Montana's 2022 Annual White-nose Syndrome Surveillance Report

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Summary

In 2019, Montana Fish, Wildlife and Parks, the U.S. Geological Survey, and the Montana Natural Heritage Program designed a plan to assess how the invasion and spread of the fungus *Pseudogymnoascus destructans* (*Pd*), which causes the disease White-Nose Syndrome (WNS), might impact bats across Montana. The resulting project involves annual, statewide surveillance for *Pd* and WNS to estimate the arrival and distribution of the disease, which is the focus of this report, and long-term acoustic monitoring to assess bat occupancy and activity. *Pd* was first detected in Montana in 2020. In 2022, we surveyed 38 sites across the state, 16 of which were *Pd*-positive. During this surveillance season, *Pd* was detected farther west in Montana than in previous years, with detections in 7 new counties including Powder River, Big Horn, Golden Valley, Blaine, Hill, Choteau, and Gallatin. *Pd* was detected on long-legged myotis for the first time in Montana. WNS was first confirmed in 2021 among little brown bats (*Myotis lucifugus*) in Fallon, Carter, and Phillips Counties.

Also, in 2021-22, Montana Fish Wildlife and Parks (FWP) participated in collection of acoustic data from 87 geographic grid cells as part of a multi-agency collaboration to monitor bat species distribution and activity as part of the North American Bat Program (NABat). Acoustic data was analyzed by The Montana Natural Heritage Program, and data detailing species ID, collection location, and call attributes have been provided to NABat personnel for analysis to meet state-wide bat monitoring goals and are expected to be appended to publicly available databases pending completion of the analysis of these data. Understanding the impacts of WNS on Montana's bats will inform decisions about how Montana pursues bat management and conservation strategies— whether it be treatments specific to WNS or ecological approaches toward offsetting the costs of disease.

Introduction

White-nose Syndrome (WNS), the disease caused by the cold-adapted fungus, *Pseudogymnoascus destructans* (*Pd),* has killed millions of North American bats since its detection in New York in 2006 (Blehert et al. 2008, Lorch et al. 2011, Frick et al. 2015). *Pd* is believed to have been introduced from Eurasia through the accidental transport of an infected bat or fungal spores (Hoyt et al. 2021). Since its arrival in 2006, national surveillance efforts have tracked the spread of *Pd* and WNS westward across North America (see updated map at whitenosesyndrome.org). In 2016, *Pd* was detected in Washington state, and more

recent detections in California indicate pathogen and disease spread from a western front. As of 2022 *Pd* has been detected in all the Rocky Mountain states, and only four western states have yet to report the fungus. WNS has driven significant and sustained population declines among numerous bat species across the eastern half of North America (Frick et al. 2010, Frick et al. 2015, Langwig et al. 2012, Nocera et al. 2019), and as a result, several bat species have been listed or petitioned for listing under the United States Endangered Species Act (Kunz and Reichard 2010, U.S. Fish and Wildlife Service 2022).

Pd thrives in cool and humid subterranean conditions (Verant et al. 2012, Langwig et al. 2012). Transmission occurs during fall and winter seasons via direct contact between bats and through contact with *Pd*-contaminated environments. Much of the transmission revolves around winter hibernacula where infected bats shed spores that infect neighboring bats, contaminate cave environments, persist throughout the year, and can reinfect bats returning to hibernate (Langwig et al. 2015). The onset and severity of disease is related to fungal load, which typically builds up in the environment over a period of years after the fungus is introduced and is influenced by hibernacula temperature and humidity, bat colony size, and species composition. *Pd*, which causes damage to wing, tail, and ear membranes on hibernating bats, causes bats to repeatedly rouse from torpor and burn through fat reserves needed to survive winter (Reeder et al. 2012). Some individuals that survive until spring mount an extreme inflammatory immune response to *Pd* which further contributes to mortality (Lilley et al. 2017, Davy et al. 2020). Individuals that survive through hibernation and spring emergence typically recover and clear infections to the point that spores and disease lesions are no longer detectable on bats by mid to late summer. Severity of disease differs among species, and appears to be related to variation in susceptibility, the immune response to infection, and hibernation behavior and ecology (Hoyt et al. 2021).

Because of the devastating impacts of WNS on North American bat populations, considerable efforts are underway to identify and test management tools to prevent infection, reduce the severity and impacts of disease, and boost overall bat survival to offset disease costs. Approaches include experimental tools aimed at directly controlling *Pd* through microbial, chemical, physical, or vaccine treatments of bats or hibernacula (e.g. Hoyt et al. 2019, Cheng et al. 2017, Cornelison et al. 2014, Turner et al. 2021, Palmer et al. 2018, Rocke et al. 2019), ecological approaches towards bolstering bat health and survival in the face of WNS (Cheng et al. 2019, Wilcox et al. 2016), or attempts to conserve habitat (Johnson & King 2018, White-nose Syndrome Conservation and Recovery Working Group 2018) and mitigate other sources of mortality such as that from wind development (Baerwald et al. 2009, Arnett et al. 2011) and anthropogenic structure loss (White-nose Syndrome Conservation and Recovery Working Group 2015). As has been carried out in other states (Szymanski et al. 2009), Montana has begun a structured decision-making exercise to identify how best to respond to the arrival of WNS to maximize bat distribution and abundance across the state and into the future.

Montana has been conducting *Pd* surveillance since 2012, with annual surveillance in at least 4-5 sites across the state since 2017. In 2019, Montana Fish, Wildlife & Parks (FWP) began collaborating with the National Wildlife Health Center (NWHC) to implement *Pd* surveillance informed by a west-wide spatial spread model (U.S. Geological Survey 2019). In 2021, FWP expanded surveillance efforts to include annual sampling across a 36-cell state-wide grid to gather the information needed to relate local *Pd/*WNS status with trends in the acoustic data collected at nearby monitored North American Bat (NABat)Program survey grid cells (Loeb et al. 2015).

Pd was detected for the first time in Montana during surveillance efforts in the spring of 2020, followed by our first detection of WNS in the spring of 2021 in eastern Montana. From work elsewhere in North America, *Pd* is known to be capable of causing WNS in seven of Montana's 15 bat species, it has been

detected in four other species that may serve as local or regional vectors and seems likely to affect at least two other Montana species due to the close relatedness of species that have been impacted to date (Maxell 2015). While observations of WNS across the eastern US have informed our predictions of what to expect in the West, important questions remain about how the disease will play out among bat populations that have very different roosting ecologies from those of their counterparts in the eastern U.S.

In 2019, FWP, the U.S. Geological Survey, and the Montana Natural Heritage Program developed a plan to document the arrival and spread of *Pd*/WNS in Montana and to understand the disease's impacts on Montana bat populations (Hanauska-Brown et al. 2019). Specifically, FWP's plan calls for (1) annual surveillance to establish the timing of *Pd*/WNS occurrence across the state, (2) statewide acoustic monitoring over time following the North American Bat Program guidelines, and (3) an analysis of long-term acoustic data for changes in occupancy and activity associated with WNS. Information from this program will be used to inform the scale of Montana's conservation efforts needed to maintain healthy bat populations well into the future. This report covers the results from the 2022 *Pd*/WNS surveillance effort.

Methods

Pd/WNS Surveillance

FWP's 2022 active surveillance sites were prioritized based on a combination of predictions from the National Wildlife Health Center's annual *Pd* spatial spread model (Figure 1, U.S. Geological Survey 2019) and an attempt to survey at least one site within each of FWP's 36 state-wide surveillance grid cells (Figure 2). Within NWHC or FWP-prioritized areas or grid cells, local biologist expertise and susceptible species-specific occupancy maps (Figure 3, Wright et al. 2018) were used to identify hibernacula, spring emergence mist-net sites, or maternity roost sites for sampling. Attempts were made to evenly distribute the survey type, including hibernacula surveys, live animal trapping, or pooled guano and environmental sampling, across the state. Hibernacula surveys involved swabbing hibernating bats, cave substrates, or collecting soil and guano. Live animal trapping involved early season mist-netting or trapping bats emerging from bat boxes between April and June (FWP Animal Care and Use Committee Agreement# FWP-07-2020). Pooled guano surveys involved collecting fresh guano and environmental swabs at early season roost sites in buildings, beneath bridges, or in bat boxes. While *Pd* would be detectable using any of these survey types, only live animal sampling or hibernacula surveys would provide opportunities to detect disease and mortality from WNS.

Following the NWHC guidance, we attempted to collect at least 25 live-bat samples (swabs of the nose and forearm) from hibernacula and live trapping sites. Species, sex, and group sizes were noted where possible. If we were unable to directly sample 25 bats at a site, we attempted to collect 2 additional environmental samples (soil, guano, or swabs of roost substrates or mist-nets) for every sample not collected from a bat, up to a maximum of 45 samples per site. At sites where we collected pooled guano, a tarp was set out for fresh guano collection (or old guano was cleared away before collection) prior to bats' return for the season (usually by May 1st). Then, after ≥ 4 weeks of guano collection, we returned to the site to gather the fresh guano, mix it together, and then subsample it for testing (either using the NWHC's pooled guano testing procedures which involved filling five 50 ml conical tubes, or by filling 45 1.8 ml cryovials with guano for individual sample testing at Oregon Veterinary Diagnostic Laboratory (OVDL)). For NWHC kits, we also collected 5 environmental swabs at pooled guano collection sites. PCR testing for *Pseudogymnoascus destructans* was conducted either at the National Wildlife Health Center or Oregon Veterinary Diagnostic Laboratory.

Where possible, bats handled during *Pd* surveillance efforts were inspected for symptoms of WNS (visible signs of the fungus, wing damage, and fluorescing lesions under a UV light). As part of our state-wide passive (opportunistic) surveillance, carcasses from bat mortality events, or individual bat carcasses with suspicious lesions, were submitted to the NWHC for WNS diagnostics (National Wildlife Health Center 2020).

To conduct acoustic monitoring of bats across Montana, detector recorder units (hereafter detectors) were deployed at 87 geographic grid cells beginning in June through the first week of August (Bachen, 2022). The beginning of the deployment window was set to coincide with the expected end of the spring migration period and the termination of deployment to coincide with the volancy of young of the year (Bachen et al. 2020). Bat call sequences were analyzed with the goal of definitively identifying individual species presence by site and cell in accordance with the Echolocation Call Characteristics of Montana Bats and Montana Bat Call Identification materials (Bachen et al. 2018).

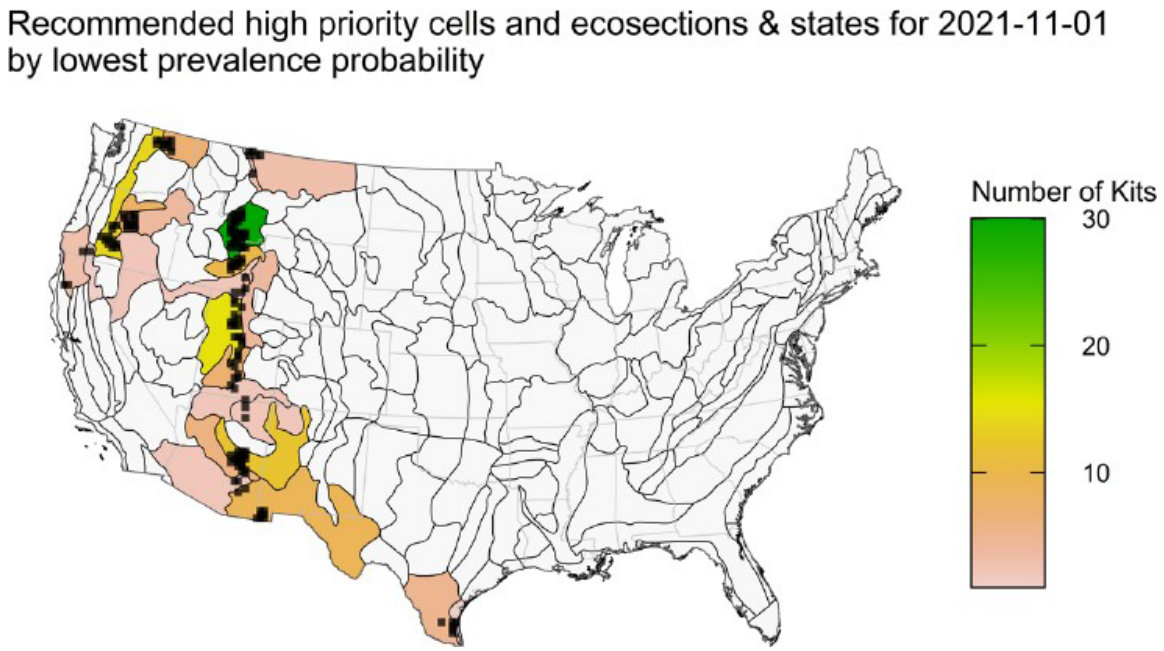

Figure 1. The top 150 highest ranking priority cells (black squares) and associated ecosections (colored polygons) where *Pd* is predicted to spread during the 2021/2022 season. The map is divided into 'eco-sections' and those eco-sections highlighted in color are areas where the leading edge of the disease was predicted to be during the winter of 2021-22. Eco-sections are color-coded by the number of target sampling locations (or 'kits') required to detect *Pd* with 95% confidence if prevalence is ≥0.15 within the sampled population. Locations of inconclusive *Pd* results are indicated by red X. Where possible, FWP sampled according to the NWHC's priorities, but also conducted additional sampling across the state's 36-cell surveillance grid (Figure 2) to document *Pd* and WNS's distribution across space and time.

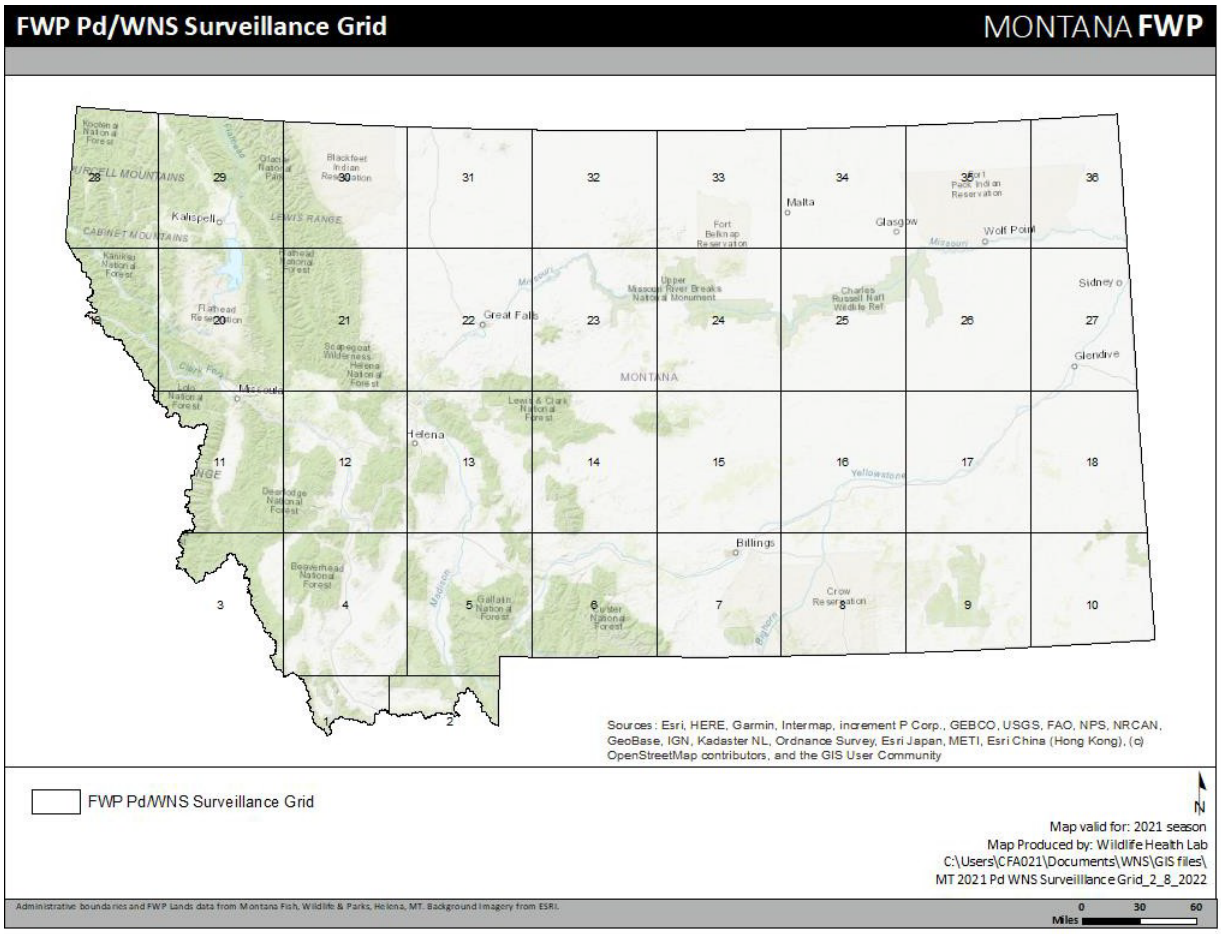

Figure 2. The state of Montana broken into 36 *Pd*/WNS sampling grid cells.

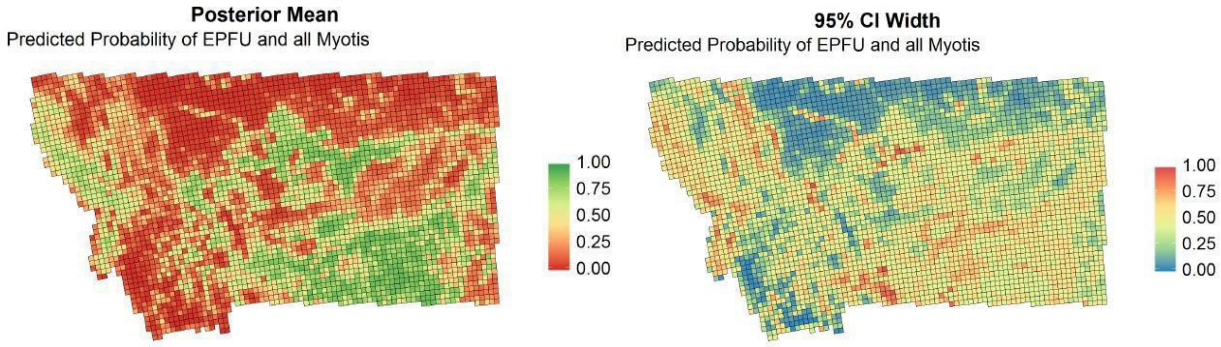

Figure 3. Estimated joint probabilities of occupancy for *Eptesicus fuscus* (EPFU; big brown bats) and all Myotis species, some of the most susceptible species to WNS. The estimates correspond to the probability that all these species are present within a grid cell (left) and the associated uncertainty (right). Reproduced from Wright et al. (2018).

Results

In 2022, FWP and partners conducted *Pd*/WNS sampling at 38 sites across Montana (Figure 4, Table 1). Sampling sites included hibernacula, roost sites (bridges, buildings, bat boxes), and landscape sites. Mist netting of live bats occurred at 16 of the 38 sites. Of the 38 sites, samples from 10 sites were submitted to the National Wildlife Health Center as part of their surveillance testing, and samples from the remaining 28 sites were tested at the Oregon Vet Diagnostic Laboratory. In addition, we submitted 1 individual bat carcass from Lewis and Clark Caverns to the NWHC for diagnostic testing as part of our passive surveillance efforts. Pd was not detected on that bat. In 2021-22, live animal sampling occurred on a range of bat species including *Myotis lucifugus* (MYLU, Little brown bats), *Myotis yumanesis* (MYYU, Yuma myotis), *Myotis ciliolabrum* (MYCI, Western small-footed myotis), *Myotis volans* (MYVO, Long- legged myotis), *Myotis evotis* (MYEV, Western long-eared myotis), *Eptesicus fuscus* (EPFU, Big brown bats) and unidentified Myotis species.

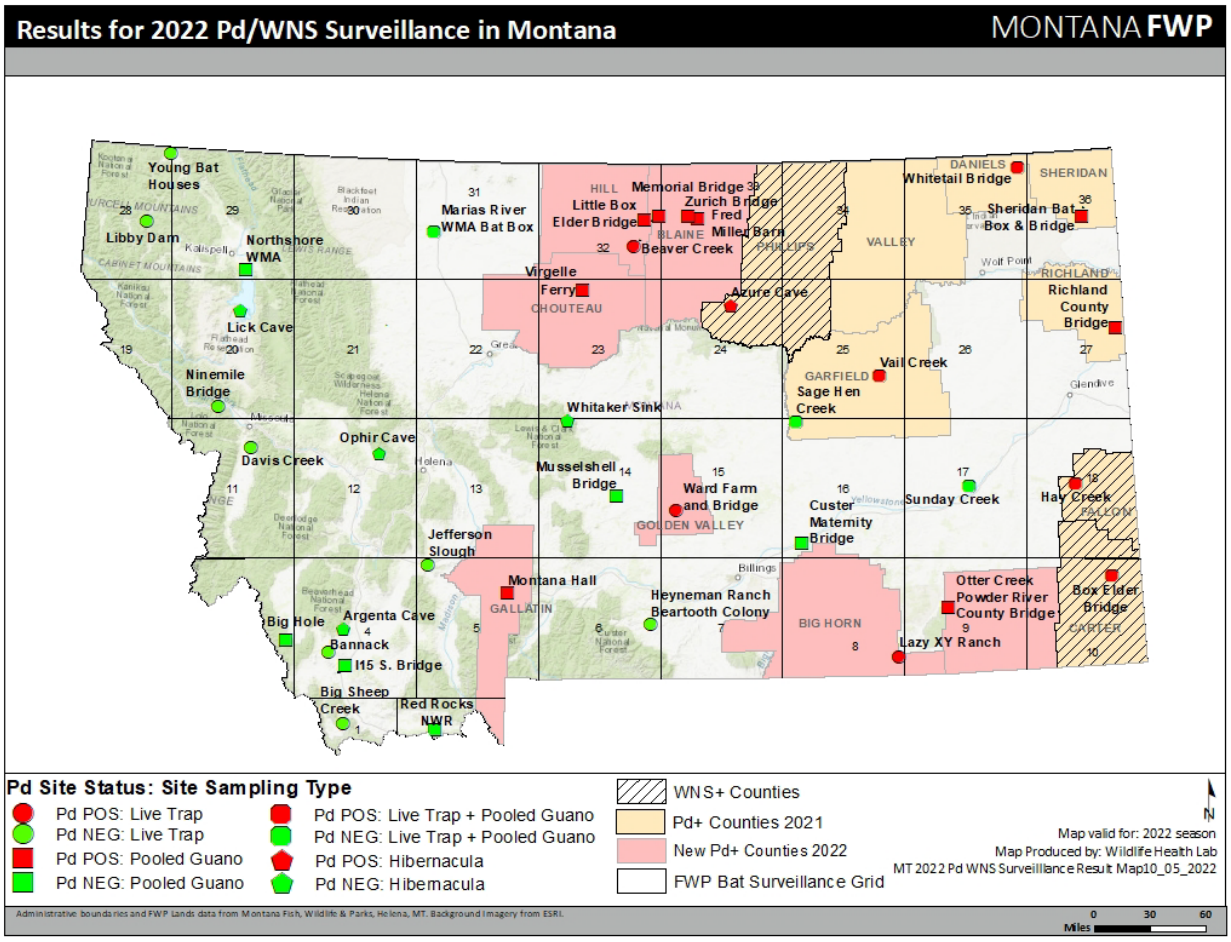

Figure 4. 2022 *Pd*/WNS surveillance sites, *Pd* status, and county-level *Pd* and WNS status. Higher-intensity sampling on the western side of the state was prioritized based on the National Wildlife Health Center's annual *Pd* spatial spread model.

Sample types collected included live bat samples (Bat Swab), bat swab paired with a fecal from the same individual anima, environmental swabs, fecal samples from the environment, and soil samples. Of the 38 sites surveyed across Montana, 16 tested positive for *Pd* by PCR on at least one bat swab, guano/pooled guano, or environmental sample collected at the site (Figure 4, Table 1). For 10 sets of paired samples from individual bats (bat swab + fecal sample), *Pd* detection results were the same regardless of sample type. For 4 sets of paired samples, results varied by sample type (Table 2). Of samples collected from live bats, *Pd* was detected on little brown bats (MYLU), long-legged myotis (MYVO), and from unidentified Myotis species. This is the first year *Pd* has been detected long-legged myotis in Montana.

Prior to the 2021-22 WNS surveillance season, *Pd* had been detected in Phillips, Valley, Garfield, Daniels, Sheridan, Richland, Fallon and Carter Counties. Samples were collected from each of these counties except for Valley and Phillips Counties during the 2021-22 season, and *Pd* was again detected in each of those resampled counties. New *Pd*-positive sites in 2022 included Beaver Creek, Fred Miller Barn, LazyXY Ranch, Little Box Elder Bridge, Memorial Bridge, Montana Hall (MSU), Otter Creek Bridge, Vail Creek, Virgelle Ferry, Ward Farm and Bridge, and Zurich Park Bridge, confirming presence of the fungus farther west than previously documented in Montana.

WNS was confirmed for the first time at Azure Cave in Phillips County during the previous surveillance season on May 21, 2021, among 3 symptomatic adult male little brown bats that were euthanized and submitted to the NWHC for testing. During the surveillance survey, an estimated 1/3-1/2 of the 2,000 hibernating bats exhibited fungal growth consistent with WNS on their noses and wings (Figure 5). When Azure Cave was surveyed on May 13, 2022 biologists found just 40 bats, an approximately 98% decline in the number of hibernating bats. Mortality events have not been observed at other sites, and *Pd* has not yet been detected at the other caves sampled in the state.

Data from 87 cells and 336 detector deployments was analyzed by Montana Natural Heritage Program. Of the 755,824calls recorded within these cells, 12,258 call sequences were to determine species presence. Analysis documented definitive calls for 13 bat species. Bat diversity at cells varied significantly across the study area, with confirmation of 1-10 species at individual cells (Bachen 2022).

Figure 5. Photograph of WNS-affected little brown bats in Azure Cave, May 21, 2021.

Table 1. Table of 2022 *Pd*/WNS surveillance sites, site types, dates of sampling, *Pd*-positive samples (P) and total sample size (N) by specimen type, bat species sampled, bat species that were *Pd*-positive, and whether WNS was detected at a site.

				Specimen Type and Number of Pd Detections (P) and Total Samples Tested (n)						
Site	**County**	**Site Type**	**Sample Date**	**Bat Swab (P/n)**	**Bat Swab + Fecal Sample (P/n)**	**Environmental Swab (P/n)**	**Fecal Sample (P/n)**	**Soil (P/n)**	**Bat Species Sampled**	**Pd Positive Bat Species**
Red Rocks NWR Buildings	Beaverhead	Building Roost	6/22/22			0/5	0/5			
I15 Southbound Bridge	Beaverhead	Bridge Roost	6/22/22			0/5	0/5			
Big Sheep Creek	Beaverhead	Landscape	6/10/22	0/24	0/1				MYSP,MYEV	
Big Hole Roost	Beaverhead	Building Roost	6/23/22			0/5	0/5			
Bannack State Park	Beaverhead	Building Roost/Landscape	6/1/22	0/10	0/1		0/28		MYSP, MYLU	
Argenta Cave	Beaverhead	Hibernacula	6/1/22	0/1			0/30	0/14	MYVO	
LazyXY Ranch	Big Horn	Landscape	6/10/22	0/4	2/13				EPFU, MYLU	MYLU
Zurich Park Bridge	Blaine	Bridge Roost	6/2/22, 6/9/22				22/22		NA	NA
Memorial Bridge	Blaine	Bridge Roost	6/19/22				20/45			
Fred Miller Barn	Blaine	Building Roost	6/24/22				21/45			
Box Elder Bridge	Carter	Bridge Roost	6/8/22	4/20	4/4		3/3		EPFU, MYLU, MYVO	MYLU, MYVO

Site	County	Site Type	Sample Date	Bat Swab (P/n)	Bat Swab + Fecal Sample (P/n)	Environmental Swab (P/n)	Fecal Sample (P/n)	Soil (P/n)	Bat Species Sampled	Pd Positive Bat Species
Virgelle Ferry	Choteau	Building Roost	6/20/22				22/45		NA	
Sunday Creek	Custer	Bridge Roost	6/22/22	0/10	0/15				EPFU, MYCL	
Whitetail Bridge	Daniels	Bridge Roost	6/21/22	4/10	6/15				MYLU	MYLU
Hay Creek	Fallon	Bridge Roost	6/3/22	0/11	4/5		0/1	0/18	EPFU, MYLU, MYVO	MYLU, MYVO
Northshore WMA	Flathead	Building Roost	6/4/22				0/45			
Montana Hall	Gallatin	Building Roost	6/21/22			1/5	5/5			
Vail Creek	Garfield	Bridge Roost	6/2/22	0/9	1/6			0/19	EPFU, MYCL, MYLU	MYLU
Sage Hen Creek	Garfield	Bridge Roost	6/1/22		0/3	0/2		0/32	MYLU	
Ward Farm and Bridge	Golden Valley	Bridge Roost	6/7/22	0/2	0/4		3/28		MYSP, MYVO	
Little Box Elder Bridge	Hill	Bridge Roost	6/24/22				4/13	0/27		
Beaver Creek	Hill	Landscape	6/8/22	5/8	1/3	2/10		1/11	MYSP	MYSP
Jefferson Slough	Jefferson	Ladscape / Bridge Roost	5/31/22	0/9	0/1	0/14	0/14		EPFU, LANO	
Whitaker Sink	Judith Basin	Hybernacula	5/15/22	0/25					EPFU, MYLU, MYSP, MYVO	
Lick Cave	Lake	Hybernacula	4/13/22	0/29					YULU	
Ophir Cave	Lewis and Clark	Hibernacula	5/10/22			0/7	0/35	0/3		

Site	County	Site Type	Sample Date	Bat Swab (P/n)	Bat Swab + Fecal Sample (P/n)	Environmental Swab (P/n)	Fecal Sample (P/n)	Soil (P/n)	Bat Species Sampled	Pd Positive Bat Species
Young Bat Houses	Lincoln	Bat Box	5/15/22	0/23	0/2		0/7		MYYU	
Libby Dam	Lincoln	Bat Box	5/14/22	0/18	0/7		0/2		MYYU	
Ninemile Bridge	Missoula	Bridge Roost	6/16/22	0/5				0/35	MYSP	
Davis Creek	Missoula	Landscape	5/23/22	0/5		0/5		0/11	EPFU, MYEV, MYVO	
Otter Creek Bridge	Powder River	Bridge Roost	5/24/22				1/11			
Richland County Bridge	Richland	Bridge Roost	5/11/22, 6/7/22				2/4	0/20		
Sheridan Bat Box and Bridge	Sheridan	Bat Box/Bridge Roost	6/9/22				44/44			
Heyneman Ranch	Stillwater	Building Roost	6/13/22	0/5				0/26	MYLU	
Marias River WMA	Toole	Bat Box	6/8/22	0/25			0/5		MYLU	
Musselshell Bridge	Wheatland	Bridge Roost	6/29/22				0/9	0/35		
Custer Bridge	Yellowstone	Bridge Roost	6/29/22				0/44			

Table 2. Paired bat swab and fecal results, *Pd* positive (P) and total sample size (n) from the same animals by sample type. In most cases *Pd* detection result was the same for each sample type; however, for asterisked sites, Pd detection result differed by sample type.

Pd Detections for Paired Samples by Sample Type			
Site	**Number of Animals with Paired Samples (n) Bat Swab + Fecal**	**Pd Detected on Fecal (P/n)**	**Pd Detected on Bat Swab (P/n)**
Bannack State Park	1	0/1	0/1
Beaver Creek*	3	0/3	1/3
Bigh Sheep Creek	1	0/1	0/1
Box Elder Bridge	4	4/4	4/4
Hay Creek*	5	0/5	4/5
Jefferson Slough	1	0/1	0/1
LazyXY Ranch*	13	0/13	2/13
Libby Dam	7	0/7	0/7
Little Box Elder Bridge	4	4/4	4/4
Sunday Creek	15	0/15	0/15
Vail Creek	6	1/6	1/6
Ward Farm and Bridge	4	0/4	0/4
Whitetail Bridge*	15	1/15	5/15
Young Bat Boxes	2	0/2	0/2

Discussion

With the help of numerous agency staff and partners, FWP significantly expanded its *Pd* and WNS surveillance effort in 2022 to include much broader and more intensive sampling across the state. We detected *Pd* at 16 of 38 sampled sites, including 11 new sites in 7 new counties extending farther west than previously documented. We did not detect any new mortalities of WNS-affected bats in 2021-22; however, in the eastern US, initial *Pd* detection is generally followed 1-2 years later by WNS and WNS-mortalities among susceptible species (Frick et al. 2017).

Although the 2021-22 NWHC *Pd* prediction model (Figure 1) identified southwestern and northwestern Montana as the highest-ranking priority sampling cells in the state, *Pd* was only detected at one site (Montana Hall, Gallatin County) in these priority cells; however, *Pd* was detected for the first time in central Montana. This suggests that either *Pd* is moving more slowly than predicted, that prevalence is still too low (<15% of the affected population), or its distribution is too patchy to detect.

Related Ongoing Work

In 2021-22, we continued our annual acoustic monitoring among 87 NABat Program grid cells to gather the information necessary to understand how species-specific occupancy and bat activity are changing in response to WNS in Montana. These data and additional data collected the same year following these protocols will be analyzed further to aid in determining the trajectory of populations of WNS susceptible species (Bachen, 2022). This is collaborative work involving numerous agency partners, land managers, and landowners. Details regarding acoustic data analysis and the NABat Program work can be found in the full report (Appendix A): Analysis of North American Bat Monitoring Program 2021 Data in Montana (2022).

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Appendix A.

Analysis of North American Bat Monitoring Program 2021 Data in Montana

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INTRODUCTION

In order to monitor bat distribution and status, Montana Fish Wildlife and Parks (FWP) and the Montana Natural Heritage program (MTNHP) have been collecting acoustic data to monitor species distributions and activity for more than 15 years. Detectors have been deployed for a variety of reasons, including to answer site-specific questions related to the impacts of wind-energy development; forest management; as part of a state-wide monitoring program for small mammals, bats and herptiles; and more recently as part of the protocol of the North American Bat Program (NABat; Loeb et al. 2015). Recently, Wright et al. (2019) analyzed some of these acoustic data to provide pre-WNS baseline estimates of state-wide species-specific occupancy and site-specific bat activity. As part of this analysis, Wright et al. (2019) conducted a power analysis to determine the sampling effort needed to detect threshold declines in occupancy and activity, and suggested improvements for statistical sampling design. In 2020, FWP coordinated the first year of acoustic monitoring, incorporating Wright et al.'s (2019) recommendations and following the NABat Program grid and guidelines. The goal of annual acoustic monitoring was to cover 100 high priority NABat grid cells. Monitoring has occurred across subsequent years to collect data to assess status and trend for the state's bat species. This report summarized data from 87 of these cells surveyed in 2021 as part of a multi-agency collaboration to monitor indices of population trends for bat species threatened by White-Nose Syndrome (WNS) and mortality at wind energy facilities.

METHODS

BAT DETECTOR/ RECORDER DEPLOYMENT

Across Montana detector recorder units (hereafter detectors) were deployed at 87 10 x 10 km^2 cells beginning in June and no later than the first week of August. The beginning of the deployment window was set to coincide with the probable end of the spring migration period and the end to coincide with the volancy of young of the year (Bachen et al. 2020). Cells were selected from the North American NABat grid and prioritized by GRTS number with lower cells determined to be of higher priority. The first 100 cells were designated as high priority and the next 80 cells as secondary priority to serve as oversamples incase the priority cells were unavailable for survey. Within each cell up to four detectors were deployed with as much spatial separation as possible give access to the site and other logistical considerations.

Within each cell detectors were deployed across a diversity of features likely to be used by bats including (1) open water for as much of the year as possible used to drink and forage; (2) rock outcrops and trees that might be used as roosts by bats; (3) habitat edges and flyways that may be used to commute between roosts, drinking sites and foraging areas; (4) areas in reasonable proximity to roads or trails to facilitate efficient survey; and (5) a low likelihood of vandalism. Across all sites we used one of three detector and microphone combinations: (1) Song Meter SM2Bat+ detector/recorder with an SMX-U1 microphone (Wildlife Acoustics Inc., Maynard, MA); (2) Song Meter SM3Bat detector/recorder with an SMX-U1 microphone (Wildlife Acoustics Inc., Maynard, MA); or (3) Song Meter SM4Bat detector/recorder with a U2 Microphone (Wildlife Acoustics Inc., Maynard, MA). Detectors were placed for a minimum of 4 nights before retrieval.

DATA MANAGEMENT & CALL ANALYSES

Bat call sequences were analyzed with the goal of definitively identifying individual species presence by site and cell in accordance with the Echolocation Call Characteristics of Montana Bats and Montana Bat Call Identification materials (Bachen et al. 2018). After initial import of call files, the Sonobat program was used to identify files containing bat call sequences. Call sequences were then analyzed to species using one or more classifier depending on the geographic location where the detector was placed to account for differences in species presence across the state. For sites west of the continental divide and in the mountains of central Montana, the "Montana West" classifier was used. For those east of the divide and outside of mountainous areas of central Montana both the "Montana Plains" and "Montana South" classifiers were used. Species identifications suggested by the classifier at a given site and cell were then vetted to confirm the presence of that species. Full spectrum sonograms were reviewed in Sonobat for call quality then attributes of search-phase call sequences were compared to criteria for definitive species ID in Bachen et al. 2018. If one or more sequences met these criteria, we considered the species present at the detector location during the period of interest.

Acoustic file recordings, in both original WAC and processed WAV formats, are stored in the Montana Bat Call Library which is housed on a series of 15-20 Terabyte Drobo 5D and 5N storage arrays at the Montana State Library as well as a secondary offsite location to protect against catastrophic loss. Acoustic analysis results were all processed and combined within SQL database tables in accordance with the general workflow pattern for data management and analysis outlined in the text and in Appendices 8-10 of Maxell (2015).

RESULTS

We analyzed data from 87 of cells containing 336 detector deployments (Figure 1). Of the 755,824calls recorded within these cells we reviewed 12,258 call sequences to determine species presence (Table 1). The automated identification algorithm in the Sonobat software initially identified sequences from all 15 species of bats found in Montana, however several species are infrequently identified using acoustics as they have a high degree of overlap with other conspecifics and these identifications were likely made in error. Through hand vetting we found definitive calls for 13 of these 15 species. Diversity at cells varied significantly across the project area with a minimum of a single species to a maximum of 10 species confirmed through hand vetting.

Data detailing species ID, collection location, and call attributes have been provided to NABat personnel for analysis to meet state-wide bat monitoring goals and are expected to be appended to publicly available databases pending completion of the analysis of these data.

CONCLUSIONS

The variation in number of calls and diversity of species recorded at each cell was not surprising. Given that deployment protocols directed placement of detectors across a wide range of habitat features, that abiotic conditions such as weather and moon phase known to impact bat activity were varied across the approximately 2-month deployment window, and that cells were randomly assigned across a landscape with varying habitat suitability, variation in the number of calls and species recorded would be expected. Furthermore, NA Bat protocols rely on short deployment periods with model prediction of species site occupancy rather than extended deployment to endure a species that is present is recorded.

Two species of bat were not confirmed within these data but may have been present within cells and not have been recorded or if recorded not identified using our methods. In particular, Pallid Bat and Northern Myotis are difficult if not impossible to distinguish from other co-occurring species. Both species have restricted

distributions within Montana (Bachen et al. 2020), so non-detection may represent true absence. Spotted Bat is rare across much of its range within the state, but calls are readily identifiable due to their low characteristic frequency (Bachen et al. 2018).

As of 2021, *Pseudogymnoascus destructans* (Pd) and the disease it causes, WNS are well established in the eastern region of the state. As the disease increasingly impacts Montana's bats, determining the extent of these impacts and implications for status and management of the species and the lands they inhabit is imperative/ These data and additional data collected the same year following these protocols will be analyzed further to aid in determining the trajectory of populations of WNS susceptible species.

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FIGURES AND TABLES

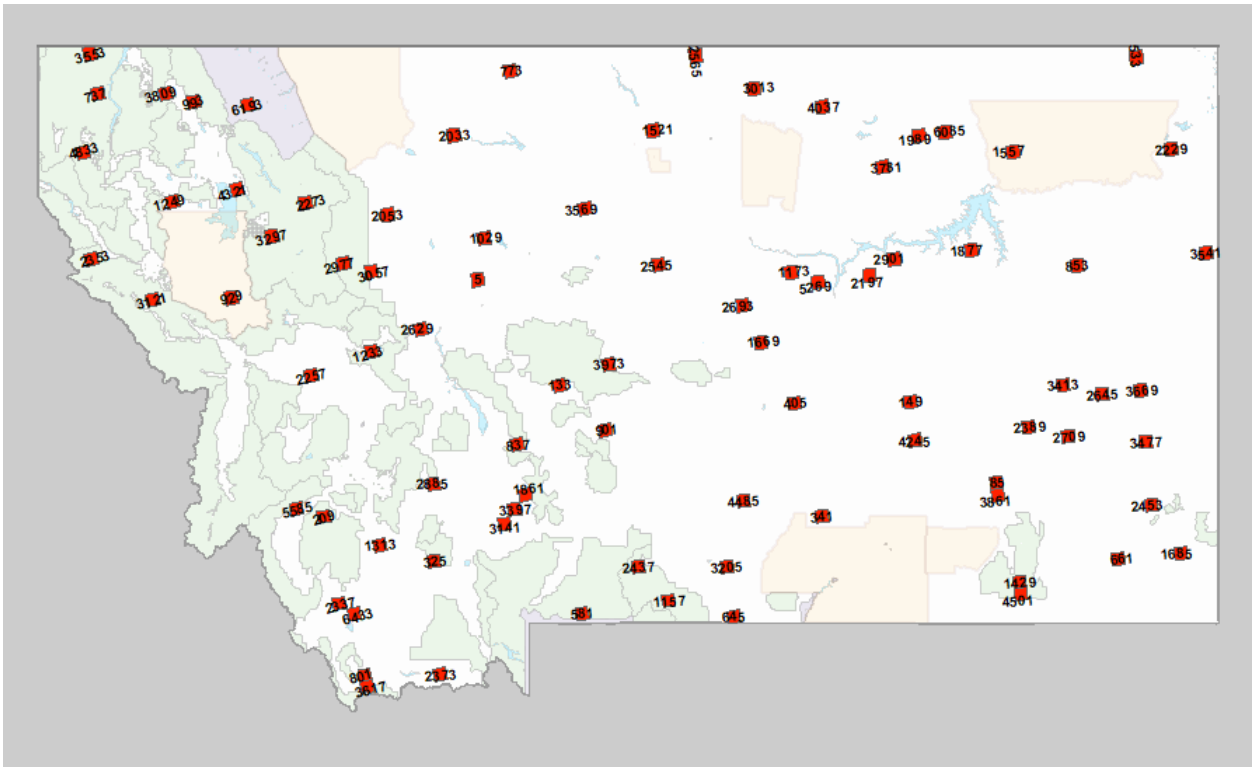

Figure 1. Cell locations (Red Squares) where data was collected labeled with GRTS IDs (Cell ID)

	Townsend's Big-eared Bat	Big Brown Bat	Spotted Bat	Eastern red Bat	Hoary Bat	Silver-haired Bat	California Myotis	Western Small-footed Myotis	Long-eared Myotis	Little Brown Myotis	Fringed Myotis	Long-legged Myotis	Yuma Myotis
Cell 661	N	P	N	N	P	N	N	P	N	P	N	N	N
Cell 1685	N	N	N	N	P	N	N	N	N	P	N	N	N
Cell 3477	N	P	N	N	P	P	N	N	N	P	P	N	N
Cell 3013	N	N	N	N	N	N	N	N	N	P	N	N	N
Cell 853	N	P	N	N	P	P	N	P	N	N	N	N	N
Cell 149	N	N	N	P	P	N	N	P	N	P	N	N	N
Cell 3413	P	P	N	N	P	P	N	P	N	N	N	N	N
Cell 4501	N	P	N	N	P	P	N	N	P	P	P	N	N
Cell 4037	N	N	N	N	P	N	N	N	N	P	N	N	N
Cell 3861	P	P	N	N	P	N	N	P	N	P	N	N	N
Cell 2885	N	N	N	N	P	N	N	N	P	P	P	N	N
Cell 3781	N	N	N	P	N	N	N	N	N	P	N	N	N
Cell 1861	N	P	N	N	P	N	N	N	P	P	P	N	N
Cell 2453	P	P	N	N	P	P	N	P	N	P	N	N	N
Cell 1557	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 3669	N	P	N	N	P	P	N	P	P	N	P	N	N
Cell 2645	P	P	N	N	P	P	N	P	P	N	P	N	N
Cell 341	N	P	N	N	P	N	N	N	N	P	N	N	N
Cell 1429	N	P	N	N	P	P	N	P	P	P	P	N	N
Cell 3541	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 581	N	P	P	N	P	P	N	N	P	P	N	N	N
Cell 2565	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 2709	P	P	N	N	P	P	N	P	P	P	N	N	N
Cell 2197	P	P	N	N	P	P	N	P	P	P	P	N	N
Cell 85	N	P	N	P	P	P	N	P	P	P	P	N	N

	Townsend's Big-eared Bat	Big Brown Bat	Spotted Bat	Eastern red Bat	Hoary Bat	Silver-haired Bat	California Myotis	Western Small-footed Myotis	Long-eared Myotis	Little Brown Myotis	Fringed Myotis	Long-legged Myotis	Yuma Myotis
Cell 4485	N	P	N	N	P	P	N	P	N	P	N	N	N
Cell 1877	P	P	N	P	P	P	N	P	P	P	P	N	N
Cell 3057	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 3569	N	P	N	P	P	P	N	N	N	P	N	N	N
Cell 4245	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 2389	P	P	N	P	P	P	N	P	P	P	P	N	N
Cell 533	N	P	N	N	P	P	N	P	P	P	N	N	N
Cell 2033	N	P	N	N	P	P	N	P	N	P	N	N	N
Cell 773	N	N	N	P	P	N	N	N	N	P	N	N	N
Cell 2901	P	P	N	P	P	P	N	P	P	P	N	N	N
Cell 4849	N	P	N	P	P	P	N	P	N	P	N	N	N
Cell 2545	N	N	N	N	P	N	N	N	N	P	P	N	N
Cell 2693	N	P	N	P	P	P	N	N	N	P	N	N	N
Cell 405	N	P	N	P	P	P	N	P	P	P	N	N	N
Cell 1669	N	P	N	P	P	P	N	N	P	P	N	N	N
Cell 2229	N	P	N	N	P	P	N	N	N	P	N	P	N
Cell 837	N	P	N	N	P	P	N	N	P	P	N	P	N
Cell 6085	N	P	N	N	P	P	N	N	N	P	N	P	N
Cell 1521	N	P	N	P	P	P	N	N	N	P	N	P	N
Cell 1173	N	P	N	P	P	N	N	P	N	P	N	P	N
Cell 5269	P	P	N	P	P	P	N	P	P	P	N	P	N
Cell 1989	N	P	N	P	P	P	N	P	N	P	N	P	N
Cell 1313	N	N	P	N	P	N	N	P	P	P	N	N	N
Cell 645	N	P	N	P	N	N	N	P	N	P	N	N	N
Cell 209	N	P	N	N	N	N	P	N	P	P	N	N	N

	Townsend's Big-eared Bat	Big Brown Bat	Spotted Bat	Eastern red Bat	Hoary Bat	Silver-haired Bat	California Myotis	Western Small-footed Myotis	Long-eared Myotis	Little Brown Myotis	Fringed Myotis	Long-legged Myotis	Yuma Myotis
Cell 3141	N	P	N	N	P	N	N	N	N	P	N	N	N
Cell 3617	N	N	N	N	P	N	N	N	P	P	N	N	N
Cell 993	N	N	N	N	P	P	N	N	N	P	N	N	N
Cell 3553	N	N	N	N	N	N	N	N	N	P	N	N	N
Cell 1249	N	N	N	N	P	P	N	N	P	P	N	N	N
Cell 3973	N	P	N	N	P	P	N	N	P	P	N	N	N
Cell 737	N	P	N	N	N	P	P	N	N	P	N	P	N
Cell 929	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 1233	N	P	N	N	P	P	N	N	P	P	N	N	N
Cell 2053	N	N	N	N	N	P	N	N	N	P	N	N	N
Cell 901	N	N	N	N	P	P	N	N	N	P	N	N	N
Cell 2257	N	P	N	N	P	P	N	N	P	P	N	N	N
Cell 5	N	P	N	P	P	P	N	P	N	P	N	N	N
Cell 3205	N	P	N	P	P	P	N	P	P	P	N	N	N
Cell 2977	N	P	N	N	P	P	N	N	P	P	N	P	N
Cell 1029	N	N	N	N	P	N	N	N	N	P	N	N	N
Cell 4833	N	P	N	N	P	P	P	N	P	P	N	N	N
Cell 5585	N	P	N	N	P	P	P	N	P	P	N	N	N
Cell 4933	N	N	N	N	P	P	N	N	P	P	N	N	N
Cell 325	N	P	N	N	N	N	N	P	P	P	P	N	N
Cell 1489	N	N	N	N	P	P	N	N	P	P	N	N	N
Cell 1157	N	P	N	P	P	P	N	P	P	P	P	P	N
Cell 3397	N	P	N	N	P	P	N	N	N	P	N	N	N
Cell 3809	N	P	N	N	P	P	N	N	P	P	N	N	N
Cell 2273	N	N	N	N	P	P	N	N	P	P	N	N	N

	Townsend's Big-eared Bat	Big Brown Bat	Spotted Bat	Eastern red Bat	Hoary Bat	Silver-haired Bat	California Myotis	Western Small-footed Myotis	Long-eared Myotis	Little Brown Myotis	Fringed Myotis	Long-legged Myotis	Yuma Myotis
Cell 2373	N	N	N	N	P	P	N	N	N	P	N	N	N
Cell 2629	N	P	N	N	P	P	N	N	P	P	P	P	N
Cell 2353	N	P	N	N	P	P	P	N	P	P	N	N	N
Cell 3121	N	N	N	N	P	P	P	P	P	P	N	N	P
Cell 2437	P	P	N	P	P	P	N	P	P	P	N	P	P
Cell 801	N	P	N	N	P	P	N	P	P	P	N	N	P
Cell 6193	N	P	N	P	P	P	P	P	P	P	N	P	P
Cell 2337	N	P	N	N	P	P	P	P	P	P	N	N	P
Cell 6433	N	P	N	N	P	P	N	P	N	P	N	N	P
Cell 133	N	P	N	P	P	P	N	N	P	P	N	N	P
Cell 4321	P	P	N	N	P	P	N	N	P	P	P	N	P
Cell 3297	P	P	N	N	P	P	P	N	P	P	P	P	P