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Chapter 12

Hibernating Bat Species in Pennsylvania use Colder Winter Habitats Following the Arrival of White-nose Syndrome

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ABSTRACT

Hibernating bat species face severe energetic challenges during winter that leave them vulnerable to human disturbance as well as changes in environmental conditions within hibernacula. The extent of this vulnerability is highlighted by bat responses to white-nose syndrome (WNS), a recently described fungal disease that has compromised winter energetic strategies, causing dramatic declines in bat populations. To improve the management of winter habitats following the onset of WNS, we studied microclimates used by hibernating bat species in Pennsylvania before and after the arrival of the disease. We focused on all six bat species that commonly hibernate in the state, with an emphasis on little brown myotis (*Myotis lucifugus*), tri-colored bats (*Perimyotis subflavus*), and big brown bats (*Eptesicus fuscus*). We found that before the arrival of WNS, little brown myotis, northern long-eared myotis (*M. septentrionalis*), Indiana myotis (*M. sodalis*), and tri-colored bats occupied hibernacula with temperatures ranging from 2–10° C during mid-winter (December–February), with larger populations of little browns found in colder hibernacula, and larger populations of tri-colored bats found in hibernacula with less variable temperatures. Big brown bats and eastern small-footed myotis (*M. leibii*) occupied the same range of winter temperatures prior to WNS, but areas within hibernacula used by these species were colder and more variable in temperature, on average, than areas of the same hibernacula used by little brown myotis and tri-colored bats. Following the arrival of WNS, we found the majority of little brown myotis, tri-colored bats, and big brown bats in sections of hibernacula that were colder than sections with the largest aggregations prior to the onset of WNS. Furthermore, after the arrival of WNS, we found members of each species in sections with colder minimum temperatures than sections reported as occupied before the arrival of WNS. These data show that at least 3 species of hibernating bats in Pennsylvania have changed their winter behavior in the years following dramatic population declines, providing management agencies with options for enhancing winter habitat since the onset of WNS.

KEY WORDS — *Eptesicus fuscus*, habitat selection, hibernation, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis*, *Myotis sodalis*, *Perimyotis subflavus*, *Pseudogymnoascus destructans*, White-nose Syndrome

Conservation and management of wildlife habitat relies upon a clear understanding of both the relationship between environmental characteristics and habitat suitability, and how this relationship varies within and among species. Such an understanding is especially important when protecting habitats that are critical for survival, such as the winter hibernacula of bats. In the northeastern United States, hibernating bat species congregate in large numbers in a relatively small number of caves and mines that provide environmental conditions suitable for survival during 6 or more months of hibernation (Davis and Hitchcock 1965). Thus, bat hibernacula represent priority habitats for conservation and management efforts. Fortunately, there is a considerable body of literature that serves as a foundation for understanding winter habitat suitability, starting with an understanding of how bats cope with temperatures inside hibernacula, which are typically <10° C (Webb et al. 1996). To survive these temperatures, in addition to the lack of adequate food sources, bats enter a state of reduced metabolism known as torpor that subsequently results in significant reductions in body temperature and energy use (Geiser 2004). Bat hibernation across winter consists of a series of torpor bouts, each up to several weeks in duration and separated by brief arousals to normothermy (Thomas et al. 1990, Humphries et al. 2003). All mammalian hibernators experience these periodic arousals, which are hypothesized to serve a number of physiological purposes (Thomas and Geiser 1997, Prendergast et al. 2002, Heller and Ruby 2004).

Species differences in body sizes, winter fat stores, and rates of heat loss through conduction and convection likely influence differences in microclimate preferences among species (Reeder and Moore 2013). Many bat species, such as the Indiana myotis (*Myotis sodalis*) rely on underground caves and mines where temperatures are relatively stable and slightly above 0° C for hibernacula (Tuttle and Kennedy 2002, Brack 2007, Kurta and Smith 2014). Other species, like big brown bats (*Eptesicus fuscus*) and eastern small-footed myotis (*Myotis leibii*) tolerate more variable environments (C. M. Butchkoski, Pennsylvania Game Commission, unpublished report) and are often found close to the entrances of caves and mines. Hibernacula with high species diversity commonly provide a range of temperatures that meet the hibernation preferences of various species throughout the hibernation season.

Although cold temperatures represent an energetic stress during normothermy, temperatures between 0 and 10° C (Heldmaier et al. 2004) are associated with the greatest reductions in metabolic rate, and, therefore, energy used, during torpor. Temperatures below freezing, as well as temperatures >10° C, are associated with higher torpid metabolic rates and higher rates of arousal from hibernation, resulting in more energy spent during hibernation, and a decrease in the potential hibernating period (Thomas et al. 1990, Buck and Barnes 2000, Geiser 2004). One study found that the relationship between temperature and rate of arousal from hibernation is important to bats with differing abilities to accumulate body fat (Boyles et al. 2007). This study found that little brown myotis (*Myotis lucifugus*) with lower body mass, and presumably lower fat reserves, hibernated in colder areas (promoting longer torpor bouts and more energy savings) than individuals with greater body mass within the same hibernaculum. Thus, hibernacula temperatures have a profound impact on bats during their winter hibernation. In Pennsylvania, average subterranean temperatures are too warm for hibernation. In rare circumstances, however, sites may slope downhill or trap cold air through other mechanisms to provide environmental conditions conducive for hibernation (Tuttle and Kennedy 2002).

Water vapor pressure, a measure of the amount of water vapor in air, is also an important microclimate feature of hibernacula because it influences the rate of evaporative water loss from the skin surface of bats during hibernation, which leads to dehydration and increased rate of arousals from torpor (Thomas and Geiser 1997, Ben-Hamo et al. 2013). Water vapor pressure in hibernacula may affect bat species differently, and this difference may in turn affect selection of roost sites. Specifically, little brown myotis and tri-colored bats (*Perimyotis subflavus*) may be particularly susceptible to evaporative water loss, and are often found in areas of hibernacula with high water vapor pressure (Twente 1955, Cryan et al. 2010).

When it comes to managing winter habitat for bats, it is important to maintain the internal microclimates of each hibernaculum and monitor the biotic and abiotic factors that influence those microclimates. This is particularly important given the variation in habitat preferences exhibited by different species and the limited resources of conservation agencies that must prioritize the conservation of multiple sites. Assessing winter habitat for bats is now further complicated by the recent introduction of a fungal pathogen of bats, *Pseudogymnoascus destructans* (*Pd*, formerly known as *Geomyces destructans*) (Coleman and Reichard 2014). *Pd* is the causative agent of the disease White-nose Syndrome (WNS) (Lorch et al. 2011, Warnecke et al. 2012), which has caused severe local- and regional-scale population declines in several bat species since it was first documented in North America in 2006 (Turner et al. 2011, Thogmartin et al. 2012, Ingersoll et al. 2013, Ingersoll et al. 2016). *Pd* is a cold-loving fungus that grows at temperatures as cold as 3° C, but optimally at temperatures from 12.5–15.8° C. Thus, *Pd* thrives at the temperatures used by bats during hibernation, especially in relatively warmer sites (Blehert et al. 2009, Verant et al. 2012). Given the temperature-dependent growth pattern of *Pd*, and its thermal optima at temperatures slightly higher than typical bat hibernation temperatures (Verant et al. 2012), it is not surprising that WNS mortality rates are positively associated with hibernacula temperatures, both in the field and in laboratory trials (Langwig et al. 2012, Johnson et al. 2014, Hayman et al. 2016). These studies show that selection of colder temperatures by hibernating bats may increase the probability of surviving WNS. As a result, data describing microclimate preferences pre-WNS may not reflect preferences in the presence of *Pd*, and the potential for shifts in winter behavior should be investigated to improve management of hibernacula for WNS-affected bat species.

Six bat species routinely hibernate in caves and mines in Pennsylvania during the winter: the big brown bat, tri-colored bat, eastern small-footed myotis, little brown myotis, Indiana myotis, and northern long-eared myotis (*M. septentrionalis*). Temperatures within hibernacula used by these bats range between 2 and 10° C (reviewed by Reeder and Moore 2013). This large range in temperatures reflects differences in the microclimate preferences among species as well as the range of hibernacula temperatures available across the geographic range of these species, making it difficult to identify trends in winter habitat selection. Here, we examine hibernacula temperatures used by all 6 common hibernating species in Pennsylvania, with an emphasis on little brown myotis, tri-colored bats, and big brown bats. Our objectives in this paper are to provide a concise understanding of: 1) how hibernacula temperature and water vapor pressure influence the number of hibernating bats; 2) how microclimate selection within hibernacula varies among bat species; and 3) how the onset of WNS and continued presence of *Pd* in hibernacula has affected winter microclimate preferences in Pennsylvania's hibernating bat species. Such an understanding can help agencies responsible for managing hibernacula predict how environmental changes may positively or negatively impact bats, and what management tools can be used to improve conditions within hibernacula for a particular species in the present WNS-affected landscape.

METHODS

Characterization of Hibernacula Microclimates

We characterized hibernacula microclimates using 2 methods. First, we recorded minimum and maximum rock surface temperatures where bats were found throughout hibernacula during winter surveys in Pennsylvania using an infrared thermometer (Model 568, Fluke-Direct, Washington). These winter surveys were conducted between mid-January and early March on a biennial or less frequent basis in an effort to survey during the coldest period of winter, when late-arriving and early-departing species such as big brown bats and eastern small-footed myotis are most likely to be present (Turner et al. 2011). These measurements were used to compare microclimate selection by bats within hibernacula before and after WNS. Second, we deployed temperature and relative humidity dataloggers (TransiTempII, MadgeTech, Inc., New Hampshire) within 9 hibernacula between 2009 and 2014 to characterize hibernacula microclimates throughout the winter (Table 1). Dataloggers were first deployed as WNS arrived in Pennsylvania. To broadly evaluate microclimate selection, dataloggers were placed in multiple areas used by bats within hibernacula: typically, at areas where bats were found closest to the entrance, at the area of highest concentration, and toward the back of the site far from any entrance. Eight of these hibernacula were consistently used by large populations of at least 1 bat species while the ninth, Dunbar Mine, was used only infrequently by tri-colored bats and *Myotis* species and is presented as an example of poor winter habitat for these species (Table 1).

Table 1. Hibernacula in Pennsylvania chosen for microclimate study between 2009 and 2014 and the maximum number of each hibernating bat species present during any winter survey between 1985 and 2015.

Hibernaculum name	Little brown myotis	N. long-eared myotis	Indiana myotis	E. small-footed myotis	Big brown bat	Tri-colored bat
Casparis Mine*	170	10	2	11	337	33
CS&M Mine	30,653	30	21	0	125	64
Dawn Mine	2,000	26	0	0	1	138
Durham Mine*	10,425	881	0	2	19	167
Dunbar Mine	6	0	0	0	79	2
Layton Fire Clay Mine*	6,489	30	11	0	592	57
Allegheny Mountain*	2,123	18	139	0	2	62
Shindle Iron Mine	2,348	35	0	0	2	62
Steifel Park Mine	1,755	0	1	0	24	35

*Indicates hibernacula included in microclimate comparisons between big brown bats and little brown myotis and tri-colored bats. Other hibernacula were only included in comparisons of relative populations of little brown myotis and tri-colored bats.

Data were collected at this site in preparation for future modifications to improve environmental conditions.

Dataloggers were programmed to record temperature (° C) and relative humidity (% saturation) at 30-min intervals throughout the winter hibernation period. We converted relative humidity to water vapor pressure because relative humidity is less informative and potentially misleading (Kurta 2014). Relative humidity is the ratio of actual water vapor pressure to the maximum amount of water vapor the air can hold, known as the saturation water vapor pressure. Relative humidity can be misleading because saturation water vapor pressure has a curvilinear relationship with air temperature, meaning that as air temperature increases, saturation water vapor pressure increases at a faster rate. Thus, the actual water vapor pressure would be different for 2 hibernacula with different air temperatures but identical relative humidity. To convert relative humidity to water vapor pressure, we calculated the saturation water vapor pressure of the air for every temperature recording according to the quadratic formula provided by Tabata (1973), and determined the actual water vapor pressure at every time point by multiplying the saturation vapor pressure by the relative humidity (Kurta and Smith 2014). We determined the daily average and minimum temperature, as well as the daily average water vapor pressure at each sampling site for each full day of sampling. These daily averages were used to generate monthly and seasonal (October through April) average and variance in winter temperature and water vapor pressure. Minimum winter temperature was the coldest temperature ever recorded at each site. We also determined the average daily temperatures outside each hibernaculum using data from National Weather Service weather stations (www.weatherunderground.com). We subtracted the daily average aboveground temperature from daily average temperature at each sampling site as a measure of how well buffered hibernacula were from aboveground conditions.

Little brown myotis was the most common species in all hibernacula surveyed except Dunbar Mine (Table 1), and we deployed one datalogger near the largest aggregation of little brown myotis at all sites. Logger data are therefore most specific to this species.

Tri-colored bats, as well as northern long-eared and Indiana myotis were also found in the rooms where we measured microclimates occupied by hibernating little brown myotis, and for this reason these logger data are also considered representative of the microclimate selected by these species. However, each hibernaculum has sections, or rooms, that possess unique microclimates and unique compositions of species occupying them. Because not all members of these other 3 species were always found near the datalogger, and because bats of each species were found hibernating at a range of temperatures within each hibernaculum during winter surveys, considerable variation may exist for these species. Big brown bats and eastern small-footed myotis were predominantly found near the entrances of several hibernacula (Table 1). Therefore, data from entrance loggers were considered representative of microclimate conditions for these 2 species, and were used in comparisons with microclimates for other species.

Statistical Analysis

All statistical analyses were performed in IBM SPSS Statistics v. 23.0. We compared the average, minimum, and variance in winter temperature, as well as average winter water vapor pressure between hibernacula with relatively larger (>5,000; $n = 3$) and smaller (<5,000; $n = 5$) winter populations of little brown myotis using t-tests on ranked data for each variable. Because the Type I error rate for these tests is approximately twice commonly accepted levels when sample sizes are ≤ 5 and variances of the groups being compared are equal (de Winter 2013), we lowered our threshold for significance to 0.025. We used Levene's test for equality of variance, which did not detect differences between any groups, before performing *t*-tests. We also compared average buffering from outside temperatures between these groups of hibernacula. This analysis was limited to temperatures collected during mid-winter (December-February) because hibernacula in Pennsylvania shift from being colder than outside air temperatures to being warmer than outside conditions during November, and shift back in March (Fig. 1). The same comparisons were conducted between hibernacula with relatively larger (>100; $n = 3$) and smaller (<100; $n = 5$) populations of tri-colored bats. Although the same 8 hibernacula are used in all comparisons, the hibernacula with relatively larger numbers of each species were not consistent across each species (Table 1). Maximum counts for each species, collected during winter hibernacula surveys by the Pennsylvania Game Commission (PGC) on a biennial or less frequent basis between 1985 and 2015, were used as the number of bats of each species occupying hibernacula. Maximum counts for each species were always observed prior to WNS. We used a Wilcoxon signed-rank test for each variable to compare microclimates in rooms occupied by little brown myotis and tri-colored bats to rooms occupied by big brown bats within the same hibernacula. These comparisons represent microclimate selection prior to the arrival of WNS.

To better understand how hibernacula microclimate selection may be affected by *Pd*, we examined 30 years of winter survey data collected by the PGC. Of the 50 hibernacula surveyed ≥5 times by the PGC, 10 had precise records of the number of bats hibernating in rooms with temperatures recorded by an infrared thermometer and had remnant populations of ≥5 little brown myotis, tri-colored bats, or big brown bats in 2014 or 2015. Using a separate Wilcoxon signed-rank test for each species, we compared rock surface temperatures where the largest aggregate of bats roosted before the arrival of WNS to tem-

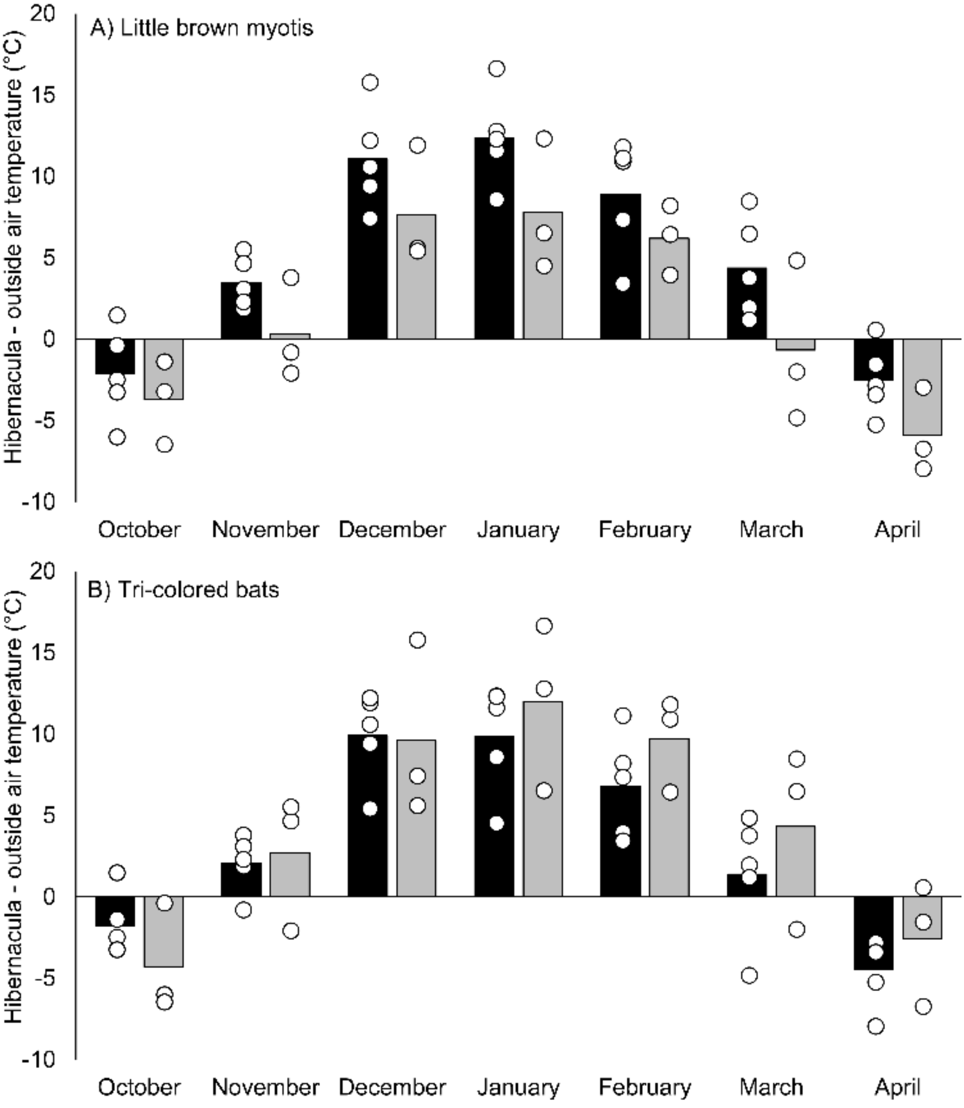

Figure 1. Insulation of hibernacula temperatures from aboveground temperatures was similar between hibernacula with relatively small (<5,000 bats, *n* = 5; black bars) and large (>5,000 bats, *n* = 3; gray bars) winter populations of little brown myotis (panel A), as well as hibernacula with relatively small (<100 bats, *n* = 5; black bars) and large (>100 bats, *n* = 3; gray bars) populations of tri-colored bats (panel B). Insulation from aboveground temperature was determined as hibernacula temperature – aboveground temperature. Circles represent average temperatures for individual hibernacula during each month.

peratures where bats roosted after the arrival of WNS within these 10 sites. Using the same statistical analysis, we compared the minimum temperatures where any member of each species was found hibernating at each site before and after the arrival of WNS. Sample sizes for each species are <10 hibernacula because not all sites housed at least 5 bats of each species. Only hibernacula with surveys occurring after 2014 were included in these comparisons. With WNS considered state-wide by the end of the winter of 2011–2012 (Turner et al. 2016) and most mass mortality at a site occurring within 2 years of WNS arrival (Turner et al. 2011), limiting our dataset in this way attempts to describe microclimate selection by WNS survivors and not bats preparing to exit the hibernaculum during WNS mass-mortality. It is important to note, however, that surveys occurring after 2014 are likely to include juveniles experiencing their first winter with WNS as well as WNS survivors.

RESULTS

Excluding the areas of hibernacula near the entrance where big brown bats and small-footed myotis predominantly hibernate, areas within caves and mines used by hibernating bats were all well-buffered (had higher minimum and lower maximum temperatures) from aboveground conditions (Table 2). Tri-colored bats, little brown myotis, northern long-eared myotis, and Indiana myotis all hibernated in these areas, although small sample sizes for Indiana and northern long-eared myotis precluded analyses specific to these species. Buffering from outside temperatures did not vary between hibernacula with >5,000 (n = 3) and <5,000 (n = 5) little brown myotis (t = -1.9, df = 6, P = 0.10, Table 2, Fig. 1a), nor did minimum winter temperature (t = -2.0, df = 6, P = 0.10, Table 2) or variance in winter temperature (t = 1.1, df = 6, P = 0.33, Table 2). Hibernacula with >5,000 little brown myotis did have lower average winter temperatures (t = -3.9, df = 6, P = 0.008, Table 2, Fig. 2a) and lower average water vapor pressures (t = -3.9, df = 6, P = 0.008, Fig. 3a) than those with <5,000 bats. All 8 hibernacula reached their temperature and water vapor pressure minima in February (Fig. 2a, 3a). In sum, little brown myotis in Pennsylvania formed larger winter colonies in hibernacula with average temperatures and water vapor pressures of 3–7° C and 0.7–1.0 kPa, respectively, during mid-winter (December–February) prior to the arrival of WNS.

Although both little brown myotis and tri-colored bats were present in all of the hibernacula we studied, we observed differing trends in which hibernacula were associated with relatively larger populations of each species. Hibernacula with >100 (n = 3) and <100 tri-colored bats (n = 5) did not differ in buffering from aboveground temperatures (t = 0.14, df = 6, P = 0.90; Table 2, Fig. 1b), and did not differ in average (t = 0.72, df = 6, P = 0.50, Table 2, Fig. 2b) or minimum winter temperature (t = 0.89, df = 6, P = 0.41, Table 2). Similarly, water vapor pressure did not differ between hibernacula (t = 0.42, df = 6, P = 0.70; Table 2, Fig. 3b). Temperature variance was the only variable that differed between hibernacula, with less variable temperatures associated with larger winter populations of tri-colored bats (t = -4.0; P = 0.007, Table 2). In sum, hibernacula in Pennsylvania with large populations of tri-colored bats prior to WNS were more thermally stable, having less variable winter temperatures (average winter variance of 0.1° C) compared to hibernacula with smaller populations (average winter variance of 1.7° C).

In comparison to little brown myotis and tri-colored bats, big brown bats occupied colder (average temperature: Z = -2.2, P = 0.03; minimum temperature: Z = -2.2, P = 0.03), more variable (Z = -2.2, P = 0.03) areas of hibernacula that were less buffered from aboveground conditions (Z = -2.2, P = 0.03) (Fig. 4, Table 2). Water vapor pressure was also lower in rooms occupied by big brown bats (Z = -2.2, P = 0.03). Average winter temperature where big brown bats roosted ranged from 3–8° C, but minimum temperatures dropped below

Table 2. Pennsylvania hibernacula included microclimate study between 2009 and 2014, along with the maximum number of each bat species present at each site during any winter survey from 1985–2015 and winter (October–April) microclimate conditions.

Comparison	Average Temp (° C)	Min temp (° C)	Temp (° C) variance	Temp (° C) difference*	Water vapor pressure (kPa)
Hibernacula with relatively larger and smaller populations of little brown myotis					
>5,000 little brown myotis	6.0 ± 1.2^{a}	4.4 ± 1.9^{a}	1.6 ± 1.7^{a}	7.3 ± 1.9^{a}	0.9 ± 0.1^{a}
<5,000 little brown myotis	8.6 ± 0.8^{b}	7.4 ± 1.9^{a}	0.8 ± 1.0^{a}	11.0 ± 1.2^{a}	1.1 ± 0.1^{b}
Hibernacula with relatively larger and smaller populations of tri-colored bats					
>100 tri-colored bats	7.8 ± 2.1^{a}	7.3 ± 2.4^{a}	0.1 ± 0.2^{a}	10.5 ± 2.5^{a}	1.1 ± 0.1^{a}
<100 tri-colored bats	7.6 ± 1.6^{a}	5.7 ± 2.4^{a}	1.7 ± 1.3^{b}	9.1 ± 1.3^{a}	1.0 ± 0.1^{a}
Rooms within hibernacula occupied by big brown bats, little brown myotis, and tri-colored bats					
Big brown bats	5.7 ± 2.4^{a}	0.9 ± 4.9^{a}	5.3 ± 4.0^{a}	6.1 ± 1.5^{a}	0.9 ± 1.7^{a}
Little brown myotis and tri-colored bats	7.7 ± 1.9^{b}	6.1 ± 2.7^{b}	1.3 ± 1.4^{b}	9.6 ± 1.2^{b}	1.0 ± 0.1^{b}

Within each comparison, rows not sharing common letter superscripts differed significantly.

* Temperature difference was defined as temperature inside the hibernacula – temperature outside. While other measures were averaged over October–April, this measure was averaged over December–February (see text).

freezing at 3 sites, and below -4° C at 2 sites. Eastern small-footed myotis occupied 2 of the big brown sites studied (average and minimum winter temperatures ranging from 4–7°, and -1–3° C. respectively), although sample sizes for this species were too low for any statistical comparisons.

Comparison of rock surface temperatures near roosting bats before and after the arrival of WNS showed that since the arrival of WNS, the majority of little brown myotis ($Z = -2.2$, $P = 0.03$), tri-colored bats ($Z = -2.5$, $P = 0.01$), and big brown bats ($Z = -2.7$, $P = 0.01$) are choosing microclimates within hibernacula with median temperatures 3, 5, and 2° C colder, respectively, than sites chosen prior to the arrival of WNS in Pennsylvania (Fig. 5a, c, and e). Additionally, little brown myotis ($Z = -2.5$, $P = 0.01$), tri-colored bats ($Z = -2.7$, $P = 0.01$), and big brown bats ($Z = -2.7$, $P = 0.01$) now also occupy areas with lower minimum temperatures than was recorded in pre-WNS surveys (Fig. 5b, d, and f). These previously unoccupied areas of hibernacula are typically close to the entrance, and have temperatures that are close to, and even below, 0° C. Conversely, some of the warmest areas of these hibernacula are no longer occupied, although it should be noted that relatively warm areas have not been completely abandoned by little brown myotis and tri-colored bats (Fig. 5a, c).

In addition to shifts in microhabitat selection within hibernacula, we also found initial evidence of bats using colder hibernacula in greater numbers since the arrival of WNS. At one railroad tunnel, where ambient temperatures range from -3 to 5° C in areas occupied by bats, the winter population of little brown myotis in 2015 ($n = 40$) was more than twice any number observed pre-WNS ($n = 15$).

DISCUSSION

We present evidence that bats hibernate in new locations within hibernacula after the introduction of *Pd* and onset of WNS. Prior to onset of WNS in Pennsylvania, hibernating

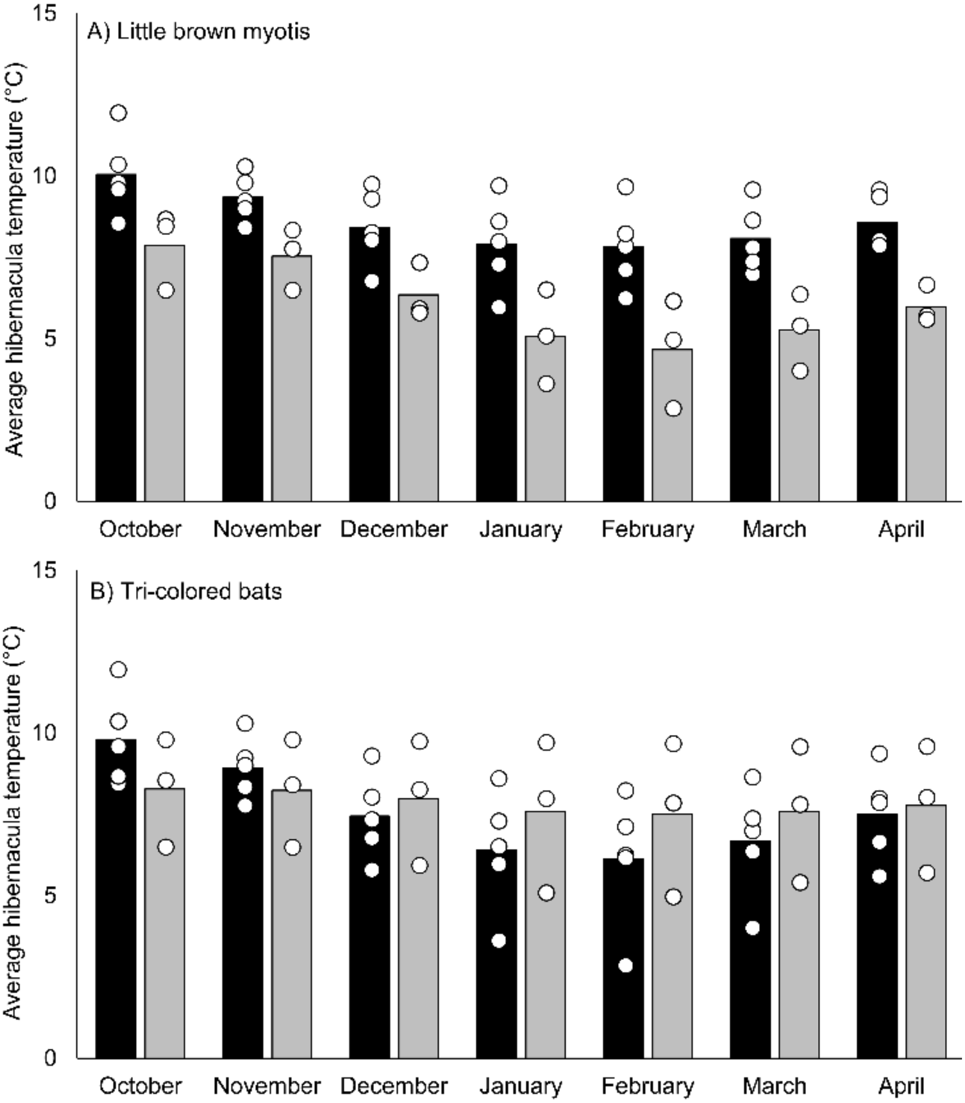

Figure 2. Hibernacula with relatively large winter populations (>5,000 bats, *n* = 3; gray bars) of little brown myotis have colder daily average temperatures during winter (October-April) than hibernacula with relatively smaller populations (<5,000 bats, *n* = 5; black bars; *P* = 0.008, panel A) while hibernacula with relatively large (>100 bats, *n* = 3; gray bars) and small (<100 bats, *n* = 5; black bars) populations of tri-colored bats did not differ in average temperature (*P* = 0.90, panel B). Circles represent average temperatures for individual hibernacula during each month.

bat species exhibited broad differences in microclimate selection. Specifically, little brown myotis formed the largest winter populations in hibernacula with relatively cold average temperatures (3–7° C) during mid-winter, whereas tri-colored bats formed their largest

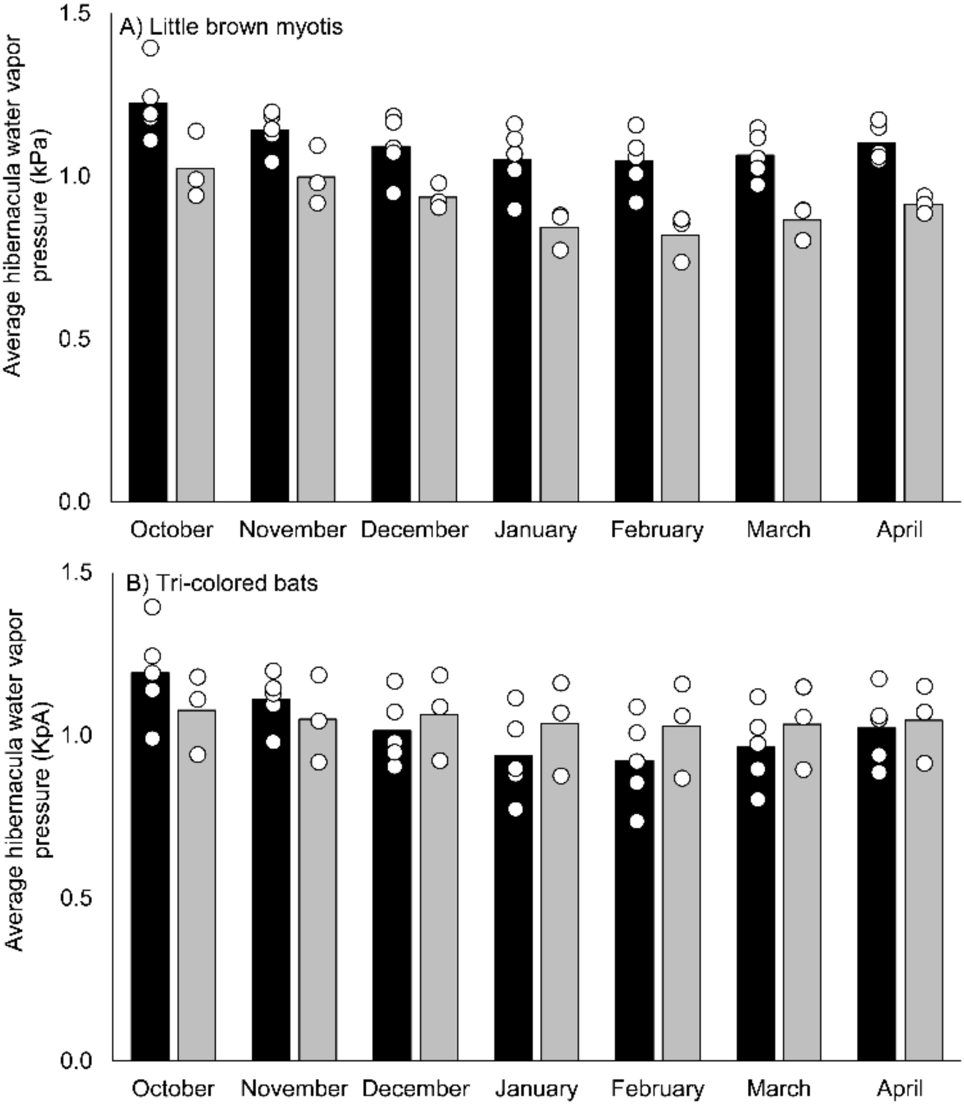

Figure 3. Hibernacula with relatively large winter populations (>5,000 bats, *n* = 3; gray bars) of little brown myotis have lower average water vapor pressure during winter (October-April) than hibernacula with relatively smaller populations (<5,000 bats, *n* = 5; black bars; *P* = 0.008, panel A) while hibernacula with relatively large (>100 bats, *n* = 3; gray bars) and small (<100 bats, *n* = 5; black bars) populations of tri-colored bats did not differ in average water vapor pressure (*P* = 0.70, panel B). Circles represent average temperatures for individual hibernacula during each month.

populations in hibernacula with stable temperatures within each site (average variance of 0.1° C), but collectively exhibited average temperatures of 5–10° C during mid-winter. In comparison to both species, big brown bats and eastern small-footed myotis occupied

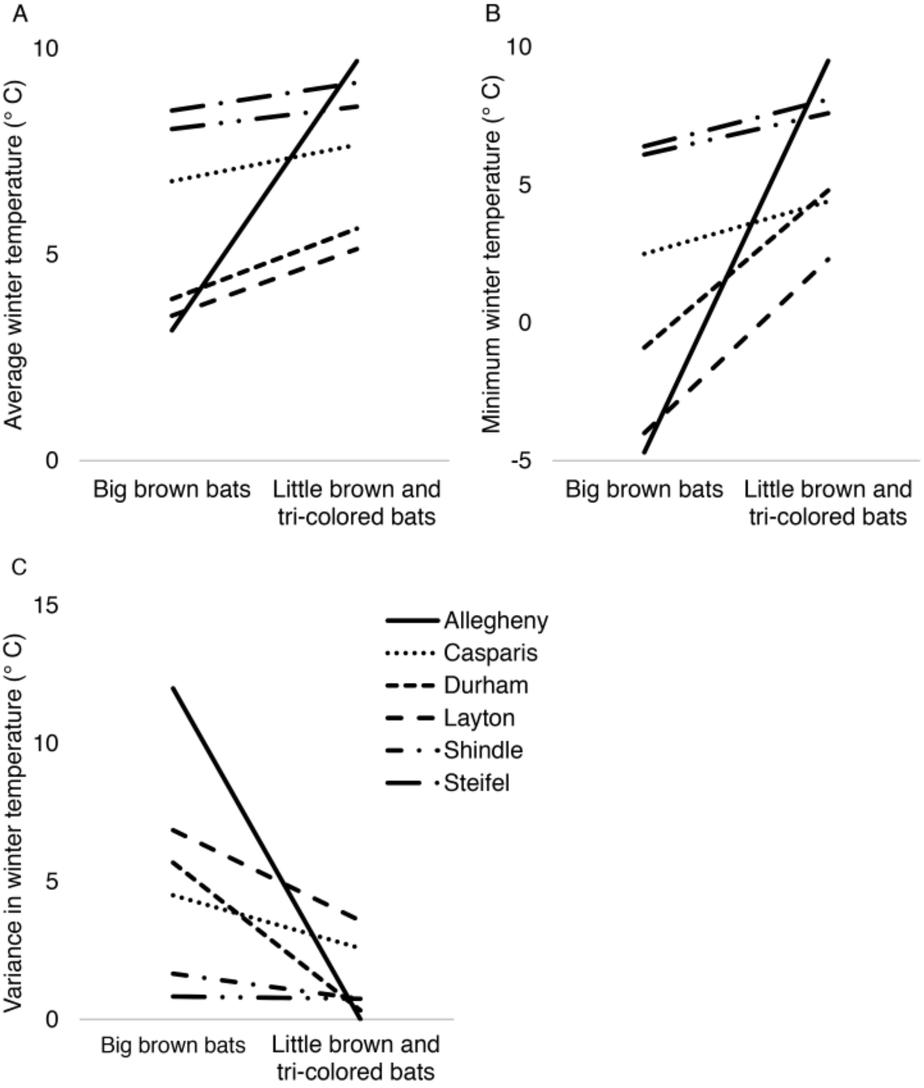

Figure 4. Big brown bats occupied areas with lower daily average (panel A, *P* = 0.03), lower minimum (panel B, *P* = 0.03), and higher variance in daily average temperature (panel C, *P* = 0.03) during winter compared to areas occupied by little brown myotis and tri-colored bats within the same hibernacula. Lines represent individual hibernacula where temperatures were monitored in rooms occupied by these species.

rooms near hibernacula entrances with more variable temperatures (average winter variance of 5° C) that reached as low as -5° C. Following the arrival of WNS, little brown myotis, tri-colored bats, and big brown bats each preferred temperatures that were 2–5° C lower than had been documented prior to the arrival of the disease. Shifts in microclimate

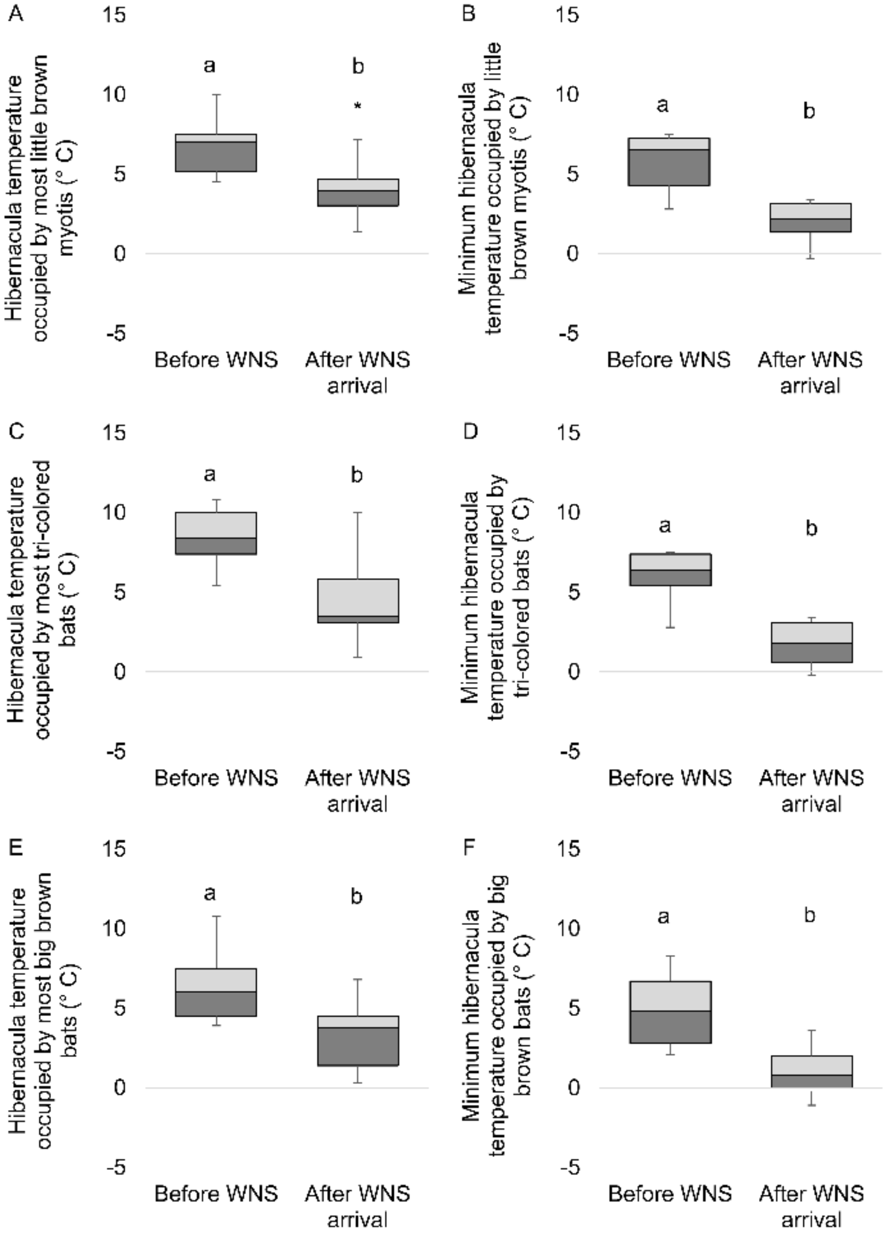

Figure 5. The majority of little brown myotis (panel A), tri-colored bats (panel C), and big brown bats (panel E) are found in areas within hibernacula with colder median temperatures since the arrival of white-nose syndrome in Pennsylvania. The minimum temperatures occupied by any little brown myotis (panel B), tri-colored bats (panel D), and big brown bats (panel F) at the same sites were also colder since the arrival of white-nose syndrome. Within each panel, box and whiskers not sharing common letters are significantly different ($P < 0.05$), and observations outside 1.5 times the interquartile range are denoted with an *.

use recorded here went beyond short-term behavioral change (e.g., via movements closer to the hibernaculum entrance; Turner et al. 2011) that had previously been documented during mass mortality events; rather these shifts were evident years after *Pd* had spread throughout the state and mass mortality had occurred at most sites.

Studies of hibernacula microclimates have shown that, even during the pre-WNS period, several species showed preference for relatively cold habitats (Nagel and Nagel 1991, Tuttle and Kennedy 2002, but see Brack 2007, Kurta and Smith 2014). This preference for temperatures close to, but above, 0° C is often discussed in the context of winter energy balance because torpid bats will expend the least energy while conforming to air temperatures just above freezing (Buck and Barnes 2000, Geiser 2004). The selective pressures faced by bats during hibernation are more complex than the need to conserve energy during torpor, however, as prolonged use of torpor incurs physiological and ecological costs that bats are believed to partially offset through arousals (Humphries et al. 2003). Prior to WNS, Boyles and colleagues (2007) hypothesized that little brown myotis balance the need to conserve energy reserves through the use of torpor yet reduce the costs of using torpor by selecting warmer hibernacula when fat reserves are relatively high and colder hibernacula when they are low. Another, not mutually exclusive hypothesis is that stable microclimates can sometimes be more important than low average or minimum temperatures. Our study includes one hibernaculum, Allegheny Mountain, that was both relatively warm (minimum temperature of 9.5° C overall, and an unusually high average of 10.6° C at Indiana myotis roosts) and extremely stable (variance of <0.002° C over the winter period), and that contained large pre-WNS bat colonies (>2,000 little brown myotis, >800 tri-colored bats, and >100 Indiana myotis). The large numbers of bats using this warm, thermally stable hibernaculum are consistent with both hypotheses for the pre-WNS period. However, since the onset of WNS, colonies of little brown myotis and tri-colored bats have almost completed disappeared at this hibernaculum (only 1 individual of each species was observed in the 2015 winter survey), which could suggest that the balance between competing energetic and physiological pressures on bats during hibernation may have shifted since the onset of WNS. Any such shift in Indiana myotis is less obvious, however, as some bats remain there (15 individuals were observed in 2015).

Kurta and Smith (2014) found that water vapor pressure deficit, the difference between saturation vapor pressure and actual vapor pressure, was a significant predictor of winter use of caves and mines in Michigan. The utility of vapor pressure deficit is that it represents a measure of evaporative water loss in hibernating bats, assuming bats are conforming to ambient conditions during this time (Kurta and Smith 2014). While this is often true, each bat likely experiences variable temperatures and water vapor pressures due to the effects of clustering, thus we chose to simply report water vapor pressure. While water vapor pressure differed between the sites used by relatively larger and smaller populations of little brown myotis, the air in all hibernacula was close to saturation vapor pressure, and the main driver of differences in water vapor pressure between the 2 hibernacula types was likely temperature differences. It is difficult to know the relative risk of evaporative water loss in these habitats without knowing the precise body temperatures of bats during hibernation, but the high water vapor pressure at all sites mirrors the findings of Kurta and Smith (2014).

Big brown bats and eastern small-footed myotis arrive at hibernacula late in the winter and leave early in the spring, spending a relatively short time in hibernacula compared to other Pennsylvania bat species (Turner et al. 2011). Previous studies have found big brown bats hibernating at temperatures close to 10° C, reflecting the ability of the species to tolerate higher winter metabolic rates and more frequent arousals from hibernation (Brack 2007, Whitaker and Gummer 1992), consistent with our data showing big brown bats choosing microclimates with temperatures as high as 10.8° C. However, our temperature logger data show that, on average, big brown bats hibernate in colder areas of hibernacula than little brown myotis and tri-colored bats, suggesting that big brown bats occupy a larger range of temperatures than other species.

The arrival of WNS in Pennsylvania during the winter of 2008–09 caused dramatic declines in bat populations (Turner et al. 2011, Ingersoll et al. 2016). Field and laboratory studies have shown that mortality of little brown myotis afflicted with WNS is greatest in relatively warm hibernacula, and that bats hibernating in relatively cold areas have higher chances of surviving the disease (Langwig et al. 2012, Johnson et al. 2014, Hayman et al. 2016). Thus, species such as the big brown bat and eastern small-footed myotis, which often hibernate in the coldest areas of caves, may be benefiting from microclimate conditions that decrease the growth rate of *Pd*. Populations of all hibernating species, however, may have since gained a survival advantage by shifting their preference towards colder microclimates. This conclusion is supported by a recent study of a little brown myotis in a WNS-affected cave in New York (Lilley et al. 2016); although all the bats in this study were infected with *Pd*, the average temperature of hibernating bats was 2° C, and there was no visible fungal growth or signs of mortality.

Thus, there is evidence that several species of hibernating bats have changed their winter behaviors in response to WNS, providing some hope that mortality could decline. These shifts in microclimate selection may allow these species to persist in the region despite widespread contamination of hibernacula by *Pd*. It is also possible that wildlife agencies could protect or enhance hibernacula using this information to enhance the survival of bats in WNS-affected hibernacula.

Implications for the Management of Hibernacula

The microclimate conditions preferred by hibernating bat species have been difficult to describe, since hibernacula with a wide range of conditions are used by each species (Reeder and Moore 2013; this study). As reported here, a range of conditions can even be used within individual hibernacula, where members of a species will occupy both relatively warmer and colder areas of the same site (Fig. 5). Despite this variation, several generalizations can be made. Prior to the arrival of WNS, little brown myotis formed larger winter populations in hibernacula with relatively colder microclimates (Fig. 2), although the species did not necessarily occupy the coldest sections of those hibernacula. Although little brown myotis have become rare in Pennsylvania, we report evidence that the species is now found in colder areas within previously used hibernacula, occupying areas that were predominantly used by big brown bats and eastern small-footed myotis prior to the onset of WNS. We found a similar trend in the habitat use of tri-colored bats.

This increased importance of colder microclimates within existing hibernacula in the presence of *Pd* presents an opportunity for management agencies to enhance winter habitat for bats. An important initial step in this regard would be placing higher conservation priority on sites containing microclimates with mid-winter temperatures of 0–5° C, followed by sites with mid-winter temperatures of 5–7° C. Sites with existing populations of hibernating bats that maintain warmer mid-winter temperatures should also be targeted for conservation, and possibly management actions that manipulate environmental conditions. Manipulation of temperatures within hibernacula has a long history in bat habitat management, frequently with the goal of trapping cold air within the site (Tuttle and Kennedy 2002, but see Kurta and Smith 2014). Our data demonstrate an increased importance for these kinds of management actions. One way microclimates can be modified is by creating mounds of earth that prevent cold air close to the hibernacula floor from migrating to the entrance of the site and leaving. At Casparis Mine, one of the hibernacula with the most variable temperatures, cold air escapes by moving down the slope and out of the entrance to the mine. In June of 2005, the PGC created several earthen mounds along this downhill slope to prevent the colder air from escaping. The number of little brown myotis at Casparis subsequently increased from 4 individuals in January 2005 to 70 in January 2006, and a high of 170 in January 2008. In addition to the number of little brown myotis increasing, total species richness at the site increased from 2 bat species in January 2005 to 4 species in 2006 and a high of 6 species in 2007. These changes in bat counts and species richness indicate a temporal concordance between immigration to the site and habitat modification. Although little brown myotis subsequently declined precipitously at this site with the arrival of WNS, these earlier changes suggest that habitat modification can modify mine microclimates in ways that attract hibernating bats. We further postulate that, if carefully planned, management efforts such as this can also effectively be used to provide microclimates associated with higher survival probabilities for remnant bat populations imperiled by WNS.

For decades, gating caves and mines has arguably been the most valuable management strategy for protecting bat populations while they are vulnerable to human disturbance during hibernation, since such disturbances may lead to energetically costly arousals (Tuttle 1977, Richter et al. 1993, Crimmins et al. 2014). With the energy-depleting effects of WNS (Reeder et al. 2012, Warnecke et al. 2012), gating hibernacula to protect bats from additional disturbance may prove even more valuable at sites with survivors. However, WNS represents a different kind of threat to bat populations, due to the high mortality rates as well as the fact that *Pd* can be introduced to new hibernacula by both people and bats. Furthermore, *Pd* remains in caves long after bat populations have declined as a result of WNS (Lorch et al. 2013), and the sediment inside hibernacula becomes a reservoir for the pathogen (Smyth et al. 2013, Hoyt et al. 2015). Because *Pd* is present in all of Pennsylvania's important hibernacula (Turner et al. 2016), new management actions need to go beyond gating sites to protect over-wintering bat populations. In Pennsylvania, prioritizing existing hibernacula with low, stable winter temperatures for conservation, and prioritizing those that may be manipulated to create the preferred microclimates of hibernating bats since the arrival of WNS are 2 strategies to increase overwinter survival of bats in the presence of *Pd*. These methods may also be useful elsewhere in the WNS-affected region of North America and regions at risk for future WNS spread, and provide management entities with new options for enhancing habitat for bats during this conservation crisis. Prioritizing sites with a diversity of temperature and humidity regimes or manipulating sites to provide preferred microclimates may also be important strategies when managing for the entire bat community of the state. Lastly, working with partners and landowners to restrict access to important hibernacula and minimize disturbance to hibernating bats should remain a high priority.

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