Montana's 2021 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 2021, we surveyed 35 sites across the state, 9 of which were *Pd*-positive. WNS was first confirmed in 2021 among little brown bats (*Myotis lucifugus*) in Fallon, Carter, and Phillips Counties. As of 2021, *Pd* and WNS detections remained restricted to the eastern half of the state. 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. Wildlife and land management agency staff in Montana are currently engaged in a structured decision-making process to understand how best to respond to WNS while trying to maximize the abundance and distribution of bats across the state.

Introduction

White-nose Syndrome (WNS), 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. The Rocky Mountains remains the last large area where *Pd* has yet to be detected, although models predict the pathogen's imminent arrival (U.S. Geological Survey, 2019, 2020). 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 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 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 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 than their counterparts back east. 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 our bat populations (Hanauska-Brown et al. 2019). Specifically, FWP's plan calls for 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 2021 Pd/WNS surveillance effort.

Methods

Pd/WNS Surveillance

FWP's 2021 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-cell state-wide surveillance grid (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) prior to bats' return for the season (usually by May 1st). Then, after ≥ 4 weeks of guano collection, we would return to the site, 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).

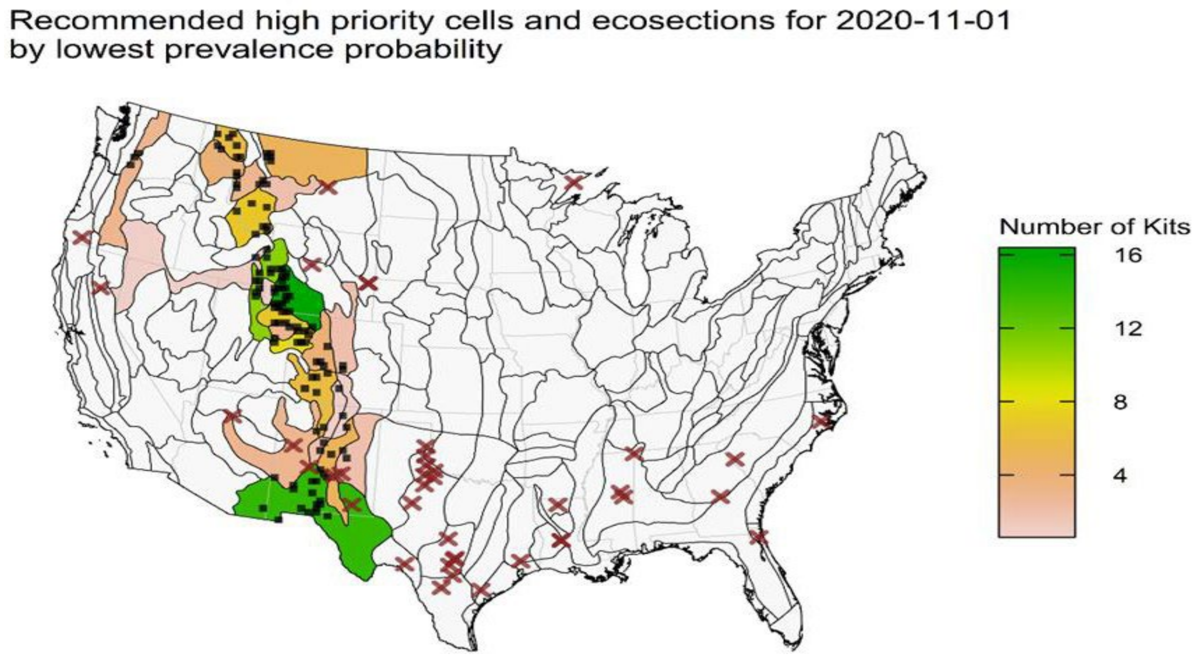

Figure 1. The National Wildlife Health Center's map of priority surveillance areas for *Pd* and white-nose syndrome for 2020-2021. The map is divided into 'eco-sections' and those eco-sections highlighted in color are areas where the leading edge of the disease was predicted to be during the winter of 2020-2021. Eco-sections are color-coded by the number of target sampling locations (or 'kits') required to detect *Pd* with 95% confidence ifprevalence 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.

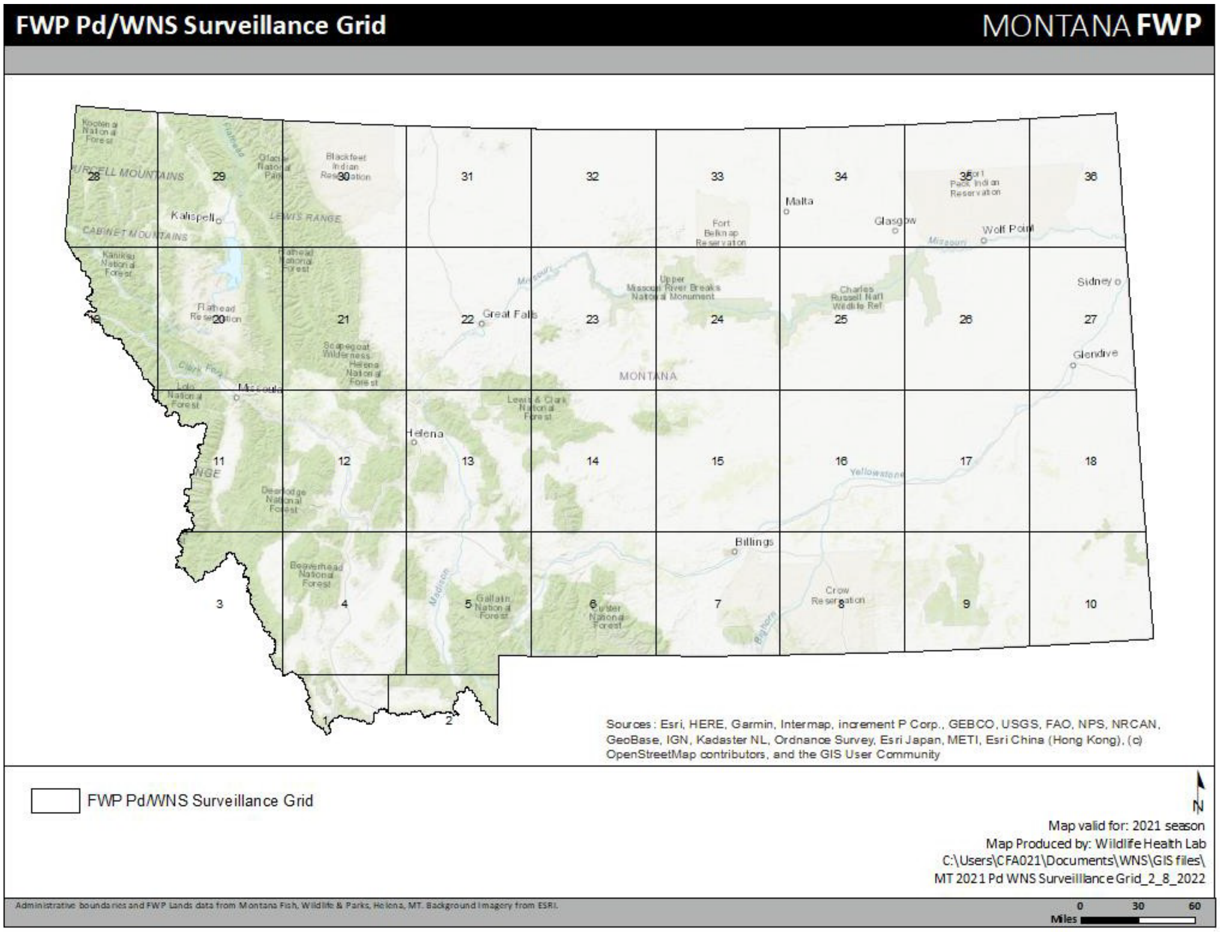

Figure 2. The state of Montana broken into 36 *Pd*/WNS sampling grid cells.

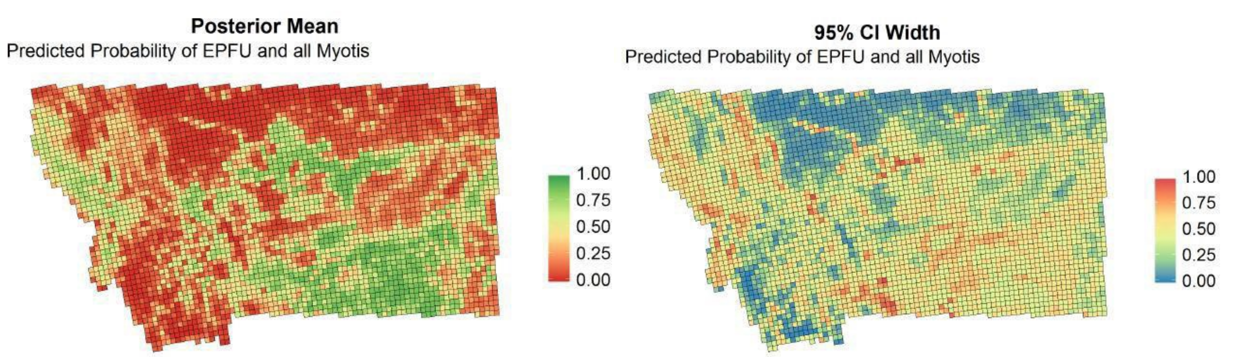

Figure 3. Estimated joint probabilities of occupancy for *Eptesicus fuscus* (EPFU; big brown bats) and all Myotis species, some of the most susceptible species to WNS. The estimates correspond to the probability that all these species are present within a grid cell (left) and the associated uncertainty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

Results

In 2021, FWP and partners conducted *Pd*/WNS sampling at 35 sites across Montana (Figure 4, Table 1). Sampling sites included 7 hibernacula, 17 summer roost/spring mist-netting sites with live-animal sampling (9 of which also received paired pooled guano sampling), and 11 sites that were only sampled via pooled guano collection at summer/maternity roosts. Of the 35 sites, samples from 27 sites were submitted to the National Wildlife Health Center as part of their surveillance testing, and samples from the remaining 8 sites were tested at the Oregon Vet Diagnostic Laboratory. In addition, we submitted individual bat carcasses from 7 sites to the NWHC for diagnostic testing as part of our passive surveillance efforts (Table 1). Live animal sampling occurred on a range of bat species including *Myotis lucifugus* (MYLU, Little brown bats), *Myotis yumanesis* (MYYU, Yuma myotis), *Myotis californicus* (MYCA, California myotis), *Myotis ciliolabrum* (MYCI, Western small-footed myotis), *Myotis volans* (MYVO, Long-legged myotis), *Myotis velifer* (MYVE, Cave myotis), *Myotis evotis* (MYEV, Western long-eared myotis), *Myotis thysanodes* (MYTH, Fringed myotis), *Corynorhinus townsendii* (COTO, Townsend's big-eared bats), *Eptesicus fuscus* (EPFU, Big brown bats) and unidentified Myotis species.

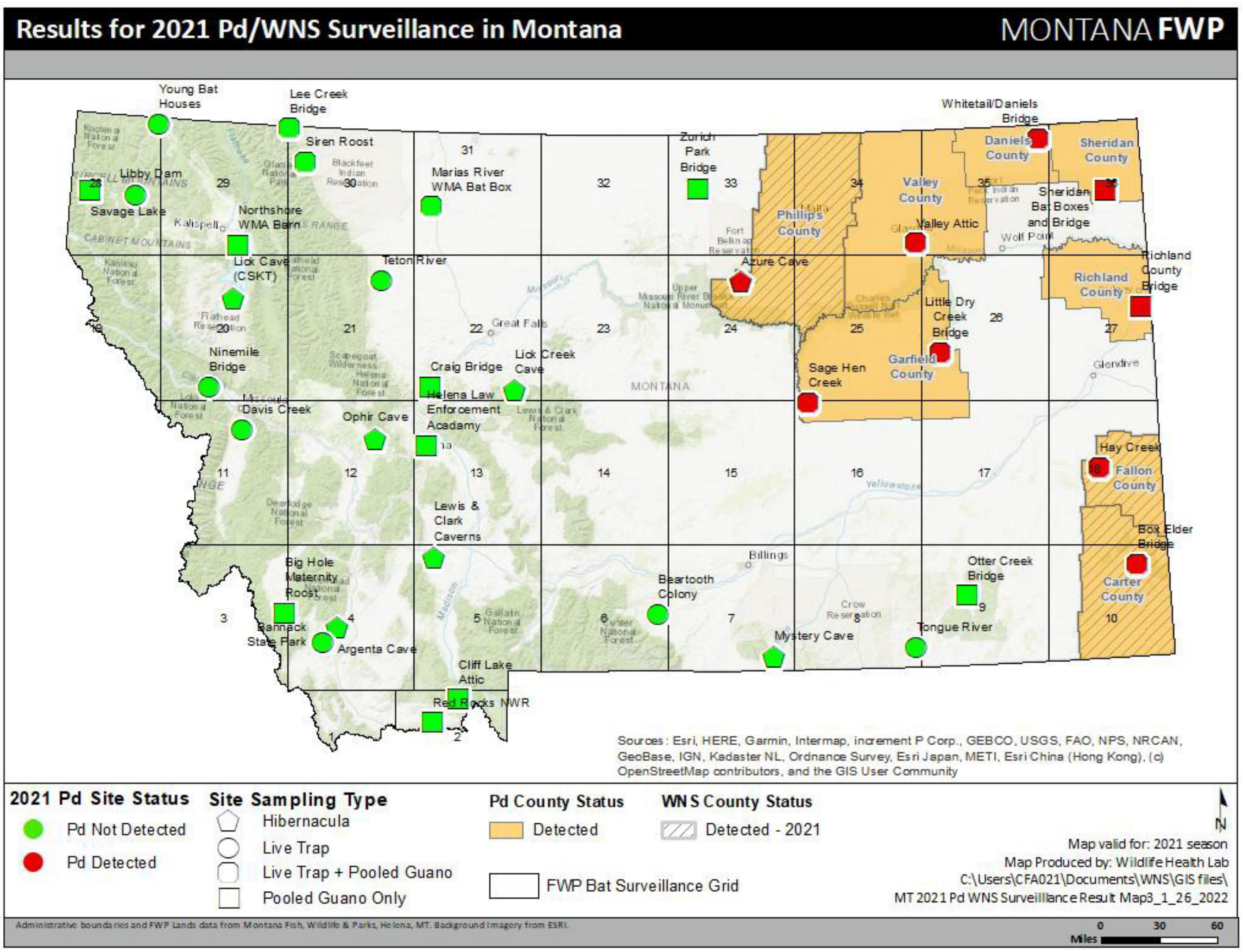

Figure 4. 2021 *Pd*/WNS surveillance sites, *Pd* status, and county-level *Pd* and WNS status. Higher-intensity sampling on the western side of the state was prioritized based on the National Wildlife Health Center's annual *Pd* spatial spread model.

Of the 35 sites surveyed across Montana, 9 sites, all in the eastern half of the state, 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). Of samples collected from live bats, *Pd* was detected on little brown bats (MYLU), Western small-footed myotis (MYCI), big brown bats (EPFU) and from unidentified Myotis species. All sites that were *Pd*-positive in 2020 (Richland County Bridge, Hay Creek, Valley Attic, Whitetail Bridge, Sheridan bat boxes and bridge, and Azure Cave) were found to be positive again when resampled in 2021. New *Pd*-positive sites in 2021 included Box Elder Bridge and Little Dry Creek Bridge. At 9 sites, where we both live-trapped and collected pooled guano and environmental swabs, we found high correspondence in the detection of *Pd* across the different approaches (Table 1), with pooled guano offering the highest rates of *Pd*-detection, at least among sites known to be infected for 2+ years. The only place where detections differed between live animal swabs and pooled guano/environmental swabs was at Little Dry Creek Bridge, where a roost of big brown bats (EPFU) were sampled and only one bat swab tested positive for *Pd* while all guano and environmental samples were *Pd*-negative.

WNS was confirmed for the first time at Azure Cave in Phillips County 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). Between March 22 and May 5, 2021, 3 little brown bats found dead, 2 in Fallon County and 1 in Carter County, were confirmed to have WNS. All three bats exhibited lesions consistent with WNS. Of the 7 sites where we opportunistically submitted dead bats, only these three sites were found to be *Pd*-positive.

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

Table 1. Table of 2021 *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	**Site Type**	**Sample Date**	**Bat Swab (P/N)**	**Bat Swab + Fecal Sample (P/N)**	**Environ-mental Swab (P/N)**	**Fecal Sample (>1) (P/N)**	**Soil (P/N)**	**Bat Carcass (P/N)**	**Bat Species Sampled**	***Pd* Positive Bat Species**	**WNS Positive/ Suspect**
Argenta Cave	Hibernacula	6/6/2021					0/45				
Azure Cave	Hibernacula	5/21/2021						3/3	MYLU	MYLU	Confirmed - MYLU
Bannack State Park	Building Roost	6/7/2021	0/7	0/10	0/4	0/9			MYCA, MYLU, Myotis Sp.		
Beartooth Colony	Building Roost	5/26/2021	0/8			0/28			MYLU		
Big Hole Maternity Roost	Building Roost	6/7/2021	0/4		0/15	0/25			Myotis Sp.		
Box Elder Bridge	Landscape/ Bridge Roost	6/7/2021	1/10	8/15	5/5	4/4			EPFU, MYLU, Myotis Sp.	MYLU, Myotis Sp.	
Cliff Lake Attic	Building Roost	6/8/2021			0/6	0/34					
Craig Bridge	Bridge Roost	5/27/2021				0/2					
Davis Creek SW of Missoula	Landscape	5/17/2021	0/1	0/12					EPFU, MYVE, MYVO		
Hay Creek	Bridge Roost	6/8/2021	6/15	1/4	3/5	5/5	0/12		EPFU, MYVO, MYLU, MYCI	MYLU, MYCI	
Helena Law Enforcement Academy	Bat Box	5/27/2021				0/2					

Site	Site Type	Sample Date	Bat Swab (P/N)	Bat Swab + Fecal Sample (P/N)	Environ-mental Swab (P/N)	Fecal Sample (>1) (P/N)	Soil (P/N)	Bat Carcass (P/N)	Bat Species Sampled	*Pd* Positive Bat Species	WNS Positive/ Suspect
Lee Creek Bridge (Glacier NP)	Bridge Roost	6/4/2021	0/25		0/6	0/4			MYLU, MYVO, Myotis Sp.		
Lewis and Clark Caverns	Hibernacula	4/9/2021	0/10		0/13	0/11	0/11		MYLU, COTO, MYCI		
Libby Dam	Landscape	5/14/2021	0/19	0/6					MYYU, MYLU		
Lick Cave - Confederated Salish and Kootenai Tribal Land	Hibernacula	3/30/2021	0/25				0/10		MYTH, COTO, Myotis Sp.		
Lick Creek Cave	Hibernacula	4/30/2021	0/24				0/2		MYEV, MYLU, MYVO, MYYU?, Myotis sp.		
Little Dry Creek	Bridge Roost	6/2/2021	1/15	0/10	0/5	0/5			EPFU	EPFU	
Marias River WMA Bat Box	Bat Box	6/3/2021	0/24	0/1	0/5	0/5			MYLU		
Mystery Cave	Hibernacula	9/23/2021				0/32	0/13				
Ninemile Bridge NW of Missoula	Bridge Roost	5/22/2021	0/15		0/1		0/9		Myotis Sp.		
North Shore WMA Barn	Building Roost	6/21/2021			0/5	0/5					
Ophir Cave	Hibernacula	5/27/2021					0/45				
Otter Creek Bridge	Bridge Roost	6/23/2021				0/45					
Red Rocks NWR Buildings	Building Roost	6/29/2021			0/5	0/5					

Site	Site Type	Sample Date	Bat Swab (P/N)	Bat Swab + Fecal Sample (P/N)	Environ-mental Swab (P/N)	Fecal Sample (>1) (P/N)	Soil (P/N)	Bat Carcass (P/N)	Bat Species Sampled	*Pd* Positive Bat Species	WNS Positive/ Suspect
Richland County Bridge	Bridge Roost	6/22/2021				1/1					
Sage Hen Creek	Bridge Roost	6/1/2021		4/8	2/5	1/1	1/24		EPFU, MYLU, MYCI	MYLU	
Savage Lake	Building Roost	6/1/2021			0/5	0/5					
Sheridan Bat Boxes and Bridge	Bat Box/Bridge Roost	6/9/2021				41/46					
Siren Roost (Glacier NP)	Building Roost	6/3/2021	0/48	0/2	0/10	0/4			MYLU		
Teton River	Landscape/ Building Roost	6/1/2021	0/9	0/11	0/5	0/2	0/2		MYLU, MYVO, Myotis Sp.		
Tongue River	Landscape	5/17/2021	0/13			0/10	0/24		MYLU, EPFU, MYCI		
Valley Attic	Building Roost	6/7/2021	5/25		2/5	3/3			Myotis Sp.	Myotis Sp.	
Whitetail/Daniels Bridge	Bridge Roost	5/31/2021	7/10	10/15	1/5	2/2			MYLU	MYLU	Suspect - MYLU
Young Bat Houses	Bat Houses/ Landscape	5/15/2021	0/25						MYLU, MYYU		
Zurich Park Bridge	Bridge Roost	6/29/2021				0/34					
Passive Surveillance Samples											
Hay Creek Bridge, Plevna, Fallon County	Landscape	5/5/2021						1/1	MYLU	MYLU	Confirmed - MYLU

Site	Site Type	Sample Date	Bat Swab (P/N)	Bat Swab + Fecal Sample (P/N)	Environ-mental Swab (P/N)	Fecal Sample (>1) (P/N)	Soil (P/N)	Bat Carcass (P/N)	Bat Species Sampled	*Pd* Positive Bat Species	WNS Positive/ Suspect
Private Residence, Baker, Fallon County	Landscape	5/3/2021						1/1	EPFU	EPFU	
Private Residence, Ekalaka, Carter County	Landscape	4/24/2021						1/1	MYLU	MYLU	Confirmed - MYLU
Sandstone Reservoir, Fallon County	Landscape	3/22/2021						1/1	MYLU	MYLU	Confirmed - MYLU
Whitehall School, Jefferson County	Landscape	11/2/2021						0/1	MYCI		
Private Residence, Florence, Ravalli County	Landscape	7/28/2021						0/2	Myotis Sp.		
Private Residence, north of Lake Helena, Lewis and Clark County	Landscape	7/2/2021						0/3	Myotis Sp.		

Discussion

With the help of numerous agency staff and partners, FWP significantly expanded its *Pd* and WNS surveillance effort in 2021 to include much broader and more intensive sampling across the state. We detected *Pd* at 9 sites, including 2 new locations, in eastern Montana and detected our first cases of WNS in Carter, Fallon, and Phillips Counties. The mortalities of WNS-affected little brown bats in Carter and Fallon Counties were likely due to the disease and point to the possibility of more substantial WNS-associated mortalities that have gone undetected. We do not know where bats are hibernating in these far eastern counties. While we did not detect any WNS-associated mortality at Azure Cave in May 2021, the presence of numerous bats exhibiting fungal growth suggests that the cave population could suffer significant mortality during the winter of 2022. This prediction is based on patterns observed in the eastern US, where initial *Pd* detection is followed 1-2 years later by WNS and WNS-mortalities among susceptible species (Frick et al. 2017).

In 2021, we were unable to locate sampling locations in the center of the state, creating a gap that has left us uncertain about the exact location of the leading edge of *Pd* this past year. However, despite the 2021 NWHC model predictions and our intensive surveillance efforts there, we did not detect *Pd* in the western half of the state. This suggests that either *Pd* is moving more slowly than predicted, or that prevalence is too low (<15% of the affected population) or its distribution is too patchy to detect yet. The NWHC model has been updated and calls for intensive sampling in northcentral and southwestern Montana in 2022 (Figure 6). In addition to trying to meet NWHC sampling requests, we plan to sample across the 36-cell surveillance grid and make extra efforts to identify and sample sites within those grid cells that we were unable to sample in 2021.

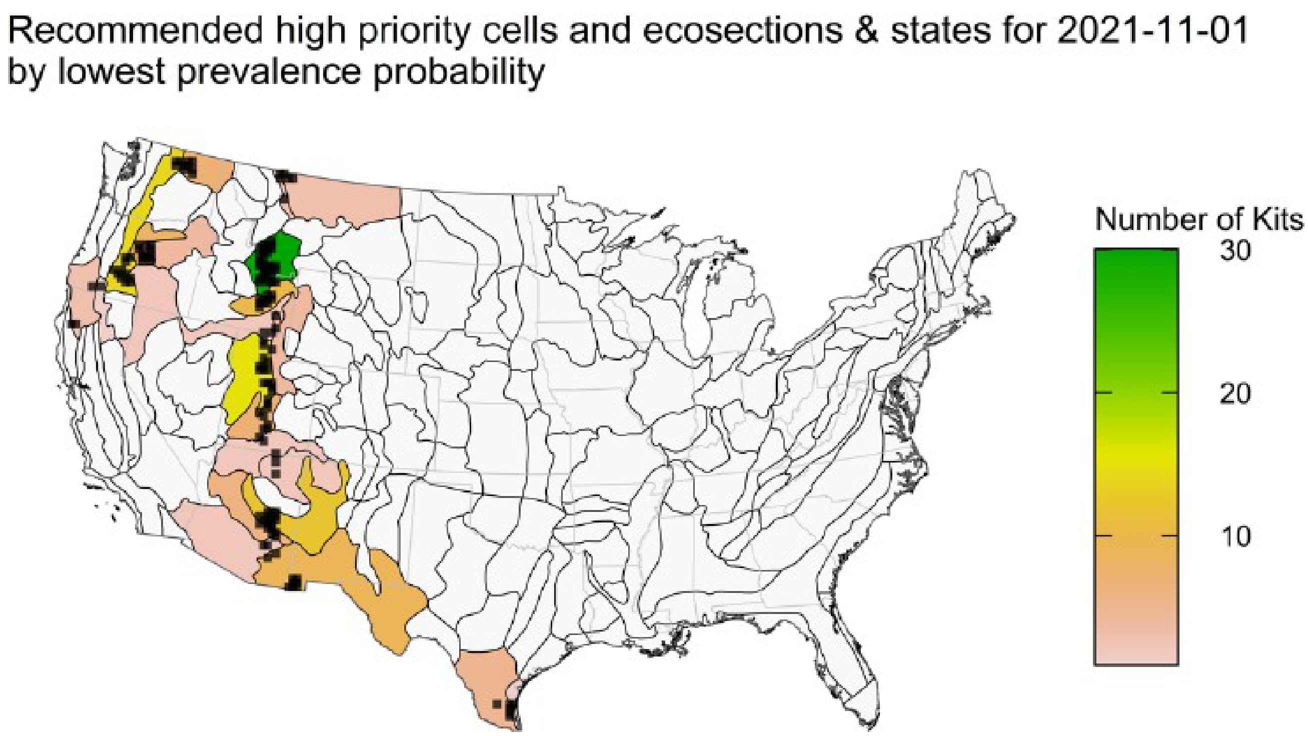

Figure 6. The top 150 highest ranking priority cells (black squares) and associated ecosections (colored polygons) where *Pd* is predicted to spread during the 2021/2022 season.

Related Ongoing Work

In 2021, we continued our annual acoustic monitoring among 87 North American Bat 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. Again, this is collaborative work involving numerous agency partners, land managers, and landowners. Automated species identification has already been completed and a subset of calls will be manually vetted this spring.

In addition, in 2022, we plan to identify a series of spring/summer roosts of WNS-susceptible bat species, distributed across the state, that can be monitored over the long-term for population counts. The intention is to have another metric of population trend/abundance that we can relate to WNS presence.

Lastly, in the fall of 2021, Vratika Chaudhary and Evan Grant with the U.S. Geological Survey, began a structured decision making exercise with representatives from FWP, the Montana Natural Heritage Program, the US Forest Service, Bureau of Land Management, National Park Service, Montana Department of Transportation, the Confederated Salish and Kootenai Tribe, Montana Department of Natural Resources and Conservation, and the U.S. Fish and Wildlife Service to determine how best to collectively respond to the arrival of WNS in Montana. The group is continuing to meet to identify how best to achieve the overall objective of maximizing the distribution and abundance of bats across the state. The results of this process will be shared in a report when the exercise is complete.

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