THESIS

ELEVATION HETEROGENEITY AND THE SPREAD OF WHITE-NOSE SYNDROME IN BATS

Submitted by

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ABSTRACT

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White-nose syndrome (WNS) has been decimating bat populations throughout North America since its discovery in New York during the winter of 2006-2007. The fungus responsible for the disease, *Pseudogymnoascus destructans*, has since been confirmed as present in Washington, over 3,700 km from the epicenter. In 2012, a stochastic discrete-time dynamical system for WNS spread was developed on a spatially structured network and used to predict the spread of this wildlife epidemic. The model uses a variable for distance and two environmental variables (cave density and winter duration) to generate spread probabilities between counties of the contiguous United States. However, predictions from the 2012 model missed several recently infected counties due to the use of a cave density variable. Major cave formations are both less frequent and poorly documented in the western U.S. Furthermore, cave density may not serve as an accurate proxy for bat hibernacula across the country considering the use of crevice and cavity roosts in rock substrates west of the Great Plains. A Terrain Ruggedness Index (TRI) can thus be calculated from elevation data and used in place of cave density to quantify elevation heterogeneity and represent crevice-dwelling bat populations. Incorporating TRI into the network spread model would generate more accurate WNS presence predictions and aid in more effective management efforts to contain the spread of this deadly bat disease.

TABLE OF CONTENTS

Chapter 1

An Overview of White-Nose Syndrome in Bats

White-nose syndrome (WNS) is one of the most severe wildlife diseases ever documented, as evidenced by the magnitude, duration, and potential damage to North America's ecosystems [1, 2]. WNS was discovered near Albany, New York during the winter of 2006-2007 after mass mortality at Howe's Cave. The most noticeable physical indication of WNS is a white fungus on bats' muzzles or other exposed skin areas [3]. Since its initial documentation in New York, WNS has been confirmed in 32 U.S. states and 5 Canadian provinces. The causative fungus, *Pseudogymnoascus destructans*, has been found in two additional states without the symptoms or mortality associated with the disease [4]. Nine species have been documented with WNS: big brown bat (*Eptesicus fuscus*), eastern small-footed bat (*Myotis leibii*), gray bat (*Myotis grisescens*), Indiana bat (*Myotis sodalis*), little brown bat (*Myotis lucifugus*), northern long-eared bat (*Myotis septentrionalis*), tri-colored bat (*Perimyotis subflavus*), southeastern bat (*Myotis austroriparius*), and Yuma myotis (*Myotis yumanensis*). Additionally, the causative fungus of WNS has been isolated from seven other species: eastern red bat (*Lasiurus borealis*), silver-haired bat (*Lasionycteris noctivagans*), Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), Virginia big-eared bat (*Corynorhinus townsendii virginiarnus*), cave bat (*Myotis velifer*), Townsend's big-eared bat (*Corynorhinus townsendii*), and Mexican free-tailed bat (*Tadarida brasiliensis*). One of these species is threatened and three are endangered [4].

Pseudogymnoascus destructans, previously named *Geomyces destructans*, is the psychrophilic (cold-loving) fungus responsible for WNS that attacks the skin tissue of hibernating bats [1]. The fungus grows in temperatures below 20°C, making underground bat hibernacula and hibernating bats suitable long-term reservoirs of the pathogen [5, 6]. DNA evidence of this fungus has been found on bodies of bats displaying clinical signs of WNS and locations of WNS mortality [6]. Laboratory studies have also confirmed that *P. destructans* creates skin lesions consistent with WNS and causes death after two to three months of hibernation [5].

Genetic evidence indicates that *P. destructans* was introduced from Europe to North America through an index site in New York [5]. However, the fungus responsible for WNS and massive bat population declines in North America has not caused mass mortality in Europe [3]. Many theories have been postulated and researched to explain the discrepancy in population effects between the two continents. One theory claims the fungus is native to North America but recently became pathogenic [6]. Alternatively, if the fungus were indeed translocated from Europe, *P. destructans* may represent a novel pathogen for bat species in North America while European populations are resistant to the disease [3]. Wild bats in Europe display the skin lesions characteristic of *P. destructans*, but European populations do not experience the mass mortality from WNS. Nonetheless, a European isolate of *P. destructans* remains fatal to North American little brown bats [5]. The longevity of the *P. destructans* presence in European bat hibernacula implies that the fungus persists after establishment [3]. Isolates of the European fungus contain a large amount of genetic variation, whereas isolates of the fungus in North America contain no such genetic variation. This indicates that *P. destructans* is spreading clonally through North America [2].

1.1 The Biology of White-Nose Syndrome

Hibernating bats conserve energy by lowering their body temperatures and suppressing their metabolism, which allows them to live on stored fat for 6-8 months [6]. Throughout most of the winter, body temperatures remain in torpor (below 10 °C) and are intermittently aroused to euthermia (between 35 °C and 38 °C) [1]. This state of torpor is when bats are most susceptible to the physiological damage of *P. destructans* [6]. WNS alters arousal patterns which reduces fat reserves, as evidenced by emaciated bats found in late winter. In fact, one arousal for a little brown bat that is hibernating at 5°C consumes the same amount of energy as 67 days in torpor [1]. The physiological functions of wing membranes may also be disrupted by skin lesions from WNS, thus affecting the carefully maintained water balance of an affected bat [3]. Dehydration from the inability to maintain water balance could partially explain the increase

in arousal frequency. One of the most heavily affected North American bat species, the little brown bat, shows significantly higher rates of water loss than a hibernating congener of similar size from Europe. This discovery further evidences the theory that disrupted water balance triggers more frequent arousals, causing fat depletion and ultimately death [5].

Behavioral signs of WNS in wild bats include forearm tremors while crawling, altered sensory thresholds, flying during daylight hours, colliding with large stationary objects, and excessive thirst. Dehydrated bats may lick snow or fly over open water for excessive lengths of time. Dehydration or electrolyte depletion may explain these behavioral markers [3]. Bats exiting their hibernacula during winter could be escaping a fungal infestation and looking for more suitable conditions elsewhere, but this phenomenon ultimately remains unexplained [6].

Recovery from WNS is possible but is physiologically demanding. Captive and wild bats have exhibited the ability to overcome an infection and heal wing lesions after emerging from their winter hibernacula. Recovery during winter is less probable. The healing process requires euthermic body temperatures that bats are not equipped to maintain during hibernation [5]. Theoretically, a bat could survive WNS by arousing from hibernation and finding a warmer habitat or a food source to combat the metabolic costs of euthermic body temperatures [6]. Unfortunately, being able to surviving the *P. destructans* infection through winter hibernation may not be enough. Evidence now suggests that the immune response is delayed until spring arousal. If the infection is significant, the inflammatory cell response may fatally overwhelm the host rather than heal the site of infection [5].

Three factors must interact for successful disease occurrence and disease spread, as outlined by the epidemiologic triad. Disease requires a susceptible host (bats), a pathogen that can facilitate spread to the susceptible hosts (*P. destructans*), and an environment that allows for the existence and interaction of the host and the pathogen (hibernacula) [6]. The dynamics of WNS spread depend on these variables and their interactions. To gain a deeper knowledge of the spread within hibernacula and rate of disease progression, these interactions must be analyzed [3]. This analysis reveals the possibility that the difference in effects of *P. destructans* in

Europe and North America may not be dependent on the characteristics of the pathogen itself, but rather the host susceptibility and habitat characteristics [5].

Environmental variables and their effects on bat traits may explain the discrepancy between the broad pattern of infection and the restricted distribution of mass mortality [6]. A hibernating bat maintains body temperatures that satisfy the optimal growth range of *P. destructans*, the bat's immune function is diminished, and the bat's hibernaculum provides a moist setting for *P. destructans* to persist [6]. Fungal growth rate is likely governed by humidity and temperature of hibernating bats' skin surfaces since conidial fungi germination depends on surface moisture. A bat's optimal hibernaculum can vary in humidity and temperature based on the species. *P. destructans* isolates display maximum growth rates in a climate from 12 to 16°C, with some variation in growth rates between geographically distinct isolates. Field data has demonstrated that humidity levels inside hibernacula connect to WNS susceptibility and that warmer hibernacula experience higher mortality rates from WNS [5]. Bats in the northeast who have have the highest susceptibility to WNS mortality also consistently use hibernacula with the highest humidity levels [1]. Three of the species in North America less severely affected by WNS (*Myotis sodalis*, *Myotis leibii*, and *Eptesicus Fuscus*) are typically found in drier areas of their hibernacula. Conversely, three of the most heavily affected species (*Myotis lucifugus*, *Myotis septentrionalis*, and *Perimyotis subflavus*) tend to choose the wettest areas of their hibernacula and frequently have visible condensation on their fur [1]. Because fungal ecology is inherently tied to WNS, the effects of humidity and temperature within and among hibernacula is an integral factor in the severity and extent of WNS [5].

In addition to climates within hibernacula, WNS spread and mortality could also be linked to the winter climate outside hibernacula [6]. Higher chances of mortality exist in high elevation areas that are cold and seasonally variable [5]. The outside environment limits the survival tactics a bat can employ after hibernation is disrupted and possibly extends the hibernation period, when bats are most susceptible to WNS. Generally, bats in North America like hibernacula with high humidity and temperatures between 3 and 15 °C, but the climate outside of those

hibernacula vary greatly and can fluctuate throughout winter. In the northeastern Appalachian mountains, where WNS has reduced bat populations most dramatically, winter conditions are harsh compared to sites south or southwest, where bat populations found with *P. destructans* have not suffered mass mortalities from WNS [6].

Even without the presence of *P. destructans*, bats are limited to specific winter lengths to survive on their energy reserves [1]. The climate outside of a hibernaculum may directly affect those bats who occasionally emerge during winter. Bats living in areas with short winter lengths or higher winter temperatures have the opportunity to emerge during the hibernation period and recharge energy reserves with food or water. This allows them to undergo an earlier euthermic immune response and makes them more likely to survive a *P. destructans* infection [5]. Insects that compose most of the food supply for bats are typically not active in extreme cold and a bat that emerges during winter must expend more energy to stay warm at colder temperatures. Additionally, if the winter conditions outside of hibernacula are cold and dry, dehydrated bats with WNS may not be able to locate water to drink [6].

To quantitatively capture these host-environment interactions, Flory *et al.* (2012) used a species-environmental matching (SEM) model to statistically bind the presence of WNS mortality to environmental predictors. The sites their model predicted to be most susceptible to WNS mortality coincided with existing high mortality sites in the Appalachian region. The predictors with the most power were land use and cover type, precipitation frequency, and annual temperature range. Elevation heterogeneity and a barren land classification (less than 15% vegetation cover) increased the probability of WNS mortality, but this result may simply be due to the land type indicative of caves and abandoned mines rather than a biological characteristic of the disease [6].

1.2 Spread Mechanisms

Direct contact between bats and with *P. destructans* spores on roosting sites are the two main spread mechanisms. Bat species that hibernate in clusters, like the little brown bat, are partic-

ularly at risk for contracting WNS through direct bat contact. Male bats use hibernacula during the summer, thus they could be contracting the disease and spreading *P. destructans* to bats or other hibernacula during fall swarming events. In fact, some bat species swarm at one hibernaculum but hibernate elsewhere, implying that those bats know the location of other hibernaculum. Bats in the later stages of WNS prematurely emerge from their hibernacula and could reenter a new hibernaculum, spreading the fungus further. The amount of viable *P. destructans* spores on an infected bat that has survived the winter has not been determined, therefore the summer and fall spread likelihood is unknown. Human facilitated spread is also likely a major factor in the large jump dispersal events that have contributed to the rapid spread of WNS. *P. destructans* spores can latch onto clothing and gear and are durable enough to survive traveling to a new site. Anthropogenic transmission via contaminated gear could explain the initial introduction of *P. destructans* into North America [3].

P. destructans first appeared in the western United States on March 11th, 2016 in King County, Washington. A dying little brown bat was discovered with symptoms of WNS and tested positive for *P. destructans*. The fungal isolate from this bat is most likely from eastern North America and does not represent an independent introduction from Europe. The little brown bat was a member of a subspecies with a distribution restricted to western North America, *M. lucifugus alascensis*. These findings imply that the infected bat did not wander from an eastern habitat, rather it contracted the disease in the west. The distance from the closest known site at the time was unprecedented at over 2,100 km, from eastern Nebraska to King County. The total spread radius by 2016 was roughly 1,900 km from the introduction site. The jump to Washington is inconsistent with previously observed spread patterns and the mechanism by which WNS jumped is not clear. Anthropogenic spread could be a possible explanation for this event [2].

1.3 Research and Response Efforts

There are many confounding factors involved in the data collection necessary for WNS research. The temporal gap between detecting visible fungus and a mass mortality event varies

greatly [3]. Locating hibernacula is also difficult, as well as finding bats within a hibernaculum [6]. Northern long-eared bats prefer roosting in deep cracks inside their hibernaculum, making them difficult to find and count [3]. These issues lead to inaccurate bat censuses and under-detection of WNS positive sites [6]. In the western U.S., management responses to WNS could be delayed because hibernacula for western bat species are unknown or difficult to reach [2]. Bat phenology can also affect winter surveys. Cold tolerant species are generally the last to enter their hibernacula and the first to emerge, the timing of which is greatly affected by average ambient temperatures. Winter surveys of WNS-positive sites are usually performed close to natural emergence times to minimize stress on bats, thus the early emerging species may have already left the site. WNS also frequently causes roost location shifts and premature emergence. Other data collection issues lie in site size, number of inaccessible passages, disturbances during hibernation, and species misidentification (particularly when bats cluster in heterogeneous groups of species or with species that have similar traits) [3].

The little brown bat is in serious danger for extinction from the northeast and WNS could infect up to 25 bat species in North America [3]. The potential infection site in the northwest could also introduce a second epicenter and catalyze spread through North America, which would dramatically decrease the amount of time available to wildlife managers to develop and enact a prevention or treatment plan [2]. The mortality differences between species has altered and will continue to alter hibernating assemblage composition and general bat biodiversity [3]. WNS may cause a shift in genetic trends, as bats with larger body sizes and bats that hibernate in drier or colder sites are more likely to avoid or survive a WNS threat [1].

Upon the discovery of an outbreak, many government entities have already developed response plans to manage the disease. Eleven states have published response plans since 2010. Alabama, Georgia, Kentucky, Michigan, Missouri, North Carolina, South Carolina, and Tennessee are eight of these such states that lie east of the Great Plains. Colorado, New Mexico, and Wyoming are the only states west of the Great Plains with a public response plan. The Canadian Wildlife Health Cooperative published Canada's national response plan, titled "A National Plan

to manage White Nose Syndrome in bats in Canada" and is coordinating the country's response efforts [4].

The United States has published a national response plan as well via the U.S. Fish and Wildlife Service, titled "A National Plan for Assisting States, Federal Agencies, and Tribes in Managing White-Nose Syndrome in Bats" [4]. The U.S. national plan aims to guide federal, state, and tribal agencies and their partners in the response to WNS. The plan provides an organized structure for action and defines the duties of individual parties. Oversight of plan implementation is designated to two committees and seven elements of the action plan are outlined: communications, data and technical information management, diagnostics, disease management, research, disease surveillance, and conservation and recovery [7]. A national plan provides structure to individual responses such that management is standardized. This will assure consistent disease and population data collection for analysis and interpretation on a larger scale [4].

Because WNS is a relatively recent epidemic, there are many research areas in great need of further development. Accurate data is one of the limiting resources in WNS research. Population counts and *P. destructans* presence data is not available for all locations and the accuracy of the data varies greatly [3]. As discussed previously, there are many confounding factors to data collection and most data collection methods appear not to be standardized. Collecting population data during the active season with acoustic surveys, counts at maternity colonies, and trapping counts during fall swarming may improve the accuracy of the current population counts and capture the population declines seen from WNS [3]. Along with accurate data collection, mechanistic models that capture and predict spread and predict species most at risk are in need for management and prevention efforts. Specifically, models relating *P. destructans* growth to both temperature and humidity and relating fungal growth to hibernation arousal frequency are in demand. Thus far humidity has only been incorporated into survival models as a variable pertaining to evaporative water loss in infected bats, not as a factor influencing humidity-dependent fungal growth rates [1].

Examining western spread of the epidemic illuminates further knowledge gaps in more fundamental areas of bat information. Wintering strategies and hibernacula are frequently unknown for bat populations west of the Great Plains and some species are thought to forage during winter. These unknown behaviors may require new pathogen and disease impact surveillance methods. Most importantly, more information and research is needed to determine how the disease is spreading west, how it will impact western populations, and how these dynamics differ from the observed epidemic in the eastern United States [2]. With more accurate models and a more thorough understanding of western bats and WNS, management and conservation can be focused on areas at high risk for WNS mortality to minimize the impact on bat populations as a whole [6].

Chapter 2

A Network Model for Spread Prediction

Modeling White-Nose Syndrome poses a unique challenge to researchers. Two types of disease spread must be considered in this application: diffusive spread and jump dispersal. Diffusive spread is expected in highly mobile hosts that transmit disease to susceptible individuals in a spatially continuous manner. Jump dispersal occurs when host species are spatially clumped together, thus creating two different dispersal rates within and between the host clusters. Cave dwelling bats in the eastern states are a patchily distributed species, but can also transmit disease during migration or seasonal mixing [8].

Maher *et al.* explored the proportion of these two dispersion mechanisms using a maximum likelihood estimation (MLE) on historical WNS spread [8]. They began with a simple diffusion model and added complexity to create a stochastic discrete-time dynamical system built on a spatially structured network. Spread was simulated for county i, where $i = 1,\ldots,N$, using a Bernoulli trial for predicting presence in each county, dependent on all previously infected counties. The output of each Bernoulli trial, $x_i(t+1)$, yields a one if the county has been infected with WNS or a zero if the county remains uninfected. Previously infected counties influence the probability that county i becomes infected through a function, $f(x_1(t), x_2(t), \ldots, x_N(t))$, that incorporates environmental or biological variables as parameters [8]. The Bernoulli model for spread thus has the form

$$x_i(t+1) \sim \text{Bernoulli}\left[f(x_1(t), x_2(t), \ldots, x_N(t))\right]. \tag{2.1}$$

For the country-level expansion of this Bernoulli expression, Maher *et al.* used a compartmental setup for their spread model. This enabled them to test multiple spread kernels without making assumptions about the inter-county spread mechanisms, as country-wide characteristics were the intended focus for this model. Three geographic characteristics dominated the

flow of disease in their most accurate model: distance, cave density, and winter duration. The model that best fit WNS data was a moderate complexity kernel including covariates based on cave density and winter duration [8].

The dispersal kernels, denoted $\tilde{p}_{ij}$, are the main component of the function f in Equation 2.1 and represent the probability that county j does not infect county i in one time step. Maher *et al.* modified these kernels to best capture the dynamics of WNS spread. The equation

$$\tilde{p}_{ij} = [1 + e^{-(\beta_0 + \beta_1 d_{ij})}]^{-1} \tag{2.2}$$

describes the dispersal kernel for a simple diffusion model. The background infection rate is denoted β_0, the distance between the centroids of counties i and j is d_{ij}, and the scaling coefficient for the effects of distance is β_1. Similar to most infectious diseases, distance has an inverse relationship with WNS spread. The probability of spread decreased when Euclidean distance between two counties increased. That is, new infections were more likely to occur when in close proximity to a county that had already been infected [8].

To explore the effects of long-distance dispersal, the distance model in Equation 2.2 was modified slightly by fitting an exponent to the distance term, β_2. The modified dispersal kernel reads

$$\tilde{p}_{ij} = [1 + e^{-(\beta_0 + \beta_1 d_{ij}^{\beta_2})}]^{-1}. \tag{2.3}$$

The parameters in Equations 2.2 and 2.3 were fit to all counties, then fit once again using only the counties with a cave density greater than zero [8].

The cave density and county area contributions to spread probability were considered using a generalized gravity model approach. The gravity model format decreases the negative effects of distance on disease spread with a weighted gravity term in the denominator. Maher *et al.* compared the effects of cave density (η_i and η_j) using

$$\tilde{p}_{ij} = \left[1 + e^{-\left(\beta_0 + \beta_1 \frac{d_{ij}}{(a_i a_j)^{\beta_2}}\right)} \right]^{-1} \tag{2.4}$$

as the dispersal kernel and county area (a_i and a_j) using

$$\tilde{p}_{ij} = \left[1 + e^{-\left(\beta_0 + \beta_1 \frac{d_{ij}}{(\eta_i \eta_j)^{\beta_2}}\right)} \right]^{-1} \tag{2.5}$$

as the dispersal kernel. Both were expressed as the product of those values for counties i and j. The magnitude of these gravity terms were fixed for each model with the exponent β_2. β_0 remains the background infection rate and β_1 is again a coefficient for the gravity model fraction [8].

Winter length (τ_i) was added to the model as an additional parameter, calculated as the estimated number of days where the temperature dipped below 10°C. The kernel

$$\tilde{p}_{ij} = \left[1 + e^{-\left(\beta_0 + \beta_1 \frac{d_{ij}}{(\eta_i \eta_j)^{\beta_2}} + \beta_3 \tau_i\right)} \right]^{-1} \tag{2.6}$$

incorporates this variable. A coefficient to this term was also included for scaling, denoted β_3. All other parameters are included in Equation 2.5 and remain unchanged [8].

Spread kernels involving other variables were also compared to the above kernels for prediction accuracy, with terms for northing, total bat species richness, and number of hibernating bat species. After adding these additional covariates to the model, some of the covariates improved the results compared to the simple dispersion kernel, but none of these alternates fit the observed data better than the cave density and winter duration model [8].

Using the spread kernels, $\tilde{p}_{ij}$, Maher *et al.* created a network model and placed a node at the centroid of each county with an assigned binary WNS presence variable. The maximum likelihood (ML) parameters in each kernel were fit using the Nelder-Mead algorithm and a Simulated Annealing search [8]. Nelder-Mead algorithms are used for unconstrained multidimensional optimization and do not require the use of derivatives [9]. Simulated Annealing optimization

searches for optima along a Markov chain and is highly effective at avoiding converging to a local optimum [10]. Disease presence was predicted in discrete one year time steps for each county using the Bernoulli trial

$$x_i(t+1) \sim \text{Bernoulli}\left[1 - \exp\left(\sum_j \ln(1-\tilde{p}_{ij})\right)\right] \tag{2.7}$$

based on the general form listed in Equation 2.1 where $x_1, ..., x_N$ are the counties counties infected by WNS at time t [8].

Spread simulations to evaluate the accuracy of each model used Schoharie county, NY as the epicenter and predicted spread for 100 years. Spread was stochastically simulated 9,999 times to asses spread characteristics and develop a county-level infection time line [8]. To assess the effects of error and the certainty of predictions, Maher *et al.* generated 9,999 parameter sets from a multivariate normal distribution around the previously estimated parameters with a Monte Carlo simulation, or repeated random sampling, to create sampling uncertainty (SU) parameters [8, 11]. The cave density and winter duration model then used these 9,999 SU parameters to quantify uncertainty. Future spread predictions were calculated using both the ML parameters and SU parameters for 95 years. The temporal predictions for each region of the country were dictated by the initial time of infection within the boundary of the region, before infection saturates the region.

White-nose syndrome presence data for 2011 was obtained from the U.S. Fish and Wildlife Service. Year of infection was assigned as the first year of observed presence, where an epidemic year is designated as May through April of the following year [8]. Cave density data was acquired from numerous sources for 37 of the contiguous states, and estimated for the remaining 11 by magnifying a cave density figure in Culver *et al.* [12]. Cave density for these states is probably underestimated because the cave location points in this figure overlap in high density areas. Individual cave points became indistinguishable when there were more than roughly 15 caves in a county. There were 22 documented counties with WNS that did not have documented caves where abandoned mines served as hibernacula. Mine presence data is available

but specific mine information is scarce. Abandoned mines were accounted for in the model by adding a single cave into these counties. Only the United States was considered in this model because cave data for Canada was unavailable [8]. Winter length averages for each county came from temperature data between January 2006 and December 2009 from NOAA's National Centers for Environmental Information [8, 13]. This data was interpolated to create a continuous temperature map using anisotropic ordinary kriging in ArcGIS, which is a method that uses directionally dependent spatial correlation to explain surface variation [8, 14]. The mean of these temperatures for each county became the winter length average [8].

Based on the observed WNS presence data, Maher *et al.* described the temporal and spatial characteristics of spread using the number of newly infected counties, the maximum distance and median distance of infected counties from the epicenter, and the convex hull area of infected counties. Most new county-wide infections were within 500 km of previously infected counties, indicating occurrences of exceedingly long distance dispersal is improbable [8].

When the model was restricted to counties with a nonzero cave density, simulations were both more efficient and more accurate. Accuracy further increased when a generalized gravity model for geographic heterogeneity and a climate covariate were incorporated. The gravity model modifies pairwise interactions between counties based on distance and cave density, while the climate covariate alters probability of infection with winter duration [8].

The negative log-likelihood (NLL) and Akaike information criterion (AIC) scores for each kernel indicated that the best fit model was the gravity model including cave density and winter length (Equation 2.6). Additionally, this was the only model that conformed to large-scale spread measurements [8].

A possible source of error lies in the assumption that WNS presence is documented within the first year of infection, but Maher *et al.* found the resulting bias levels from incomplete detection simulations to be low. Under-detection will likely only affect the rate at which gaps are filled within the convex hull of the infected region, not the outward spread to new geographic regions. Delayed detection increased the speed of spread to western counties by less than five

years and reduced predicted spread to southeastern and southwestern counties. This model from 2012 predicted WNS to spread to the Rocky Mountains by 2017, then progress slowly to the west coast, as is visually depicted in the following Figure [8].

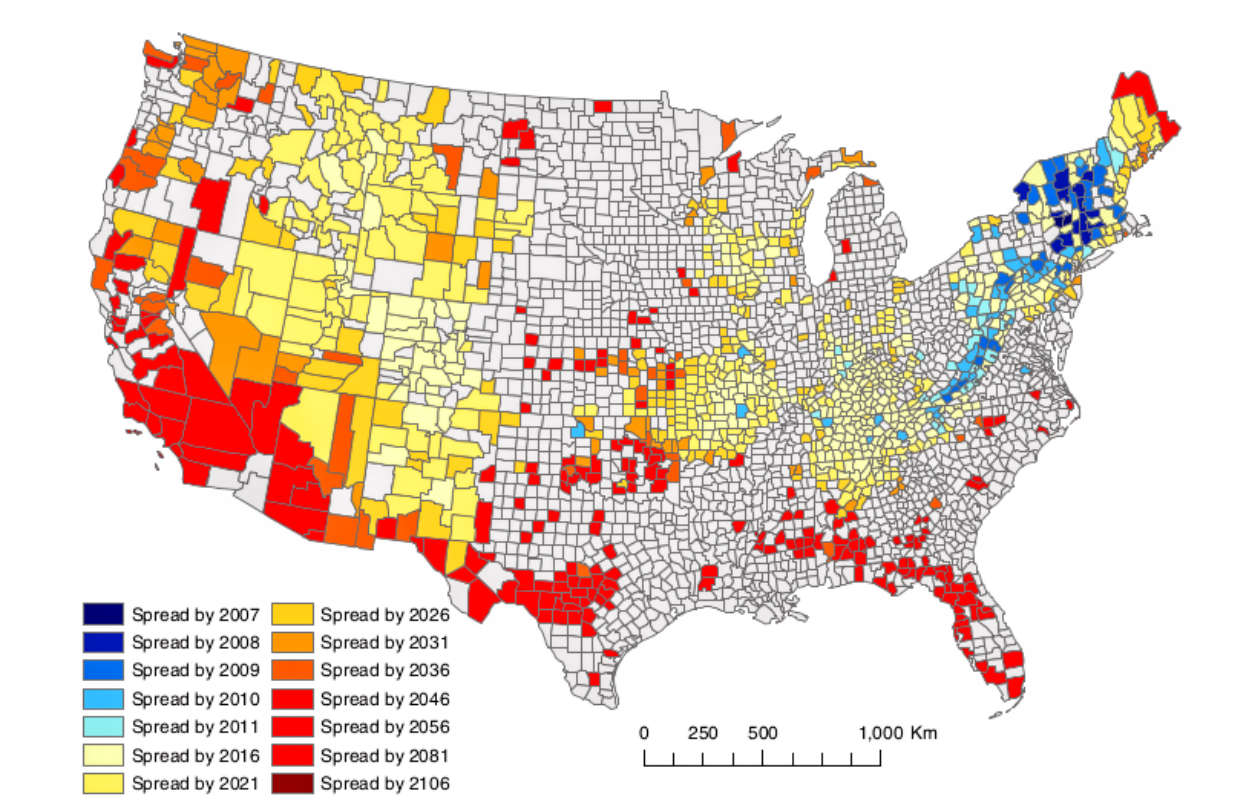

Figure 2.1: Mean year of infection estimated with the cave density and winter length dispersal kernel (Equation 2.5). Mean was calculated based on simulations using the ML parameter set and predictions were made through year 2106 based on the 2010-2011 WNS presence data. Counties in gray either did not have any reported caves or were not predicted to contract WNS within the time range [8].

Counties with caves in close proximity create geographic corridors that facilitate the spread of WNS, causing a consistently positive coefficient on inter-county distance. The exponent on the cave density term was always positive, indicating that spread is density-dependent. Higher hibernacula availability suggests a larger bat population, which increases the probability of infection. Additionally, colder counties were shown to have an increased probability of transmission, as evidenced by a negative coefficient on the winter length parameter. This trend may be due to the biological traits of *P. destructans* or the effects of longer winters on infected bats. This

corroborates the conclusion that WNS mortality can be predicted using environmental characteristics [8].

As with all large scale stochastic spread models, localized predictions are not reliably accurate. Anthropogenic hibernacula were not considered in the model by Maher *et al.*, which could facilitate spread through areas with low or non-existent cave density values. This effect is likely minimal, as mines and other man-made hibernacula are not effective reservoirs of the pathogen. As discussed previously, under-detection did not have a significant effect on the model. Moreover, detection typically occurs within the first two years of infection due to high WNS awareness [8].

The purpose of the Maher *et al.* model was to extrapolate beyond June 1, 2011 to explore how WNS will spread using the most supported model (cave density and winter length) with ML and SU parameter sets. These predictions provide insight into the general characteristics of spread and can provide more specific county level predictions. Short term predictions are the most accurate and relevant from a management perspective [8].

Models that describe the biological mechanisms controlling spread on an individual basis would help refine large scale spread models, but the data and information needed to create these models is underdeveloped. Using discrete-year time steps allows the Maher *et al.* model to encapsulate the possibility of multiple spread mechanisms. Susceptible-Infected-Recovered (SIR) models and further research into the effects of cave microclimates on fungus growth and bat mortality could clarify some of these issues and remaining uncertainties not resolved by the Maher *et al.* model [8].

The predictions in Figure 2.1 from Maher *et al.*, [8], estimate county-level WNS spread through the year 2106. Counties in gray either did not have any documented caves or were not predicted to contract the disease within a century of the first case of WNS. As shown in Figure 2.2, *P. destructans* was detected in Goshen County, Wyoming during 2018. Maher *et al.* predicted spread to Goshen County by 2031, thirteen years later than the actual year of occurrence. Itasca, Becker, and Saint Louis counties in Minnesota all tested positive for the fungus between

2015 and 2017. A proactive WNS screening of Badlands National Park in Jackson County, South Dakota showed *P. destructans* presence in 2018. All three Minnesota counties listed and Jackson County, SD were not predicted to contract WNS before 2106. Although many of the counties infected after 2012 were captured in the Maher *et al.* model, the examples described above and the examples evident between Figures 2.1 and 2.2 highlight an important flaw in this network model.

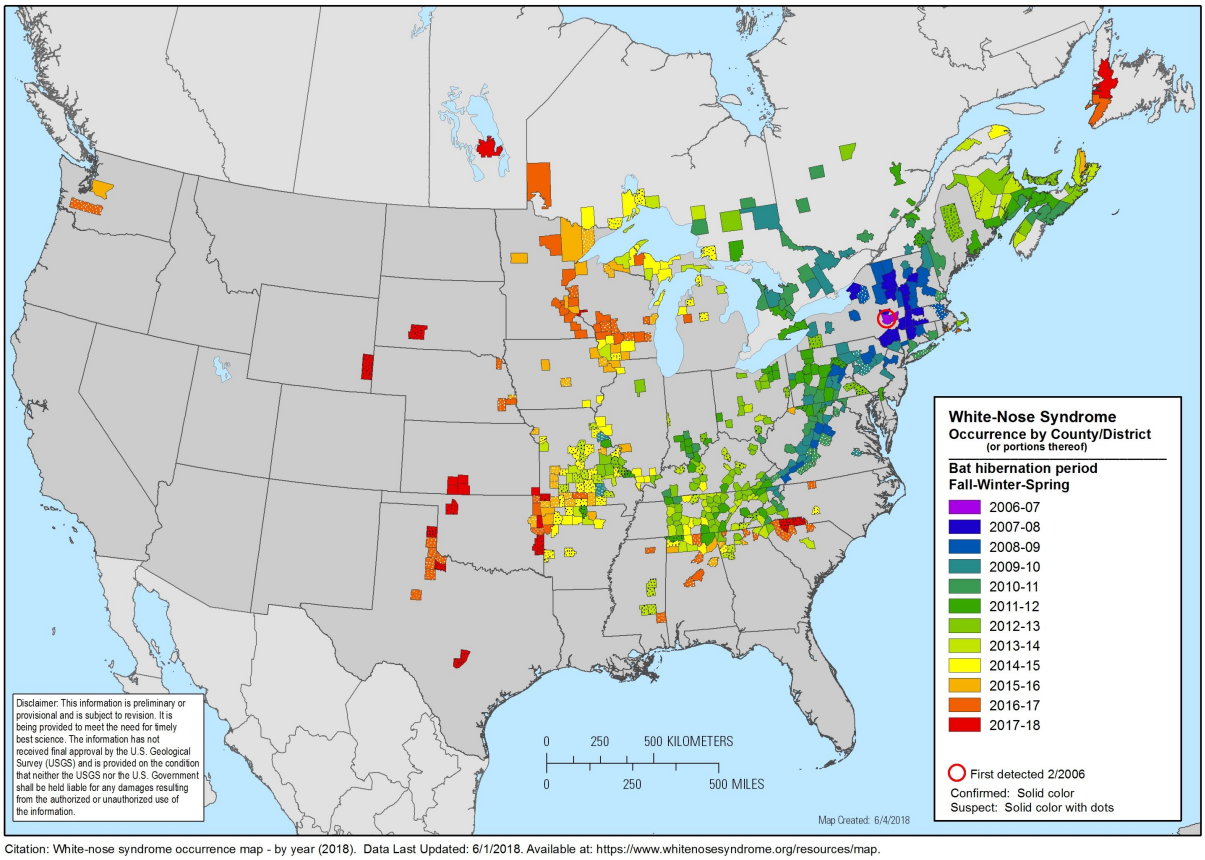

Figure 2.2: WNS presence map from June 1, 2018 [4].

Depicted below is a map of documented caves in the U.S. from 1999, where each dot on the map represents one cave [12]. The regions densely covered with dots include the Appalachian Mountains, which span the eastern side U.S. and are home to the initial infection site of WNS, along with most of the infection sites discovered since 2006. Notice the counties discussed

previously in Minnesota and South Dakota that were not predicted to contract WNS. None of these counties have any documented caves, which likely explains the lack of predicted infection. These results suggest that cave density is not well documented or cave density is not a suitable proxy for bat hibernacula for all regions of the U.S., probably both. As numerical methods for filling gaps in cave presence data would presumably involve elevation or other geological data, these problems can both be addressed through modifying the cave density variable.

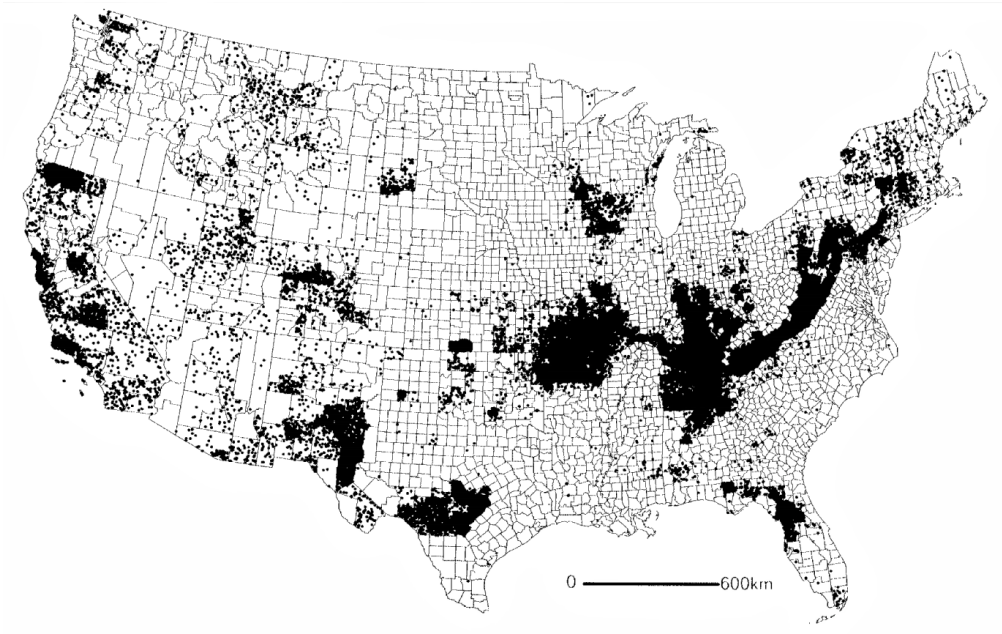

Figure 2.3: Map of U.S. cave counts from Culver *et al.* (1999) [12]. The country is sectioned into counties and each dot represents one cave.

Chapter 3

Crevice and Cavity Roosting Bats

Of the forty five bat species in the United States, twenty seven are mostly found in the western U.S. and six are distributed throughout both the eastern and western U.S. However, bat species distributions are shifting due to climate and habitat change, consequently altering these numbers. Bat diversity has been linked to the level of roost variety such that a higher number of distinct roost structures implies a higher number of bat species. This connection may explain the high level of species diversity in the West compared to other regions of the U.S. Among those described as western species, a substantial portion roost in crevices and cavities at some point during their lives. A list of western species and their WNS status can be found in Table 3.1. Bat roosts may be found in caves, crevices, and cavities in trees, tree bark, rocks, and man-made structures. Species belonging to the vespertilionidae and molossidae families (known as the evening bats and free-tailed bats, respectively) are highly likely to adopt crevice roosts in arid and semiarid regions [15].

Solick *et al.*, [16], defines a narrow fissure in rock substrate layers as a crevice and a gap in an aggregation of at least three rocks as a cavity. Bats roosting in shallow rock crevices enjoy warmer temperatures overall, but are more exposed to weather and a variable microclimate. Deep crevice roosts are well buffered from the environment and host a consistent microclimate, but generally maintain colder temperatures. An example of a crevice-dwelling species lies in western long-eared bats (*Myotis evotis*), which inhabit a large range of western North America and roost in crevices, cavities, or larger habitats (caves, mines, or man-made structures) during the warmer months. Solick *et al.* studied the summer habitats of a rock-roosting population of these bats in the Rocky Mountains of Alberta. All but six of the seventy nine roosts found in this study were in rock crevices or cavities. The six other roosts were found in standing dead trees, but the two females using the tree roosts also occupied rock roosts.

Table 3.1: Western U.S. bat species known to use crevices or cavities at some point in their annual life cycle [15], including their WNS status [4]. *Based on preliminary evidence. (T) Threatened.

Scientific Name	Common name	WNS Status
Antrozous pallidus	Pallid Bat	
Choeronycteris mexicana	Mexican Long-tongued Bat	
Corynorhinus townsendii	Townsend's Big-eared Bat	Pd Positive
Eptesicus fuscus	Big Brown Bat	Confirmed
Euderma maculatum	Spotted Bat	
Eumops perotis	Greater Mastiff Bat	
Idionycteris phyllotis	Allen's Big-eared Bat	
Lasionycteris noctivagans	Silver-haired Bat	Pd Positive
Leptonycteris curasoae	Lesser Long-nosed Bat	
Leptonycteris nivalis	Greater Long-nosed Bat	
Myotis auriculus	Southwestern Bat	
Myotis californicus	California Bat	
Myotis ciliolabrum	Western Small-footed Bat	Pd Positive
Myotis evotis	Long-eared Bat	
Myotis keenii	Keen's Bat	
Myotis lucifugus	Little Brown Bat	Confirmed
Myotis occultus	Occult Bat	
Myotis septentrionalis	Northern Long-eared Bat	Confirmed (T)
Myotis thysanodes	Fringed Bat	
Myotis volans	Long-legged Bat	
Myotis yumanensis	Yuma Bat	Confirmed
Nyctinomops femorosaccus	Pocketed Free-tailed Bat	
Nyctinomops macrotis	Big Free-tailed Bat	
Pipistrellus hesperus	Western Pipistrelle Bat	
Tadarida brasiliensis	Mexican Free-tailed Bat	Pd Positive*

3.1 Research Challenges

Understanding the roosting ecology of western bats is still a challenging endeavor. Underwood's bonneted bat (*Eumops underwood*), Keen's myotis (*Myotis keenii*), and cave myotis (*Myotis veliger*) are all species known to use cliffside crevice roosts in the U.S., but count data of these species inhabiting crevices are nonexistent. In fact, Bogan *et al.*, [15], noted in 2003 that bats using crevice and cavity roosts represented only 6% of 1,513 western bat observations in the U.S. Geological Survey Bat Population Database (BPD). The paucity of crevice and cavity roost data illuminates the longstanding emphasis on studying large groups of bats in caves or mines as well as the physical and technical challenges of attempting to study small groups of bats in rock crevices.

Some western hibernating bats are readily found in the summer, but entirely disappear in the winter to unknown hibernacula [17]. Species known to roost in cavities and crevices may utilize more spacious habitats during hibernation or remain in smaller crevice roosts. The warm season roosts of the little brown bat are usually composed of crevices in rocks, trees, or buildings, yet the species hibernates in caves and mines. However, many species likely over-winter in these cavity and crevice structures [15].

Even in the warm season, there are serious obstacles confounding attempts to monitor bat populations that inhabit cavities or crevices. One of these obstacles is low roost fidelity. Most bat species switch roosts at least once during a summer season. Roost fidelity directly relates to roost permanency and inversely relates to roost availability. Thus, bat populations residing in areas with a large quantity of temporary roosts would most likely display very low roost fidelity whereas bat populations in areas with a small number of long-lasting habitats would show high fidelity. Crevice-dwelling bats frequently use multiple roosts and switch between them, presumably due to the abundant and ephemeral nature of crevices and cavities. Fortunately for researchers, bats that move among tree and rock crevices typically do so within a small area. Exploring the roost characteristics common to the variety of structures inhabited by these bats

could illuminate their low fidelity behavior and make future monitoring attempts more efficient [15].

Bogan *et al.*, [15], and Lewis *et al.*, [18], agree that frequent roost switching may help curb parasitic infestations in bat populations. Bats may desert an infested roost before parasite eggs hatch, leaving the parasite with no food source. Similarly, low roost fidelity could decrease white-nose syndrome mortality by enabling bats to abandon infected habitats and find a more suitable roost. However, the case may also be made that lower roost fidelity will simply increase the spread rate of WNS, as the fungus does not require a bat host to persist in a roost. Additionally, Lewis *et al.* found that bat species with high roost fidelity occasionally visit other roosts, allowing the bats to readily locate a new roost in the event that their old roost becomes uninhabitable [18]. This behavior could easily catalyze WNS spread, as WNS may create uninhabitable roosts, pushing the current residents to new roosts, carrying large loads of *P. destructans* spores. Although, as noted in chapter one, it is unknown how many viable spores are carried by infected bats that have survived the winter, thus the magnitude of this spread mechanism may be negligible.

Currently, abundance estimates for bats roosting in crevices or cavities require either looking into the roost or counting the number of bats exiting the roost since bats roosting in these locations typically cannot be seen from the outside. The least invasive method is a visual emergence count. Visual counts are usually not highly accurate, however, due to the following reasons: bats tend to emerge in the evening at low light levels, researchers are frequently positioned far away from the roost, counting a large group of emerging bats is difficult, and species identification confirmation is impossible. Capture methods yield more precise data, as species identification and colony demographics can be easily and accurately recorded, but these methods are very invasive and may affect future attempts at monitoring. Since the late 1950's, most of the life history information on bats roosting in crevices and cavities has come from capturing those bats in mist nets positioned over water sources. In the western U.S., this method

is particularly effective and popular because water sources are typically isolated, which likely concentrates bat populations [15].

Miniature radio transmitters have revolutionized bat research and are commonly used to track bat movement in roost studies. Bogan *et al.*, [15], recommends exploring the uses of integrated-transponder tags, infrared or thermal imaging cameras, ultrasonic bat detectors, and miniaturized camera probes (for looking into roosts). More research in roosting habits of crevice-dwelling bat populations in general is in high demand, but research on roosting habits over extended periods of time would be exceptionally useful for studying the movements and site fidelity of those species on a larger scale.

Chapter 4

A Terrain Ruggedness Index

A key predictor variable for a species' niche is terrain heterogeneity, which is often incorporated into habitat distribution models for wildlife dependent on relatively rough or smooth landscapes [19]. Because crevice and cavity roosts are frequently located in rock faces or clusters, terrain heterogeneity serves as a proxy for possible bat roosts and hibernacula. Incorporating a numerical representation for terrain heterogeneity could improve the Maher *et al.*, [8], model by allowing WNS to spread into and through mountainous or otherwise geologically rough counties with a cave density value equal to zero. Much of the mountainous terrain in western U.S. states has a sparse distribution of documented caves (Figure 2.3), yet support large bat populations with a large variety of possible bat habitats. Using terrain heterogeneity in place of or in addition to cave density may help capture westward WNS spread more accurately.

Riley *et al.*, [19], developed a terrain ruggedness index (TRI) based on USGS digital elevation models (DEMs) to quantify the terrain heterogeneity descriptor. Equation 4.1 below details the calculation of this index. The TRI value is found for a group of nine cells using the Euclidean distance between the value of the center elevation cell (x_{00}) and the values in the eight surrounding cells, depicted in the grid to the left of Equation 4.1.

$X_{-1,-1}$	$X_{0,-1}$	$X_{1,-1}$
$X_{-1,0}$	$X_{0,0}$	$X_{1,0}$
$X_{-1,1}$	$X_{0,1}$	$X_{1,1}$

$$\text{TRI} = \sqrt{\sum_{i,j}(x_{ij} - x_{00})^2} \qquad (4.1)$$

Looping this calculation through a bounded region yields a dataset of geographical values that highlights areas of high elevation variability. A map of TRI values can be displayed using

GIS software for a visual representation of terrain heterogeneity or used in a computational model as a terrain variable [19].

Figure 4.1 is an image of elevation data for all U.S. states west of the Great Plains from the Shuttle Radar Topography Mission (SRTM) in 2007 with a pixel resolution of 30 meters [20]. This data was downloaded through Google's Earth Engine Explorer and the image was generated using Esri's ArcGIS Desktop. Note that in this image, elevation values appear relatively continuous.

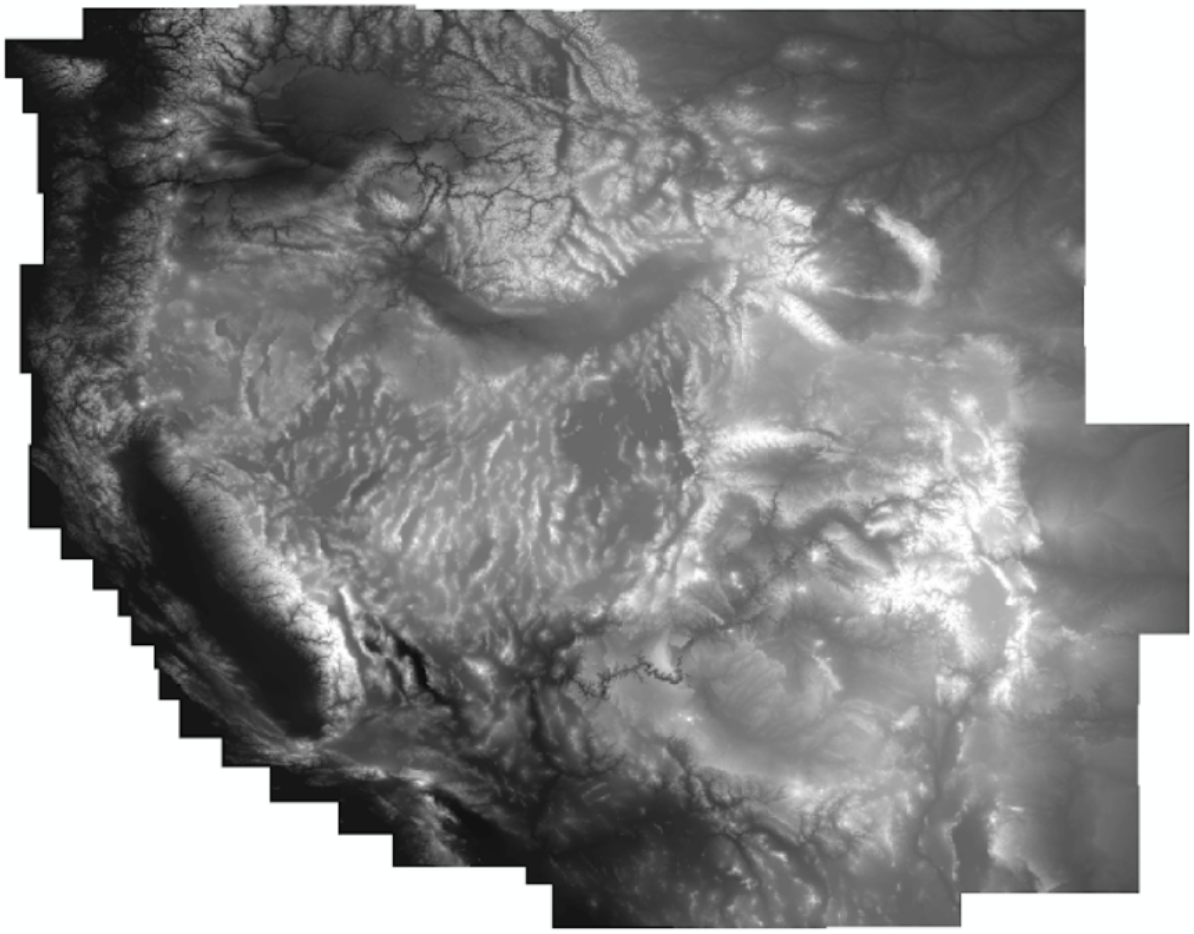

Figure 4.1: A digital elevation model (DEM) of U.S. states west of the Great Plains. Data is from the Shuttle Radar Topography Mission at a 30 meter pixel resolution and was downloaded from the Google Earth Engine Explorer [20]

After applying Equation 4.1 to the DEM in Figure 4.1, TRI values for the western U.S. were obtained and are displayed in Figure 4.2. This image highlights the mountain ranges discussed previously and other areas with high elevation heterogeneity (areas appearing to be rippled or cracked in Figure 4.1). The data in the TRI image also appears more coarse and discrete. This is due to the intended separation of low terrain variability from high terrain variability.

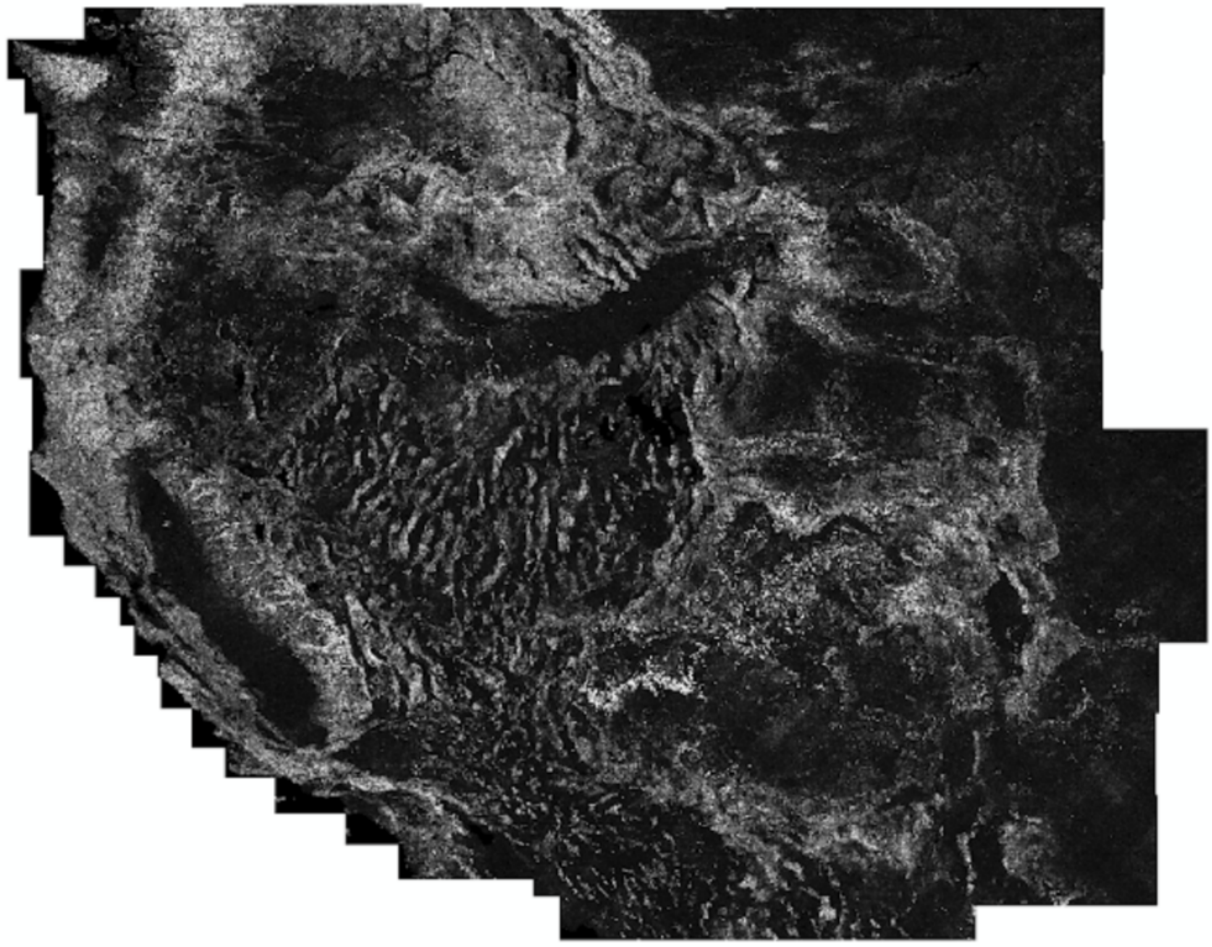

Figure 4.2: Terrain Ruggedness Index values of U.S. states west of the Great Plains, calculated from the elevation data in Figure 4.1 and the TRI algorithm in Equation 4.1.

Creating Figure 4.2 first required downloading elevation data for the region. During this stage, each state was split into smaller sections with landmarks as reference points on Google's Earth Engine Explorer and downloaded individually. A Python script created by Yiran Li, [21],

and modified for this project (see appendix) read in each DEM raster, applied Equation 4.1, and output a raster of TRI values for that selection. After all DEM rasters for the western U.S. were downloaded and transformed into TRI rasters, the regions were combined into one raster using the "Mosaic to New Raster" tool in Esri's ArcGIS Desktop with the mosaic operator set to mean.

To apply Equation 4.1 to the DEM pictured in Figure 4.1, elevation pixels are grouped into overlapping grids of nine and Equation 4.1 is applied directly to those groups. This application assigns TRI values to all of the center pixels in the grids, leaving the border of the chosen region blank at a width equivalent to the pixel resolution. In this DEM, pixels are 30 meters by 30 meters, thus this algorithm groups pixels into 90 meter square blocks and leaves an empty boundary of 30 meter width. To compensate for this empty boundary, the DEM regions downloaded were intentionally selected with a significant overlap that prevented gaps in the final TRI raster.

Chapter 5

Future Directions

The Terrain Ruggedness Index outlined by Riley *et al.*, [19], provides a promising alternative or addition to the cave density metric used in the Maher *et al.*, [8], network model for WNS spread. In order to be compatible with the cave density and WNS presence data available, the TRI raster displayed in Figure 4.2 must be converted into a county-level metric. Using an overlay of U.S. county boundaries and the spatial analyst tool in Esri's ArcGIS Desktop, TRI values can be averaged for each county, producing a county-wide terrain heterogeneity value which could be incorporated into the network model from Maher *et al.*.

There are two paths that could be taken when implementing this modification. Cave density could be replaced altogether by TRI data, which requires TRI values for all counties in the contiguous U.S. While this approach may simplify the model, it may also decrease the accuracy of spread through the eastern U.S. Alternatively, cave density could be used exclusively in eastern states (η_i and η_j) and TRI in western states (γ_i and γ_j) through the dispersal kernel

$$\tilde{p}_{ij} = \left[1 + e^{-\left(\beta_0 + \beta_1 \frac{d_{ij}}{h_i h_j} + \beta_3 \tau_i\right)}\right]^{-1} \tag{5.1}$$

where, for any i,

$$h_i = \eta_i^{\beta_2} + \gamma_i^{\beta_4}. \tag{5.2}$$

In this scenario, calculated TRI values are assigned to western states and TRI values for eastern states are set to zero, while cave density values are assigned to eastern states and set to zero for western states. For two counties that lie on the same side of the Great Plains, the same variable in each h term becomes zero and Equation 5.1 simplifies to Equation 2.6. In contrast, if county i and county j are on different sides of the Great Plains, the denominator of the gravity

term in Equation 5.1 includes both metrics. The exponents on each cave density and TRI term provide additional flexibility in the effects of the two hibernacula proxies.

Both methods of incorporating TRI discussed in this section present possible obstacles, but with some experimentation and analysis, the discrepancy between eastern and western U.S. terrain could be bridged. A model that includes terrain heterogeneity as a variable contributing to bat suitability would likely generate more accurate predictions of westward WNS spread. More accurate spread predictions would help land and wildlife managers increase their preparedness and efficiency to prevent and contain this deadly bat epidemic.

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Appendix A

Python Script for Terrain Ruggedness Index (TRI)

```
### Title: Calculate Terrain Ruggedness Index (TRI) on DEM
### Author: Yiran Li
### Date: 2017
### Available at
### https://www.arcgis.com/home/item.html?id
### =334346db638844039dc1c4abf5dd8d00
###
### Modified by Catherine Read on June 6, 2018

import arcpy
import numpy as np
import numpy.ma as ma
import os
import math

# Set path and file names for reading in DEM and outputting TRI files.
os.chdir("C:/Users/read/Documents/ArcGIS/DEMs/WUS_DEMs/CA")
DEMin = os.path.join(os.getcwd(),"CA1.tif")
TRIout = os.path.join(os.getcwd(),r"CA1.tif")
# Dimension of square elevation grid used to calculate TRI values.
winsize = 3


try:
```

```
    demRaster = arcpy.Raster(DEMin)
    arcpy.env.overwriteOutput = True
    arcpy.env.outputCoordinateSystem = DEMin
    arcpy.env.cellsize = DEMin

# Assign radius of elevation grid (radius=1 here), dims of total region,
# and min/max extent.
    rad = int(winsize/2)
    demcols = demRaster.width
    demrows = demRaster.height
    mx = demRaster.extent.XMin
    my = demRaster.extent.YMin

    print("Reading␣the␣input␣DEM...")
    demArray = arcpy.RasterToNumPyArray(demRaster,arcpy.Point(mx,my),
    demcols,demrows)
    maskDEMArray = ma.masked_where(demArray == -9999.0, demArray)

# Initialize blank array for TRI values.
    newshape = (int(np.ceil(demrows/winsize)),
    int(np.ceil(demcols/winsize)))
    TRIArray = np.zeros(newshape)

    print("Calculating␣TRI␣index...")
    trirow = 0

# Calculate TRI for each cluster of cells.
```

```
    for i in range(rad,demrows-rad,winsize):
        tricol = 0
        for j in range(rad,demcols-rad,winsize):
            diff = 0.0
            if maskDEMArray[i, j] is ma.masked:
                TRIArray[trirow, tricol] = -9999.0
            else:
                winArray = maskDEMArray [i-rad:i+rad+1, j-rad:j+rad+1]
                diff = (winArray - maskDEMArray[i, j])
                diff =np.array(diff, dtype='int64')
                squares = (diff**2)
                sumDiff = np.sum(squares)
                TRIArray[trirow, tricol] = math.sqrt(sumDiff)
            tricol = tricol+1
        trirow = trirow+1

# Save TRI values to a raster and output to .tif file.
    print("Saving␣the␣TRI␣index␣map...")
    TRIRaster = arcpy.NumPyArrayToRaster(TRIArray,arcpy.Point(mx,my),
    winsize*demRaster.meanCellWidth,
    winsize*demRaster.meanCellHeight,-9999.0)
    TRIRaster.save(TRIout)

    del TRIRaster
    del demRaster
    print("All␣done!")
except arcpy.ExecuteError:
```

```
print(arcpy.GetMessages())
```