



White-Nose Syndrome Surveillance and Bat Monitoring Activities in North Coast and Cascades Network Parks 2016–2024



Mount Rainier.

OREGON STATE UNIVERSITY / JENNY URBINA

White-Nose Syndrome Surveillance and Bat Monitoring Activities in North Coast and Cascades Network Parks 2016–2024

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Abstract

Pseudogymnoascus destructans (Pd), the causative agent of white-nose syndrome (WNS) in bats, has caused serious declines in bat populations across North America. We conducted WNS surveillance in five different park units in the North Coast and Cascades Network (NCCN) from 2016 to 2024, following the initial detection of Pd and WNS in Washington State in 2016. We captured and swabbed bats, swabbed roost materials, and collected guano and tested these samples for Pd DNA using qPCR. We confirmed WNS through histopathology of tissue samples and carcasses. We detected Pd at five locations in Mount Rainier National Park, starting in 2017. We confirmed WNS at four of these locations, with the first clinical signs detected in 2022. We detected Pd for the first time in Olympic and North Cascades National Parks in 2024. From these efforts, we generated information that can be compared to other datasets, helping us advance our knowledge of WNS/Pd epidemiology. We also conducted three field and laboratory-based experiments to inform early detection/rapid response (EDRR) planning. The first was a field experiment using non-infective Pd DNA to evaluate the rate of DNA degradation and the probability of detecting Pd DNA in the field. Experimental degradation rates for Pd DNA ranged from 1.6% to 8.2% and were lower in protected sites. The second was a laboratory-based experiment to understand Pd growth on four different substrates. We detected increasing levels of Pd in autoclaved guano and in plywood, suggesting these substrates may be environmental reservoirs. Pd remained stable in fresh guano but in soil it decreased, suggesting microbial interactions that may influence Pd growth in these substrates. We also collected wood shavings from a Pd positive bat box in June and August to evaluate viable Pd persistence in wood in a summer roost. Despite the characterization that Pd required cold conditions to persist, viable Pd was present in wood shavings collected during the summer season. Finally, we evaluated the National White-Nose Syndrome Decontamination Protocol through experiments. We found that ethanol was not effective as a sporicidal agent in any of the concentrations we tested and that a 1:10 dilution of bleach did not kill Pd spores, though higher concentrations did. These findings resulted in changes to the national protocol.

Executive Summary

Pseudogymnoascus destructans (Pd), the causative agent of white-nose syndrome (WNS) in bats, is a leading factor explaining declines in bat populations across North America. Eleven bat species occur in western Washington and six are known to be susceptible to WNS, while three are suspected to be carriers of Pd but have not been detected with the disease. Here, we report on disease surveillance and monitoring based on standardized protocols across multiple national parks in Washington state. We generated data suitable for comparison to other datasets, helping us advance our knowledge of WNS/Pd epidemiology. We evaluated our protocols in experimental field-based and laboratory settings to inform practitioners and managers of detection probabilities under different conditions and the limits of interpretation.

The North Coast and Cascades Network (NCCN) monitors natural resources in seven park units located in Washington and Oregon, primarily west of the Cascade Range, spanning from the Columbia River in the south to the Canadian border in the north. From 2016 to 2024, the National Park Service (NPS), U.S. Geological Survey (USGS), and Oregon State University (OSU) conducted WNS surveillance and surveyed for Pd in six different park units including Ebey's Landing National Historical Reserve, Mount Rainier National Park, North Cascades National Park Service Complex, Olympic National Park, Lewis and Clark National Historical Park, and San Juan Island National Historical Park. Mount Rainier National Park is situated approximately 30 miles south of the location where Pd and WNS were first detected in Washington in 2016. The NCCN parks were identified in the NPS Pacific West Region (PWR) WNS Response Plan as the highest risk for rapid disease spread. Because of this proximity to the disease center and the predicted rates of WNS spread, the NCCN responded with urgency to conduct active and passive disease surveillance by swabbing bats and collecting guano in the field. We also conducted field and laboratory-based experimental work to inform early detection/rapid response (EDRR) planning. Here we summarize bat monitoring and disease surveillance conducted in the NCCN parks, as well as laboratory experiments conducted to explore Pd growth, detection and degradation of Pd DNA on different substrates, and to develop genetic species identification tools.

We coordinated our disease surveillance efforts with the Washington Department of Fish and Wildlife (WDFW) to ensure consistency with the direction of the state wildlife veterinarian. Following the first WNS detection in 2016, we began a data gathering effort to identify known or suspected bat roosts in NCCN parks but did not conduct any bat captures on the landscape per WDFW direction. From 2016 through 2024, we collected a total of 1,198 swab samples from 12 locations, 545 guano samples from 36 locations, and 21 environmental samples from 4 locations. In 2017, we began screening bats at maternity colonies and spring roost sites by sampling bats directly and collecting fresh guano. That year, at one site in Mount Rainier National Park (Lewis County, WA), four out of 25 *Myotis* bats (likely *Myotis lucifugus*) tested positive for Pd but did not show clinical signs of WNS disease using UV illumination of wing membranes. We had no other Pd positive samples during the first four years of sampling (2016–2019). In 2020, we detected Pd from guano samples collected from the same site at Mount Rainier National Park where the four bats

tested Pd positive in 2017. The detection of Pd in guano at this site in 2020, and from all subsequent sampling efforts, confirmed that bats using the site were still being exposed to Pd, but we did not observe clinical signs of WNS disease in bats at that site until 2024. We also detected Pd at a total of four additional sites at Mount Rainier in Lewis and Pierce Counties during the 2021 and 2023 sampling seasons. Park staff submitted tissue samples (wing biopsies or whole carcasses) to the National Wildlife Health Center (NWHC) for histopathology from three of these sites in 2022 and 2023, which confirmed that specimens from all three locations had fungal hyphae invading tissues, the pathologic indication for WNS disease. A bat can have Pd spores on its skin resulting in detection of the fungus in a swab sample, but only histopathologic examination of the tissues can confirm WNS disease. The first Pd positive samples were collected from Olympic National Park and North Cascades National Park Complex in spring of 2024. As of spring 2024, we had not yet found evidence of Pd or WNS at any of the other NCCN parks.

Our experimental work was conducted in three parts. The first was a field experiment using extracted DNA (non-infective) to evaluate the probability of detecting Pd DNA at known bat colonies and the rate of DNA degradation in guano samples. This experiment was deployed in the field at Mount Rainier National Park in 2018. This work aimed to evaluate the accuracy of non-invasive protocols for collecting guano, which are used in an early detection/rapid response framework by State and Federal agencies to monitor Pd spread. Additionally, we used DNA extracted from skin swabs, guano, and wing punches to generate a baseline framework to identify bat species from each of these sample types. The second was a laboratory-based experiment to understand Pd growth on different substrates including bat guano, soil, and plywood, which is a common material used for artificial bat roosts (i.e., bat boxes). These laboratory experiments helped direct field-based studies aimed at identifying and mitigating environmental reservoirs, and informed surveillance plans using non-invasive monitoring for early detection and rapid response. In a follow-up to these experiments, we collected wood shavings from a Pd positive bat box at Mount Rainier in June and August to evaluate viable Pd persistence in wood in a summer roost. Despite the characterization that Pd required cold conditions to persist, viable Pd was present in wood shavings collected during both summer season sampling bouts. Finally, we evaluated the National White-Nose Syndrome Decontamination Protocol that recommended bleach and ethanol to decontaminate equipment. We found that ethanol was not effective as a sporicidal agent in any of the concentrations we tested (60%, 70% and 80%), and that a 1:10 dilution of household bleach did not kill Pd spores, though higher concentrations did. These findings resulted in changes to the protocol where our findings were adopted by White-nose Syndrome Disease Management Working Group in February 2024 and added to the National White-nose Syndrome Decontamination Protocol.

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We owe thanks to the bat monitoring teams and maintenance staff at Mount Rainier National Park, North Cascades National Park Service Complex, San Juan Island National Historical Park, Lewis and Clark National Historical Park, Ebey's Landing National Historical Reserve, and Olympic National Park for their efforts to support bat sampling in the parks, for their input, and for assistance identifying sites and collecting samples. Thank you to the Levi and Garcia labs at Oregon State University for the use of their laboratory equipment and to the members of the Levi laboratory for support during sample analysis. We also thank the Royal BC Museum (British Columbia, Canada) and the Burke Museum of Natural History and Culture (Seattle, Washington) for providing access to bat tissue samples. We thank Anne Ballmann and Kyle George from the USGS National Wildlife Health Center for guidance on sample collection and laboratory analyses. We sincerely appreciate peer-reviewers Michelle Verant, Alice Chung-MacCoubrey, and Allen Calvert for providing comments that improved the manuscript and Saylor Moss and Miranda Terwilliger, the handling editors for this manuscript. Funding for this work was provided by the National Park Service and the U.S. Geological Survey. This work was carried out by the National Park Service, U.S. Geological Survey, and Oregon State University. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Introduction

Emerging infectious diseases (EIDs) caused by fungi are recognized as a threat to global health, food security and ecosystems (Fisher, Gow and Gurr 2016; Ghosh, Fisher and Bates 2018). Species faced with multiple stressors such as habitat loss, human development, and exposure to contaminants are at additional risk to EIDs (Schmeller, Courchamp and Killeen 2020). Among vertebrates affected by EIDs, bats have suffered unprecedented mortality rates across eastern North America (Frick et al. 2010; Reeder and Moore 2013). Recent bat declines in North America are attributed to white-nose syndrome (WNS), a disease caused by the fungus *Pseudogymnoascus destructans* (Pd) that threatens several bat species during hibernation and was responsible for an estimated 5–6 million bat deaths annually in the northeastern United States during the initial years of the outbreak (USFWS 2019). WNS vulnerable species have been exposed to additional stressors including loss of habitats, specifically large roosting trees and snags; direct mortality from wind farm development; and exposure to pesticides, which can have direct effects on individuals and populations and indirect effects on insect food resources (Bernard and Grant 2019; Bernard et al. 2020).

In North America, Pd was first reported in 2006 in New York (Frick et al. 2010) and as of this report, has spread across 35 states and seven Canadian provinces (www.whitenosesyndrome.org). Pd has been reported in twelve bat species, including two endangered and one threatened species (www.whitenosesyndrome.org). The first detection of WNS in western North America occurred in 2016 in King County, WA, approximately 30 miles north of Mount Rainier National Park (Lorch et al. 2016). In 2016, we began studying the current distribution of WNS disease in the National Parks of the North Coast and Cascades Network (NCCN; Figure 1) and its causative agent Pd, as well as the bat species most likely to be affected by the disease. WNS affects bats primarily during the winter, when they are hibernating. The only roost sites identified in the NCCN that could be monitored were spring and summer roosts, where bats who survived winter WNS infections could be screened for disease during the spring before they recover from winter infections to the point that evidence of the disease can no longer be detected.

Monitoring bats in the NCCN has been challenging because bats are inherently difficult to study. Bats can travel long distances (Moussy et al. 2013); are small, cryptic, and mainly nocturnal; and can be difficult to identify in the hand due to few visual morphological differences in certain species (Norberg and Rayner 1987). Correct identification relies on cranial or dental characteristics (Nagorsen and Brigham 1993) that cannot be observed in live bats. Non-invasive techniques like skin swabs and fecal pellet analysis are often used to determine the presence of pathogens in hosts (Kriger, Hero, and Ashton 2006; Oehm et al. 2011), and these samples can also be used to identify the host species through DNA analysis (Höss et al. 1992; Kurose, Masuda and Tatara 2005). Our monitoring approach relied on non-invasive techniques to sample for Pd in bats and their environments.

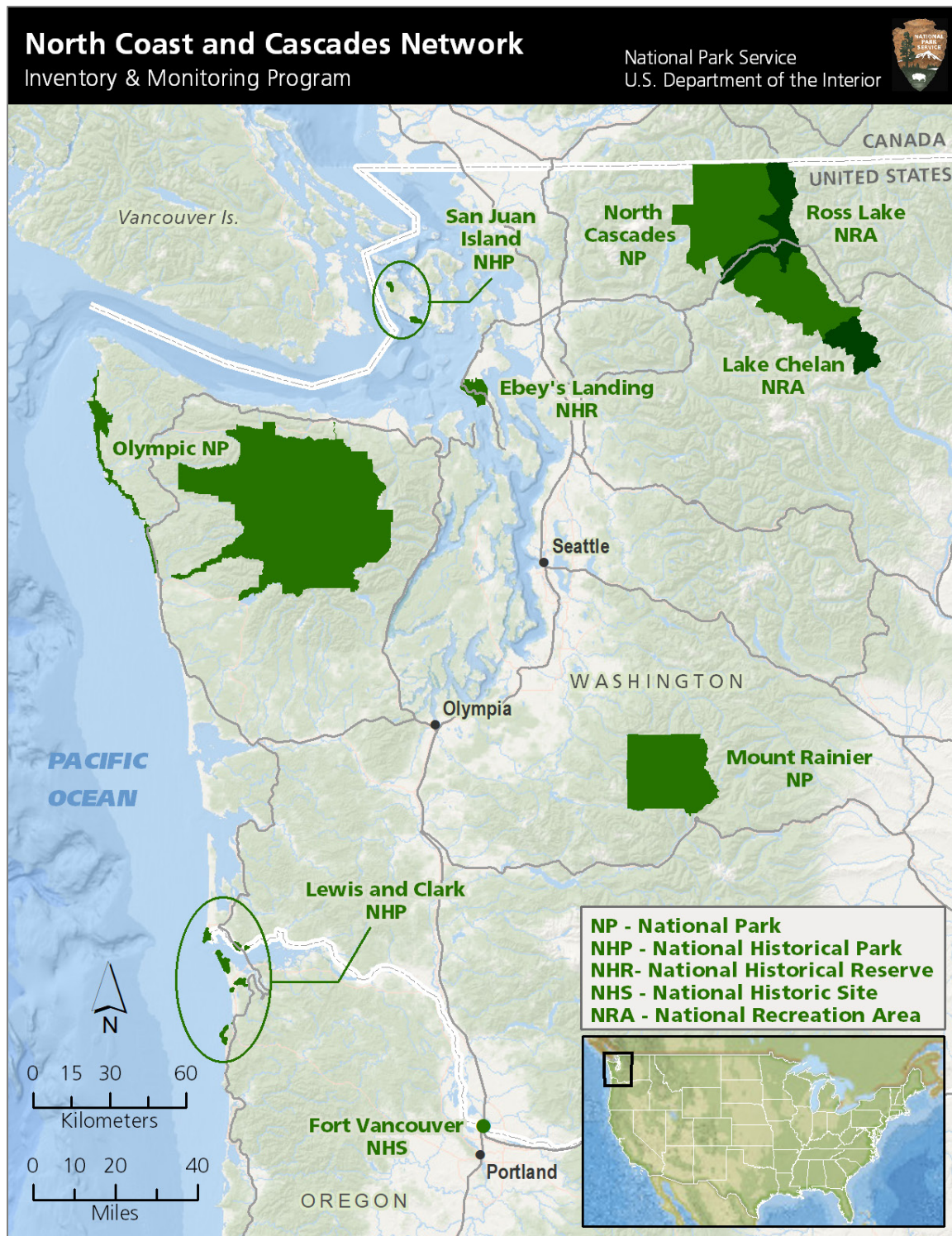


Figure 1. North Coast and Cascades Network (NCCN) includes national park units on the west side of the Cascade Mountains in Washington and Oregon from the Columbia River to the Canadian border. From 2016 to 2024, white-nose syndrome surveillance occurred in five of eight park units: Olympic National Park, Ebey's Landing National Historical Reserve, San Juan Island National Historical Park, Mount Rainier National Park, and North Cascades National Park Service Complex.

NPS

DNA isolated from feces (molecular scatology) has been used for more than 20 years to study diverse aspects of the biology of various species (Kohn and Wayne 1997). The use of molecular scatology for bats is promising as a source of DNA to ensure correct identification of species, characterization of their diet, and potential exposure to pathogens (Puechmaille, Mathy and Petit 2007; Walker et al. 2016). Molecular scatology of bats has been used successfully to identify co-occurring species (Zinck, Duffield and Ormsbee 2004; Korstian et al. 2015), and recent studies have highlighted the benefits of using these non-invasive techniques to reduce stress due to capture (Walker et al. 2016; Player et al. 2017). We incorporated molecular scatology into several aspects of our monitoring efforts and experimental work.

Interagency Coordination

In March 2016, the first case of WNS was reported from a little brown bat (*Myotis lucifugus*) near North Bend, WA (King County) (Lorch et al. 2016). In response, the NPS Pacific West Region (PWR) formed a working group of subject matter experts with experience in bat biology, disease ecology, veterinary medicine, WNS epidemiology, park management, and communications. This working group drafted a bat monitoring and WNS response plan for PWR parks which included five priority areas: communication, training and resources, WNS surveillance, bat monitoring, and collaboration and coordination.

Following the initial WNS detection, WDFW directed all their scientific collection permittees to temporarily stand down bat handling while they conducted a risk assessment of bat handling and developed best practices to prevent the unintentional spread of the pathogen by researchers. Although NPS managers are not required to obtain state permits, we mirrored the guidance WDFW provided to their permittees for WNS surveillance in the NCCN to have a unified WDFW/NPS response.

To implement the priorities outlined in the PWR WNS response plan, Mount Rainier National Park hosted an interagency workshop for bat practitioners throughout Washington, Oregon, Idaho, and northern California in August 2016. This workshop provided training to State, Tribal, and Federal partners on bat monitoring, disease surveillance, best practices for decontamination, and identified knowledge gaps and opportunities for future research that would inform and improve regional WNS response and coordination. This report represents one of several outcomes from this workshop, where we collaborated with partners at the National Wildlife Health Center (NWHC), other NPS Inventory and Monitoring networks, and Oregon State University (OSU) to implement activities identified as high priority (e.g., assessing Pd and WNS status in bats, guano, and bat roosts in the NCCN) and fill knowledge gaps through novel research (e.g., using molecular and observational techniques to advance non-invasive methodologies to improve bat species identification, Pd detection, and interpretation of positive Pd results, and increase understanding of Pd ecology in the absence of bat hosts).

In 2023, implementation of the Pd/WNS surveillance program transferred from NPS to the U. S. Geological Survey (USGS) Olympic Field Station (Forest and Rangeland Ecosystem Science Center), which had been collaborating on this and other bat research efforts in the NCCN since 2018.

Herein, we report on our efforts in the NCCN from 2016 to 2024 to:

1. determine the presence of the pathogenic fungus (Pd) in bats from colonies in NCCN parks,
2. quantify the infection load of wing-swab and guano samples,
3. generate a molecular scatology framework for bat species identification,
4. experimentally evaluate degradation rates of genomic Pd DNA in guano at summer bat roosts, and
5. evaluate Pd persistence and growth in different substrates associated with bat roosts.

Bat Monitoring and Disease Surveillance

To identify potential study sites in NCCN parks, we conducted an exhaustive search of records of bat detections from previous park inventories and followed up on incidental or informal reports (e.g., from Integrated Pest Management specialists and maintenance workers who incidentally observed bats in attics or other structures). We visited potential sites in early spring to determine if the site was occupied in the current year and if it was a large enough roost to collect swab samples from at least 25 bats, the minimum number of swabs recommended by the NWHC for disease surveillance. If site occupancy was uncertain, we placed a plastic sheet under the suspected roost to collect guano to inform seasonal occupancy by bats.

In 2016–2017, we initially identified 13 potential study sites, some of which were dropped in subsequent years because bat exclusion efforts were successful (Table 1). Several of the sites in buildings posed a human health hazard, and there were active efforts to exclude bats during times of the year when the site was not occupied (i.e., attic sites that were summer colonies abandoned in winter). Six sites had consistent bat populations that were accessible for capture, which served as our primary capture locations for annual WNS surveillance capture efforts. Systematic searches and reports from NPS and USGS staff in subsequent years identified 30 additional locations, which have been sampled for guano and/or opportunistic capture. As of winter 2024, a total of 36 sites were sampled at least once. All the sampled sites were artificial roosts including bat houses, buildings, bridges, and abandoned mine sites (Table 1).

Table 1. North Coast and Cascades Network (NCCN) sites that have been sampled for *Pseudogymnoascus destructans* disease surveillance from 2016 through 2024. Park unit abbreviations: EBLA = Ebey's Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	Roost Type	Description and Maintenance History	Bat Species Observed
Primary Capture Locations	MORA	Ohanapecosh Bat Box	Bat box	Colony previously occupied furnace room in building, bat boxes installed on nearby building exteriors	• <i>Myotis lucifugus</i>
	MORA	Tahoma Woods	Bat box and buildings	Bat box mounted on shed, tarp tents, adjacent building eaves	• <i>Myotis evotis</i>, • <i>M. lucifugus</i>, • <i>M. californicus</i>, • <i>Eptesicus fuscus</i>, • <i>Lasionycteris noctivagans</i>
	NOCA	Hozomeen	Historic maintenance building with open access	Bats occupy attic and nearby bat box	• <i>Myotis yumanensis</i>, • <i>M. lucifugus</i>
	NOCA	Stehekin	Historic house	Bats occupy attic	• <i>Myotis yumanensis</i>
	OLYM	Hoh Ranger Station	Historic building	Bats occupy attic and siding	• <i>Myotis yumanensis</i>
	SAJH	English Camp	Bat box	Bat box installed away from buildings to encourage occupancy of bat box	• <i>Myotis yumanensis</i>, • <i>M. lucifugus</i>
Guano and Opportunistic Capture Sites	EBLA	Ebey's Landing	Historic maintenance building with open access	Renovated in 2018 to exclude bats to reduce human-wildlife conflict	• <i>Myotis californicus</i>
	EBLA	Ferry House	Historic building	Very little guano, no bats observed, being renovated	• Unknown
	EBLA	Sheep Barn	Historic barn	Very little guano, no bats observed	• Unknown
	LEWI	Netul Landing	Building	Bats occupy bus shelter eaves and shed	• <i>Myotis yumanensis</i>

Table 1 (continued). North Coast and Cascades Network (NCCN) sites that have been sampled for *Pseudogymnoascus destructans* disease surveillance from 2016 through 2024. Park unit abbreviations: EBLA = Ebey's Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	Roost Type	Description and Maintenance History	Bat Species Observed
Guano and Opportunistic Capture Sites (continued)	MORA	Carbon Mine	Mine	Abandoned mine adit tunnel near the Carbon River. Mine gate non-functional. Very little guano, no bats observed	Unknown
	MORA	Carbon River	Historic building	Windows replaced in 2018; efforts made to exclude bats to reduce human-wildlife conflict	<i>Myotis evotis</i>, <i>M. lucifugus</i>, <i>M. yumanensis</i>, <i>Lasionycteris noctivagans</i>
	MORA	Eagle Peak Mines	Mine	Three abandoned mine adit tunnels on the slope of Eagle Peak, all gated.	<i>Corynorhinus townsendii</i> (winter)
	MORA	Fryingpan Creek Bridge	Steel web girder bridge	Very little guano, no bats observed	Unknown
	MORA	Laughingwater Bridge	Steel and concrete box girder bridge	1–2 bats regularly seen roosting in summer and fall	<i>Corynorhinus townsendii</i>, <i>Myotis</i> spp.
	MORA	Longmire Community Building	Historic building	Lone bat found during upstairs renovation 2022	<i>Myotis evotis</i>
	MORA	Longmire Storage Bays	Roost discovered during roof replacement in 2018	Colony did not reoccupy building after roof replacement	<i>Myotis evotis</i>
	MORA	Longmire Warehouse	Historic building	Bats occupy attic crawlspace, attached bat box is unoccupied	<i>Myotis evotis</i>
	MORA	Nisqually Entrance	Historic building	One carcass found incidentally	<i>Myotis</i> spp.
	MORA	Nisqually Glacier Bridge	Steel and concrete box girder bridge	A single bat (<i>Corynorhinus townsendii</i>) regularly roosts here. <i>Myotis</i> guano is present, but no other bats have been directly observed.	<i>Corynorhinus townsendii</i>, <i>Myotis</i> spp.

Table 1 (continued). North Coast and Cascades Network (NCCN) sites that have been sampled for *Pseudogymnoascus destructans* disease surveillance from 2016 through 2024. Park unit abbreviations: EBLA = Ebey's Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	Roost Type	Description and Maintenance History	Bat Species Observed
Guano and Opportunistic Capture Sites (continued)	MORA	Ohanapecosh Ranger Station	Historic building	Exclusion mostly successful, single bats occasionally found in building	• <i>Myotis evotis</i>
	MORA	Ohanapecosh Water Treatment	Water treatment and maintenance building	Collected guano around exterior, no bats observed	• <i>Eptesicus fuscus</i>
	MORA	White River Bat Box	Bat box	Bat box attached to exterior of White River dorm building	• <i>Myotis</i> spp.
	MORA	White River Dorm	Park housing unit	Bats primarily occupy attic, few use attached bat box	• <i>Myotis evotis</i>
	MORA	White River House	Park housing unit	Guano in attic, no bats observed	• <i>Myotis</i> spp.
	NOCA	Diablo	Decommissioned Housing	Decommissioned park housing area, several buildings. Demolition in progress. Very little guano, no bats observed	• Unknown
	NOCA	Newhalem Amphitheater	Campground amphitheater	Very little guano, no bats observed	• Unknown
	NOCA	Newhalem Bat Box	Bat box	Bat box mounted on pole near Seattle City Lights housing area	• <i>Myotis yumanensis</i>
	NOCA	Newhalem VC	Visitor Center	Collected guano around exterior, no bats observed	• Unknown
	OLYM	Elwha	Historic house	Bats occupied attic; exclusion installed in 2016	• <i>Myotis</i> spp.
	OLYM	Kalaloch	Historic building	Building demolished after bats were no longer occupying	• <i>Myotis</i> spp.
	OLYM	Mora Housing	Park housing unit	Bats occupied attic and external siding. Exclusion planned for winter 2024/25	• <i>Myotis</i> spp.

Table 1 (continued). North Coast and Cascades Network (NCCN) sites that have been sampled for *Pseudogymnoascus destructans* disease surveillance from 2016 through 2024. Park unit abbreviations: EBLA = Ebey's Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	Roost Type	Description and Maintenance History	Bat Species Observed
Guano and Opportunistic Capture Sites (continued)	OLYM	Olympic	Historic building	Building demolished after bats were no longer occupying	• <i>Myotis</i> spp.
	OLYM	Quinault	Historic building	Building demolished after bats were no longer occupying	• <i>Myotis</i> spp.
	OLYM	Staircase Bridge	Bridge	Very little guano, no bats observed	• Unknown
	OLYM	Staircase Ranger Station	Historic building	Bats roost around exposed pipe on exterior eave of building	• <i>Myotis yumanensis</i> , • <i>M. lucifugus</i>

Bat Monitoring and Disease Surveillance Methods

We collected bat skin swab, guano, and environmental swab samples for Pd screening on bats and their roosts; wing tissue biopsies for WNS diagnosis; and guano and wing tissue for bat species identification. We submitted carcasses found during winter or spring for testing by the NWHC. All of our animal handling techniques were approved by the National Park Service Institutional Animal Care and Use Committee (IACUC SOP <https://www.nps.gov/orgs/1103/upload/NPS-IACUC-Bat-SOP-Final-1.pdf>).

Bat and Guano Sampling

Our initial goal was to swab 25 bats per site to achieve a 95% chance of detecting the pathogen if it was present when disease prevalence was greater than 15% (Cameron and Baldock 1998). The NWHC WNS surveillance program was only able to provide resources to sample 10 sites per state at this sampling rate. Once a county was determined to be positive for Pd or WNS was confirmed, NWHC deprioritized samples from that county to focus their limited surveillance resources on counties that were not yet positive. Given the large size and the ecological differences of some counties where national parks occur, NCCN was interested in sampling at a higher rate to detect Pd and WNS when the prevalence was lower so we could respond using an early detection/rapid response framework. For example, Olympic National Park occurs in Clallam and Jefferson County and is roughly the size of Rhode Island. The park spans the entire east/west gradient of the Olympic Peninsula which has widely variable habitats ranging from coastal rainforests in the west to high elevation alpine environments to drier mixed conifer forests in the east. Pd and WNS detections on the east side of these counties may not be informative to the west side of the counties because the differences in climate and habitat may influence bat activity patterns and behavior in ways that could be meaningful to Pd and WNS mitigation efforts.

Samples collected in 2016 and 2017 were processed by the NWHC. When NPS WNS funding was secured in 2017, we leveraged our funds to increase the number of sites that could be sampled statewide so that all the NWHC resources (10 sites in Pd negative counties) could be allocated to WDFW. We increased our goal to swab 60 bats per site (or all bats when fewer than 60 were present) to achieve a 95% chance of detecting the pathogen when it was present and the disease prevalence was 5% (Cameron and Baldock 1998). We collected duplicate swabs from each bat so if we detected Pd in a bat from a new park location or county in the OSU lab, we could send the duplicate sample to NWHC for independent verification and include it as part of the official WNS spread map for the United States and Canada (whitenosesyndrome.org/where-is-wns). We shared all positive results with WDFW so those results could be incorporated into the WDFW spread map (wdfw.wa.gov). In 2024, we began collecting samples at a subset of our sites for the NWHC's Pd Surveillance 3.0, which tracks Pd range expansion, and Pd Surveillance 4.0, which provides long-term monitoring of WNS progression in western bat species. These latter efforts are ongoing.

In March 2017, we began to monitor bat roosts for phenology and occupancy by placing 3.0 mil clear plastic sheeting under bat roosts to collect fresh guano. We checked the plastic for evidence of bat presence (accumulated guano and urine) approximately once per week and recorded the date evidence was first observed. This provided us with information on seasonal phenology so we could

best plan our monitoring efforts. We allowed guano to accumulate throughout the season and collected up to five samples in 2.0 ml Eppendorf tubes from the surface of the accumulated guano at each sampling event. Sites were typically occupied in early May, and we collected at least two repeated samples throughout the season. Guano collection frequency varied based on availability of staff and ceased when bats left the roost in the autumn.

In May and June of each year, we conducted emergence counts and captured bats using mist nets and funnel traps once their numbers were between 25 and 60. Funnel traps (Appendix B) were extremely effective in capturing bats at colonies in bat boxes. Like other species, bats are very smart animals who can become conditioned to capture methods and change their behavior to avoid capture. In general, bats were captured once each year using these methods. However, at our Ohanapecosh site, we captured twice a year as part of a mark-recapture banding effort. We saw some evidence of trap avoidance at this site, with low recapture numbers and banded bats observed to remain in the box while the trap was in place. Varying capture methods can help to minimize this kind of capture avoidance.

From 2016 through 2024, we collected swabs, guano, or both to detect the presence of Pd and occurrence of WNS from a total of 36 different locations in the NCCN (Table 2). Four sites (Ohanapecosh, Tahoma Woods, the Hoh Ranger Station, and English Camp) encompassing three parks were sampled consistently using live captures and guano collection from 2017 to 2024. An additional two sites (Hozomeen and Stehekin) were sampled annually with exceptions: sampling at Stehekin did not begin until 2018 and the Hozomeen site was not accessible due to COVID-19 pandemic travel restrictions and a road washout, in 2020 and 2021, respectively. In 2020, we were not able to handle or swab bats due to animal handling restrictions related to the COVID-19 pandemic. To compensate for this, we collected additional guano samples from sites where we would normally have captured and sampled bats. Additionally, we collected 16 wing punches from two different locations (Ohanapecosh and English Camp) to develop local primers for species identification.

Table 2. Sampling history at 36 sites in National Park Service North Coast and Cascades Network (Washington and Oregon State) park units where we conducted surveillance for *Pseudogymnoascus destructans* (Pd) from bat guano, bat skin swabs, and environmental swabs from 2016 to 2024. The number of samples tested for the presence of Pd DNA and number of Pd positive samples (in parentheses) are reported, a dash (–) indicates no samples were collected that year. Primary capture locations have been surveyed regularly as access permitted. All samples in 2020 were guano only due to COVID 19 bat-handling restrictions. For more detailed real-time quantitative polymerase chain reaction (qPCR) results by sample type, refer to Appendix A. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	# Samples Collected (# Positive) By Year								
			2016	2017	2018	2019	2020	2021	2022	2023	2024
Primary Capture Locations	MORA	Ohanapecosh Bat Box	–	30(4)	105(0)	65(0) ^A	39(3)	61(7) ^A	37(21) ^A	41(40) ^A	31(26 ^B) ^A
	MORA	Tahoma Woods	–	2(0)	17(0)	28(0)	14(0)	3(0)	3(0)	18(11 ^B) ^A	10(6 ^B) ^A
	NOCA	Hozomeen	18 (0)	28(0)	35(0)	41(0)	–	–	64(0)	61(0)	62(0)
	NOCA	Stehekin	–	–	17(0)	19(0)	20(0)	18(0)	66(0)	13(0)	6(1)
	OLYM	Hoh Ranger Station	–	2(0)	27(0)	38(0)	22(0)	31(0)	24(0)	37(0) ^A	41(0)
	SAJH	English Camp	–	26(0)	33(0)	45(0)	19(0)	70(0)	60(0)	61(0)	31(0)
Guano and Incidental Capture Locations	EBLA	Ebey’s Landing	7 (0)	19(0)	1(0)	–	–	–	–	–	–
	EBLA	Ferry House	–	–	–	–	–	–	1(0)	–	–
	EBLA	Sheep Barn	–	–	–	–	–	–	1(0)	–	–
	LEWI	Netul Landing	–	–	–	–	–	–	4(0)	–	–
	MORA	Carbon Mine	–	–	–	–	–	2(0)	–	1(0)	–
	MORA	Carbon River	–	4(0)	12(0)	3(0)	–	3(1)	6(4 ^B) ^A	1(1 ^B)	–
	MORA	Eagle Peak Mines	–	–	–	–	–	6(0)	1(0)	–	–
	MORA	Fryingpan Creek Bridge	–	–	–	–	–	1(0)	–	–	–
	MORA	Laughingwater Bridge	–	–	–	–	–	–	1(0)	–	–
	MORA	Longmire Community Building	–	–	–	–	–	–	2(0)	–	–
	MORA	Longmire Storage Bays	–	–	29(0)	–	–	–	–	–	–
	MORA	Longmire Warehouse	–	–	–	–	–	3(0)	–	–	–

^A One or more samples had an inconclusive result.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed white-nose syndrome (WNS).

Table 2 (continued). Sampling history at 36 sites in National Park Service North Coast and Cascades Network (Washington and Oregon State) park units where we conducted surveillance for *Pseudogymnoascus destructans* (Pd) from bat guano, bat skin swabs, and environmental swabs from 2016 to 2024. The number of samples tested for the presence of Pd DNA and number of Pd positive samples (in parentheses) are reported, a dash (–) indicates no samples were collected that year. Primary capture locations have been surveyed regularly as access permitted. All samples in 2020 were guano only due to COVID 19 bat-handling restrictions. For more detailed real-time quantitative polymerase chain reaction (qPCR) results by sample type, refer to Appendix A. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	# Samples Collected (# Positive) By Year								
			2016	2017	2018	2019	2020	2021	2022	2023	2024
Guano and Incidental Capture Locations (continued)	MORA	Nisqually Entrance	–	–	–	–	–	–	–	1(1 ^B)	–
	MORA	Nisqually Glacier Bridge	–	–	–	–	6(0) ^A	3(1)	5(0)	–	–
	MORA	Ohanapecosh Ranger Station	–	–	–	–	–	–	2(0)	–	–
	MORA	Ohanapecosh Water Treatment	–	–	–	–	–	–	1(0)	–	–
	MORA	White River Bat Box	–	–	–	–	–	–	–	1(0)	2(0)
	MORA	White River Dorm	–	–	–	–	–	3(0)	1(0)	1(0)	–
	MORA	White River House	–	–	–	–	–	–	–	1(0)	–
	NOCA	Diablo	–	–	–	–	–	3(0)	–	–	–
	NOCA	Newhalem Amphitheater	–	–	–	–	–	1(0)	–	–	–
	NOCA	Newhalem Bat Box	–	–	–	–	–	–	–	–	31(0) ^A
	NOCA	Newhalem VC	–	–	–	–	–	3(0)	–	–	–
	OLYM	Elwha	–	1(0)	–	–	–	–	–	–	–
	OLYM	Kalaloch	–	1(0)	–	–	–	–	–	–	–
	OLYM	Mora Housing	–	–	–	–	–	–	–	–	2(0) ^A
	OLYM	Olympic	–	4(0)	–	–	–	–	–	–	–
	OLYM	Quinault	–	2(0)	–	–	–	–	–	–	–

^A One or more samples had an inconclusive result.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed white-nose syndrome (WNS).

Table 2 (continued). Sampling history at 36 sites in National Park Service North Coast and Cascades Network (Washington and Oregon State) park units where we conducted surveillance for *Pseudogymnoascus destructans* (Pd) from bat guano, bat skin swabs, and environmental swabs from 2016 to 2024. The number of samples tested for the presence of Pd DNA and number of Pd positive samples (in parentheses) are reported, a dash (–) indicates no samples were collected that year. Primary capture locations have been surveyed regularly as access permitted. All samples in 2020 were guano only due to COVID 19 bat-handling restrictions. For more detailed real-time quantitative polymerase chain reaction (qPCR) results by sample type, refer to Appendix A. Park unit abbreviations: EBLA = Ebey's Landing National Historical Reserve; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Site Type	Park Unit	Site Name	# Samples Collected (# Positive) By Year								
			2016	2017	2018	2019	2020	2021	2022	2023	2024
Guano and Incidental Capture Locations (continued)	OLYM	Staircase Bridge	–	–	–	–	–	2(0)	1(0)	–	–
	OLYM	Staircase Ranger Station	–	–	–	–	–	2(0)	29(0)	1(0)	7(7)

^A One or more samples had an inconclusive result.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed white-nose syndrome (WNS).

Upon capture, we recorded bat sex, age, and diagnostic measurements. We used datasheets provided by the USGS NWHC with all relevant metadata included. Additionally, we evaluated wing condition and examined exposed skin for potential fungal infections using an ultraviolet (UV) light. If we observed orange fluorescence, which suggests WNS infection, we collected a tissue sample using a sterile 4 mm punch biopsy. We submitted biopsies to the NWHC for WNS diagnosis. Swabs were prepared following standardized NWHC protocols with 150 μ l of nuclease-free water. We collected duplicate swab samples from the wing and muzzle of up to 60 bats and collected guano from the roost site and capture bags. Prior to shipping, all samples were stored in a -20°C non-frost-free freezer to ensure samples were kept at a stable temperature and not subjected to freeze/thaw cycles of a frost-free freezer. We shipped swab and guano samples overnight in coolers with frozen gel packs to Oregon State University for analyses. We sent duplicate swabs to the NWHC in instances of positive or ambiguous results from samples processed at OSU, or when bats appeared unhealthy. Starting in 2024, we sent a subset of samples directly to NWHC for processing as part of their Pd Surveillance 3.0 and 4.0 projects, and the remaining samples were processed at OSU. Results reported here include the combined results from all samples processed by NWHC and OSU. In instances where duplicate samples from a single bat were tested at both laboratories, we report the NWHC results.

Pd Detection

We used real-time quantitative polymerase chain reaction (qPCR) to test for the presence of Pd in all swab and guano samples collected (Verant et al. 2016). We extracted DNA from swabs using the DNeasy Blood and Tissue Kit (QIAGEN, MD, USA), and extracted DNA from guano samples using the DNeasy PowerLyzer Powersoil Kit (QIAGEN, MD, USA), following the manufacturer's instructions with the addition of lyticase as recommended in published protocols for Pd detection (Verant et al. 2016). DNA extractions included blanks as negative extraction controls. The total volume of reactions was 25 μ l, which included 12.5 μ l Taqman Environmental master mix 2.0 (Life Technologies, Carlsbad, CA), forward primer nu-IGS-0169-5' Gd and reverse primer nu-IGS-0235-3' Gd) at a final concentration of 200 nM, TaqMan FAM-labeled probe at 200 nM, and 5 μ l of DNA template. We prepared standards using genomic DNA using serial dilutions of 1:10 for final concentrations of 1,000 femtogram ($\text{fg} = 10^{-15}$ g), and the lowest standard containing 1 fg DNA. We ran each standard in triplicate to generate a reference curve. Each 96-well plate included standards as a control for consistency across plates. All analyses were done using an ABI PRISM 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA) with the following cycling conditions: initial activation 95°C for 10 min; denaturation 95°C for 15 s and annealing, and extension 60°C for 60 s, with a total of 40 cycles. We considered a positive result as suspect if the target DNA did not amplify until cycle 37 or later and labeled these as inconclusive. As the number of cycles increases the chance of primer dimers also increases, when two primers bind to each other instead of the target DNA, resulting in false positives.

Information on the laboratory and diagnostic protocols used by the National Wildlife Health Center for Pd and WNS surveillance is available online at whitenosesyndrome.org.

Bat Monitoring and Disease Surveillance Results

From 2016 through 2024, we sampled a total of 1,198 individual bats and collected 566 environmental and guano samples. We also sent 10 wing punches and eight carcasses to NWHC for histopathology to confirm WNS in bats that were UV positive or in carcasses found in winter or spring. Species captured were confirmed to be *Myotis californicus*, *M. evotis*, *M. lucifugus*, and *M. yumanensis* by identification in-the-hand using morphometrics, release calls, and molecular methods. Molecular species ID from pooled guano samples also included *Myotis volans*, *M. ciliolabrum*, *Eptesicus fuscus*, and *Corynorhinus townsendii*, although these species were not directly captured and sampled for Pd.

Samples from five sites at Mount Rainier National Park have tested positive for the presence of Pd DNA. Samples from four of these sites have also confirmed WNS through histopathology of tissue samples or bat carcasses completed at the NWHC (Table 2, Figure 2). Species with confirmed WNS included *M. evotis* and *M. lucifugus*. The other site at Mount Rainier that had a Pd-positive sample was only sampled through guano collection, so no tissue samples from that location have been tested.

No samples from sites at any of the other NCCN parks tested positive for Pd from 2016 through 2023. There have been inconclusive sample results, which may indicate that DNA amplification was due to an artifact of the PCR process (primer dimer), rather than true presence of the target DNA. It could also mean that the target organism was present in very small amounts, but specific criteria were not met to be considered a true positive. We prioritized sites with inconclusive results for ongoing, early season surveillance in the event the latter was true. In 2024, Pd was detected in Olympic and North Cascades National Parks. Mount Rainier, Olympic, and North Cascades National Parks are the only NCCN units where we have detected Pd as of spring 2024.

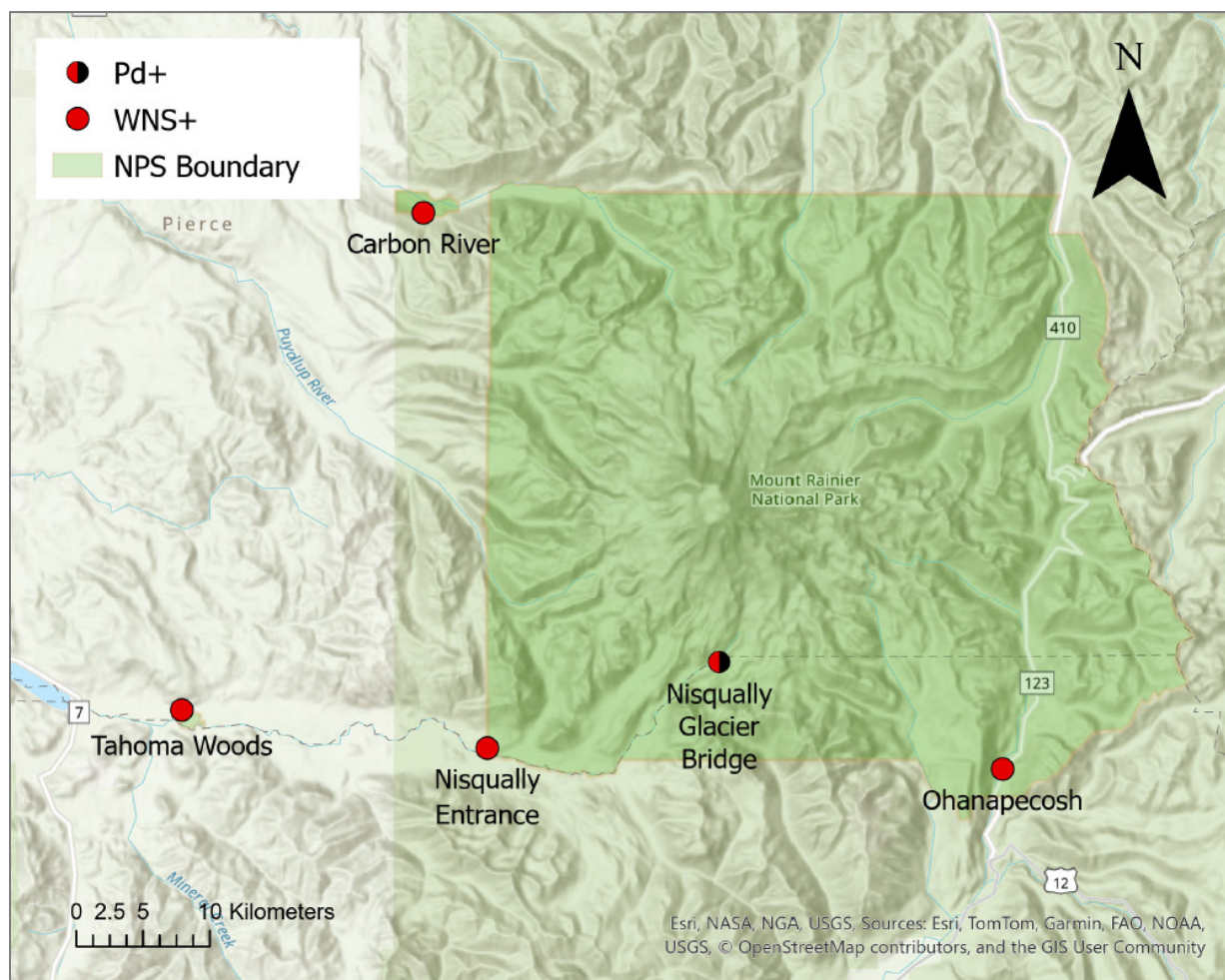


Figure 2. Locations at Mount Rainier National Park in Washington State from 2016–2024 where the fungal bat pathogen *Pseudogymnoascus destructans* (Pd) was detected, indicated as Pd+, and where histopathology confirmed white-nose syndrome disease, indicated as WNS+. All WNS+ bats that could be identified were either *Myotis evotis* or *Myotis lucifugus*. NPS = National Park Service. Please refer to Figure 1 for the larger geographical context of these parks.

USGS

Ohanapecosh, Mount Rainier

In 2017, NPS staff swabbed 24 bats at the Ohanapecosh colony in Mount Rainier and submitted samples to the USGS NWHC for screening. Four (17%) of these bats tested Pd positive as reported by the NWHC. This was the first detection of Pd at any of the NCCN parks. All four individuals were likely *Myotis lucifugus*. Although identification was not confirmed with a release call or genetic testing in 2017, all subsequent captures at Ohanapecosh have found only *M. lucifugus* occupying this roost. Guano and swab samples from this colony in 2018 and 2019 did not test positive for the Pd DNA, although two of 10 guano samples sent to the NWHC in 2019 had an inconclusive result. A sample from the Ohanapecosh site tested positive again in 2020 (guano only, as no swab samples were taken in the first year of the pandemic to protect bats from potentially being exposed to asymptomatic animal handlers who were infected with SARS-COV2). All subsequent swab and

guano sampling efforts at Ohanapecosh have had Pd positive results, with increasing proportions of positive samples (Table 2). In 2021, 11% (6 of 56) of bats sampled were Pd positive. In 2022, 53% (18 of 34) were Pd positive, and in 2023 and 2024, nearly all the bats we sampled were Pd positive (40 of 41 bats in 2023 and 26 of 31 with three additional inconclusive results in 2024). Despite the increasing Pd prevalence at this site, we did not see clinical signs of WNS in the Ohanapecosh bats until spring of 2024, when a carcass we submitted to NWHC was confirmed to have WNS lesions through histopathology and we observed several bats at Ohanapecosh with wing tissue damage (Figure 3).

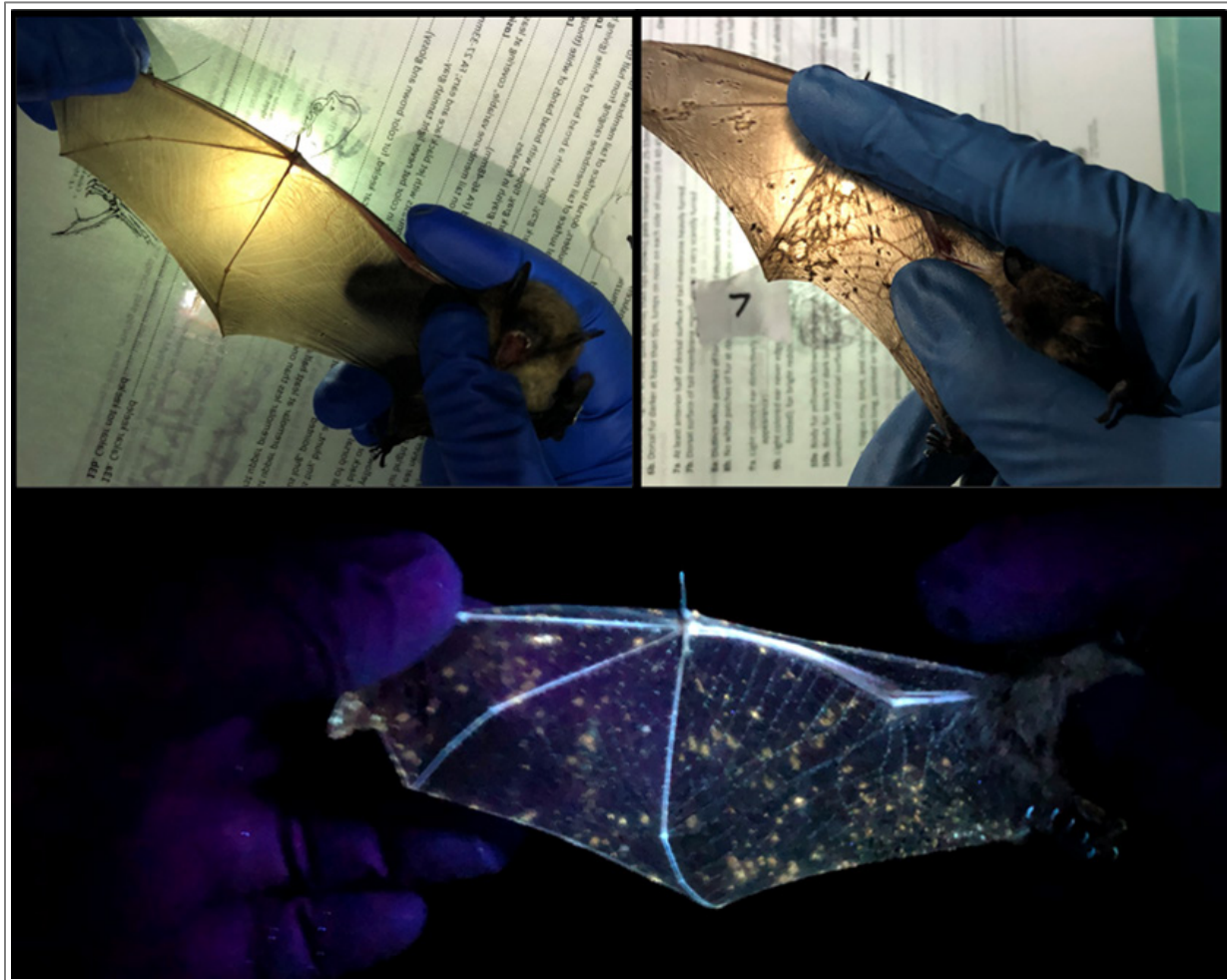


Figure 3. Comparison of healthy and damaged *Myotis lucifugus* wings. A healthy wing from the Ohanapecosh sampling location in Mount Rainier National Park is top left, a damaged wing from Ohanapecosh, potentially caused by white-nose syndrome (WNS), is top right, and the bottom image is a wing from Tahoma Woods with orange fluorescence under UV light consistent with WNS pathology. All photographs were taken in spring 2024.

USGS / MICHAEL HANSEN

After the initial detection of Pd at Ohanapecosh in 2017, samples from four additional sites tested positive for Pd at Mount Rainer: Nisqually Glacier Bridge, Carbon River, Tahoma Woods, and the Nisqually Entrance (Table 3, Figure 2).

Table 3. Sampling timeline of the five sites in Mount Rainier National Park, one site in North Cascades National Park, and one site at Olympic National Park (Washington State) where we detected the presence of *Pseudogymnoascus destructans* (Pd) DNA in bats and guano from 2017 to 2024. A dash indicates that no sampling was done in a given year, Pd- indicates that there was no evidence of Pd DNA during sample testing, Pd+ indicates that Pd DNA was detected in one or more samples, but no tissue samples were collected, or tissue samples did not have diagnostic signs of white-nose syndrome (WNS) disease. Pd & WNS+ indicates that Pd DNA was detected, and tissue or carcass samples were submitted for histopathology by the National Wildlife Health Center and confirmed to have WNS. The species of bat is indicated unless the positive result was from guano or environmental samples.

Park Unit	Site Name	2017	2018	2019	2020	2021	2022	2023	2024
MORA	Ohanapecosh Box	Pd+ (<i>M. lucifugus</i>)	Pd-	Pd- ^A	Pd+ (guano)	Pd+ (<i>M. lucifugus</i>)	Pd+ (<i>M. lucifugus</i>)	Pd+ (<i>M. lucifugus</i>)	Pd & WNS+ (<i>M. lucifugus</i>)
MORA	Tahoma Woods	Pd-	Pd-	Pd-	Pd-	Pd-	Pd-	Pd & WNS+ (<i>M. lucifugus</i> , <i>M. evotis</i>)	Pd & WNS+ (<i>M. lucifugus</i> , <i>M. evotis</i>)
MORA	Carbon River	Pd-	Pd-	Pd-	–	Pd+ (guano)	Pd & WNS+ (<i>M. lucifugus</i> , <i>M. evotis</i>)	Pd & WNS+ (<i>M. evotis</i>)	–
MORA	Nisqually Glacier Bridge	–	–	–	Pd- ^A	Pd+ (guano)	Pd- (guano)	–	–
MORA	Nisqually Entrance	–	–	–	–	–	–	Pd & WNS+ (<i>Myotis</i> sp.)	–
NOCA	Stehekin	–	Pd-	Pd-	Pd-	Pd-	Pd-	Pd-	Pd+ (guano)
OLYM	Staircase Ranger Station	–	–	–	–	Pd-	Pd-	Pd-	Pd+ (environmental swab)

^A One or more samples had an inconclusive result.

Glacier Bridge, Mount Rainier

Guano samples from Glacier Bridge had inconclusive results from guano collected in 2020. One guano sample showed some amplification for Pd but only in one of three PCR replicates in two sample runs. Although the PCR product did amplify, we only consider a sample positive when a minimum of two of three PCR replicates amplify in two separate runs. In 2021, one sample did have a positive result, but no samples amplified in 2022. Genetic species identification from pooled guano at this site has identified sequences from western *Myotis evotis*, *M. lucifugus*, *Eptesicus fuscus*, and *Corynorhinus townsendii*, but only *C. townsendii* have been observed roosting under the bridge. Only guano samples have been collected from this site.

Carbon River, Mount Rainier

Guano samples from the Carbon River site first tested positive in 2021. The following winter, park staff collected three carcasses at the Carbon River Ranger Station (one *M. lucifugus* and two *M. evotis*). Two swabs from these bats tested positive, and those two carcasses were sent to the NWHC. The *M. lucifugus* was confirmed to have diagnostic signs of WNS, and the *M. evotis* result was WNS suspect. Tissue samples from all three bats tested positive for Pd. This was the first WNS confirmation in Mount Rainier National Park. An additional *M. evotis* carcass was found at the Carbon River site in winter 2022/2023 and submitted to the NWHC. This carcass was also confirmed to have WNS.

Tahoma Woods, Mount Rainier

The Tahoma Woods site also tested positive for Pd and WNS for the first time in 2023. This site is a collection of structures that bats of multiple species and both sexes use, rather than a maternity colony in a specific roost, like the other regularly sampled site with positive WNS results, Ohanapecosh. Roosts at Tahoma Woods where bats have been captured include a shed with a bat box mounted to the outside. *Myotis evotis* usually roost inside the shed and *M. lucifugus* and *M. californicus* occupy the bat box and some nearby tarp storage tents. Individual bats of all three species are occasionally found on adjacent buildings roosting in gable vents and roof eaves. Staff collected carcasses of two *M. evotis* and one *M. lucifugus* from the Tahoma Woods site in 2023, all of which tested positive for Pd and WNS. Swabs from seven of the 12 live bats from this location also tested positive for Pd in 2023. In spring of 2024, four more bat carcasses were found at Tahoma Woods (one *M. evotis* and three *M. lucifugus*), and all tested positive for WNS. There were few live bats present at the time of capture in 2024, and we were only able to swab six bats. Two swabs tested positive (one *M. evotis* and one *M. lucifugus*), three swabs from *M. lucifugus* were inconclusive, and the sole negative swab was from an *M. californicus* roosting on a different building. We had sampled at Tahoma Woods regularly prior to 2023, with no positive results or observed disease. The first detection of WNS and concurrent mortalities at the same time as the initial detection of Pd contrasts with the slower disease progression following initial Pd detection at the Ohanapecosh colony.

Nisqually Entrance, Mount Rainier

The carcass of an unidentified *Myotis* was collected by NPS staff near the Nisqually Entrance and sent to the NWHC in spring of 2023. This carcass was also confirmed to be positive for WNS through histopathology, but there is no roost location associated with this detection.

Staircase Ranger Station, Olympic

In 2024, Olympic National Park had its first detection of Pd from five environmental swabs and 2 guano samples taken from the Staircase Ranger Station (Table 2, Table 3, Figure 4). The Staircase Ranger Station has been sampled using guano and captures since 2021. Only female *M. yumanensis* have been captured at this roost, but *M. lucifugus* sequences have been detected in pooled guano samples. No bats have been captured at this site since the detection of Pd in 2024, so it is unknown if any bats that roost at this site have WNS. Carcasses have not been observed at this location either.

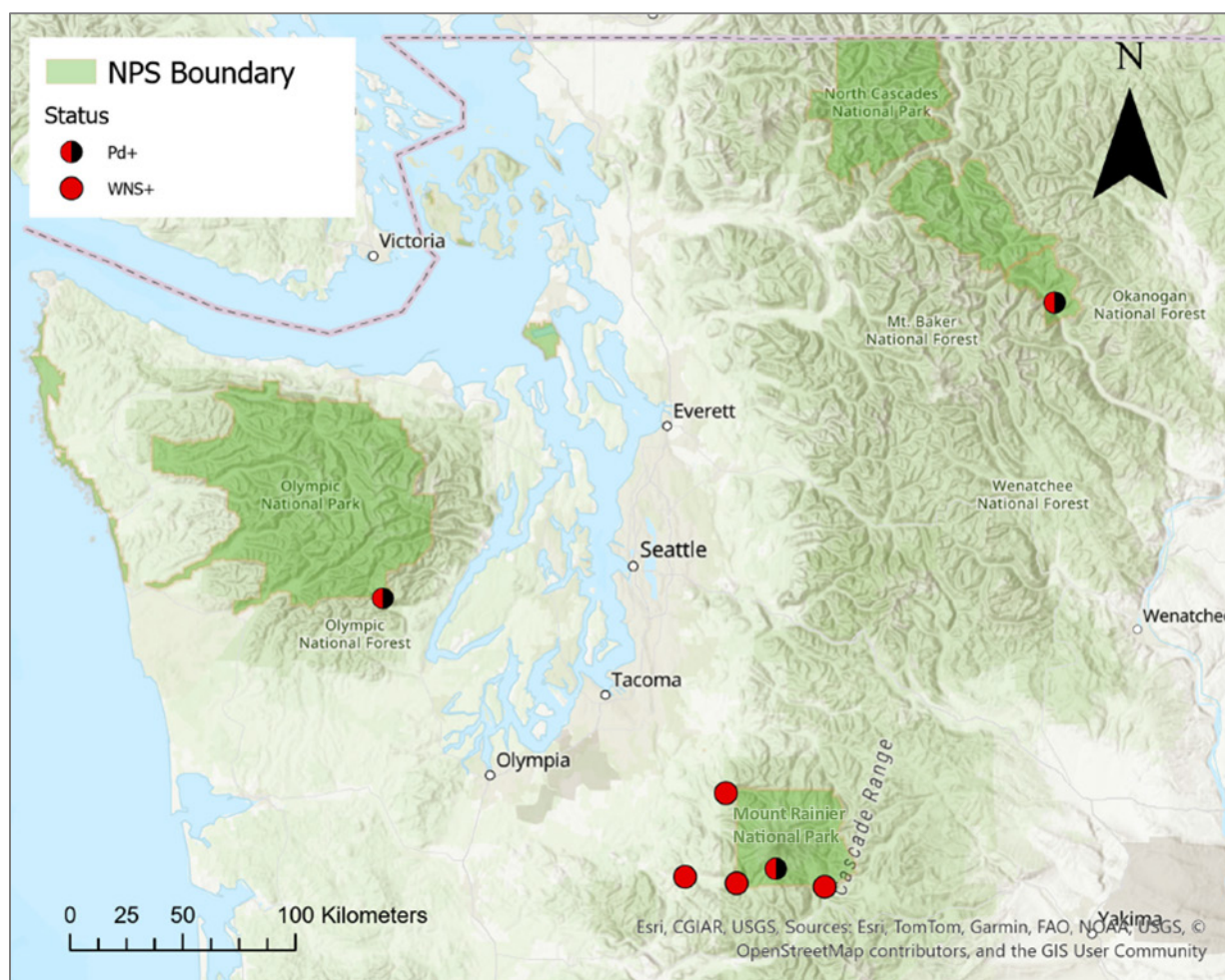


Figure 4. Locations across the North Coast and Cascades Network (NCCN) where the fungal bat pathogen *Pseudogymnoascus destructans* (Pd) has been detected, indicated as Pd+, and where histopathology confirmed white-nose syndrome, indicated as WNS+, as of spring 2024. NPS = National Park Service. Please refer to Figure 1 for the larger geographical context of these parks.

USGS

Stehekin, North Cascades

North Cascades also had its first positive detection in an NPS housing unit in Stehekin, WA in 2024. Environmental swabs from around the building tested negative, but a pooled guano sample tested positive. The demographic composition of this colony has varied across sampling events. Most years

we captured 10–20 mixed sex individuals, and during one sampling event captured over 100 females. We have only identified *M. yumanensis* during captures at Stehekin, but *M. lucifugus* and *Eptesicus fuscus* sequences have been detected in pooled guano samples. No bats have been captured at this site since the detection of Pd in 2024, so it is unknown if any bats that roost at this site have WNS, although no mortalities have been observed.

Experimental Work

i. Tracking Pd Genomic DNA Degradation Rates

Study Area

We selected six different locations in Mount Rainier National Park that were known to be occupied by bats. We set up the experiment outside of buildings and structures occupied by bats where guano would be exposed to sunlight, rain, wind and other weather. Sites were categorized as exposed or protected according to the presence of structures that shielded guano samples from direct exposure to solar radiation or rain. One site was removed from the analysis because the samples were disturbed at the site. At these sites, we set up our experiment by randomly placing petri dishes in identical trays. We placed 1 g of autoclaved guano (SD = 0.018 g) in each petri dish with 1 ml of inoculum, at five concentrations of genomic DNA (fg) of Pd (Table 4). A single strand of DNA has a mass of approximately 6.5 fg. Each concentration was plated in three replicates for a total of 15 samples per site. Prior to initiating the experiment, we consulted with WDFW and NPS wildlife veterinarians to receive concurrence that non-infective, genomic DNA posed no threat to bats that occupy these sites.

Table 4. Concentrations of *Pseudogymnoascus destructans* genomic DNA used to evaluate degradation through time in Mount Rainier National Park.

Concentration	DNA Amount (fg)
Control	0
1	1,000,000
2	100,000
3	10,000
4	1,000

Sample Collection

We collected samples from the petri dishes during six sampling events from July 10th, 2018 to September 17th, 2018. At each site, we wore a new pair of nitrile gloves to collect guano pellets in each concentration treatment to avoid cross-contamination among different concentrations. We collected 15 samples per each of the five sites with a total of 450 guano samples (mean = 0.13 g $\pm$ 0.06 g/ sample). Samples were placed inside 2.0 ml Eppendorf safe-lock tubes and stored on ice. After each sampling event, samples were shipped overnight to Oregon State University to prevent damage or degradation. Samples were stored at -20°C until DNA extraction.

Quantifying Genomic DNA Degradation Rates

To quantify the amount of DNA on the guano collected we used real-time qPCR. All guano samples were weighed, and DNA was extracted using a DNeasy PowerLyzer Powersoil Kit following the manufacturer's instructions. Details of reactions and amplification conditions followed the protocols reported above under the heading "Pd detection."

We evaluated the rate of DNA degradation using log-transformed DNA quantity measured with qPCR as the dependent variable in a linear model with normal error distribution. To facilitate log-transformation, we added 0.195 fg to all DNA concentrations, which corresponded to the lower limit of detection for this experiment. To evaluate the change in the probability of detection, we used logistic regression with Pd positive/negative as the dependent variable. Our predictors for both models included: initial quantity of inoculum, field site, sampling time, mass of guano sampled, and an interaction term for site and sampling time to account for any variation in the degradation rate of DNA by site. We determined the best fit model using Akaike's Information Criterion corrected for small sample size (AICc) and performed statistical analyses in R (Version 3.4.1, 2017-06-30) using packages *plyr*, *magritt*, *ggplot2* and *cowplot* (Wickham 2011, 2016; Bache and Wickham 2014; Wilke 2019). This research has been published in Urbina et al. (2020).

ii. Bat Species Identification from DNA

Study Area

Samples for bat identification were collected at different locations across the NCCN (Table 2). We used the samples collected during the sampling events of 2018–2020 as swabs, guano, and wing punches from the same sites to facilitate comparison of results among sample types.

Sample Collection

All swabs and guano were preserved following standard protocols described above and were stored in a –20 °C non-frost-free freezer to prevent degradation. All samples were shipped to Oregon State University after completion of sampling events to be analyzed by Dr. Urbina in the Levi laboratory.

Species Identification

Accurate identification of bat species based on morphological features is sometimes difficult, particularly for species classified in the same genus (e.g., *Myotis*). Collection of skin swabs is a less invasive method to collect DNA from bats and prevents injuries generated by wing biopsies (Faure, Re and Clare 2009). Similarly, bat guano can be used to obtain good quality DNA that can be used to identify bat species. By using DNA extracted from the skin swabs, guano, and wing punches collected during the sampling events, we worked to generate a framework to facilitate identification of species from samples collected across the NCCN, with Mount Rainier as the primary reference site.

Four different genes were considered to generate the best framework for species identification: 12s, 16s, cytochrome b (cytb) and cytochrome c oxidase I (COI). For each of the genes, we compiled all sequences reported for 15 species from areas in and around Mount Rainier National Park.

Information came from two main database repositories, GenBank and Barcode of Life Data Systems (<https://boldsystems.org/>).

We selected records of sequences from species reported in California, Oregon, Washington, Nevada, Idaho, Montana, British Columbia, and Alberta. These filtered datasets for the different genes were useful for selection of appropriate sequences that could work as forward and reverse primers.

For primer validation and assessment, we used extractions originated from skin swabs and wing biopsies for at least five species to determine if primers amplified properly and to select the best primer combination. We tested several sets of selected primers for all the genes. Generally, we used a temperature gradient PCR, with conditions ranging from 46 to 56°C to cover the temperature reported on the forward and reverse primers description.

We visualized amplification of PCR products on 1% agarose gels under UV light. If products amplified properly, then we purified PCR products using the ExoSAP-IT protocol (Affymetrix, Santa Clara, CA, USA). Once all PCR products were cleaned, we submitted them for sequencing using the Sanger method at the OSU Center for Genome Research and Biocomputing. After Sanger sequencing, the resultant sequences were blasted at <https://blast.ncbi.nlm.nih.gov/Blast.cgi> to verify them as bats species sequences. All obtained sequences were then analyzed using the Geneious software (2018) to evaluate if the sets of primers selected were appropriate to identify the species from where the DNA was extracted.

Using tissue samples from museum specimens with confirmed species identification, we also tested our different sets of selected primers for all genes, following the same protocols mentioned above. Similarly, we used a temperature gradient polymerase chain reaction (PCR), to cover the temperature reported in the forward and reverse primers description.

iii. Pd Growth in Different Substrates

There is great interest to conduct Pd surveillance using non-invasive techniques, that do not involve bat handling, to facilitate rapid detection of this fungus. However, the role played by different substrates as environmental reservoirs is not fully understood. Importantly, we sought to understand if Pd detections using non-invasive methods could be from live fungus persisting in substrates, which would inform decontamination and management actions. We evaluated whether Pd would grow on guano (fresh or sterilized), soil, and plywood, which are environmental substrates present in bat hibernacula or roosting locations. We assessed 1) Pd growth and persistence on all these substrates, 2) differences in growth rates of Pd among different substrates and 3) viability of conidia retrieved from remainder substrates after the end of the experiment. This experiment has been published in Urbina et al. (2021).

In a Biosafety Level 2 laboratory at OSU, we inoculated four replicates of fresh guano, autoclaved guano, plywood, and soil held in individual petri dishes by pipetting 1 ml of inoculum (460,000 Pd conidia) on top of 1.06 ($\pm$ 0.0066) gm of substrate. Inoculation of the plywood pieces (5.08 cm side, ~3 mm thick) was done by dividing the piece of wood into five equal parts that were assigned a random order to be sampled. We inoculated each part of the plywood by pipetting five drops of 40 ul per part (200 ul), so the total piece of wood had the same amount of conidia as the other substrates. Further details on the experimental setup can be found in Urbina et al. (2021).

Sample Collection

We performed the first sampling event (time zero) immediately after adding the Pd inoculum to all the substrates. We collected 0.18 ($\pm$ 0.05) gm of autoclaved guano, fresh guano, and soil to quantify

the amount of Pd for each substrate replicate petri dish. For the plywood sampling, sterile rayon swabs (MVE, Medical Wire, UK) were rolled into the length of the plywood within a row allocated for each sampling event to avoid swabbing the same portion of wood repeatedly. We continued collecting samples from each substrate at four regular intervals every 16 days until day 64. All petri dishes holding the substrates were kept in a refrigerator at 9°C without a particular light/dark cycle. Samples were stored in vials at –20°C prior to DNA extraction for no longer than two weeks. We quantified Pd using a probe-based qPCR assay targeting the intergenic spacer region (IGS) of the fungus following standard protocols described above. To confirm conidia viability, we soaked the remaining material from each substrate in 5 ml of phosphate buffered saline for 15 minutes, then collected the liquid. We used a binocular compound microscope (40x magnification, Model B120C, AmScope) to count the number of conidia in the harvested liquid using a hemocytometer. We plated 10 ul of the harvested liquid using the droplet method in two Sabouraud Dextrose Agar petri dishes (150 mm × 15 mm) divided into four quadrants. We also used the streak method to plate two additional petri dishes for each sample using a sterile rayon swab that was submerged in the harvested liquid. We visually observed and photographed the plates to assess whether harvested spores were viable two weeks later. We confirmed the fungus was Pd using quantitative PCR (qPCR). Further details can be found in Urbina et al. (2021).

iv. Pd Viability in Wood Collected from a Pd Positive Bat Box

The two previous experiments led us to investigate whether Pd could survive in natural settings, where bats that tested positive in the spring occur during the summer months. Pd is often described as cryophilic (thriving in cold temperatures), and little is known about its seasonal ecology. We sought to evaluate if bat boxes could be environmental reservoirs for Pd by collecting wood from the surfaces bats come into physical contact with when they roost. If Pd can survive the high summer temperatures in the wooden substrate, then bat boxes and other wooden structures that bats occupy in the summer months could be potential candidates for treatments to reduce bat exposure and Pd spread to hibernacula, where it can develop into WNS in bats exposed to the fungus.

In 2023, we collected wood shavings using a stainless steel 4 mm No.7 woodworking gouge from the bat box at Ohanapecosh, Mount Rainier used by Pd-positive bats. Our goal was to determine if Pd remained viable in the wooden substrate of the bat box through ambient summer temperatures. Samples were collected at two times while bats occupied the box, June 22 and August 22. To avoid disturbing roosting bats, we collected the samples in the evening, after they left the roost for the night. We avoided disturbing the young of the year by sampling early in the season, before bats were expected to be born, and again after the young were expected to be volant. We continually monitored temperature using a HOBO U23 Pro v2 External Temperature/ Relative Humidity Data Logger (Onset Computer Corporation, Bourne, MA) set to record at 2-hour intervals from November 2021 to September 2023. Samples of wood were placed in 2.0 ml Eppendorf tubes or whirl-pac bags, then refrigerated prior to transport to the Biosafety Level 2 laboratory at OSU.

We isolated fungi in the wood shavings by culturing on petri plates. Once in the lab, the material from individual bags or vials were used to start isolating fungus using petri plates containing Sabouraud Dextrose Agar (SDA). A wood chip was added directly to SDA plates. Using 2 ml vials,

wood chips were resuspended in 500 ul of liquid media (malt broth) or ultrapure water. We prepared slides to check microscopically for Pd conidia immediately after resuspension and again two days later. Then, we used four drops of 30 ul each from the broth and the water to start cultures in petri plates with SDA.

One month later, we did a second isolation procedure taking portions of the growth cultures with a sterile agar punch per type of pure culture or using a dilution of the original material prepared. We then evaluated growth and viability of the fungi observed on the plates. From those live cultures, we did DNA extractions to definitively identify as Pd the fungal species we observed growing using quantitative PCR. We used the same DNA extraction and qPCR techniques as described in the previous section (Muller et al. 2013).

v. Decontamination Using Ethanol and Bleach

In our final experiment, we evaluated the 2020 National White-Nose Syndrome Decontamination Protocol that recommended bleach and ethanol to decontaminate sampling equipment (WNSDMWG 2020). Following an observation in the laboratory, where Pd cultures appeared to still be growing after following the decontamination protocol using ethanol, we reviewed the references cited in the protocol to ensure we were using the proper procedures. We found that one reference was to a proposal, not a completed study, and that reference was not publicly available: “J.A. Glaeser and C. Kunze – Further Evaluation of Decontamination Products to Minimize Human-based Transmission of *Pseudogymnoascus destructans*. In prep. These products were tested by the Northern Research Station, under USDA Forest Service Cooperative Agreement 13-IA-11242310-036 (U.S. National Park Service and U.S. Forest Service) and 16IA11242316017 (U.S. Fish and Wildlife Service and U.S. Forest Service).” Upon reading the second reference, Shelley et al. (2013), we found that the recommendations in the national protocol were contrary to their findings. Specifically, Shelley et al. stated, “Alcohol did not demonstrate any effective killing of fungal spores in these assays (Table 4).”

We ran our experiments following methods similar to those of Shelley et al. (2013) to evaluate the action of ethanol and bleach. For ethanol, we considered different concentrations (60, 70, and 80%) and bleach at concentrations of 1:10, 1:5, 1:2, and undiluted. We checked viability by plating cultures at two different times after exposure (15 minutes and 24 hours). We did not evaluate the action of bleach or ethanol on fabrics or other surfaces (instruments or tools) that can be in contact with contaminated surfaces or bats.

Ethanol is preferred by many practitioners in the field because it is easier to manage and apply (wipes are readily available). Ethanol also does not damage and corrode equipment and clothing like bleach does. In the laboratory setting, household bleach is an affordable and accessible product that can easily be used to decontaminate items that cannot be autoclaved. From a biosafety perspective, it is essential that recommended procedures for decontamination are effective to prevent the spread of pathogens.

Experimental Results

i. Tracking Genomic DNA Degradation Rates

Quantity of genomic DNA decreased through time with varying decay rates across sites. Over the duration of the experiment, degradation rates ranged from 1.6% (Nisqually site) up to 8.2% (Ohanapecohsh site) and were generally lower in protected sites that had overhead cover from sunlight, wind, and rain. Samples inoculated with the highest quantities of Pd DNA maintained detectable levels of Pd during all sampling events at all sites, and 60% of samples inoculated with the lowest quantity of DNA maintained detectable levels of Pd in four out of five sites by the end of the experiment. The probability of detection varied according to site and the interaction with sampling time. Sample mass had a significant effect on the amount of DNA detected and in the positive detection of samples. The minimum amount of Pd that we were able to detect was 0.195 fg, which is similar to the amount reported by Verant et al. (2018) from hibernacula sediments. Further results are detailed in Urbina et al. (2020).

ii. Bat Species Identification

We collected 140 skin swabs, 124 pellet samples, and 16 wing biopsies in 2018. After evaluating and validating the different alignments and primers for the four different genes, we found that 16s, 12s, and cytochrome b (cytb) were not the best candidates to identify different species of bats, particularly those in the same genus.

We evaluated the primers LGL284 and LGL384 (Zinck, Duffield and Ormsbee 2004), but those primers did not work for our experiments. As the annealing temperature could explain this failure, we re-ran temperature gradient PCRs, but we could not obtain any product. Primers selected for cytb did not work properly either. Mitochondrial divergence does not always reflect morphological differences across species (Table 5). The National Wildlife Health Center has also developed a promising method to identify bat species using nuclear DNA.

Table 5. List of bat species that cannot be 100% identified according to sequences aligned from different genes.

Gene	Species ^A	Source	Year
COI	• MYCI/ MYCA	Vonhof, Russell and Miller-Butterworth 2015	2015
	• MYEV/ MYTH	Walker et al. 2016	2016
	• MYLU/ MYOC		
Cytb	• MYLU/ MYTH	Ruedi and Mayer 2001	2001
	• MYCA/ MYCI	Rodriguez and Ammerman 2004	2004
	• MYEV/ MYKE ^B	Lausen et al. 2018	2018
	• MYKE ^B / MYTH	This report	2019
	• MYCA/ MYU		

^A MYCA = *Myotis californicus*; MYCI = *Myotis ciliolabrum*; MYEV = *Myotis evotis*; MYKE = *Myotis keenii*; MYLU = *Myotis lucifugus*; MYTH = *Myotis thysanodes*; MYVO = *Myotis volans*.

^B Recent evidence suggests MYKE and MYEV are the same species (Lausen et al. 2019).

Table 5 (continued). List of bat species that cannot be 100% identified according to sequences aligned from different genes.

Gene	Species ^A	Source	Year
UCE	• MYEV/ MYTH	Morales et al. 2019	2019
	• MYEV/ MYTH	Morales et al. 2016	2016
Nuclear genes	• MYCA/ MYCI • MYEV/ MYKE ^B / MYTH	Stadelmann et al. 2007	2007
12s	• MYCA/ MYCI • MYEV/ MYLU • MYEV/ MYKE ^B • MYEV/ MYTH • MYKE ^B / MYLU • MYKE ^B / MYVO • MYKE ^B / MYTH • MYLU/ MYTH	This report	2019
16s	• MYCA/ MYCI • MYEV/ MYKE ^B • MYEV /MYTH • MYLU/ MYEV • MYLU/ MYKE ^B • MYKE ^B / MYTH	This report	2019

^A MYCA = *Myotis californicus*; MYCI = *Myotis ciliolabrum*; MYEV = *Myotis evotis*; MYKE = *Myotis keenii*; MYLU = *Myotis lucifugus*; MYTH = *Myotis thysanodes*; MYVO = *Myotis volans*.

^B Recent evidence suggests MYKE and MYEV are the same species (Lausen et al. 2019).

iii. Pd Growth in Different Substrates

Our laboratory experiment examined Pd growth on four different substrates that were inoculated with Pd in a laboratory setting; plywood, potting soil, fresh guano, and autoclaved guano (Urbina et al. 2021). We detected Pd DNA in all substrates during all sampling events. In autoclaved guano and plywood, the quantity of Pd detected increased over time and was greater than the inoculum dose, suggesting these substrates may be environmental reservoirs. In fresh guano, Pd remained stable and the quantity of Pd detected decreased in soil, suggesting microbial interactions that may influence Pd growth in these substrates. Pd grew from liquid obtained by soaking wood, soil, and autoclaved guano in phosphate-buffered saline. There was no evident growth of Pd in plates inoculated from harvesting liquid from fresh guano (Urbina et al. 2021).

iv. Pd Viability in Wood Collected from a Pd Positive Bat Box

Our results provided evidence that Pd is persistent and remains viable throughout the summer in a wooden structure (bat box) where bats seasonally occur in a maternity colony. Mean temperature inside the bat box in June was 16°C with a maximum of 38°C and minimum of 5°C. Mean temperature inside the bat box in August was 22°C with a maximum of 44°C and a minimum of 10°C. Pd was present in the wood shavings we collected from the maternity colony during both sampling events (June 22 and August 22, 2023). We observed viable fungus that grew in culture, and we confirmed that the fungus was Pd using proven molecular methods.

v. Decontamination Using Ethanol and Bleach

Similar to Shelley et al. (2013), we found that ethanol was not an effective sporicide in any of the concentrations we tested (60%, 70% and 80%). Contrary to Shelley et al. (2013), we found that a 1:10 dilution of household bleach did not kill Pd spores. The 1:5 dilution killed some, but not all spores and the effectiveness varied across replicates. The 1:2 dilution and the undiluted bleach was effective at killing Pd spores.

Discussion

In our work across the NCCN from 2016–2024, we documented the spread of Pd from one to several sites in Mount Rainier, the first detection of Pd in Olympic and North Cascades National Parks, and the continued Pd-negative status in other monitored NCCN parks. These data provide a useful baseline for continued monitoring of disease spread throughout this park system, which can be combined with broader surveillance efforts conducted across the state to assess impacts of Pd and WNS spread on Washington’s WNS-susceptible bat species. This effort addressed the priority objective of WNS surveillance as outlined in the PWR WNS Response plan, as well as the objective of collaboration and coordination across regions and agencies. Disease surveillance was conducted in coordination with WDFW and NWHC, and results were disseminated to those partners to be incorporated into the Washington State and North American WNS distribution data and map products. Participation in the NWHC Pd Surveillance 3.0 and 4.0 efforts is ongoing. Further, our collaborative experimental work on Pd DNA degradation rates, Pd growth on different substrates, and Pd persistence in wood substrate from a sampled bat box advance our understanding of 1) the feasibility of non-invasive sampling for Pd, and 2) where and how Pd can persist in the environment, with implications for disease spread. Finally, our work testing two chemicals included in decontamination protocols for Pd documented the ineffectiveness of these chemicals, which resulted in updates to the national WNS decontamination protocol. Together, our work advances understanding of both where and how we test for Pd on the leading edge of the disease and can inform future protocols and management recommendations going forward.

Bat Monitoring and Disease Surveillance

Our surveillance work over the past eight years documented a lag between initial and subsequent detections of Pd, years of detecting Pd on bats at a summer roost before any observations of WNS disease in the population, and a slow but steady geographic spread of Pd in the NCCN. These observations follow the larger pattern of slower spread of Pd and WNS in bat populations west of the Rocky Mountains (Blejwas et al. 2023). We detected Pd from skin swabs collected at the Ohanapecosh colony in Mount Rainier National Park in 2017, but guano samples from this site did not test positive for Pd until 2020, three years later. We detected Pd at Ohanapecosh again in 2020, and proportions of positive samples increased each year to near-saturation in 2023 and 2024, with the first signs and confirmation of WNS in spring of 2024. Several factors could have contributed to the lag in consistent detections and delay in observed signs of disease in the Ohanapecosh colony.

Time gaps of up to a year have previously been observed between the initial detection of Pd from skin swabs before detection in environmental substrates (Verant et al. 2018, Langwig et al. 2015), and our observations show a similar, but prolonged delay of three years before pooled guano samples tested positive. Some of the bats that tested positive for Pd during spring captures may not have had infections over winter but may have picked up fungus after emergence from other bats or infected roost material in the bat box. Bats do not return to this colony until later in spring, so mild infections may have cleared and no longer been detectable during spring captures. Due to the apparently dispersed nature of hibernation sites in western populations, as opposed to large congregations in

caves and mines found in the eastern North America (Blejwas et al. 2023; Weller et al. 2018), it is likely that this population scatters to several different hibernacula in the winter, and it may take longer for the pathogen to spread and become established across many different locations. From the limited observations that have been made of the conditions inside hibernacula used by western *Myotis* species, microclimates may be less conducive to Pd growth than cavern hibernacula (Blejwas et al. 2023). Fungal loads in the environment play a major role in WNS infection, and infection severity can mirror the progression of Pd establishment and growth in hibernacula (Hoyt et al. 2020; Langwig et al. 2015; Langwig et al. 2016). As such, if it took several seasons for the fungus to spread widely to many hibernacula and then accumulated slowly to infectious levels in the environment, that could explain the lag between Pd detection and signs of disease. That said, the initial detection of Pd at the Tahoma Woods site was concurrent with WNS disease and mortality, so there is obviously some variation in disease progression among local populations or our ability to detect Pd and WNS at these sites. With only a handful of known hibernacula locations for *M. lucifugus* across western North America, and no known hibernacula for populations at Mount Rainier, it is impossible to observe these bats or their habitat during the winter when infections occur. Unless we can locate and access hibernation sites, we can only speculate as to why Pd and WNS infections progress as they do in these and other western bat populations.

The rate of geographic spread for Pd and WNS across the NCCN and Washington State has been slow relative to the spread during the initial outbreak in eastern North America, likely due to differences in winter ecology between these populations (Blejwas et al. 2023). We did not detect Pd at a second location until four years after the initial detection of Pd at Ohanapecosh. Between 2021 and 2024, samples from four additional locations across a wide area of Mount Rainier tested positive for Pd, and three of those have also tested positive for WNS. We had our first detection of Pd in Olympic National Park in 2024, although Pd was detected elsewhere on the Olympic Peninsula in 2021, and WNS was confirmed on the Peninsula in 2023 (WDFW 2024). We also had our first detection of Pd in North Cascades National Park in 2024. Over the last two years, sampling by other agencies has detected Pd near the boundaries of Ebey's Landing National Historical Reserve and North Cascades National Park Service Complex (WDFW 2024). Despite the relatively slow spread of Pd and WNS in the west, wildlife managers across the NCCN should anticipate the arrival of Pd to their bat populations in the coming years.

Experimental Work

We sought to use an early detection/rapid response framework to detect Pd in bat colonies when disease prevalence was low so we could prioritize response actions. We were successful in achieving this goal through a series of laboratory experiments that directed our case study field investigations. To our knowledge, our work provides the first evidence that Pd is viable in wooden substrates that bats occupy in the summer months. Our work also provides a path to develop protocols to implement early detection/rapid response that targets Pd growth and does not affect bats.

The probability of detecting a pathogen when it is present is not perfect or linear: detection depends on several variables including population size, the number of samples collected, and infection prevalence and the sensitivity of the testing methods used (Cameron and Baldock 1998). For

example, the probability of detecting Pd at the Mount Rainier colony is 95% when disease prevalence is 15% and 25 individuals within the population are sampled, whereas 60 individuals needed to be sampled for the same detection probability when disease prevalence is 5% (Cameron and Baldock 1998). Given the rapid spread of Pd in eastern USA and Canada (Hallam and McCracken 2011) and new cases reported in the Pacific Northwest, tools that enable early detection and identification of potential environmental reservoirs—such as those used in this study—could support rapid response to disease mitigations efforts.

Using non-invasive techniques that do not require direct animal handling as part of a disease surveillance program can be cost- and time-effective. Non-invasive sampling increases the number of samples that can be collected from a particular site and does not require specialized animal handling training or rabies vaccinations. However, it is important to have clearly stated study objectives to determine if bat handling is required or if non-invasive methods will meet objectives. If a study objective is to determine WNS status in a bat colony, bat handling is required; alternatively, if a study objective is to determine Pd presence in a bat colony, non-invasive sampling such as pooled guano collection or sampling substrates may suffice. Minimally invasive swabs can be collected to test for presence of Pd in only a couple of minutes per bat but does not confirm WNS disease. A wing biopsy is required to confirm presence of WNS lesions if clinical signs of the disease are observed (i.e., orange fluorescence on skin under UV light). If a study objective is to simply determine the presence of Pd, non-invasive methods may be appropriate, but the type of material sampled (e.g., pooled guano, substrates bats encounter) affects the probability of detection. It is important to note the three-year lag in Pd detection between bats and pooled guano sampled at the first site with positive results at Mount Rainier, which suggests that, in some cases, bats may need to have a high infection prevalence before the pathogen can be detected using non-invasive methods such as pooled guano.

Our work provides evidence to inform the design of non-invasive sampling protocols to minimize false negative results and identifies potential substrates to target for treatment. Collecting fresh guano simplifies sample collection; however, it is important to consider that DNA obtained from these samples can degrade through time. Our experimental work suggested that minute amounts of extracellular Pd DNA may be detectable in guano for up to 42 days (mass of 1,000 fg), an extremely small amount of DNA that is a fraction of a single genome in many Ascomycete fungi. Estimates indicated DNA can be detected for approximately 100 days when initial inoculation quantities are higher than 100,000 fg. This information can be used to develop a protocol that includes estimates of detection probability and the number of samples required to detect Pd DNA when it is present given the time since the specimen was deposited. Following standardized protocols can prevent this degradation. Importantly, in this experiment, we could not use live Pd at colonies with live bats and it is likely that DNA degradation would occur more quickly when not protected within conidia, so our results may overestimate true persistence of detectable Pd DNA in natural samples.

The viability of Pd on the environmental substrates we tested suggests that Pd-contaminated substrates (e.g., by infected bats contacting the surface, spores suspended in the air that land on surfaces, or researchers not properly decontaminating equipment) can potentially be an

environmental reservoir and remain infectious even when bats are not occupying the structure. The viability on plywood may indicate that human made structures could be playing a role in disease spread, particularly if the fungus invades and persists in wood. The time of year when these structures are used by bats is relevant for development of WNS. Although bats are not affected by WNS in the summer, it is possible that they could carry viable spores back to their winter roosts and hibernacula, where they and other bats who share the site could develop infections. Our study of Pd growth on different substrates validates the utility of experiments to understand Pd viability and Pd persistence through time in different environmental reservoirs. Importantly, our laboratory experiments, which identified wood as a substrate Pd could survive in, provided direction for our follow-up field work investigating Pd viability in a Pd positive colony.

Our observation that Pd can survive in a wooden substrate used by bats in the summer months has serious implications for bats that are vulnerable to WNS and their conservation. It also identifies a pathway to implement an early detection/rapid response for treatment that targets the fungus in wooden substrates that bats use. We are currently exploring RNA interference as a potential treatment to mitigate Pd, a treatment that targets Pd specifically and no other organisms that share their habitat.

Throughout the NCCN study area, all the known bat colonies were in buildings and structures such as attics, roofs, and bat boxes, all of which have wooden substrates that the bats come into contact with. We have documented Pd on adult bats that occupy the Ohanapecosh bat box as a maternity roost in the spring but have not tested them in the late summer to determine if they are carrying Pd spores on them when they leave the roost for the season; however, it is a reasonable assumption that this is possible. We acknowledge that this is a case study of one bat box and that the patterns across the landscape may be different than at this one site. Future work could expand to include multiple roosts made of different materials (i.e., concrete from bridge roosts, wood from bat boxes, etc.) that Pd positive bats occupy in summer. Roosts could be sampled repeatedly to determine if Pd is consistently viable in roost substrate throughout summer and bats could be sampled in the late summer to determine if they are carrying Pd from summer roosts to hibernacula and whether transmission of Pd to juvenile bats is occurring within maternity roosts. Concurrently measuring the range of conditions inside roost structures (e.g., temperature, relative humidity, microbial community) could also further refine our understanding of factors affecting the growth and persistence of Pd in the environment.

Our investigation into the national WNS decontamination protocol (WNSDMWG 2020) found that the protocol's recommendations for using ethanol or household bleach were not well substantiated and contained contradictory statements. For example, the protocol made recommendations that were contrary to Shelley et al.'s (2013) study findings that ethanol was ineffective in killing Pd. Our experimental results also determined that ethanol was not effective in killing Pd. Contrary to Shelley et al. (2013), we also found that 1:10 household bleach was not effective in killing Pd, though higher concentrations were. Importantly, protocols should clearly state measurements, as there are often differences between fields about ratios. For example, 1:10 in some fields indicates 1 part to 10 parts so the final solution volume equals 11 parts, while in other fields 1:10 indicates 1 part to 9 parts so

the final solution volume equals 10. Explicit protocols can help users avoid confusion, which could lead to ineffective decontamination. Our work resulted in changes to the national WNS decontamination protocol that were adopted by White-nose Syndrome Disease Management Working Group in the February 2024 protocol (WNSDMWG 2024).

Conclusions

We documented Pd presence and spread in the NCCN over an eight-year period since it was first detected at Mount Rainier in 2017, following initial detection of the fungus in Washington State in 2016. Our results shed light on the rate and extent of spread in the NCCN to date, with implications for ongoing surveillance in the region. We also conducted a series of experiments that inform how and where to test for Pd as well as the effectiveness of decontamination methods. Below we provide concluding recommendations based on our work.

Our work highlights the critical importance of network-wide surveillance for Pd and WNS in the region. Wildlife disease surveillance for any species can be improved through coordination with state officials and the intended diagnostic laboratories to ensure samples can be processed and assays are available to achieve desired results. Surveillance strategies focused on maximizing the probability of detection will improve our chances of early detection of Pd in bats and potential emergence in new areas. Timely sharing of information among response partners, particularly any new detections of Pd or WNS in new areas or species, can facilitate coordination of additional surveillance and other management actions as appropriate.

Quantitative PCR is a highly sensitive tool for detecting Pd. Use of standardized and qualified methods for sample collection and analysis, and use of diagnostic laboratories that participate in the national WNS diagnostic network, are important to ensure reliable results. Our results from the experiment evaluating degradation rates of non-infectious genomic DNA of Pd in guano at summer roosts provide insights into sampling timing and improve existing monitoring protocols. There is still a need to determine if the source of Pd detected in environmental samples such as guano and on substrates correspond to extracellular DNA fragments, intracellular dead Pd cells, or viable Pd cells, as those characteristics can affect the time of degradation, the probability of detection, and because the risk of disease spread is different; i.e., extracellular DNA fragments and intracellular dead Pd indicate former presence of the pathogen but no infection transmission risk. Our observation that Pd isolated from the wood substrate of a summer roost grew in culture suggests that decontamination measures and additional management actions, such as targeted treatment of Pd positive maternity colonies, may help to mitigate disease spread.

Effective methods for identification of bat species found within and near the NCCN require further validation. We were not able to accomplish that in this study but have identified additional genes that may provide more effective targets for differentiating *Myotis* spp. The COI gene has been suggested as the best candidate for species identification in bats (Walker et al. 2019). Nuclear DNA has also shown promise in designing assays to differentiate some bat species that cannot be reliably identified with mitochondrial DNA (Stadelmann et al. 2007). The information obtained from alignments of genetic sequences is fundamental to generate a baseline that leads to proper identification of species and to differentiate species of the same genus.

Public awareness and decontamination protocols for WNS have been focused on cave environments, but the public may also encounter potentially infectious roost material in sheds, attics, or the soil and

guano surrounding bat boxes. We suggest further study into the persistence of viable Pd in natural settings on substrates that bats come into contact with and how that persistence changes as substrates are exposed to different seasonal conditions. This would include persistence of viable Pd throughout summer, both inside Pd positive roosts and in the surrounding environment.

Importantly, summer roost sites have been assumed to be low risk for Pd spread because the high temperatures they experience during the summer months are assumed to kill this “cold-loving” fungus. We observed viable Pd collected from the interior of a wooden bat box at a Pd positive roost in early and late summer. The next steps could be to expand this to examine substrates from additional Pd positive locations and test soil or other nearby material in transects around the roost. Understanding the seasonality and spatial extent of viable Pd in and around human structures could inform mitigation methods to protect against unintentional spread of viable Pd by people unknowingly contacting infected material, such as pedestrians who walk near infected roosts.

Finally, we suggest the National WNS Decontamination Protocol continue to be reexamined and refined as needed to ensure all recommended protocols are effective against Pd. Although having a dedicated set of equipment and gear for each site is the only way to completely prevent unintentional transport of the fungus or other potential pathogens or organisms between sites, effective and reliable means of decontamination can substantially reduce the risk in situations where dedicated equipment is not practical.

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Appendix A. Supplemental Table

A complete summary of disease surveillance work in the North Coast and Cascades Network parks from 2016 to 2024 is presented in Table 6.

Table 6. Disease surveillance sample results by year, park unit code, site name, and sample type for all locations where we conducted surveillance for *Pseudogymnoascus destructans* in the National Park Service North Coast and Cascades Network in Oregon and Washington State. Samples are divided by type: samples collected directly from bats (skin swabs and/or tissue) and samples from the environment surrounding a roost (guano unless specified as environmental swabs). The number of samples collected, and number of positive samples (in parentheses) are reported. A dash (–) indicates no samples were collected that year. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Year	Park Unit	Site Name	# Bat Samples Collected (# Positive)	# Environmental Samples Collected (# Positive)
2016	EBLA	Ebey’s Landing	7(0)	–
	NOCA	Hozomeen	15(0)	3(0)
2017	EBLA	Ebey’s Landing	10 (0)	9 (0)
	MORA	Carbon River	–	4 (0)
	MORA	Ohanapecosh Box	24 (4)	6 (0)
	MORA	Tahoma Woods	–	2 (0)
	NOCA	Hozomeen	25 (0)	3 (0)
	OLYM	Elwha	–	1 (0)
	OLYM	Hoh Ranger Station	–	2 (0)
	OLYM	Kalaloch	–	1 (0)
	OLYM	Olympic	–	4 (0)
	OLYM	Quinault	–	2 (0)
	SAJH	English Camp	21 (0)	5 (0)
2018	EBLA	Ebey’s Landing	1 (0)	–
	MORA	Carbon River	–	12 (0)
	MORA	Longmire Storage Bays	24 (0)	5 (0)
	MORA	Ohanapecosh Box	30 (0)	75 (0)
	MORA	Tahoma Woods	2 (0)	15 (0)
	NOCA	Hozomeen	25 (0)	10 (0)

^A One or more samples had an inconclusive result not included in the count of positive results.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed symptomatic white-nose syndrome (WNS).

^C Includes results from environmental swabs in addition to guano samples.

Table 6 (continued). Disease surveillance sample results by year, park unit code, site name, and sample type for all locations where we conducted surveillance for *Pseudogymnoascus destructans* in the National Park Service North Coast and Cascades Network in Oregon and Washington State. Samples are divided by type: samples collected directly from bats (skin swabs and/or tissue) and samples from the environment surrounding a roost (guano unless specified as environmental swabs). The number of samples collected, and number of positive samples (in parentheses) are reported. A dash (–) indicates no samples were collected that year. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Year	Park Unit	Site Name	# Bat Samples Collected (# Positive)	# Environmental Samples Collected (# Positive)
2018 (cont.)	NOCA	Stehekin	17 (0)	–
	OLYM	Hoh Ranger Station	17 (0)	10 (0)
	SAJH	English Camp	26 (0)	7 (0)
2019	MORA	Carbon River	–	3 (0)
	MORA	Ohanapecosh Box	25 (0)	40 (0) ^A
	MORA	Tahoma Woods	11 (0)	17 (0)
	NOCA	Hozomeen	25 (0)	16 (0)
	NOCA	Stehekin	6 (0)	13 (0)
	OLYM	Hoh Ranger Station	21 (0)	17 (0)
	SAJH	English Camp	25 (0)	20 (0)
2020	MORA	Nisqually Glacier Bridge	–	6 (0) ^A
	MORA	Ohanapecosh Box	–	39 (3)
	MORA	Tahoma Woods	–	14 (0)
	NOCA	Stehekin	–	20 (0) ^C
	OLYM	Hoh Ranger Station	–	22 (0)
	SAJH	English Camp	–	19 (0)
2021	MORA	Carbon Mine	–	2 (0)
	MORA	Carbon River	–	3 (1)
	MORA	Eagle Peak Mines	–	6 (0)
	MORA	Fryingpan Creek Bridge	–	1 (0)
	MORA	Longmire Warehouse	–	3 (0)
	MORA	Nisqually Glacier Bridge	–	3 (1)
	MORA	Ohanapecosh Box	57 (6) ^A	4 (1) ^A
	MORA	Tahoma Woods	–	3 (0)

^A One or more samples had an inconclusive result not included in the count of positive results.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed symptomatic white-nose syndrome (WNS).

^C Includes results from environmental swabs in addition to guano samples.

Table 6 (continued). Disease surveillance sample results by year, park unit code, site name, and sample type for all locations where we conducted surveillance for *Pseudogymnoascus destructans* in the National Park Service North Coast and Cascades Network in Oregon and Washington State. Samples are divided by type: samples collected directly from bats (skin swabs and/or tissue) and samples from the environment surrounding a roost (guano unless specified as environmental swabs). The number of samples collected, and number of positive samples (in parentheses) are reported. A dash (–) indicates no samples were collected that year. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Year	Park Unit	Site Name	# Bat Samples Collected (# Positive)	# Environmental Samples Collected (# Positive)
2021 (cont.)	MORA	White River Dorm	–	3 (0)
	NOCA	Diablo	–	3 (0)
	NOCA	Newhalem Amphitheater	–	1 (0)
	NOCA	Newhalem VC	–	3 (0)
	NOCA	Stehekin	12 (0)	6 (0)
	OLYM	Hoh	26 (0)	5 (0)
	OLYM	Staircase Bridge	–	2 (0)
	OLYM	Staircase Ranger Station	–	2 (0)
	SAJH	English Camp	64 (0)	6 (0)
2022	EBLA	Ferry House	–	1 (0)
	EBLA	Sheep Barn	–	1 (0)
	LEWI	Netul Landing	–	4 (0)
	MORA	Carbon River	3 (2 ^B) ^A	3 (2)
	MORA	Eagle Peak Mines	–	1 (0)
	MORA	Laughingwater Bridge	–	1 (0)
	MORA	Longmire Community Building	–	2 (0)
	MORA	Nisqually Glacier Bridge	–	5 (0)
	MORA	Ohanapecosh Box	34 (18) ^A	3 (3)
	MORA	Ohanapecosh Ranger Station	–	2 (0)
	MORA	Ohanapecosh Water Treatment	–	1 (0)
	MORA	Tahoma Woods	–	3 (0)
	MORA	White River Dorm	–	1 (0)

^A One or more samples had an inconclusive result not included in the count of positive results.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed symptomatic white-nose syndrome (WNS).

^C Includes results from environmental swabs in addition to guano samples.

Table 6 (continued). Disease surveillance sample results by year, park unit code, site name, and sample type for all locations where we conducted surveillance for *Pseudogymnoascus destructans* in the National Park Service North Coast and Cascades Network in Oregon and Washington State. Samples are divided by type: samples collected directly from bats (skin swabs and/or tissue) and samples from the environment surrounding a roost (guano unless specified as environmental swabs). The number of samples collected, and number of positive samples (in parentheses) are reported. A dash (–) indicates no samples were collected that year. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Year	Park Unit	Site Name	# Bat Samples Collected (# Positive)	# Environmental Samples Collected (# Positive)
2022 (cont.)	NOCA	Hozomeen	60 (0)	4 (0)
	NOCA	Stehekin	60 (0)	6 (0)
	OLYM	Hoh Ranger Station	20 (0)	4 (0)
	OLYM	Staircase Bridge	–	1 (0)
	OLYM	Staircase Ranger Station	28 (0)	1 (0)
	SAJH	English Camp	58 (0)	2 (0)
2023	MORA	Carbon Mine	–	1 (0) ^C
	MORA	Carbon River	1 (1 ^B)	–
	MORA	Nisqually Entrance	1 (1 ^B)	–
	MORA	Ohanapecosh Box	41 (40) ^A	–
	MORA	Tahoma Woods	15 (10 ^B)	3 (1) ^A
	MORA	White River Bat Box	–	1 (0)
	MORA	White River Dorm	–	1 (0)
	MORA	White River House	–	1 (0)
	NOCA	Hozomeen	60 (0)	1 (0)
	NOCA	Stehekin	11 (0)	2 (0)
	OLYM	Hoh Ranger Station	36 (0) ^A	1 (0)
	OLYM	Staircase Ranger Station	–	1 (0)
	SAJH	English Camp	60 (0)	1 (0)
2024	MORA	Ohanapecosh Box	31 (26 ^B) ^A	–
	MORA	Tahoma Woods	10 (6 ^B) ^A	–
	MORA	White River Bat Box	–	2 (0)
	NOCA	Hozomeen	60 (0)	2 (0)
	NOCA	Newhalem Bat Box	29 (0) ^A	2 (0)

^A One or more samples had an inconclusive result not included in the count of positive results.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed symptomatic white-nose syndrome (WNS).

^C Includes results from environmental swabs in addition to guano samples.

Table 6 (continued). Disease surveillance sample results by year, park unit code, site name, and sample type for all locations where we conducted surveillance for *Pseudogymnoascus destructans* in the National Park Service North Coast and Cascades Network in Oregon and Washington State. Samples are divided by type: samples collected directly from bats (skin swabs and/or tissue) and samples from the environment surrounding a roost (guano unless specified as environmental swabs). The number of samples collected, and number of positive samples (in parentheses) are reported. A dash (–) indicates no samples were collected that year. Park unit abbreviations: EBLA = Ebey’s Landing National Historical Reserve; LEWI = Lewis and Clark National Historical Park; MORA = Mount Rainier National Park; NOCA = North Cascades National Park Service Complex; OLYM = Olympic National Park; SAJH = San Juan Island National Historical Park.

Year	Park Unit	Site Name	# Bat Samples Collected (# Positive)	# Environmental Samples Collected (# Positive)
2024 (cont.)	NOCA	Stehekin	–	6 (1) ^C
	OLYM	Hoh Ranger Station	66 (0)	5 (0)
	OLYM	Mora Housing	–	2 (0) ^A
	OLYM	Staircase Ranger Station	–	7 (7) ^C
	SAJH	English Camp	28 (0)	3 (0)

^A One or more samples had an inconclusive result not included in the count of positive results.

^B Tissue sent to the U.S. Geological Survey’s National Wildlife Health Center (NWHC) for histopathology confirmed symptomatic white-nose syndrome (WNS).

^C Includes results from environmental swabs in addition to guano samples.

Appendix B. Funnel Traps

We visually estimated the circumference of the bat box opening and the distance of the bat house to the ground to minimize disturbance to bats occupying the roost. We assembled a tube from clear 3-mil plastic with duct tape (purchased from hardware store) and turned the tube inside out to ensure the bats did not come into contact with the sticky side of the tape. Approximately one hour before dusk, we attached the tube to the opening of the bat box using bungee cords and duct tape and monitored from a close distance for emergence (Figure 5).

We attached a cotton fabric bag (e.g., pillowcase) to the bottom of the tube with duct tape. The cotton fabric bag had an access hole sewn into it with a Velcro closure so bat handlers could access individual bats while keeping animals safe and contained. Once bats began to emerge, bat handlers removed individual bats from the cotton fabric bag for immediate processing. Bats appeared to remain calm and could move around within the bag for warmth or to keep cool (Figure 6).

Once bats appeared to have finished emerging or an hour had lapsed (whichever came first), the clear 3-mil plastic tube was removed and inspected to ensure there were no bats still inside the tube. To reduce the chances of bats learning to avoid emerging after the funnel trap is deployed, we are interested in exploring other slippery translucent materials that are more breathable, may be available at fabric stores, and do not restrict airflow as much as plastic.



Figure 5. Funnel trap tube attached to bat house to capture emerging bats.

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Figure 6. Inside view of pillowcase with captured bats.

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