending south into Mexico and north into southern British Columbia (Kunz and Martin 1982). Two isolated subspecies occur in the eastern United States (*C. townsendii virginianus* and *C. t. ingens*) but we did not consider these subspecies in our study. *C. townsendii* often hibernates in caves and mines, but is generally thought to select colder, drier, and more variable sites for hibernation than many other species (Kunz and Martin 1982). Populations may migrate short distances from maternity colonies to hibernacula, but *C. townsendii* sometimes uses caves or mines as maternity roosts and may even use separate areas of one cave for maternity roosting and hibernation (Sherwin et al. 2000). Despite its widespread distribution, *C. townsendii* hibernation has received comparatively (compare with *M. lucifugus*, below) little study (e.g., Hughes 1968; Humphrey and Kunz 1976; Sherwin et al. 2000; Ingersoll et al. 2010).

Myotis lucifugus (little brown myotis) is also a small (approximately 6 – 12 g body mass) insectivorous bat (family Vespertilionidae) and has one of the largest geographic distributions among North American bats. The species regularly occurs from Georgia in the southeast to Alaska in the northwest (Fenton and Barclay 1980). There has been extensive study of hibernation in *M. lucifugus*, though primarily focused in the northeast (e.g., Fenton 1969; Thomas et al. 1990; Thomas and Cloutier 1992a; Kunz et al. 1998; McGuire et al. 2009) and Manitoba (e.g., Jonasson and Willis 2012; Czenze et al.

2013; Norquay and Willis 2014; McGuire et al. 2017). *Myotis lucifugus* may travel several hundred kilometers from summer maternity roosts to winter hibernacula (Fenton 1969; Norquay et al. 2013). Hibernacula are typically caves or mines with stable, cool temperatures, and consistently high humidity (Fenton and Barclay 1980).

We collected physiological measurements from free-living *C. townsendii* at hibernacula over a region that spanned 1,200 km north to south, and 1,200 km from east to west, including one of the northernmost hibernacula known for the species (**Figure 1**). We conducted fieldwork at sites in Colorado, Utah, Nevada, Oregon, and British Columbia. Similarly, we collected measurements from *M. lucifugus* at hibernacula in Montana, Alberta, and Northwest Territories, including the most northerly known hibernaculum for the species (Figure 1; Wilson et al. 2014). The *M. lucifugus* study sites represented a latitudinal gradient of approximately 1,500 km. None of our sites were affected by white-nose syndrome (Willis 2015) at the time of our fieldwork.

We characterized the climate at each of our study sites based on 1981 – 2010 climate normals (Arguez et al. 2012; Environment and Climate Change Canada 2020). Although it was not our explicit aim to test for variation along specific environmental gradients, the *M. lucifugus* sites spanned a wide temperature gradient and *C. townsendii* sites included both arid sites and much more mesic sites (**Table 1**). Microclimates can be highly variable within sites (Perry 2013; McClure et al. 2020), and bats move within sites during hibernation (Ransome 1968; Boyles et al. In review), therefore we did not attempt to relate measured physiological variables (see below) to the conditions experienced within a site.

Data Collection– We captured bats at hibernacula during pre-hibernation swarming or during midwinter over three field seasons from 2017 to 2019. Swarming bats were captured in mist-nets over several days. In mid-winter we made a single entry into the hibernaculum to minimize disturbance (coordinating with planned surveys where possible) and captured bats by hand from the walls of the hibernaculum.

We recorded body mass (±0.1 g; Ohaus Corporation USA), forearm length (±0.1 mm; Avinet Inc.) and sex. We excluded any bats that were suspected to be young of the year (Brunet-Rossinni and Wilkinson 2009) and make the assumption that all of the bats in our study were adults.

We selected a subset of up to 14 bats each night to be held for respirometry measurements, preferentially maintaining an even sex ratio and including the heaviest bats available to avoid additional stress to bats in relatively poor condition. We used open flow respirometry to measure torpid metabolic rate (TMR) and evaporative water loss (EWL) across a range of temperatures. At all American field sites respirometry measurements were performed on site in a mobile laboratory. Animal chambers (350 mL glass jars) had wire lattice as a roosting surface, mineral oil to the bottom of the chamber to prevent bat excretions from biasing water vapor pressure (WVP) measurements, and 0.2 μm in-line filters (Cole-Parmer, #15945-42) to prevent possible *Pseudogymnoascus destructans* contamination (the causative agent of white-nose syndrome). Chambers were housed inside a dark, temperature-controlled (±0.5°C) cabinet (PELT-5, Sable Systems International). We used calibrated thermocouple probes (TC-2000; Sable Systems International) hanging within each metabolic chamber to continuously monitor ambient temperature throughout experimental trials. Incurrent air was scrubbed with Drierite® (W.A. Hammond Drierite Co. LTD, Xenia, Ohio) to remove water vapor, soda lime (Sigma-Aldrich cat no: 72073) to remove CO_2, and finally magnesium perchlorate (Sigma-Aldrich cat no: 222283) to remove additional water vapor released from the CO_2 scrub. We settled bats at 10 °C for 12 h prior to the start of measurements, maintaining humidity (~85% RH; DG-4 dew point generator, Sable Systems) during the settling phase. We maintained flow rate (flowbar FB-8; Sable Systems) at approximately 300 mL/min during settling, and reduced flow rate to 100 - 120 mL/min when bats entered torpor. We sequentially monitored each bat (RM-8 multiplexer, Sable Systems) at 10 min intervals. Following 12 h of settling, we switched to a stream of dry air to avoid potential condensation at the low saturated WVP associated with low temperatures. We cycled through bats and the baseline reference channel at 3 min intervals

(accounting for washout period) during the measurement phase, starting at 10 °C and sequentially reducing temperature to 8, 5, and 2 °C. Each temperature treatment was maintained for approximately 3 h, corresponding to 7 – 8 measurements of TMR and EWL per bat at each temperature. Excurrent air was subsampled at 75 mL/min for measurement of WVP and excurrent CO_2 (Field Metabolic System; Sable Systems International). Respirometry gas analyzers were regularly calibrated with custom certified span gas (AirGas Cuevas Distribution Inc, USA or Praxair Canada Inc.).

Respirometry procedures in Canada followed the same general protocol, with some minor differences. Rather than conducting measurements on site in a mobile lab, bats in Canada were transported < 50 km to a local field station (approximately 30 minutes, bats held in individual cloth bags during transport). Animal chambers were of similar design but 250 mL instead of 350 mL, and chambers were maintained in a dark custom temperature cabinet (± 0.5°C; modified from 6 cubic foot mini-fridge, Danby Products Ltd.). During the settling phase bats were provided ambient air that did not have a precisely controlled WVP, but was > 85% RH to match measurements made at American sites. Instead of an FB-8 flowbar, in Canada we used a subsampler (SS-4; Sable Systems International) and factory calibrated flow controllers (MFC-2; Sable Systems International) to maintain flow at the same rates used at American field sites. Instead of an FMS multi-analyzer system, in Canada we used separate analyzers to measure WVP (RH-300; Sable Systems International) and CO_2 (FoxBox Respiratory System; Sable Systems International). We do not anticipate that any of differences in respirometry measurement would impact measurements used in our analysis.

The total respirometry protocol took approximately 24 h, during which time bats were continuously monitored. If a bat showed signs of increasing metabolic rate (i.e., not in steady state torpor) we increased flow rate for that bat until the bat returned to torpor. Bats that refused to settle were removed from the experiment to prevent unnecessary stress and depletion of fat stores. Upon

completion of measurements we offered water and either mealworms or a high calorie nutritional supplement (Nutrical, Tomlyn Veterinary Science) to each bat before release at the site of capture.

We processed respirometry recordings in Expedata (v1.9.13; Sable Systems). We corrected for lag and drift and then calculated $\dot{V}CO_2$ using equation 10.5 from Lighton (2018) and evaporative water loss using equation 10.9 from Lighton (2018). We converted $\dot{V}CO_2$ to metabolic rate (in mW) assuming fat oxidation (respiratory quotient = 0.7; Price and Mager 2020). We quality checked all measurements and excluded values from bats that were not torpid or if there was an erroneous measurement (values < 0.25 mW g^{-1} or > 2 mW g^{-1}) and selected the minimum TMR measurement at each temperature for each individual for further analyses. We report mass-specific metabolic rates (mW g^{-1}) for ease of comparison with other literature and other accompanying studies, but the qualitative outcomes were not affected whether using mass-specific or whole animal measurements.

Data Analysis– We used linear mixed models to analyze TMR or EWL following procedures described by Zuur et al. (2009), with separate models for *C. townsendii* and *M. lucifugus*. We suspected we might observe greater variance in TMR or EWL at either the highest or lowest temperatures due to individual physiological thresholds. Therefore, we included a variance structure in our analysis which allowed for a heterogeneous variance among temperatures (varIdent variance structure in nlme package of R statistical software; Zuur et al. 2009; R Core Team 2020; Pinheiro et al. 2021). We included a random effect of individual to account for repeated measures and tested for the main effects of temperature, sex, season (pre-hibernation or mid-winter), site, and the interactions of temperature and site and temperature and sex (in case bats from different sites or sexes responded differently to temperature). We compared models with likelihood ratio tests, visually assessed residual plots, and used Tukey's post-hoc tests for pairwise comparisons of significant effects. For all main effects we assessed statistical significance at $\alpha = 0.05$, but in post-hoc comparisons we considered pairwise differences if $p < 0.10$. All

statistical analysis was conducted in R (v 3.6.3; R Core Team 2020) including packages ggmap (Kahle and Wickham 2013), dplyr (Wickham et al. 2021), lubridate (Grolemund and Wickham 2011), nlme (Pinheiro et al. 2021), and multcomp (Hothorn et al. 2008).

Permits and Ethics– All field activities were conducted under permits from the respective state or provincial agencies. Although none of our sites were affected by white-nose syndrome at the time we conducted our fieldwork, we followed US Fish and Wildlife Service protocols for fieldwork and decontamination (US Fish and Wildlife Service 2018). All field methods were approved by the Institutional Animal Care and Use Committee at Texas Tech University (all sites in the United States), the National Park Service (Oregon), Alberta Environment and Parks, British Columbia Ministry of Forest Lands Natural Resource Operations and Rural Development, Northwest Territories Department of Environment and Natural Resources, and Parks Canada.

Results

C. townsendii– Our analysis included data for 152 *C. townsendii* from 6 different sites (4 mines, 2 caves; n = 10 – 47 bats per site). Our dataset included a similar sex ratio among sites (test for equality of proportions; χ^2=10.0, df = 5, p = 0.07) with a female bias across sites (number females ≥ males at all sites; binomial test p = 0.0001). Models of torpid metabolic rate were better supported when they included heterogeneous variance among temperatures (likelihood ratio = 32.38, df = 3, p < 0.0001) and the random effect of individual (likelihood ratio = 100.92, df = 1, p < 0.0001). Our dataset includes samples from both pre-hibernation and mid-winter at four sites, and mid-winter only at two sites. Torpid metabolic rate was related to temperature (likelihood ratio = 12.84, df = 3, p = 0.005) but the effect of temperature did not vary among sites (site:temperature interaction, likelihood ratio = 22.30, df = 15, p = 0.10) or between sexes (sex:temperature interaction, likelihood ratio = 1.70, df = 3 p = 0.64). Torpid metabolic rates measured in winter were slightly greater (0.1 ± 0.07 mW g^{-1}) than when

measured during swarming (likelihood ratio = 4.85, df 1, p = 0.03), but given that no similar effect was observed for EWL and no seasonal effects were observed for *M. lucifugus* (below), this may be a spurious result. Torpid metabolic rate did not vary among sites (likelihood ratio = 6.85, df = 5, p = 0.23) or between sexes (likelihood ratio = 0.15, df = 1, p = 0.70). Torpid metabolic rate was greater at 2°C than either 5 or 8°C and TMR at 10°C was intermediate, suggesting decreasing TMR to a minimum metabolic rate (0.33 ± 0.03 mW g^{-1}) over the range of 5 – 8°C (**Figure 2a**) and minimum defended temperature between 2 and 5°C.

Trends for EWL generally followed those observed for TMR. For EWL, there was better support for models that included heterogeneous variance among temperatures (likelihood ratio = 107.10, df = 3, p < 0.0001) and the random effect of individual (likelihood ratio = 57.51, df = 1, p < 0.0001). Evaporative water loss varied across temperatures (likelihood ratio = 19.03, df =3, p = 0.0003) but the effect of temperature did not differ among sites (site:temperature interaction; likelihood ratio = 15.84, df = 15, p = 0.39) or between sexes (sex:temperature interaction; likelihood ratio = 4.49, df = 3, p = 0.21). Evaporative water loss did not differ between seasons (likelihood ratio = 2.60, df = 1, p = 0.11) or sexes (likelihood ratio = 2.74, df = 1, p = 0.10). Minimum EWL (0.010 ± 0.0007 mg H_2O min^{-1} g^{-1}) was measured at 5 - 8°C (**Figure 2b**), the same temperature range for minimum TMR. There was one pairwise difference in EWL among sites (likelihood ratio = 13.05, df = 5, p = 0.02), with lower EWL measured in British Columbia compared to Colorado (Tukey's post-hoc p = 0.01). There were no other differences among sites.

M. lucifugus– Our analysis included data for 99 *M. lucifugus* from 3 different caves (sample size ranged from 20 – 40 bats per site). Our dataset included males and females at all sites, but sex ratio varied among sites (test for equality of proportions; χ^2=12.6, df = 2, p = 0.001), with heavy male bias in Montana and Northwest Territories (binomial test; p < 0.0001) and an even sex ratio in Alberta (binomial test: p = 0.87). In Montana we conducted fieldwork during pre-hibernation swarming and mid-

winter hibernation, but at the Alberta and Northwest Territories sites we only conducted pre-hibernation fieldwork. In an analysis of just data from Montana, TMR did not differ between fall and winter (likelihood ratio = 0.83, df = 1, p = 0.36). Therefore, we pooled swarming and hibernation data in Montana and did not include season in comparison among the three sites. As for the results of *C. townsendii*, there was better support for models that included a random effect of individual (likelihood ratio = 27.33, df = 1, $p < 0.0001$) and allowed for differences in variance among temperatures (likelihood ratio = 22.17, df = 3, p = 0.0001). Torpid metabolic rate varied among temperatures (likelihood ratio = 15.21, df = 3, p = 0.002), but the effect of temperature did not vary among sites (site:temperature interaction; likelihood ratio = 7.06, df = 6, p = 0.32) or between sexes (sex:temperature interaction; likelihood ratio = 6.04, df = 3, p = 0.11). There were no differences in torpid metabolic rate among sites (likelihood ratio = 3.21, df = 2, p = 0.20) or between sexes (likelihood ratio = 0.04, df = 1, p = 0.84). Torpid metabolic rate was greater at 10°C than at any of 8, 5, or 2°C (**Figure 3a**). Minimum metabolic rate was 0.30 ± 0.02 mW g^{-1} and the absence of an increase in metabolic rate at the lower tested temperatures indicates the minimum defended temperature is < 2°C, the coldest temperature we tested at.

Evaporative water loss models that included the random effect of individual and heterogeneous variance structure were better supported (likelihood ratio = 19.74, df = 1, $p < 0.0001$; likelihood ratio = 67.74, df = 3, $p < 0.0001$). There was a marginally significant interaction between site and temperature (likelihood ratio = 12.96, df = 6, p = 0.044) but upon inspection the interaction was driven only by slightly greater EWL at the Alberta site at 8°C and did not indicate any broader patterns of variation among temperatures or sites. After excluding the site by temperature interaction, EWL did not differ between sexes (likelihood ratio = 1.95, df = 1, p = 0.16) but was related to temperature (likelihood ratio = 19.75, df = 3, p = 0.0002) with greater evaporative water loss at 10°C than either 8 or 5°C (**Figure 3b**). As for *C. townsendii* there was one site effect; bats from Montana had greater EWL than either of the other two

sites (likelihood ratio = 13.81, df = 2, p = 0.001; **Figure 3b**). Minimum EWL (calculated at 5 and 8 °C across all sites) was 0.018 ± 0.001 mg H_2O min^{-1} g^{-1}.

Discussion

Despite large geographic distances (> 1,000 km) and climatic variation (Table 1) among our study sites, we found no evidence of intraspecific variation, or local adaptation, in TMR. Torpid metabolic rate did not vary among sites, nor did the relationship between TMR and ambient temperature. There are few studies that have previously considered similar comparisons, with mixed results. In two studies which contrasted bat populations across evaporative water loss gradients, there was little to no variation in metabolic rate among populations (Klüg-Baerwald and Brigham 2017; Gearhart et al. 2020), consistent with the findings in our study. Notably, Dunbar and Brigham (2010) found variation in torpid metabolic rate among populations of hibernating *Eptesicus fuscus* (big brown bats). However, the differences in metabolic rates among populations were only observed at the coldest and warmest temperatures tested, both of which were outside the range of temperatures we considered in our study. In the range of 5 – 10°C, torpid metabolic rate did not vary among the populations considered by Dunbar and Brigham (2010), similar to our findings. Our objective was to test hibernation physiology over a range of biologically relevant temperatures that might be regularly experienced by hibernating bats. We might have observed intraspecific variation if we included temperatures farther below the minimum defended temperature. However, assuming preferred microclimates are available, hibernators should select temperatures slightly above those that result in minimum metabolic rate (Boyles and McKechnie 2010; Boyles et al. 2020), but note that additional factors affect hibernation strategies, including sex (Jonasson and Willis 2011; Czenze et al. 2017) and body condition (Boyles et al. 2007).

352 Therefore, we conclude that within the range of temperatures which are likely to be preferred during
353 hibernation, there is no evidence for intraspecific variation in TMR.

354 The conclusion for EWL was generally consistent with the finding for TMR, but notably there
355 were site differences in water loss. Among *C. townsendii* the relationship between water loss and
356 temperature was consistent across populations, except for one pairwise combination of sites (British
357 Columbia and Colorado) that differed in evaporative water loss (by 0.009 ± 0.003 mg H_2O min^{-1} g^{-1}).
358 Similarly, for *M. lucifugus*, evaporative water loss was greater in Montana than at the other sites (by
359 0.007 ± 0.002 mg H_2O min^{-1} g^{-1}). Other than these specific comparisons, there was no variation in water
360 loss among sites for either species. It is notable that the only pairwise differences in EWL were observed
361 among the most distant sites: the British Columbia and Colorado sites for *C. townsendii* are separated by
362 approximately 1,400 km, and the Montana *M. lucifugus* site is separated from the other two sites by
363 approximately 800 and 1,500 km respectively. High rates of gene flow among nearby populations
364 reduces phenotypic differentiation among populations, and differences may only become apparent at
365 greater distances (Rousset 1997). Considering biologically relevant conditions, the evidence for
366 intraspecific variation in EWL is even less compelling. We measured EWL in dry air, partially because of
367 methodological constraints (the challenge of maintaining humidity below saturation at low
368 temperatures) but also because measuring in dry air exacerbates potential differences in EWL among
369 groups (Klüg-Baerwald and Brigham 2017; McGuire et al. 2017). In natural arid conditions, free-living
370 animals would experience less water stress than in our measurement conditions, and the potential
371 differences among populations would be further diminished under real world conditions. Indeed, this
372 was exactly the finding in a study of hibernating *E. fuscus* that only observed a difference in EWL
373 between an arid and a mesic population when measured in dry air and not when measured under higher
374 humidity (Klüg-Baerwald and Brigham 2017). Taken together, the fact that differences were only

observed among the most distant sites, and when measured under unnatural extreme conditions, we argue that there is likely to be little biologically relevant variation in EWL among free-living populations.

The potential for more pronounced differences in EWL (even if minimal) than TMR is consistent with previous studies (Klüg-Baerwald and Brigham 2017; Gearhart et al. 2020). Furthermore, variation in EWL but not TMR may reflect a physical difference among populations rather than a difference in a physiological process (Klüg-Baerwald and Brigham 2017). We measured total evaporative water loss, which is comprised of both respiratory and cutaneous water loss. Respiration is related to metabolic rate, and therefore differences in total evaporative water loss in the absence of differences in metabolic rate likely reflect differences in cutaneous water loss (Klüg-Baerwald and Brigham 2017; McGuire et al. 2017; Gearhart et al. 2020). Total water loss during torpor is thought to be primarily driven by cutaneous water loss (Thomas and Cloutier 1992b), due to low respiration rates and large surface area due to wing and tail membranes and large ears. Studies of hibernating bats often consider evaporative water loss as an important driver of hibernation energetics and success (Thomas and Geiser 1997; Willis et al. 2011; Ben-Hamo et al. 2013; Boratyński et al. 2015; Klüg-Baerwald and Brigham 2017; Haase et al. 2019) and this may be an important avenue for future studies considering intraspecific variation in hibernation physiology.

In general, we did not find strong evidence for local adaptation and intraspecific variation in hibernation physiology, despite studying two species with very large geographic ranges encompassing a wide gradient of environmental challenges. We suggest it is likely that rather than being locally adapted to different climate conditions, hibernating bats may be able to find suitable hibernacula with generally consistent microclimate across their range. Furthermore, some species mitigate environmental influences by clustering during hibernation (Boratyński et al. 2015). The combined effects of migration, microclimate selection, and clustering may represent an example of the Bogert effect, the phenomenon of behaviour reducing the effects of selection and therefore reducing local adaptation (Buckley et al.

2015). This is particularly plausible for hibernating bats because of their ability to fly long distances in search of suitable hibernacula (Griffin 1970). Furthermore, copulation occurs during pre-hibernation swarming (Fenton 1969) and the long-distance movements to swarming sites and hibernacula result in extensive gene flow among populations (Burns et al. 2014). Local adaptation typically requires restricted gene flow (Kawecki and Ebert 2004) and therefore the combination of seasonal movement patterns and behavioural thermoregulation (selecting preferred hibernation conditions, clustering) may contribute to a lack of local adaptation in hibernation physiology. Behavior may be more important than physiology and bats may be able to behaviorally select suitable hibernacula rather than physiologically specializing on locally variable conditions during hibernation.

Our data suggests that within preferred microclimate conditions, there is little evidence of intraspecific variation in hibernation physiology. However, it is important not to extrapolate this finding. Extreme populations, hibernation strategy, global climate change, and disease may all affect hibernation. As environmental conditions extend beyond the range of preferred conditions (e.g., at range margins; Humphries et al. 2002), differences among widespread populations may become apparent. We included populations of both species at their northern limits but it is possible that there are more extreme unknown populations which are forced to hibernate outside their preferred range of conditions. Furthermore, subterranean hibernacula are buffered from surface conditions (Perry 2013) but some species select hibernacula that are more exposed to surface weather (Dunbar and Brigham 2010; Perry et al. 2010) and therefore may experience stronger gradients of environmental conditions. Finally, there may be future changes in both extrinsic environmental conditions (global climate change) and intrinsic hibernator phenotypes (e.g., disease consquences of white-nose syndrome; Auteri and Knowles 2020).

Overall, we conclude that there is a general lack of intraspecific variation in hibernation physiology among widespread bat populations across broad environmental gradients when measured

423 within the range of environmental conditions likely to be selected during hibernation. We suggest that
424 highly mobile bats are able to seek out preferred microclimates for hibernation, regardless of local
425 macroclimate conditions, emphasizing the importance of both behavior and physiology when studying
426 how animals are adapted to their environments. However, there are multiple plausible scenarios (more
427 extreme environmental gradients, populations closer to the extremes of the range margin, global
428 climate change, disease consequences) which may reveal intraspecific variation in hibernation
429 physiology. Variations in phenotype and genotype among populations provide the potential for natural
430 selection. Such variation among populations may be important for speciation processes, adaptation to
431 changing climates, or source material for evolutionary rescue from zoonotic disease.

TABLES

Table 1. We collected measurements from *Corynorhinus townsendii* at 6 sites and from *Myotis lucifugus* at 3 sites. Sites for *M. lucifugus* had a notable temperature gradient, while precipitation varied widely among sites for *C. townsendii*. Among *C. townsendii* sites, the most mesic site received approximately 6.5 times more precipitation than the most arid site. Among *M. lucifugus* sites, temperature decreased with latitude and the mean annual temperature at the most northern site was below freezing. All climate data from 1981 – 2010 climate normals. Hibernation duration estimates are calculated from Hranac et al. (Accepted). We do not report precise locations of these sensitive sites, but general locations are illustrated in Figure 1.

Site	Annual Precipitation (mm)	Mean Annual Temperature (°C)	Coldest Month[1] (°C)	Warmest Month[2] (°C)	Predicted Hibernation Duration (days)
Corynorhinus townsendii sites					
Nevada[3]	248	7.2	-11.7	30.9	120
Colorado	338	10.3	-9.5	33.9	89
Utah	501	9	-8.4	31.1	127
British Columbia	535	8	-7.2	28.7	139
Oregon	1597	13.2	0.6	34.6	81
Myotis lucifugus sites					
Montana	563	6.3	-9.7	27.5	154
Alberta	599	4.1	-11.7	22.7	170
Northwest Territories	354	-1.8	-27	23.3	205

1. Mean of daily minimum temperature for the coldest month of the year, based on 1981 – 2010 climate normals
2. Mean of daily maximum temperature for the warmest month of the year, based on 1981 – 2010 climate normals.
3. The two sites in Nevada were in relatively close proximity and are characterized by a single weather station.

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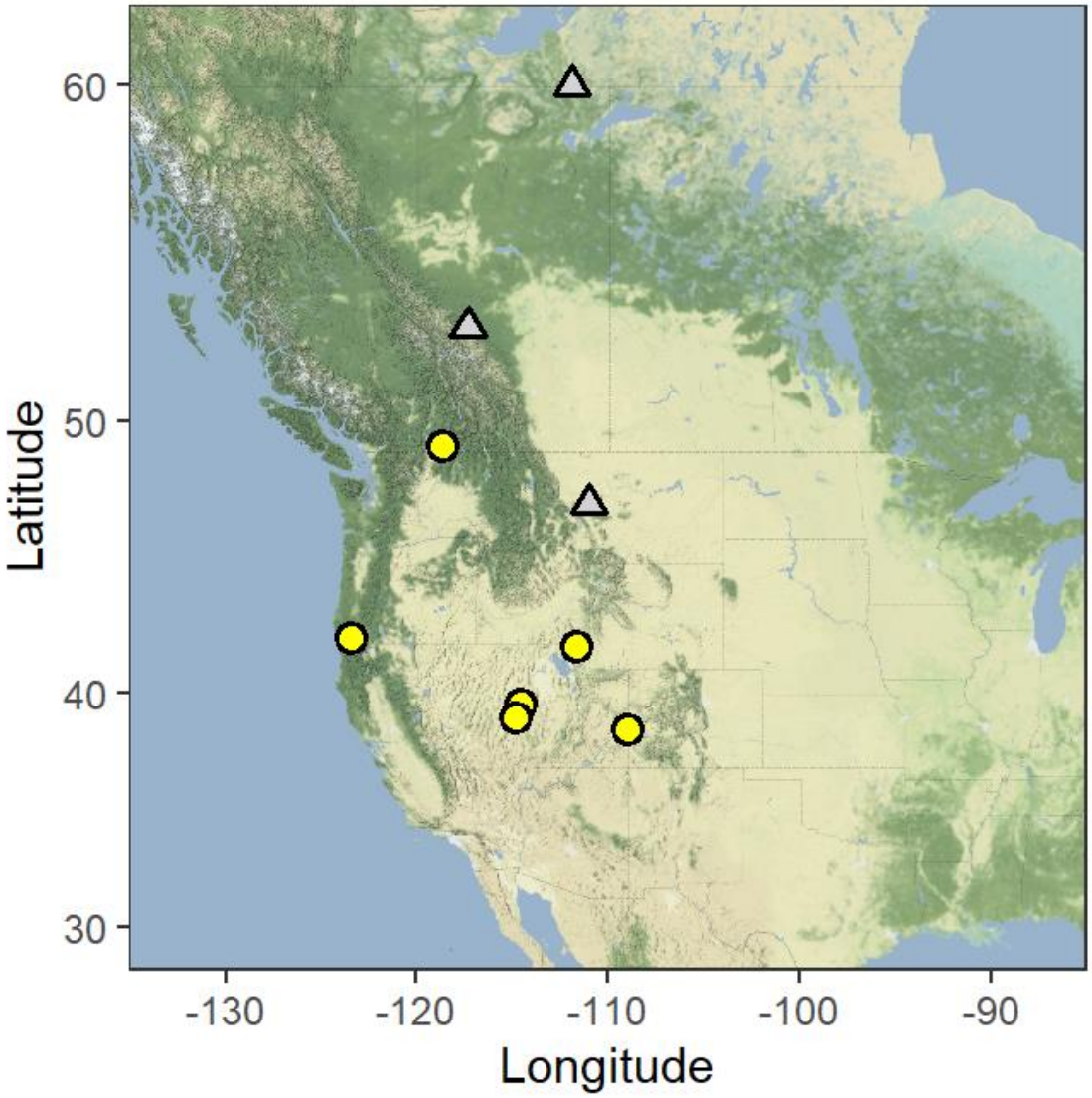


585

586 **Figure 1.** We collected data from bats across western North America. Study sites for *Myotis lucifugus* are
587 indicated in grey triangles and span approximately 1,500 km (approximately 13 degrees of latitude).
588 Study sites for *Corynorhinus townsendii* are indicated in yellow circles and span approximately 1,200 km
589 east to west, and 1,200 km north to south. For both species, the most northern sites are among the
590 most northerly known hibernacula. Map created with map tiles from Stamen Design (maps.stamen.com;
591 CC BY 3.0).

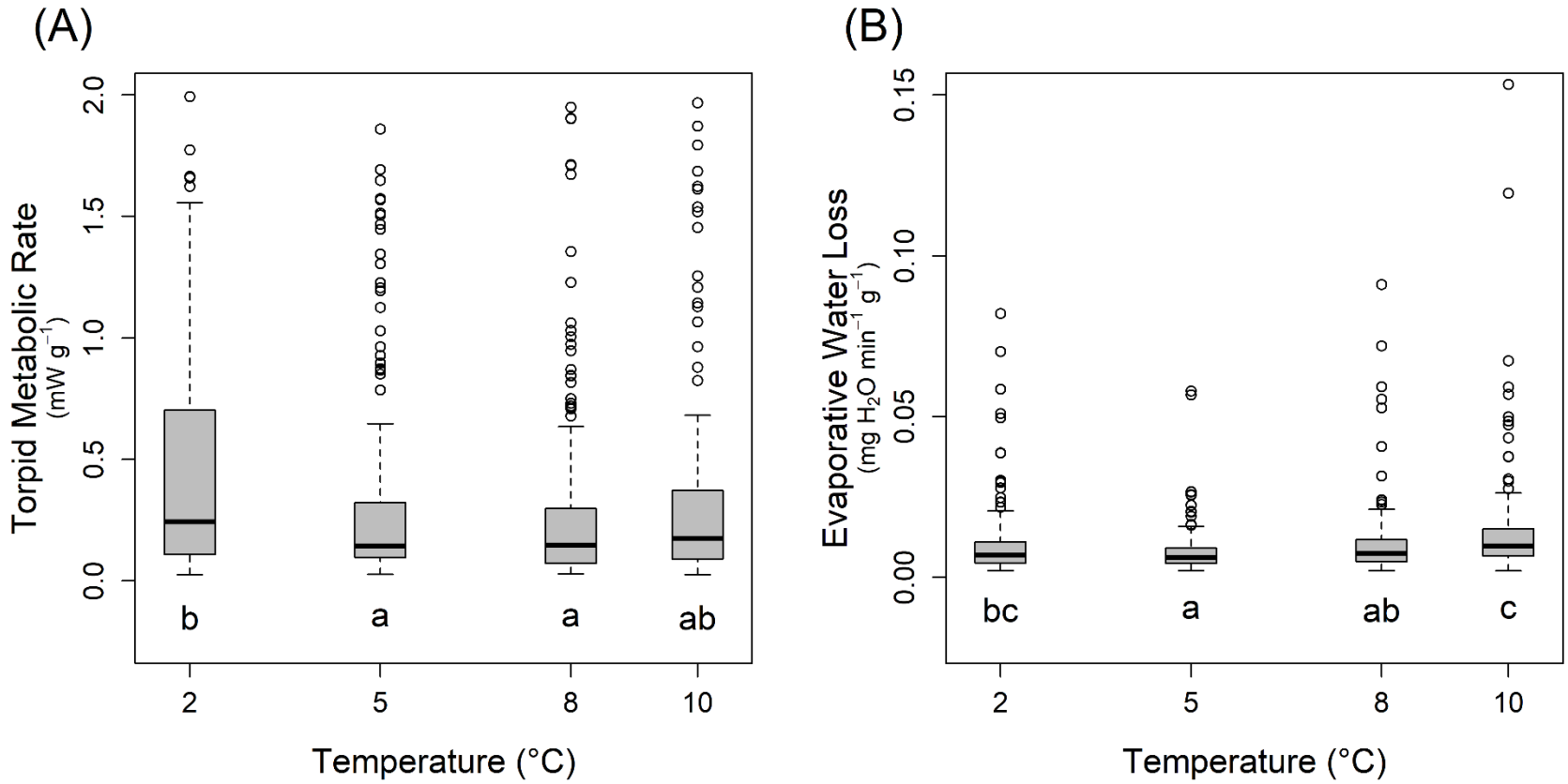
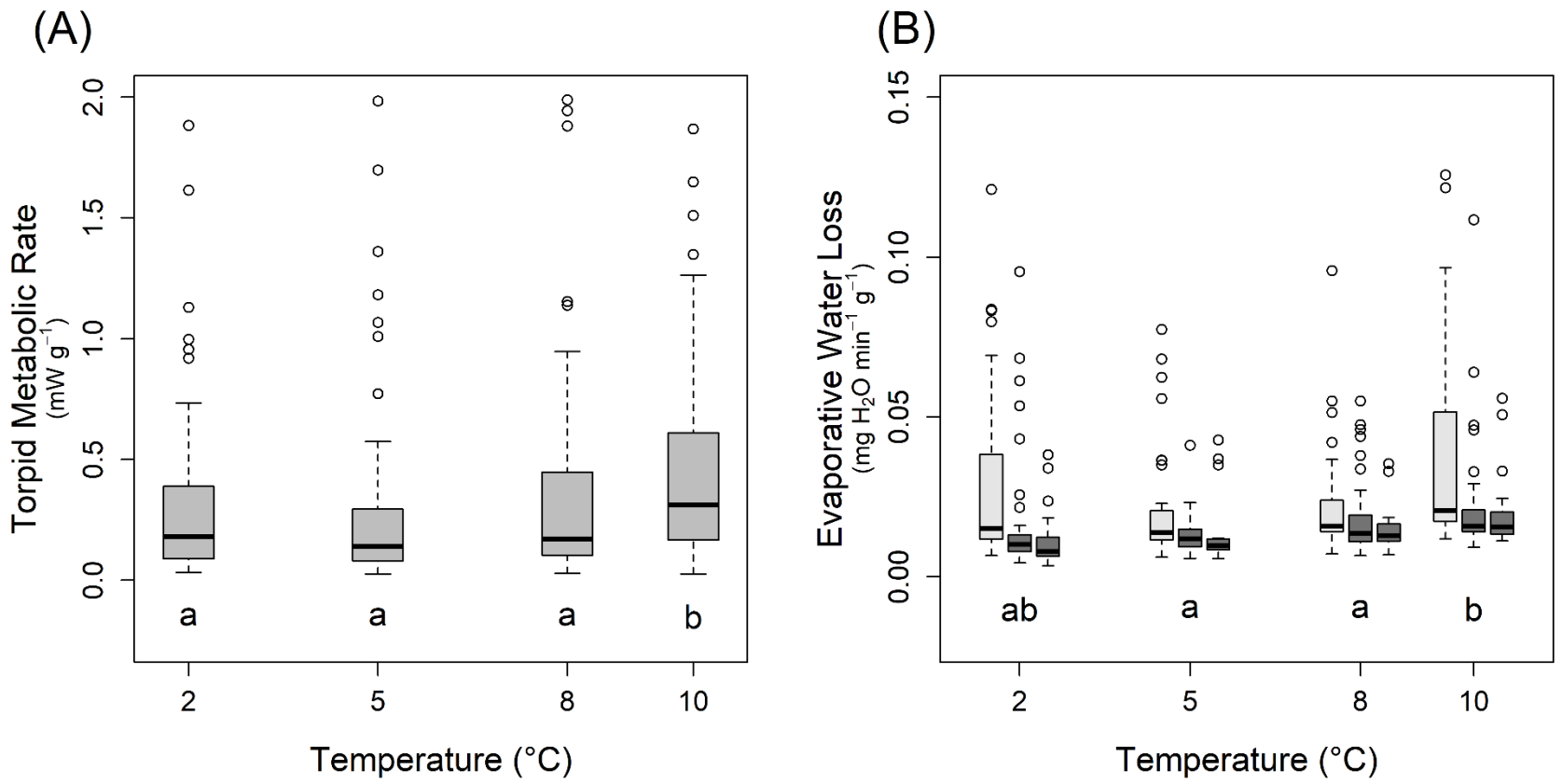


Figure 2. (A) *Corynorhinus townsendii* (n = 152) torpid metabolic rate did not vary across 6 sites, nor did the relationship between site and temperature vary across sites. Minimum metabolic rate was measured between 5 – 8°C and metabolic rate increased at 2°C. Measurements made during mid-winter were greater than measurements made during pre-hibernation swarming, but both seasons have been combined here for visual simplicity and comparison with *M. lucifugus*. **(B)** A similar pattern was observed for evaporative water loss. Minimum evaporative water loss was measured between 5 – 8°C. There were generally no differences among sites, except for one pairwise difference between sites in British Columbia and Colorado, but sites have been combined here for visual simplicity. In both plots, temperatures indicated with the same

600 letter did not differ. Boxplots indicate median, interquartile range, and outliers (open circles) as determined by standard boxplots in software
601 package R.



602

603 **Figure 3. (A)** *Myotis lucifugus* (n = 99) torpid metabolic rate did not vary across 3 sites, nor did the relationship between site and temperature
604 vary across sites. Minimum metabolic rate was measured between 2 – 8°C. **(B)** Minimum evaporative water loss was measured between 5 – 8°C.
605 Evaporative water loss was greater in Montana (light grey) than either Alberta or Northwest Territories (dark grey). In both plots, temperatures
606 indicated with the same letter did not differ. Boxplots indicate median, interquartile range, and outliers (open circles) as determined by standard
607 boxplots in software package R.

608