Incorporating evaporative water loss into bioenergetic models of hibernation to test for relative influence of host and pathogen traits on white-nose syndrome

Short title: Modelling the dehydration hypothesis with WNS

Catherine G. Haase,[1*+] Nathan W. Fuller,[2] C. Reed Hranac,[3] David T. S. Hayman,[3] Liam P. McGuire,[2] Kaleigh J. O. Norquay,[4] Kirk A. Silas,[5] Craig K. R. Willis,[4] Raina K. Plowright,[1]† Sarah H. Olson[5]†

[1] Department of Microbiology and Immunology, Montana State University, Bozeman, USA.
[2] Department of Biological Sciences, Texas Tech University, Lubbock, USA.
[3] Molecular Epidemiology and Public Health Laboratory, Hopkirk Research Institute, Massey University, NZL.
[4]Department of Biology, University of Winnipeg, Winnipeg, CAN.
[5] Wildlife Conservation Society, Wildlife Health Program, Bronx, USA.
†RKP and SHO are joint senior authors

*Corresponding author haasec@apsu.edu

[+]Current address:
Department of Biology, Austin Peay State University, Clarksville, USA

Abstract

Hibernation consists of extended durations of torpor interrupted by periodic arousals. The 'dehydration hypothesis' proposes that hibernating mammals arouse to replenish water lost through evaporation during torpor. Arousals are energetically expensive, and increased arousal frequency can alter survival throughout hibernation. Yet we lack a means to assess the effect of evaporative water loss (EWL), determined by animal physiology and hibernation microclimate, on torpor bout duration and subsequent survival. White-nose syndrome (WNS), a devastating disease impacting hibernating bats, causes increased frequency of arousals during hibernation and EWL has been hypothesized to contribute to this increased arousal frequency. WNS is caused by a fungus, which grows well in humid hibernaculum environments and damages wing tissue important for water conservation. Here, we integrated the effect of EWL on torpor expression in a hibernation energetics model, including the effects of fungal infection, to determine the link between EWL and survival. We collected field data for *Myotis lucifugus,* a species that experiences high mortality from WNS, to gather parameters for the model. In saturating conditions we predicted healthy bats experience minimal mortality. Infected bats, however, suffer high fungal growth in highly saturated environments, leading to exhaustion of fat stores before spring. Our results suggest that host adaptation to humid environments leads to increased arousal frequency from infection, which drives mortality across hibernaculum conditions. Our modified hibernation model provides a tool to assess the interplay between host physiology, hibernaculum microclimate, and diseases such as WNS on winter survival.

Keywords: dehydration hypothesis, hibernation, *Myotis lucifugus*, *Pseudogymnoascus destructans*, torpor, white-nose syndrome

In periods of food scarcity, hibernators conserve energy by entering torpor, during which body temperature (T_b) is maintained near hibernaculum temperature and metabolic rate is lowered to reduce energy demands [1]. There are several hypotheses proposed to explain periodic arousals, but two of the most prominent are linked to water balance: 1) the dehydration hypothesis [2,3]; and 2) the need to excrete metabolic byproducts [4]. The dehydration hypothesis suggests that hibernators arouse periodically after a threshold of total body water is reached [5,6]. The metabolic byproducts hypothesis suggests that bats accumulate byproducts from biochemical reactions during torpor, and these byproducts need to be excreted as waste as they can be damaging to cellular function [4]. Both of these hypotheses are affected by microclimate, which is supported by empirical evidence of the relationship between hibernaculum temperature and relative humidity and torpor bout duration [3,5,7,8]. Hibernators do not normally defecate or urinate during torpor [9], thus water lost during inactive periods of hibernation is assumed to be from evaporation. Evaporative water loss (EWL) is comprised of respiratory and cutaneous water loss [10,11] and is driven by the difference in water vapor pressure between the surface of an animal and the surrounding air, which, in turn, is determined by the saturation of the air given air temperature [12]. The dehydration hypothesis is supported by correlations between torpor bout duration/arousal frequency and hibernaculum temperature and relative humidity in both free living and laboratory conditions [2,5,13].

Though arousals only make up a small portion of hibernation time, these periods account for the majority of the winter energy budget [1,14]. Therefore, the influence of microclimate on arousal frequency is critical for over-winter fat loss. However, the influence of microclimate has recently become an important question in the context of white-nose syndrome (WNS). WNS is a rapidly spreading infectious disease that has led to high mortality rates in hibernating bats across

eastern and central North America. It has been proposed that increased EWL from infection could be a trigger of increased arousals associated with WNS [13,15].

The causal agent of WNS is a psychrophilic fungus, *Pseudogymnoascus destructans*, which erodes wing tissue [16,17]. Mechanistic models and empirical evidence connecting *P. destructans* infection to altered torpor-arousal cycles suggest that ulceration of the highly vascularized wing tissues causes increased fluid and water loss [13,18–21]. The growth rate of *P. destructans* is linked to both ambient temperature [22] and humidity [23], with higher fungal growth rates in environmental conditions frequently found in bat hibernacula.

Although studies have linked arousal frequency with survival, and hibernaculum microclimate and EWL with arousal frequency, none have explicitly considered the effect of EWL on survival to our knowledge. Hibernation energetic models are commonly used to understand energy consumption over winter but have yet to account for water balance and its effect on arousal behavior. With the current threat of WNS, a disease that potentially directly impacts water balance, it is important to understand the implications surrounding the association of EWL, energy consumption, and survival. We therefore developed a hibernation energetics model that incorporates water balance to assess the effects of the dehydration hypothesis on survival of *Myotis lucifugus*, a wide-ranging species that is heavily impacted by WNS. Using WNS as a study system, we tested the hypothesis that increased EWL from fungal infection results in greater energy consumption due to increased arousal frequency and, therefore, reduced survival. We predicted that hibernaculum conditions that reduce EWL (cold temperatures, high relative humidity) would increase our modeled survival rates. We also predicted that model parameters that influence EWL (surface area, area-specific rate of EWL), would have greater effects on modeled survival rates compared to other parameters.

Building on equations developed by Thomas et al. [1], Humphries et al. [24], and Hayman et al. [25], we included the effects of hibernaculum microclimate on fungal growth, EWL, torpor bout duration, and total fat loss. We parameterized the model using morphometric and physiological characteristics collected from *M. lucifugus* captured in the field. We validated the modified model components using a variety of data sources, determined the most influential parameters using a sensitivity analysis, and predicted fat loss over a range of hibernaculum conditions for both healthy and *P. destructans*-infected bats. Finally, in the context of winter duration, we inferred the impact of WNS on survival by comparing pre-hibernation fat stores to fat loss estimated by the energy expenditure model.

Methods

Ethics statement

All procedures were approved by the Texas Tech University Institutional Animal Care and Use Committee (protocol 16031-05) and followed guidelines of the Guide for the Care and Use of Laboratory Animals. We obtained proper permits from the Montana Department of Fish, Wildlife & Parks (permits 2016-104, 2017-018, and 2018-008).

Study species

M. lucifugus is a common insectivorous bat species found across most of North America [26]. The hibernation behavior of *M. lucifugus* is well-studied. During the winter, *M. lucifugus* hibernate in caves and abandoned mines, often in large colonies [26]. Most hibernacula have stable microclimates, with high humidity (≥ 90 % RH) and temperatures ranging from 2 to 8 °C [8,27,28]. Many energetic models have determined energy expenditure during hibernation in response to microclimate selection, sex, and location [1,8,24,29–35]. Energetic models have been used to predict energy expenditure from WNS with alterations to arousal frequency [21,32].

M. lucifugus is also one of the most studied species in terms of WNS impacts. Since the discovery of WNS in 2006, millions of *M. lucifugus* have died across the species' range and have faced upwards of 99% mortality rates [28,36,37]. Populations across eastern and mid-western North America affected by WNS remain at severely reduced population sizes and reduced population growth rates [36,38,39].

Field data collection for model parameters

We captured *M. lucifugus* during the pre-hibernation (September-November) swarming and mid-hibernation (January-February) periods from 2016-2018 at a cave in central Montana. We used mist nets placed at the cave entrance to capture bats during swarming and hand-captured bats from hibernaculum walls during mid-hibernation. We transported bats in cloth bags to a mobile laboratory at the field site location for morphometric measurements. We weighed each bat (± 0.1 g) and used quantitative magnetic resonance (Echo-MRI-B, Echo Medical Systems, Houston, TX) to measure fat mass and lean mass [40]. We measured torpid metabolic rate (TMR) and EWL using open-flow respirometry at 2, 5, 8, and 10 °C (Supplementary Materials; [41]). We calculated the mean body mass, fat mass, and lean mass across all fall field seasons and the mean of mass-specific TMR across both seasons among all individuals across to use as parameters in the hibernation model (Table 1).

131 Table 1. Parameters for the energetics model for the little brown bat (*Myotis lucifugus*), their units, and the reference.

Parameter Name	Parameter	Value	Units	Reference
Basal metabolic rate	BMR	2.6	ml O_2 g^{-1} h^{-1}	Calculated from [55]
Minimum torpid metabolic rate	TMR_{min}	0.14	ml O_2 g^{-1} h^{-1}	Measured in this study
Lower defended temperature during torpor	$T_{tor\ -\ min}$	2	°C	[48–50]
Lower critical temperature	T_{lc}	32	°C	[48–50]
Euthermic body temperature	T_{eu}	37	°C	[1,48–50]
Change in torpid metabolism	Q_{10}	$1.6 + 0.26\ T_a - 0.006T_a^2$	-	[48]
Torpid thermal conductance	C_t	0.20	ml O_2 g^{-1} $°C^{-1}$ h^{-1}	Calculated from [73]
Euthermic thermal conductance	C_{eu}	0.26	ml O_2 g^{-1} $°C^{-1}$ h^{-1}	Calculated from [27]
Wing surface area	SA_{wing}	19.68	cm^{-2}	Calculated in this study
Body surface area	SA_{body}	39.26	cm^{-2}	Calculated from [53]
Area-specific rate of evaporative water loss for wing	$rEWL_{wing}$	0.33	mg hr^{-1} ΔWVP^{-1} cm^{-2}	Calculated in this study
Area-specific rate of evaporative water loss for body	$rEWL_{body}$	0.10	mg hr^{-1} ΔWVP^{-1} cm^{-2}	Calculated in this study
Time in euthermia per arousal	t_{eu}	1.10	h	[31,74,75]
Maximum time in torpor	$t_{tor\text{-}max}$	1300	h	[51]
Specific heat of tissue	S	0.173	ml O_2 g^{-1} $°C^{-1}$	[14]
Rewarming rate	WR	0.80	°C min^{-1}	[44,58,76,77]
Body mass	M_b	7.80	g	Measured in this study
Proportion of lean mass	pLean	0.58	g	Measured in this study
Proportion of fat mass	pFat	0.26	g	Measured in this study
Proportion of body water threshold	pMass	0.027	mg	Calculated in this study
Humidity-dependent fungal growth parameter	μ_1	$1.51\ x10^{-4}$	-	[25]
Humidity-dependent fungal growth parameter	μ_2	$-9.92\ x\ 10^{-3}$	-	[25]
Temperature-dependent fungal growth parameter	β_1	$1.15\ x\ 10^{-3}$	-	[25]
Temperature-dependent fungal growth parameter	β_2	0.27	-	[25]

We measured hibernaculum temperature and relative humidity over each hibernation period using HOBO (Model U23-001, ± 0.001 °C, ± 0.001% RH, Onset Computer Corporation) and iButton (temperature only; Model DS1921Z-F5, ± 0.05 °C, Maxim Integrated Products) dataloggers. We placed four HOBO and ten iButton dataloggers throughout the hibernaculum in the fall and recorded conditions at 3 h intervals. We determined the main winter roosting location from personal communication with U.S. Forest Service and Montana Department of Fish, Wildlife, and Parks personnel. We placed two HOBO loggers in the main roost, a large cathedral room at the back of the cave (one logger at the far end, one at the entrance), one within 3 m of the entrance of the cave, and one attached to a tree immediately outside the cave entrance (< 10 m). We spaced the iButtons evenly throughout the cave system from the entrance to the cathedral room. We suspended HOBO loggers with copper wire and used pantyhose to attach each iButton to a projected rock to suspend the logger in the air column. We collected loggers from the hibernaculum in the spring of each year.

We estimated winter duration for central Montana by acoustically monitoring bat activity at the entrance to the cave (Anabat Roost Logger RL1, Titley Scientific). The acoustic logger operated between 30 min before sunset and 30 min following sunrise. We used AnaLookW software (v4.3) to digitize calls and count the number of bat passes per day [42]. We were not interested in species-specific calls, but rather use the calls as an index of winter duration so we counted passes that contained calls of *Myotis* species (minimum frequency [f_{min}] > 30 kHz) to filter out noise [43]. We were also not interested in the number of individual bats passing the detector, but rather if there was general activity outside the cave; we thus used a threshold of 50 passes day^{-1}, defining the lower end of the 95% of bat counts, to determine the onset and end of the hibernation period [43].

Incorporating evaporative water loss into the hibernation energetics model

We revised the hibernation energetics model first described by Thomas et al. [1] and Humphries [24], and then modified to include fungal growth by Hayman et al. [25]. The model estimates the amount of fat consumed during hibernation as a summation of the energy expended during multiple torpor-arousal bouts across a winter period (full model presented in Supplementary Materials). We derived estimates of the energy required during torpor (E_{tor}), euthermia (E_{eu}), and the warming (E_{warm}) and cooling (E_{cool}) periods between torpid and euthermic temperatures. We estimated the period of each arousal spent within euthermia from literature (Table 1) and the time to warm and cool were calculated given published warming and cooling rates, respectively [44].

We incorporated a mechanistic link between EWL and torpor bout duration. We estimated torpor bout duration (t_{tor}) in two ways: 1) as a function of torpid metabolic rate in response to ambient temperature (T_a) as described in Hayman et al. [25], and 2) as a function of EWL. Our revised model uses the shorter of the two estimates given hibernaculum conditions (Supplementary Figure S1), either arousing as a consequence of EWL or TMR, whichever comes first. By including both calculations in our estimates of torpor bout duration, we considered both the effect of EWL and metabolism on torpor physiology [45,46].

To estimate torpor bout duration as a function of metabolic rate (t_{torTMR}), we modified the existing equations developed by Hayman et al. [25] that scale maximum possible time in torpor (t_{torMax}) by the effects of metabolic rate given T_a:

$$t_{torTMR} = t_{torMax} \Big/ Q^{\left(\frac{T_a - T_{torMin}}{10}\right)} \quad \text{if } T_a > T_{torMin} \tag{1}$$

$$t_{torTMR} = \frac{t_{torMax}}{1 + (T_{torMin} - T_a) \cdot \left(\frac{C_t}{TMR_{min}}\right)} \quad \text{if } T_a \leq T_{torMin} \tag{2}$$

where Q_{10} is the change in metabolism with a 10°C change in temperature [47], T_{torMin} is the minimum defended T_b in torpor, TMR_{min} is the associated metabolic rate at T_{torMin}, and C_t is the thermal conductance during torpor. Minimum defended T_b [48–50] and the maximum time in torpor (t_{torMax}) were estimated from literature [51], and minimum torpid metabolic rate and thermal conductance were measured in the field using respirometry (Supplementary Materials).

To calculate torpor bout duration as a function of EWL (t_{torEWL}), we assumed bats arouse when the total body water pool was depleted to a threshold [5]. The hourly rate of total EWL (mg H_2O h^{-1}) is comprised of both cutaneous and respiratory rates of EWL and is dependent on the water vapor pressure deficit between the bat and the surrounding environment. The hourly rate of cutaneous evaporative water loss (CEWL; mg H_2O h^{-1}) is a function of the difference between water vapor pressure at the surface of the bat and the environment (ΔWVP):

$$\Delta WVP = WVP_{bat} - WVP_{air} \quad (3)$$

where WVP_{bat} is the water vapor pressure at the skin surface and WVP_{air} is the water vapor pressure of the surrounding air (both in kPa). We assumed WVP_{bat} was at saturation, which can be calculated as:

$$WVP_{bat} = 0.611 \cdot e^{\left[\frac{17.503 \cdot T_b}{(T_b + 240.97)}\right]} \quad (4)$$

where T_b is the body temperature of the bat in torpor [52]. We then calculated WVP_{air} at T_a and given relative humidity. We modeled cutaneous EWL as a function of ΔWVP and the area-specific rate of EWL from bodily tissue (rEWL; mg H_2O h^{-1} cm^{-2} per ΔWVP^{-1}) across the surface area (SA; cm^2) of the bat:

$$CEWL = SA \cdot rEWL \cdot \Delta WVP \quad (5)$$

We used a surface area scaling equation [53] to calculate body surface area (SA_{body}) and photos of bat wings to estimate the total surface area of the wings and tail (SA_{wing}; Supplementary

Materials). Assuming that a furred body and naked wing have biophysical differences that would affect cutaneous EWL, we used different values of the area-specific rate of EWL for the body ($rEWL_{body}$) and wing ($rEWL_{wing}$), estimated from respirometry (Supplementary Materials). Therefore, we rewrote Equation 5 as:

$$CEWL = [(SA_{body} \cdot rEWL_{body}) + (SA_{wing} \cdot rEWL_{wing})] \cdot \Delta WVP \quad (6)$$

Respiratory EWL (REWL; mg H_2O h^{-1}) is a function of the saturation deficit between inspired and expired air. We assumed that inspired air is at T_a and is expired as saturated air at torpid T_b [5]. Therefore, we calculated respiratory EWL as:

$$REWL = respired\ air\ volume \cdot saturation\ deficit \quad (7)$$

The volume of air that a bat breathes per hour was calculated as a function of the respiration rate of oxygen (i.e. TMR_{min}) in ml O_2 g^{-1} h^{-1} and body mass:

$$respired\ air\ volume = \frac{TMR_{min} \cdot M_b}{0.2095 \cdot 0.30 \cdot 10^3} \quad (8)$$

assuming the fractional concentration of oxygen in air is 0.2095 and that oxygen extraction efficiency is 30% [5]. Using the ideal gas law [52], we converted the water vapor pressure deficit (ΔWVP; Equation 3) from kPa to mg L^{-1} to determine the saturation deficit.

We validated the rate of total EWL (cutaneous EWL and respiratory EWL) by comparing modeled EWL (from Equations 5 and 7) to measured EWL from each individual during our respirometry procedures. We used individual body mass (Equations 5-6), metabolic rate (Equations 7-8), area-specific rate of EWL (Equations 5-6), and predicted surface area given body mass (Equations 5-6). We modeled the hourly rate of total EWL given the measured T_a and WVP experienced by each individual. We used linear regression to compare modeled EWL to measured EWL rates, assuming that if the model was accurate, the slope of the relationship should be equal to 1.

Given total EWL, we calculated torpor bout duration (t_{torEWL}) based on the reduction of the total body water pool, setting the threshold at 2.7% of lean mass (assuming no body water in fat stores):

$$t_{torEWL} = \frac{0.027 \cdot Lean\ Mass \cdot 1000}{CEWL + REWL} \quad (9)$$

Including the effects of fungal growth on hibernation

We further adjusted the hibernation model by including a link between fungal growth and reduced torpor bout duration through an increase in both metabolic rate and EWL (modifying Equations 1-2, 9). We first altered the estimation of torpor bout duration from T_a (t_{torTMR}; Equations 1 and 2) by scaling t_{torTMR} by the proportion the bat wing surface affected by the fungus. When fungal growth > 0, t_{torTMR} was calculated as:

$$t_{torTMR} = \left[\frac{t_{torMax}}{Q^{\left(\frac{T_a - T_{torMin}}{10}\right)}_{10}}\right] \Big/ \left(\frac{area_{Pd}}{SA_{wing}}\right) \quad \text{if } T_a > T_{torMin} \quad (10)$$

$$t_{torTMR} = \left[\frac{t_{tor-max}}{1 + (T_{torMin} - T_a) \cdot \left(\frac{C_t}{TMR_{min}}\right)}\right] \Big/ \left(\frac{area_{Pd}}{SA_{wing}}\right) \quad \text{if } T_a \leq T_{torMin} \quad (11)$$

where $area_{Pd}$ is the area (cm^2) of fungal growth calculated as a function of T_b and relative humidity given equations from Hayman et al. [25].

We adjusted the calculation of torpor bout duration in response to EWL (t_{torEWL}; Equation 9) by increasing CEWL and REWL as a function of fungal growth. We used data from McGuire et al. [20], who directly measured an increase in TMR and EWL in *M. lucifugus* infected with *P. destructans* (Supplementary Materials). We increased CEWL by including a linear increase to the rate of EWL of bat wings ($rEWL_{wing}$) in response to the proportion the bat wing surface affected by the fungus (from Equation 6):

$$CEWL_{wing} = \left[(SA_{body} \cdot rEWL_{body}) + \left(SA_{wing} \cdot \left[rEWL_{wing} + \left(0.16 \cdot \frac{area_{Pd}}{SA_{wing}} \cdot 100\right)\right]\right)\right] \cdot \Delta WVP \quad (12)$$

where 0.16 is the rate of increase in $rEWL_{wing}$, given the proportion the bat wing surface affected by the fungus, determined from data presented in McGuire et al. [20] (Supplementary Materials). REWL also is hypothesized to increase in response to fungal growth with an increase in TMR; we included this linear increase by adjusting Equation 8:

$$respired\ air\ volume = \frac{\left[TMR_{min} + \left(0.015 \cdot \frac{area_{Pd}}{SA_{wing}} \cdot 100\right)\right] \cdot M_b}{0.2095 \cdot 0.30 \cdot 10^3} \quad (13)$$

where 0.015 is the linear increase of torpid metabolic rate given the proportion the bat wing surface affected by the fungus (Supplementary Materials).

To validate the adjustment to the estimation of torpor bout duration in response to fungal growth (Equations 10-11,13), we used an independent dataset of skin temperature measurements from a captive hibernation study by McGuire et al. [54]. Skin temperature data were measured from thirteen *M. lucifugus* infected with *P. destructans* prior to hibernating in a controlled environment (T_a = 7 °C, relative humidity = 98%). Using methodology from Jonasson and Willis [31], we defined torpor and arousal periods based on cut-off temperatures and calculated the total time in each hibernation phase. We then estimated torpor bout duration (Equations 10-13) at each measured torpor bout from each individual given individual morphometric parameters (initial body mass, predicted surface area). We estimated TMR from body mass and T_b [55] and allowed for variation in lean mass (to determine threshold of body water) by sampling from a normal distribution with mean and standard deviation from our capture data. We predicted fungal growth area at each torpor bout given the time since inoculation and equations 2-4 in Hayman et al [25]. We then used a linear model to compare modeled torpor bout duration to measured torpor bout duration, assuming that if the prediction was accurate, the slope of the relationship

should be equal to 1. To determine if including EWL improved our description of torpor expression, we also predicted torpor bout duration without the contribution of EWL using only Equations 10-11. We then compared these predictions to measured bout duration to determine model accuracy. Finally, we compared the R^2 values of both fitted relationships to determine which model had better precision in predicting torpor bout duration.

Estimation of total fat loss and survival for M. lucifugus *in Montana*

Using our modified hibernation model and model parameters obtained from our field captures and literature (Table 1), we estimated time until total fat exhaustion for *M. lucifugus* over the range of hibernaculum microclimate conditions measured at our field site. Torpor bout duration changes with body condition and fungal growth so we used differential equations to estimate energy consumption over the winter. We assumed that bats require energy to arouse at the end of hibernation and to leave the hibernaculum in order to obtain food. Therefore, we included energy required to warm (E_{warm}) and spend 24 h in euthermia (24 x E_{eu}) at the end of winter hibernation. We used the lsoda function of the *deSolve* package, which allowed torpor bout duration to change over time given fungal growth, bat parameters (Table 1), and hibernaculum microclimate. We converted total energy consumed over time from ml O_2 g^{-1} to the amount of fat expended (g) as:

$$fat_{winter} = (E_{winter} \cdot 19.6)/(37.6 \cdot 1000) \quad (14)$$

assuming that 1 ml O_2 releases 19.6 J of energy and the energy content of fat is 37.6 J mg^{-1} [30]. We calculated time until fat exhaustion (t_{fatEx}) as the time when total fat exhaustion (fat_{winter}), became greater than mean fat stores measured during our fall field captures. Finally, we compared the estimated t_{fatEx} for both healthy and infected bats over the range of hibernaculum conditions to the duration of winter for central Montana estimated from our acoustic data. We

assumed that mortality would occur if t_{fatEx} was less than winter duration; that is, the mean fat stores did not provide enough fat for a bat to survive through winter, as measured above.

We validated the entirety of the hibernation energetic model by comparing measured mass loss from 56 free-living hibernating *M. lucifugus* (Norquay and Willis, unpublished data, but see [56] for description of capture methodology and locations) to predicted fat loss from our model. We used this dataset because data from captive animals may not accurately reflect field conditions of free-living animals. We used individuals in which mass was measured during both swarming (August-September) and emergence (April-May). We estimated fat loss using the bioenergetic model for the time between swarming and emergence capture dates, given the hibernaculum conditions where each bat was captured [57,58]. We took the mean and standard deviation of T_a and water vapor pressure of each capture location and sampled random values from a normal distribution for each individual. We estimated TMR from body mass and T_b [55] and allowed for variation in lean mass by sampling from a normal distribution set at the mean and standard deviation from our capture data. We compared estimated fat loss with measured mass loss (assuming all mass change is due to fat loss) using linear regression, assuming if the two values were the same, the slope of the relationship would be no different than 1. We also predicted fat loss for the validation dataset given the hibernation model without the inclusion of EWL; more specifically, we only included Equations 1-2 in our calculations of torpor bout duration. We compared these predictions to measured mass loss and determined both model accuracy (slope = 1) and precision (R^2) to compare against our modified model including EWL.

Following Hayman et al. [25], we used a multi-parameter sensitivity analysis to assess the impact of each parameter on estimations of time until mortality. Using Latin hypercube sampling in R package *lhs*, we created 100 random parameter sets sampled from a uniform

311 distribution of potential values ranging from 10% lower or higher than the default value (Table
312 1). By constraining the minimum and maximum values of the parameters, and including a joint
313 distribution within the Latin hypercube sampling, we considered the potential for correlations
314 between parameters. We determined the relative importance of each variable by comparing
315 partial rank correlation coefficients (PRCC) values. Positive PRCC values indicate an increase in
316 the model output with an increase in the parameter value, while negative PRCC values indicate a
317 decrease in the model output with an increase in parameter value [25].

318 **Results**

319 We captured 219 *M. lucifugus* over the capture periods of 2016-2018 (176 during fall, 43 during
320 winter; Table 2). There was minimal variation in hibernaculum microclimate measured by the
321 HOBO and iButton loggers within the hibernaculum (temperature: mean = 4.80 ± 0.60 °C, range
322 = 2.77 – 5.68 °C; water vapor pressure deficit: mean = 0.11 ± 0.26 kPa, range = 0.00 – 2.57 kPa)
323 across winters (Figure 1). We found all bats roosting in the cathedral room, where hibernaculum
324 microclimate was stable throughout the winter (Ta = 4.8 °C, RH = 100%). Activity decreased <
325 50 passes day^{-1} by mid-October (mean date among years 14 October) and increased beyond 50
326 passes day^{-1} by mid-April (mean date among years 13 April). We therefore concluded that
327 hibernation duration in central Montana was 181 days.

328

329 Table 2. Morphometric and physiological data measured from *Myotis lucifugus* captured at a
330 hibernaculum in central Montana. *N* = sample size, TMR: mass-specific torpid metabolic rate,
331 EWL: mass-specific evaporative water loss.

Variable	Value ± SD	*N*
Body mass (g)	8.30 ± 0.98	176
Fat mass (g)	2.09 ± 0.74	65

Lean mass (g)	4.56 ± 0.72	65
TMR (ml O_2 g^1 h^{-1})	0.03 ± 0.02	49
EWL (mg H_2O g^{-1} h^{-1})	0.93 ± 0.60	49

Figure 1. (A) Hibernaculum temperature (°C) and (**B**) water vapor pressure deficit (kPa) deep within the hibernaculum where *Myotis lucifugus* are found during hibernation (black line), at the entrance of the hibernaculum (purple line), and outside the hibernaculum entrance (blue line). Both the entrance (purple) and inside the hibernaculum (black) were at saturation the entire winter period.

Measured EWL from our respirometry procedures in dry air (0% relative humidity) ranged from 0.31 to 1.53 mg H_2O h^{-1} g^{-1} (mean: 0.71 ± 0.25 mg H_2O h^{-1} g^{-1}) depending on temperature and individual. Our model accurately predicted EWL for *M. lucifugus* in Montana ($F_{1,61}$ = 570.3, $p < 0.001$, *slope* = 0.97 [0.89, 1.06]; Figure 2a). Given the hibernaculum conditions measured at the roosting location (T_a = 4.8 °C, RH = 100%), we predicted EWL from *M. lucifugus* as 0.06 ± 0.40 mg H_2O h^{-1} g^{-1} in healthy bats (Supplementary Figure S2). *P. destructans* had no impact on EWL early in infection, but by late hibernation had increased EWL to 2.19 mg H_2O h^{-1} g^{-1} (Supplementary Figures S2).

Figure 2. Comparison of measured and modeled (**A**) evaporative water loss (EWL), (**B**) torpor bout duration, and (**C**) fat loss in *Myotis lucifugus*. EWL and fat loss were measured/modeled in healthy bats, while torpor bout duration was measured/modeled in bats that were inoculated with *P. destructans*. Dashed lines represent one-to-one line and solid lines represent fitted relationship with 95% confidence intervals (shaded blue).

353 Our model accurately estimated torpor bout duration in captive bats infected with *P.*
354 *destructans* ($F_{1,32}$ = 18.64, p = 0.0001, *slope* = 0.65 [0.43, 1.16]; Figure 2b), but the estimates
355 had a wide variance and lacked precision (only 25% of the variation in the data was explained by
356 the model). Without the inclusion of EWL, however, the model did not accurately describe
357 torpor bout duration ($F_{1,32}$ = 0.40, p = 0.53, *slope* = -0.15 [-0.59, 0.30]) and did not describe
358 variation in the data (R^2 = 0.02). We therefore predicted torpor bout duration using our modified
359 model including EWL. For healthy bats, torpor bouts lasted 16.10 ± 5.04 days within the
360 microclimate conditions of the hibernaculum at the field site (range: 4.54 – 18.3 days;
361 Supplementary Figure S3). Torpor bouts ranged from < 1 day to 18.3 days (mean: 6.20 ± 5.40
362 days) for bats infected with *P. destructans* (Supplementary Figure S3).
363 Our modified hibernation model accurately predicted mass loss in healthy wild bats ($F_{1,47}$
364 = 74.38, p < 0.0001, *slope* = 0.87 [0.67, 1.07]; Figure 2c). Though there was a lack of individual
365 metabolic rate and EWL data for the bats used in this validation procedure, our model still
366 explained 62% of the variation in the dataset. Our model was also more precise than the
367 hibernation model that lacked EWL, which was not accurate ($F_{1,47}$ = 1.04, p = 0.84 , *slope* = -
368 0.02 [-0.18, 0.15]) and described less than 1% of the variation in the data. Using the model with
369 EWL, the mean time until total fat exhaustion for healthy *M. lucifugus* predicted in the
370 hibernaculum microclimate conditions at our field site in Montana was 317.5 ± 105.50 days
371 (Figure 3a) at a rate of 0.006 ± 0.002 g day^{-1}. Bats were predicted to survive for over 360 days in
372 the microclimate selected for roosting (T_a = 4.8 °C, RH = 100%). The shortest time until fat
373 exhaustion (176 days) was at the warmest temperature available in the hibernaculum (5.5 °C)
374 and lowest humidity (90%). Almost all other available microclimate conditions within the

hibernaculum (2-5 °C and > 90% RH) result in predicted hibernation duration greater than winter duration (181 days).

Figure 3. Predicted number of days until fat exhaustion for (**A**) healthy and (**B**) *P. destructans* - infected little brown bats (*Myotis lucifugus*) over a range of hibernaculum temperature (°C) and water vapor deficit (kPa) values. Contours represent hibernaculum conditions that allow survival for specific winter duration (in months); dark black contour indicates 6 months, the estimated hibernation duration at our study site in central Montana. White area bounded by grey line represents impossible parameters space for each temperature (e.g. at 2 °C, air is saturated at 0.50 kPa and cannot hold more water).

Within the hibernaculum conditions available at our field site, we predicted a higher and more variable rate of fat loss (range: 0.006 – 0.32 g day^{-1}) for infected bats. In the specific hibernaculum conditions selected for roosting within these conditions, infected bats lost 0.01 ± 0.001 g day^{-1} at the beginning of hibernation (< 14 days) while the rate of fat loss increased to 0.03 ± 0.01 g day^{-1} at the end of hibernation (181 days). Almost all microclimate conditions available at our field site resulted in mortality for infected bats as time until fat exhaustion was less than hibernation duration (mean: 131.23 ± 38.40 days; Figure 3b). The only available microclimate conditions that permitted survival were at the lowest temperatures (2 – 3 °C) and highest humidity conditions (96 – 100%) but these locations were not selected by any healthy bats within this hibernaculum. Bats selected saturated environments that were within the temperature range that allowed fungal growth, resulting in increased energy expenditure and ultimately decreased time until total fat exhaustion.

Our sensitivity analysis revealed that fat loss was influenced by host-specific parameters, including body mass, the proportions of body mass comprised of fat and lean mass, and parameters that influenced EWL, including wing surface area and the area-specific rates of cutaneous EWL (Figure 4). Model parameters that were most influential to survival were physical traits that vary both within and among species. There was little effect of metabolic rate during torpor or euthermia, nor time spent euthermic.

Figure 4. Sensitivity analyses for model calculating total fat exhaustion in hibernating bats infected with *P. destructans*. Dashed lines signify confidence intervals ($\alpha = 0.05$). Positive PRCC values indicate an increase in predicted time until total fat exhaustion with an increase in parameter value; negative values indicate a decrease in predicted time until fat exhaustion with an increase in parameter value.

Discussion

With the continued spread of WNS, it is imperative to understand the effects of hibernaculum microclimate (temperature and humidity) on fungal growth, host physiology, and winter survival. A model that includes effects of EWL on arousal frequencies within the study system of WNS, can improve understanding of the role of EWL on the evolution of hibernation and the interplay of host physiology with the environment. We showed that host parameters, particularly body mass, fat mass, and area-specific rate of EWL, were important drivers of torpor bout duration. Our results suggest that factors associated with EWL and arousal frequency are key elements for predicting the effects of WNS on hibernating bats.

Our modified hibernation bioenergetic model predicted torpor behavior similar to torpor-arousal behavior observed in wild *M. lucifugus* populations. For instance, Reeder et al. [59] measured torpor bouts of 16.32 ± 6.65 days and Czenze et al. [35] measured bouts of 16.2 ± 11.4 days in similar conditions where we predicted bouts of 16.1 ± 5.04 days in the Montana cave system (Supplementary Figure 2b). Observations of torpor behavior in WNS-affected bats corroborated our predictions of torpor bout duration (6.20 ± 5.40 days; Supplementary Figure 2b): wild populations of *M. lucifugus* remained in torpor for 7.93 ± 2.49 days [59], while captive populations averaged 6.48 ± 0.76 days [60]. Similarly, Reeder et al. [59] determined a negative relationship between wing damage due to fungal growth and torpor bout duration, which is aligned with how we incorporated the effect of infection in our model. Overall, the fidelity of the model implies that our prediction of torpor bout duration as a function of EWL is biologically relevant and representative of hibernation physiology and behavior.

We showed complete survival capacity (100% survival) in the entire microclimate space inhabited by healthy *M. lucifugus* in a cave system in central Montana (Figure 3a). Unfortunately, these hibernaculum temperatures and predicted torpor bout durations are comparable to hibernacula inhabited by highly impacted *M. lucifugus* populations in WNS-affected regions [28,35,39]. We predicted almost complete mortality (11% survival) for *M. lucifugus* within the current hibernaculum conditions in this cave system, in part because the high humidity selected by hibernating bats also results in high fungal growth [22,23]. Our predictions are consistent with population trends observed in WNS-affected regions in eastern North America, where similar hibernaculum microclimates have resulted in high mortality (80-98%) [39]. However, our model predicts a small window of microclimate space that would allow for survival, where cooler temperatures and moderate humidity reduce fungal growth, resulting

443 in longer torpor bout duration and decreased arousal frequency (Figure 3b; Supplementary
444 Figure S2). Our model predictions are consistent with observations of WNS-affected bats
445 roosting in colder temperatures compared to unaffected bats [59,61]. These observations, in
446 conjunction with our predictions, suggest that *M. lucifugus* within the Montana cave system
447 would be highly impacted by WNS, but could potentially survive if individuals seek out cooler
448 microclimates. Alternatively, if there are cooler microclimates available, those individuals that
449 already prefer these conditions will survive while others will not. If microclimate preference is
450 heritable, there is the potential for evolutionary rescue [62,63].

451 Our modified hibernation energetics model relies on measurements of the response of *P.*
452 *destructans* to temperature from the lab and modeled response to relative humidity based on
453 previous work [25]. Multiple studies reported diverse *P. destructans* responses to microclimate
454 conditions [22,23,37,64,65], potentially due to subtle differences in laboratory procedures, and
455 thus our predictions of fungal growth may not perfectly represent wild conditions. Additionally,
456 parameters we used to estimate the increase in both metabolic rate and the rate of cutaneous
457 EWL were derived from a single captive study [20]. However, results of studies of the effects of
458 WNS on torpor patterns in wild and free-ranging bats are similar [59,66]. Additionally, our
459 sensitivity analysis indicates that the predictions did not change significantly in response to
460 changes in the temperature and humidity-dependent fungal growth rates or the increase to
461 metabolic rate and EWL (Figure 4). Although future research into humidity-dependent fungal
462 growth rate parameters on wild bats within natural conditions is warranted to increase our
463 understanding of these dynamics, our predictions are consistent with the data currently available.

464 Evidence of at least some *M. lucifugus* populations with greater fat stores persisting post-
465 WNS [67–71] corroborates our findings from our sensitivity analysis that fat mass is a major

466 driver of WNS-survival (Figure 4). Large fat stores allow for increased arousal frequency
467 associated with infection with *P. destructans* and extend the time until total fat exhaustion.
468 Currently, we assess the costs of infection on the mean parameters – that is, body mass, fat mass,
469 and lean mass represent the center of the distribution of morphometric characteristics if we
470 assume a symmetrical distribution. Given evidence of the importance of body mass and fat, we
471 would expect that some individuals from the Montana population would survive in the sampled
472 hibernaculum if they had greater fat stores. It is therefore important that we further our research
473 on the drivers of intra- and interspecific variation in overwintering survival from WNS.

474 The relationship between water vapor pressure and fungal growth indicates the potential
475 for mitigation of WNS impacts if bats roost in microclimates below saturation – that is, infected
476 individuals may trade-off water conservation with energy minimization. In healthy bats,
477 maximum survival was at saturation (100% RH; Figure 3a). As saturation leads to negligible
478 EWL [5], bats can remain in torpor longer before dehydration leads to arousal [46]; thus,
479 roosting in saturated microclimates minimizes energetic costs. However, because *P. destructans*
480 growth increases with water vapor pressure [23], infected bats had lower survival at saturation
481 compared to less humid environments (Figures 3b). This hypothesis is supported by evidence of
482 a relationship between increased population growth rate in multiple species and decreased
483 relative humidity in regions post-WNS infection [39]; hibernacula with less than 90% relative
484 humidity were the only microclimates to have population growth rates above zero, which aligns
485 with our predictions of reduced survival at saturation.

486 Our model supports the role of EWL as a driver of periodic arousals in hibernation, and
487 contributes to addressing one of the longest-standing questions in hibernation biology. It also
488 showcases how interactions between host and pathogen physiology, and the environment can

exacerbate or mitigate the costs of a disease. The relationship between EWL, fungal growth, and humidity suggests that bats found in some parts of western North America, where hibernacula are often drier than eastern hibernacula, may not be as impacted by WNS as eastern populations. Additionally, species and populations that inhabit more arid environments tend to have lower rates of EWL [72] due to adaptations to allow maintenance of water balance in sub-optimal conditions, and thus may not experience high WNS-related mortality [23]. The non-linear interplay of temperature, humidity, and behavior (selecting roosting conditions) needs further analysis, and our model provides a tool to address these questions. The model allows for species-specific parameterization and interspecific variation in morphometrics, physiology, and roosting habitats, suggesting that morphometric and physiological data from western bat species is needed. With this modified hibernation energetics model, we now have the tool to assess the potential impact of WNS on populations that have different hibernation behaviors than previously impacted species.

Acknowledgments: We thank Quinn Fletcher and other members of the U. of Winnipeg bat lab for help with field data on mass loss of hibernating bats. We thank L. Hanauska-Brown for helping obtain permits and B. Maxwell and D. Bachen for site identification. We appreciate the field help from H. D. Bobbitt, D. Taylor, D. Jones, D. Crowley, E. Brandell, G. Botto, E. Lee, K. Smucker, and D. Bishop. This study was supported with equipment from Texas Tech University. This project has been funded in whole with Federal funds from the Department of Defense Strategic Environmental Research and Development Program, under Contract Number W912HQ-16-C-0015. RP was supported by DARPA D16AP00113, NSF DEB-1716698, NIH P20GM103474, and NIH P30GM110732. DTSH is supported by RDF-MAU1701. CKRW and KJON were supported by NSERC, Canada, USFWS, and Species at Risk Research Fund of

Ontario. **Author contributions:** CGH wrote the manuscript and performed the analyses. CGH, NWF, KAS, KJON obtained field data. CGH, NWF, CRH, DTSH, SHO, and LPM developed the model. RKP, SHO, DTSH, and LPM acquired funding and designed the study. CGH, CRH, DTSH, NWF, LPM, RKP, CKRW, and SHO edited the manuscript.

Competing interests: The authors declare that they have no competing interests. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the Government.

Data and materials availability: All data needed to evaluate the conclusions of the paper are available in the paper. Additional data or code related to this paper may be requested from the authors.

References

1. Thomas DW, Dorais M, Bergeron J-M. Winter energy budgets and cost of arousals for hibernating little brown bats, *Myotis lucifugus*. Journal of Mammalogy. 1990;71: 475–479. doi:10.2307/1381967
2. Ben-Hamo M, Muñoz-Garcia A, Williams JB, Korine C, Pinshow B. Waking to drink: rates of evaporative water loss determine arousal frequency in hibernating bats. Journal of Experimental Biology. 2013;216: 573–577. doi:10.1242/jeb.078790
3. Thomas DW, Geiser F. Periodic arousals in hibernating mammals: is evaporative water loss involved? Functional Ecology. 1997;11: 585–591. doi:10.1046/j.1365-2435.1997.00129.x
4. Fisher KC. On the mechanism of periodic arousal in the hibernating ground squirrel. *Annales Academiae scientiarum fennicae* Ser A. 1964;71: 143–156.
5. Thomas DW, Cloutier D. Evaporative water loss by hibernating little brown bats, *Myotis lucigugus*. Physiological Zoology. 1992;65: 443–456.

6. Kallen FC. Some aspects of water balance in the hibernating bat. *Annales Academie Scientiarum Fennicae*. Suomalainen Tiedeakatemia; 1964. pp. 259–267.

7. Humphries MM, Thomas DW, Speakman JR. Climate-mediated energetic constraints on the distribution of hibernating mammals. Nature. 2002;418: 313–316. doi:10.1038/nature00828

8. Boyles JG, McKechnie AE. Energy conservation in hibernating endotherms: Why "suboptimal" temperatures are optimal. Ecological Modelling. 2010;221: 1644–1647. doi:10.1016/j.ecolmodel.2010.03.018

9. Nelson RA. Nitrogen turnover and its conservation in hibernation. Living in the Cold. Paris: John Libbey Eurotext; 1989. pp. 299–307.

10. Herreid CF, Schmidt-Nielsen K. Oxygen consumption, temperature, and water loss in bats from different environments. Am J Physiol. 1966;211: 1108–1112.

11. Morris S, Curtin AL, Thompson MB. Heterothermy, torpor, respiratory gas exchange, water balance and the effect of feeding in Gould's long-eared bat *Nyctophilus gouldi*. Journal of Experimental Biology. 1994;197: 309–335.

12. Schmidt-Nielsen K, editor. Animal Physiology: Adaptation and Environment. 3rd ed. London; New York: Cambridge University Press; 1987.

13. Cryan PM, Meteyer CU, Blehert DS, Lorch JM, Reeder DM, Turner GG, et al. Electrolyte depletion in white-nose syndrome bats. Journal of Wildlife Diseases. 2013;49: 398–402.

14. Wang LCH. Energetics and field aspects of mammalian torpor: the Richardson's ground squirrel. In: Wang LCH, Hudson JW, editors. Strategies in the Cold. New York: Academic Press; 1978. pp. 109–145.

15. Willis CKR, Menzies AK, Boyles JG, Wojciechowski MS. Evaporative water loss is a plausible explanation for mortality of bats from white-nose syndrome. Integr Comp Biol. 2011;51: 364–373. doi:10.1093/icb/icr076

16. Cryan PM, Meteyer CU, Boyles JG, Blehert DS. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology. 2010;8: 135. doi:10.1186/1741-7007-8-135

17. Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, Shearn-Bochsler V, et al. Histopathologic criteria to confirm white-nose syndrome in bats. Journal of Veterinary Diagnostic Investigation. 2009;21: 411–414.

18. Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, et al. Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. Biology Letters. 2013;9: 20130177. doi:10.1098/rsbl.2013.0177

19. Verant ML, Meteyer CU, Speakman JR, Cryan PM, Lorch JM, Blehert DS. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. BMC Physiology. 2014;14: 10. doi:10.1186/s12899-014-0010-4

20. McGuire LP, Mayberry HW, Willis CKR. White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. Am J Physiol Regul Integr Comp Physiol. 2017;313: R680–R686. doi:10.1152/ajpregu.00058.2017

21. Boyles JG, Willis CK. Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white-nose syndrome? Frontiers in Ecology and the Environment. 2010;8: 92–98. doi:10.1890/080187

578 22. Verant ML, Boyles JG, Waldrep W, Wibbelt G, Blehert DS. Temperature-dependent
579 growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. PLOS
580 ONE. 2012;7: e46280. doi:10.1371/journal.pone.0046280
581 23. Marroquin CM, Lavine JO, Windstam ST. Effect of humidity on development of
582 *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome. Northeastern
583 Naturalist. 2017;24: 54–64. doi:10.1656/045.024.0105
584 24. Humphries MM, Thomas DW, Speakman JR. Climate-mediated energetic constraints on
585 the distribution of hibernating mammals. Nature. 2002;418: 313–316.
586 doi:10.1038/nature00828
587 25. Hayman DTS, Pulliam JRC, Marshall JC, Cryan PM, Webb CT. Environment, host, and
588 fungal traits predict continental-scale white-nose syndrome in bats. Science Advances.
589 2016;2: e1500831. doi:10.1126/sciadv.1500831
590 26. Fenton MB, Barclay RMR. Myotis lucifugus. Mammalian Species. 1980; 1–8.
591 doi:10.2307/3503792
592 27. Fenton MB. Population studies of *Myotis lucifugus* (Chiroptera: Vespertilionidae) in
593 Ontario. Toronto, Canada: The Royal Ontario Museum; 1970. Available:
594 https://www.biodiversitylibrary.org/item/111399
595 28. Langwig KE, Frick WF, Hoyt JR, Parise KL, Drees KP, Kunz TH, et al. Drivers of
596 variation in species impacts for a multi-host fungal disease of bats. Phil Trans R Soc B.
597 2016;371: 20150456. doi:10.1098/rstb.2015.0456
598 29. Frederico P. Bat Population Dynamics: An Individual-based Model Approach. Ph.D.
599 Dissertation, University of Tennessee. 2007.

600 30. Boyles JG, Brack V. Modeling survival rates of hibernating mammals with individual-
601 based models of energy expenditure. J Mammal. 2009;90: 9–16. doi:10.1644/08-MAMM-
602 A-205.1
603 31. Jonasson KA, Willis CKR. Hibernation energetics of free-ranging little brown bats. J Exp
604 Biol. 2012;215: 2141–2149. doi:10.1242/jeb.066514
605 32. Ehlman SM, Cox JJ, Crowley PH. Evaporative water loss, spatial distributions, and survival
606 in white-nose-syndrome-affected little brown myotis: a model. J Mammal. 2013;94: 572–
607 583. doi:10.1644/12-MAMM-A-111.1
608 33. Burles DW, Fenton MB, Barclay RM, Brigham RM, Volkers D. Aspects of the winter
609 ecology of bats on Haida Gwaii, British Columbia. Northwestern Naturalist. 2014;95: 289–
610 299. doi:10.1898/12-32.1
611 34. Wilcox A, Willis CK. Energetic benefits of enhanced summer roosting habitat for little
612 brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. Conservation
613 Physiology. 2016;4: cov070.
614 35. Czenze ZJ, Jonasson KA, Willis CKR. Thrifty females, frisky males: Winter energetics of
615 hibernating bats from a cold climate. Physiological and Biochemical Zoology. 2017;90:
616 502–511. doi:10.1086/692623
617 36. Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, et al. An
618 emerging disease causes regional population collapse of a common North American bat
619 species. Science. 2010;329: 679–682. doi:10.1126/science.1188594
620 37. Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, et al. Bat
621 white-nose syndrome: An emerging fungal pathogen? Science. 2009;323: 227–227.
622 doi:10.1126/science.1163874

623 38. Dzal Y, McGuire LP, Veselka N, Fenton MB. Going, going, gone: the impact of white-nose
624 syndrome on the summer activity of the little brown bat (*Myotis lucifugus*). Biol Lett.
625 2011;7: 392–394. doi:10.1098/rsbl.2010.0859
626 39. Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Marm Kilpatrick A. Sociality,
627 density-dependence and microclimates determine the persistence of populations suffering
628 from a novel fungal disease, white-nose syndrome. Ecol Lett. 2012;15: 1050–1057.
629 doi:10.1111/j.1461-0248.2012.01829.x
630 40. McGuire LP, Guglielmo CG. Quantitative magnetic resonance: a rapid, noninvasive body
631 composition analysis technique for live and salvaged bats. J Mammal. 2010;91: 1375–1380.
632 doi:10.1644/10-MAMM-A-051.1
633 41. Fuller NW, Haase CG, Silas KA, Olson SH, McGuire LP. Tradeoffs between energy
634 conservation and the costs of torpor: hibernation of Myotis velifer at low latitudes. Journal
635 of Comparative Physiology B. In press;
636 42. Corben C. AnaBat (version 6.0) and AnaLook (version 4.9 j)[computer programs]. 2004.
637 43. Lausen CL, Barclay RMR. Winter bat activity in the Canadian prairies. Can J Zool.
638 2006;84: 1079–1086. doi:10.1139/z06-093
639 44. Haase CG, Fuller NW, Hranac CR, Hayman DTS, Olson S, Plowright R, et al. Bats are not
640 squirrels: revisiting the cost of cooling in hibernating mammals. Journal of Thermal
641 Biology. 2019;81: 185–193.
642 45. Geiser F, Kenagy GJ. Torpor duration in relation to temperature and metabolism in
643 hibernating ground squirrels. Physiological Zoology. 1988;61: 442–449.
644 46. Thomas DW, Geiser F. Periodic arousals in hibernating mammals: is evaporative water loss
645 involved? Functional Ecology. 11: 585–591. doi:10.1046/j.1365-2435.1997.00129.x

646 47. Geiser F. Metabolic rate and body temperature reduction during hibernation and daily
647 torpor. Annu Rev Physiol. 2004;66: 239–274.
648 doi:10.1146/annurev.physiol.66.032102.115105
649 48. Hock RJ. The metabolic rates and body temperatures of bats. The Biological Bulletin.
650 1951;101: 289–299. doi:10.2307/1538547
651 49. Hanus K. Body temperatures and metabolism of bats at different environmental
652 tempereatures. Physiol Bohemoslov. 1959;8: 250–259.
653 50. Speakman JR, Webb PI, Racey PA. Effects of disturbance on the energy expenditure of
654 hibernating bats. Journal of Applied Ecology. 1991;28: 1087–1104. doi:10.2307/2404227
655 51. Brack V, Twente JW. The duration of the period of hibernation of three species of
656 vespertilionid bats. I. Field studies. Can J Zool. 1985;63: 2952–2954. doi:10.1139/z85-442
657 52. Campbell GS, Norman JM. An Introduction to Environmental Biophysics [Internet]. New
658 York: Springer-Verlag; 1998. Available: http://www.springer.com/us/book/9780387949376
659 53. Gouma E, Simos Y, Verginadis I, Lykoudis E, Evangelou A, Karkabounas S. A simple
660 procedure for estimation of total body surface area and determination of a new value of
661 Meeh's constant in rats. Laboratory Animals. 2012;46: 40–45. doi:10.1258/la.2011.011021
662 54. McGuire LP, Mayberry HW, Fletcher QE, Willis CKR. An experimental test of energy and
663 electrolyte supplementation as a mitigation strategy for white-nose syndrome. Conserv
664 Physiol. 2019;7. doi:10.1093/conphys/coz006
665 55. Speakman JR, Thomas DW. Physiological Ecology and Energetics of Bats. In: Kunz TH,
666 Fenton MB, editors. Bat Ecology. First. Chicago and London: University Of Chicago Press;
667 2003. pp. 430–490.

668 56. Norquay KJO, Martinez-Nuñez F, Dubois JE, Monson KM, Willis CKR. Long-distance
669 movements of little brown bats (Myotis lucifugus). J Mammal. 2013;94: 506–515.
670 doi:10.1644/12-MAMM-A-065.1
671 57. Bilecki LC. Bat Hibernacula in the Karst Landscape of Central Manitoba: Protecting
672 Critical Wildlife Habitat while Managing for Resource Development. M.S. Thesis,
673 University of Manitoba. 2003.
674 58. Czenze ZJ, Willis CKR. Warming up and shipping out: arousal and emergence timing in
675 hibernating little brown bats (Myotis lucifugus). J Comp Physiol B, Biochem Syst Environ
676 Physiol. 2015;185: 575–586. doi:10.1007/s00360-015-0900-1
677 59. Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A, Britzke ER, et al. Frequent
678 arousal from hibernation linked to severity of infection and mortality in bats with white-
679 nose syndrome. PLOS ONE. 2012;7: e38920. doi:10.1371/journal.pone.0038920
680 60. Brownlee-Bouboulis SA, Reeder DM. White-nose syndrome-affected little brown myotis
681 (Myotis lucifugus) increase grooming and other active behaviors during arousals from
682 hibernation. J Wildl Dis. 2013;49: 850–859. doi:10.7589/2012-10-242
683 61. Storm JJ, Boyles JG. Body temperature and body mass of hibernating little brown bats
684 Myotis lucifugus in hibernacula affected by white-nose syndrome. Acta Theriol. 2011;56:
685 123–127. doi:10.1007/s13364-010-0018-5
686 62. Maslo B, Fefferman NH. A case study of bats and white-nose syndrome demonstrating how
687 to model population viability with evolutionary effects. Conservation Biology. 2015;29:
688 1176–1185. doi:10.1111/cobi.12485
689 63. Gonzalez Andrew, Ronce Ophélie, Ferriere Regis, Hochberg Michael E. Evolutionary
690 rescue: an emerging focus at the intersection between ecology and evolution. Philosophical

691 Transactions of the Royal Society B: Biological Sciences. 2013;368: 20120404.
692 doi:10.1098/rstb.2012.0404
693 64. Bandouchova H, Bartonička T, Berkova H, Brichta J, Kokurewicz T, Kovacova V, et al.
694 Alterations in the health of hibernating bats under pathogen pressure. Scientific Reports.
695 2018;8: 6067. doi:10.1038/s41598-018-24461-5
696 65. Chaturvedi V, Springer DJ, Behr MJ, Ramani R, Li X, Peck MK, et al. Morphological and
697 molecular characterizations of psychrophilic fungus *Geomyces destructans* from New York
698 bats with white nose syndrome (WNS). PLOS ONE. 2010;5: e10783.
699 doi:10.1371/journal.pone.0010783
700 66. Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, et al. Inoculation of
701 bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the
702 origin of white-nose syndrome. PNAS. 2012;109: 6999–7003.
703 doi:10.1073/pnas.1200374109
704 67. Cheng TL, Gerson A, Moore MS, Reichard JD, DeSimone J, Willis CKR, et al. Higher fat
705 stores contribute to persistence of little brown bat populations with white-nose syndrome.
706 Journal of Animal Ecology. 2019;88: 591–600. doi:10.1111/1365-2656.12954
707 68. Dunbar MB, Brigham RM. Thermoregulatory variation among populations of bats along a
708 latitudinal gradient. J Comp Physiol B. 2010;180: 885–893. doi:10.1007/s00360-010-0457-
709 y
710 69. Speakman JR, Racey PA. Hibernal Ecology of the Pipistrelle Bat: Energy Expenditure,
711 Water Requirements and Mass Loss, Implications for Survival and the Function of Winter
712 Emergence Flights. Journal of Animal Ecology. 1989;58: 797–813. doi:10.2307/5125

713 70. Jonasson KA, Willis CKR. Changes in Body Condition of Hibernating Bats Support the
714 Thrifty Female Hypothesis and Predict Consequences for Populations with White-Nose
715 Syndrome. PLOS ONE. 2011;6: e21061. doi:10.1371/journal.pone.0021061
716 71. Boyles JG, Dunbar MB, Storm JJ, Brack V. Energy availability influences microclimate
717 selection of hibernating bats. Journal of Experimental Biology. 2007;210: 4345–4350.
718 doi:10.1242/jeb.007294
719 72. Klüg-Baerwald BJ, Brigham RM. Hung out to dry? Intraspecific variation in water loss in a
720 hibernating bat. Oecologia. 2017;183: 977–985. doi:10.1007/s00442-017-3837-0
721 73. McNab BK. On estimating thermal conductance in endotherms. Physiological Zoology.
722 1980;53: 145–156.
723 74. French AR. Allometries of the durations of torpid and euthermic intervals during
724 mammalian hibernation: A test of the theory of metabolic control of the timing of changes
725 in body temperature. J Comp Physiol B. 1985;156: 13–19. doi:10.1007/BF00692921
726 75. French AR. Effects of temperature on the duration of arousal episodes during hibernation. J
727 Appl Physiol Respir Environ Exerc Physiol. 1982;52: 216–220.
728 76. Geiser F, Baudinette RV. The relationship between body mass and rate of rewarming from
729 hibernation and daily torpor in mammals. J Exp Biol. 1990;151: 349–359.
730 77. Hirshfeld JR, O'Farrell MJ. Comparisons of differential warming rates and tissue
731 temperatures in some species of desert bats. Comp Biochem Physiol A Comp Physiol.
732 1976;55: 83–87.

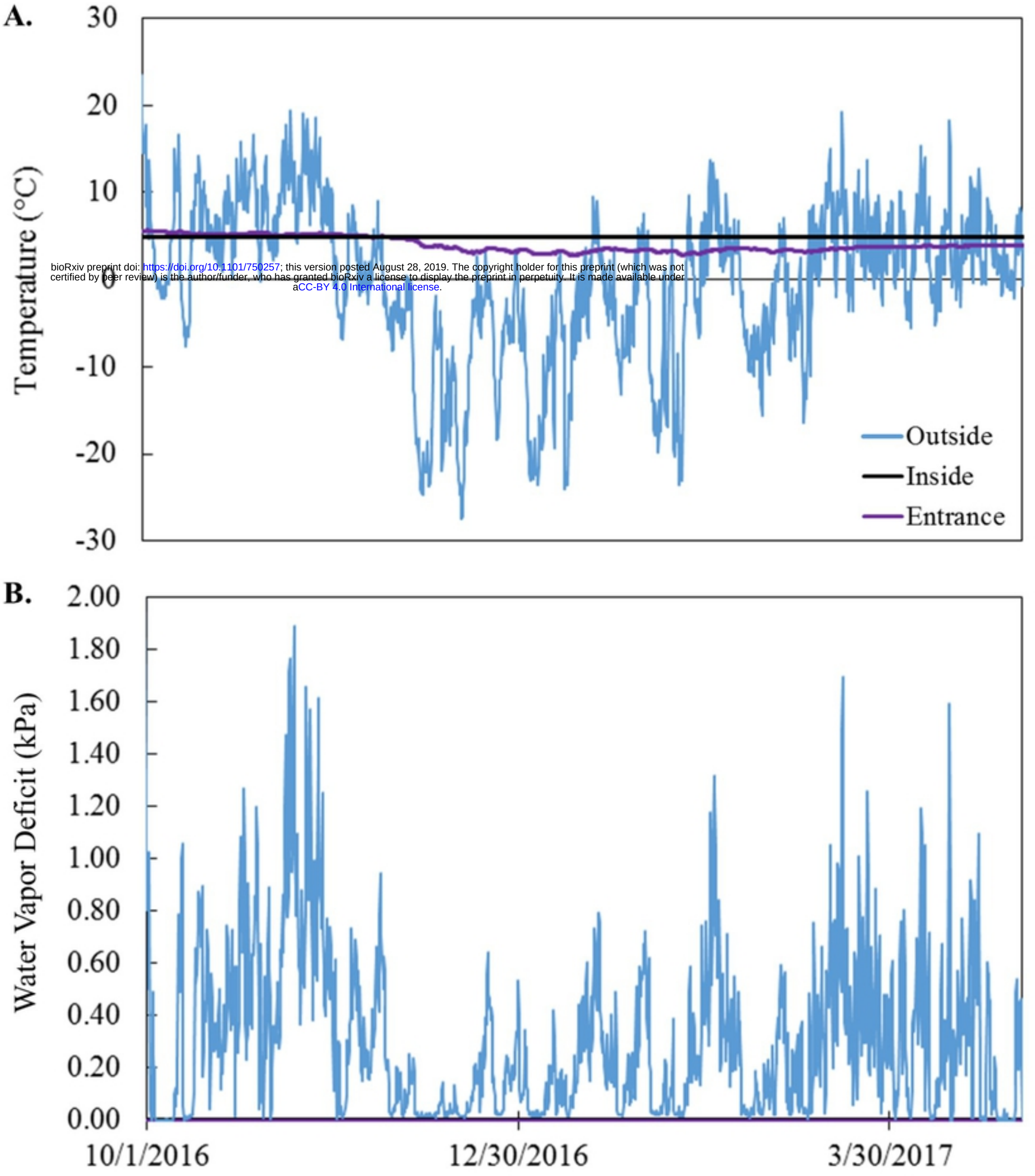
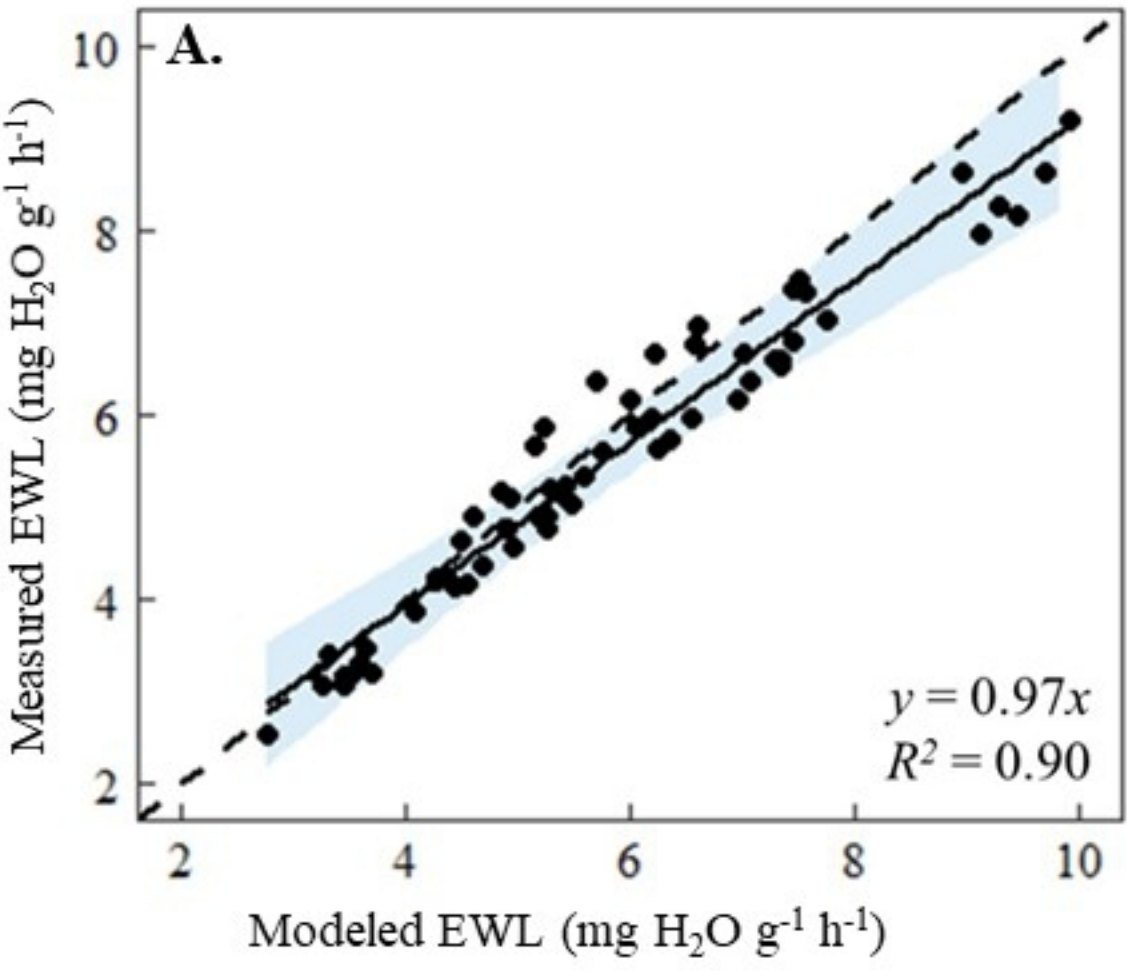
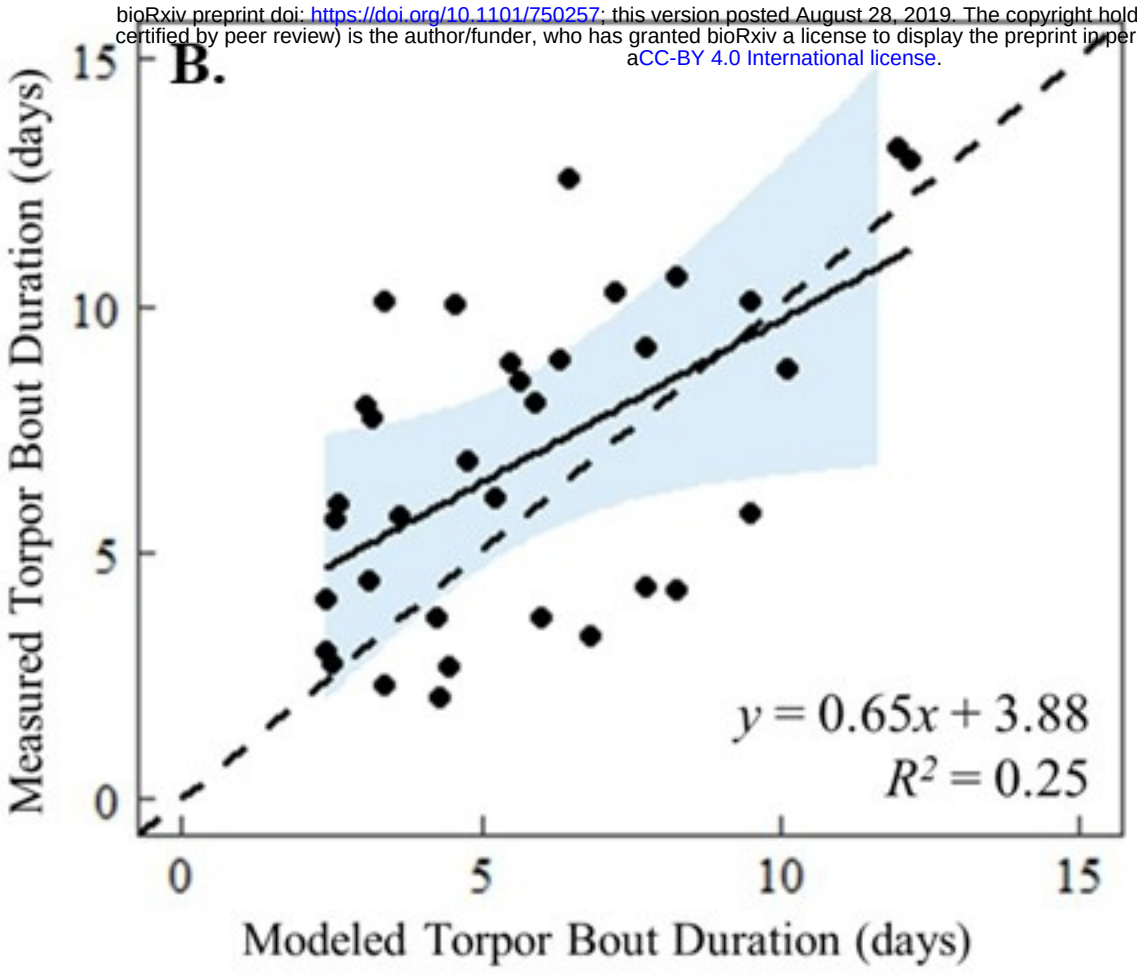


Figure1





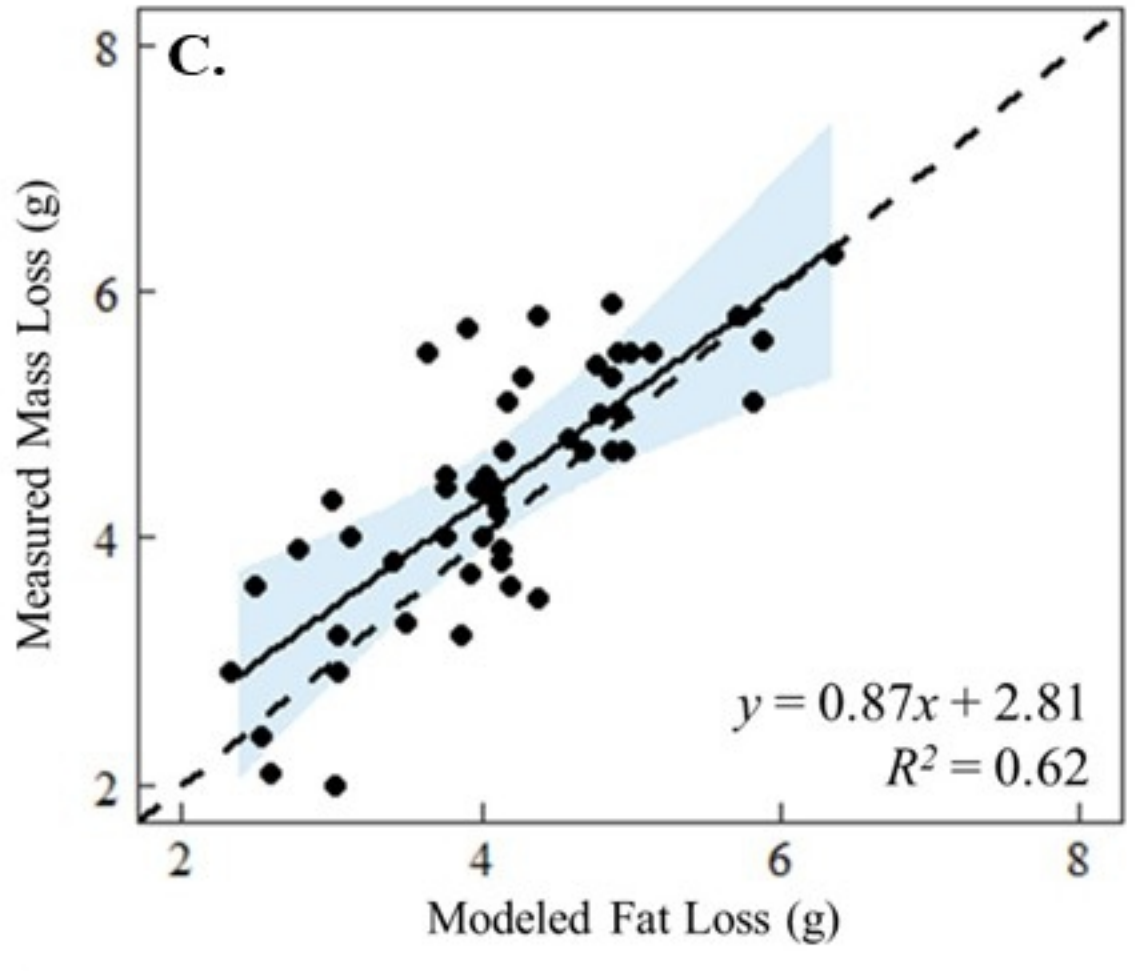


Figure2

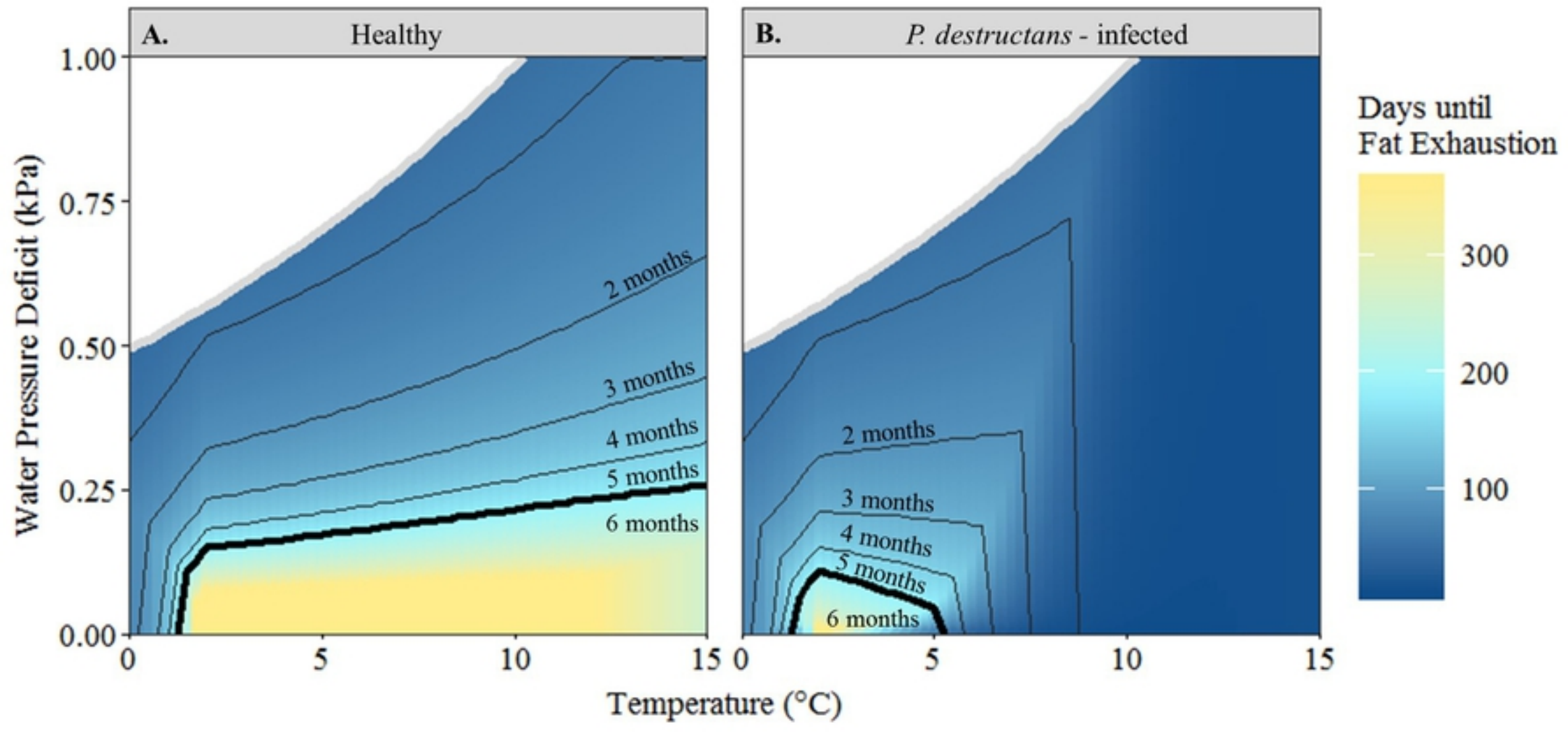

A.
Healthy
B.
P. destructans - infected
Water Pressure Deficit (kPa)
1.00
0.75
0.50
0.25
0.00
0
5
10
15
Temperature (°C)
2 months
3 months
4 months
5 months
6 months
Days until
Fat Exhaustion
300
200
100

Figure3

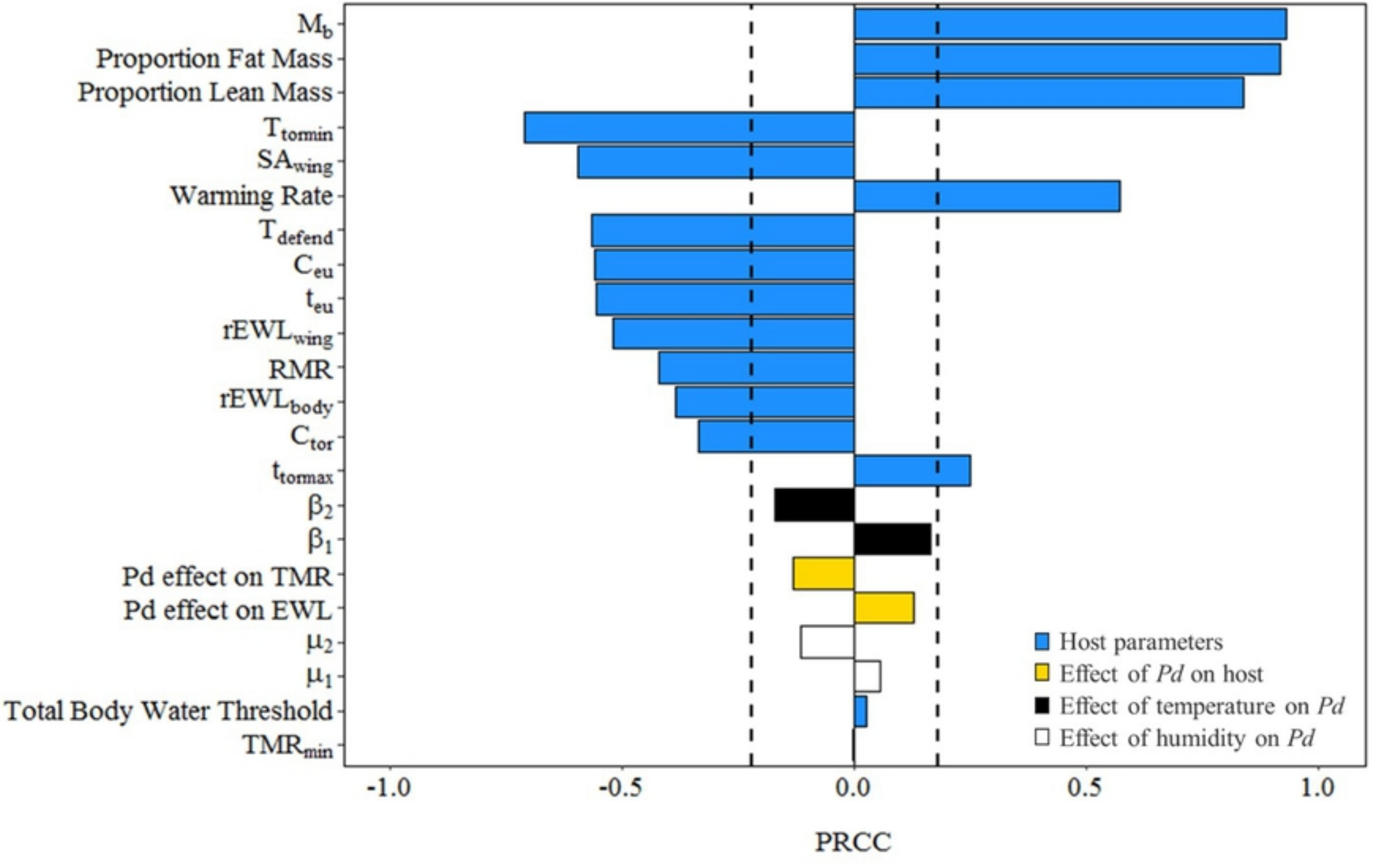

M_b
Proportion Fat Mass
Proportion Lean Mass
T_{tormin}
SA_{wing}
Warming Rate
T_{defend}
C_{eu}
t_{eu}
$rEWL_{wing}$
RMR
$rEWL_{body}$
C_{tor}
t_{tormax}
β_2
β_1
Pd effect on TMR
Pd effect on EWL
μ_2
μ_1
Total Body Water Threshold
TMR_{min}
-1.0
-0.5
0.0
0.5
1.0
PRCC
Host parameters
Effect of *Pd* on host
Effect of temperature on *Pd*
Effect of humidity on *Pd*

Figure4