Leveraging Abandoned Railroad Tunnels for Bat Conservation

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16. Abstract

This report summarizes a study on abandoned tunnels' importance to bat populations in Ohio. We located 50 tunnels and surveyed 40 of these for one or more winter season. Little brown myotis, big brown bats, and tricolored bats were the most commonly observed species, with varying preferences for tunnel features. Key predictors of bat use such as tunnel length, temperature range, and minimum temperatures were identified, with different patterns across species. Trends analysis showed growing little brown myotis colonies, stable tricolored and big brown bat populations, and low rates of infection with *Pseudogymnoascus destructans*, the fungus that causes white-nose syndrome. Recommendations focus on improving hibernacula conditions, reducing temperature variability, and prioritizing tunnel enhancements based on species importance and habitat suitability. This report highlights the critical role of abandoned tunnels as winter habitats for Ohio's bats and provides actionable conservation recommendations.

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1.0 Problem Statement and Introduction

The fungal disease white-nose syndrome (WNS) poses an extreme, pervasive threat to several North American bat species (Cheng et al. 2021). WNS is caused by a fungus, *Pseudogymnoascus destructans*, that is widespread in Europe and Asia but only introduced to North America by humans around 2006 (Blehert et al. 2009, Puechmaille et al. 2011, Warnecke et al. 2012). *Pseudogymnoascus destructans* can grow on a variety of substrates, including, but not limited to, bats (Lorch et al. 2013; Reynolds and Barton 2014). The survival and reproduction of the fungus is therefore not tied to the survival of any host species, potentially resulting in host extirpations (Kuris et al. 2014). Declines of bat species susceptible to WNS, such as the little brown myotis (*Myotis lucifugus*), northern myotis (*M. septentrionalis*), and tricolored bat (*Perimyotis subflavus*) reach nearly 100% in some areas (Cheng et al. 2021; Vanderwolf and McAlpine 2021), supporting concerns of regional extirpations and extinctions (Frick et al. 2010).

Despite the dramatic declines in bat populations in the years immediately following arrival of WNS, there are notable hibernacula and summer colonies with stable or increasing populations (Dobony and Johnson 2018; Turner et al. 2022). For several species, survival in the WNS-endemic regions of North America includes a shift in winter habitat selection, with trends varying across species. For example, while little brown myotis populations in Pennsylvania select colder habitats after the arrival of WNS (Johnson et al. 2016; Turner et al. 2022) populations of Indiana myotis (*M. sodalis*) exhibit higher survival in warmer habitats (Grimaudo et al. 2023). Understanding these changes in winter habitat selection are essential to supporting the recovery of bat populations threatened by WNS and may require changes to local conservation strategies and priorities (Sewall et al. 2016). This is certainly true in Ohio, where WNS is considered endemic and several bat species susceptible to WNS have been extirpated from several caves and mines with important winter colonies pre-WNS (Johnson and Johnson 2024).

During the winter of 2016–2017, a winter colony of little brown myotis was found hibernating in a railroad tunnel in Harrison County, Ohio. Unfortunately, bats were later found dead in the tunnel, having been shot with an air gun (Chris Staron, Ohio Department of Transportation, *personal communication*). This event suggested an opportunity for conservation and management through conventional means such as use of bat friendly gates, but also indicated that a larger inquiry into the importance of similar habitats in Ohio. **Our goal, therefore, was to locate as many potential tunnel hibernacula as possible and to provide recommendations for managing these sites in the interest of bat conservation**. We proposed to the following objectives to meet this goal:

1. Locate abandoned tunnels throughout Ohio
2. Inventory of tunnels that serve as suitable hibernacula
3. Describe the habitat features that are important predictors of bat use of tunnels
4. Determine trends of growth or decline in winter colonies hibernating in inventoried tunnels
5. Assess the health of bats hibernating in tunnels
6. Provide recommendations for enhancing used hibernacula to better protect the bats
7. Provide recommendations for making unused tunnels more suitable for bats
8. Matrix indicating prioritization of which tunnels to pursue for future enhancement for improved use by bats
9. Quantify the conservation value of these tunnels to bat conservation in Ohio

2.0 Research Background

Before the onset of WNS, abandoned limestone mines housed the largest documented populations of tricolored bats, little brown myotis, northern myotis, and Indiana myotis in Ohio (Brack, 2007). A recent survey of nearly all historically important caves and limestone mines, with the notable exception of a critical hibernacula in western Ohio, found that these habitats were no longer used by significant numbers of little brown myotis, northern myotis, or tricolored bats (Johnson and Johnson 2024). Since WNS has become endemic, abandoned railroad tunnels have larger populations of little brown myotis and tricolored bats than caves or mines, making these sites critical for the Ohio's bat population and important sites for conservation (Johnson and Johnson 2024). Inactive coal mines have also served as hibernacula (Lacki and Bookhout, 1983), but they have been deemed too hazardous for human survey in Ohio, leading to many closures or seals for public safety, and uncertainty about the number of bats that use these sites and their long-term viability.

In addition to some railroad tunnels providing critical habitat for bats in Ohio, unused tunnels are ideal sites for management efforts aimed at mitigating the effects of WNS. For example, Turner and colleagues (2022) manipulated the air flow within an abandoned railroad tunnel in Pennsylvania to change the temperature within the hibernaculum and improve overwinter survival in hibernating bats. This management strategy leverages knowledge of growth rates in *Pseudogymnoascus destructans*, which is greatest at 12–15.8 °C (Verant et al. 2012). Turner and colleagues (2022) therefore sought to cool sites to slow the growth of fungus and improve conditions for bats. Cooling in this study was limited to 6 sites but resulted in significant increases in little brown myotis and tricolored bats. Turner and colleagues (2022) noted, however, that cooling is unlikely to be an appropriate management action in all settings. For example, where hibernacula temperatures are already cold or variable, stabilizing underground temperatures may be more appropriate. Furthermore, the railroad tunnels modified by Turner and colleagues (2022) were not important hibernacula prior to WNS and the authors advocated against manipulating temperatures at sites that were important to bats pre-WNS in the event that bats adapt to the disease and return to those habitats.

Perspectives on managing hibernacula to mitigate the effects of WNS are not without controversy. Boyles and colleagues (2023) believe that science and practice of managing hibernacula is not sufficient, and that the work of Turner and colleagues (2022) has the potential to create ecological traps, habitats that appear attractive but are inferior. This argument is based on models of energetic and non-energetic physiological costs of hibernation, which increase at temperatures close to freezing and at temperatures that are highly variable (Boyles et al. 2020). Boyles and colleagues (2023) believe that counts of hibernating bats alone are insufficient to demonstrate quality of habitat and that caution should be taken when basing management decisions on winter counts. Managers seeking to counter this critique must therefore collect additional data to support their actions. In the region of North America where WNS has become endemic, non-lethal measures of *Pseudogymnoascus destructans* infection (Turner et al. 2014) and measures of body condition such as mass can be used to confirm that overwintering bats are not simply attracted to risky habitats. If measured during winter, body mass provides a clear indication of whether WNS has depleted animals' fat reserves, an important part of WNS pathophysiology (Verant et al. 2014), or if bats are otherwise in poor health. Measuring *Pseudogymnoascus destructans* infection alongside body mass offers further clarification on the health of bats in the weeks before spring emergence, and by proxy, a confirmation of whether bats in these hibernacula are at high risk.

Despite varying perspectives on how habitats can be managed, the importance of railroad tunnels as potential sites for conservation is increasingly acknowledged (Loeb and Winters, 2022; Turner et al., 2022; Johnson and Johnson 2024). Although these sites may offer opportunities for recovery from WNS, they are not without their own conservation threats. As the mortality event at the tunnel in Harrison County, Ohio, showed, abandoned tunnels are vulnerable to human disturbance. Tunnel locations are often posted on the internet and are destinations for adventure-seekers. Although tunnels are often known to the public, state and federal wildlife agencies have not been aware of tunnel locations or use by bats. Interest in identifying tunnel locations is currently stymied by the lack of any database containing their locations in Ohio. An additional conservation risk occurs when organizations seeking to convert abandoned rail lines into bicycle trails pass through tunnels. Ohio has several rail-trail tunnels, such as the National Road Bikeway, and on August 20, 2021, two additional tunnels were acquired by way of a Governor's deed (originally proposed as part of Section 753.10(B)(1) of House Bill 74). Both sites were hibernacula used by little brown myotis and tricolored bats, including the fourth largest winter colony of little browns at the time (Johnson and Johnson, *in press*). Unfortunately, at least one of these two tunnels has been purposed for future conversion to trails (proposed trail maps currently available at https://www.railstotrails.org/greatamericanrailtrail/route/).

Thus, there is an urgent need to locate abandoned railroad tunnels in Ohio, survey them for hibernating bats, determine the characteristics that influence the size of winter colonies, and evaluate the health of bats in these sites. With these data in hand, management agencies can embark on conservation strategies specific to bats in the region to best aid in the recovery of populations threatened by WNS.

3.0 Research Approach

We developed specific methods for each of our research objectives as outlined below.

Objective 1: Locate abandoned tunnels throughout Ohio

We located abandoned railroad tunnels by searching the internet, reviewing old topographical maps, and examining digital elevation models (DEMs). We targeted internet websites and message boards devoted to local Ohio history, train and tunnel enthusiasts, and adventuring. For our map review, we downloaded U.S. Geological Survey topographical maps and examined the path of all marked rail lines in Ohio. We noted the location of all marked tunnels as well as instances where rail lines appeared to pass through hills and would have required a tunnel for train passage. Similarly, we used QGIS to examine the paths of rail lines in relation to DEMs. This allowed for a second opportunity to discover evidence of tunnels in the form of rail lines passing through rapid changes in elevation, which would not be possible without at one point creating a tunnel.

Objective 2: Inventory of tunnels that serve as suitable hibernacula

We attempted to visit all potential tunnels identified during completion of Objective 1. We first determined the landowner using tax maps hosted by county websites in Ohio and attempted to call or visit those landowners to acquire permission to survey. When permission was granted, we visited tunnels from mid-December through mid-March to survey the site for hibernating bats. This survey period is typical for bats in Ohio (Johnson and Johnson 2024). We surveyed each tunnel during ≥1 winter over four winters beginning in December 2020 and ending during March 2024. We also used data from previous research (Johnson and Johnson 2024). We counted all bats seen during surveys, identifying each animal to species.

Objective 3: Describe the habitat features that are important predictors of bat use

We collected a suite of habitat variables inside and outside of each tunnel to determine which features best drive the number of hibernating bats. We deployed temperature dataloggers (HOBO models MX2301A and MX2202, Onset Computer Corporation, Bourne, MA), recorded the internal materials that composed the tunnel walls (concrete, stone, brick, or a combination of these materials), tunnel height, and tunnel length in all surveyed tunnels. Temperature dataloggers were placed in 1-3 locations inside each tunnel and programmed to record temperature at 15-min intervals. At a minimum, dataloggers were placed in the middle of each tunnel. Dataloggers were also placed near the entrances of tunnels that served as important hibernacula to capture more data on the variation within each site. Outside of each tunnel, we measured the height, width, and total area of each entrance. We also used QGIS to collect landscape-scale data that may influence bat use. Together, these variables represented the full suite of features that we assessed as possible features that could be used to predict bat use of tunnels (Appendix 1).

Because we collected a relatively large number of predictor variables (18), we analyzed these data in two-phases. Within each phase we analyzed data for the three species most commonly found in tunnels (little brown myotis, tricolored bats, and big brown bats) separately. Phase 1 consisted of using machine learning to limit the set of potential predictor variables from 18 to further analysis. Phase 2 consisted of creating a suite of statistical models predicting bat counts for each species and using model selection to determine which model most parsimoniously explained counts. For phase 1, we used the r package randomForestSRC to determine 1) how well the full suite of variables predicted counts of each species, and 2) which variables had the greatest contribution to the overall model. For phase 2, we took the top 5 variables identified for each species in phase 1 and constructed a suite of generalized linear mixed models (GLMMs) using the package glmmtmb. For each model, the number of bats was the response variable, and a negative binomial distribution was used. We used all possible combinations of the top 5 predictor variables unless variables were correlated with each other. Pairs of variables did not occur in the same model if they had pairwise correlations of >0.3. We then ranked the candidate models within Akaike's Information Criterion adjusted for small sample sizes.

Objective 4: Determine trends of growth or decline in winter colonies

We quantified trends in bat counts over time using statistical and qualitative approaches. For our statistical approach, we included *Winter of* as a predictor variable in our phase 1 and phase 2 analyses for each species. *Winter of* was defined as a winter hibernation season, lasting December—March, and named from the calendar year when the winter began. Values ranged from 2017—2023. We included *Winter of* in the phase 2 approach regardless of the importance of that variable in phase 1 to allow us to determine if winter counts increased or decreased over time. We also used a simple, qualitative approach to displaying trends over time: plotting the raw data of counts over time. This simple approach allows for visualization of the number of bats in important hibernacula regardless of whether we found that winter had a statistical effect on counts or not.

Objective 5: Assess the health of bats hibernating in tunnels

We sought to assess the health of hibernating bats using both a passive study of periodic arousals from hibernation and an active study of *Pseudogymnoascus destructans* infection in bat wings. The frequency of periodic arousals from hibernation is an important element of WNS mortality; bats dying of WNS experience more frequent arousals than they can sustain, whereas surviving populations have less frequent arousals (Lilley et al. 2016, Reeder et al. 2012, Warnecke et al. 2012). We therefore sought to determine if arousal rates were similar to populations dying from WNS or similar to populations surviving despite the presence of *Pseudogymnoascus destructans*. To conduct this part of the study, we deployed an array of 14 thermal infrared cameras (model A65, Teledyne FLIR, Wilsonville, OR) deployed within one tunnel in Harrison County. We chose this site because it was the largest known hibernaculum at the time; therefore, it presented the greatest promise of success. The camera array was deployed during autumn, prior to bats migrating into the site, and focused on regions of the tunnel wall where bats have been found hibernating. Cameras were powered by solar panels and a battery bank at the entrance of the tunnel. Ethernet cables stemming from the battery bank provided power to the cameras and brought video feed back to Microsoft Surface tablets to be recorded. Camera placement was changed slightly each year depending on the previous year's success. Cameras were programmed to record video whenever temperatures in the field of view were ≥15 C°.

We also quantified the severity of WNS in a subset of bats hibernating three tunnels using UV photos. Whereas quantifying rates of periodic arousals was intended to provide an assessment of health based on a well-known symptom of WNS, UV photos will directly quantify infection (Turner et al. 2014). We took photos by entering these hibernacula late in hibernation, during the first week of March, and capturing bats by removing them from the tunnel walls by hand. Bats were placed in paper bags, weighed, and photographed. Each bat was photographed while the wings were trans-illuminated by UV light as described by (Turner et al. 2014). This process takes approximately two minutes per bat and consisted of stretching each wing over a custom-made UV light box (WayTooCool, LLC., Glendale, AZ). Following UV photography, bats were placed back on the tunnel wall where they were collected. We later determined the percentage of the wing infected by *Pseudogymnoascus destructans* as described by Sewall and colleagues (2023).

Objective 6: Provide recommendations for enhancing used hibernacula to better protect the bats

The observation of bats shot in the Harrison County tunnel provided clear evidence that tunnels used by bats as hibernating bats require management to protect the wildlife resource. However, gating is only one of several possible ways to enhance habitats where bats are present (Johnson et al. 2021). To provide recommendations for enhancing currently used habitat, we considered the results of the analysis conducted in Objective 3 alongside any other important observations we made in the field. Specifically, we documented human disturbance to habitats using trail cameras placed outside of important hibernacula and used our professional experience to identify any potential threats to the site (e.g., flooding potential, collapse, and signs of human use such as graffiti, fires, etc.).

Objective 7: Provide recommendations for making unused tunnels more suitable for bats

We used the results from the analyses conducted in Objective 3 to provide recommendations for how to make unused tunnels more suitable. This was done by examining the variables in the GLMM model that best explained bat counts and determining if the variables included in that model could be modified though management actions. Should a variable in the model represent something land managers could manipulate at unused tunnels, we then examined the statistical relationship between that variable and bat counts. Finally, we provided guidance on how to modify existing sites to more closely resemble suitable conditions based on our experience managing hibernacula (Turner et al. 2022).

Objective 8: Prioritize which tunnels to pursue for future enhancement

We used structured decision-making with a three-step process to prioritize tunnels for management. In step one, we created a matrix containing a tiered ranking of the **current** conservation value of each site based upon the bat species already present and the vulnerability of those species to WNS. Each tunnel was therefore placed into one of three tiers. Tier 1: sites currently used by one or more state or federally endangered species during hibernation. Tier 2: sites currently occupied by bats, but not any state or federally endangered species. And Tier 3: sites not used by any bats during winter. To allow for the next two steps in the decision-making process, sites were scored for conservation priority. Tier 1 tunnels received a score of 10 points, sites in Tier 2 received 5 points, and sites in Tier 3 received 0 points.

In step two, we amended the scores of each site based on how easily they can be managed to resemble suitable conditions (Objective 3) or otherwise enhanced (Objective 6). Specifically, we examined the current conditions in each tunnel in relation to habitat variables included in the GLMMs that best explained counts of little brown myotis and tricolored bats (n = 3). For each of the two habitat variables in those models, each tunnel was awarded 2 points if conditions were already suitable, 2 points if conditions could be easily modified to be suitable, and 0 points if conditions could not be modified. Sites with a total score ≥10 were placed in Tier 1, sites with scores ≥5 were placed in Tier 2, and all other sites were placed in Tier 3. Thus, sites originally placed in Tiers 2 and 3 could be uplisted to Tiers 1 and 2, respectively, if they could be easily managed. At this stage, any site initially identified as Tier 1 remained in Tier 1.

In step three, we considered potential barriers to management based on land ownership. In this step, we subtracted 5 points from sites located on properties where the landowner declined permission for our surveys at any point during the study. As a result, the presence of a known barrier to management resulted in sites being downgraded exactly one tier. This three-step process resulted in a ranking matrix where each site was designated as being either a high priority from management (Tier 1), a moderate priority (Tier 2), or a low priority (Tier 3).

Objective 9: Quantify the conservation value of these tunnels to bat conservation in Ohio

We quantified the overall conservation value of abandoned railroad tunnels to bat conservation in Ohio by comparing the number of little brown myotis and tricolored bats occupying tunnels to the numbers occupying caves and mines (Johnson and Johnson 2024). We did not quantify the potential conservation value of tunnels that are currently not suitable for bats, but which could be modified to increase their suitability.

4.0 Research Conclusions and Findings

The results for Objectives 1–5, which focused on locating, surveying, and assessing the importance of tunnels to bats, are outlined below. Objectives 6-9, which focused on management recommendations, are discussed in Section 5.

Objective 1: Locate abandoned tunnels throughout Ohio

We located 49 railroad tunnels and 1 subway tunnel that are either currently abandoned or have been abandoned and since collapsed or repurposed to some other use. The locations of these tunnels are provided in supplemental appendices to protect the locations of sensitive wildlife. Because sensitive wildlife inhabit many of these tunnels, locations are not included in this report.

Objective 2: Inventory of tunnels that serve as suitable hibernacula

Of the 50 tunnels located, we were able to confirm the current status (collapsed versus intact) of 48 tunnels during one or more field surveys. We were unable to survey one of the remaining 2 tunnels due to a lack of landowner permission. The status of the last tunnel could not be confirmed because we believe it to be underwater. Of the 48 tunnels we were able to survey in the field, 4 (8%) had collapsed, 1 (2%) had been filled in, and 1 (2%) site had either collapsed, buried, or filled. Further, 1 tunnel (2%) was converted into a tourist site and 1 tunnel (2%) was being used as a landfill. Thus, 8 of the 48 tunnels could not be surveyed for bats, reducing our sample to 40 sites. We surveyed the 40 remaining tunnels a total of 166 times over the course of four winters. Of the tunnels we surveyed, 26 (65%) were occupied by big brown bats (*Eptesicus fuscus*) during one or more winter, 12 (30%) were occupied by little brown myotis, 11 (28%) were occupied by tricolored bats, 2 (5%) were occupied by northern myotis, and 1 site (3%) was occupied by silver-haired bats (*Lasionycteris noctivagans*). Numbers of big brown bats ranged 1–146, little brown myotis 1–806, tricolored bats 1–49, and no more than 1 northern myotis was ever observed in a single tunnel. Full survey results are provided in Appendix 2.

Objective 3: Describe the habitat features that are important predictors of bat use

The full suite of habitat measurements was successfully collected from 32 of the 40 surveyed tunnels. We were unable to gather temperature data from the remaining 8 sites because dataloggers were repeatedly stolen during the winter. Temperature variables were subsequently treated as missing data for these sites in the random forest analyses.

A total of 10,000 decision trees were built in the random forest model for little brown myotis, with a terminal node size of 6. On average, each tree had 10.8 terminal nodes. At each split, the algorithm considered 6 variables out of the total of 17 available predictors. The trees were built using the 'swor' resampling method (**s**ampling **w**ith**o**ut **r**eplacement), with a resample size of 97. The splitting rule was based on mean squared error (MSE) with 10 random split points. The out-of-bag (OOB) R-squared value, a measure of model performance, was found to be 0.752, indicating that the model explains 75.2% of the variance in the response variable. The requested performance error, as measured by mean squared error, was approximately 2817. These results suggest that the random forest model demonstrates **good** predictive performance in explaining the relationship between the predictor variables and counts. The most important variables in this analysis were tunnel length (hereafter, *Length*), the range of temperatures during winter (T_{range}), minimum winter temperature (T_{min}), total portal area (*TotPortalArea*), and average winter temperature (T_{avg}) (Figure 1).

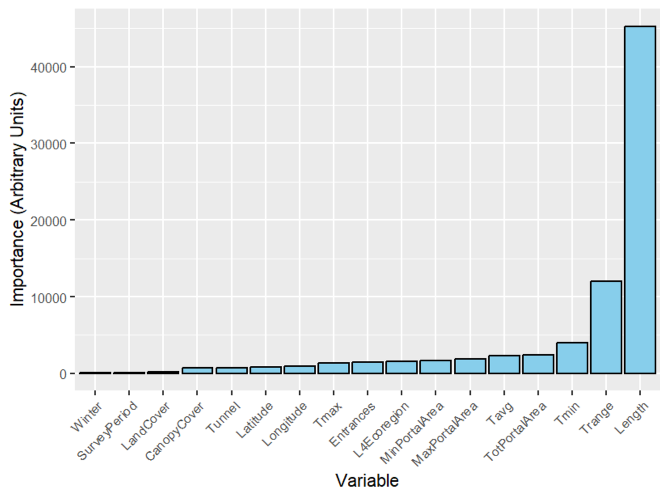


Figure 1. Importance of predictor variables included in the analysis of little brown myotis counts in abandoned railroad tunnels. The units on the y-axis are arbitrary, representing the decrease in prediction accuracy when the values of a given predictor variable are randomly permuted.

A total of 10,000 decision trees were built in the random forest model for tricolored bats, with a terminal node size of 5. On average, each tree had 9.7 terminal nodes. At each split, the algorithm considered 6 variables out of the total of 17 available predictors. The trees were built using the 'swor' resampling method, with a resample size of 97. The splitting rule was based on MSE with 10 random split points. The OOB R-squared value was 0.617, indicating that the model explains 61.7% of the variance in the tricolored bat counts. The performance error was 21.38. These results suggest that the random forest model demonstrates **moderate** predictive performance in explaining the relationship between the predictor variables and the response variable. The most important variables were *Length*, T_{range}, T_{min}, the maximum winter temperature (T_{max}), and *TotPortalArea* (Figure 2).

A total of 10,000 trees were built in the random forest model for big brown bats, with a terminal node size of 5. On average, each tree had 17.7 terminal nodes. At each split, the algorithm considered 6 variables out of the total of 17 available predictors. The trees were built using the 'swor' resampling method, with a resample size of 97. The splitting rule was based on MSE with 10 random split points. The OOB R-squared value was found to be 0.787, indicating that the model explains approximately 78.7% of the variance in big brown bat counts. The requested performance error was approximately 148.9. These results suggest that the random forest model demonstrates **good** predictive performance. The most important variables in this analysis were T_{max}, *Latitude*, *Longitude*, the tunnel (*Tunnel*), and T_{range} (Figure 3).

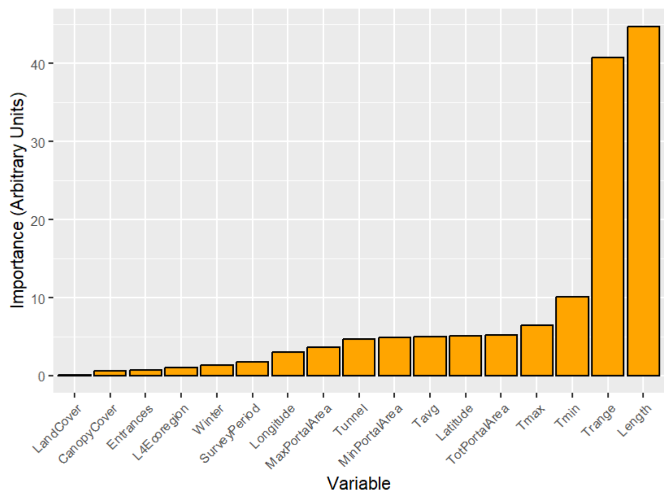


Figure 2. Importance of predictor variables included in the analysis of tricolored bat counts in abandoned railroad tunnels. The units on the y-axis are arbitrary, representing the decrease in prediction accuracy when the values of a given predictor variable are randomly permuted.

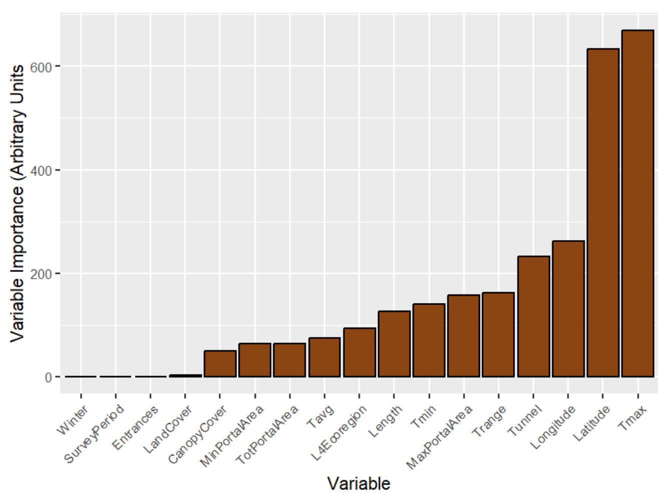


Figure 3. Importance of predictor variables included in the analysis of big brown bat counts in abandoned railroad tunnels. The units on the y-axis are arbitrary, representing the decrease in prediction accuracy when the values of a given predictor variable are randomly permuted.

All of the temperature variables (T_{min}, T_{max}, T_{range}, and T_{avg}) were correlated with each other and with *TotPortalArea* (pairwise correlations > 0.6). As a result, only one of these variables were ever used in a single GLMM model. A GLMM with *Length*, T_{range}, and *Winter of* best predicted counts of little brown myotis (*Length* = 1.8 ± 5.0, $p < 0.001$; T_{range} = -1.9 ± 7.1, $p < 0.001$; *Winter of* = 3.0 ± 4.4, $p = 0.007$; conditional pseudo-R2 = 0.68) (Figure 4). One additional model also had a ΔAICc score <2 and should therefore be considered a plausible alternative to the top model. This model was identical to the top model except that it included T_{min} instead of T_{range} (*Length* = 1.8 ± 5.2, $p < 0.001$; T_{min} = 2.0 ± 8.4, $p = 0.02$; *Winter of* = 3.0 ± 4.3, $p < 0.001$; conditional pseudo-R2 = 0.67) (Figure 5). In these models, **counts of little brown myotis were larger over time, in the longest tunnels, in tunnels with the smallest amount of temperature variation during winter, and in tunnels with higher minimum temperatures.** T_{range} and T_{max} were strongly correlated and it is therefore impossible to disentangle the effects of the two variables on little brown myotis (Figure 6). However, *Length* and T_{range} were only weakly correlated (pairwise correlation = 0.25), as long tunnels had variable temperatures when the entrances were open.

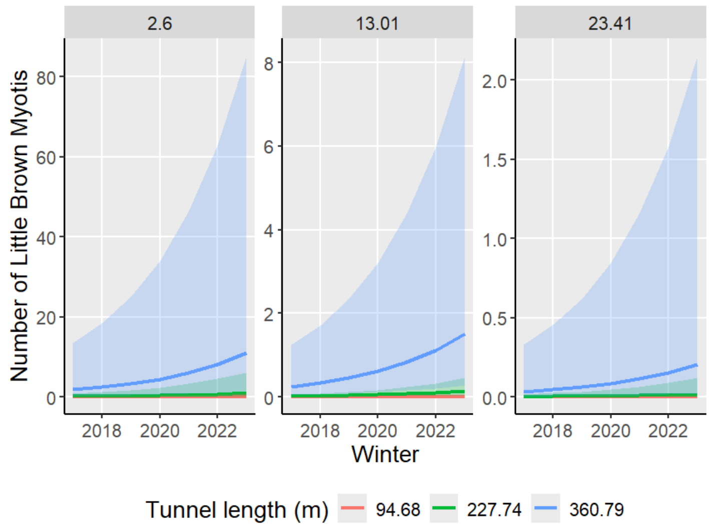


Figure 4. Winter counts of little brown myotis were greater in longer tunnels, in tunnels with a smaller range of winter temperatures (top facets based on quartiles in the data), and increased over time. Lines represent predicted values derived from the top generalized linear mixed model surrounded by 95% confidence intervals.

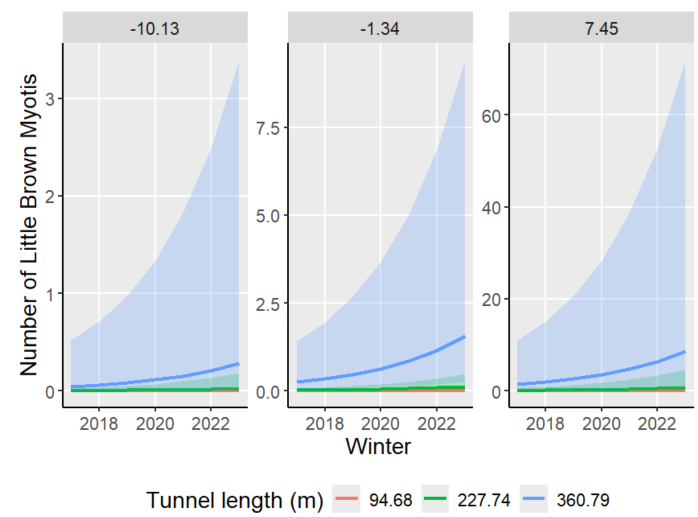


Figure 5. Winter counts of little brown myotis were greater in longer tunnels, in tunnels with higher minimum winter temperatures (top facets based on quartiles in the data), and increased over time. Lines represent predicted values derived from a competing generalized linear mixed model surrounded by 95% confidence intervals.

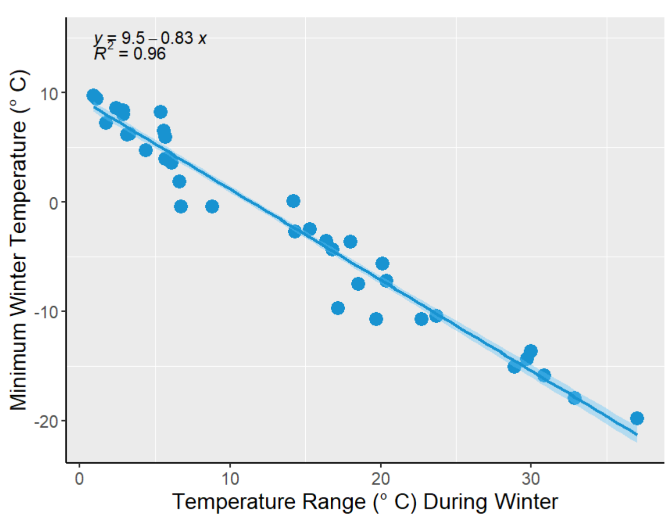


Figure 6. Minimum winter temperature within tunnels was negatively correlated with range of temperatures recorded during that time, with thermally stable sites also being the only tunnels with winter temperatures that did not fall below freezing.

A GLMM with T_{range} and *Winter of* best predicted counts of tricolored bats (T_{range} = -0.43 ± 0.087, $p < 0.001$; *Winter of* = 0.32 ± 0.087, $p < 0.001$; conditional pseudo-R2 = 0.64) (Figure 7). One other model had ΔAICc scores < 2. This model also included T_{range} and *Winter of*, but also included *Length* (T_{range} = -0.40 ± 0.83, $p < 0.001$; *Winter of* = 0.33 ± 0.86, $p <$ 0.001; *Length* = 0.004 ± 0.003, $p = 0.18$;conditional pseudo-R2 = 0.64). The latter model therefore included an additional parameter with a *p*-value of 0.18. Because the inclusion of *Length* did increase the log-liklihood score of the model and was more complex than the top model, the simpler model was superior. That model showed that **counts of tricolored bats were greatest in tunnels with the smallest amount of temperature variation during winter, and that counts increased over time.**

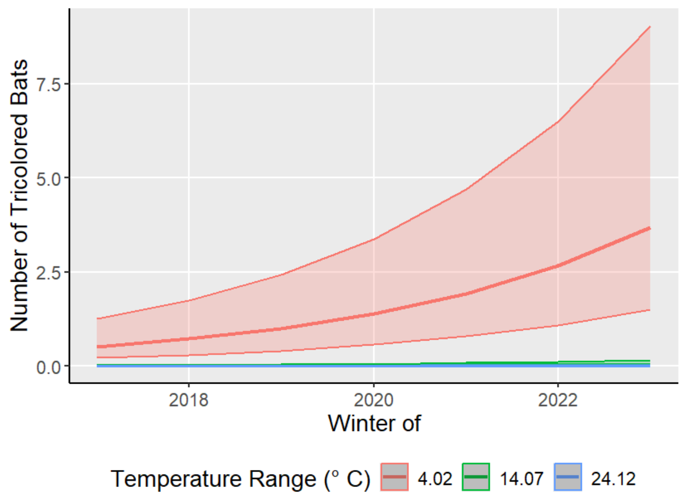


Figure 7. Winter counts of tricolored bats were greater in tunnels with a smaller range of winter temperatures and increased over time. Lines represent predicted values derived from the top generalized linear mixed model surrounded by 95% confidence intervals.

The GLMM that most parsimoniously predicted counts of big brown bats contained T_{range} and *Winter of* (T_{range} = 0.03 ± 0.04, $p = 0.019$; *Winter of* = 0.18 ± 0.08, $p = 0.017$; conditional pseudo-R2 = 0.55) (Figure 8). No other model had a ΔAICc score < 2 (Appendix 3). The top model indicates that **counts of big brown bats were higher in tunnels with a greater range of temperatures during winter and increased in tunnels over time**.

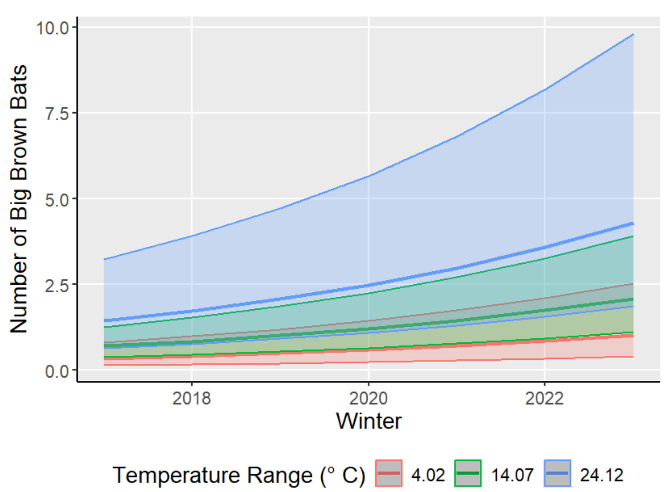


Figure 8. Winter counts of big brown bats were greater in tunnels with lower minimum temperatures. Lines represent predicted values derived from the top generalized linear mixed model surrounded by 95% confidence intervals.

Overall, the results from this Objective show that the three bat species most often observed hibernating in abandoned railroad tunnels in Ohio exhibit different patterns of winter habitat selection. While big brown bats were found in greater numbers in sites with a wide range of temperatures during winter, tricolored bats and little brown myotis were found in larger numbers in more thermally stable environments. As a result, tunnels with larger winter colonies of big brown bats were found in tunnels where both original entrances remained unmodified whereas little brown myotis and tricolored bats were found in sites where the entrances had been sealed and later partially re-opened (Figure 9). These small openings restricted the movement of air in and out of the tunnel, decreasing temperature variability and making the sites more suitable for imperiled bats. Although *TotPortalArea* was not in the top models predicting bat counts (Figure 10), it was correlated with T_{range} and provides a starting point for future efforts to modify tunnels to make them more suitable for imperiled bats.

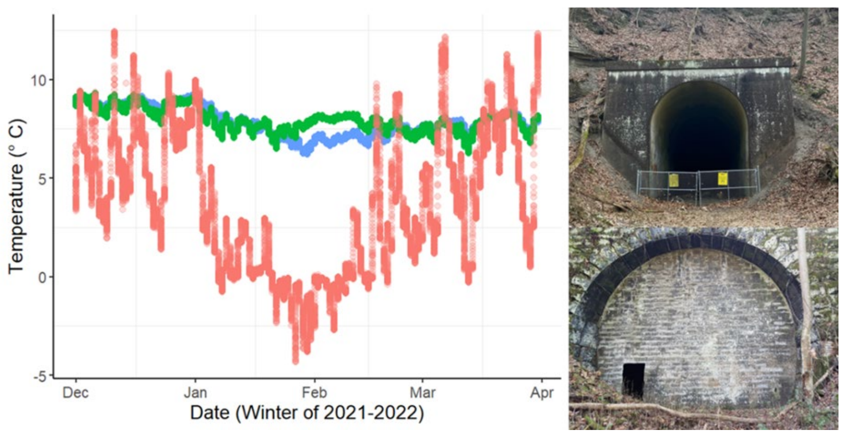


Figure 9. Winter temperature within three abandoned railroad tunnel hibernacula in Ohio (left), including two occupied by tricolored bats and little brown myotis (shown with blue and green circles) and one used only by big brown bats (red circles). Top right: an example of an abandoned tunnel occupied by only big brown bats (top) and a tunnel occupied by tricolored bats and little brown myotis (bottom right).

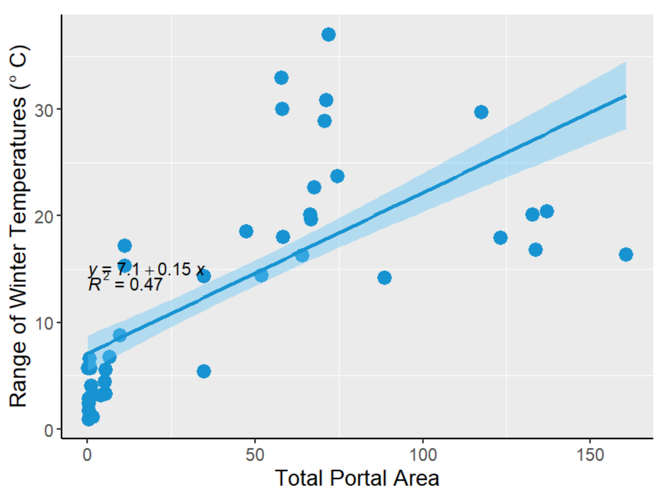
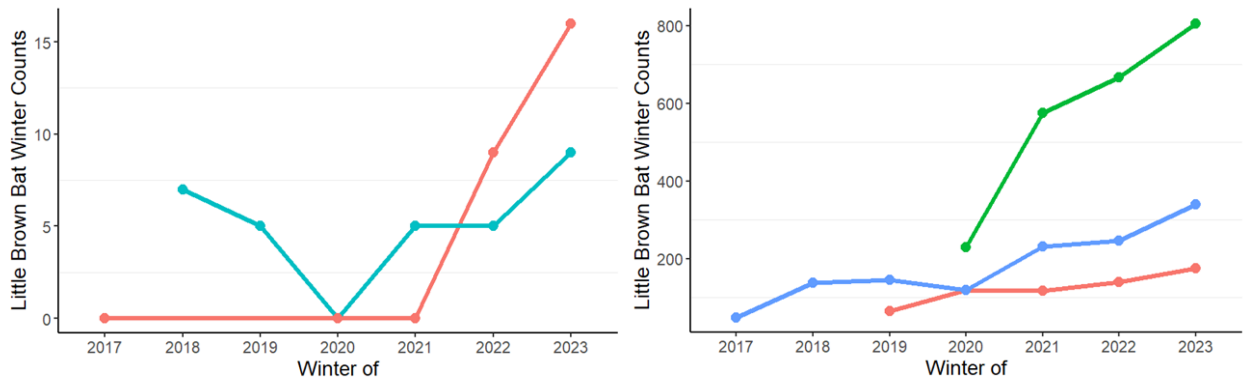


Figure 10. The range of winter temperatures within tunnels was positively correlated with the total area of the portals into these habitats.

Objective 4: Determine trends of growth or decline in winter colonies

The GLMM analyses of all three species found a significant, positive effect of *Winter of* on winter bat counts, meaning that **winter colonies of each species grew over time when controlling for the T_{range} and *Length***. These statistical results are confirmed in the qualitative assessment of counts of these three species. Little brown myotis counts at hibernacula with >1 bat, and that we were able to survey over multiple winters (*n* = 5), increased dramatically over time (Figures 11 and 12).

Figure 11. Counts of little brown myotis at the five most important railroad tunnel hibernacula found in Ohio (site names are omitted to protect sensitive wildlife) during this study. Sites with >15 bats are presented on the right panel to prevent obscuring trends at sites with smaller populations (left panel).



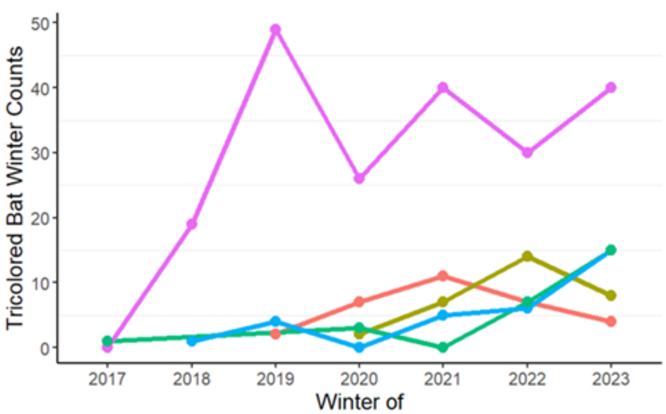


Figure 12. Winter counts of tricolored bats at the five most important railroad tunnel hibernacula in Ohio (site names are omitted to protect sensitive wildlife).

Objective 5: Assess the health of bats hibernating in tunnels

Our assessment of bat health met with mixed results. We took UV-illuminated photographs of 144 little brown myotis at 3 tunnels since the winter of 2020. With the exception of 5 bats (3%), the percentage of the wing determined to be infected with *Pseudogymnoascus destructans* was found to be <10% of the wing (1.9% ± 2.7 SD), with many animals exhibiting <1% fluorescence (Figure 13). These numbers are markedly lower than observed when WNS was causing high mortality throughout the northeastern United States (Turner et al. 2014) and demonstrate that these bats are less infected than populations several years ago.

Our infrared camera array met with less success. Contrary to our expectations, bats exhibited little fidelity to regions within the tunnel for roosting. Thus, although we recorded hundreds of videos of hibernating and active bats, these videos almost always contained bats arousing from hibernation, taking flight, and never returning to the camera's field of view. Thus, we were unable to use these recordings to assess arousal rates or bat health. However, we did record instances of bats emerging from cracks in the tunnel walls that led to spaces that researchers could not survey. While not the original intention of our work, this finding provides the important insight that **our surveys underestimate that true number of bats hibernating in these locations**.

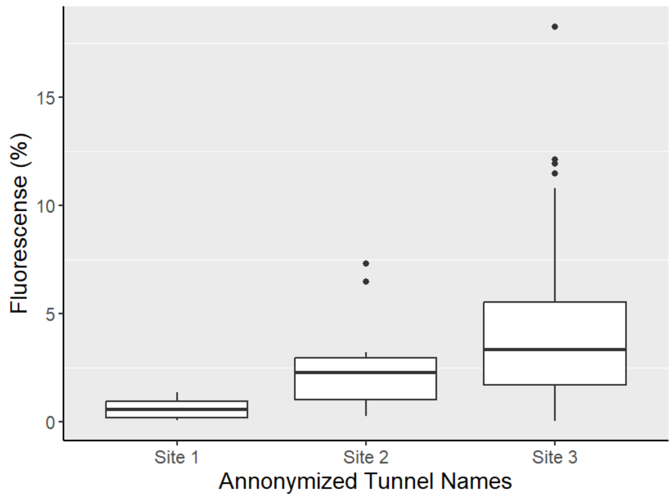


Figure 13. *Pseudogymnoascus destructans* infection in bats hibernating in three Ohio tunnels. Locations are omitted to protect sensitive wildlife.

5.0 Recommendations for Implementations

The findings detailed above demonstrate that tunnels are important hibernacula for bats in Ohio, and that bats overwintering in these sites are in good health at the end of winter despite the presence of *Pseudogymnoascus destructans*. Our recommendations for leveraging these tunnels for bat conservation, based on the results above, are detailed below.

Objective 6: Provide recommendations for enhancing used hibernacula to better protect the bats

There are currently 15 abandoned tunnels where we have documented use by 1 or more state listed species (little brown myotis) or federally listed (northern myotis) or proposed (tricolored bat) species. Recommendations for improving conditions for bats at these locations are provided in Appendix 3. Although an analysis of the effect of gating tunnels was not a formal objective of this study, the trends in winter counts before and after gating the Harrison County tunnel where little brown bats were shot in 2017 clearly demonstrate the potential benefits of gating (Appendix 4).

Objective 7: Provide recommendations for making unused tunnels more suitable for bats

The results from Objective 3 found 1 habitat feature that can be managed at tunnels: T_{range}. Fortunately for managers, this variable had a similar effect on counts of both little brown myotis and tricolored bats: **as T_{range} declines, winter counts increase** (Figure 14). Thus, we recommend reducing temperature variability during winter at unused and little-used tunnels to improve habitat quality for little brown myotis and tricolored bats. Because counts of both species were greatest at sites with <5 °C variability in winter, we recommend this as a target condition. Reducing total portal area, which would decrease flow of air into and out of the tunnels, is likely the easiest way to decrease the range of temperatures experienced within a tunnel during winter. Unfortunately, it is not clear from our data what this area should be, total portal area was not perfectly correlated with T_{range}. However, we recommend using the conditions at the 3 largest hibernacula in Ohio as target conditions. Notably, these tunnels also include large bodies of water. While the presence of water was not an important predictor variable for bat counts, water has a stabilizing effect on air temperature, and we recommend creating or maintaining water within unused tunnels to promote bat use.

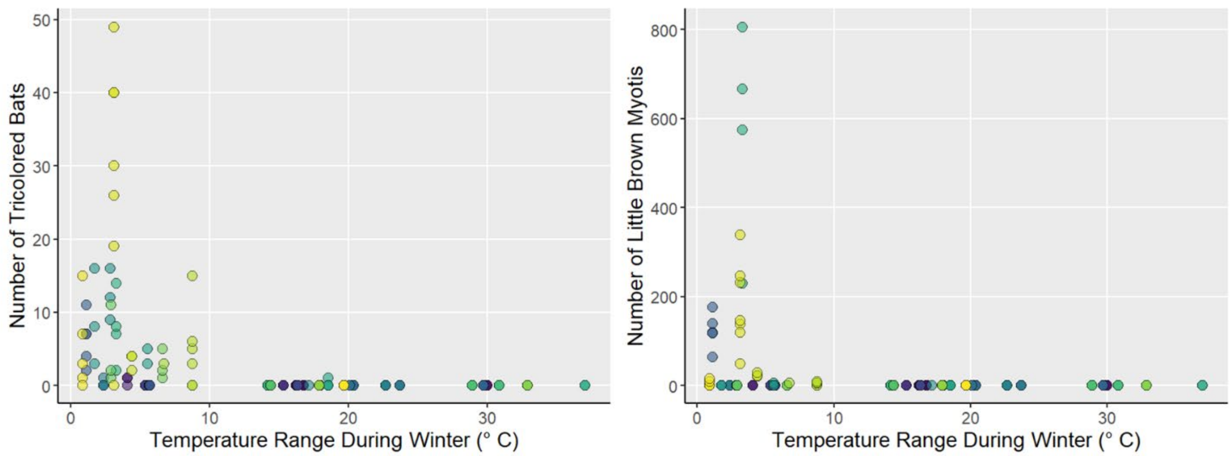


Figure 14. Counts of hibernating tricolored bats (left panel) and little brown myotis (right panel) were greatest in tunnels where the range of winter temperatures was <5 ˚C (right panel). Counts from the same tunnel will appear shown in the same colors and appear stacked over the same temperature on the x-axis.

Objective 8: Prioritize which tunnels to pursue for future enhancement

Results of a structured decision-making process are shown below. Sites are prioritized by tier, but not within each tier.

Table 1. Tier 1 (high priority) tunnels for bat conservation.

Tunnel†	Species Present	Length (m)	Temperature Range (˚ C)	Comments
Tunnel 5	Big brown, tricolored	278	4.1	Max count of 1 tricolored; unclear why more bats are not present based on conditions
Tunnel 6	Big brown, little brown, silver-haired	256	18	Max count of little brown myotis was 1; large numbers of non-listed species
Tunnel 13	Little brown, northern, tricolored	412	1.1	Currently an important hibernaculum; needs gating
Tunnel 14	Little brown, big brown	412	29.7	Max little brown myotis count of 1
Tunnel 17				Length and relative ease for stabilizing temperature
Tunnel 22	Tricolored	143	18.5	Max count of tricolored bat count of 1

Tunnel	Species Present	Length (m)	Temperature Range (˚ C)	Comments
Tunnel 26	Tricolored, little brown	481	3.3	Currently an important hibernaculum that needs erosion control
Tunnel 35	Tricolored, little brown, big brown	382	8.8	Currently an important hibernaculum
Tunnel 37	Little brown, northern, tricolored, big brown	436	3.1	Currently an important hibernaculum that needs monitoring against vandalism
Tunnel 38	Little brown, tricolored	307	0.9	Rising populations of little brown myotis and tricolored bats, along with suitable temperatures
Tunnel 39				Length and relative ease for stabilizing temperature

†Tunnels were assigned random numbers to differentiate sites and to protect the locations of vulnerable wildlife.

Table 2. Tier 2 (medium priority) tunnels for bat conservation.

Site†	Species Present	Length (m)	Temperature Range (˚ C)	Comments
Tunnel 1	Big brown	145	16.3	
Tunnel 2	Big brown	71	Undetermined	Currently an unpaved public trial
Tunnel 3	Big brown	142	16.8	
Tunnel 8	Big brown	118	14.3	
Tunnel 9	Big brown	275	16.4	
Tunnel 10	Big brown	98	Undetermined	Tunnel is unsealed and likely has highly variable temperatures
Tunnel 11	Big brown	143	5.7	
Tunnel 12	Big brown	99	5.7	
Tunnel 15	None	82	20.4	Public ownership and nearby to large little brown myotis maternity roost
Tunnel 18	Tricolored	265	2.4	Heavy disturbance. Owner plans to erect a bat-friendly gate with no entrance
Tunnel 21	Little brown, tricolored	Undetermined	5.5	Structurally unsound tunnel that likely floods to the ceiling some years. Potential hazard to bats.
Tunnel 25	Big brown	61	37.0	

Site	Species Present	Length (m)	Temperature Range (˚ C)	Comments
Tunnel 27	Big brown	319	14.2	New landowner has renovated tunnel to exclude bats
Tunnel 28	Little brown, big brown	36.5	30.1	Max little brown myotis count of 1
Tunnel 30	Big brown	220	28.9	
Tunnel 31	Big brown	30.4	14.4	
Tunnel 32	Big brown	222	32.9	
Tunnel 33	Little brown, big brown	78.6	17.9	Max little brown myotis count of 1
Tunnel 34	Little brown, tricolored	140	6.7	Landowner is hostile towards bats
Tunnel 36	Little brown, tricolored, big brown	285	4.4	Landowner is hostile towards bats

†Tunnels were assigned random numbers to differentiate sites and to protect the locations of vulnerable wildlife.

Table 3. Tier 3 (low priority) tunnels for bat conservation.

Site†	Species Present	Length (m)	Temperature Range (˚ C)	Comment
Anonymous Tunnel 4	None	36.6	30.0	Popular hiking trail with locals
Anonymous Tunnel 7	None	96.0	15.3	
Anonymous Tunnel 16	None	101	20.1	Being demolished
Anonymous Tunnel 19	None	108	22.7	Hiking trail, no use by bats
Anonymous Tunnel 20	None	238	17.2	Structurally unsound
Anonymous Tunnel 23	None	73.4	Undetermined	Hiking trail
Anonymous Tunnel 24	None	157	Undetermined	Bike trail
Anonymous Tunnel 29	None	75.3	Undetermined	
Anonymous Tunnel 40	None	Undetermined	Undetermined	

†Tunnels were assigned random numbers to differentiate sites and to protect the locations of vulnerable wildlife.

Objective 9: Quantify the conservation value of these tunnels to bat conservation in Ohio

Although data on winter habitat use by bats in Ohio pre-WNS are scarce, a recent study found that tricolored bats were absent from 11 of 16 (69%) sites with pre-WNS records and little brown myotis were absent from all sites with pre-WNS survey records (n = 5), including 1 mine with a historical population of >1,000 bats (Johnson and Johnson 2024). No more than 2 little brown myotis were documented at other caves and mines surveyed by Johnson and Johnson (2024). It is important to note that the Prebble County mine has not been surveyed for years, since the relationship between the landowner and the Ohio Division of Wildlife deteriorated, and it is unclear if that site remains an important hibernaculum. In the absence of data on Prebble county and other unknown sites, however; one can only conclude that abandoned railroad tunnels are the most important winter habitats for little brown myotis that we currently know about in Ohio.

The relative importance of abandoned railroad tunnels for tricolored bats in Ohio is less clear, although these habitats are undoubtedly important. The largest winter colony of tricolored bats observed in a statewide survey of caves and mines in Ohio was 8 (Johnson and Johnson and Johnson 2024), compared to 49 in a Harrison County railroad tunnel. Similar to little brown myotis, those results are further confirmed in the present study. However, tricolored bats inhabit non-traditional hibernacula in Ohio (Johnson et al. 2024) and the presence of this species across the landscape is likely underestimated. Thus, although tricolored bats seem less reliant on abandoned tunnels than little brown myotis, these habitats are where most tricolored bats have been found in Ohio in recent years.

Conclusions

We present the most extensive assessment of abandoned railroad tunnels as winter habitat for bats to date. We found that most abandoned tunnels provide winter habitat for one of more of Ohio's bat species, with big brown bats being the most frequently observed. To a lesser, but still significant extent, abandoned railroad tunnels were used by the imperiled little brown myotis, northern myotis, and tricolored bat. While these bats are infected by the fungus that causes WNS while hibernating in tunnels, they are not severely afflicted and winter populations have grown over time, especially at gated sites. Beyond gating, our results suggest that simple modifications to tunnels can improve habitat for bats and contribute to the recovery of populations decimated by WNS.

In addition to our recommendations for management, we suggest several opportunities for follow-up studies that could further aid bat conservation. First, we suggest investigating the use of these tunnels by northern myotis. Although we observed few northern myotis during our surveys, these bats were captured during spring and fall harp trap surveys at several tunnels (J. Johnson, unpublished data). We suggest that these bats hibernate behind the walls of the tunnel, out of sight during winter surveys. While a precise count may not be required for conservation efforts, inability to accurately assess the value of these tunnels may lead to reduced incentives, such as mitigation credits, for protecting and enhancing these habitats.

We also suggest that there are currently unknown hibernacula within Ohio, and that finding these locations is a high priority effort for conservation. We observed counts of little brown myotis double between consecutive winters at several tunnels, something implausible to happen by summer reproduction alone. This observation suggests that some bats changed their hibernacula between winters. As none of the sites we monitored experienced declining counts during the course of our study, this suggests bats switched from a hibernaculum unknown to our research team, quite likely in Ohio. We suggest radio-tracking bats during the fall swarm to locate these additional hibernacula.

Finally, it is essential to recognize that effective bat conservation requires stewardship of summer and transitional habitats as well as hibernacula. For example, winter colonies hibernating in abandoned railroad tunnels will only continue to increase if these populations successfully reproduce on their summer range. We recommend that attempts track bats migrating from the tunnels to their summer roosts so that partnerships can be forged with agencies or private landowners that own and manage those maternity roosts. We also encourage identification of areas used by foraging bats during the fall swarm. Populations of little brown myotis that persist despite WNS have larger fat reserves that bats that died from WNS when the disease first erupted in the northeastern United States (Cheng et al. 2019). It therefore stands to reason that foraging habitat during the fall swarm, where bats feed and prepare for hibernation, are critical for the habitat. We recommend telemetry studies of nocturnal habitat use and movements of imperiled bats that swarm at abandoned railroad tunnels.

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Appendices

Appendix 1. Full list of variables used in the random forest analysis of abandoned railroad tunnels.

Variable Name Used in Report	Variable Description and Relevance
Tunnel	The name of an abandoned railroad tunnel, capturing the potential for site-specific effects.
Winter of	As a variable, *Winter of* was coded with the year they began during. For example, winter 2023 was the period beginning in December 2023 and ending in March 2024.
Survey period	A categorical representation of when during the winter the survey occurred. Specifically, we divided the winter season in two-week periods beginning with December 1 and ending with March 31.
Longitude	The east-west location of the tunnel in decimal degrees.
Latitude	The north-south location of the tunnel in decimal degrees.
Land cover	A categorical description of the landcover surrounding the entrance to a tunnel, as determined using the National Landcover Dataset.
Canopy cover	The percentage of canopy cover surround this entrance to the tunnel, as determined using the National Landcover Dataset.
L4 Ecoregion	The level 4 ecoregion where a tunnel was located; a potential landscape scale effect.
Entrances	The number of open entrances to the tunnel, which influences airflow into the site and, therefore, temperature.
MaxPortalArea	The size of the largest entrance to the tunnel, which influences airflow into the site and, therefore, temperature.
MinPortalArea	The size of the smallest entrance to the tunnel, which influences airflow into the site and, therefore, temperature.
TotalPortalArea	The total area of open entrances into a tunnel, which influences airflow into the site and, therefore, temperature.
Length	The length of the tunnel, in meters.
T_{range}	The range of temperatures recorded inside a tunnel from December 1-March 31, in degrees Celsius.
T_{min}	The minimum temperature recorded inside a tunnel from December 1-March 31, in degrees Celsius.
T_{max}	The maximum temperature recorded inside a tunnel from December 1-March 31, in degrees Celsius.
T_{avg}	The average temperature recorded inside a tunnel from December 1-March 31, in degrees Celsius.
Material	

Appendix 2. Full survey results.

Tunnel†	Survey Date	Big Brown Bats	Little Brown Myotis	Tricolored Bats	Silver-haired Bats	Northern Myotis
Tunnel 1	01/06/22	5	0	0	0	0
Tunnel 1	01/07/23	4	0	0	0	0
Tunnel 2	12/23/21	0	0	0	0	0
Tunnel 2	03/11/23	3	0	0	0	0
Tunnel 3	02/02/21	0	0	0	0	0
Tunnel 3	12/14/21	1	0	0	0	0
Tunnel 3	03/12/23	11	0	0	0	0
Tunnel 4	02/03/21	14	0	0	0	0
Tunnel 4	01/10/22	16	0	0	0	0
Tunnel 4	12/12/22	14	0	0	0	0
Tunnel 4	03/12/23	23	0	0	0	0
Tunnel 5	12/20/21	3	0	1	0	0
Tunnel 5	02/10/23	3	0	0	0	0
Tunnel 6	03/04/21	41	0	0	0	0
Tunnel 6	01/08/22	146	1	0	0	0
Tunnel 6	12/27/22	127	0	0	0	0
Tunnel 6	02/01/23	104	0	0	1	0
Tunnel 7	02/01/21	0	0	0	0	0
Tunnel 7	01/14/22	0	0	0	0	0
Tunnel 7	02/11/23	0	0	0	0	0
Tunnel 8	02/26/20	2	0	0	0	0
Tunnel 8	02/23/21	6	0	0	0	0
Tunnel 8	01/14/22	1	0	0	0	0
Tunnel 8	02/11/23	0	0	0	0	0
Tunnel 8	02/26/20	4	0	0	0	0
Tunnel 8	02/23/21	1	0	0	0	0
Tunnel 8	01/14/22	1	0	0	0	0
Tunnel 8	02/11/23	0	0	0	0	0
Tunnel 9	02/01/21	3	0	0	0	0
Tunnel 9	01/14/22	4	0	0	0	0
Tunnel 9	02/11/23	2	0	0	0	0
Tunnel 10	03/11/23	0	0	0	0	0
Tunnel 11	02/26/21	0	0	0	0	0
Tunnel 11	12/27/21	1	0	0	0	0
Tunnel 11	01/08/23	2	0	0	0	0
Tunnel 12	01/31/21	6	0	0	0	0
Tunnel 12	01/12/22	6	0	0	0	0

Tunnel 12	12/17/22	3	0	0	0	0
Tunnel 13	12/22/20	0	119	7	0	0
Tunnel 13	03/17/20	0	64	2	0	0
Tunnel 13	03/10/22	0	118	11	0	0
Tunnel 13	03/04/23	0	139	7	0	1
Tunnel 13	03/04/24	0	176	4	0	0
Tunnel 14	03/17/20	0	0	0	0	0
Tunnel 14	12/22/20	0	0	0	0	0
Tunnel 14	03/10/22	3	0	0	0	0
Tunnel 14	03/04/23	3	1	0	0	0
Tunnel 15	02/26/21	0	0	0	0	0
Tunnel 15	12/12/21	0	0	0	0	0
Tunnel 15	1/32/2023	0	0	0	0	0
Tunnel 16	01/12/21	0	0	0	0	0
Tunnel 16	12/18/21	0	0	0	0	0
Tunnel 17	01/30/21	2	0	0	0	0
Tunnel 17	03/06/22	0	0	0	0	0
Tunnel 17	01/08/23	3	0	0	0	0
Tunnel 18	02/27/20	0	0	0	0	0
Tunnel 18	03/04/21	0	0	0	0	0
Tunnel 18	01/05/22	0	0	1	0	0
Tunnel 18	12/12/22	0	0	0	0	0
Tunnel 19	01/16/20	2	0	0	0	0
Tunnel 19	12/08/20	0	0	0	0	0
Tunnel 19	12/15/21	1	0	0	0	0
Tunnel 19	03/07/23	2	0	0	0	0
Tunnel 20	01/15/22	4	0	0	0	0
Tunnel 21	02/04/21	0	6	3	0	0
Tunnel 21	02/01/22	0	0	5	0	0
Tunnel 22	02/02/21	0	0	1	0	0
Tunnel 22	12/14/21	0	0	0	0	0
Tunnel 22	01/17/23	0	0	0	0	0
Tunnel 23	12/08/20	0	0	0	0	0
Tunnel 23	12/15/21	0	0	0	0	0
Tunnel 23	03/07/23	0	0	0	0	0
Tunnel 24	12/18/21	0	0	0	0	0
Tunnel 24	03/12/23	0	0	0	0	0
Tunnel 25	02/12/21	0	0	0	0	0
Tunnel 25	03/14/22	11	0	0	0	0
Tunnel 26	02/28/21	0	230	2	0	0

Tunnel 26	12/21/21	0	575	7	0	0
Tunnel 26	12/15/22	0	667	14	0	0
Tunnel 26	12/21/23	0	806	8	0	0
Tunnel 27	01/09/20	13	0	0	0	0
Tunnel 27	12/17/21	5	0	0	0	0
Tunnel 27	03/10/23	10	0	0	0	0
Tunnel 28	12/21/21	15	0	0	0	0
Tunnel 28	01/10/23	32	1	0	0	0
Tunnel 29	12/21/21	0	0	0	0	0
Tunnel 30	02/12/21	3	0	0	0	0
Tunnel 30	12/22/21	3	0	0	0	0
Tunnel 30	02/07/23	0	0	0	0	0
Tunnel 31	01/09/22	1	0	0	0	0
Tunnel 31	03/07/23	5	0	0	0	0
Tunnel 32	02/12/21	6	0	0	0	0
Tunnel 32	01/10/22	10	0	0	0	0
Tunnel 32	01/31/23	3	0	0	0	0
Tunnel 33	02/02/21	1	0	0	0	0
Tunnel 33	12/18/21	0	0	0	0	0
Tunnel 33	01/17/23	1	1	0	0	0
Tunnel 34	03/07/18	0	0		0	0
Tunnel 34	12/22/20	0	5	3	0	0
Tunnel 35	03/05/21	47	0	0	0	0
Tunnel 35	03/04/22	81	5	5	0	0
Tunnel 35	12/20/22	45	5	3	0	0
Tunnel 35	03/04/23	60	5	6	0	0
Tunnel 35	03/02/24	36	9	15	0	0
Tunnel 36	02/27/19	0	20	2	0	0
Tunnel 36	03/05/20	1	22	4	0	0
Tunnel 36	03/05/21	4	28	4	0	0
Tunnel 37	12/19/17	0	49	0	0	0
Tunnel 37	02/27/19	0	138	19	0	0
Tunnel 37	03/05/20	1	146	49	0	0
Tunnel 37	03/05/21	2	119	26	0	0
Tunnel 37	03/04/22	16	232	40	0	0
Tunnel 37	03/04/23	0	247	30	0	0
Tunnel 37	03/02/24	0	339	40	0	1
Tunnel 38	03/07/18	0	0	1	0	0
Tunnel 38	12/22/20	0	0	3	0	0
Tunnel 38	12/12/21	0	0	0	0	0

Tunnel 38	02/22/23	0	9	7	0	0
Tunnel 38	02/29/24	0	16	15	0	0
Tunnel 39	03/17/20	0	0	0	0	0
Tunnel 39	02/10/21	5	0	0	0	0
Tunnel 39	12/27/21	0	0	0	0	0
Tunnel 39	01/08/23	10	0	0	0	0

[†]Tunnels were assigned random numbers to differentiate sites and to protect the locations of vulnerable wildlife.

Appendix 3. Recommendations for management at tunnels currently used by ≥1 state or federally listed bat species.

Site†	Tier	Recommended Action	Justification
Tunnel 5	1	Continue monitoring	Conditions appear suitable for bats, but no more than 1 tricolored bat has ever been observed. Bats simply may not have discovered this site as of yet.
Tunnel 6	1	Gate	Only 1 little brown myotis has ever been documented here, but a large population of big brown bats and some silver-haired bats have been observed. The habitat for these species should be preserved as-is.
Tunnel 13	1	Gate, manage vegetation	Currently among the most important sites in Ohio but is ungated. Additionally, recent tree clearing in the area has allowed for dense vegetation to grow around the entrance to the tunnel, which may prevent bats from entering and exiting the site over time. Erosion may also be a concern here.
Tunnel 14	1	Block entrances to reduce airflow, gate	Tunnel is longer than 300 m but is wide open on both ends, resulting in highly variable temperatures.
Tunnel 18	2	None	Current owner intends (and may have already) to build an entrance-less gate, making the site impossible to monitor and assess any conservation action.
Tunnel 22	1	Block entrances to reduce airflow, gate	Only 1 tricolored bat ever documented here. Site is open on both ends and has a wide range of temperature fluctuation
Tunnel 26	1	Stabilize banks from erosion, keep gate clear of debris eroding from hillside	Currently the largest hibernaculum for little brown myotis in Ohio, but the hillside surrounding the tunnel is eroding rapidly and will block the entrance to the tunnel in coming years.
Tunnel 21	1	None	This site appears dangerous to humans and bats, with the ceiling beginning to cave in near the entrance
Tunnel 28	2	Block entrances to reduce airflow, gate	Only 1 little brown bat has ever been documented here. Site is open at both ends.
Tunnel 33	2	Block entrances to reduce airflow, gate	Only 1 little brown bat has ever been documented here, and this is the second shortest tunnel in Ohio. Both ends are currently wide open and the internal temperatures are highly variable.
Tunnel 34	2	None	Ownership changed to a hostile landowner in 2021.

Site[†]	Tier	Recommended Action	Justification
Tunnel 35	1	None	Conditions were manipulated prior to the start of this study and populations of little brown myotis and tricolored bats seem to be slowly increasing
Tunnel 36	2	None	Ownership changed to a hostile landowner in 2021.
Tunnel 37	1	Continue monitoring	This site has had an increasing population of tricolored bats and little brown myotis since gating but has had persistent vandalism issues, including someone cutting through the gate
Tunnel 38	1	Continue monitoring, gate	This site has recently experienced a substantial increase in the number of little brown and tricolored bats

[†]Tunnels were assigned random numbers to differentiate sites and to protect the locations of vulnerable wildlife.

Appendix 4. The effect of gating Tunnel 37 (site name chosen randomly) on winter populations of little brown and tricolored bats.