Articles in PresS. Am J Physiol Regul Integr Comp Physiol (August 23, 2017). doi:10.1152/ajpregu.00058.2017

1 Running Head: White-nose syndrome increases metabolic rate and water loss
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3 # White-nose syndrome increases torpid metabolic rate and
4 # evaporative water loss in hibernating bats
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25 ## Abstract

26 Fungal diseases of wildlife typically manifest as superficial skin infections but can have devastating
27 consequences for host physiology and survival. White-nose syndrome (WNS) is a fungal skin disease that
28 has killed millions of hibernating bats in North America since 2007. Infection with the fungus
29 *Pseudogymnoascus destructans* causes bats to rewarm too often during hibernation, but the cause of
30 increased arousal rates remains unknown. Based on data from studies of captive and free-living bats,
31 two mechanistic models have been proposed to explain disease processes in WNS. Key predictions of
32 both models are that WNS-affected bats will show (1) higher metabolic rates during torpor (TMR), and
33 (2) higher rates of evaporative water loss (EWL). We collected bats from a WNS-negative hibernaculum,
34 inoculated one group with *P. destructans* and sham-inoculated a second group as controls. After four
35 months of hibernation, we used respirometry to measure TMR and EWL. Both predictions were
36 supported and our data suggest that infected bats were more affected by variation in ambient humidity
37 than controls. Furthermore, disease severity, as indicated by the area of the wing with UV fluorescence,
38 was positively correlated with EWL but not TMR. Our results provide the first direct evidence that
39 heightened energy expenditure during torpor, and higher EWL, independently contribute to WNS
40 pathophysiology with implications for the design of potential treatments for the disease.
41

42 **Keywords**: *Myotis lucifugus*, hibernation, *Pseudogymnoascus destructans*, respirometry

43 # Introduction

44 Fungal diseases of wildlife are on the rise worldwide (13). In contrast to viral and bacterial
45 pathogens, which often lead to systemic infections, fungal pathogens of animals often manifest as
46 superficial skin infections, especially among poikilothermic species. Although typically limited to
47 infecting skin, fungal pathogens can lead to devastating physiological impacts, and fatal disease, across a
48 range of taxa (1, 4, 5, 35, 36). A mechanistic understanding of pathogenesis in fungal diseases of wildlife
49 is critical for understanding and predicting population-level impacts and developing safe and effective
50 mitigation and management strategies.

51 White-nose syndrome (WNS) is a recently emerged disease of hibernating bats (5) caused by the
52 fungus *Pseudogymnoascus destructans* (25, 42). Since its discovery in 2007, millions of bats have been
53 killed in eastern and central North America, leading to dramatic population declines (16) and the
54 possibility of regional extinctions (15). Recent reviews have summarized our understanding of disease
55 mechanisms in WNS (17, 45). A number of putative virulence factors have now been identified (14, 29)
56 and studies of both captive (42) and free-living bats (33) indicate that the disease causes increased
57 frequency of arousals from torpor during hibernation, emaciation, and death. Infected bats also exhibit
58 signs of altered fluid, electrolyte and pH balance (10, 11, 26, 41, 43) leading to development of two
59 complementary mechanistic models of WNS pathophysiology (41, 43).

60 Symptoms of WNS develop in a progressive manner (26), and the most pronounced symptoms
61 are only apparent relatively late in hibernation (41, 43). Increased arousal frequency, and arousal
62 cascades that may reflect conspecific disturbances (40), in later stages of infection lead to dramatic
63 increases in energy expenditure and are thought to be a primary cause of emaciation (43). This pattern
64 is described in a pathophysiological model proposed by Warnecke *et al.* (43). The model proposes that
65 lesions in wing tissue, which occur in later stages of fungal infection, lead to altered blood chemistry and
66 haematology, increased water loss, respiratory rate, and energy consumption. More recently, however,

67 Verant *et al.* (41) found evidence of increased energy turnover at an earlier stage of disease, before a
68 detectable increase in arousal frequency was observed. They proposed a model of earlier stage disease
69 based on these findings suggesting that increased metabolic rate following initial tissue invasion,
70 combined with reduced excretion of CO_2, initiates the cascade of physiological responses observed by
71 Warnecke *et al.* [15] in the final stages of WNS [16]. Two key elements of both models are increased
72 energy expenditure, and disruption of osmotic homeostasis (41, 43). Although the cause of increased
73 arousal frequency is unknown, observations of electrolyte and fluid depletion (10) led to the
74 dehydration hypothesis (11, 46) that fluid loss across fungal lesions on the skin increases rates of water
75 loss, resulting in increased arousal frequency and energy depletion. In healthy hibernators, ambient
76 humidity and evaporative water loss (EWL) affect torpor bout duration (3, 38), which suggests the
77 possibility that increased EWL due to wing damage could trigger increased arousal frequency and
78 mortality in WNS (46). Thus, understanding the impacts of WNS on energy expenditure and water loss,
79 as predicted by both pathophysiological models of WNS published to date, is a critical step in
80 understanding the mechanism by which fungal infection leads to bat mortality.
81 We conducted an experimental inoculation to test the hypothesis that WNS causes increased
82 energy expenditure and EWL during torpor bouts, as predicted by mechanistic models of WNS (41, 43).
83 Specifically, we predicted that bats inoculated with *P. destructans* would have (1) higher torpid
84 metabolic rate (TMR), and (2) increased EWL compared to healthy controls. We also measured TMR and
85 EWL in both dry and humidified air to assess the impact of environmental conditions on WNS
86 pathophysiology.

87 Methods

88 *Animal Collection and Housing*

89 We collected 28 male little brown bats (*Myotis lucifugus*) from a WNS-negative hibernaculum in central
90 Manitoba, Canada on 7 November 2013 and transported them to the University of Winnipeg. We

91 randomly assigned bats to either a control (n = 14) or treatment group (n = 14) matched for body
92 condition. Bats in the treatment group were inoculated with *P. destructans* and controls were sham
93 inoculated as described previously (42). The two groups were placed in separate nylon mesh cages (23 x
94 38 x 38 cm; modified from Exo Terra, Rolf C Hagen Inc., Montreal, Quebec, Canada) in an environmental
95 chamber (model 6040-1, Caron, Marietta OH) maintained at 7°C and 98% relative humidity (RH) (i.e.,
96 water vapour pressure = 0.982 kPa). *Ad libitum* water was provided in a dish that was flushed weekly
97 with fresh water using tubes that ran outside the chamber to avoid disturbance. We used infrared
98 motion-activated video cameras (Model HT6501RVFHQ; Speco Technologies, New York, NY, USA) to
99 monitor hibernating bats. We minimized potential confounding cage effects by placing the two cages
100 beside each other (within a few cm) in a single environmental chamber. Thus any disturbances or
101 environmental fluctuations that could have influenced our results were experienced equally by both
102 groups. Within the first week of hibernation there was an unavoidable disturbance to repair one of the
103 cages, but this repair was completed as quickly and as quietly as possible. The only other potential
104 disturbances occurred when individual animals met humane intervention criteria for assessment (i.e.,
105 isolated from other bats, low in the cage with wings spread out; (42)). Three bats in the inoculated
106 group met these criteria before termination of the experiment and were removed. We always replicated
107 any disturbance in both experimental cages and, apart from these events, hibernating bats were not
108 disturbed for >113 days. We tested for *P. destructans* DNA by swabbing the forearm and muzzle of each
109 bat (as in (9)) to confirm all bats were WNS-negative prior to our study.
110 We used infrared videos to identify arousals of individual bats. In multiple previous experiments,
111 when we have recorded skin temperature alongside video recording, our motion-activated video system
112 has never failed to detect an arousal by any hibernating bat in this experimental setup (6, 44). We used a
113 linear mixed effects model to test for differences in torpor bout duration (and hence arousal frequency)
114 between inoculated and control groups. We recorded the duration of all torpor bouts that occurred in

115 the final 50 days of hibernation prior to respirometry (fewer days for bats removed for humane
116 intervention), and tested for effects of date and treatment, including individual as a random effect to
117 account for repeated measures. We compared rate of mass loss between infected and control bats
118 using a t-test.
119
120 *Respirometry*

121 All bats were kept under the same environmental conditions for the duration of captive
122 hibernation (7° C, 98% RH). After approximately 4 months of hibernation we removed bats from the
123 environmental chamber (details below) to measure TMR and EWL by open flow respirometry. We
124 measured TMR and EWL under two conditions: humidified air and dry air. The comparison of dry and
125 humidified air conditions allowed us to assess potential impacts of changes in environmental humidity
126 on bats suffering from WNS, either by change in microclimate selection by the bat, or perhaps
127 environmental manipulation as a mitigation strategy.

128 Our open-flow respirometry system was configured to record up to 7 animal chambers plus a
129 baseline channel. The building pressurized air system was connected to a CO_2 adsorbing system (PCDA
130 series, PureGas, Broomfield, CO) and passed through a column of $MgClO_4$ to provide dry, CO_2-free air for
131 the respirometry system. Air was saturated by flowing through a bubbler, and reduced to approximately
132 65% relative humidity (0.7059 kPa) with a custom dew point generator (Polystat 12101-00, Cole-
133 Parmer). A series of valves allowed us to supply either dry (bypassing bubbler and dew point generator)
134 or humidified air to the animal chambers. A flow-controller (FB8, Sable Systems) regulated flow to each
135 chamber, and all chamber temperatures were monitored with thermocouples (TC-2000, Sable Systems).
136 Animal chambers (370 mL volume) were housed in a temperature controlled cabinet (8°C). Hardware
137 cloth (1/2”) was used to provide a roosting surface inside each chamber, and separate bats from a layer
138 of mineral oil in the bottom of the chamber to prevent introduction of humidity from excreta. In-line

139 HEPA syringe filters were placed before and after each animal chamber to prevent any *P. destructans*
140 contamination. Animal chambers were sequentially subsampled with a multiplexer (MUX, Sable
141 Systems). The subsampled excurrent airflow was analyzed for water vapor (RH-300, Sable Systems)
142 before passing through a gas dryer (ND2, Sable Systems) and $MgClO_4$ column. Finally, gas analyzers
143 recorded CO_2 (CA-10, Sable Systems) and O_2 (FC-2, Sable Systems, operating in differential mode). All
144 instruments were calibrated prior to measurements. Prior to measuring animals we determined the
145 washout time for our chambers and set measurement durations accordingly (washout time < 60 s,
146 measurement time = 10 min, flow rate = 100 mL/min) (23).

147 We transferred groups of 5 – 7 bats from their cage in the environmental chamber to individual
148 respirometry chambers to measure TMR and EWL. Thus hibernation duration was 114 or 118 days for
149 inoculated bats, and 121 or 125 days for controls. We transferred bats in the evening and allowed bats
150 to return to torpor overnight under the humidified airstream. Recording TMR and EWL under the
151 humidified air condition (excurrent WVP = 0.8474 ± 0.010 kPa, 81.9% RH) began the following morning,
152 and continued for 24 h. On the second morning we switched to a dry incurrent air stream. Dry air
153 measurements continued overnight except for one group of 5 inoculated bats. Bats in this group began
154 to arouse and therefore we ended the experiment after 6 hours of dry air measurement. All bats were
155 weighed (± 0.1 g) before and after respirometry measurements, and we recorded rectal temperature (±
156 0.1°C; model 80005, Sper Scientific, Scottsdale AZ, USA) of each bat upon removal from respirometry.
157 We used a UV lightbox (9-W, 368 nm wavelength, model BM100, Way Too Cool LLC, Glendale, AZ, USA)
158 and overhead UV light to take photographs of the wings of each bat at the end of hibernation to
159 document fluorescence characteristic of WNS (26). We measured the area of the wing affected by
160 fluorescence in imageJ (v1.50i) as described in (26), and compared measured values to those reported in
161 McGuire et al. (26) to provide a qualitative indication of disease severity for bats in our study. Following
162 UV photos, we provided all bats with dilute Pedialyte® (1:1 dilution of unflavored Pedialyte; Abbott

163 Nutrition, Abbott Laboratories, Columbus, OH, USA) and Nutrical (Albrecht, Germany) nutrient
164 supplement. All bats were then transferred to a different area of the captive colony for rehabilitation
165 and a separate study of recovery and healing.
166
167 *Analysis*
168 We analyzed all respirometry files in Expedata (V 1.3.0 Sable Systems). We corrected for lag and
169 drift, and then calculated $\dot{V}CO_2$ using equation 10.4 from (23). We converted to TMR in mW assuming
170 fat oxidation (RQ = 0.71; see supplementary materials). We calculated EWL using equation 10.9 from
171 (23). We used repeated measures ANOVAs to test for effects of treatment (inoculated or control),
172 condition (wet air or dry air), and body mass on TMR and EWL.
173 EWL will increase as a result of increasing TMR (31), but we wanted to test whether inoculation
174 had a direct effect on EWL, independent of the effect of increased TMR. Therefore, we used structural
175 equation modeling (lavaan package in R; (34)) to compare models with only indirect (i.e., via increased
176 TMR) and both indirect and direct effects of inoculation on EWL. Model specification and selection
177 followed (19, 20), using the lavaan.survey package (30) to adjust for repeated measures.
178 Finally, we used linear regression to test whether TMR or EWL was related to disease severity,
179 as indicated by the % of wing area with UV fluorescence at the end of hibernation. All statistical analysis
180 was performed in R (37). The experiment was approved by the University of Winnipeg Animal Care
181 Committee, and conducted under Manitoba Conservation Wildlife Scientific Permit WB15396.
182

183 Results

184 All inoculated bats exhibited the obvious UV fluorescence characteristic of *P. destructans*
185 infection (19.2 ± 4.5% of visible wing area, max = 47.8%). This suggests bats in our study were in
186 relatively late stages of infection with many observed fluorescence values among the highest reported in

187 McGuire et al. (26). All control bats hibernated normally, and none had UV fluorescence. Inoculated bats
188 had shorter torpor bouts, and hence aroused more frequently than controls (**Table 1**; likelihood ratio =
189 18.10, df = 1, $p < 0.0001$). The difference in rate of mass loss between inoculated and control bats
190 approached significance (one-tailed t-test, $t_{21.9}$, $p = 0.10$) with inoculated bats tending to lose mass at a
191 greater rate than controls.

192 Respirometry data were compromised for one control bat due to an air leak, and two inoculated
193 bats never entered deep, steady-state torpor, based on their elevated and variable CO_2 production
194 during dry air measurements. Therefore we excluded those values from our analysis. Bats inoculated
195 with *P. destructans* had higher TMR than controls ($F_{1,20} = 23.75$, $p < 0.0001$), and TMR was also higher in
196 humidified air ($F_{1,20} = 31.54$, $p < 0.0001$)(**Fig. 1a**)(**Table 1**). Inoculated bats also had higher EWL than
197 controls ($F_{1,20} = 7.06$, $p = 0.015$) and, not surprisingly, EWL was higher in dry air ($F_{1,20} = 44.97$, $p <$
198 0.0001)(**Table 1**). A treatment*condition interaction ($F_{1,20} = 8.15$, $p = 0.010$) indicated that the increase
199 in EWL for inoculated bats in dry air was greater than that for control bats (**Fig. 1b**).

200 The structural equation model that included both direct and indirect effects of treatment on
201 EWL (**Fig. 2)** was better supported than a model in which EWL was affected only by TMR (ΔAICc = 9.69,
202 χ^2_{diff}= 10.1, df = 1, $p = 0.0015$). There was not strong support for including a relationship between TMR
203 and EWL in our structural equation model ($z = 1.11$, $p = 0.26$) but we elected to retain this relationship
204 because of its likely biological importance. This analysis supports the conclusion that bats infected with
205 *P. destructans* suffered greater evaporative water loss than would be expected due to increased TMR
206 alone.

207 We used the area of the wing showing UV fluorescence as an index of disease severity (as in
208 (26)) to test for the influence of severity on TMR and EWL. We report the relationships for the dry air
209 condition where EWL effects were more pronounced, but the relationships were qualitatively similar for
210 both dry and humid air conditions. We found a positive relationship between levels of disease severity,

211 based on UV fluorescence, and EWL ($F_{1,7}$ = 11.56, p = 0.01, slope = 0.21, intercept = 12.80, R^2 = 0.62; **Fig.**

212 **3a**) but not TMR ($F_{1,7}$ = 0.19, p = 0.68, R^2 = 0.03; **Fig. 3b**).

213 See the Online Data Supplement for the complete dataset.

214

215 # Discussion

216 Our data support the hypothesis that WNS causes increased TMR and EWL for hibernating bats,

217 consistent with predictions arising from two complementary models of WNS pathogenesis (41, 43). As

218 predicted, TMR and EWL were both greater in bats inoculated with *P. destructans*. Furthermore,

219 infected bats were more sensitive than controls to changes in ambient humidity, with a greater increase

220 in EWL in dry relative to humidified air. Importantly, our analysis confirms that increased EWL is a direct

221 result of infection and not simply an indirect consequence of increased TMR and any associated increase

222 in respiratory rate. This result points to wing damage as the cause of dehydration, consistent with the

223 dehydration hypothesis (11, 46).

224 We measured total EWL, which is comprised of respiratory EWL and cutaneous EWL. Disruption

225 of passive gas exchange leading to increased respiratory frequency (and respiratory EWL) has been

226 proposed as a possible mechanism underlying increased EWL in bats with WNS (11), but our data, and

227 those of Carey and Boyles (8), do not lend support to this hypothesis. Based on our structural equation

228 modeling, the link between increased TMR (associated with increased respiratory rate) and EWL was not

229 supported, but a direct link between infection and EWL had strong support. Increased respiratory rate

230 may contribute to elevated EWL in relatively early stages of WNS, but in the later stage of disease

231 exhibited by bats in our study, the relationship between TMR and EWL was not as important as the

232 direct effect of inoculation on EWL, likely via fluid loss across wing lesions. As tissue invasion becomes

233 more pronounced, and larger areas of the wing are affected (increased fluorescence), cutaneous EWL is

234 increased. EWL could also be affected by changes in surface lipid composition (2, 28) but the

235 relationship between UV fluorescence and EWL, and not TMR, further supports the importance of wing
236 lesions from WNS as a driver of EWL. If dehydration is a strong trigger underlying periodic arousals (38,
237 39) arousal frequency should increase as rate of EWL increases (46). As infection proceeds and wing
238 lesions grow, the rate of EWL will increase further, resulting in more frequent arousals as observed for
239 bats with WNS in the lab (42) and field (33). Bats can drink during arousals to offset water loss, but they
240 cannot replenish electrolytes from hibernaculum water sources. If water loss from wing lesions includes
241 loss of electrolytes and other constituents of body fluids, this could lead to the hypotonic dehydration
242 observed in previous studies (10, 43).

243 Increased TMR could also exert important influence on arousal frequency in WNS. As predicted
244 by both the Warnecke *et al.* (43) and the Verant *et al.* (41) models, we observed increased TMR in our
245 captive inoculation experiment. The same result has also recently been confirmed in studies of naturally
246 infected bats (TE Tomasi, pers. comm.). Increased TMR is consistent with the increased energy
247 consumption observed (41) in the absence of increased arousal frequency. Much of the emphasis of
248 WNS pathophysiology research has focused on increased arousal frequency as a leading cause of
249 mortality, but our results indicate that energy expenditure is increased even during the torpid phase of
250 hibernation. Increased TMR during early stages of infection could result from a small but persistent
251 immune response as bats initially attempt to fight off the fungal infection (12, 24, 27, 32). This elevation
252 in TMR might not have a pronounced effect on overall energy expenditure, but it could strongly
253 influence arousal dynamics. For example, at higher TMR (due to higher ambient temperature), bats are
254 known to have shorter torpor bouts and arouse more frequently possibly because higher TMR leads to
255 faster accumulation of metabolic wastes (18). Thus increased TMR could indirectly increase energy
256 consumption by increasing arousal frequency.

257 The treatment*condition interaction we observed for EWL has implications for understanding
258 the influence of environmental conditions on bats with WNS (21, 22). Our results suggest that WNS

259 exacerbates potentially detrimental effects of reduced humidity on bats, with EWL increasing
260 disproportionately for bats from our infected group in dry air. Thus, not only does WNS increase EWL,
261 but it also appears that changes in humidity inside hibernacula could disproportionately affect bats with
262 WNS compared to healthy bats. Hibernaculum microclimate has long been recognized as a critical factor
263 defining suitable hibernacula. Increased water loss in drier conditions may be an important driver of
264 observed differences in the severity and impact of WNS among species and sites with exacerbation of
265 EWL caused by WNS disproportionately affecting species such as *M. lucifugus* that have relatively high
266 rates of EWL (46) and depend on high humidity in hibernation (38). Disproportionate increases in EWL
267 with reduced humidity could interact with behavioral responses of bats to WNS, to influence disease
268 pathophysiology. Bats hibernating in clusters have lower EWL than solitary individuals (7) yet one
269 characteristic response of bats with WNS is to reduce clustering and isolate themselves (22, 42). By
270 isolating themselves (22) bats might reduce the chance of acquiring new points of infection on their
271 wings from other infected colony-mates (17) and slow the growth of *P. destructans* in drier conditions,
272 but any benefits of this response could be overshadowed by the consequences of increased EWL. In
273 drier conditions, the impacts of increased EWL may become more pronounced, contributing to the rapid
274 escalation of pathophysiology observed late in hibernation (26). More work is needed to understand
275 whether the isolation behavior shown by bats with WNS is an adaptive or maladaptive response.
276 Our results provide experimental support of key predictions made by mechanistic models of
277 WNS. Support and revision of these models is a key step in understanding WNS and developing "theory
278 to physiology to populations" (45) strategies for future research and management. Understanding the
279 underlying physiological processes of hibernation and how they are perturbed by infection is critical to
280 developing effective mitigation strategies. For example, microclimate modification of hibernacula is one
281 approach that has been suggested to help mitigate impacts of WNS. Drier conditions in hibernacula
282 could limit fungal growth rate but, at the same time, our results indicate that dry conditions will cause a

283 particularly large increase in EWL for bats with WNS which could make their situation worse. Therefore
284 any mitigation that affects ambient humidity conditions inside hibernacula requires careful
285 consideration. Similarly, a range of chemical and/or biological treatments have been proposed to help
286 slow growth, invasion or spread of the fungus and a number are now being laboratory and field-tested
287 (https://www.whitenosesyndrome.org/us-fish-wildlife-service-funded-research-projects). Our results
288 suggest that topical treatments which increase EWL across the skin could have unintended negative
289 consequences. Ultimately, effective mitigation strategies require understanding of the underlying host
290 physiology and disease pathophysiology.

291 PERSPECTIVES AND SIGNIFICANCE

292 White-nose syndrome continues to spread across North America, invading new regions and infecting
293 new species. While many research efforts focus on identifying potential treatment options, the
294 underlying pathophysiology of the disease is still not fully understood. Our experimental results support
295 key predictions of mechanistic models of the disease. Importantly, our study suggests that WNS affects
296 both TMR and EWL independently in infected bats. Understanding interacting effects of microclimate
297 and behavior is an important future direction that will help develop an integrated understanding of the
298 pathophysiology of WNS. As treatment and mitigation options are tested and considered, experiments
299 addressing the pathophysiology of WNS are necessary for a more complete understanding of the
300 underlying physiological processes involved in hibernation and disease.

301 Acknowledgements

302 Thanks to D. Baloun, T. Cheng, M.-A. Collis, C. Davy, A. Menzies, K. Muise, K. Norquay, Q. Webber, and A.
303 Wilcox for assistance conducting experiments. We are especially grateful to M. Wojciechowski for advice
304 and assistance in planning respirometry. Thanks to J. Foster and K. Parise for conducting qPCR analysis
305 and the University of Winnipeg Animal Care staff for assistance maintaining animals in captivity.

Grants

All authors were involved in project planning, execution, analysis, and manuscript preparation. Funding was provided by grants to CKRW from the Natural Sciences and Engineering Research Council (NSERC, Canada) and the U.S. Fish and Wildlife Service. LPM was supported by a post-doctoral fellowship from NSERC.

Disclosures

The authors have no competing interests, financial or otherwise.

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437

438 **Table 1.** Summary of experimental results. All values are reported as mean ± SE. Final body mass is reported when bats were removed from
439 hibernation for respirometry. Late hibernation torpor bouts were quantified in the 50 days prior to respirometry measurements (day 63 – 113 of
440 hibernation). In late hibernation, inoculated bats had shorter torpor bouts and hence aroused more frequently than controls. There was no
441 difference in initial body mass, but the rate at which inoculated bats lost mass approached significance ($p = 0.10$).

Treatment	Capture Body Mass (g)	Final Hibernation Body Mass (g)	Late Hibernation Torpor Bout Duration (days)	Respirometry Condition	Torpid Metabolic Rate (mW)	Evaporative Water Loss (mg H_2O hr^{-1})
Inoculated	10.2 ± 0.2	7.7 ± 0.2	6.4 ± 0.6	Wet (n = 11)	3.90 ± 0.28	6.14 ± 0.61
				Dry (n = 9)	3.74 ± 0.25	16.57 ± 1.37
Control	10.1 ± 0.2	7.6 ± 0.2	9.2 ± 0.3	Wet (n = 13)	3.24 ± 0.13	4.00 ± 0.27
				Dry (n = 13)	2.34 ± 0.17	10.67 ± 0.56
Sig.	$p = 0.22$	$p = 0.59$	$p < 0.0001$		Treatment $p < 0.0001$ Condition $p < 0.0001$	Treatment $p = 0.015$ Condition $p < 0.0001$ Interaction $p = 0.010$

442

443 # Figure Legends

444

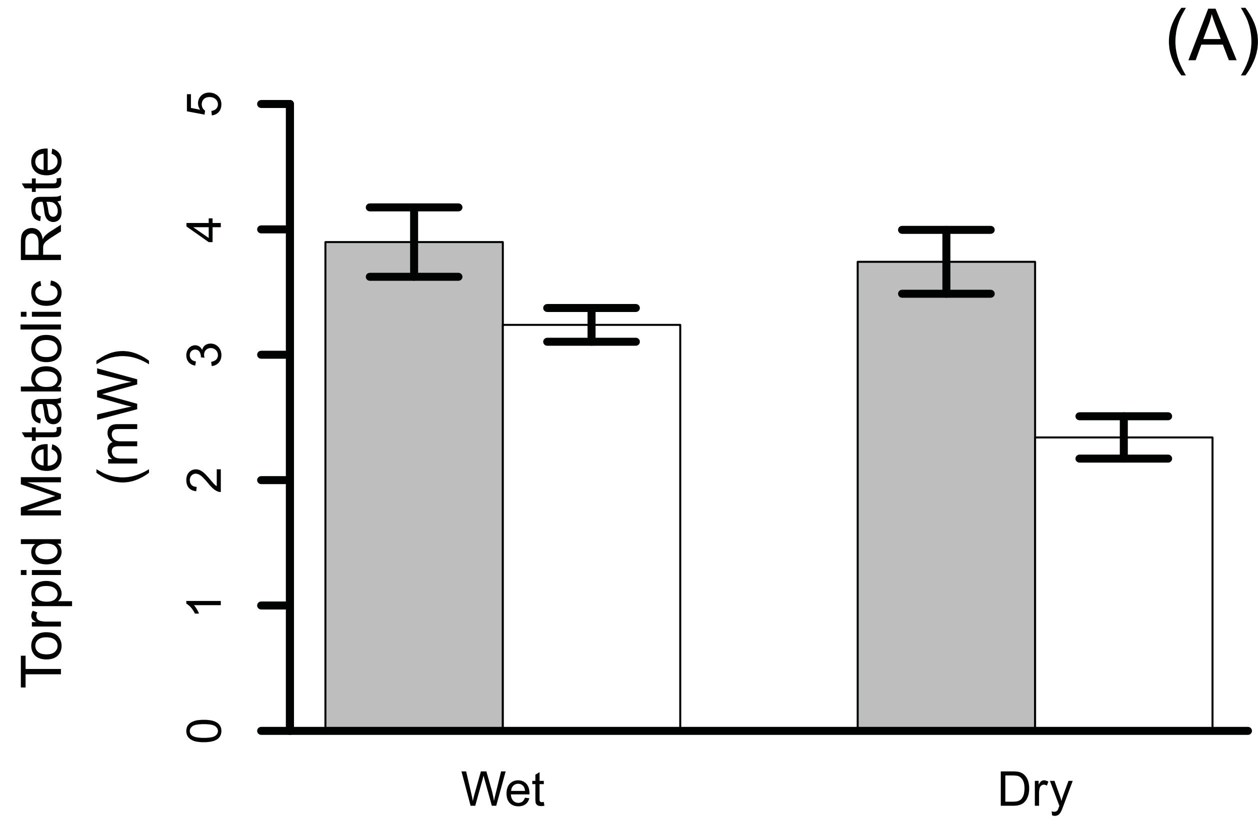
445 **Figure 1.** Physiological responses of hibernating bats to *P. destructans* infection. (A) Bats inoculated with
446 *P. destructans* (grey bars) had higher torpid metabolic rate (mean ± se) than controls (white bars), and
447 metabolic rate was higher under the wet air condition than dry air. (B) Inoculated bats similarly had
448 higher evaporative water loss (mean ± se) than controls, but an interaction indicated a greater increase
449 in evaporative water loss in dry air for inoculated bats. Sample sizes are indicated inside the bars.

450

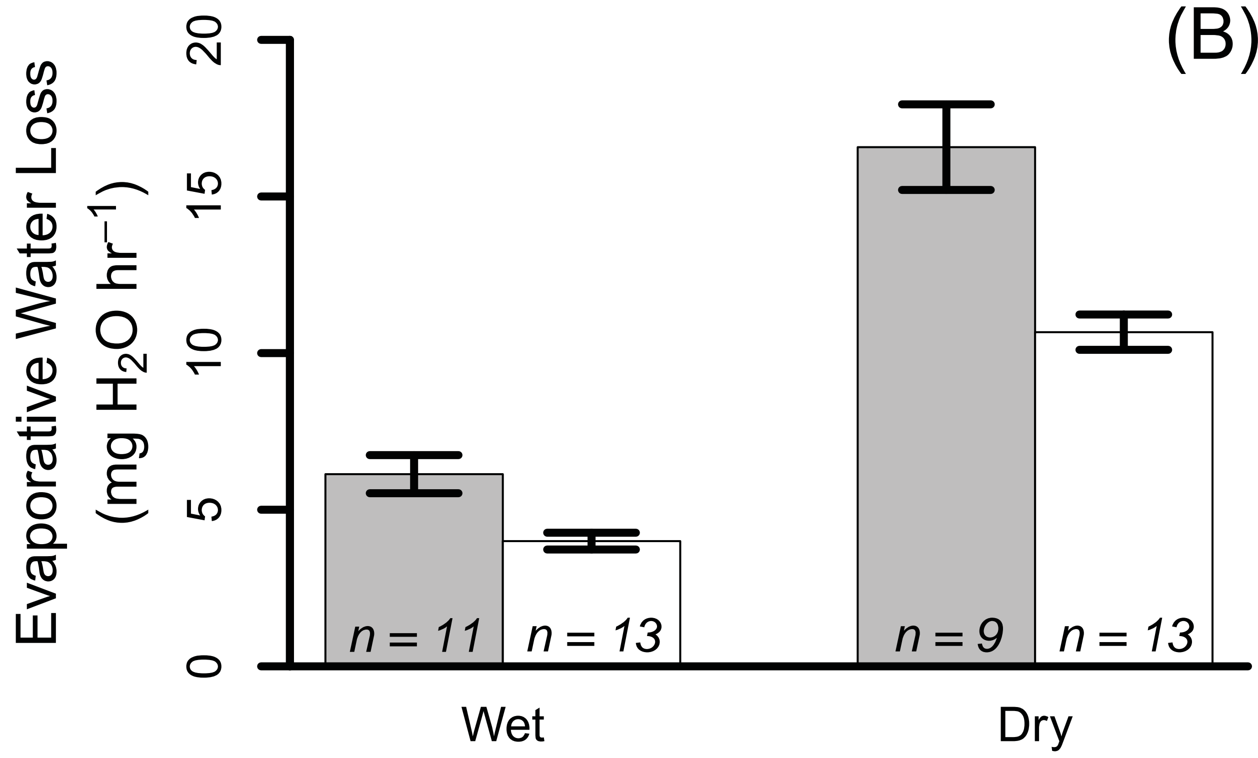
451 **Figure 2.** Structural equation modeling indicated that increased EWL for inoculated bats was not simply
452 an indirect effect caused by increased TMR, but that infection status directly affected EWL. Values
453 indicate standardized parameter estimates with positive relationships indicated in black (e.g., inoculated
454 bats have higher torpid metabolic rate), and negative relationships indicated in grey (e.g., increasing
455 humidity leads to a decrease in EWL). The width of connecting arrows are drawn proportional to the
456 magnitude of the relationship.

457

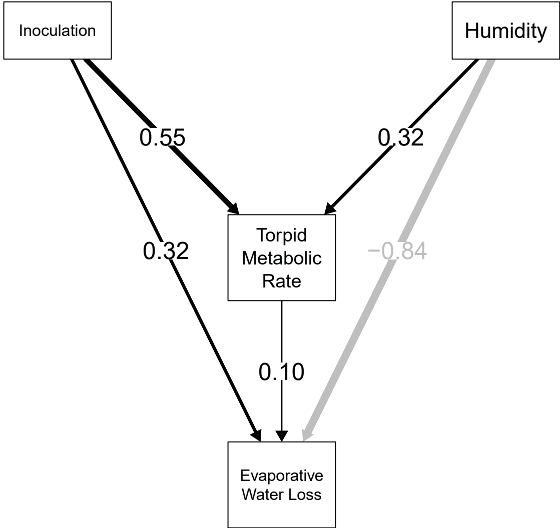
458 **Figure 3.** Relationship between disease severity and physiological responses to *P. destructans* infection
459 (n =9). (A) Bats with larger areas of the wing affected by WNS lesions (indicated by UV fluorescence) had
460 higher rates of EWL but (B) TMR was not affected by disease severity. This suggests that fluid loss across
461 wing lesions as the primary driver of EWL in bats suffering from WNS.

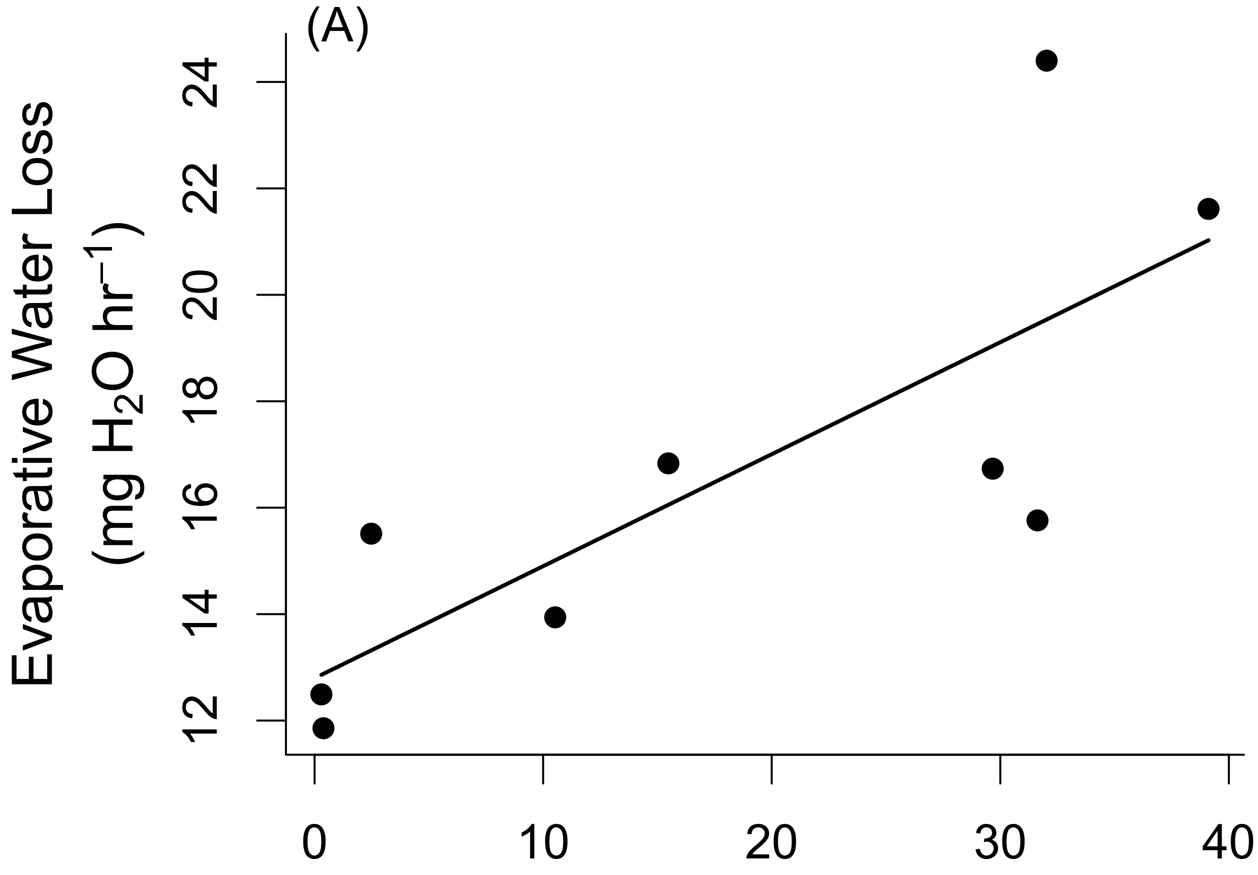


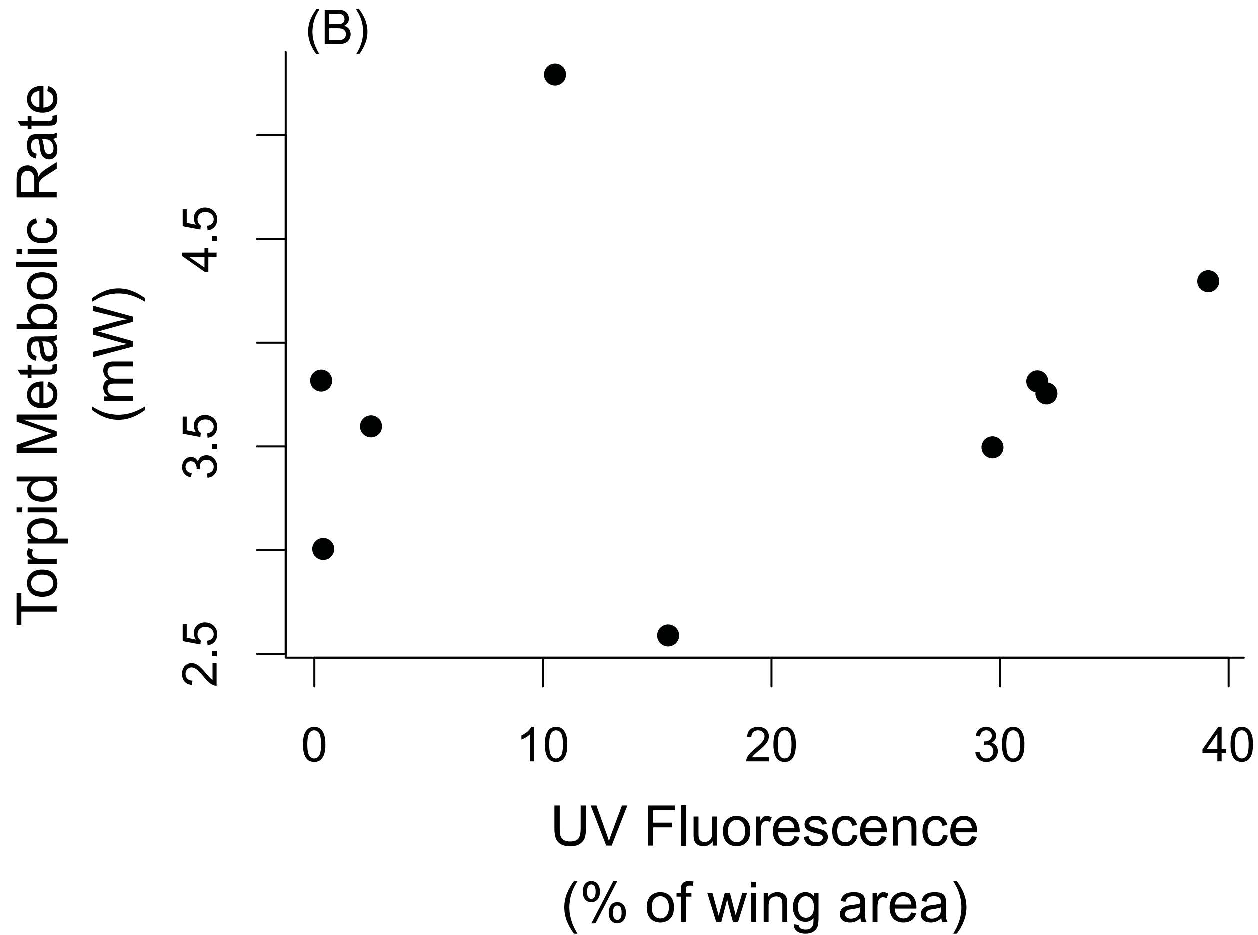
(A)
Torpid Metabolic Rate
(mW)
0
1
2
3
4
5
Wet
Dry



(B)
Evaporative Water Loss
(mg H_2O hr^{-1})
0
5
10
15
20
n = 11
n = 13
n = 9
n = 13
Wet
Dry


Inoculation
Humidity
0.55
0.32
Torpid
Metabolic
Rate
0.32
−0.84
0.10
Evaporative
Water Loss


(A)
Evaporative Water Loss
(mg H_2O hr^{-1})
12 14 16 18 20 22 24
0 10 20 30 40


(B)
Torpid Metabolic Rate
(mW)
2.5 3.5 4.5
0 10 20 30 40
UV Fluorescence
(% of wing area)