Preregistration

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

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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 *Pseudogymnoascus destructans* 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 (Francl, 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 from the Cypress Hills Interprovincial Park 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 WNS in Cypress Hills, in addition to constructing population projection models under WNS 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 (See Figure 1), in line with similar trends found in affected eastern North American bat populations. Specifically:

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

Directional: We expect to see population **increases** in silver-haired bats (*Lasionycteris noctivagans*) and big brown bats (*Eptesicus fuscus*) 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

Design: Case-control design equivalent to a two-group design.

Assumption: No two same individual bats were sampled before and after *P. destructans* detection. Therefore, we will be using unpaired t-test (design). We plan on using this design because our study is a before/after study of a bat population affected by the pathogen *P. destructans.* We assume that we will not have any recaptures and therefore, we will use an unpaired t-test. We recognise that this can be mixed design but since we make the above assumption we decide to use only unpaired design.

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 access to the dataset has been limited because, all team members except Ms. Hannah Wilson come from other departments and laboratories with no prior studies conducted on bats. Ms. Wilson is a new student to the bat research group to the University of Regina and started in September 2021. The dataset we plan to use for our study has been collected by multiple trained researchers using the above protocols since 1991. Ms. Wilson has seen the dataset with data from 1991-1992 and ca. 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 the 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 population to the fungal disease we will collect samples of *P. destructans* by swabbing wings and muzzle of the bat follwing (**Zakul?**) et al. 2016. We will analyse swabs using qPCR ((**Zakul?**) et al. 2016).

We will also measurements temperature, cloud cover and wind speed while sampling

for bats. 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 at Battle creek, the genus and species of bat being captured and initials of the personnel collecting the capture

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Sample size

Our target sample size is 2000 individuals. However, we do not have a 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. Therefore, from anecdotal knowledge and previous experience we estimate that we will to capture anywhere between 100-200 bats. After twenty years of data collection we will have collected around 2000 bats.

Stopping rule

Data collection will end each year in mid-August. The field 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

The project overall will end in 2030. White-Nose Syndrome was first detected in Saskatchewan in 2021. Ending the project 10 years after WNS 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.

Variables

Manipulated variables 'Raw' 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 White-nose Syndrome. We will measure this by counting how many bats of each we catch in mist nets each year.

Indices Not applicable.

Analysis Plan

It's hypothesized that the community would undergo similar changes as eastern coast populations. Following equations aim to describe changes in the size of populations of different bat species. Some coefficients should be estimated based on Saskatchewan bat community data from 1990-2021 (growth rate, carrying capacity and competition coefficients) and some from previous studies in east coast(transmission rate)populations and then predication can be made for Saskatchewan bat community.

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

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 coefficent 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 dye before next time point. This parameter is calculated as follow:

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

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

Estimation of coefficients: r, K and α: They should be calculated based on the data gathered sofar (1991-2021) using genetic algorithm (or any other optimization method?).

d: depends 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 probably 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 method as 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

The model provides some predictions for population of different species at different time points and we can test our hypothesis using a chi-2 goodness of fit test.Besides, power analysis will be conducted using `pwr` package. 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

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Inference criteria

Data exclusion

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Missing data

We might expect bat records not to have a species identifier. As this is the data of interest these records will have to be removed as there is no feasible way to impute the missing data.

Exploratory analyses (optional)

If the test finds significant difference, then an exploratory test should be conducted 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.

Other

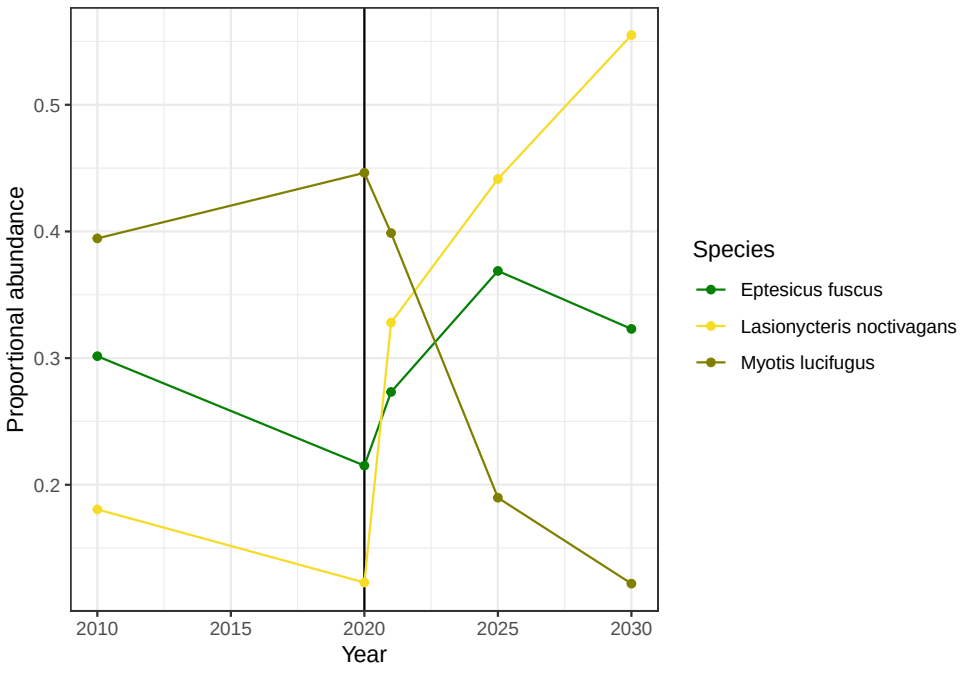

Figure 1: Expected changes in bat species frequencies after the introduction of white nose syndrome in 2019 the Cypress hill region of Saskatchewan Province.

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