

Preregistration

Preregistration: Effects of white-nose syndrome (WNS) on bat community structure in Cypress Hills, Saskatchewan, Canada.

Sophia Fan¹, Keerthi Krutha³, Javad Meghrazì¹, Tanya Strydom², Hannah Wilson³

¹ University of British Columbia

² University of Regina

³ Université de Montréal

06. October 2021

Study Information

Title	Preregistration: Effects of white-nose syndrome (WNS) on bat community structure in Cypress Hills, Saskatchewan, Canada.
--------------	--

Description	White-nose syndrome (WNS) is an infectious fungal disease caused by <i>Pseudogymnoascus destructans</i> targeting hibernating bats. First detected in New York City in the winter of 2006, it has since affected and killed more than 6 million hibernating bats in eastern North America Jachowski et al. (2014a). Notably, the disease
--------------------	--

has had a pronounced effect on the little brown bat, *Myotis lucifugus*, where it's predicted that regional population collapse and extirpation could occur as soon as within the next 16 years Frick et al. (2010). Other bat species responded variably: some studies show that silver-haired bat (*Lasionycteris noctivagans*) detection rates increased following WNS (Jachowski et al., 2014b; Nocera, Ford, Silvis, & Dobony, 2019a), while results for big brown bats (*Eptesicus fuscus*) are mixed, but most studies seem to show that their numbers increased following competitive relaxation from other declining bat species (Francel, Ford, Sparks, & Brack, 2012; Jachowski et al., 2014c; Morningstar, Robinson, Shokralla, & Hajibabaei, 2019; Nocera, Ford, Silvis, & Dobony, 2019b). Because WNS affects bat species differentially, community-level monitoring studies are needed to fully appreciate and mitigate consequences for community structure and ecosystem function Hoyt, Kilpatrick, & Langwig (2021). In 2021, the first instance of *P. destructans* with no bat mortality was reported in Saskatchewan, Canada (Wilson pers. comm., Lausen-WCS Canada pers. comm.), indicating westward disease spread into central Canada. This study aims to inform local bat conservation efforts by compiling and comparing bat community data from before and after the introduction of *P. destructans* in the Cypress Hills, in addition to constructing population projection models to predict species survivorship into the future.

Hypotheses We expect to see white-nose syndrome affect bat species differently in the Cypress Hill region of Saskatchewan, Canada, in line with similar trends found in affected eastern North American bat populations (Figure 1). Specifically:

Directional: We expect to see pronounced population **declines** in little brown bats (*Myotis lucifugus*) after *P. destructans* introduction.

Directional: We expect to see population **increases** in silver-haired bats (*Lasionycteris noctivagans*) and big brown bats (*Eptesicus fuscus*) after *P. destructans* introduction due to reduced interspecific competition.

Design Plan

Study type	Observational Study. Data is collected from study subjects that are not randomly assigned to a treatment. This includes surveys, natural experiments, and regression discontinuity designs.
Blinding	No blinding is involved in this study.
Study design	<p>Design: Case-control study design (Mann, 2003). The study group will be the bat community in Saskatchewan. Our control group would be the bat community in the Cypress Hills before the detection of <i>P. destructans</i>.</p> <p>Assumption(s): No two same individual bats were sampled before and after <i>P. destructans</i> detection. Proportion of each species captured are equal to their proportion in nature. Other factors that may impact species decline are minimal.</p>
Randomization	Not applicable

Sampling Plan

Existing data	Registration prior to analysis of the data. As of the date of submission, the data exist and you have accessed it, though no analysis has been conducted related to the research plan (including calculation of summary statistics). A common situation for this scenario when a large dataset exists that is used for many different studies over time, or when a data set is randomly split into a sample for exploratory analyses, and the other section of data is reserved for later confirmatory data analysis.
Explanation of existing data	Our dataset will be from 2010-2030, and the dataset from 2010-2021 already exists. Our access to the dataset has been limited because, all team members except HW come from other departments and laboratories with no prior studies conducted on bats. HW is a new student to the bat research group to the University of Regina and started in September 2021. The 2010-2021 dataset we plan to use for our study

has been collected by multiple trained researchers who are not connected with our project. HW has seen the dataset with data from 2019 but has not conducted any form of statistical analysis with it. Also, as authors of this study, none of us are aware of any patterns or summary statistics relating to the effect of a fungal disease on bats of this dataset even though there are publications/results from previous studies conducted by other researchers. We plan to only conduct any detailed analysis with the data only after 2030 once all data collection is complete.

**Data collection
procedures**

Study site: West Block, Cypress Hills Provincial Park, Saskatchewan, Canada.

Study locations: Previously established capture locations for bats along Battle Creek, established by the University of Regina (Wilson pers. comm.).

Sampling period: Mid-June to mid-August every year. Samples will be collected until August 2030. Data points from this same period will be used from records prior to *P. destructans* incidence starting from August 2010.

Data collection methods: We will set up 1-3 mist nets at one site each night. Nets will be set up across Battle Creek. Nets will be at least 1m tall, and wide enough to cover the width of the creek. We will raise nets for three hours after last light and check the nets every 10min in the first hour after last light and every 15min in the subsequent two hours. We will capture and handle bats with nitrile-gloves. Once a bat is caught it will be moved away from the net in order to avoid attracting other individuals to the captured bat's distress call. We will record the age, sex, mass and species of the bat.

In order to attribute the possible difference in bat populations due to the fungal disease we will collect samples of *P. destructans* by swabbing the wings and muzzle of the bat following Zukal et al. (2016). We will analyse swabs using qPCR techniques Zukal et al. (2016) and record whether the individual bat has contracted *P. destructans*.

We will also measure ambient temperature, cloud cover, and wind speed every night at each sampling site. In case of rain or if wind speed is above 20km/s, we will stop sampling. When recording a capture, we will record the date of sampling in the ISO format along with a three-letter code of the sampling location, the genus and species of bat being captured and initials of the personnel collecting the capture.

Sample size	Our target sample size is 2000 individuals. However, we do not have a definitive pre-determined count and cannot control the number of bats we capture each year.
Sample size rationale	We do not have control over how many bats will be captured in a night. From anecdotal knowledge and previous experience we estimate that we will to capture anywhere between 100-200 bats per summer. Therefore, after twenty years of data collection we will have collected around 2000 bats.
Stopping rule	<p>The station where researchers live during the field season is owned by the University of Regina, who use the station for an undergraduate class during the last week of August. The field station is closed after that, so data collection will stop mid-August.</p> <p>The project overall will end in 2030. <i>P. destructans</i> was first detected in Saskatchewan in 2021. Ending the project 10 years after the fungus was first detected allows us to observe bat community structure at the start of the disease, directly after it should have killed the largest number of bats, and ten years later when it should have become endemic to the population.</p>
Variables	
Manipulated variables	'Raw' bat species abundance records will be transformed to a proportional abundance which will be used in the modelling process.
Measured variables	We are going to be measuring the population size of the bat species in the Cypress Hills before and after the detection of <i>P. destructans</i> . We will measure this by counting how many bats of each species we catch in mist nets each year and recording instances of infection.
Indices	Not applicable.

Analysis Plan

Are there significant changes in population size for bat species in Cypress Hills before and after the introduction of *P. destructans*?

Unpaired student t-tests will be used to compare population size estimates for each species before and after infection. Specifically, between 2021 (summer before detection) and 2023 (two winters later), as well as at any increments of interest up to 2030. The test should be one tail. In addition, power analysis should be conducted using `pwr` package in R. We expect to see significant differences in population size before and after infection.

Predictive model of bat community composition change

See Figure 1 for how we expect model output to potentially look

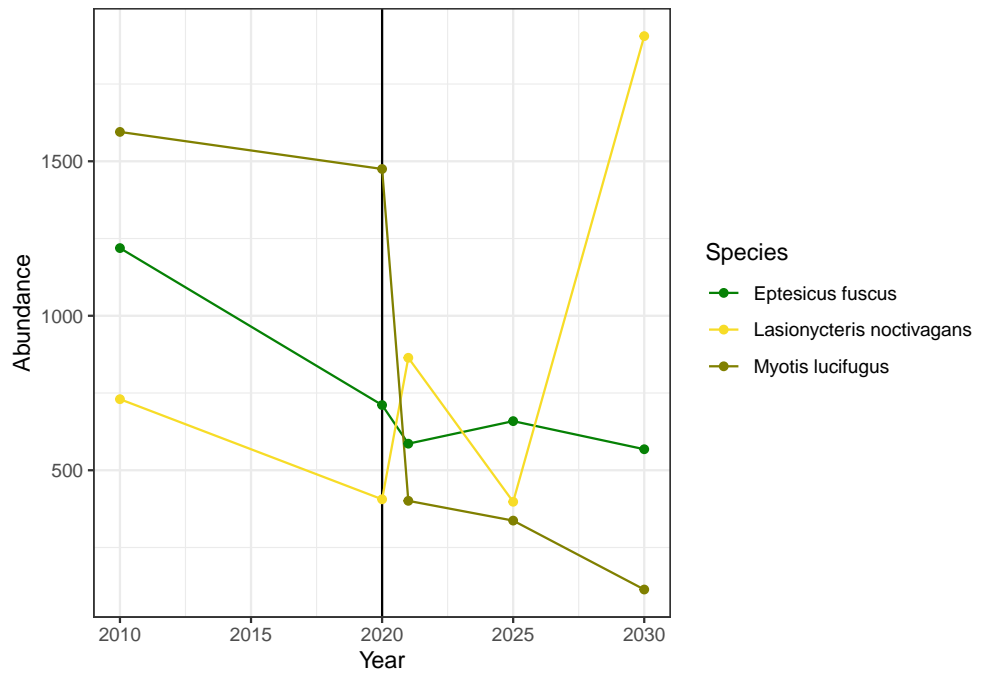


Figure 1: Expected changes in bat species frequencies after the introduction of white nose syndrome in 2019 the Cypress hill region of Saskatchewan Province.

To investigate effect of WNS on bat community as a whole instead of investigating species individually, we have also developed a mathematical framework to compare

Cypress Hills data with existing eastern North American data. The following equations aim to describe changes in the size of populations of different bat species. The aim of the model is to simulate bat population data for Cypress Hills following infection. Some coefficients should be estimated based on Saskatchewan bat community data from 2010-2021 (growth rate, carrying capacity and competition coefficients) and some from previous studies in east coast (transmission rate) populations and then predictions can be made for Saskatchewan bat community.

$$N_{i,t+1} = N_{i,t}r_i(1 + \frac{K_i - N_{i,t} - \sum_{j=1}^n \alpha_{i,j}N_{j,t}}{K}) - I_{i,t}$$

Where: $N_{i,t}$ is the population size of species i at time t .

r_i is the intrinsic growth rate of species i .

K_i is the carrying capacity for species i .

$\alpha_{i,j}$ is the coefficient for competition effect of species j on species i .

$I_{i,t}$ is the number of individuals infected with WNS at time t . It's assumed that all of these individuals will die before next time point. This parameter is calculated as follows:

$$I_{i,t+1} = d_i I_{i,t} \frac{N_{i,t}}{k_i}$$

Where d_i is the transmission rate of WNS in species i . It's assumed that transmission of disease is density dependent.

Estimation of coefficients: r , K and α : These should be calculated based on the data gathered up to 2021 using a genetic algorithm.

d : Will depend on the objective of study: 1) If we are going to make predictions for future dynamics of populations, previous studies should be used to estimate this parameter. It should be considered that d likely won't stay constant over time and evolutionary dynamics of host and parasite would affect it. 2) If we are going to estimate d in each of these populations after the end of study, similar methods from other coefficients can be used.

Note that in both cases an initial number of infected individuals at year 2021 is required ($I_{i,t=2021}$).

Statistical models
Predictive model of bat community composition change

The model provides some predictions for populations of different species at different time points. We can then use this data to test whether community dynamics are similar to declines in eastern North America using a chi-2 goodness of fit test. Power analysis will also be conducted. A single test should be conducted for all for species over 2021-2030 period. If the test finds significant difference, then an exploratory test should be conducted (further details explained in next sections) to determine new values for the coefficients using the data from Saskatchewan bat community 2021-2030 using a genetic algorithm optimization method. Then it should be determined if the sample size can support the estimated effect size and again another round of power analysis should be conducted.

Transformations	N/A
------------------------	-----

Inference criteria	For all analysis type I error threshold will be set to 0.05. Alpha error correction will be done using the Bonferroni method to address the effect of conducting multiple tests\simultaneously on type I error.
---------------------------	---

Data exclusion	See missing data.
-----------------------	-------------------

Missing data	We might expect bat records not to have a species identifier (e.g. not able to identify species in field, etc). As this is data of interest these records will have to be removed as there is no easy way to re-examine an individual once released.
---------------------	--

References

Francel, K. E., Ford, W. M., Sparks, D. W., & Brack, V. (2012). Capture and Reproductive Trends in Summer Bat Communities in West Virginia: Assessing the Impact of White-Nose Syndrome. *Journal of Fish and Wildlife Management*, 3(1), 33–42. doi:[10.3996/062011-JFWM-039](https://doi.org/10.3996/062011-JFWM-039)

- Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., . . . Kunz, T. H. (2010). An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species. *Science*, 329(5992), 679–682. doi:[10.1126/science.1188594](https://doi.org/10.1126/science.1188594)
- Hoyt, J. R., Kilpatrick, A. M., & Langwig, K. E. (2021). Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology*, 19(3), 196–210. doi:[10.1038/s41579-020-00493-5](https://doi.org/10.1038/s41579-020-00493-5)
- Jachowski, D. S., Dobony, C. A., Coleman, L. S., Ford, W. M., Britzke, E. R., & Rodrigue, J. L. (2014a). Disease and community structure: white-nose syndrome alters spatial and temporal niche partitioning in sympatric bat species. *Diversity and Distributions*, 20(9), 1002–1015. doi:[10.1111/ddi.12192](https://doi.org/10.1111/ddi.12192)
- Jachowski, D. S., Dobony, C. A., Coleman, L. S., Ford, W. M., Britzke, E. R., & Rodrigue, J. L. (2014b). Disease and community structure: white-nose syndrome alters spatial and temporal niche partitioning in sympatric bat species. *Diversity and Distributions*, 20(9), 1002–1015. doi:[10.1111/ddi.12192](https://doi.org/10.1111/ddi.12192)
- Jachowski, D. S., Dobony, C. A., Coleman, L. S., Ford, W. M., Britzke, E. R., & Rodrigue, J. L. (2014c). Disease and community structure: white-nose syndrome alters spatial and temporal niche partitioning in sympatric bat species. *Diversity and Distributions*, 20(9), 1002–1015. doi:[10.1111/ddi.12192](https://doi.org/10.1111/ddi.12192)
- Mann, C. J. (2003). Observational research methods. Research design II: cohort, cross sectional, and case-control studies. *Emergency Medicine Journal*, 20(1), 54–60. doi:[10.1136/emj.20.1.54](https://doi.org/10.1136/emj.20.1.54)
- Morningstar, D. E., Robinson, C. V., Shokralla, S., & Hajibabaei, M. (2019). Interspecific competition in bats and diet shifts in response to white-nose syndrome. *Ecosphere*, 10(11), e02916. doi:[10.1002/ecs2.2916](https://doi.org/10.1002/ecs2.2916)
- Nocera, T., Ford, W. M., Silvis, A., & Dobony, C. A. (2019a). Patterns of acoustical activity of bats prior to and 10 years after WNS on Fort Drum Army Installation, New York. *Global Ecology and Conservation*, 18, e00633. doi:[10.1016/j.gecco.2019.e00633](https://doi.org/10.1016/j.gecco.2019.e00633)
- Nocera, T., Ford, W. M., Silvis, A., & Dobony, C. A. (2019b). Patterns of acoustical activity of bats prior to and 10 years after WNS on Fort Drum Army Installation, New York. *Global Ecology and Conservation*, 18, e00633. doi:[10.1016/j.gecco.2019.e00633](https://doi.org/10.1016/j.gecco.2019.e00633)

Zukal, J., Bandouchova, H., Brichta, J., Cmokova, A., Jaron, K. S., Kolarik, M., . . . Martínková, N. (2016). White-nose syndrome without borders: *Pseudogymnoascus destructans* infection tolerated in Europe and Palearctic Asia but not in North America. *Scientific Reports*, *6*, 19829. doi:[10.1038/srep19829](https://doi.org/10.1038/srep19829)