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SYMPOSIUM

Evaporative Water Loss Is a Plausible Explanation for Mortality of Bats from White-Nose Syndrome

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Synopsis White-nose syndrome (WNS) has caused alarming declines of North American bat populations in the 5 years since its discovery. Affected bats appear to starve during hibernation, possibly because of disruption of normal cycles of torpor and arousal. The importance of hydration state and evaporative water loss (EWL) for influencing the duration of torpor bouts in hibernating mammals recently led to "the dehydration hypothesis," that cutaneous infection of the wing membranes of bats with the fungus *Geomyces destructans* causes dehydration which in turn, increases arousal frequency during hibernation. This hypothesis predicts that uninfected individuals of species most susceptible to WNS, like little brown bats (*Myotis lucifugus*), exhibit high rates of EWL compared to less susceptible species. We tested the feasibility of this prediction using data from the literature and new data quantifying EWL in Natterer's bats (*Myotis nattereri*), a species that is, like other European bats, sympatric with *G. destructans* but does not appear to suffer significant mortality from WNS. We found that little brown bats exhibited significantly higher rates of normothermic EWL than did other bat species for which comparable EWL data are available. We also found that Natterer's bats exhibited significantly lower rates of EWL, in both wet and dry air, compared with values predicted for little brown bats exposed to identical relative humidity (RH). We used a population model to show that the increase in EWL required to cause the pattern of mortality observed for WNS-affected little brown bats was small, equivalent to a solitary bat hibernating exposed to RH of ~95%, or clusters hibernating in ~87% RH, as opposed to typical near-saturation conditions. Both of these results suggest the dehydration hypothesis is plausible and worth pursuing as a possible explanation for mortality of bats from WNS.

Introduction

White-nose syndrome (WNS) has caused alarming population declines of hibernating bats throughout eastern North America since 2005/2006 (Dzal et al. 2010; Frick et al. 2010; Foley et al. 2011). Annual mortality rates range from 30% to 99% averaging 73%, and conservative estimates suggest more than a million animals have died, making this one of the fastest declines of wild mammal populations ever observed (Cryan et al. 2010; Frick et al. 2010). Mechanisms underlying the mortality of bats have still not been confirmed but WNS is consistently associated with cutaneous infection by a cold-adapted, ascomycete fungus, *Geomyces destructans*. Consistent with an infectious epizootic, WNS has spread rapidly from the presumed site of origin in New York State and now occurs throughout eastern North America (Blehert et al. 2009; Gargas et al. 2009; Meteyer et al. 2009; Foley et al. 2011). Susceptibility to infection by *G. destuctans* has been confirmed in nine North American species but little brown bats (*Myotis lucifugus*), along with northern long-eared bats (*Myotis septentrionalis*), appear most adversely affected (Cryan et al. 2010;

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Frick et al. 2010; Foley et al. 2011). Infection with *G. destructans* has also been confirmed in at least five species of hibernating bats in Europe but with no evidence of large-scale mortality (Martinkova et al. 2010; Puechmaille et al. 2010; Wibbelt et al. 2010). Combined with the progressive spread of WNS in North America, apparent tolerance of, or resistance to *G. destructans* by European bats has led to the hypothesis that the fungus is an invasive species from Europe (Puechmaille et al. 2011). Under this scenario, there may be some physiological, morphological, or ecological difference between hibernating bat species in Europe versus North America. However, alternative hypotheses cannot yet be ruled out. For example, *G. destructans* may have been historically present on both continents but recently rendered more pathogenic in North America due to mutation or environmental change (Martinkova et al. 2010; Puechmaille et al. 2010; Wibbelt et al. 2010).

WNS is named for conspicuous growth of fungal conidia on the muzzles of bats but the most biologically important manifestation of the disease appears to be infection of wing tissue (Reichard and Kunz 2009; Cryan et al. 2010). The fungus causes significant lesions to wing membranes by digesting and eroding wing epithelia, sebaceous and apocrine glands, muscle and connective tissue, and blood and lymphatic vessels (Meteyer et al. 2009; Reichard and Kunz 2009; Cryan et al. 2010). These lesions can lead to significant wing damage which likely impairs the ability to fly as well as a range of physiological functions in which wing tissue plays an important role (Cryan et al. 2010).

Bats affected by WNS tend to be emaciated and many appear to die of starvation, which could reflect how their energy reserves are expended during hibernation (Blehert et al. 2009; Boyles and Willis 2010; Cryan et al. 2010). All hibernators express a characteristic pattern of body temperature (T_b) and metabolism during winter consisting of long bouts of torpor interspersed with brief arousals to normothermic T_b. During torpor, T_b can approach ambient temperature (T_a, ~2–10°C for little brown bats) and metabolic rate can fall to <1% of normothermic levels resulting in enormous energy savings (Geiser 2004). However, at some interval, all hibernators periodically arouse to high T_b for periods lasting from about an hour to a day or more, depending on the species (Thomas et al. 1990; Thomas and Geiser 1997; Geiser 2004). Arousals usually account for <1% of total hibernation time during winter but can require 80% or more of winter energy reserves because of the high cost of metabolic heat production during the arousal phase (Thomas et al. 1990). Analyses based on a bio-energetic model for little brown bats revealed that either an increase in average frequency of arousal (i.e., shorter torpor bouts) or average duration of arousal could result in population-level patterns of mortality similar to those observed in WNS-affected populations (i.e., 75–80% with a mortality peak in late February/early March, Boyles and Willis 2010). Preliminary field evidence also supports the hypothesis that WNS-affected bats arouse from torpor more often during hibernation compared to uninfected conspecifics (Turner and Reeder 2009).

A number of hypotheses have been put forward to explain periodic arousals in hibernating mammals and restoration of a variety of physiological functions, inconsistent with low T_b, may be important (Thomas and Cloutier 1992; Thomas and Geiser 1997). One particularly compelling explanation was provided in a landmark study by Thomas and Cloutier (1992) who demonstrated strong relationships between relative humidity (RH) in the hibernation environment, evaporative water loss (EWL) in torpid bats, and torpor bout duration. Bats exposed to drier air had higher rates of EWL and expressed significantly shorter torpor bouts than bats in more humid air and there was a strong predictive relationship between RH and torpor bout duration. Moreover, even in nearly saturated air (99.4% RH), solitary little brown bats in long bouts of deep, steady-state torpor still lost water more rapidly than the rate of metabolic water production. Even in hibernacula this humid, little brown bats incur a water deficit that would require them to arouse to drink. Subsequent analyzes on both ground squirrels and bats also implicate dehydration as the best predictor of arousal frequency (Thomas and Geiser 1997). This relationship, along with the observation that species most adversely affected by WNS hibernate in the most humid sites led Cryan et al. (2010) to suggest the ''dehydration hypothesis''. They proposed that cutaneous infection of bats' wings with *G. destructans* could lead to an increase in cutaneous EWL (and hence an increase in total EWL) resulting in clinically significant dehydration. Dehydration could cause mortality directly or could kill bats indirectly via increased frequency of arousal as bats become dehydrated during torpor bouts. Affected bats that warm up more frequently to drink would prematurely deplete their fat reserves putting them at greater risk of starvation before spring (Cryan et al. 2010). Thus, the observation that bats affected by WNS appear to die of starvation (Blehert et al. 2009; Storm and Boyles 2011) may be something

of a red herring if starvation represents a clinical sign rather than an underlying cause.

In the absence of data comparing affected versus unaffected bats, our objective was to use a comparative analysis of EWL, as well as an energetically-informed population model from Boyles and Willis (2010), to test the plausibility of Cryan et al.'s (2010) dehydration hypothesis as an explanation for mortality of bats due to WNS. One prediction of the dehydration hypothesis is that species most susceptible to mortality from WNS are also the species most susceptible to dehydration prior to infection because of intrinsically high rates of EWL. These species would be most sensitive to any additional dehydration caused by cutaneous infection with the fungus. Therefore, we used data from the literature, and new data on EWL from a European bat species, to test whether little brown bats, a species highly susceptible to WNS (Frick et al. 2010), have relatively high rates of EWL compared to: (1) bats in general, and (2) an ecologically similar European species (*Myotis nattereri*) which is sympatric with *G. destructans* but not known to be affected by WNS. We also used Thomas and Cloutier's (1992) relationship between EWL and frequency of arousal to inform Boyles and Willis' (2010) individual-based population model and investigate how a change in EWL might influence survival in bat populations via its effects on arousal frequency. We aimed to generate testable predictions quantifying the increase in EWL required to bring about the pattern of mortality observed for WNS-affected bats in the field.

Methods

Comparative analysis

We obtained values of EWL and body mass from the literature, as well as values of water vapor pressure (WVP) and ambient temperature (T_a) at the time of measurement when these conditions were reported. When values for any variable of interest were not reported but available in published figures we used XY Extract software (v. 5.1, Perrera da Silva 2004) to digitize graphs and obtain datapoints. To ensure that values of EWL we analyzed were as comparable as possible to conditions reported for little brown bats by Thomas and Cloutier (1992), we only included values in our analysis if recordings of torpid bats occurred at a T_a of 15°C or less and it was clear from metabolic data or wording in the text that bats were indeed torpid at the time of measurement. We were similarly cautious for our analysis of normothermic EWL, including only values from the literature for which it was clear from metabolic data or wording in the text that bats were not in torpor. We excluded values from reproductive females because of possible seasonal or reproductive effects on EWL (Cryan and Wolf 2003) and excluded measurements from clusters of bats. When multiple values were reported in the literature for a given species we analyzed average values for that species.

Respirometry

All experimental procedures were approved by the Local Committee for Ethics in Animal Research and by the General Directorate of Environmental Protection, Poland. On two occasions during the hibernation season we captured Natterer's bats ($n = 6$ male, 1 female) from the walls of a 19th century fortification in Toruń, Poland and transported them ~5 km to a laboratory at Nicolas Copernicus University. Within 1 h of capture bats were sealed in a 350 ml transparent metabolic chamber housed in a temperature-controlled cabinet. We recorded data from three (December 9, 2010) or four (January 18, 2011) bats simultaneously. Bats were placed in the metabolic chambers in the afternoon and recordings lasted for ~48 h. We recorded body mass before and after measurements and assumed a linear decrease in mass during recordings for calculation of mass-specific rates. Outside air (i.e., with 20.95% O_2, 0.04% CO_2) was pumped into the chambers at a rate of ~200 ml/min. To prevent the influence of evaporation from bat excreta on our measurements, we filled the bottom of each chamber with paraffin oil to serve as a trap for excreta.

We first measured EWL of bats exposed to relatively wet air (i.e., $RH = 67.0 \pm 10.4\%$, $T_a = 6.1 \pm 0.3$°C), which is within the range of RH to which little brown bats were exposed in Thomas and Cloutier's (1992) study of little brown bats, although much lower than the 90–99% RH in which little brown bats normally hibernate. We then dried the incurrent airstream using Drierite® (W. A. Hammond Drierite Co. Ltd, Xenia, OH, USA) to quantify EWL of bats in dry air ($RH = 4.0 \pm 1.3\%$, $T_a = 6.2 \pm 0.2$°C). Mass-flow meters (FlowBar-4, Sable Systems Int., Las Vegas, NV, USA) were used to measure flowrate upstream of the respirometry chambers. Air from each chamber was sequentially subsampled at ~100 ml/min using a computer-controlled multiplexer (RM-4 Multiplexer, Sable Systems Int.). We recorded from each animal chamber for 15 min then switched to a reference airstream for 5 min between each chamber. Water vapor pressure in each excurrent airstream was first measured using a water vapor analyzer

(RH-300, Sable Systems Int.). The airstream was then dried using magnesium perchlorate (POCh, S.A. Gliwice, Poland) before gas concentrations were measured using the gas analyzer. In the first series of experiments we measured only O_2 concentration in the excurrent air with a dedicated O_2 analyzer (FC-10, Sable Systems Int.) while in the second series we measured both CO_2 and O_2 concentrations with an integrated gas analyser (Foxbox C, Sable Systems Int.). CO_2 was not removed from the airstream for either of the experimental setups. Data from the water vapor analyzer and O_2 and CO_2 analyzers were recorded and later baseline- and lag-corrected using ExpeData (ver. 1.3, Sable Systems Int.). EWL was calculated using equation 10.9 from Lighton (2008). For calculation of $\dot{V}O_2$ from bats for which we only measured O_2 concentration we used eq. 10.2 from Lighton (2008) assuming RQ = 0.8 (Koteja 1996). $\dot{V}O_2$ and $\dot{V}CO_2$ of bats for which O_2 and CO_2 concentrations were measured were calculated using equation 10.6 and 10.7 from Lighton (2008). For this study, metabolic data were used only to identify bouts of torpor from metabolic traces.

From each bat's recording trace we identified the minimum average value for EWL during a stable, 5-min period of measurement (i.e., variation of <15% across five consecutive measurements), and used these minimum, stable average values for further analysis. We excluded data recorded during the first hour of each trial to minimize the influence of the stress of captivity on our results. We identified torpor bouts in the metabolic recordings as periods of stable and markedly reduced metabolic rate and/or EWL following an abrupt decline from normothermic levels (Willis 2007). We were able to extract up to three datapoints per individual; one each for torpid EWL in dry air, torpid EWL in wet air, and normothermic EWL in wet air. All bats entered steady-state torpor, although one individual did not exhibit stable rates during exposure to wet air, so we were able to record torpid EWL from seven bats in dry air and six in wet air. Three bats remained normothermic long enough to allow steady-state recordings in wet air but none remained normothermic long enough during exposure to dry air.

Individual-based population model

We combined Boyles and Brack's (2009) individual-based, bio-energetic population model (IBM) with Thomas and Cloutier's (1992) model of the relationship between EWL and the duration of torpor bout to investigate how disruption in EWL could cause the pattern of mortality resulting from WNS. Details of the model are outlined in detail elsewhere (Boyles and Brack's 2009; Boyles and Willis 2010) but, briefly, it estimates survival of individual bats in a hypothetical population of 1000 individuals. Each individual is assigned values for a range of traits (e.g., body mass, fat mass, torpor about duration, arousal duration, torpid metabolic rate, normothermic metabolic rate, etc.). These values are drawn from published distributions or can be manipulated in the model to explore the consequences of each parameter for survival. The model also accounts for the energetic benefits of clustering where bats in a cluster are assumed to exhibit a 60% reduction in resting metabolic rate (Canals et al. 1997). A bat in the hypothetical population survives if it starts hibernation with a larger energy reserve (i.e., fat mass) than the amount of energy it consumes over a 200-day winter.

Boyles and Willis (2010) used Boyles and Brack's (2009) IBM to show that an increase in time spent normothermic during hibernation was a more likely explanation for the pattern of mortality observed for bats with WNS than was a change in pre-hibernation fat reserves. We employed a similar approach here but, this time, assumed that the duration of torpor bouts (i.e., frequency of arousals) was controlled only by the relationship between EWL and torpor bout duration as determined by Thomas and Cloutier (1992). Under this assumption it is straightforward to calculate the proportion of a hibernating population likely to have sufficient fat reserves to survive winter. We assumed that in healthy bat populations at least 95% of individuals survive a 200-day winter while in WNS-affected populations only ~20% of individuals survive (Boyles and Willis 2010). Thus, we quantified the average rate of EWL that would result in an increase in the frequency of arousal for individuals such that only 20% of individuals in the population would still be alive at the end of a 200-day winter.

Statistical analysis

All statistical analyzes were conducted in Systat (v. 11, Systat Software Inc.). Non-normal data were log-transformed as necessary and normality was confirmed with Shapiro–Wilks tests. We used general linear models to test for the influence of T_a and body mass on torpid and normothermic rates of EWL across species. If one of the predictor variables was non-significant ($P > 0.05$) we removed it and re-ran the model. Since there was a large effect of body mass on EWL across species, we used

mass-independent residuals from the general linear models to compare little brown bats to other species (i.e., little brown bats versus other bat species and little brown bats versus Natterer's bats specifically). Significance was assessed at an α-level of 0.05 and values are expressed as the mean $\pm$ SD.

Results

Comparative analysis and respirometry

There are surprisingly few data in the literature quantifying EWL of bats during steady-state normothermia or steady-state torpor that also satisfied our criteria for selection (i.e., non-reproductive, nonclustering, $T_a < 15^\circ$C for torpid bats). In total, we analyzed values of EWL during torpor for nine bat species and EWL during normothermia for seven species, including values for little brown bats and our new data (see below) from Natterer's bats (Table 1). Across species there was no effect of measurement T_a on log-transformed, torpid EWL ($t = 0.037$, df $= 2.6$, $P = 0.97$) so we excluded T_a from the model and assessed the influence of log-body mass alone using least-squares regression. There was a significant effect of log-body mass on log-EWL during torpor (Fig. 1, $F_{1,7} = 10.9$, $P = 0.13$, $r^2 = 0.55$) and the relationship was described by the equation log-EWL during torpor $= (1.011 \pm 0.306) \times$ log-body mass $-(1.939 \pm 0.357)$, where values equal parameter estimates $\pm$ 1 SE. When we compared the log-body mass – log-EWL residual value from this regression analysis for little brown bats versus residuals for all other bat species there was no significant difference (one-sample t-test, $t = 1.3$, $P = 0.25$, df $= 7$).

Data for normothermic bats were obtained under comparable conditions in terms of humidity across studies (water vapor pressure $= 1.35 \pm 0.81$ kPa at $23.1 \pm 7.7^\circ$C) so were potentially more reliable than values for EWL during torpor. As for torpid rates, there was no significant effect of T_a on log-EWL during normothermia ($t = 0.40$, $P = 0.71$, $n = 7$), so we excluded T_a from the model and assessed the influence of log-body mass using least squares regression. Again, there was a significant influence of log-body mass across species (Fig. 2, $F_{1,5} = 8.8$, $P = 0.031$, $r^2 = 0.57$) and the relationship was described by the equation log-EWL during normothermia $= (1.156 \pm 0.389) \times$ log body mass -1.338 ± 0.418. This time, the log-body mass $-$ log-EWL residual value for little brown bats was significantly higher than residual values for all other bat species (one-sample t-test, $t = 4.6$, $P = 0.006$, df $= 5$). In other words, mass-corrected rates of normothermic EWL were significantly higher for little brown bats than for the other bat species for which comparable data were available. For the subset of species we analyzed (i.e., those for which rates of EWL during both torpor and normothermia were available), we also found a significant relationship between torpid and normothermic rates of EWL ($F_{1,4} = 18.8$, $P = 0.012$, $r^2 = 0.78$). This means normothermic EWL was a reliable predictor of torpid EWL, at least for this sample of bat species.

We were able to obtain respirometry measurements of EWL during torpor from seven hibernating Natterer's bats exposed to dry air and six of those bats in wet air (rates for 1 individual did not stabilize during measurements in wet air and were excluded). Average EWL during torpor was 0.221 ± 0.739 mg/min in dry air ($n = 7$, mass $= 8.5 \pm 0.7$ g) and 0.065 ± 0.022 mg/min in wet air ($n = 6$, mass $= 8.6 \pm 0.6$ g). EWL during normothermia was 0.703 mg/min for the one individual (mass $= 7.7$ g) which provided usable data in dry air and 0.5864 ± 0.0434 mg/min in wet air ($n = 3$, mass $= 8.7 \pm 0.9$ g). Natterer's bats had significantly lower rates of mass-specific EWL during torpor than values predicted by Thomas and Cloutier's (1992) equation for little brown bats exposed to dry air with RH equivalent to that experienced by bats in our study (Fig. 3A, predicted EWL $= 0.242$ mg/min, one-sample t-test, $t = 4.9$, $P = 0.003$). Natterer's bats also had significantly lower rates of mass-specific EWL during torpor compared to predicted values for little brown bats exposed to wet air (predicted EWL $= 0.070$ mg/min, $t = 5.5$, $P = 0.003$). Average mass-specific EWL during torpor for Natterer's bats was only 63% that of the predicted value for little brown bats in dry air of equivalent RH and 72% that of little brown bats in wet air of equivalent RH (Fig. 3A and B).

Individual-based population model

Based on the IBM combined with Thomas and Cloutier's (1992) model, before accounting for the energetic benefits of clustering, average EWL of $\sim$0.004 mg/min would correspond with an arousal frequency leading to a 95% survival rate. (Fig. 4, Boyles and Brack 2009). Even at this very low rate of water loss, corresponding with relatively long bouts of torpor, 5% of solitary bats still failed to survive a 200-day winter. To reduce survival rate to $\sim$20%, as observed with WNS, EWL would only need to increase to $\sim$0.013 mg/min on average (Fig. 4). Based on Thomas and Cloutier's (1992) model, this is equivalent to a bat moving from a

Table 1 Average values of body mass, ambient temperature (T_a) during measurement, and EWL during torpor and normothermia for bats, including our new data from *M. nattereri*

Species	Family	Mass (g)	T_a (°C)	WVP (kP_a)	EWL ± SD[a] (mg/min)	*N*	Source
Torpid values							
Tadarida brasiliensis	Molossidae	11.2	10.0	–	0.3448 ± 0.4108	2	Carpenter (1969)
Tadarida teniotis	Molossidae	33.4	15.0	–	0.2900	6	Marom et al. (2006)
Eptescius fuscus	Vespertilionidae	13.3	10.0	–	0.2553 ± 0.1696	4	Carpenter (1969)
Lasiurus cinereus	Vespertilionidae	32.5	8.3	–	0.5384 ± 0.3255	5	Cryan and Wolf (2003)
Nyctophilus geoffroyi	Vespertilionidae	8.0	7.5	0.104	0.0686 ± 0.0477	4	Hosken and Withers (1997)
Otonycteris hemprichii	Vespertilionidae	27.1	15.0	–	0.2300	6	Marom et al. (2006)
Nyctophilus gouldi	Vespertilionidae	9.3	5.3	0.564	0.0608	17	Morris et al. (1994)
Myotis nattereri	Vespertilionidae	8.5	6.1	0.631	0.0654 ± 0.022	6	This study
Myotis lucifugus	Vespertilionidae	6.4	3.0	0.345	0.0933	31	Thomas and Cloutier (1992)
Normothermic values							
Tadarida brasiliensis	Molossidae	11.2	27.5	1.400	0.9159 ± 0.0504	17	Carpenter (1969)
Eptescius fuscus	Vespertilionidae	13.3	20.0	1.400	1.2592 ± 0.3999	13	Carpenter (1969)
Lasiurus cinereus	Vespertilionidae	32.5	25.0	–	2.3706 ± 0.6570	30	Cryan and Wolf (2003)
Nyctophilus geoffroyi	Vespertilionidae	8.0	25.0	0.322	0.3446 ± 0.0476	17	Hosken and Withers (1997)
Nyctophilus gouldi	Vespertilionidae	9.3	30.0	2.643	0.3021	17	Morris et al. (1994)
Myotis nattereri	Vespertilionidae	8.7	7.2	0.675	0.5864 ± 0.0434	3	This study
Myotis lucifugus	Vespertilionidae	7.8	27.3	1.642	0.9039 ± 0.2791	140[b]	Proctor and Studier (1970)

Note: See methods for selection criteria.
[a]Where values for SD are missing they were not reported in the text of the original source.
[b]This value represents a sample size for number of measurements, not individuals. Sample sizes for number of individuals could not be obtained from the original source for this species.

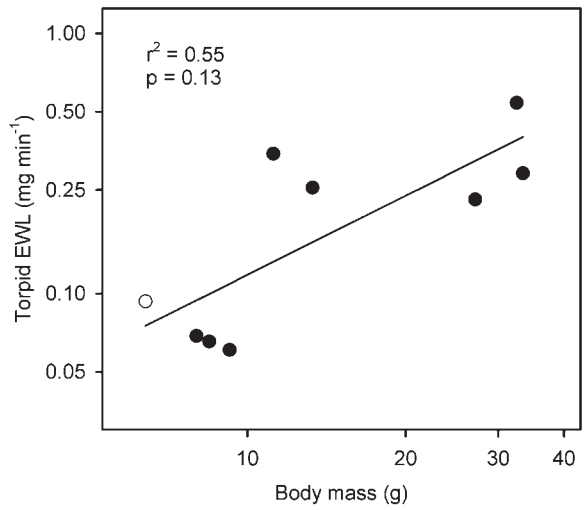

Fig. 1 Relationship between body mass and torpid EWL for nine bat species including our new data for Natterer's bats. Both axes are plotted on a log scale. Little brown bats, the species hardest hit by WNS, are represented by the empty circle. Body mass—EWL residuals were not significantly higher for little brown bats than the average for all species but few studies reported ambient humidity during measurement for other species which could have introduced unexplained variation. See text for regression results.

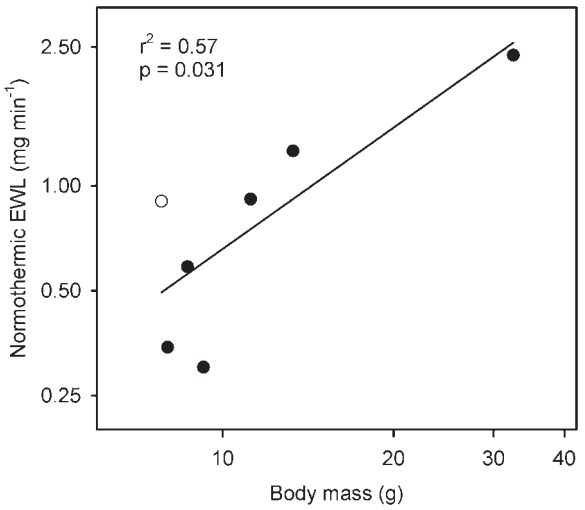

Fig. 2 Relationship between body mass and normothermic EWL for seven bat species, including our new data for Natterer's bats. Both axes are plotted on a log scale. Little brown bats, the species hardest hit by WNS, are represented by the empty circle. Ambient humidity during EWL measurement was comparable across species in this analysis and body mass—EWL residuals were significantly higher for little brown bats than the average for all other species. See text for regression results.

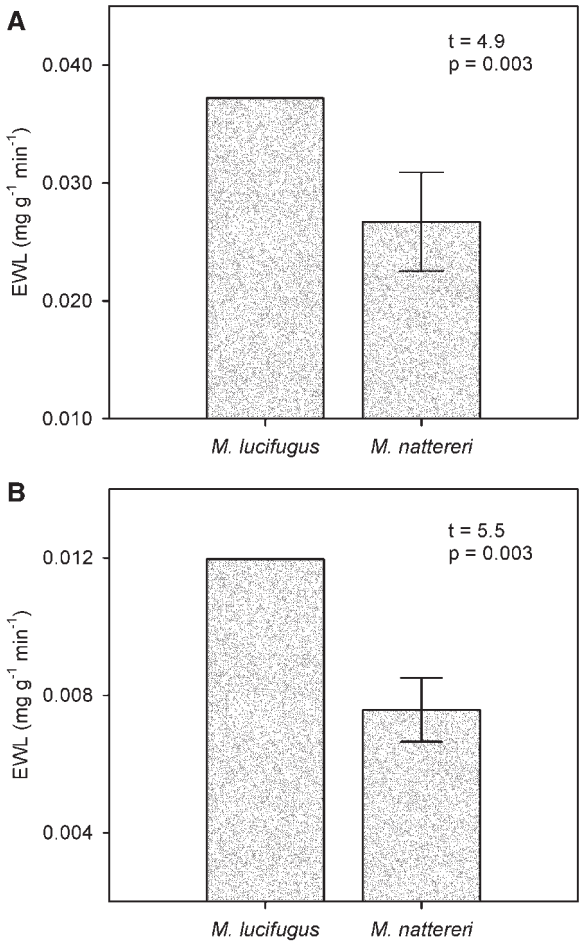

Fig. 3 Values for mass-predicted, torpid EWL of little brown bats and Natterer's bats in relatively dry (**A**, RH = 4.0 ± 1.3%), and relatively humid air (**B**, RH = RH = 67.0 ± 10.4%). Values for little brown bats are predicted from Equation (3) of Thomas and Cloutier (1992) based on the ambient humidity in which we measured EWL for Natterer's bats. Values for Natterer's bats were significantly lower than the predicted values for little brown bats. See text for statistical results.

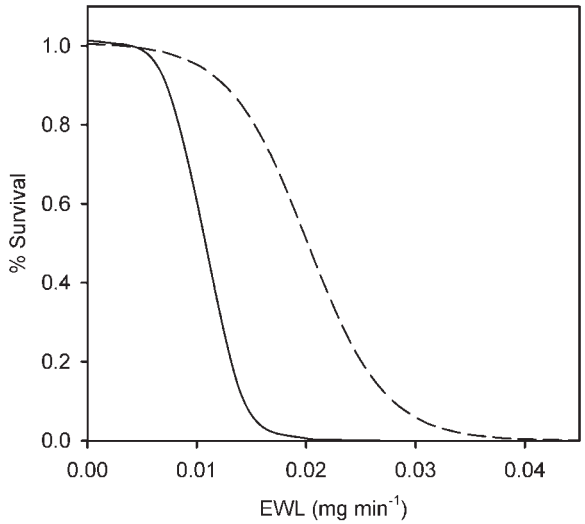

Fig. 4 Proportions of bat populations surviving based on changes in the average rates of EWL for individuals in the simulated populations, based on the hypothesis that infection with *G. destructans* causes an increase in EWL. The dashed line represents survival for bats which have the opportunity to cluster and the solid line represents survival for bats roosting solitarily. Based on Thomas and Cloutier's (1992) findings, to result in a level of mortality similar to that observed with WNS, (~20% survival), average EWL would need to increase in magnitude equivalent to the effect of reducing RH in the hibernaculum from 99% to 93% for solitary bats and from 99% to 87% for clustered bats.

hibernaculum with nearly saturated air (RH ≤ 99%) to a hibernaculum with air at ~93% RH. When we accounted for the energetic benefits of clustering during arousals, rates of EWL had to be higher to result in significant mortality. Average EWL of ~0.010 mg/min resulted in average frequency of arousal leading to 95% survival (Fig. 4). For clustered bats, to reduce survival to 20%, EWL would only need to increase to ~0.025 ml/min (Fig. 4), the equivalent of a bat moving from a hibernaculum with nearly saturated air to one with RH of ~87%. Thus, the model indicates that even very small increases in EWL could have pronounced implications for over-winter survival that are consistent with rates of mortality observed for WNS-affected populations in the wild.

Discussion

Our results support the plausibility of Cryan et al.'s (2010) hypothesis that dehydration is a consequence of cutaneous infection with *G. destructans*. Our findings are consistent with one prediction of the dehydration hypothesis: that bat species most affected by WNS should be the species already most susceptible to dehydration during hibernation. Little brown bats represent such a species with among the highest rates of mortality from WNS (Frick et al. 2010) and our findings suggest that they also have relatively high rates of EWL. After controlling for body mass, healthy individuals of this species exhibited significantly higher rates of EWL during normothermia compared to other bats, although we caution that our sample size was small due to a lack of comparable data on EWL. We also found a strong correlation between rates of EWL during normothermia and torpor (i.e., species with high rates during torpor also exhibited high rates during normothermia) which suggests that data from normothermic bats can be used to predict EWL in torpid bats. We did not find a significant difference between EWL during torpor of little brown bats versus rates

during torpor for all other species, which is not consistent with the dehydration hypothesis. However, few studies reporting EWL during torpor also reported RH during measurement, despite the important influence of RH on EWL (Thomas and Cloutier 1992). Due to the difficulty of controlling humidity at low T_a, especially prior to the availability of digital water vapor sensors, the values we analyzed for EWL during torpor may have been obtained across a wide range of RH which could have introduced considerable unexplained variation. We are more confident in our analysis of EWL during normothermia because we could confirm that recordings occurred in reasonably comparable conditions across species. Also compelling is our finding that predicted values of EWL for little brown bats both in wet and dry air were significantly higher than rates for a morphologically and ecologically similar European bat species, the Natterer's bat, which is sympatric with *G. destructans* but is not affected by WNS (Martinkova et al. 2010; Puechmaille et al. 2010; Wibbelt et al. 2010). Clearly, a range of other factors could influence differences in EWL between little brown and Natterer's bats and more data on EWL from additional European species are needed. However, taken together our results are consistent with predictions of the dehydration hypothesis.

As reviewed by Cryan et al. (2010), cutaneous infection with *G. destructans* could degrade the natural waterproofing of the wings and actively wick water from wing tissues via invasion of glands and microvasculature. Cutaneous infection could also reduce the potential for cutaneous respiration across the wings, which can be a significant source of gas exchange for bats during torpor (e.g., Mankanya and Mortola 2007). This, in turn, could lead to increased pulmonary respiration to compensate for impaired cutaneous gas exchange, further elevating overall EWL (Cryan et al. 2010). Thus, species which have intrinsically high rates of EWL during torpor, prior to infection with *G. destructans*, could be most at risk from any additional increase in water loss. In addition to this direct physiological consequence of an increased risk of dehydration, bat species like little brown bats with relatively high rates of EWL may also be more likely to become infected in the first place because of their likely dependence on humid microhabitats which favor fungal growth, as well as their tendency to cluster in large groups to reduce energetic costs and EWL (Cryan et al. 2010). In addition to increasing the risk of infection, reliance on humid microclimates could also speed the progression of the disease by allowing more rapid growth of *G. destructans*. Species with lower rates of EWL may also become infected but, if the fungus grows more slowly in drier microenvironments, they could still survive until spring. If WNS is the result of an invasive species from Europe, differences in EWL and habitat selection of European bats could help explain differential survival (Cryan et al. 2010). These physiological and behavioral traits may have been subject to strong selection during a past outbreak of *G. destructans*, leaving Europe with species and populations of bats with lower rates of EWL and a preference for, or tolerance of, drier hibernacula.

Thomas and Cloutier (1992) showed that even slightly drier hibernacula (e.g., 95% RH instead of 99%) can have a large impact on EWL and, therefore, on arousal frequency and survival. Although hypothetical, our population model further illustrates this point and was also consistent with the dehydration hypothesis. The increase in EWL needed to increase the frequency of arousals enough to cause starvation for 80% of individuals in a population was readily plausible. Indeed, even for clustering bats, the required increase in EWL was within, if slightly lower than, the normal range of RH experienced by hibernating bats in the wild (e.g., from 99% to 87% RH). Moreover, for clustering bats the model likely overestimates the required increase in EWL because, in the absence of reliable data for generating parameter estimates, it only accounts for the energetic benefits of clustering and not for the added benefits of reduced EWL arising due to clustering (Boyles and Brack 2009). As a result, an even smaller increase in EWL than that predicted by our model would likely correspond to 80% mortality in little brown bat populations. Thus, two independent lines of evidence (i.e., relatively high rates of EWL in little brown bats compared with other bats and a similar European species, and model output indicating that only a small increase in EWL is required to cause high rates of mortality) lend circumstantial support to the dehydration hypothesis.

Our results, and the dehydration hypothesis, could also help explain some of the abnormal behaviors observed for bats with WNS. As pointed out by Cryan et al. (2010), affected bats may emerge early to restore water balance, rather than energy balance, if their rates of EWL become high enough to exceed their ability to obtain water from condensation inside hibernacula. Affected bats also tend to move toward the entrances of hibernacula as winter progresses which could reflect energy limitation resulting from increased frequency of arousal. There is a growing appreciation that many heterothermic mammals rely on passive arousal and basking to arouse from torpor at reduced energetic cost (e.g., Geiser and

Drury 2003; Warnecke et al. 2008; Warnecke and Geiser 2010). As bats with WNS deplete their energy reserves, they may concentrate near entrances to hibernacula because larger fluctuations in T_a could allow them to reduce T_b during torpor, enhancing energy and water savings, while access to solar radiant energy and fluctuations in T_a could allow them to arouse at a markedly reduced energetic cost. If their fat reserves are significantly depleted this could be an emergency response providing at least a chance to rewarm passively in the event that insects become available.

Clearly our results do not confirm the dehydration hypothesis but, in the absence of data from affected versus unaffected bats, they suggest important avenues for future research. Controlled inoculation trials are needed for both European and North American species, first, to determine if *G. destructans* is indeed the cause of mortality and whether it disrupts cycles of torpor and arousal, assumptions that have yet to be confirmed experimentally. Inoculation trials should also aim to quantify rates of EWL in affected versus unaffected bats under controlled conditions. Results of our modeling exercise could be used to generate effect sizes for power analyzes to ensure that future experiments have large enough sample sizes to detect significant effects while minimizing the impact of over-sampling on wild populations. Also critical are inoculation trials to assess the clinical signs and progression of disease in bats exposed to varying controlled microclimates during infection. It is essential that we quantify the influence of T_a and RH in hibernacula on progression of disease for infected bats. Although there are limitations of studying captive populations, and husbandry of hibernating bats can be challenging, we encourage establishment of captive breeding colonies of little brown bats and other species to help minimize impacts of research on wild populations. In addition to laboratory studies, also critical are more data on relationships between hibernaculum microclimates preferred by bats, EWL, and torpor—arousal cycles for wild-captured bats, both in North America and Europe. It is important to determine whether European bats do indeed tend to roost in drier microclimates or if other aspects of their behavior or physiology (e.g., social behavior or immune function) increase either their tolerance of, and/or resistance to, *G. destructans* or slow the spread of the disease. Determining the heritability of traits associated with energy balance, EWL, and microhabitat preferences during hibernation, as well as other traits that might afford protection to bats (e.g., potential immune responses to infection) is also critical for determining if natural selection can operate strongly enough to allow bat populations to survive and rebound from WNS in the future.

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