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Meet you at the local watering hole? No use of an artificial water resource, and evidence of dehydration in hibernating bats in the prairies

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While torpid, small hibernators experience negative water balance due to evaporative water loss. The use of humid hibernacula and ability to drink during periodic arousals allows most hibernators to manage this deficit over the course of a winter. Some populations of big brown bats (*Eptesicus fuscus*) hibernate in relatively dry rock-crevices that do not contain free standing water. We monitored the winter behaviour and physiology of one such population in the Canadian prairies. Due to the semi-arid climate, we hypothesized that these bats would experience relatively high evaporative water loss and make frequent mid-winter flights to find water. We measured serum ion concentrations and hematocrit to assess level of dehydration in bats captured during winter. We also provided a heated water tank enriched in deuterium (^{2}H) and used stable isotope analysis to test for elevated hydrogen isotope ratios (^{2}H/^{1}H; herein δ^2H) in the blood of bats to determine if individuals drank from the tank. We also used passive acoustic monitoring, video surveillance, and passive integrated transponder (PIT) tags to determine if bats visited the heated water tank. We found evidence of hypertonic dehydration (elevated hematocrit and concentrations of some serum ions) in bats as winter progressed. Blood δ^2H of bats was similar to that of water on the landscape, and acoustic and video surveillance did not indicate any visits by bats to the water tank. Post-arousal dehydration is not uncommon in hibernators, which agrees with our observation that the water tank did not represent a water resource, despite it being the only open (not frozen) water available. It is unknown whether bats may exploit frozen sources of water (e.g., snow) to supplement metabolic water produced from fat catabolism.

Key words: bats, dehydration, hibernation, stable isotopes, torpor, water developments, arousals

Introduction

Hibernation allows animals to survive prolonged periods of food shortage by greatly reducing energy expenditure (Ruf and Geiser, 2015). Despite the obvious energetic benefits, there are non-energetic costs to hibernation that lead to periodic arousals in virtually all hibernators (Humphries *et al*., 2003). During these arousals, metabolic rate and body temperature return to near normal values expected in a non-hibernating individual. The reason(s) for these arousals remain poorly understood (Willis, 1982; French, 1985). Among many hypotheses proposed, access to food or water has been suggested. Arousal patterns facilitate foraging for some small mammals overwintering in areas with mild winters (e.g., French, 1977; Körtner *et al*., 1998; Hope and Jones, 2012). Some hibernators may exit their den, burrow, or roost to find water (Thomas and Geiser, 1997) because metabolically produced water may not fully compensate for evaporative water loss during hibernation (Thomas and Cloutier, 1992).

The need to periodically drink during hibernation may be exacerbated by the microclimate of the hibernacula (temperature and humidity). In bats, the combination of relatively large lungs compared to birds and terrestrial mammals of similar size (Maina, 2000) and large surface area of the wings contribute to high rates of total evaporative water loss (TEWL; e.g., Kurta *et al*., 1989; Hosken and Withers, 1997, 1999). Potential hibernacula for bats in the North American prairies (e.g., rock crevices) are small, dry, and thermally variable (Klüg-Baerwald and Brigham, 2017; Klüg-Baerwald *et al*., 2017) compared to most known cavernous hibernacula (Webb *et al*., 1996; Perry, 2012), and these microclimate conditions may increase the risk of dehydration. Common winter flights made by individuals from prairie bat-populations despite the unavailability of insect prey (Lausen and Barclay, 2006; Klüg-Baerwald *et al*., 2016) leads to the

prediction that these flights could represent a search for water.

To contend with a lack of free standing water, arid-adapted species rely on dietary water intake and or physiological or behavioural adaptations, but often still experience water deficits (McKechnie and Wolf, 2010). Artificial provision of water is commonly used as a management tool for wildlife and livestock in many arid regions (Rosenstock *et al*., 1999). A common technique to track the use of these structures involves the analysis of stable isotope ratios. Consumption of water with experimentally enriched concentrations of deuterium (^{2}H) — a stable isotope of hydrogen — has been used to track water use by wildlife and through trophic levels (e.g., McKechnie *et al*., 2004; McCluney and Sabo, 2010; Smit *et al*., 2019). Comparison of the δ^2H in an individual's tissues relative to that of their isoscape (all potential foods or drinking sources) allows for the approximation of the relative contribution of each source (Ben-David and Flaherty, 2012).

We hypothesized that prairie bats would supplement their water balance to contend with the high rates of TEWL caused by their dry hibernacula microclimate We predicted that individuals would make frequent mid-winter flights to find water to drink (McKechnie and Wolf, 2010). We thus predicted that bats captured in mid-winter flight would exhibit physiological signs of dehydration, such as increased serum ion concentrations and elevated hematocrit. We provided a heated water tank as an open source of water all winter and predicted that bats would drink from this structure relative to other sources of water, such as the creek or river, which were typically frozen for most of the winter when bat activity occurred. We enriched the tank water with ^{2}H and used stable isotope analysis of blood samples to test for elevated δ^2H in the blood of bats captured in winter. To supplement our isotope analysis, we also used passive acoustic monitoring, video surveillance, and passive integrated transponder (PIT) tags to determine if bats visited the heated water tank to drink.

Materials and Methods

Study Site and Species

Our study took place in Dinosaur Provincial Park (DPP), Alberta, Canada (50°45'09"N, 111°31'03"W) during October through March of 2012–2015. The park is comprised of riparian and prairie habitat with an extensive network of creeks and drainages, and has a semi-arid climate (Bailey, 1979). Three species — the big brown bat (*Eptesicus fuscus*), Western small-footed myotis (*Myotis ciliolabrum*), and long-eared myotis (*M. evotis*) — are known to overwinter within the park and are active during the winter when the weather is favourable (Lausen and Barclay, 2006; Klüg-Baerwald *et al*., 2016). In 2008, we installed a modified hot tub (1,700 l, 2 m in diameter) as an open water source available year round for use by bats (Fig. 1). The tank was located ca. 500 m from the three known rock-crevice hibernacula in the park (Klüg-Baerwald *et al*., 2017) and ca. 25 m from the Little Sandhill Creek, which is the only permanent creek in the park and the only location where bats can be reliably captured in mist nets during winter. We captured bats in mist nets set across this creek and recorded sex, age, and morphometric measurements (e.g., mass and forearm length).

We collected 50–75 µl of blood from the interfemoral vein of each captured *E. fuscus*. We used ca. 25 µl in a handheld blood gas analyzer (iStat®; Abaxis North America, Union City, California) to analyze Na^+, K^+, and Cl^- concentrations (mmol/l) and hematocrit (Hct; % packed cell volume [PCV]). We transferred the remaining portion (ca. 50 µl) to a 200 µl o-ring microcentrifuge tube filled with 95% ethanol (K. Hobson, personal communication) and kept it frozen until analysis at the University of Regina. Before release, we permanently marked all *E. fuscus* with a 0.1 g passive integrated transponder (PIT) tag (Trovan ID100 nano-transponders; EIDAP Inc., Sherwood Park, AB Canada) injected under the skin of the lower back. After tagging, we used tissue adhesive (Vetbond™; 3M Canada, London, Ontario) to close the injection site and then monitored each bat during the next hour for signs of distress or injury and to ensure proper insertion of the PIT tag.

The University of Regina President's Committee on Animal Care approved all methods and procedures (Animal Use Protocol #12-12), which conformed to the guidelines for animal care and use outlined by the American Society of Mammalogists (Sikes *et al*., 2016). We performed fieldwork under research and collection permits issued by Alberta Sustainable Resource Development and Alberta Tourism, Parks and Recreation Division.

Stable Isotopes

At our study site, the annual mean of streamflow δ^2H values (‰ Vienna Standard Mean Ocean Water [VSMOW]; Bowen and Revenaugh, 2003) is -120‰ to -140‰ (Gibson *et al*., 2020). To trace the bat's use of the supplied water (heated tank), we treated the tank water with small amounts (100–200 ml) of enriched (99.8%) deuterium oxide (^2H_2O). We took a 100 ml sample of water from the tank every month from October through April, as well as other potential sources of drinking water (i.e., snow and river water). We stored water samples in airtight collection vials at cool (but above freezing) temperatures and blood samples at subzero temperatures to minimize ^{2}H exchange between water and the storage solution (i.e., kinetic and equilibrium fractionation, respectively; Ben-David and Flaherty, 2012). We transported samples on ice to the Institute of Environmental Change and Society (IECS) at the University of Regina for analysis. Prior to analysis, we freeze-dried blood samples. We performed stable isotope analyses on a Thermo Finnigan Delta plus XL isotope ratio mass spectrometer (precision: ± 0.5‰; Thermo Electron Corporation Canada, Gormley, ON Canada) that was coupled to a conversion/elemental analyzer (TC/EA). We standardized isotopic values of blood to Kudu Horn Standard (KHS) and Caribou Hoof Standard (CBS),

FIG. 1. Photograph of the 1,700 l heated water tank installed in Dinosaur Provincial Park from 2008–2015 as a continuously accessible source of water all winter. Tank water was enriched with deuterium (^{2}H) to levels of +400–925‰

and those of water to Picarro standards Zero, Mid, High, and DI water. We report all isotope results in delta notation in permil (‰) representing:

$$\delta^{j/i}X = [(^jX/^iX)_{sample}/(^jX/^iX)_{standard}] - 1,$$

where R is the ratio of heavy to light isotopes in the sample and standard, respectively (Bond and Hobson, 2012).

Acoustic and PIT-tag Monitoring

We used an Anabat (Titley Electronics, Ballina, NSW, Australia) detector to record acoustic bat activity at the water tank every night from an hour before sunset to an hour after sunrise. We housed the detector in a custom built waterproof box mounted on the side of the tank and powered with a 12 V, 12 Ah sealed lead-acid battery coupled to a 10 W solar panel. We calibrated the detector (see Larson and Hayes, 2000) to record activity in a small volume of space directly above the water. We used AnalookW software (version 3.9c; C. Corben, Columbia, MS, USA) with a custom filter to separate background noise (e.g., insects and wind) from bat calls (for details see Lausen *et al*., 2014). We used the presence of "feeding buzzes" (segments of rapidly produced short duration pulses; (Fenton, 2003) to indicate bats possibly drinking from the tank. On nights when we anticipated high bat activity (Klüg-Baerwald *et al*., 2016), we also used a handheld camcorder with high definition night vision capability (HDR-CX730; Sony Corp., Tokyo, Japan) to record activity at the tank beginning 3–4 h after sunset. We also used a PIT-tag decoder (Trovan 650, EIDAP Inc.) connected to a waterproof plate antenna (IP68; EIDAP Inc.) placed just below (ca. 2 cm) the surface of the water to record the time and date when any PIT-tagged *E. fuscus* came within range of the antennae (ca. 5 cm).

Statistical Analyses

We used linear models to assess the influence of hibernation date on serum electrolyte concentrations (Na^+, K^+, Cl^-) and hematocrit (Hct). We defined hibernation date as the number of days since the first day of the season with a daytime high $T_a \leq 0$°C (23 October 2012, 28 October 2013, and 9 November 2014). We also used linear models to produce baseline regressions with 95% confidence intervals (CI) of δ^2H of surface and tank water over the course of the sampling period (October–March). We compared δ^2H of blood sampled from bats during winter to the values from surface and tank water, and considered any points above the 95% CI of surface water as indicative that those bats had consumed at least some water from the tank. We conducted all statistical analyses using R (R Core Team, 2016) and present all data as means ± *SD*.

RESULTS

We captured 102 *E. fuscus* during the three years of our study and PIT-tagged each one. We collected 31 blood samples for serum electrolyte and hematocrit analysis, and 54 blood samples for stable isotope analysis. We pooled data across years and did not use data from recaptured individuals ($N = 2$) in either analysis. Hibernation date was positively correlated with Hct (R_2 = 0.2577, $F_{1,28}$ = 9.72, P = 0.004 — Fig. 2) and the serum electrolyte concentration of Na^+ (R_2 = 0.4219, $F_{1,28}$ = 20.43, $P < 0.001$) and Cl^- (R_2 = 0.5282, F = 31.34,

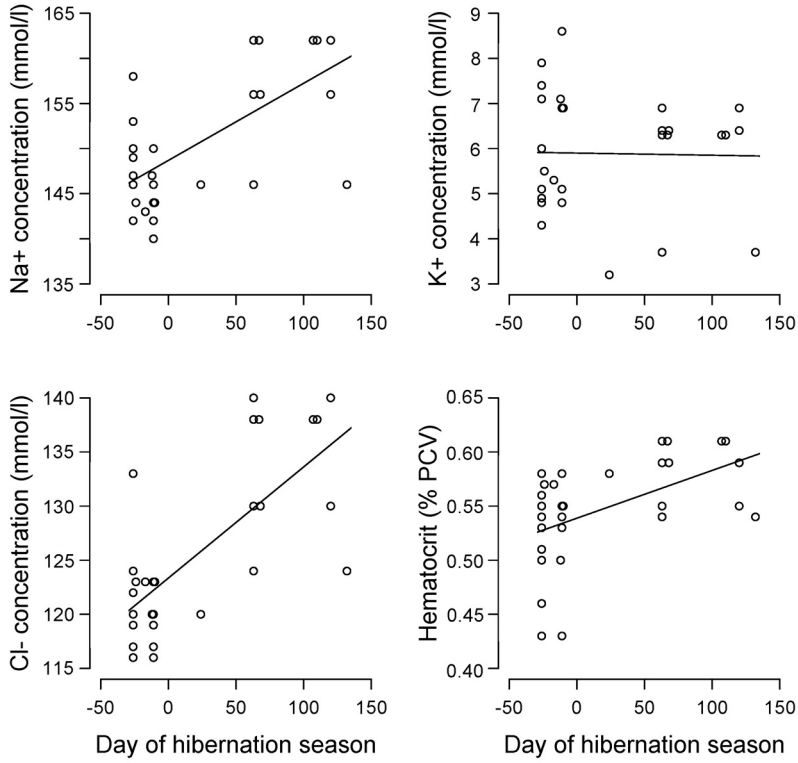

FIG. 2. Serum electrolyte concentrations in blood collected from *E. fuscus* captured mid-flight in Dinosaur Provincial Park, Alberta during the winters of 2012–2015

$P < 0.001$), but not K^+ ($R_2 = 0.0004$, $F_{1,28} = 0.01$, $P = 0.914$). From pre-hibernation (i.e., October) to late hibernation (i.e., February and March), mean Hct rose from 0.53 ± 0.05 to 0.58 ± 0.03%, mean Na^+ concentration rose from 147 ± 4.3 to 158 ± 7.0 mmol/ml, and mean Cl^- concentration rose from 121 ± 3.9 to 134 ± 6.8 mmol/ml. Mean δ^2H of blood samples taken from bats (δ^2H bat) was -157 ± 7.7‰ VSMOW. All δ^2H bat data points fell within the 95% CI of surface water δ^2H (mean δ^2H surf = -143 ± 33.6‰ VSMOW — Fig. 3), considerably lower than that of the water tank (mean δ^2H tank = 719 ± 180.2‰ VSMOW).

The acoustic detector recorded 33 bat echolocation calls over the surface of the water tank, but we did not identify any as a 'feeding buzz'. In 41 h of video footage over nine nights (4.5 ± 1.08 h night-1), we did not record any bats visit the tank. The PIT-tag reader did not record the presence of any tagged *E. fuscus* over the surface of the water tank.

DISCUSSION

Winter bat activity is of considerable interest given the emergence of white-nose syndrome as a threat to hibernating bats (Frick *et al*., 2015). Initially thought of as atypical, we now know that bats in many areas exhibit some activity throughout hibernation and mid-winter flights are not uncommon (Boyles *et al*., 2006). Although the exact reasons for winter activity remain unclear (Willis, 1982), in arid areas such as the prairies, it is logical to hypothesize that evaporative water loss and negative water balance may drive bats to arouse and seek water. In such cases, the provision of water may become an important management tool for bat conservation. Other studies have documented the use of artificial water developments for a range of animals, including bats in arid environments (Adams and Hayes, 2008), but we believe ours is the first to investigate this during winter.

Our data show hypertonic dehydration occurs in free-ranging *E. fuscus* as hibernation progresses. Hematocrit and all serum electrolyte concentrations except K^+ increased from pre-hibernation (i.e., October) to late hibernation (i.e., February–March). Other studies have investigated hematological changes during hibernation with mixed results depending on species and depth of hibernation (Riedesel and Folk, 1958). Both serum K^+ concentrations and Hct decrease within days of *E. fuscus* entering hibernation (Riedesel and Folk, 1958) but

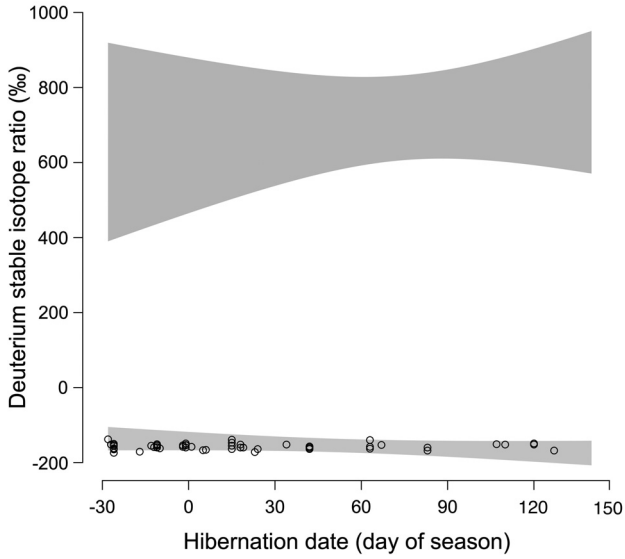

FIG. 3. Plot of deuterium stable isotope ratios detected in the blood of *E. fuscus* (δ^2H_{bat}; open circles) over time in Dinosaur Provincial Park, Alberta. Shaded areas represent 95% confidence intervals of δ^2H detected in surface water (snow and melt; light grey) and a heated water tank enriched with 99.8% ^2H_2O (dark grey). Data points lying above the 95% CI limit for surface water would suggest at least some use of the water tank by bats

no study has evaluated changes in blood composition of this species over the course of an entire hibernation period. Despite hibernating for < 24 h and not likely being dehydrated, hematocrit and K^+ concentration were higher for hibernating little brown bats (*Myotis lucifugus*) than active pre-hibernating controls (Riedesel and Folk, 1958). Thus, observations of increased Hct and serum electrolyte concentrations may reflect a normal state of dehydration in late hibernation. It would be difficult to parse out the added effect of aridity on the hydration state of bats in our study area given the inconsistent results of other serum electrolyte studies and our limited dataset. The homeostasis of serum K^+ in our samples is not entirely unexpected. Even bats experimentally infected with the fungus that causes white-nose syndrome (*Pseudogymnoascus destructans*) show little change in serum K^+ concentrations from healthy control groups, despite hypotonic dehydration and fluctuations in most other blood parameters (Warnecke *et al*., 2013). The limited information on the hydration status of hibernating bats is an important gap that requires further study.

Contrary to our prediction, we found no evidence for the use of the water tank by bats in DPP. Although we detected bats in the vicinity of the water tank, we did not identify any acoustic signs (i.e., feeding buzzes) indicating bats actually drank from the tank. Further, we did not detect any PIT-tagged bats within range of the submersed antenna, nor did we collect any video evidence of visitation by bats to the water tank. Most conclusively, we did not find evidence of elevated δ^2H bat, which would be indisputable, as even uncommon drinking from the water tank would have been easily detected in blood samples given the large differences in δ^2H between the water tank (400–900‰ VSMOW) and ground water (annual mean level of -120‰ to -140‰ VSMOW — Gibson *et al*., 2020). The lack of foraging in winter means δH of body tissues is likely not influenced by the contribution of dietary lipids and should reflect that of environmental water (Soto *et al*., 2013). Conversely, the low metabolic rate of hibernating bats (Geiser, 2004) would result in isotopic values of consumed prey persisting in the blood for months after ingestion (Storm-Suke *et al*., 2012). The δ^2H values we detected likely reflect consumption of surface water or water released from fat stores.

In summary, we found no evidence that bats in DPP used the artificial water source. Bat activity occurred at subzero temperatures (as low as -10.4°C — Klüg-Baerwald *et al*., 2016), and we were unaware of any other source of open (not frozen) water in the study area, particularly in mid to late winter during freezing winter conditions when bats are still detected flying in this area (Lausen and Barclay, 2006; Klüg-Baerwald *et al*., 2017). While thirst has been suggested as reason for hibernal arousals of bats (Ben-Hamo *et al*., 2013); in our study area, we found that bats emerged and took flight outside of hibernacula only 36% ($N = 27$) of the times that they aroused (Klüg-Baerwald *et al*., 2017), suggesting that arousal is not always associated with water seeking. Dehydration status may still be driving emergence from hibernacula in winter, but in our study, given the lack of use of the water tank, this assumes that another water source was available, if in fact bats were obtaining water. The increasing dehydration that we saw in bats as winter progressed suggests that if water is being obtained, it may be in smaller quantities that would be needed to keep Hct and blood ions at normal levels. For example, snow accumulations in the river valley could provide a water source, though large consumption could lower body temperatures beyond that conducive to flight. Snow would not have been immediately available outside the crevice hibernacula due to the steep cliff features in which hibernacula were located (Klüg-Baerwald *et al*., 2017).

We found increased levels of hypertonic dehydration in late winter; however, the influence of

arid conditions on dehydration is unclear, as is the amount of water that would need to be consumed by hibernating *E. fuscus* in our area. Bats have been reported to quickly find and exploit artificial water provisions (Adams and Hayes, 2008), and the water tank at our study site was functional for several years as a reliable resource located close to the hibernacula during a time in which access to water is constrained. If bats are flying to find water in our study area during cold winter flights, it seems unlikely that none would make use of this readily and continuously available resource. Further research is needed into the causes of winter bat-activity, the requirements of winter water intake during hibernation for the variety of hibernating bat species across varied landscapes, and the possible use of artificial water developments to mitigate dehydration, especially as climate change and WNS add to the challenge of maintaining water balance faced by hibernating bats in some areas.

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