Supporting Information

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SI Materials and Methods

Animal Procedures and Housing. All bats were obtained from three clusters within a 5-m^2 area on the ceiling of the cave. The bats were held in cotton bags suspended within a small cooler maintained at ~7 °C and immediately transported to the Western College of Veterinary Medicine, Animal Care Unit, at the University of Saskatchewan, Saskatoon, Canada. Each bat was given a numbered, lipped forearm band (Porzana Limited) and outfitted with a temperature-sensitive device (see below).

Bats were housed in nylon mesh and sheet plastic enclosures (37 × 37 × 104 cm modified Reptarium; Apogee) contained within separate, dark, temperature-controlled environment chambers (VWR BOD 2020; VWR International) kept at ambient temperature (T_a) = 6.9 ± 0.03 °C [North American isolate of *Geomyces destructans* (*Gd*), or *NAGd*], 6.9 ± 0.07 °C (European isolate of *Gd*, or *EUGd*) and 7.0 ± 0.02 °C (sham-inoculated control group, *CO*). To mimic hibernation conditions that are ideal for *Myotis lucifugus*, relative humidity (RH) was maintained at >97% (1) using a custom-built misting system (Ecologic Technologies), which was programmed to moisten the air at regular time intervals (ON 12 s, OFF 60 s). Cotton sheets were used to shield bats from direct spray from the misters and ultrasonic noise was dampened using polystyrene foam. We attached an infrared, waterproof security camera (VL650IRVFS; Speco Technologies) to the ceiling of each reptarium and recorded video to a motion-detecting digital video recorder (SHR-3040; Samsung Techwin). Bats were not fed, but we provided a water dish (filled with marbles to prevent bats from accidentally drowning) that was filled through plastic tubing weekly from outside the environment chamber to avoid disturbing the bats.

At the conclusion of the study in March 2011, all bats were removed from the environmental chambers, allowed to arouse from torpor, anesthetized using isoflurane in oxygen (5%), and humanely euthanized. Some bats reached moribund status before the end of the study, determined based on changes in activity (avoidance of clusters, roosting in isolation near the bottom of the cage, wings partly unfolded during rest). These bats were immediately removed from the environmental chambers and humanely euthanized as described above.

Inoculation. For the inoculation, PBS-Tween-20 solution was pipetted onto the dorsal surface of each wing, between the innermost finger and the body, and spread evenly across the area. Conidia were harvested as previously described in Lorch et al. (2) except that fungal colonies were gently scraped with a sterile, disposable plastic loop to loosen conidia before flooding the plate. Resuspended conidia were frozen in 50-μL aliquots at −80 °C for 2 d during transport to the facility where the bats were held; colony counts demonstrated that freezing did not affect viability of the conidia.

Skin Temperature Recordings. To monitor torpor patterns we noninvasively recorded skin temperature (T_{skin}), which is closely correlated with body temperature (T_b) in bats (3), using two different types of devices: temperature-sensitive radio transmitters (LB-2NT; Holohil Systems; n = 6 each group) and data loggers (DS1922L-F5 Thermochron iButton, Maxim, n = 9 each group; and iBBat, Alpha Mach, n = 3 each group). We used transmitters to allow us to monitor T_{skin} for one-third of each group in real time so we could intervene if bats failed to re-enter hibernation when introduced into the environmental chambers. We used data loggers for most individuals because these devices allowed more reliable data collection within a "noisy" environment because of radio interference. The iButtons were modified after Lovegrove (4), wrapped in cling wrap, and coated with a plastic sealant (Plasti Dip; Plasti Dip International). A small patch of fur was trimmed between the shoulder blades and the T_{skin} device was attached using a latex-based adhesive (Osto-Bond; Montreal Ostomy Centre, Vaudreuil-Dorion, QC, Canada). Transmitter signals were recorded at 15-min intervals using a small dipole antenna connected to a receiver/logger (SRX 400; Lotek Wireless), which was downloaded twice weekly. Data loggers were also programmed to record every 15 min: they were downloaded at the conclusion of the study with a success rate of 89% for the modified iButtons (24 of 27) and 100% for the iBBats.

Behavioral Observations. Behavioral observations allowed us to count the total number of arousals from torpor per group although individual bats could not be identified in the video. We validated these counts using 76 d of continuous T_{skin} data for the *CO* group. The number of arousals recorded per day based on activity was identical to the number of arousals measured via T_{skin} on all but one day, when two arousals were missed because seven bats aroused at the same time (i.e., video observations failed to account for only 2 of 71 arousals). We counted the total number of arousals for each group within each interval (Fig. 2*B*) and these data were clearly consistent with T_{skin} (compare Fig. 2 *A* and *B*; the lower values in period 1 in Fig. 2*B* are because of the omitted induced arousal, see below). Hence, behavioral data proved useful for monitoring torpor patterns in this captive hibernation study.

Histopathology and Fungal Culture. We followed Meteyer et al. (5) for fixation of tissues and histopathological examination of multiple sections from the left wing, as well as nose and ear. Tissues were fixed in formalin immediately after bats were euthanized and later stained for examination using the periodic-acid Schiff method. All treatment bats exhibited the histopathological lesions of *Gd* infection (5). In addition, lung, liver, kidney, spleen, and brain were collected from all bats. These tissues were fixed in formalin, processed routinely for histology, and stained with H&E. In five *Gd*-infected bats there was evidence of a terminal bacteremia or postmortem bacterial overgrowth in lung and liver, which is similar to findings for some dead bats with *Gd* infections collected from natural die-offs. Other than infection with *Gd* in exposed bats, no other significant disease processes were identified histologically. We also subjected sections of the right wing skin to fungal culture techniques (2) to confirm the presence of viable *Gd* on inoculated bats. Sixteen of 18 samples from *NAGd* were culture-positive for *Gd* and 8 of 17 received samples from *EUGd* bats were positive. All control bats were culture-negative for *Gd*.

Statistical Analyses. To analyze torpor patterns, we divided the entire study period into four intervals of 26.3 d each, starting 1 wk after inoculation to allow sufficient time for bats to adjust to the new environmental conditions: Interval 1: December 4–30, 2010; Interval 2: December 30, 2010 to January 25, 2011; Interval 3: January 25 to February 21, 2011; Interval 4: February 21 to March 19, 2011. The memory capacity of T_{skin} data loggers was filled by February 21, 2011, 4 d before termination of *EUGd*. Therefore, most of our analyses focus on the first 3 mo of the experiment.

Within each interval, we tabulated the number of arousals from torpor for each individual to generate mean values for each treatment group. This process was more appropriate than analyzing the duration of torpor bouts because most torpor bouts were long and spread across two intervals. The frequency of arousals was calculated as the number of arousals divided by the number of recording days for a given individual within each interval. To be conservative and avoid overestimating arousal frequency, we only counted arousals if we had collected more than 6.6 d of continuous T_{skin} data (i.e., measurement every 15 min) within an interval for a particular individual. We chose this threshold as it approximates the minimum average torpor bout duration for any of the groups. The "arousal duration" began when T_{skin} had clearly stabilized after the characteristic steep increase that occurred during onset of arousal. Arousal duration ended when T_{skin} exhibited an obvious, abrupt decline as bats reentered torpor (6) (Fig. 1).

The power supply to the *EUGd* environment chamber failed on day 25, causing a brief decrease in RH to 87% and an increase in T_a to 15.7 °C. We immediately replicated these conditions for the other two groups to ensure all bats experienced the same pattern of disturbance and responded identically to it. This disturbance induced an arousal in almost all bats (i.e., only 2 of the 39 individuals for which continuous T_{skin} data were available on that day did not arouse), so it did not affect our comparison of the three groups.

Statistical analysis of torpor patterns was conducted using *statistiXL* v7.0 and survival analysis was conducted in Systat v11.0. Data are presented as mean ± 1 SE; *n* denotes the number of individuals. We used full-factorial ANOVA to analyze differences in torpor bout duration and arousal duration among groups within each interval. Student-Newman-Keuls (SNK) post hoc tests were used for pair-wise comparisons following a significant ANOVA result. To test for an effect of time on torpor patterns (i.e., arousal frequency and arousal duration), we also used repeated-measures ANOVA testing for differences among the first three intervals (i.e., for as long as data loggers were recording) within each group. We conducted this analysis separately for each group. We used a Breslow–Gehan survival analysis to test for differences in the time to mortality/moribund status for the three groups, with a Bonferroni correction to account for multiple comparisons between each pair of groups.

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