



Montana's 2023 Annual White-nose Syndrome Surveillance and Bat Monitoring Report

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Summary

Since 2019, Montana Fish, Wildlife and Parks, the U.S. Geological Survey, and the Montana Natural Heritage Program (MTNHP) have been collaborating on a project designed to measure the spread and impact of white-nose syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans* (*Pd*), on Montana's bats. This project involves annual, statewide surveillance for *Pd* and WNS to estimate the distribution of the fungus and disease, coupled with annual acoustic monitoring to assess bat occupancy and activity. We first detected *Pd* in Montana in 2020, followed by the disease, WNS, in 2021; both detections occurred in the eastern portion of the state. In 2023, we surveyed 31 sites spanning the state, with an emphasis on western Montana, 9 of which were *Pd*-positive. While we documented the continued spread of *Pd* and WNS, we have yet to detect either in the western-most portion of the state, including west of the Continental Divide. To date, *Pd* has been detected in four species across 16 counties within Montana. WNS has been documented in three species within six of those counties.

In 2023, state, federal, tribal, and non-governmental partners collaborated to collect acoustic data from approximately 112 geographic grid cells to monitor bat species distribution and activity as part of the North American Bat Monitoring Program (NABat). MTNHP is compiling and analyzing acoustic data collected during this effort and will upload data and results to the NABat database. Here we present results from the 2022 and 2023 acoustic survey effort, including average nightly activity and species richness across surveyed cells.

The first analysis of the impacts of *Pd* and WNS on bat occupancy and activity in Montana has been accepted for publication (Stratton et al. *In press*). Notably, this analysis demonstrated a modest and variable, but detectable effect of *Pd* presence on acoustic activity, which we assume is related to abundance, of WNS-susceptible bat species. Understanding the impacts of WNS on Montana's bat populations will inform decisions related to management and conservation strategies, including potential use of treatments specific to WNS or ecological approaches toward offsetting the costs of disease.

We conclude the report with updates on related ongoing projects: a structured decision making exercise to inform WNS and bat management in Montana, a project to collect summer colony counts, and the development of an updated bat acoustic call library.

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 <https://www.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 2023, *Pd* has been detected in all 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, Langwig et al. 2012, Frick et al. 2015, Nocera et al. 2019, Cheng et al. 2021), and as a result, several bat species have been listed, or petitioned for listing, under the United States Endangered Species Act (ESA) (Kunz and Reichard 2010, U.S. Fish and Wildlife Service 2022a & b).

Pd thrives in cool and humid subterranean conditions (Langwig et al. 2012, Verant et al. 2012). Transmission occurs during fall and winter seasons via direct contact between bats and *Pd*-contaminated environments. Most transmission revolves around winter hibernacula where infected bats shed spores that infect neighboring bats, contaminate cave environments, and persist throughout the year, and surviving spores 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 hibernation until spring emergence mount an extreme inflammatory immune response to *Pd* which further contributes to mortality (Lilley et al. 2017, Davy et al. 2020). Surviving individuals 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, 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. Cornelison et al. 2014, Cheng et al. 2017, Palmer et al. 2018, Hoyt et al. 2019, Rocke et al. 2019, Turner et al. 2022); ecological approaches towards bolstering bat health and survival in the face of WNS (Wilcox et al. 2016, Cheng et al. 2019); 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 Fish, Wildlife & Parks (FWP) and partners have conducted *Pd* surveillance since 2012, with annual surveillance in at least 4-5 sites across the state since 2017. In 2019, 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 statewide grid of 36 surveillance cells to gather information needed to relate local *Pd* and WNS status to trends in acoustic data collected at nearby North American Bat Monitoring Program (NABat) 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 the first detection of WNS in the spring of 2021 in eastern Montana. Work elsewhere in North America indicates that *Pd* can cause WNS in 7 of Montana's 15 bat species. Nationally, *Pd* has been detected in four other species that occur within the state, and these species may serve as local or regional vectors. Additionally, *Pd* 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 eastern North America 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 East.

In 2019, FWP, the U.S. Geological Survey (USGS), and the Montana Natural Heritage Program (MTNHP) developed a plan to document the arrival and spread of *Pd* and WNS in Montana and to understand the disease's impacts on Montana bat populations (Hanuska-Brown et al. 2019). Specifically, this plan calls for (1) annual surveillance to establish the timing of *Pd* and WNS occurrence across the state, (2) statewide acoustic monitoring over time following NABat guidelines, and (3) an analysis of long-term acoustic data for changes in occupancy and activity associated with WNS. Information gained from this effort 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 2023 *Pd* and WNS surveillance effort, the 2022 and 2023 acoustic monitoring season, and the analysis of *Pd* presence and bat occupancy and activity to date.

Methods

***Pd* and WNS Surveillance and Monitoring**

In this report, we distinguish *surveillance*, or the effort to search for the pathogen or disease where it is not yet known to exist, from *monitoring*, which is designed to track changes in pathogen or disease presence and burden over time after it is detected. In 2023, we adjusted our *Pd* and WNS surveillance and monitoring goals to 1) focus our efforts on areas of the state where *Pd* had yet to be detected, 2) initiate long-term monitoring of fungal loads and disease severity among bats at several *Pd*-positive sites as part of a new project called *Surveillance 4.0*, led by the NWHC, and 3) continue passive surveillance by testing carcasses found in the winter through early summer.

We moved away from attempting to survey all 36 surveillance/monitoring grid cells because this approach created an unsustainable workload, and it was increasingly difficult to capture and sample at some sites in eastern Montana where *Pd* had been present for several years and where some species/roosts had noticeably declined. Furthermore, the questions we had hoped to explore behind the epidemic front, regarding *Pd* loads and disease severity among species, are now part of the objectives of NWHC's *Surveillance 4.0*, in which we decided to participate. Thus, FWP's 2023 active surveillance/monitoring sites were selected based on 1) predictions from the NWHC annual *Pd* spatial spread model (Fig. 1, U.S. Geological Survey 2019); 2) an attempt to survey any of FWP's 36 surveillance grid cells where *Pd* has not yet been detected (Fig. 2); and 3) participation in NWHC's *Surveillance 4.0*. Within the NWHC or FWP-prioritized areas or grid cells, local biologist expertise and susceptible species-specific occupancy maps (Fig. 3, Wright et al. 2018) were used to identify hibernacula, spring emergence mist-net sites, or maternity roost sites for sampling.

At surveillance and monitoring sites across the state, we used a range of survey types, including hibernacula surveys, live animal trapping, or pooled guano and environmental sampling. Survey type was determined by the logistical feasibility of site access and potential for capturing and sampling live animals. Hibernacula surveys involved swabbing hibernating bats, cave substrates, and 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 #FWP04-2023). Pooled guano surveys involved collecting fresh guano (either 5

50-ml conical tubes or 45 2-ml cryovials filled ¾ full) and environmental swabs at early-season roost sites in buildings, beneath bridges, or in bat boxes. Environmental sampling involved collecting soil, guano, or swabs of roost substrates or mist-nets. While *Pd* would be detectable using any of these survey types, only live animal sampling, hibernacula surveys, or the opportunistic collection of dead bats would provide opportunities to detect disease and mortality from WNS.

Recommended high priority cells and ecosections & states for 2022-11-01 by lowest prevalence probability

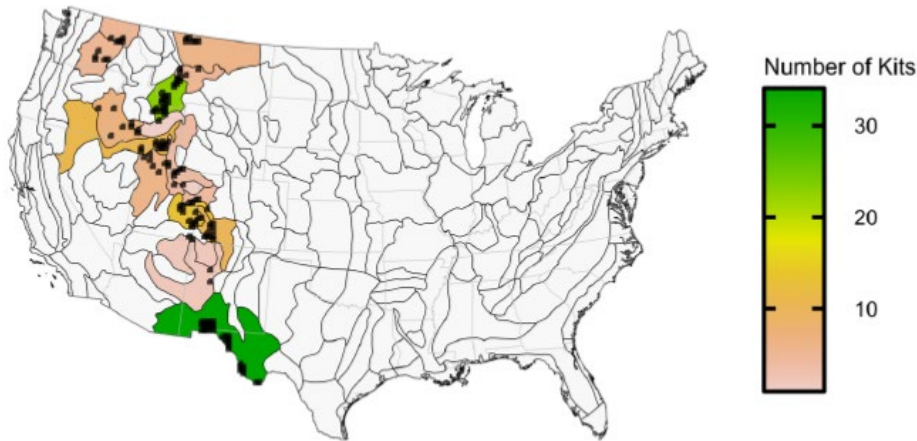


Figure 1. The top 143 highest ranking priority cells (black squares) and associated eco-sections (irregularly shaped polygons) where *Pseudogymnoascus destructans* (*Pd*) was predicted to spread during the 2022/2023 season. Eco-sections highlighted in color are areas where the leading edge of white nose-syndrome (WNS) was predicted to be during the winter of 2022-23. 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. Where possible, Montana Fish, Wildlife & Parks sampled according to the National Wildlife Health Center's priorities, but also conducted additional sampling across the state's 36-cell surveillance grid (Fig. 2) to document *Pd* and WNS's distribution across space and time.

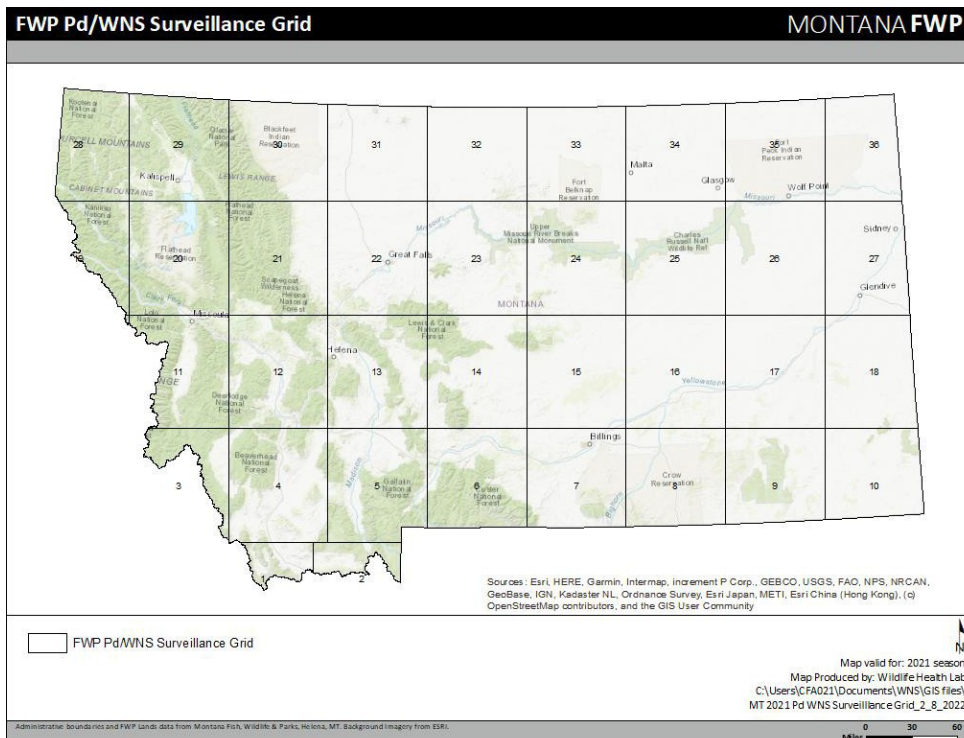


Figure 2. The state of Montana broken into 36 *Pseudogymnoascus destructans* (*Pd*) and white-nose syndrome (WNS) sampling grid cells. In 2023, Montana Fish, Wildlife & Parks prioritized the sampling of grid cells where *Pd* had not yet been detected.

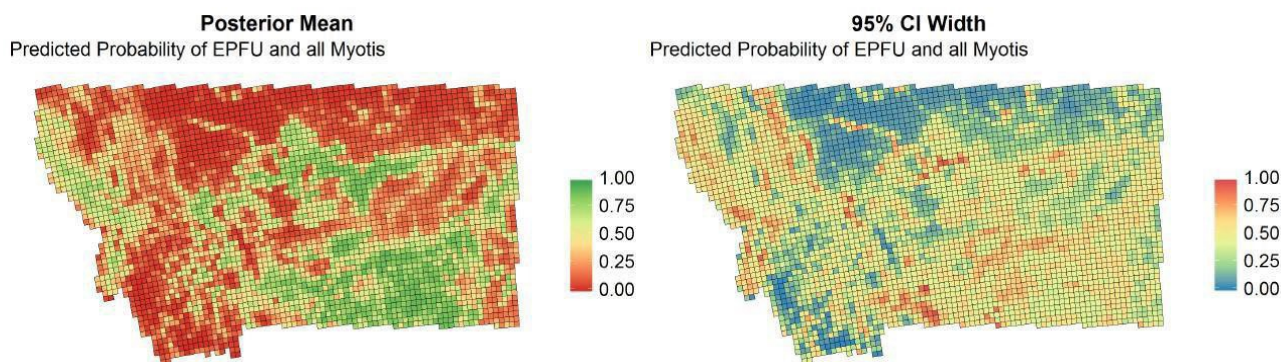


Figure 3. Estimated joint probabilities of occupancy for *Eptesicus fuscus* (EPFU; big brown bat) and all *Myotis* species, the most susceptible species group to white-nose syndrome. Estimates correspond to the probability that these species are present within a grid cell (left) and associated uncertainty (right). Reproduced from Wright et al. (2018).

At hibernacula and live trapping sites, biologists followed NWHC guidance and attempted to collect samples from at least 25 bats (i.e., swabs rolled back and forth five times on the nose and forearm). We recorded species, sex, morphometric measurements, and group sizes, where possible. If we were unable to directly sample 25 bats at a site, we attempted to collect 2 additional environmental samples (i.e., 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. Bats handled during *Pd* surveillance efforts were inspected and scored for symptoms of WNS, including visible signs of the fungus, wing damage (Reichard and Kunz 2009), and orange-fluorescing lesions under a UV light (Turner et al. 2014). Wings of bats captured and handled as part of *Surveillance 4.0* were photographed under UV and white light. We also prioritized the opportunistic testing of symptomatic or dead bats found in the winter through early summer as part of our statewide passive 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).

At sites where pooled guano was collected, 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). After a minimum of four weeks of guano collection, we gathered the fresh guano, mixed it together, and subsampled it for testing, either using the NWHC's pooled guano testing procedures, which involved filling 5 50-ml conical tubes, or by filling 45 1.8-ml cryovials with guano for individual sample testing at Oregon Veterinary Diagnostic Laboratory (OVDL). Polymerase chain reaction (PCR) testing for *Pd* was conducted either at the NWHC or OVDL.

Acoustic Monitoring

Acoustic monitoring was carried out in accordance with NABat, which provides a prioritized, spatially-balanced selection of 10- by 10-km grid cells for sampling (Loeb et al. 2015). Within each cell, we deployed four stationary acoustic bat detectors at spatially-balanced sites selected to maximize the quality and quantity of echolocation recordings (Loeb et al. 2015). Each detector recorded for a minimum of four consecutive nights (Wright et al. 2019). Detectors turned on 30 minutes before sunset and recorded until 30 minutes after sunrise. The sampling window, June through July, is set to occur after the end of the spring migration and before young of the year become volant. At each detector site, we collected information on environmental characteristics that can influence recording quality, such as clutter and distance to water (Loeb et al. 2015).

Bat echolocation call sequences were processed by MTNHP and assigned a species identification using the acoustic autoclassification software (Sonobat, version 4.1, <https://sonobat.com>). A subset of auto-classified echolocation call sequences were manually vetted by experts to confirm species presence at a detector site and within a cell.

Analysis of Bat Activity in Relation to *Pd* Presence

Through a partnership with USGS, Postdoctoral Researcher, Christian Stratton, and Research Statistician, Kathi Irvine, led the analysis of acoustic data collected between 2020-2022 in relation to the detection of *Pd*, as well as other biotic and abiotic site-level covariates. The full analysis and methods can be found in an article entitled, "Joint spatial modeling bridges the gap between disparate disease surveillance and population monitoring efforts informing conservation of at-risk bat species," currently in publication at the Journal of Agricultural, Biological, and Environmental Statistics. Stratton et al. (*In press*) used a "joint" statistical modeling framework designed to accommodate spatial and temporal misalignment of the acoustic and *Pd* surveillance datasets. In this report, we summarize the key findings from this study's analysis of *Pd* and acoustic data from WNS-susceptible bat species, distinguished as having echolocation calls with characteristic frequencies > 34 kHz.

Results

***Pd* and WNS Surveillance and Monitoring**

In 2023, FWP and partners conducted *Pd* and WNS sampling at 31 sites across Montana, 9 of which were found to be *Pd* positive, and 4 of which were confirmed/suspected WNS-positive (Fig. 4; Table 1). Some of these sites were surveyed previously (Fig. 5), whereas others were surveyed for the first time in 2023. We detected *Pd* for

the first time in Carbon, Cascade, Jefferson, Judith Basin, Meagher, and Stillwater counties, and WNS for the first time in Carbon and Chouteau counties. This was the first year that we detected *Pd* on *Myotis evotis* (long-eared myotis) and WNS among *M. evotis* and *M. volans* (long-legged myotis) in Montana. To date, *Pd* has been detected on four of Montana's bat species, including *M. lucifugus* (little brown myotis), *Eptesicus fuscus* (big brown bat), *M. evotis*, and *M. volans*, across 16 counties. WNS has been documented in three species, including *M. lucifugus*, *M. evotis*, and *M. volans*, among six counties.

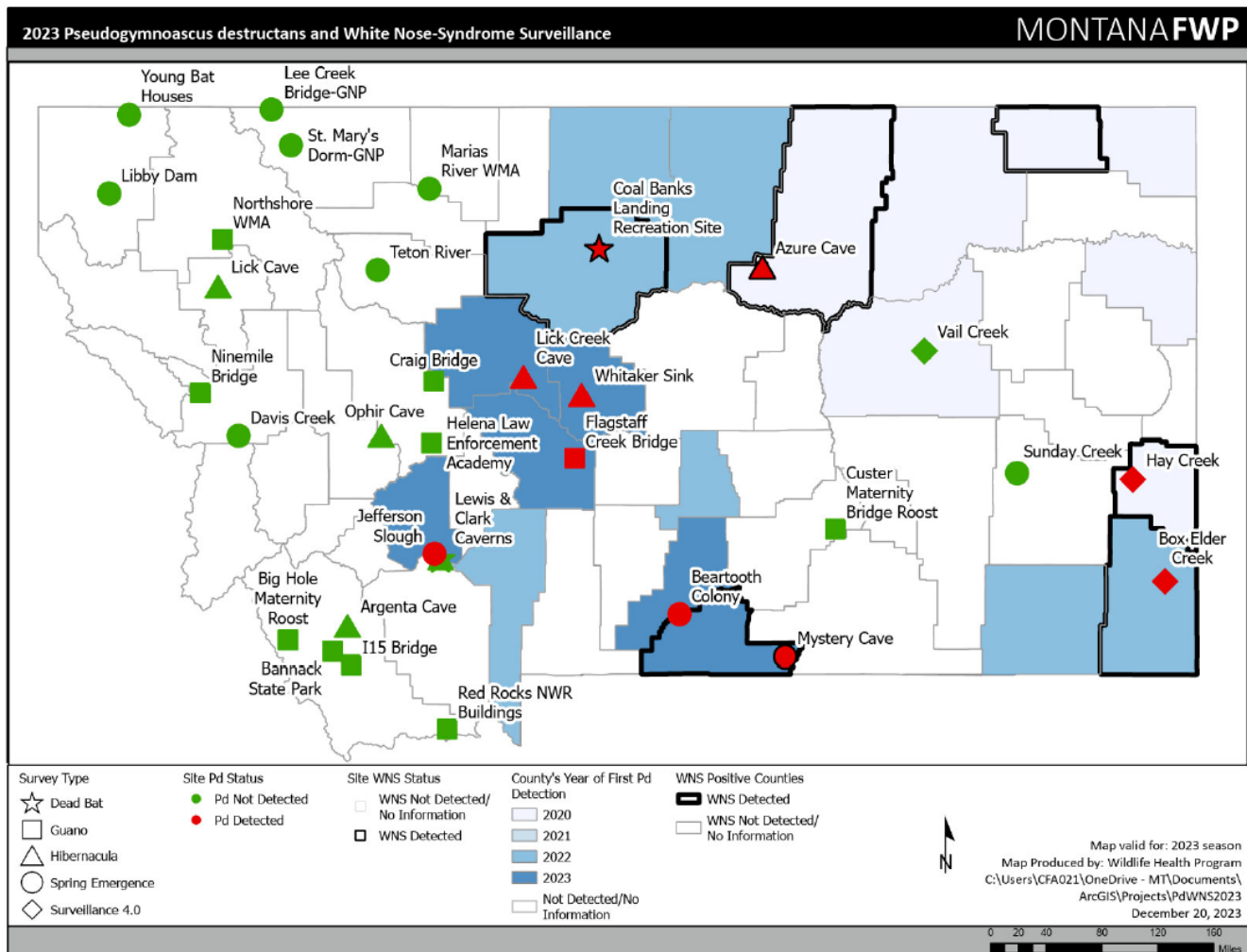


Figure 4. 2023 *Pseudogymnoascus destructans* (*Pd*) and white nose-syndrome (WNS) surveillance sites, *Pd* status, and county-level *Pd* and WNS status in Montana. Higher-intensity sampling on the western side of the state was prioritized based on the National Wildlife Health Center's annual *Pd* spatial spread model.

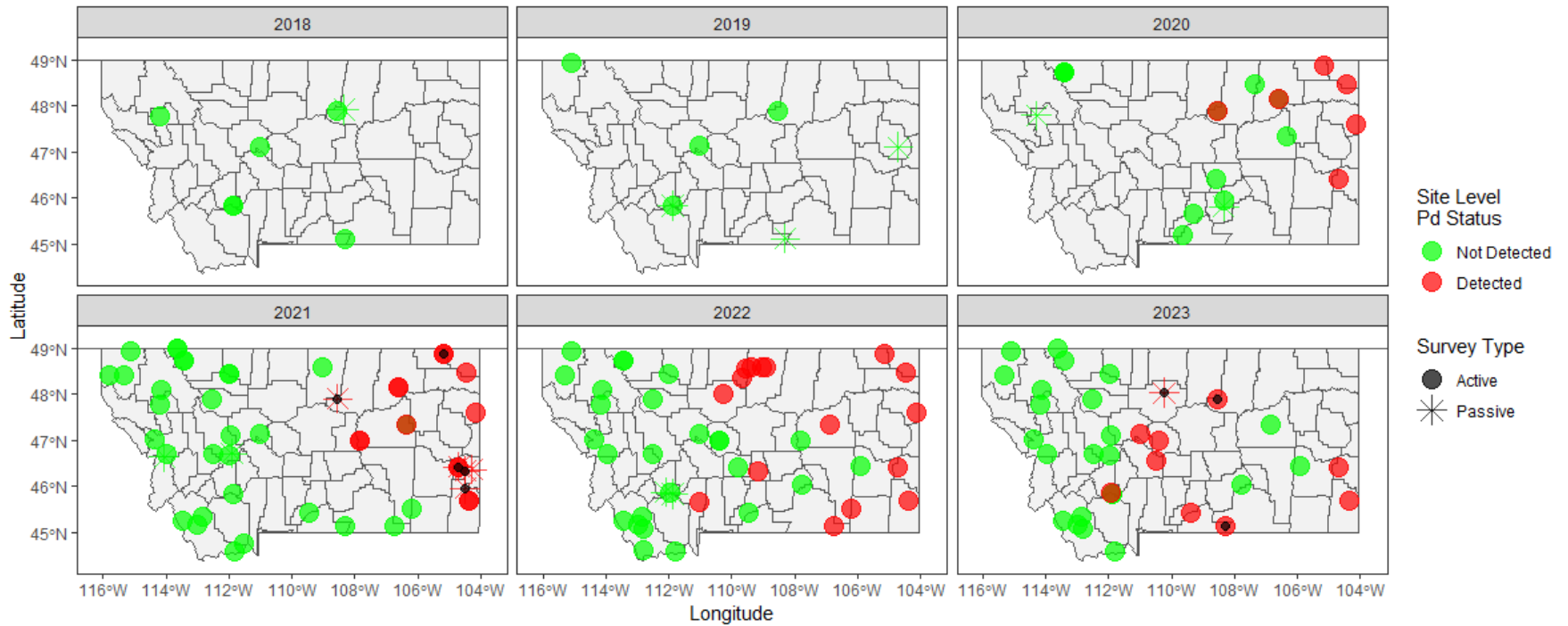


Figure 5. Annual *Pseudogymnoascus destructans* (*Pd*) and white nose-syndrome (WNS) surveillance sites across Montana from 2018-2023, and their corresponding *Pd* status. WNS detections at a site are denoted with a black dot (•). Sites included active surveys for *Pd* and WNS as well as passive surveys, where we opportunistically tested dead or symptomatic bats found in late winter through the end of June.

In 2023, FWP biologists and partners sampled 7 hibernacula, 10 pooled guano sites, and live trapped bats at 14 landscape sites (Fig. 4; Table 1). Live animal sampling occurred on a range of bat species including *M. lucifugus*, *M. yumanesis* (Yuma myotis), *M. ciliolabrum* (Western small-footed myotis), *M. volans*, *M. evotis*, *E. fuscus*, *Corynorhinus townsendii* (Townsend's big-eared bat), and unidentified *Myotis* species (Table 2). Where *Pd* was detected, prevalences were highest among *Myotis* species, with significantly lower prevalences among *E. fuscus*, and no detections among *C. townsendii* (Table 2). Bat swabs and guano samples remained the most valuable sample types for *Pd* detection (Table 1).

In 2023, we had two significant *Pd*/WNS detections within our known hibernacula, including our first detection of *Pd* at Lick Creek Cave in Cascade County and our first detection of *Pd* and WNS at Mystery Cave in Carbon County. These two hibernacula are currently the largest two remaining winter *Myotis* colonies in the state, and they are likely to experience significant disease-induced declines within the next 1-2 years.

We submitted samples from 25 sites to the NWHC to be tested as part of their national surveillance or *Surveillance 4.0*, and samples from the remaining 6 sites were tested at OVDL. In addition, we submitted one individual bat carcass from Coal Banks Landing Recreation Site to the NWHC for diagnostic testing as part of our passive surveillance efforts. This *M. lucifugus* was found to be positive for *Pd* and WNS, representing our first WNS detection in Chouteau County.

Summary of Surveillance 4.0

FWP captured and sampled 48 bats across three sites in eastern Montana (Box Elder Creek, Vail Creek, and Hay Creek) as part of the NWHC's *Surveillance 4.0* (Fig. 4; Table 1). *E. fuscus* were the most commonly captured species, followed by *M. lucifugus* and *M. ciliolabrum*. Only two *M. lucifugus*, one each from Hay Creek and Box Elder Creek, showed any UV fluorescence and/or wing damage (Table 2). *Pd* prevalence ranged from 75-100% among *M. lucifugus*, 0% among *M. ciliolabrum*, and 0-10% among *E. fuscus* (Table 2). *Pd* loads (reported as PCR copy number) were orders of magnitude higher among *M. lucifugus* at both Hay Creek (mean = 465.0 copies of target DNA, SE = 518.0, n = 4) and Box Elder Creek (mean = 30.3 copies of target DNA, SE = 22.6, n = 3) than among *E. fuscus* (Hay Creek: mean = 3.9 copies of target DNA, SE = NA, n = 1; Box Elder Creek: mean = 3.3 copies of target DNA, SE = NA, n = 1) at the two sites. Photographs of bats' wings were submitted to the NWHC for analysis.

Acoustic Monitoring

In 2023, with the help of over 45 participating individuals, we surveyed 445 detector sites across 112 NABat cells. MTNHP is currently processing and analyzing these data. In 2022, partners surveyed 336 detector sites spanning 87 NABat cells. Analysis of these data documented definitive echolocation sequences for 13 bat species within the state. Bat activity (Fig. 6) and diversity (Fig. 7) varied significantly across NABat cells, with confirmation of 1-11 species at individual cells.

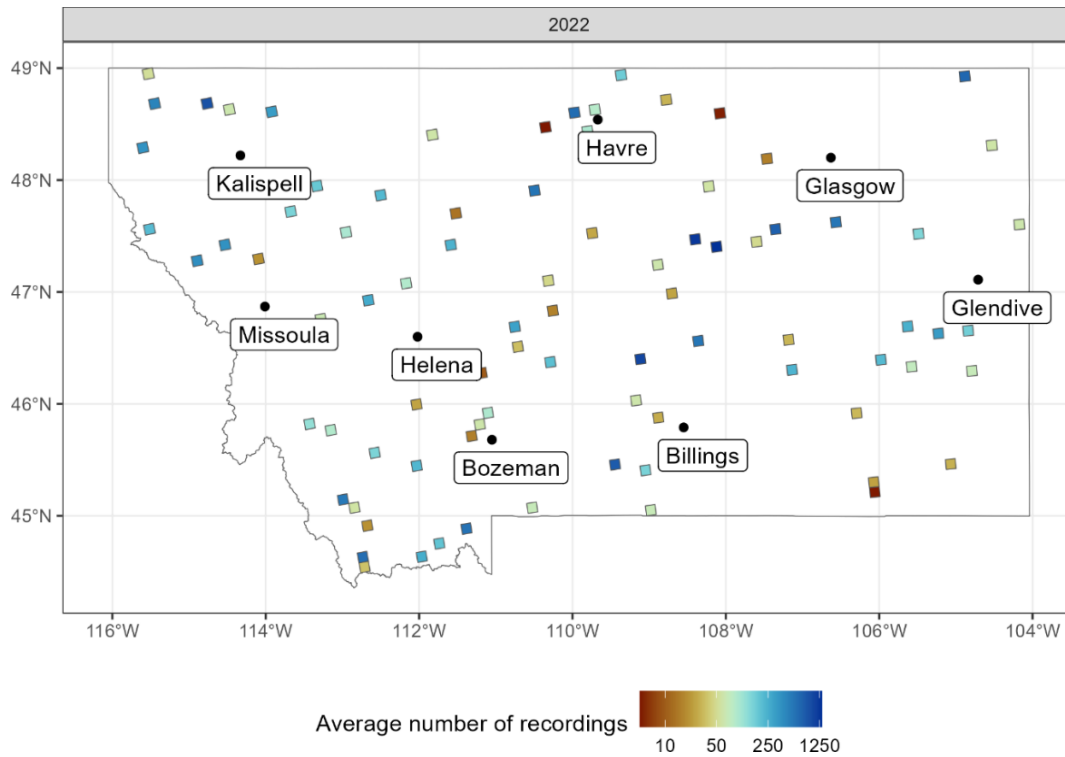


Figure 6. Map of the average number of bat recordings per 10- by 10-km (100 km²) North American Bat Monitoring Program grid cell surveyed in 2022. Red hues denote cells with lower mean counts and blue hues denote cells with higher mean counts. The sampled grid cells represent a spatially balanced sample from all of Montana.

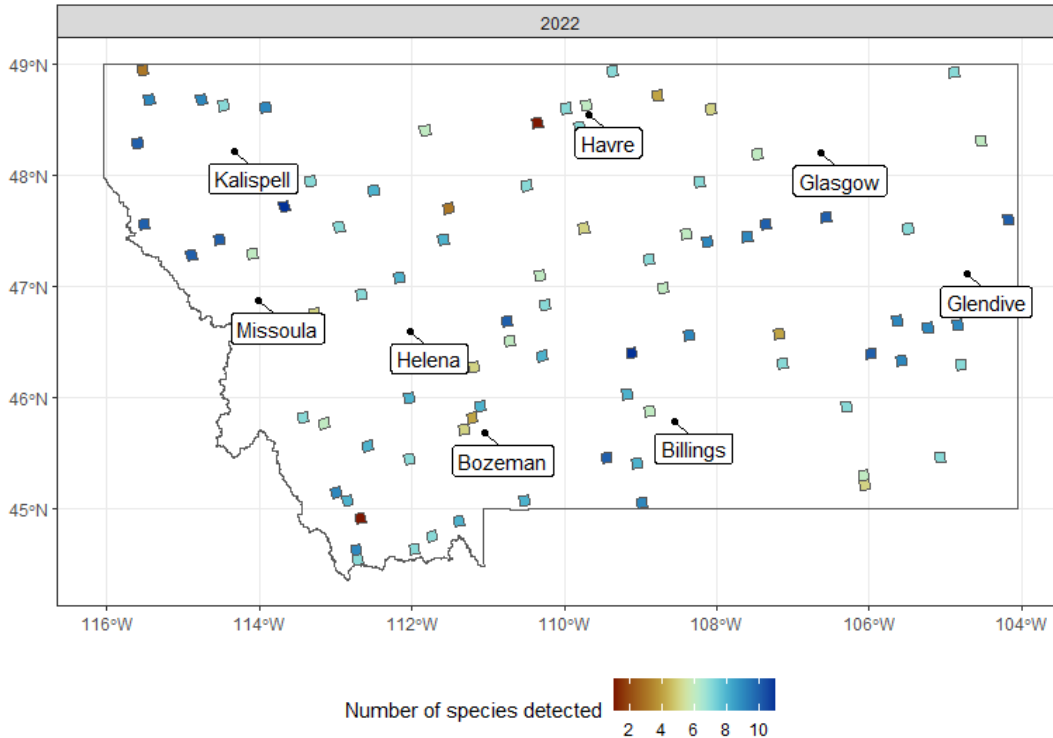


Figure 7. Map of the number of bat species recorded per 10- by 10-km (100 km²) North American Bat Monitoring Program grid cell surveyed in 2022. Red hues denote cells with fewer species counted and blue hues denote cells with greater numbers of species counted. The sampled grid cells represent a spatially balanced sample from all of Montana.

Analysis of Bat Activity in Relation to *Pd* Presence

Stratton et al. (*In press*) used a novel “joint” statistical modeling framework to estimate the relationship between the detection of *Pd* at sites across Montana and the acoustic activity of our WNS-susceptible bat species, defined as species exhibiting characteristic frequencies greater than 34 kHz. This model was designed to accommodate the fact that our acoustic and *Pd* surveillance data are collected at different spatial scales and locations on the landscape, leading to data that is spatially mis-aligned. The model was also designed to account for the uncertainty in *Pd* status of unsampled areas across the state.

Stratton et al.’s analysis confirmed a strong signal of westward spread of *Pd* across Montana between 2020-2022, and found evidence for a negative association, albeit one characterized by high uncertainty, between the annual detection of *Pd* and bat acoustic activity. Their analysis also found evidence for strong positive associations between bat activity and site covariates, including distance to water and roosting structures and activity during the previous night. Future analyses of this dataset may explore how assumptions about the persistence of *Pd* at a site, which may not be monitored every year, as well as lagged effects of *Pd* exposure, may affect the strength of associations with bat activity. Although WNS presence, as opposed to *Pd* presence on the landscape, may be a better predictor of bat activity, disease data are far less complete and more difficult to collect as this requires the live capture of bats.

Table 1. Prevalence of *Pseudogymnoascus destructans* (*Pd*) and corresponding sample sizes, in parentheses, among sample types and across surveillance sites sampled in 2023. Bolded site names were those sites where *Pd* was detected. Kit type varied depending on the site and included hibernacula kits for use in caves, guano kits designed for pooled guano sampling, spring emergence kits for live animal trapping and sampling, and *Surveillance 4.0* kits for live animal sampling at known *Pd* positive sites. “Bat Swabs” were collected by rolling moistened swabs back and forth five times on a bat’s nose and forearm; “Paired Fecal Samples” were fecal samples collected from a swabbed bat; “Fecal Sample” indicated a sample of up to several guano pellets collected from a hibernacula or roost site; “Fecal Sample (> 1)” indicated 2-50 mL of pooled guano collected at a roost site; “Soil” indicated soil samples collected within a hibernacula or at a roost site; “Environmental Swabs” were collected from sites with bat use; and “Carcass” indicated a bat carcass swabbed or submitted for testing. The National Wildlife Health Center issued invalid test results for several samples from Argenta Cave, Ophir Cave, and Whitaker Sink because the internal positive controls did not perform as expected on some of the test plates; these are not included in the final sample sizes reported in this table.

Site	Kit Type	Bat Swab	Paired Fecal Sample	Fecal Sample	Fecal Sample (>1)	Soil	Environ-mental Swab	Carcass
Argenta Cave	Hibernacula			0 (19)		0 (11)	0 (1)	
Azure Cave	Hibernacula	0.67 (6)						
Bannack State Park	Guano	0 (1)			0 (2)		0 (4)	
Beartooth Colony	Spring Emergence	0.39 (18)		0 (6)				
Big Hole Maternity Roost	Guano				0 (5)		0 (5)	
Box Elder Creek	Surveillance 4.0	0.22 (23)						
Craig Bridge	Guano				0 (2)			
Custer Maternity Bridge Roost	Guano			0 (45)				
Davis Creek	Spring Emergence	0 (25)	0 (15)					
Flagstaff Creek Bridge	Guano				0.38 (45)			
Hay Creek	Surveillance 4.0	0.36 (14)						
Helena Law Enforcement Academy	Guano				0 (3)			
115 Bridge	Guano				0 (5)		0 (5)	
Jefferson Slough	Spring Emergence	0 (14)	0 (10)	0.2 (10)			0 (5)	
Lee Creek Bridge	Spring Emergence	0 (25)	0 (8)					
Lewis and Clark Caverns	Hibernacula	0 (23)			0 (2)	0 (4)	0 (3)	0 (1)
Libby Dam	Spring Emergence	0 (25)	0 (9)					
Lick Cave - Confederated Salish and Kootenai Tribal Lands	Hibernacula	0 (17)				0 (16)		
Lick Creek Cave	Hibernacula	0.5 (12)		0.1 (10)		0.11 (9)	0 (7)	
Marias River WMA	Spring Emergence	0 (25)						
Mystery Cave	Spring Emergence	0.71 (24)						
Ninemile Bridge	Guano			0 (44)				
Northshore WMA	Guano				0 (4)			
Ophir Cave	Hibernacula					0 (21)		
Red Rocks NWR Buildings	Guano				0 (5)		0 (5)	

Site	Kit Type	Bat Swab	Paired Fecal Sample	Fecal Sample	Fecal Sample (>1)	Soil	Environmental Swab	Carcass
St. Mary Men's Dorm - Glacier National Park	Spring Emergence	0 (15)	0 (4)	0 (1)			0 (1)	
Sunday Creek	Spring Emergence	0 (10)	0 (8)			0 (20)	0 (10)	
Teton River	Spring Emergence	0 (25)						0 (1)
Vail Creek	Surveillance 4.0	0 (6)						
Whitaker Sink	Hibernacula			1 (3)				
Young Bat Houses	Spring Emergence	0 (25)	0 (10)					

Table 2. Prevalence of *Pseudogymnoascus destructans* (*Pd*) and sample sizes (in parentheses) among swabbed live bats or bat carcasses found at surveillance sites in 2023. Abbreviations for bat species include: MYLU = *Myotis lucifugus*; YULU = potentially *Myotis yumanensis* or *Myotis lucifugus*; MYCI = *Myotis ciliolabrum*; MYEV = *Myotis evotis*; MYVO = *Myotis Volans*; MYSP = unknown *Myotis* species; EPFU = *Eptesicus fuscus*; COTO = *Corynorhinus townsendii*; and UNK = unknown bat species. Bolded site names indicate *Pd* was detected at the site in 2023. **Double asterisks denote cases where bats were both *Pd* positive and had at least one symptom consistent with white-nose syndrome (WNS), including UV fluorescence, visible fungus, wing damage consistent with WNS, or histopathology conducted at the lab.

Site	MYLU	YULU	MYCI	MYEV	MYVO	MYSP	EPFU	COTO	UNK
Azure Cave	1 (2)**			1 (1)**	1 (1)**			0 (2)	
Bannack State Park						0 (1)			
Beartooth Colony	0.41 (17)								0 (1)
Box Elder Creek	0.75 (4)**					1 (1)	0.06 (18)		
Davis Creek				0 (5)	0 (2)		0 (17)		0 (1)
Hay Creek	1 (4)**						0.1 (10)		
Jefferson Slough	0 (8)		0 (3)	0 (1)	0 (1)		0 (1)		
Lee Creek Bridge	0 (12)				0 (13)				
Lewis and Clark Caverns				0 (2)		0 (1)		0 (21)	
Libby Dam		0 (25)							
Lick Cave - Confederated Salish and Kootenai Tribal Lands		0 (14)						0 (3)	
Lick Creek Cave	0.43 (7)			0.5 (2)	0.67 (3)				
Marias River WMA	0 (17)					0 (8)			
Mystery Cave	0.64 (14)**			0.75 (4)	0.8 (5)**				1 (1)
St. Mary Men's Dorm - Glacier National Park	0 (15)								
Sunday Creek			0 (1)	0 (1)			0 (8)		
Teton River	0 (5)	0 (21)							
Vail Creek			0 (2)				0 (4)		
Young Bat Houses		0 (25)							

Discussion

With the help of numerous agency staff and partners, FWP continued its intensive sampling for *Pd* and WNS across the state. This year, our efforts were concentrated on the western half of the state where *Pd* had yet to be detected. Additionally, we participated in the NWHC's *Surveillance 4.0* aimed at surveying known *Pd*-positive sites to understand *Pd* loads and disease severity among various bat species. Through these efforts, we detected *Pd* at 9 of 31 actively sampled sites, including six new sites in six new counties, and at sites farther west than previously documented. Similarly, confirmed/suspected WNS cases were detected at four actively surveyed sites and one passively/opportunistically sampled site, including at two new sites in two new counties. Despite additional detections farther west than before, we have yet to detect *Pd* west of the Continental Divide within the state.

Notably, in 2023 we discovered that our 2 largest remaining *Myotis hibernacula* at Lick Creek Cave and Mystery Cave, became *Pd* and WNS positive, respectively, suggesting that we are likely to witness large declines in these populations in the coming years. Both sites are gated, limiting public access, which should help minimize disturbance during the critical window of hibernation. Furthermore, FWP, MTNHP, Bureau of Land Management (BLM) and the U.S. Forest Service (USFS) have issued press releases and conducted additional outreach requesting that the public and caving community avoid entering caves known to contain bats during the critical window of hibernation (i.e., October-May) to minimize disturbance. FWP and partners have reiterated the importance of decontaminating gear used in caves to prevent the inadvertent spread of *Pd* to new locations (White-nose Syndrome Disease Management Working Group 2020).

In 2023, we continued our annual acoustic monitoring of bats among 112 NABat Program grid cells to gather the information necessary to understand how bat activity is changing in response to *Pd* and WNS in Montana. Furthermore, Stratton et al. (*In press*) conducted the first analysis of our *Pd* surveillance and acoustic data collected from 2020-2022. This effort has detected a negative association between *Pd* and the acoustic activity of our WNS-susceptible bat species, suggesting that WNS is causing detectable population declines in the eastern half of the state. This is consistent with data from the rest of the U.S. (Cheng et al. 2021), as well as with observed declines in *Myotis* populations at Azure Cave in 2021 and among some of the summer roosts sampled by field staff in *Pd*/WNS positive counties in the state. This initial analysis of our data has also clarified the importance of finding opportunities to co-locate some of our *Pd*/WNS surveillance in the same locations where we collect acoustic data for NABat, to improve our ability to relate pathogen/disease status to localized bat activity. To this end, both our participation in *Surveillance 4.0* and additional efforts to monitor summer roosts should provide opportunities to link trends in roost counts over time to *Pd* loads and disease severity among our WNS-susceptible species.

Related Ongoing Work

Structured Decision Making to Inform WNS and Bat Management in Montana

In 2021, FWP, MTNHP, BLM, USFS, the Montana Department of Natural Resources and Conservation, the Confederated Salish and Kootenai Tribes, the National Park Service, and the U.S. Fish and Wildlife Service began a partnership with the USGS to use structured decision making (SDM) to help inform how Montana should respond to *Pd* and WNS and manage Montana's bats to maximize their distribution and abundance into the future. This process is underway, and we anticipate management recommendations from the effort within the next year.

Summer Colony Counts

The monitoring framework provided by NABat includes four survey types: stationary acoustics, mobile acoustics, winter colony counts, and summer colony counts (Loeb et al 2015). Stationary acoustic surveys allow us to estimate occupancy, and in turn, distribution (Loeb et al 2015). However, inferences on distribution can be bolstered with winter and/or summer colony counts, and under strict assumptions, these data can be used to

estimate population size. Although winter colony counts would meet most of these assumptions and allow us to better estimate populations and monitor trends in relation to WNS, they are not feasible at a statewide scale in Montana. Unlike many WNS-susceptible bat species in eastern North America, most of the state's bats do not hibernate in caves or mines. It is likely that bats use other rock features, such as talus, rock outcrops, or cliffs, but to what degree is largely unknown. This, coupled with logistical difficulty in accessing the few caves used as hibernacula in winter, reduces the feasibility of winter colony counts as a metric to estimate population abundance or trend. However, there are numerous maternity roosts distributed across the state, and many of these are accessible during the summer.

In 2023, FWP expanded a pilot project to coordinate and conduct summer colony counts at maternity roosts, including bat boxes, buildings, and bridges around the state. This effort involved agency staff and partners, as well as volunteers, who were asked to conduct at least 2 colony counts between June 1 and July 15 at a given roost site; we assumed multiple visits within this window occurred before pups were volant and counts represented adult bats. We aimed for 2 or more observers per survey (i.e., 2 independent observations). Most surveys were visual emergence counts, where observers counted bats that they saw as they emerged from roosts. However, at one maternity roost, we explored using infrared cameras to conduct a visual emergence count. For visual emergence counts, observers surveyed 30 minutes prior to sunset until the last bat emerged or until it was too dark to efficiently count bats. In addition to visual emergence counts, we explored internal roost counts, both visually and with infrared, at one site where visual emergence counts are difficult. During these surveys, we entered the maternity roost and attempted to count bats that were roosting during the day by photographing clusters; we assume that this is a stable colony and took care to minimize disturbance during entry.

During all surveys, we noted the roost type (e.g., barn, bridge, tree, bat box), location within the roost structure (e.g., attic, siding, roof), number of known roosts at site, and roost ownership (i.e., private or public). When possible, we identified the species using the roost and noted the first observation of pups and evaluated their development stage (i.e., pink and hairless or furred). Lastly, we collected data on conditions that can influence emergence and roost occupancy (e.g., moon phase, temperature, and cloud cover).

Over 60 people participated in assisting with emergence counts at 22 individual roosts across the state. Although we are working to digitize and explore data collected during this pilot effort, results from 2023 will provide baseline data on what accessible maternity roosts look like across the state and inform method refinement. Future efforts include: 1) exploring data collected during 2023 field season, 2) refining count methods at larger and difficult-to-count roosts using infrared technology, 3) narrowing down parturition dates across the state, 4) meeting with National WNS coordinators to refine our sampling scheme and prioritize monitoring sites, and 5) reaching out to partners and citizen scientists to assist in summer colony counts. Colony count data are imperfect, and population size estimates rely on strong assumptions. However, in the absence of winter colony counts these surveys, in addition to acoustic surveys, produce the best available data for monitoring population trends as WNS spreads. Summer colony count data will also bolster inferences made with NABat efforts. Lastly, colony counts may also provide a nexus for public and partner outreach, as they can be performed by citizen scientists with minimal training.

Bat Acoustic Call Library

In 2023, FWP and MTNHP began systematically recording acoustic echolocation sequences from released, mist-netted bats that had been identified to species as part of our *Pd* and WNS surveillance or other bat-related research. The purpose of this effort, which is expected to take up to three years, is to generate acoustic recordings of echolocation sequences from known-species individuals across a gradient of environmental clutter (i.e., habitat structure ranging from forest to open meadow). Clutter, defined as “the density of obstacles in the

flight environment” (Fenton 1990), such as shrubs, branches, tree trunks, or water, can influence the structure of echolocation calls emitted by bats and our overall ability to identify echolocation sequences to species (Loeb et. al 2015). Results from this effort will be used to update and improve the accuracy of our auto-species-classification software, Sonobat 4.0. Additionally, results will improve our understanding of acoustics in relation to western species that forage in cluttered environments, such as the newly ESA-listed *M. septentrionalis* (Northern myotis), and allow us to make acoustic processing recommendations, not only for Montana, but the West as a whole.

In 2023, echolocation sequences were recorded at 15 sites around the state. Across sites, we obtained recordings from several hundred individual bats among species including *Lasionycteris noctivagans* (silver-haired bat), *Lasiurus borealis* (eastern red bat), *Lasiurus cinereus* (hoary bat), *Antrozous pallidus* (pallid bat), *E. fuscus* (big brown bat), *M. lucifugus*, *M. lucifugus/M. yumanesis*, *M. septentrionalis*, *M. volans*, *M. ciliolabrum*, and *M. evotis*. MTNHP are currently processing these data.

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