Washington Department of Fish & Wildlife

White-Nose Syndrome Strategy Plan

Washington Department of Fish and Wildlife
Wildlife Diversity Division
Wildlife Program

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Cover photo: Yuma myotis roosting under a bridge / photo: S. Thomas

NEED AND PURPOSE

Washington Department of Fish and Wildlife (WDFW) decided a White-nose Syndrome (WNS) Strategy Plan was needed to clearly identify and prioritize strategies that ensures we are using resources effectively in our WNS response. The plan describes WDFW's bat conservation and disease management actions and guides future research and monitoring. WDFW will use this plan to coordinate and collaborate with partners and stakeholders including state, tribal and federal agencies, non-governmental organizations, and education institutions on the WNS response in Washington. The plan includes our first effort to prioritize issues and actions related to WNS.

This plan is subject to change as actions are implemented, we learn more about WNS in Washington, and new science becomes known. Plan review will occur approximately every 2 years or as needed to reassess each action and its priority and ensure WDFW management goals and objectives are consistent with the latest WNS research and disease occurrence information in Washington.

One of WDFW's bat conservation management goals is to preserve and protect Washington's bat populations by understanding emerging threats such as WNS. The actions and priorities described in this plan reflect WDFW goals and objectives. Using WDFW's Bat Conservation Plan (Hayes and Wiles 2013) and the National Plan for Assisting States, Federal Agencies, and Tribes in Managing White-Nose Syndrome in Bats (USFWS 2011) as guidance, these are WDFW's WNS response objectives:

(1) Coordinate and communicate internally and with partners and stakeholders on WNS response activities in Washington;
(2) Develop and implement outreach and education programs for bats and WNS;
(3) Investigate and understand the distribution and ecology of WNS in Washington;
(4) Understand the natural history of Washington bat species to effectively monitor populations;
(5) Limit the spread of WNS in Washington;
(6) Mitigate effects of WNS, to the degree possible, by managing habitat and mitigating other threats to maintain and enhance bat species diversity and abundance; and,
(7) Conduct and facilitate research related to bats and WNS.

BACKGROUND

WNS is a fungal disease in bats caused by the epidermal cellular infection of a psychrophilic fungus, *Pseudogymnoascus destructans* (Pd). WNS was first identified in North America near Albany, New York in 2006 and is believed to have originated from Europe (Puechmaille et al. 2010, Wibbelt et al. 2010). As of January 2018, WNS had spread to 31 states and 5 Canadian Provinces (Fig. 1). WNS has killed millions of hibernating bats in eastern North America by invading skin cells on their wings, muzzle, tail, and ears. This causes many bats to rouse from hibernation, burning critical fat reserves needed to survive through the winter, causing severe tissue damage and disrupting essential physiological mechanisms, such as fluid balance and gas exchange (Reichard and Kunz 2009, Cryan et al. 2010). Because Pd is a psychrophilic fungus, its optimal growing conditions (3 – 20°C, 90-100% relative humidity) are usually within the same climatic conditions present at some hibernacula; thus the impacts on some species can be devastating (Blehert et al. 2009). Currently, WNS has been confirmed in 9 species in North America, 3 of which are found in Washington, the little brown bat (*Myotis lucifugus*), Yuma myotis (*Myotis yumanensis*) and big brown bat (*Eptesicus fuscus*). There are, however, 15 species of bats in Washington, 14 of which presumably hibernate during the winter or part of the winter. It is possible then that these hibernating species may be susceptible to WNS (Table 1). The winter roosting ecology for most species found in Washington is largely unknown, making inferences about the impacts of WNS difficult (Table 2).

Many hibernacula either have been poorly documented or are undiscovered because of the difficulties with accessing and locating roosts; especially those in remote and obscure locations (e.g., talus slopes, cliffs, abandoned mines).

In March 2016, the first detection of WNS west of the continental divide occurred in King County, Washington. This initial detection occurred in a little brown bat. As of March 2018, 7 other detections of WNS or Pd in King County, and 1 detection of Pd in Lewis County have occurred. These additional detections indicate that WNS/Pd may be more widespread and environmental reservoirs are within or near the immediate area of these detections. Landscape level surveillance can be problematic due to technical and logistical restraints (e.g., locating roosting bats) and limitations on surveillance and diagnostic methods. Despite our limited understanding of bats in Washington, it is reasonable to assume that WNS has the potential to cause significant mortalities among some species and perhaps species imperilment or local extirpations in Washington.

It is not known how WNS reached Washington. The causative agent, Pd, is primarily transmitted by bat-to-bat or bat-to-environment contact. However, it is possible for humans to inadvertently carry Pd on clothing or equipment. WNS/Pd can persist year round in some environments such as caves and mines; thus, the risk of spreading the disease is possible throughout the year in those environments (Ballmann et al. 2017). Humans and other animals are not known to contract the disease (Frick et al. 2010). Bats are important ecologically and economically because they consume a vast amount of night-flying insects many of which are agricultural and forest pests (e.g., moths and beetles) and potential disease vectors (e.g., mosquitos). Protecting the stability and viability of Washington's bat population by limiting the spread and impacts of WNS is a vital part of WDFW's WNS strategy plan.

WDFW conserves and protects native species, including bats, and is the lead agency for coordinating, developing and implementing bat management activities and monitoring statewide. Therefore, it is WDFW's authority and responsibility to develop management actions to limit the spread and impacts of WNS on Washington's bat population. The US Fish and Wildlife Service is responsible for national and regional-level WNS leadership and WDFW seeks their advice and review during the development of WNS response plans and activities.

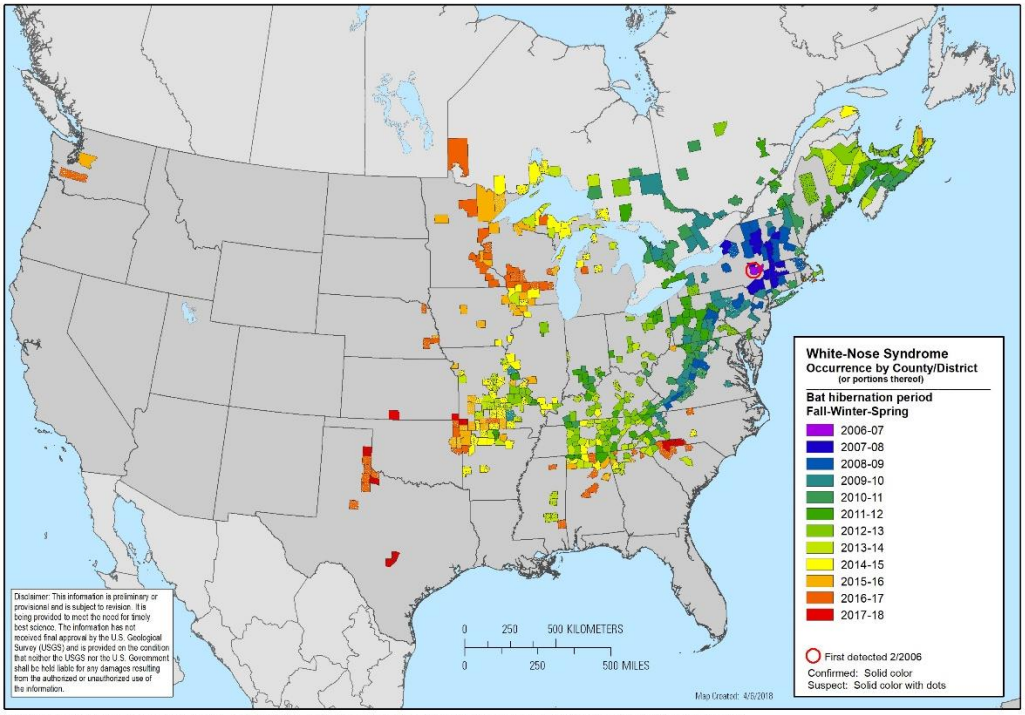

Figure 1. Current distribution of WNS in North America. *Citation: White-nose syndrome occurrence map – by year (2018). Date last updated: 4/6/2018. Available at https://www.whitenosesyndrome.org/resources/map.*

Table 1. List of the 15 bat species in Washington, including legal and conservation status, winter strategies documented in Washington, species confirmed with WNS, and species documented with Pd but no diagnostic sign of WNS.

Species	USFWS Species of Concern	WDFW Species of Greatest Conservation Need	Hibernating Species	Migrating Species	Active in Winter	Confirmed with WNS	Confirmed with Pd only
Antrozous pallidus (pallid bat)			X				
Corynorhinus townsendii (Townsend's big-eared bat)	X	X	X		X		X
Eptesicus fuscus (big brown bat)			X		X	X	
Euderma maculatum (spotted bat)		X	X				
Lasionycteris noctivagans (silver-haired bat)		X	X	X	X		X*
Lasiurus cinereus (hoary bat)		X		X			
Myotis californicus (California myotis)			X		X		
Myotis ciliolabrum (western small-footed myotis)	X		X				
Myotis evotis (western long-eared myotis)	X		X				

Species	USFWS Species of Concern	WDFW Species of Greatest Conservation Need	Hibernating Species	Migrating Species	Active in Winter+	Confirmed with WNS	Confirmed with Pd only
Myotis keenii (Keen's myotis)		X	X				
Myotis lucifugus (little brown bat)			X			X*	
Myotis thysanodes (fringed myotis)	X		X				
Myotis volans (long-legged myotis)	X		X				
Myotis yumanensis (Yuma myotis)			X			X*	
Parastrellus hesperus (canyon bat)			X				

*Species confirmed with WNS or Pd in Washington.

Table 2. Winter roost use for little brown bat (*Myotis lucifugus*), Yuma myotis (*Myotis yumanensis*) and big brown bat (*Eptesicus fuscus*) in Washington summarized from WDFW's centralized database. These species have been confirmed with WNS. Yuma myotis and little brown bat roosts are summarized together due to the difficulty in distinguishing between these species; observations were assumed not to have had genetic or acoustic analysis completed to confirm species.

Species	Total Number of Winter Roosts	Roost Type	Number of Bats in Roost	Survey Year(s)	County(s)
Myotis lucifugus (little brown bat) / *Myotis yumanensis* (Yuma myotis)	7	Caves	1 - 7	1965, 1967, 1970, 1971, 1972, 1999, 2001, 2003, 2017	Skamania, Yakima
	1	Buildings	1	2000	Island
	1	Abandoned mines	3	1989	Okanogan
Eptesicus fuscus (big brown bat)	1	Caves	1 - 2	1998, 2000, 2001	Yakima
	1	Bridges	1	2000	Thurston
	2	Buildings	1 - 2	2003, 2011	Island, Thurston
	2	Abandoned mines	1	2006, 2010	Stevens, Pend Oreille
	1	Talus caves	1	2012	King

Left: Big brown bat roosting under a bridge in western Washington / photo: C. Anderson
Right: Myotis species hibernating in an abandoned mine in western Washington / photo: S. Thomas

STRATEGIES, ACTIONS AND TASKS

Working with partners and stakeholders WDFW will implement a statewide WNS response. Strategies, actions and tasks priorities are identified in Table 3 as guidance and are ranked by urgency and feasibility of implementation over the next 2 years.

We will implement activities in phases. *Phase I* includes strategies and actions identified as critical. These are considered an essential component to our WNS response and should be implemented within the 2-year period. *Phase II* includes strategies and actions identified as a high priority. These are also considered an important component of our WNS response and should be attempted to be implemented within the 2-year period as well. Phase I and II can coincide when staff capacity and resources allow, however, this should not occur if it would affect the implementation of any of Phase I actions. *Phase III* includes strategies and actions identified as medium or low priority. These are considered not an essential component to our WNS response at this time and should be implemented only if resources allows. Actions are prioritized relative to all actions in the plan. Tasks are listed under each action and should be implemented relative to their prioritization. New tasks will be identified and reprioritized with implementation and more information.

Table 3 will be reevaluated collaboratively among WDFW and key partners to identify new actions and tasks every 2 years, or as needed based on effectiveness of the actions we take, disease occurrence, and current WNS research. Prioritization helps us meet management goals and objectives consistent with WNS and bat research as well as disease occurrence in Washington. Partners are encouraged to participate in actions regardless of personnel designations in Table 3. WDFW will communicate in a timely and effective manner on the status of implementation of the plan with partners and stakeholders.

Table 3. Washington Department of Fish and Wildlife's (WDFW) white-nose syndrome (WNS) response strategies, actions and tasks. Actions and tasks are prioritized to reflect current management needs for responding to WNS in Washington. These will be evaluated biennially or as needed to facilitate meeting management goals and objectives consistent with WNS and bat research and disease occurrence in Washington.

Objective 1: Coordinate and communicate internally and with partners and stakeholders on WNS response activities

Strategy	Action	Priority[1]	Survey Type[1]	Time Period[1]	Focal Area[1]	Survey Effort[1]
Communication	A. Communicate with partners and stakeholders in a timely and effective manner on WNS response activities and latest disease occurrence	Critical	On-going	Year round	Statewide	Coordinated / as needed
	Tasks				**Priority**	**Personnel** [1]
	1. Participate in regional and national calls and meetings.				Critical	WDFW-Hdqrts
	2. Develop WNS strategy actions with state, federal and tribal agencies, and nongovernmental organizations.				Critical	WDFW-Hdqrts
	3. Coordinate WNS activities with appropriate land management agencies.				Critical	WDFW
	4. Review and modify, if necessary, the WNS communication plan.				High	WDFW (and Public Affairs)
	B. Provide education and coordinate internally on bat conservation and WNS activities	High	New	Year round	Statewide	Coordinated
	Tasks				**Priority**	**Personnel** [1]
	1. Meet with Habitat and Fish programs, and Lands Division to discuss WNS strategy plan and how WDFW programs can contribute and coordinate on activities.				High	WDFW-Hdqrts

Objective 2: Develop and implement outreach and education programs for bats and WNS

Strategy	Action	Priority[1]	Survey Type[1]	Time Period[1]	Focal Area[1]	Survey Effort[1]
Education and Outreach	A. Conduct outreach to encourage reporting bat observations (e.g., aggregations, sick and dead bats)	Critical	On-going	Year round	Statewide	Coordinated / as needed
	Tasks				**Priority**	**Personnel** [1]
	1. Develop a WNS outreach implementation plan.				Critical	WDFW (and Public Affairs)
	2. Conduct outreach through social media and/or other media options focused in the fall and spring, to inform the public about WNS and solicit public observations of bats via WDFW online link (http://wdfw.wa.gov/conservation/health/wns/).				Critical	WDFW-Hdqrts
	3. Laminate and post WNS fact sheet and poster (Appendix 1) at trailheads, outdoor stores, visitor centers, campgrounds, climbing gyms, etc. Obtain landowner permission prior to implementing.				Critical	WDFW-Regions and Partners

	4. Present information about locating bat colonies and reducing WNS transmission to partners and stakeholders (e.g., Department of Transportation, Department of Health, wildlife control operators, Natural Resources Conservation Service, Port of Seattle and Tacoma, National Speleological Society).					High	WDFW
	5. Coordinate outreach activities with bat organizations and stakeholders (e.g., Bats Northwest, National Speleological Society, Washington Bat Working Group).					High	WDFW
	6. Update WDFW website with current WNS information and links.					High	WDFW-Hdqrts
	7. Update and develop new outreach materials (e.g., WNS and bat reporting poster and postcard, videos, training tutorials).					Medium	WDFW-Hdqrts
	8. Distribute information on bats and WNS at public events and meetings (e.g., Washington Trail Association, The Mountaineers, Washington's Sportsmen's Show, county fairs, farmers markets, library events, National Hunting and Fishing Day Fair).					Medium	WDFW and Partners
	B. Conduct outreach to foster a supportive community for bat conservation	Medium	On-going	Year round	Statewide	Coordinated / as needed	
	Tasks					**Priority**	**Personnel**
	1. Evaluate the success of new outreach strategies and feasibility of implementing in Washington (e.g., Climbers for Bat Conservation program in Colorado [www.climbersforbats.colostate.edu]).					High	WDFW and Partners
	2. Prepare presentations about WNS and bats for use by WDFW staff.					Medium	WDFW-Hdqrts
	3. Provide information about ecological services provided by bats and realistic threats to public health on WDFW website.					Medium	WDFW-Hdqrts
	4. Present information about WNS and bats at primary, secondary and higher education institutions.					Low	WDFW and Partners
	5. Develop materials for teachers to use in classrooms (e.g., presentations, educational bat trunk).					Low	Partners
	C. Provide education on bat ecology and WNS to key partners and stakeholders	Critical	On-going	Annually	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Provide WNS guidelines and/or trainings (e.g., wildlife rehabilitators, Department of Health, scientific collection permit holders, collection museums (Appendices 2.1, 2.2), wildlife control operators [https://www.whitenosesyndrome.org/sites/default/files/resource/wns_nwco_amp_1_april_2015.pdf]).					Critical	WDFW-Hdqrts
	2. Work with partners to raise awareness on bat-friendly management practices (e.g., humane bat euthanasia at county health departments, bat-friendly maintenance and construction of bridge/road infrastructure (www.bati.institute), humane bat exclusion techniques for Master Builders Association and/or construction companies, and deployment of mitigation bat housing or creation of natural roost habitat).					High	WDFW and partners
	3. Work with industry partners (e.g., wind energy facilities) and biological consultants to incorporate WNS activities into their projects (e.g., assess carcasses during mortality surveys at wind farms).					High	WDFW and partners
	D. List WNS as an invasive species in Washington	Low	On-going	Tbd	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. WNS is currently listed on Washington Invasive Species Council's Top 50 Invasive Species List, but need to determine if listing WNS as an invasive species in Washington is warranted.					Medium	WDFW-Hdqrts

Objective 3: Investigate and understand the distribution and ecology of WNS in Washington						
Strategy	**Action**	**Priority[1]**	**Survey Type[1]**	**Time Period[1]**	**Focal Area[1]**	**Survey Effort[1]**
WNS Surveillance	A. Conduct active surveillance at hibernacula and/or maternity roosts to determine geographic distribution and environmental reservoirs of WNS (see Appendix 2 for detailed surveillance plan)	Critical (emphasis/contracted areas) Medium (statewide)	On-going	Annually (Winter and Spring)	Emphasis area/ Statewide	Coordinated
	Tasks				**Priority**	**Personnel**
	1. Identify and prioritize focal species, hibernacula and maternity roosts for winter and spring surveillance.				Critical	WDFW
	2. Conduct annual WNS surveillance.				Critical	WDFW and Partners
	3. Locate new hibernacula and/or maternity roosts statewide to include in surveillance (see below under Bat Monitoring Action B).				Critical	WDFW and Partners
	4. Identify and acquire equipment needs for each district and region participating in surveillance.				Critical	WDFW
	5. Determine spring emergence to identify survey window for surveillance at maternity roosts (e.g., acoustic monitoring).				High	WDFW and Partners
	6. Provide guidelines (Appendix 2) and trainings on surveillance methods (e.g., swabbing bats, environmental sample collection, trapping techniques).				High	WDFW-Hdqrts
	7. Determine appropriateness of using landscape trapping for surveillance (e.g., Pd exposure risk to bats).				High	WDFW-Hdqrts
	8. Monitor winter use at maternity roosts and conduct surveillance when activity documented.				Medium	WDFW and Partners
	B. Conduct passive surveillance to determine geographic distribution and identify species susceptible to WNS	Critical (Nov-Jun) Medium (Jul-Oct)	On-going	Year round	Statewide	Coordinated
	Tasks				**Priority**	**Personnel**
	1. Respond to public reports using guidelines (Appendix 2.3) and collect carcasses when appropriate/feasible.				Critical	WDFW
	2. Coordinate sample collection and submission with partners (e.g., wildlife rehabilitators, Department of Health, wind energy facilities, bat research biologists).				Critical	WDFW-Hdqrts
	3. Monitor bat intake/submissions to wildlife rehabilitation facilities and Department of Health to track any unusual mortality events or an increase in sick or injured bats in one location which will inform WNS surveillance.				High	WDFW-Hdqrts
	C. Evaluate vulnerability of bat species to WNS to help prioritize sites and focal species for surveillance	High	On-going	Annually (Winter)	Statewide	Coordinated
	Tasks				**Priority**	**Personnel**
	1. Determine winter activity at a variety of landscape features (e.g., talus slopes) and use of hibernation using acoustic monitoring (e.g., bats active in winter may be less vulnerable to WNS).				High	WDFW and Partners

	2. Characterize microclimate conditions at known or suspected hibernacula by deploying temperature and relative humidity data loggers and compare data to optimal growth conditions of Pd to rank relative risk of WNS persistence.					High	WDFW and Partners
	D. Collect baseline health data in presumably uninfected areas to track progression of WNS and other impacts to bat health (e.g., loss of genetic diversity and recovery)	Medium	New	Annually	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Develop Bat Health Team to determine appropriate strategy to implement this action.					Medium	WDFW-Hdqrts

Objective 4: Understand the natural history of Washington bat species to monitor populations effectively							
Strategy	**Action**	**Priority[1]**	**Survey Type[1]**	**Time Period[1]**	**Focal Area[1]**	**Survey Effort[1]**	
Bat Monitoring	A. Identify and evaluate new maternity roosts to include in surveillance and population monitoring	Critical	On-going	Annually (Summer)	Statewide	Coordinated / as needed	
	Tasks					**Priority**	**Personnel**
	1. Follow up on reports from the public, partners and stakeholders (see above under Education and Outreach Action A) to determine species composition, colony size and feasibly to include in monitoring and surveillance activities.					Critical	WDFW and Partners
	2. Identify and acquire equipment for districts and regions participating in maternity colony site evaluations.					Critical	WDFW
	3. Provide any necessary trainings and/or materials (e.g., bat identification keys, acoustic analysis guidelines) needed to complete action (e.g., acoustic detector use and call analysis, trapping techniques).					High	WDFW
	B. Identify and evaluate hibernacula to include in surveillance and long-term population monitoring	High (emphasis area) Medium (statewide)	New	Annually	Emphasis area / Statewide	Coordinated / as needed	
	Tasks					**Priority**	**Personnel**
	1. Develop and implement project(s) to locate new hibernacula. Potential methods include, a) radio telemetry, b) acoustic monitoring, c) internal surveys at caves, inactive mines, buildings, bridges, etc., and d) transect or grid searches on talus slopes or cliffs using visual observation or canine assistance.					Critical	WDFW and Partners
	2. Compile known cave and mine survey data including information on location (accurate latitude/longitude), access, site condition (e.g., open, gated, destructively closed), wildlife use, and survey type needed (e.g., internal, external).					Medium	WDFW and Partners
	C. Assess population status to determine long-term trends	Critical (maternity) Medium (hibernacula)	On-going	Annually (Summer)	Statewide	Coordinated / as needed	
	Tasks					**Priority**	**Personnel**
	1. Prioritize sites to include in long-term population monitoring.					Critical	WDFW
	2. Re-evaluate maternity colony emergence count protocol with WDFW staff, partners and statistician to ensure feasibility and statistical power of protocol (Appendix 3).					Critical	WDFW

	3. Conduct maternity colony emergence counts annually.					Critical	WDFW and Partners
	4. Identify and acquire equipment for districts and regions participating in population monitoring.					Critical	WDFW
	5. Assess approaches used in long-term population monitoring and which would be most effective in Washington (e.g., landscape netting to document capture rates and reproductive status [Pettit and O'Keefe 2017], NABat, emergence counts, demographic study).					High	WDFW-Hdqrts
	6. Re-evaluate use of citizen scientists for maternity colony emergence counts (e.g., Neighborhood Bat Watch [www.batwatch.ca], BC Community Bat Program).					Medium	WDFW
	7. Determine if post-volancy counts are feasible and necessary to look at potential impacts of WNS on fecundity and juvenile recruitment.					Medium	WDFW-Hdqrts
	8. Develop a training video or tutorial for maternity colony emergence counts.					Medium	WDFW-Hdqrts and Partners
	D. Implement NABat acoustic monitoring to document species distribution and population trends	Medium	New	Annually (Summer)	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Determine WDFW involvement and scope of study for future management actions.					High	WDFW
	E. Conduct mark-recapture study (e.g., banding, PIT tagging, genetic markers, freeze branding) to determine survival rates, abundance and recruitment rates.	High (emphasis area) Medium (statewide)	New	Annually	Emphasis area	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Assess the use of mark-recapture methods to evaluate demographics and WDFW involvement in studies.					Critical	WDFW-Hdqrts
	F. Determine migratory patterns of bat species to understand population connectivity and disease spread patterns in Washington and the Pacific Northwest	Medium	New	Annually	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Determine best approach to understand the migratory patterns of bat species, their flight routes, and transient and seasonal roost use in Washington (e.g., acoustic monitoring grid system).					High	WDFW-Hdqrts
	G. Assess the genetic structure of bat populations in Washington to understand the risk of genetic diversity loss and recovery	Medium	New	Tbd	Tbd	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Determine best approach and WDFW involvement with this study.					High	WDFW-Hdqrts

Objective 5: Limit the spread of WNS in Washington

Strategy	Action	Priority[1]	Survey Type[1]	Time Period[1]	Focal Area[1]	Survey Effort[1]
WNS Decontamination	A. Implement WNS decontamination to prevent human-assisted spread of WNS	Critical	On-going	Year round	Statewide	Coordinated

Tasks	Priority	Personnel
1. Distribute WNS decontamination guidelines (Appendix 4) and provide trainings to WDFW staff, scientific collection permit holders, and partners (e.g., wildlife control operators, wildlife rehabilitation facilities and practitioners).	Critical	WDFW-Hdqrts
2. Identify and acquire necessary equipment for regions working with bats.	Critical	WDFW
3. Develop and coordinate WNS decontamination education and strategy for the public at show caves.	Critical	Partners
4. Post WNS decontamination guidelines on WDFW webpage.	High	WDFW-Hdqrts
5. Conduct outreach and/or present information to organizations that may spread WNS (e.g., miners, cavers, industrial cleaning services [bat guano removal], transportation services [see above under Education and Outreach Action A]).	High	WDFW-Hdqrts and Partners
6. Determine amount of visitation at Washington show caves (e.g., trail counters) and/or track where visitors are from or traveling to assess risk of human assisted spread within and beyond Washington.	Low	Partners

WNS Treatment	A. Evaluate treatment options and determine viability of use in Washington	Low[2]	New	Year round	Tbd	Coordinated

Tasks	Priority	Personnel
1. Consult the literature and researchers on the effectiveness of treatment options.	High	WDFW-Hdqrts
2. Conduct risk analysis for treating bats in rehab and/or environments in Washington using current treatment and/or mitigation options, some of which include; a) seasonal closures at bat roosts, b) preventing winter roost switching, c) deploying heated bat houses for spring and summer use (Craig Willis Bat Lab), and d) supplementing diet in fall.	Medium	WDFW-Hdqrts and Partners

	B. Identify and prioritize sites and species to include in treatment research and trials	Low[2]	New	Annually	Tbd	Coordinated

Tasks	Priority	Personnel
1. Collaborate with researchers and laboratories to determine efficacy of treatment options on western bat species and in Washington's environments.	High	WDFW-Hdqrts and Partners
2. Use field-based qPCR (Biomeme) to test treatment options on infected bats with partners (i.e., wildlife rehabilitators).	Medium	WDFW-Hdqrts and Partners
3. Prioritize sites for treatment research.	Medium	WDFW-Hdqrts

Objective 6: Mitigate effects of WNS by managing habitat and mitigating other threats to maintain and enhance bat species diversity and abundance							
Strategy	**Action**	**Priority[1]**	**Survey Type[1]**	**Time Period[1]**	**Focal Area[1]**	**Survey Effort[1]**	
Assess Threats	A. Identify and mitigate threats (other than WNS) to bats to enhance population stability and resilience	Medium	New	Year round	Statewide	Coordinated/as needed	
	Tasks					**Priority**	**Personnel**
	1. Consult the literature and researchers to identify threats to Washington's bat population. This may include threats from wind farm facilities, habitat disturbances (e.g., development, recreation, climate change, fire, abandoned mine reclamation, active mining), bat exclusion from buildings, and forest and agriculture practices (e.g., logging, pesticide use).					Medium	WDFW and Partners
Habitat Enhancement	A. Provide alternative roosting habitat for bats to mitigate against habitat loss and degradation, disease impacts and roost disturbances	Medium	New	Year round	Statewide	Coordinated/as needed	
	Tasks					**Priority**	**Personnel**
	1. Assess information for successful artificial bat roosts.					Medium	WDFW-Hdqrts
	2. Identify partnerships and resources needed to complete action and the monitoring strategy to determine success of artificial roosts.					Medium	WDFW-Hdqrts
	3. Construct and deploy artificial bat roosts (e.g., bat houses or condos) in degraded habitat (e.g., logged forest, urban areas) and at buildings scheduled for bat exclusion.					Medium	WDFW and Partners
	B. Identify and preserve, protect and restore bat roosting and foraging habitat through partnerships	Medium	New	Year round	Statewide	Coordinated/as needed	
	Tasks					**Priority**	**Personnel**
	1. Collaborate with partners on the Abandoned Mine Land Reclamation Program to ensure use of bat-compatible closures and effectiveness of closure (e.g., continued bat use, gate integrity; Tobin and Chambers 2017).					Medium	WDFW and Partners
	2. Work with partners and stakeholders to identify conservation needs (e.g., wetland preservation, snag retention) and develop strategy that identifies approaches, partnerships and resources needed to preserve and protect foraging and roosting locations (e.g., bridges, caves) for bats .					Low	WDFW and Partners
	C. Ensure best management practices for bat protection are being incorporated to new and existing industry projects (e.g., wind energy, tower construction, transition lines)	Medium	New	Year round	Statewide	Coordinated/as needed	
	Tasks					**Priority**	**Personnel**
	1. Develop and distribute best management practices for new and existing industry projects.					High	WDFW

Objective 7: Conduct and facilitate research related to bats and WNS							
Strategy	**Action**	**Priority[1]**	**Survey Type[1]**	**Time Period[1]**	**Focal Area[1]**	**Survey Effort[1]**	
Facilitate Research	A. Continue to support and encourage research related to bat natural history and WNS in Washington	High	On-going	Year round	Statewide	As needed	
	Tasks					**Priority**	**Personnel**
	1. Identify research needs by consulting the literature, researchers and partners. Examples include; a) Pd persistence in guano in non-subterranean habitat, b) year round detectability of Pd, c) winter roost selection, d) disease model to determine rate of spread and risk of site contamination, e) population survivorship and resilience models, and f) population connectivity and genetic structure.					High	WDFW and Partners
	2. When appropriate and needed, provide support to researchers.					Low to Critical[3]	WDFW and Partners
Data Management	A. Develop centralized database that captures bat and WNS survey data	Critical	On-going	Year round	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Identify data management needs.					Critical	WDFW-Hdqrts
	2. Conduct data solicitation with new partners and stakeholders.					High	WDFW-Hdqrts
	B. Write annual report to document, analyze, and synthesize all bat and WNS work completed each fiscal year	High	New	Annually (Winter)	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Collect all survey data regarding bats and WNS in Washington from regional WDFW staff and partners.					High	WDFW-Hdqrts
	2. Ensure all data are in the database (See above under Data Management Action A).					High	WDFW-Hdqrts
	3. Synthesize, analyze and provide an overview, written as an annual report, on bat and WNS activities. This should include epidemiological analyses of surveillance efforts, disease ecology and pathogen spread and data-based guidelines and recommendations for subsequent efforts. This report will be distributed internally and externally.					High	WDFW-Hdqrts
Capacity Building	A. Obtain additional funds to support Washington's WNS response	Critical	On-going	Year round	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Identify and apply for funding opportunities.					Critical	WDFW and Partners
	B. Develop and provide trainings and education on WNS and bat monitoring techniques	High	On-going	Year round	Statewide	Coordinated	
	Tasks					**Priority**	**Personnel**
	1. Promote technical training workshops for WDFW staff and partners (e.g., acoustic analysis, trapping techniques).					High	WDFW
	2. Engage with education institutions to increase research interest and opportunities with bats and WNS.					Medium	WDFW

[1] **Priority** = Critical, High, Medium, Low. *Critical* = activity is vital and should be implemented as soon as possible. *High* = activity is important and should be implemented within a timely manner. *Medium* = activity provides important information but does not need to be implemented within a timely manner. *Low* = activity provides interesting information but is not critical part of response. Prioritization of actions are relative to all actions included in the table and tasks are prioritized relative to other tasks under the same action and not among all tasks; **Survey Type** = New, or On-going. *New* = activities that have not been developed or implemented. *On-going* = activities have been implemented but require continual survey efforts; **Time Period** = length of project and/or time line for activities to occur; **Focal Area** = Statewide, Emphasis area, or Tbd. *Statewide* = activity should occur throughout Washington. *Emphasis area* = activity should occur within a 100-mile radius of the initial WNS detection this includes Districts 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17. *Tbd* = to be determined; **Survey Effort** = As needed, Coordinated, or Coordinated/as needed. *As needed* = activity will occur periodically and does not require statewide coordination. *Coordinated* = activity requires statewide coordination. *Coordinated/as needed* = activity initially requires statewide coordination, but subsequent tasks may arise that do not require this level of coordination; **Personnel** = WDFW-Hdqrts, WDFW-Regions, WDFW, Partners. W*DFW-Hdqrts* = WDFW headquarters in Olympia, WA are responsible for implementing and completing task. *WDFW-Regions* = WDFW regions are responsible for implementing task. *WDFW* = WDFW headquarter and regional staff are responsible for implementing and completing task. *Partners* = partners such as, U.S. Fish and Wildlife Service, U.S. Forest Service, Washington Department of Natural Resources, Bureau of Land Management, National Park Service, Washington State Parks, have a role in implementing task.

[2] Treatment options for WNS are currently unavailable therefore this action is lower priority. If and when viable treatment options are available, the prioritization of this action may change.

[3] Priority of task will vary depending on the value of research proposal.

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Appendix 1: Examples of WDFW's WNS outreach products

WHITE-NOSE SYNDROME IN WASHINGTON

REPORT BAT SIGHTINGS

Report sick/dead bats or a group of live bats by phone or online.

360.902.2515
WDFW.WA.GOV/BATS

LEARN MORE

www.whitenosesyndrome.org

Washington Department of FISH and WILDLIFE

What is white-nose syndrome?

- White-nose syndrome is a fungal disease that has killed millions of hibernating bats, mostly in eastern North America.
- The disease is confirmed in 31 states and five Canadian provinces since it was first documented in 2006.
- In March 2016, the first case of the disease was confirmed in Washington, about 30 miles east of Seattle.
- The disease is primarily spread by bat-to-bat contact, but bats can also contract the disease from an area where the fungus is present.
- White-nose syndrome does not pose a threat to humans, pets or other animals.

How does white-nose syndrome affect bats?

- The fungus *Pseudogymnoascus destructans* can grow on the nose, wings and ears of an infected bat during winter hibernation, giving it a white, fuzzy appearance. After bats wake from hibernation, this fuzzy white appearance goes away.
- Even though the fungus may not be visible, it invades and damages deep skin tissues. Infected bats arouse more often during hibernation which causes them to use crucial fat reserves, leading to possible starvation and death.
- Infected bats may also die from wing damage, inability to regulate body temperature, breathing disruptions, and dehydration.

Little brown bat found in western Washington in March 2016. The fungus damaged the bat's wings making it unable to fly.
Photos courtesy of Progressive Animal Welfare Society (PAWS)

How can I help?

- Whenever possible, avoid entering areas where bats may live to limit the potential of spreading the disease and disturbing roosting bats.
- If you come into contact with caves, mines, or other areas where bats are living, please clean your gear. When possible, use the decontamination guidelines at www.whitenosesyndrome.org.
- Improve bat habitats. Reduce lighting around your home, minimize tree clearing, and protect streams and wetlands. For more information on how to conserve bat habitat, visit wdfw.wa.gov/living/bats.html.

Front

Wild Washington
Mammals
05
Did you know?
A single little brown bat can eat up to 1,000 insects in an hour!
Photo: Merlin Tuttle
LITTLE BROWN BAT

Back

Wild Washington
Photo: Merlin Tuttle
Little brown bat
Myotis lucifugus
Washington Department of FISH and WILDLIFE
WDFW.WA.GOV/CONSERVATION
SIZE: Weigh less than half an ounce and have a wing span of 8 to 11 inches
EATS: Insects including moths and mosquitos
HABITAT: Near streams and lakes in most of the U.S. and Canada. In winter, little brown bats may hibernate in caves, mines, trees, or old buildings.
THREATS: A bat disease called white-nose syndrome has killed millions of hibernating bats. The first case in Washington was confirmed in 2016.
FUN FACTS:
• 15 bat species live in Washington
• Baby bats are called pups
• Many bats use echolocation to capture prey

Appendix 2: Implementation plan for WNS surveillance in Washington

Washington Department of Fish and Wildlife

Implementation Plan for White-nose Syndrome Surveillance in Washington

BACKGROUND

White-nose syndrome (WNS) continues to advance across North America, decimating the local hibernating bat populations. This disease is caused by the psychrophilic fungus *Pseudogymnoascus destructans* (Pd) and has resulted in >90% mortality at some hibernacula. Pd invades the tissues on hairless areas of hibernating bats (e.g., wings, muzzle, ears) and disrupts physiological processes (e.g., thermoregulation) causing the bat to rouse excessively from hibernation, prematurely using critical fat reserves that are required to survive through the winter. It is estimated that millions of bats in eastern North America have been killed from WNS, contributing to the listing of species under the US Endangered Species Act. In March 2016, the first western detection of WNS occurred in Washington State. The first confirmation of WNS was from a little brown bat (*Myotis lucifugus*) that was found in King County. As of January 2018, 5 other detections of WNS or Pd, all within King County, have occurred: (1) Pd detected on a deceased silver-haired bat (*Lasionycteris noctivagans*), (2) Pd detected in a guano sample collected under a bridge, (3) Pd detected in a guano sample collected from a maternity roost, (4) a WNS-positive Yuma myotis (*Myotis yumanensis*) found deceased on a trail, and (5) a WNS-positive Yuma mytois/little brown bat (species has not been confirmed) found in same location as the WNS-positive Yuma myotis.

Washington Department of Fish & Wildlife (WDFW) is collaborating with state, federal, local and tribal agencies and non-governmental organizations to understand how widespread WNS is in the state and the potential effects of WNS on bat populations. To better understand the epidemiology and management of WNS/Pd in Washington, WDFW will coordinate surveillance activities and work with partners to implement this WNS surveillance implementation plan. Surveillance involves assessing bats for clinical signs of WNS and/or collecting bat or environmental samples for WNS/Pd diagnostic testing. WDFW implements two forms of surveillance, active and passive, both described in detail in the sections below. Passive and active surveillance are a key part of WDFW WNS Strategy Plan; this plan outlines and prioritizes actions WDFW will implement over the next few years in response to WNS. WDFW will strive to implement WNS surveillance annually in the winter and spring.

PURPOSE

This document provides an overview of the WNS surveillance strategies WDFW is implementing to understand the ecology and distribution of WNS in Washington. In addition, this plan provides the prioritization scheme WDFW is using for implementing active surveillance. Surveillance provides insight to the geographic extent of WNS, helps inform management decisions such as decontamination requirements and may provide opportunities for future implementation of treatment options. This plan reflects the current understanding of WNS distribution and epidemiology and will be revised when necessary to incorporate any advances in our knowledge of WNS or Pd.

OBJECTIVES

1. Determine geographic distribution of WNS/Pd in Washington. This will help us understand the geographic scope of the challenge, and should allow us to focus on key landscapes or populations of bats.
2. Identify environmental reservoirs for WNS/Pd in Washington. This will help us understand which environments bat are at risk in, where Pd can persist, and allow us to target sites for future implementation of treatment options.
3. Determine bat species susceptible to WNS. This will allow us to determine which species are at risk for population declines, and thus which species to prioritize for surveillance and monitoring activities.
4. Collect baseline information in intermediate and at-risk areas (pre-WNS). This will allow for early disease detection, a better understanding of the rate of spread and may provide insights to mechanisms that facilitate the spread.

PASSIVE SURVEILLANCE

Passive surveillance involves working with partners, stakeholders and the public to collect data for WNS surveillance. WDFW will provide trainings and/or guidelines to partners on WNS passive surveillance and coordinate sample collection (e.g., bat carcasses, bat swabs) and submission for WNS/Pd diagnostic testing. Passive surveillance provides the opportunity for broad spatial coverage, a variety of species to be included for testing, and enhanced ability for early detection of WNS in unaffected areas. Information from passive surveillance may not provide specific information on the location of environmental reservoirs of WNS/Pd, but provides information on species that are susceptible and areas to focus future active surveillance. All passive surveillance activities should be coordinated with WDFW's WNS Coordinator. We have identified five general approaches to passive surveillance, as outlined below.

State Rabies Lab – WDFW is collaborating with the Department of Health (DOH) state rabies lab to screen bats collected throughout Washington for WNS clinical signs (e.g., orange UV fluorescence, wing damage), to submit carcasses that show clinical signs for testing and swab asymptomatic bats for testing between December and June (Appendix 2.1). These samples are submitted to the USGS National Wildlife Health Center (NWHC) for diagnostics. In addition, WDFW works with DOH to document the number of bat submissions and the areas these are coming from. In WNS-infected areas, the rabies labs have documented an increase in winter submissions, therefore, tracking submissions in Washington may increase the likelihood of early detection of WNS and allow us to identify areas to focus active surveillance.

Wildlife Rehabilitators – Wildlife rehabilitation facilities are usually the first entities to receive calls from the public about dead, sick or injured bats and therefore play a vital role in educating the public about bats and WNS as well as early detection of WNS. WDFW is collaborating with rehabilitation facilities to ensure that all bats are assessed for WNS clinical signs and that any with WNS symptoms are euthanized and submitted for diagnostics (Appendix 2.2). Once treatment options become viable, bats with WNS clinical signs may be able to be rehabilitated (genes of bats that survive WNS should remain for population resistance and resilience). Asymptomatic bats are required to be swabbed for WNS/Pd testing but can be rehabbed as long as the bat is appropriately quarantined and decontamination procedures are followed before confirmation is received that the bat is WNS/Pd-negative. WDFW also works with wildlife rehabilitation facilities to track the number of bats taken into care which may help in early detection of WNS, as well as detecting trends in bat morbidity and mortality.

Public Reports – WDFW developed an online reporting system (http://wdfw.wa.gov/conservation/health/wns/) for the public to submit reports on bat observations. Reports can include observations of dead, potentially sick bats, and apparently healthy bats, including groups of roosting bats, or bats displaying unusual behaviors (e.g., flying in cold, inclement weather or daytime; Appendix 2.3). These reports provide valuable information on previously unknown colony locations that can be included in future monitoring and assist with passive surveillance. WDFW collects carcasses from the public between November and June to be tested for WNS/Pd. Outside of this time period, dead bat reports are tracked to detect increasing trends of mortality in certain areas.

Scientific Collection Permittees/Researchers – WDFW requires any holder of a scientific collection permit who will capture and handle bats to assess each for clinical signs of WNS (e.g., wing damage), to swab bats captured between November and June, and to follow decontamination procedures. Currently, WDFW asks any permittee to euthanize a bat with clinical signs of WNS until further notice. Once treatment options become viable, bats with WNS clinical signs could be submitted to rehabilitation facilities for treatment.

Museum Collections – WDFW is collaborating with the Burke Mammalogy Collection located in Seattle to screen bats in their collection for WNS clinical signs. Any bat that has questionable WNS signs will be submitted for diagnostic testing. In addition, WDFW will seek collaborations with other museums in Washington to have their repositories of bats screened for WNS clinical signs (e.g., Slater Museum of Natural History in Tacoma, Conner Museum of Natural History in Pullman).

ACTIVE SURVEILLANCE

Active surveillance is the intentional collection of WNS/Pd data which involves using either invasive or non-invasive approaches to detect clinical signs of WNS, obtain samples for diagnostic testing and to assess the general physical condition of bats. Invasive approaches involve handling bats, whereas the non-invasive approaches rely on visual observations or environmental sampling. These approaches provide information on the geographic distribution of WNS/Pd, identifies environmental reservoirs and may identify which species are susceptible to WNS/Pd. The NWHC and the National Disease Surveillance Implementation Plan (in draft) recommends a hierarchy for active surveillance locations and timing (ordered from higher to lower priority) which include, (1) internal hibernacula surveys, (2) spring emergence at hibernacula, (3) captures in early spring at maternity colonies or on the landscape, and (4) environmental sampling at bat roosts. In all surveillance activities no matter the approach used, disturbance to bats, especially during hibernation, will be minimized by using trained surveyors who are prepared to conduct sampling, using appropriate survey methods, and limiting the amount of time in the site or handling bats. All active surveillance activities should be coordinated with WDFW's WNS Coordinator. The general approaches to active surveillance are outlined below.

Invasive approach – This approach involves capturing and handling bats to detect WNS clinical signs, collect wing/muzzle swabs and/or UV-guided wing biopsies, and to assess the physical condition of individual bats. Despite the potential for disturbance, this method provides a robust way to detect WNS and assess the health of the colony.

> *Spring Emergence at Hibernacula:* Sampling hibernacula is optimal because WNS is more detectable during this time of year. However, little is known about hibernacula for many bat species in Washington, which makes targeting spring emergence at hibernacula impractical. WDFW is working to understand the winter roosting ecology and identify hibernacula for most

of Washington's bat species so given time, surveillance activities may target these locations in the future.

Spring Emergence Captures at Roosts: WDFW has information on numerous summer bat roost locations; therefore, capturing bats should focus at these colonies in the spring. Spring is the best time to focus these efforts as opposed to summer, because of the increased chances of detecting Pd from skin swabs and wing damage associated with WNS earlier in the season. Bats will begin to clear the infection and heal damaged tissue soon after they emerge from hibernation. The NWHC recommends timing surveillance within 4 weeks after bats emerge from hibernation to increase detectability of WNS or Pd. Several techniques can be used to determine when bats return to roosts, some of which include placing drop cloths to detect new guano accumulation, and using infrared beam counters, remote video cameras or acoustic detectors at egress/ingress points. Techniques to capture bats at roosts can include using mist nets or harp traps placed at ingress/egress points or in flight routes near roosts, funnel traps, other types of traps (e.g., hand nets) and hand captures.

Captures on Landscape: Landscape captures is another technique used in WNS surveillance and involves capture of bats at foraging or other high-use sites that are not associated with colonies. This approach has not yet been used by WDFW due to potential, but unknown risk of increasing the rate of spread between bat species that come into contact in a net or trap that generally may not interact except at the site where they are captured. This is especially important to consider during the swarming period in the fall when bats are most at risk of contracting Pd before hibernation. Although the argument can be made that during swarming, which is a period of high interaction, any bats captured would already have some potential for having come in contact with other bats using the same site or feature. Landscape captures in the spring may pose a much less risk to bats since they are active and able to fight a WNS infection. However, the risks are not fully understood and it may be possible for bats to transmit Pd to new environments during this time. Despite these risks, landscape captures may be the most productive option to determine the geographic distribution of WNS/Pd and which species are susceptible to WNS. This is especially true when roosts are not known for certain species. Before landscape captures are implemented, the risks must be assessed, advantages of the samples should outweigh the risks, and methods to mitigate against risks should be assessed. Acoustic monitoring can aid with timing of landscape captures and where to conduct captures by detecting when target species are active and areas where activity is high.

<u>*Non-invasive approach*</u> – This approach involves collecting environmental substrate (e.g., sediment) and/or guano samples to detect the presence of Pd. Additionally, this method can involve visually observing WNS clinical signs on roosting bats (e.g., white fungal growths on the wings, muzzle, uropatagium, and ears) in the winter and spring without handling.

Internal Hibernacula Surveys: Sampling hibernacula is optimal since WNS is more detectable during this time; however, little is known about hibernacula locations for many species in Washington. Therefore, conducting surveys to document clinical signs of WNS on bats or collect environmental samples at hibernacula is not possible currently. WDFW is working to identify hibernacula for Washington's bat species, and future surveillance activities may target these locations. WDFW has information on hibernacula for Townsend's big-eared bat (*Corynorhinus townsendii*), but this is not a priority species currently since subspecies in eastern North America (e.g., Rafinesque's big-eared bat [*Corynorhinus rafinesquii*], Virginia big-eared bat [*Corynorhinus*

virginianus]) have not been documented with WNS. Although these species have been found to carry Pd, which could contribute to spread of Pd in Washington. Because of this, some Townsend's big-eared bat hibernacula may be considered for surveillance.

Environmental Sampling: Collecting environmental samples at bat roosts provides information that may reveal use by infected bats or the presence of Pd in the environment. Environmental sampling can occur at any site, and would be the primary sampling technique when bats cannot be captured or observed. How samples are collected will vary depending on the type of roost and the availability of samples. At sites where guano is prevalent, tarps or similar collection techniques can be used to collect fresh guano. The fresher the guano sample, the more likely Pd can be detected using polymerase chain reaction (PCR). Using this technique may require repeated visits over a short period of time. At sites where substrate (e.g., soil) is the only available sample type, one visit may be sufficient because tarps or other collection techniques are not involved.

Some environments provide optimal conditions for Pd persistence (e.g., microclimate within 12.5 -15.8^{o}C; Verant et al. 2012); substrate samples from such sites could provide valuable information about risk of site being an environmental reservoir for Pd. Abandoned mines and caves in eastern North America have been documented as the primary environmental reservoir for Pd. Other environments, however, may offer similar microclimate conditions such as talus slopes or crevices in cliffs that allow for Pd persistence. There has been limited research on bat use in these environments, and it is unclear whether (1) there is potential for these to be Pd reservoirs, (2) surveillance techniques will effectively detect Pd, and (3) diagnostic results from such efforts are informative for management (e.g., false-negative rates). Despite the limited information, WDFW seeks to understand the extent of bat use and the existence, location and use of potential environmental reservoirs. WDFW will pursue guidance from diagnostic and research labs on the best surveillance strategy in these environments (e.g., environmental sampling, mist netting) and will conduct acoustic monitoring to identify sites with high bat activity that are better suited for surveillance.

Site Prioritization – WDFW is summarizing roost data (e.g., maternity, hibernacula, swarming, day) from available databases (e.g., WDFW, USFS, BLM) to prioritize sites for monitoring and surveillance efforts. Additional roosts will be added to the database as new sites are discovered or verified (e.g., public reports of groups of bats). Roost data will be prioritized for surveillance using criteria adapted from the National Disease Surveillance Implementation Plan (in draft) to meet WDFW goals and objectives for surveillance. Each site will be rated according to the criteria listed below. Sites with a larger value are higher priority for WNS surveillance. Criteria that are considered in site prioritization but not listed below include the proximity of sites to one another and the geographic distribution of sites. In order, to meet surveillance objectives, sites that are in close proximity to one another will not necessarily both be included in surveillance even if both have a high prioritization value. There may be other circumstances where exceptions to this prioritization (e.g., based on previous diagnostic results) would be necessary.

The prioritization criteria for site selection includes (1) presence of bat species that are vulnerable to WNS, (2) size of colony, (3) number of species at the site, (4) roost type, (5) proximity of the site to WNS-infected areas, (6) whether the site is accessible (e.g., landowner permission, accessible during sampling period), (7) the amount of human use (e.g., recreation), and (8) the type of surveillance approaches that can be used. Species that are considered vulnerable to WNS include little brown bat, Yuma myotis, big brown bat (*Eptesicus fuscus*) and other *Myotis species*. It is unknown which other *Myotis species* may be

susceptible to WNS, so all will be considered relevant for surveillance until more information becomes available. The size of the colony and the number of species present have implications on the rate of spread of WNS/Pd, which makes these important factors to consider when evaluating sites. Sites that are relatively close in proximity to WNS-infected areas (i.e., 100 miles) may already be infected or are at a distance that they could easily become infected over time due to natural movements of bats. Sites must be accessible for sampling to occur; therefore, it is an important factor to consider in site selection. Additionally, sites with higher human use are at risk for human-assisted spread. Lastly, use of both types of active surveillance sampling approaches (i.e., invasive and non-invasive) can increase detectability of WNS/Pd and allow for all surveillance objectives to be met. The list below contains the prioritization criteria and the point value for each criterion:

1. Species that are vulnerable to WNS are present. Yes – 2, No - 0
2. Colony size is >50. Yes – 1, No – 0
3. More than 2 species have been documented in the roost. Yes – 1, No – 0
4. The site is either a hibernacula or maternity colony. Yes – 1, No - 0
5. Site is within 100 miles of WNS detections. Yes – 2, No - 0
6. Site is accessible. Yes – 1, No - 0
7. Human use is high. Yes – 1, No - 0
8. Both invasive and non-invasive approaches can be used. Yes – 1, No – 0

Site Selection – Using site prioritization and results from the previous surveillance efforts, sites will be placed into 1 of 4 groups; ***core***, ***non-core, other*** or ***follow-up***. Any new sites that are identified through public reporting or other means will be added to one of these groups depending on the site conditions and its prioritization value. Prioritization within the 4 groups may be necessary to account for specific objectives, funding or staffing limitations, engagement by partners, or other factors.

Core group consists of sites that have the highest prioritization values. These sites will be included in WNS surveillance each year unless other factors remove them from this group (e.g., proximity to another core group site). A limited number of sites will be included in this group to ensure that the minimum amount of surveillance can be achieved annually despite any changes in staff and lab capacity.

The *non-core group* includes all other known bat roosts. Within this group, sites will be placed in 1 of 3 tiers based on their prioritization: high (7-9 points), medium (6 points), and low (0-5 points). Sites will be selected from each tier each year to complete the number of sites that will be sampled during the active surveillance season. Generally, the proportion of sites will reflect the importance of each tier (i.e., tier 1 is most important, tier 3 is least important). The number selected from each tier will vary based on lab capacity and funding each year.

Other group consists of sites that may not be documented as bat roosts but have the potential to be environmental reservoirs such as show caves and abandoned mines. These sites will be divided into 2 tiers; show caves (those that WDFW can access) and technical sites (i.e., abandoned mines, vertical caves) that would require collaboration with partners to complete sample collection, such as Bat Conservation International's Subterranean Program. Sites will be selected from each tier each year, if possible, to be sampled during the active surveillance season. The number selected from each tier will vary each year depending on lab capacity, funds, WDFW objectives, and partnerships.

Follow-up group sites include those in the previous active surveillance effort that require additional sampling, following surveillance efforts indicating WNS-positive or Pd present at the site. Environmental samples typically will not be needed at these sites but bats may need to be captured to assess the health of bats (e.g., wing damage, tissue damage using UV) and potential progression of WNS in the colony. This will include collecting skin swabs, UV screening and wing biopsies. Any sites with equivocal WNS or Pd results should be sampled (e.g., environmental or bat swabs) again within the same season, if possible, and included in the next surveillance season.

WNS DIAGNOSTICS

Diagnostic tests for WNS include PCR, histopathology (identifying fungal hyphae in skin tissue), and/or fungal culture for Pd. Sample type submitted for testing will vary depending on time of year, if the bat is alive or dead and accessibility of the site for sampling. Sample type includes wing/muzzle swabs, fungal tape-lift (visible fungi collected with tape from bat), guano, substrate, environmental swab, UV-guided wing biopsy and/or whole body carcass. For a sample to be diagnosed as WNS-positive, both WNS lesions from histopathology and detection of Pd by PCR or fungal culture must be confirmed (https://www.nwhc.usgs.gov/disease_information/white-nose_syndrome /Case%20Defintions%20for%20WNS.pdf).

The USGS NWHC conducts WNS diagnostics in support of the national WNS surveillance plan. Unfortunately, the capacity of NWHC is limited and cannot accept the number of samples WDFW collects through passive and active surveillance. Because of this, additional labs have been identified and have become partners for diagnostic testing. Current options include: University of California Davis (Kevin Keel lab), USFS Northern Research Station (Dan Lindner lab), and Oregon State University College of Veterinary Medicine. The type of sample collected and method of collection varies depending on which lab conducts the diagnostic testing. Because of this WDFW developed guidelines for the 2017 and 2018 active surveillance seasons to facilitate sample collection (Appendix 2.4). Each year WDFW will assess and adjust these guidelines, as needed, to accommodate differences in lab capacity and site selection.

WNS DECONTAMINATION

Participation in surveillance activities requires adherence to WNS decontamination procedures after working with bats or in bat roosts. Decontamination guidelines were developed by WDFW using the National WNS protocol (https://www.whitenosesyndrome.org/sites/default/files/resource/ national_wns_decon_protocol_04.12.2016.pdf) as guidance to help facilitate cooperation and participation with national decontamination efforts (Appendix 4).

REPORTING

WDFW will write an annual report summarizing the surveillance efforts including roost types and bat species sampled, number of sites and samples collected, type of samples collected, timing of sample collection, and diagnostic results. WDFW will share this report with partners and discuss how those results will influence subsequent WNS surveillance efforts, including adjustments to this strategy. Additionally, WDFW developed talking points to communicate surveillance activities and diagnostic results with landowners (Appendix 2.5).

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Verant, M. L., J. G. Boyles, W. Waldrep Jr, G. Wibbelt, and D. S. Blehert. 2012. Temperature-dependent growth of Geomyces destructans, the fungus that causes bat white-nose syndrome. *PLoS One*, *7*(9), e46280.

Appendix 2.1: White-nose syndrome surveillance guidelines for the Department of Health

Washington Department of Fish and Wildlife

White-nose Syndrome Surveillance Guidelines for Department of Health

OVERVIEW

White-nose Syndrome (WNS) is a deadly fungal disease, caused by *Pseudogymnoascus destructans* (Pd) that has killed millions of hibernating bats in eastern North America. The fungus grows on the hairless areas (e.g., wings, muzzle, ears) of hibernating bats and disrupts physiological processes (e.g., thermoregulation) causing the bat to rouse from hibernation, burning critical fat reserves that are required to survive through the winter. In March 2016, the first western case of WNS was confirmed in Washington State on a little brown bat (*Myotis lucifugus*). As of January 2018, the Yuma myotis (*Myotis yumanensis*) has also been confirmed with WNS in Washington now. Additionally, the silver-haired bat (*Lasionycteris noctivagans*) has been confirmed with Pd, but not WNS. Washington Department of Fish & Wildlife (WDFW) is collaborating with state, federal, and tribal agencies and non-governmental organizations to determine how widespread WNS is in the state and the effects of disease on the bat population. To achieve this, we will monitor bat populations and conduct WNS surveillance. In particular, WDFW has been collaborating with wildlife rehabilitators and the Department of Health (DOH) to collect samples from bats submitted for rehabilitation or rabies testing. With this collaboration we will be able to track the spread of WNS and identify susceptible species, allowing us to continue making comprehensive strides in understanding the potential effects of WNS on Washington's bat populations.

PURPOSE

WDFW has asked DOH to submit carcasses and/or swabs from bats submitted for rabies testing in order to track the spread of WNS and determine species that are susceptible to WNS/Pd. We are asking that all carcasses be screened for clinical signs of WNS and samples be collected from **December to June**, the time period when WNS can most readily be detected.

WHITE-NOSE SYNDROME CLINICAL SIGNS

General clinical signs often associated with WNS in bats include:

- Bats exhibiting yellow-orange fluorescence on hairless skin under long-wave UV light
- Excessive/unexplained bat mortality or population decline
- Aberrant bat behaviors (e.g., found on ground, increased bat activity during freezing temperatures or daytime)
- Thin body condition and/or dehydrated (wrinkled and flaky appearance of hairless areas)
- Moderate to severe wing damage (e.g., membrane thinning, depigmented areas, holes, tears, flaky appearance, sticky membranes; Reichard 2008)
- White or gray powdery fungus seen around the muzzle, ears, wings and/or uropatagium – note that as the season progresses and bats are roused from hibernation, this white fungus is no longer visible on the hairless areas.

WHITE-NOSE SYNDROME SAMPLING

UV Screen Bat Carcasses

- Examine wings, ears, tail membranes under UV light (380-385 nm) for signs of pinpoint to coalescing areas of yellow-orange fluorescence (Turner et al. 2014). NOTE: mite ectoparasites

may also fluoresce yellow-orange but are often observed to be moving. Other fluorescent colors (blue or green) may be observed on wings or claws but are not associated with WNS.

- Carcasses that are UV-positive should be submitted to lab (e.g., USGS National Wildlife Health Center [NWHC]) or WDFW, regardless of which County they were collected in or time of year.

Target Species for Sample Collection

Species that should be targeted for sample collection include: big brown bat (*Eptesicus fuscus*), silver-haired bat, Townsend's big-eared bat (*Corynorhinus townsendii*) and all *Myotis species*. Swabs or carcasses should be collected from these species for WNS testing. The type of sample will depend on which county the bat was found in and whether clinical signs of WNS are present (see below). All other species that are not included in this list should still be assessed for clinical signs of WNS. If any have questionable signs of WNS, then the carcass should be submitted for testing. Any asymptomatic non-target bat species can be sent to the Burke Museum for archiving.

Sample Collection for King County

- Carcasses collected in King County from December to June that are species <u>already confirmed</u> with WNS (i.e., little brown bat, Yuma myotis), should have a swab collected following NWHC protocol (Appendix A). Freeze swabs (-20°C) and ship to lab or WDFW for long-term storage within four weeks of collection. Or store samples in a -80 °C freezer for long-term storage and send at end of season (June) to lab or WDFW. The carcasses can then be sent to the Burke Museum for archiving.
- Carcasses collected in King County from December to June that show clinical signs of WNS and are species <u>NOT confirmed</u> with WNS or Pd, should be submitted to NWHC. Carcasses can be stored in freezer (-20°C) and sent at end of season (June) to lab or WDFW.
- Carcasses collected in King County from December to June that do NOT show clinical signs of WNS and are species <u>NOT confirmed</u> with WNS, should have a swab collected following NWHC protocol (Appendix A). Freeze swabs (-20°C) and ship to lab or WDFW for long-term storage within four weeks of collection. Or store samples in a -80 °C freezer for long-term storage and send at end of season (June) to lab or WDFW. The carcasses can then be sent to the Burke Museum for archiving.
- Decontamination should be followed after each bat is processed to prevent any inadvertent spread of Pd (https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf). This includes changing gloves after each carcass, using new sampling supplies and wiping any equipment or surfaces with an approved solution after processing each bat (e.g., isopropyl alcohol wipe).

Sample Collection outside of King County

- Carcasses collected from December to June outside of King County that show clinical signs of WNS should be submitted to NWHC for WNS/Pd testing. Carcasses can be stored in freezer (-20°C) and sent at end of the season (June) to lab or WDFW.
- Carcasses collected outside of King County from December to June that do NOT show clinical signs of WNS, should have a swab collected following NWHC protocol (Appendix A). Freeze swabs (-20°C) and ship to lab or WDFW for long-term storage within four weeks of collection. Or store samples in a -80 °C freezer for long-term storage and send at end of season (June) to lab or WDFW. The carcasses can then be sent to the Burke Museum for archiving.

- Decontamination should be followed after each bat is processed to prevent any inadvertent spread of Pd (https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf). This includes changing gloves after each carcass, using new sampling supplies and wiping any equipment or surfaces with an approved solution after processing each bat (e.g., isopropyl alcohol wipe).

LITERATURE CITED

Reichard, J. D. 2008. Wing-damage index used for characterizing wing condition of bats affected by white-nose syndrome. https://www.fws.gov/northeast/PDF/Reichard_Scarring%20index%20bat%20wings.pdf

Turner, G.G., Meteyer, C.U., Barton, H., Gumbs, J.F., Reeder, D.M., Overton, B., Bandouchova, H., Bartonička, T., Martínková, N., Pikula, J. and Zukal, J., 2014. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases*, *50*(3), pp.566-573.

For additional information please contact WDFW WNS Coordinator.

Appendix 2.2: Bat rehabilitation facilities guidelines for WNS passive surveillance

Washington Department of Fish and Wildlife
Bat Rehabilitation Facilities
Guidelines for White-Nose Syndrome Passive Surveillance

WHITE-NOSE SYNDROME CLINICAL SIGNS
General clinical signs often associated with white-nose syndrome (WNS) in bats include:

- Bats exhibiting yellow-orange fluorescence on hairless skin under long-wave UV light
- Excessive/unexplained bat mortality or population decline
- Aberrant bat behaviors (e.g., found on ground, increased bat activity during freezing temperatures or daytime)
- Thin body condition and/or dehydrated (wrinkled and flaky appearance of furless areas)
- Moderate to severe wing damage (e.g., membrane thinning, depigmented areas, holes, tears, flaky appearance, sticky membranes; Reichard 2008)
- White or gray powdery fungus seen around the muzzle, ears, wing/limbs, and/or tail – note that as the season progresses and bats are roused from hibernation, this white fungus is no longer visible on the wings or nose.

CALLS FROM THE PUBLIC
Please see Washington Department of Fish and Wildlife (WDFW) public bat report guidelines.

If caller observes live or dead bats (single or multiple individuals in a single location) that are downed or appear ill or infected with disease report the incident to WDFW's WNS Coordinator.

- Advise public NOT to handle or touch the bat; a downed bat or one in torpor may appear dead.
- A wildlife rehabilitator or WDFW staff will be sent to collect the bat if live.
- If public wants to bring in carcass:
    - Pick up carcass wearing leather gloves;
    - Double bag the carcass;
    - Label with date of observation, location of collection, contact information.
- Instruct public to photograph bat on the ground and the surrounding area.
- Instruct public to report bat observations on WDFW's online bat reporting form: http://wdfw.wa.gov/conservation/health/wns/reporting.html.

NON-LETHAL SAMPLING
As of 2017, WDFW has asked that **all bats that have questionable signs of WNS be euthanized immediately** and shipped to a diagnostic lab for *Pseudogymnoascus destructans* (Pd) testing, the fungus that causes WNS. Please coordinate with WDFW's WNS Coordinator.

- All species of bats brought to your facility must be sampled from **November to June** (e.g., UV screen, wing punch biopsy, wing/muzzle swab and/or feces) for submission to lab for WNS testing. Please coordinate with WDFW's WNS Coordinator for shipping samples to the lab.
- Sampling kits, instructions, and training will be provided by WDFW.
- Please store collected samples at -20°C and ship within four weeks of collecting. Or store in a -80°C freezer and ship at the end of the season (June).

INTAKE OF LIVE BATS

- Record all bats admitted to your facility on your Daily Ledger.
- Provide a monthly update to WDFW's WNS Coordinator, with the number of bats submitted to rehab and their status (still in care, dead, released, etc.).
- If the public brings in a bat, record all of their contact information on your facility's intake form and detailed information on where and when the bat was found. Coordinate with WDFW's WNS Coordinator to have the carcass (if dead) shipped to WDFW or the appropriate lab.
    - Ask if there is any possibility of rabies exposure.
    - If there was rabies exposure, bats MUST be euthanized and sent to the County Health Department – they are cooperating with WDFW for WNS screening.
- On intake, keep bat isolated from all other bats. If bat has questionable signs of WNS, euthanize immediately.
    - Quarantine all bats and ensure proper biosecurity measures when handling bats. Change gloves between all bats, and disinfect all surfaces that come in contact with a bat. Use products that have shown efficacy against Pd. (https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf).
- If a bat appears healthy on intake (e.g., collected from torpor) you may attempt rehabilitation and release; keep isolated from any other bat in your facility, provide supportive care, and release as soon as possible.
- Bats may be euthanized upon intake for humane reasons. Follow American Veterinary Medical Association's (AVMA) guidelines for humane euthanasia (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf)
    - Freeze carcass and submit for WNS/Pd testing between November and June. Coordinate submission with WDFW's WNS Coordinator.
- Collect swabs for WNS/Pd testing on intake. Freeze swabs (-20°C) and ship to lab or WDFW for long-term storage within four weeks of collection. Coordinate this with WDFW's WNS Coordinator.
- If a bat dies within 72 hours of admission and has only received supportive care, freeze the carcass and submit it along with the associated swab and any wing biopsies collected at intake. Clearly label & group together all samples associated with the same individual bat.
    - Record individual bat information and samples collected on the datasheet provided. Include internal reference (case) number that will allow you to match external lab results to the correct case. Submit samples to appropriate lab with help of WDFW's WNS Coordinator.
- Bats with WNS may develop moderate to severe wing necrosis after 1-2 weeks after initiating supportive care due to a hyperimmune response to the infection. Bats that die or are euthanized at this time may also be submitted for diagnostic evaluation for WNS. Coordinate the submission of the carcass (either directly to the appropriate lab or WDFW) with WDFW's WNS Coordinator.

UV Screen Live Bats on Intake

- Examine wings, ears, tail membranes under UV light (380-385 nm) for signs of pinpoint to coalescing areas of yellow-orange fluorescence (Turner et al. 2014). NOTE: mite ectoparasites may also fluoresce yellow-orange but are often observed to be moving. Other fluorescent colors (blue or green) may be observed on wings or claws but are not associated with WNS.
- If UV+ (yellow-orange fluorescence present) lesions are observed, euthanize without bringing it into the hospital population.

Note that as of 2018, WDFW is asking that **all bats that have yellow-orange UV fluorescence be euthanized**. In future years, if WNS is more prevalent in Washington, bats with fluorescent lesions will **not** be euthanized and the following samples will be collected:

- A wing/muzzle swab
- Two UV-guided wing punch biopsies that include fluorescent areas within the first 48 hours of admission, once the bat is stable, into sterile vial containing ~1.5 ml 10% buffered neutral formalin. Label sterile vial with bat's internal reference number and biopsy date.

Acceptable Methods of Euthanasia for Bats

These methods will not interfere with WNS diagnostics:

- Inhalant anesthesia overdose followed by cervical dislocation
- Injectable barbituate overdose (IV, IP) – can interfere with full necropsy/histologic examination of internal organs
- When euthanizing bats, be sure to follow the AVMA guidelines for humane euthanasia: https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

Potential Rabies Exposure

If there is a rabies exposure concern, submit the carcass to the County Health Department with instructions to save for submission to USGS National Wildlife Health Center (NWHC). The Department of Health is cooperating in WNS surveillance. Do not collect wing/muzzle swabs on the carcass; this will be done at the County Health Department.

TREATMENT

As of 2018, WDFW is asking that all bats with clinical signs of WNS/Pd be euthanized. However, moving forward we will seek treatment for infected bats. The protocol for this will be an ongoing discussion.

RELEASE AFTER TREATMENT FOR WNS/Pd

For discussion, once treatment protocols are in place.

LITERATURE CITED

Reichard, J. D. 2008. Wing-damage index used for characterizing wing condition of bats affected by white-nose syndrome.
https://www.fws.gov/northeast/PDF/Reichard_Scarring%20index%20bat%20wings.pdf

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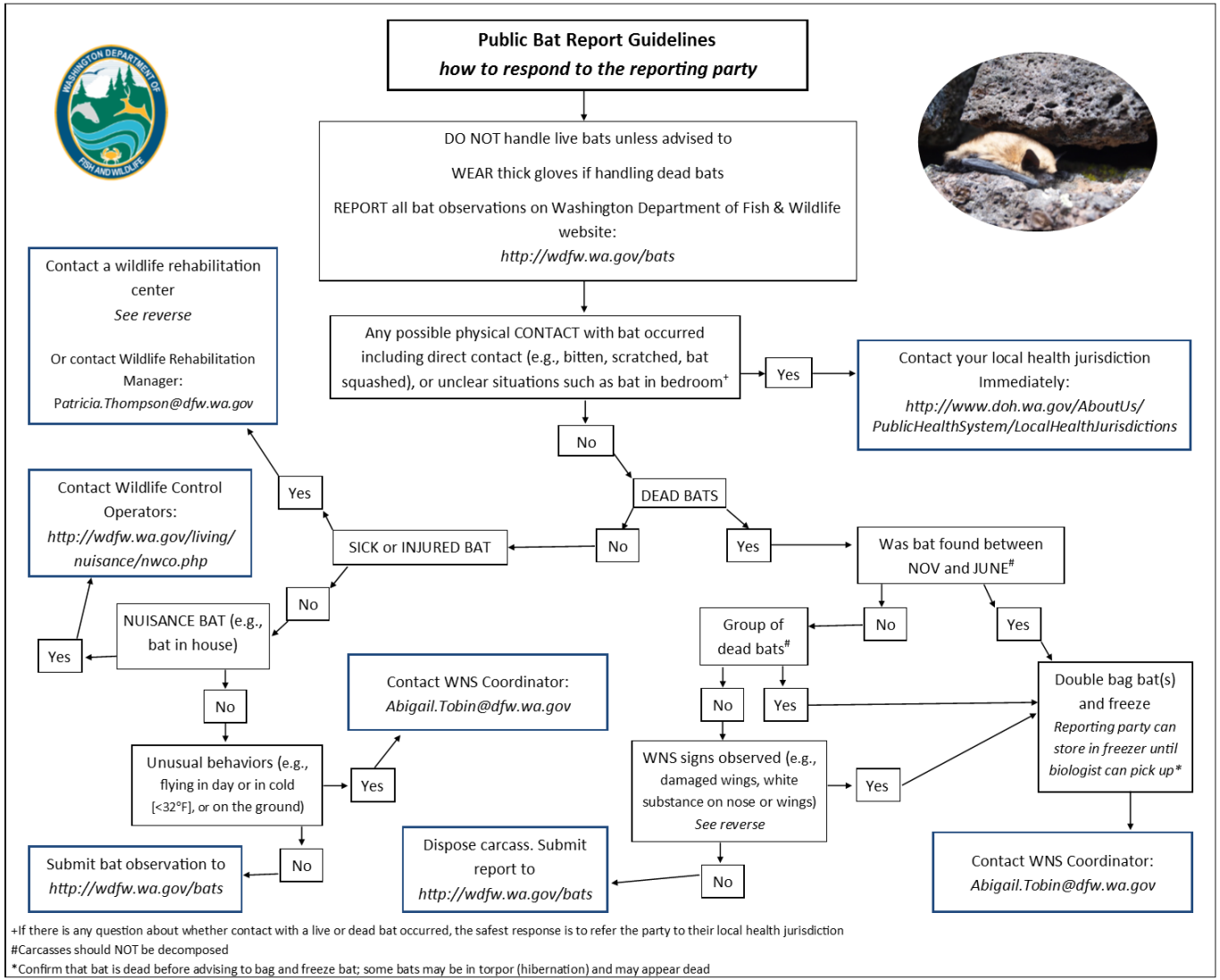
For additional information please contact WDFW WNS Coordinator.

Appendix 2.3: Guidelines for responding to public bat reports.



More Information

Wildlife Rehabilitation Centers in Washington (*http://wdfw.wa.gov/conservation/health/rehabilitation/*):

1. PAWS Wildlife Center, Lynnwood, WA—425-412-4040
2. Happy Valley Bats, Stanwood, WA—360-652-7690
3. West Sound Wildlife Shelter, Bainbridge Island, WA—206-855-9057
4. Wolf Hollow Wildlife Rehabilitation Center, Friday Harbor, WA—360-378-5000
5. Center Valley Animal Rescue, Quilcene, WA— 360-765-0598
6. Exotics and Wildlife Department, Washington State University, Pullman, WA—509-335-0711
7. Dr. Jessica Adams, Chewelah Veterinary Clinic, Chewelah, WA— 509-935-6424

Outside Washington (bats cannot be transported over state lines):

1. Idaho Department Game and Fish: 208-454-7638
2. Idaho wildlife rehabilitator: Ryan Law, Palouse Wildlife Rescue and Rehabilitation, Moscow, ID, 208-614-2273
3. Oregon Department of Fish and Wildlife: http://www.dfw.state.or.us/wildlife/health_program/WNS/reporting.asp

Example of white-nose syndrome (WNS) signs:

Fungal growth on wing

Fungal growth on nose and wings

Damaged wing

Note: Fungal growth will only be observable on bats who are hibernating or in torpor

Please have party submit photos with report if observe any WNS signs

If you have any questions, please contact: Abby Tobin, Abigail.Tobin@dfw.wa.gov, 360-902-2523 (office), 360-999-7958 (cell)

Appendix 2.4: White-nose syndrome active surveillance guide for sample collection ***in 2018***

Washington Department of Fish and Wildlife

White-nose Syndrome Active Surveillance Guidelines for 2018

PURPOSE OF DOCUMENT: Facilitate sample collection for white-nose syndrome (WNS) surveillance in Washington. Washington Department of Fish and Wildlife (WDFW) and partners identify high priority sites for surveillance and diagnostic labs for testing each year. In the past, WDFW has collaborated with the USGS National Wildlife Health Center (NWHC), USFS Northern Research Station and University of California Davis for diagnostic testing. This year (2018), the NWHC has capacity to test samples from 10 sites and the USFS Northern Research Station can test samples from 10 sites although they can bank samples for future testing if we exceed 10 sites. Site selection and lab capacities will be re-evaluated each year before WNS surveillance commences.

HOW TO USE THIS DOCUMENT: Locate the site name in the list below to identify the lab we are partnering with for diagnostic testing. Find the header on the following pages with the lab name and follow the guidelines. The site list will change each year depending on site prioritization, staff, and lab capacities.

WDFW District	County	Site name	Lab
1	Pend Oreille	Site 1	USGS NWHC
2	Whitman	Site 2	USGS NWHC
6	Okanagan	Site 3	USGS NWHC
7	Chelan	Site 4	USGS NWHC
7	Douglas	Site 5	USGS NWHC
8	Yakima	Site 6	USGS NWHC
9	Skamania	Site 7	USFS Northern Research Station
9	Skamania	Site 8	USGS NWHC
11	Thurston	Site 9	USGS NWHC
11	Pierce	Site 10	USFS Northern Research Station
11	Pierce	Site 11	USFS Northern Research Station
12	King	Site 12	USGS NWHC
12	King	Site 13	USFS Northern Research Station
12	King	Site 14	USFS Northern Research Station
12	King	Site 15	UC Davis[1]
13	Snohomish	Site 16	USFS Northern Research Station
13	Snohomish	Site 17	USFS Northern Research Station
14	Skagit	Site 18	USFS Northern Research Station
14	Whatcom	Site 19	USFS Northern Research Station
15	Kitsap	Site 20	USFS Northern Research Station
16	Clallam	Site 21	USGS NWHC

[1]Follow guidelines for USGS NWHC

USFS Northern Research Station

The USFS Northern Research Station lab requires collection of only environmental samples (i.e., guano and/or sediment). The steps below provide guidance for environmental sample collection, but please *read the USFS sample collection protocol for more details and record data on the USFS datasheet.* If there is staff capacity, it is feasible to collect swabs from bats, and the site is not already being sampled for the USGS NWHC, then capture bats to acquire wing/muzzle swabs. Capturing and swabbing bats at these sites are not a priority so any additional samples collected from bats will be banked and eventually sent to UC Davis. Even though these samples will not be sent to USGS NWHC, please follow the USGS NWHC sample collection protocol and use the datasheet to record data. Samples should be collected by the end of May.

Follow the steps below to determine which type of sample to collect and how many samples to collect per site.

1. Are you able to enter or access the inside of the site?
   No – go to step 2.
   Yes – go to step 3.
2. Place a tarp or use another collection method near entry/exit points of roost to collect guano. Sample tubes should be filled to the 1.0 or 1.5 mL mark; collect as many samples as possible. Note: If entry/exit points are unknown consider observing emergence prior to sample collection. If species using site is unknown consider deploying an acoustic detector during survey to aid with species identification.
3. Do you know where bats roost in site?
   No – go to step 4.
   Yes – go to step 5.
4. Conduct an internal survey to locate roosting bats and bat sign (e.g., guano, moth wings/insect parts). Proceed to step 5.
5. Is guano present at site?
   Yes – follow steps 6-8.
   No – follow steps 9-10.
6. Collect up to 25 guano samples that are at least 12 inches apart from under roosting areas; if roosting areas are unknown, evenly space samples in order to cover the entire site (mark sample locations on a map/drawing of site on datasheet). Attempt to collect guano that appears fresh (e.g., not desiccated). The tube should be filled to the 1.0 or 1.5 mL mark. Note: If the site is small and collecting 25 samples that are 12 inches apart is not possible then collect as many samples as possible.
7. Collect 5 sediment samples from under roosting areas, when possible. If roosting areas are unknown, evenly space samples in order to cover the entire site (mark sample locations on a map/drawing of site on datasheet). The tube should be filled to the 1.0 mL mark. Note: If sediment is unavailable, collect 5 additional guano samples instead and note that on the data sheet.
8. Collect 1 negative control sample (see USFS sample collection protocol). Proceed to step 11.
9. Collect up to 30 sediment samples that are at least 12 inches apart from under roosting areas; if roosting areas are unknown, evenly space samples in order to cover the entire site (mark sample locations on a map/drawing of site on datasheet). The tube should be filled to the 1.0 mL mark. Note: If the site is small and collecting 30 samples that are 12 inches apart is not possible then collect as many samples as possible.
10. Collect 1 negative control sample (see USFS sample collection protocol). Proceed to step 11.

11. OPTIONAL: If samples are not already being collected for USGS NWHC, collect samples from bats by either swabbing them in place (does not require handling) or capturing bats inside roost using hand nets or outside roost using mist nets or harp traps. Dip the swab in the tube with sterile water then roll swab over the surface of skin on forearm (three passes back and forth) then use the same swab to roll across the muzzle 3 times. When possible, use a UV light to examine the wings for questionable fluorescence (orange) and take a UV guided wing biopsy of those areas. When necessary, mark captured bats after sampling to ensure not sampling the same individual again (e.g., mark wing or fur with marker).

USGS National Wildlife Health Center

The USGS NWHC prefers to have samples collected from bats, but environmental samples (i.e., sediment, guano) can be supplemented to reach the total sample size when bats are not present or present in fewer numbers. The steps below provide guidance for sample collection, but p*lease read the USGS NWHC sample collection protocol for more details and record data on their datasheet.* Samples should be collected by the end of May.

Follow the steps below to determine which type of sample to collect and how many samples to collect per site.

1. Are you able to enter or access the inside of the site?
   No – go to step 2.
   Yes – go to step 3.
2. Place mist nets or harp traps near entry/exit points to roost or in the bat's fly way in order to capture bats. Collect swabs from 25 bats; if 25 bat swabs are not possible then collect as many as possible from bats then supplement the remaining number with environmental samples. Dip swab into tube with sterile water then roll swab over the surface of skin on forearm (three passes back and forth) then use the same swab to roll across the muzzle 3 times. Use a UV light, when possible, to examine the wings of those bats that are captured. If any have questionable fluorescence (orange), take a UV guided wing biopsy of area. When necessary, mark captured bats after sampling to ensure not sampling the same individual again (e.g., mark wing or fur with marker). A tarp or other collection method can be used to collect guano near entry/exit points to roost; fill tube to the 1.5 mL mark.
3. Do you know where bats roost in site?
   No – go to step 4.
   Yes – go to step 5.
4. Conduct an internal survey to locate roosting bats and bat sign (e.g., guano, moth wings/insect parts). Proceed to step 5.
5. Are bats present?
   No – go to step 6.
   Yes – go to step 7.
6. Collect 30 environmental samples; prefer sediment/guano samples, but ceiling/wall swabs on visible roost stain is acceptable. Collect sediment/guano samples below roosting areas that are at least 12 inches apart; if roost areas are not known, evenly space out the samples in order to cover the entire site (mark sample locations on a map/drawing of site on datasheet). If collecting guano, collect ones that appear fresh (e.g., not desiccated). Fill the tube to the 1.5 mL mark. For ceiling/wall samples, dip the swab in the tube with sterile water then roll the swab over an area the size of a palm 3-5 times.
7. Are bats within hands reach or within range of hand/butterfly net?

No – go to step 9.
Yes – go to step 8.

8. Collect 25 swabs from bats; if 25 bat swabs are not possible then collect as many as possible from bats then supplement the remaining number with environmental samples. Dip the swab in the tube with sterile water then roll swab over the surface of skin on forearm (three passes back and forth) then use the same swab to roll across the muzzle 3 times. When possible, use a UV light to examine the wings of those bats that are captured. If any have questionable fluorescence (orange), take a UV guided wing biopsy of area. When necessary, mark captured bats after sampling to ensure not sampling the same individual again (e.g., mark wing or fur with marker). If necessary, collect sediment/guano samples from under roosting areas that are at least 12 inches apart; fill tube to the 1.5 mL mark. Note: When capturing or swabbing bats inside roost, it is recommended to conduct this work closer to sunset in order to avoid bats exiting roost during the day.
9. Is it possible to capture bats outside roost using mist nets or harp traps?
   No – go to step 11.
   Yes – go to step 10.
10. Collect 25 swabs from bats; if 25 bat swabs are not possible then collect as many as possible from bats then supplement the remaining number with environmental samples. Dip swab in tube with sterile water then roll swab over the surface of skin on forearm (three passes back and forth) then use the same swab to roll across the muzzle 3 times. When possible, use a UV light to examine the wings of those bats that are captured. If any have questionable fluorescence (orange), take a UV guided wing biopsy of area. When necessary, mark captured bats after sampling to ensure not sampling the same individual again (e.g., mark wing or fur with marker). If necessary, collect sediment/guano samples from under roosting areas that are at least 12 inches apart; fill tube to the 1.5 mL mark.
11. Collect 30 environmental samples; prefer sediment/guano samples, but ceiling/wall swabs on visible roost stain is acceptable. Collect sediment/guano samples below roosting areas that are at least 12 inches apart; if roost areas are not known, evenly space out the samples in order to cover the entire site (mark sample locations on a map/drawing of site on datasheet). Fill the tube to the 1.5 mL mark. For ceiling/wall samples, dib the swab in tube with sterile water then roll the swab over an area the size of a palm 3-5 times.

If you have any questions, please contact WDFW's WNS Coordinator.

Appendix 2.5: White-nose syndrome surveillance talking points.

Washington Department of Fish and Wildlife

White-nose Syndrome Surveillance Talking Points

The information below provides guidance on how to interpret white-nose syndrome (WNS) results and presents Washington Department of Fish and Wildlife (WDFW) perspective of highest priority management actions following certain WNS results. This information is intended for WDFW staff to share with partners, stakeholders, and private landowners and is appropriate for those audiences who have some familiarity of WNS. For those who are less familiar with WNS, please refer to http://whitenosesyndrome.org, or http://wdfw.wa.gov/bats, for more background information.

WNS case definitions from National Wildlife Health Center (NWHC):

(https://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/Case%20Defintions%20for%20WNS.pdf)

Positive for WNS – histologic lesions of WNS present on bat and *Pseudogymnoascus destructans* (Pd), causative agent of WNS, is detected by PCR or fungal culture.

Suspect for WNS – Either 1) histologic lesions of WNS present but Pd is not detected, 2) one or more clinical signs of WNS observed (e.g., fungal growth, characteristic UV fluorescence) and Pd is detected, OR 3) multiple clinical signs of WNS are observed (e.g., unusual mortality, fungal growth on skin, UV fluorescence).

Negative for WNS – histologic lesions are not present and Pd is not detected by PCR or fungal culture.

Pd present – Pd detected by PCR or fungal culture in an environmental sample or from a bat with no other clinical signs of WNS observed. A site is considered contaminated with Pd when 1 sample is found positive.

Pd not detected – Pd is not detected by PCR or fungal culture in an environmental sample or from a bat with no other clinical signs of WNS observed. Note: A negative PCR result indicates the amount of Pd is below the level of detection for the test but does not guarantee that the site or bat is free of Pd.

Equivocal for Pd (not included in NWHC definitions) – The PCR test was neither clearly positive nor negative for Pd. This happens when a sample contains amounts of Pd DNA but is so small that it cannot be reliably detected with the PCR test. This can happen when the sample actually contains a small amount of Pd DNA (from a single spore) or when a small amount of lab-based DNA contaminates the sample.

Management options based on surveillance results:

Options outlined below represent WDFW perspective on 'highest level' management actions. Options are not in order of priority and multiple options can and likely will be selected per site.

Negative for WNS/Pd not detected

- *Option A*: No response.

- *Option B*: Re-sample site annually or biennially (whichever is practical) for early disease detection. This will include environmental sampling (e.g., sediment, guano) and/or swabbing bats. Sample type (i.e., swab, environment) will depend on which diagnostic lab we work with and the availability of sample type at site.
- *Option C*: Provide WNS education to those who use site (e.g., formal programs, interpretative signs).
- *Option D*: Screen those who visit site to prevent any human-assisted spread of Pd. This may involve using cleaning stations or dedicated gear at the site. For those sites with limited staff presence, interpretative signs or self-cleaning stations can be used.
- *Option E*: Elucidate bat use of site (e.g., roost type, species interaction, inter/intra-seasonal use) to determine the potential exposure risk to Pd.

Positive for WNS/Pd present

- *Option A*: Collect additional samples from bats to assess physical condition and to document extent and severity of disease in colony or site.
- *Option B*: Monitor bat population to track changes in colony size.
- *Option C*: Clarify bat use at site (e.g., roost type, species interaction, inter/intra-seasonal use) to predict rate of spread and exposure risk.
- *Option D*: Implement WNS educational programs for those who visit site (e.g., formal programs, interpretative signs).
- *Option E*: Screen those who visit site to prevent or minimize human-assisted spread of Pd. This would involve setting up cleaning or decontamination stations. For those sites with limited staff presence, interpretative signs or self-cleaning stations could be established and used.
- *Option F*: Use dedicated gear/equipment at site to prevent any human-assisted spread of Pd.
- *Option G*: Close site seasonally or permanently to public use to prevent human-assisted spread of Pd. This option should only be considered when options D-F are not feasible.

Suspect for WNS/equivocal for Pd

- *Option A*: Re-sample site to clarify results.
- *Option B*: Provide WNS education to those who use site (e.g., formal programs, interpretative signs).
- *Option C*: Screen those who visit site to prevent or minimize human-assisted spread of Pd. This could involve using cleaning stations or dedicated gear at the site. For those sites with limited staff presence, interpretative signs or self-cleaning stations can be used.
- *Option D*: Elucidate bat use of site (e.g., roost type, species interaction, inter/intra-seasonal use) to determine the potential exposure risk to Pd (e.g., microclimate).

Appendix 3: Bat colony emergence count protocol *(version updated April 2018).*

Washington Department of Fish and Wildlife

Bat Colony Emergence Count Protocol

OVERVIEW

White-nose syndrome (WNS) is a deadly fungal disease that has killed millions of hibernating bats in eastern North America. Unfortunately, in March 2016, the first western detection of this disease was found in King County, Washington on a little brown bat (*Myotis lucifugus*). To evaluate the impact of WNS and monitor the overall health of bat populations in Washington it is important to understand the natural history of bats (e.g., roost locations), and to assess population trends. Many bat species group together in the summer months, forming maternity colonies, where females give birth and raise their young (pups). Monitoring these colonies will provide an index of colony size and contribute to understanding population trends. Assessing population trends will allow us to track declines that may be related to WNS and will guide future management activities. Washington Department of Fish and Wildlife (WDFW) staff, partners, and stakeholders with bat experience who plan to conduct emergence counts at known bat colonies should use this protocol.

HOW TO USE THIS PROTOCOL

We developed a decision flow diagram (Fig. 1) to help determine the best method for counting bats at a colony. We deemed it necessary to provide this level of guidance due to the varying conditions encountered at bat roosts. The lead surveyor at each site should work through this flow diagram to determine the best approach to conduct a count and then find this approach in the "METHODS" section for detailed instructions. Some roost conditions make it difficult to adhere to this protocol; in those cases, these can still be counted to obtain a colony size estimate, but such surveys are not currently being prioritized and resulting data would not be included in the population trend analysis.

SCHEDULE

Bat colonies should be monitored at least **twice in a season** during the dates listed below to acquire information on population trends. The two counts should occur 3-7 days apart; this timing is to minimize the possibility that counts would be influenced by short-term weather events or other environmental conditions, but close enough that the counts are representative of the colony.

Myotid bats and big brown bats

June 15 to July 15

Note: This range is based on 10 years of data collected from several colonies in western Washington. This range, however, may shift as we learn more about new colonies in the state.

Townsend's big-eared bats

July 1 to July 22

Note: WDFW is not focusing monitoring efforts on this species currently since the vulnerability of this species to WNS is assumed low.

EQUIPMENT LIST

- ☐ Handheld tally counter (at least 1 per surveyor) or smartphone tally app
- ☐ Watch or phone
- ☐ Headlamp with extra batteries
- ☐ Handheld weather meter
- ☐ Geographic positioning system (GPS)
- ☐ Binoculars or scope
- ☐ Camera

- ☐ Personal protective equipment
- ☐ WNS decontamination supplies

Optional:

- ☐ *Acoustic detector setup (e.g., detector, tripod or pole, microphone and cable, SD card, batteries)*
- ☐ *Night vision googles*
- ☐ *Infrared or thermal video camera setup (e.g., camera, infrared lights, tripod, SD card, batteries)*
- ☐ *Red spotlight*

EQUIPMENT DESCRIPTION

- A handheld tally counter or app is used to keep track of the number of bats exiting a roost. It is recommended that a surveyor use two counters; one to keep track of the bats exiting ('outs') and one to keep track of the number entering ('ins') the roost. At roosts where this behavior is not common, keeping track of the number of bats entering without a counter is acceptable. For those roosts, that have a large number of bats emerging at once, one tally can represent 10 or 20 bats. In these cases, be sure to be consistent with you counting method in order to not misrepresent the colony size.
- Watch or phone (on silence) is needed to keep track of when the emergence count starts and ends, and when the first and last bat was observed leaving the roost.
- Headlamp or flashlight with extra batteries is needed when leaving the roost and returning to vehicle in the dark.
- Handheld weather meter (e.g., Kestrel) is needed to record the temperature at sunset.
- GPS or other navigation system is used to record latitude and longitude of roost if a street address is not available.
- Binoculars or scope can improve visibility in the fading daylight making bats easier to detect. They can be particularly of use at sites where a concentration of bats emerges from one spot. Binoculars are not as effective when bats emerge from many different spots. At the latter sites, it is recommended not to use binoculars as this would limit the scope of view and potentially result in some emerging bats going undetected.
- A camera is needed to document roost location, where bats emerge from roost, and where equipment is set up if used.
- WNS decontamination supplies should be available in case any surveyors come in contact with bats or with the roosting environment (e.g., guano). These supplies can consist of, but not limited to, boot covers, disinfectant wipes, spray bottle with disinfectant solution (e.g., isopropyl), trash bags.
- Personal protective equipment such as helmets, respirators, or protective clothing may be necessary at some sites. Consult with WDFW to determine if there are any special equipment needs.
- Optional specialized equipment can be used to determine species composition of colony (e.g., acoustic detectors) and to improve the detectability of bats as they emerge (e.g., night vision google, infrared or thermal video camera, red spotlight). This gear may not be available for use for some surveys and requires technical knowledge of their use. If specialized equipment is used be sure to use it for subsequent counts in order to not bias counts within and between years.

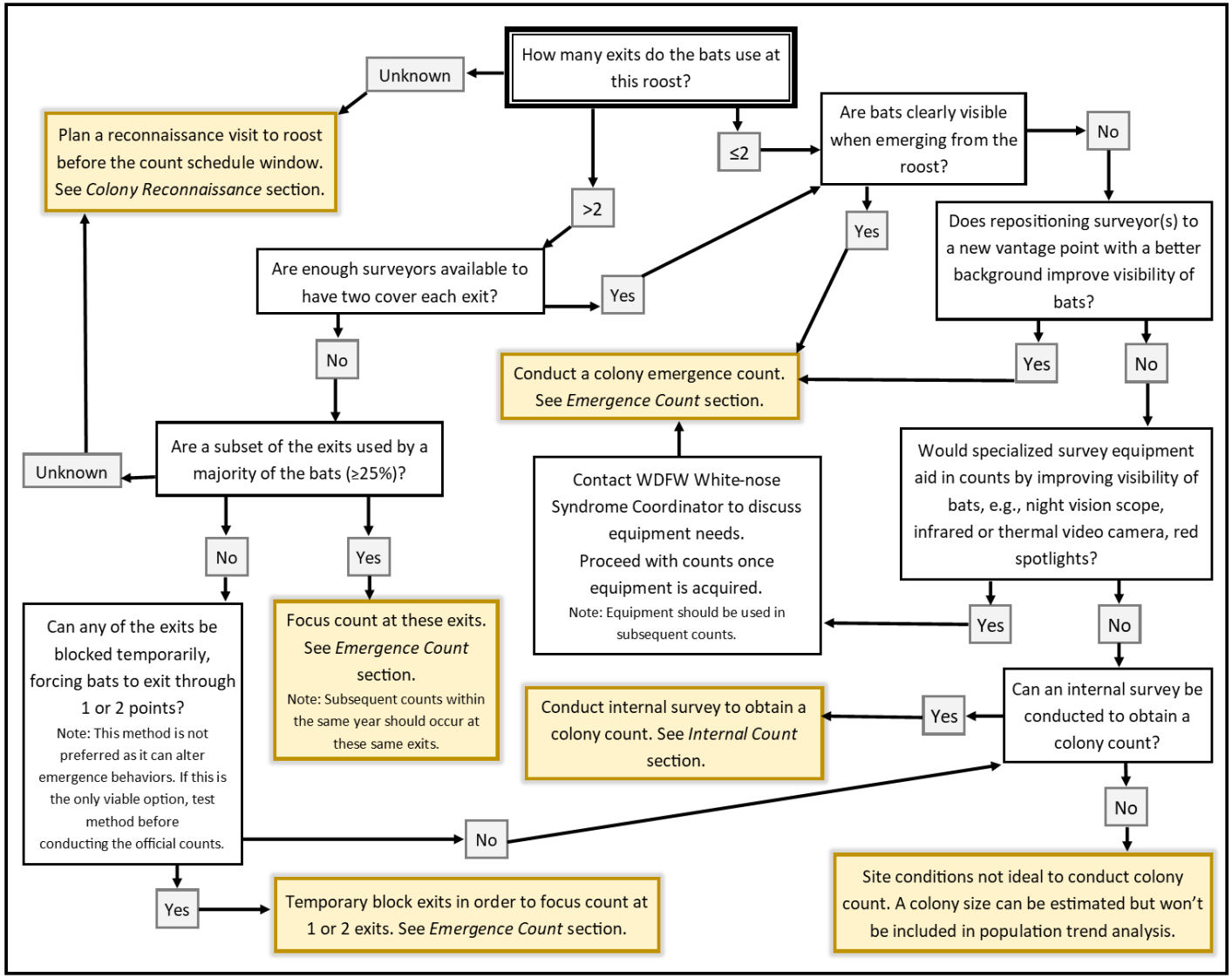


Figure 1: Decision flow diagram to determine best approach to obtain a bat colony count.

METHODS

Colony Reconnaissance

Colonies that are new, have not been visited within the last year or two, or are suspected to have had changes occur to the roosting structure or with bat behavior, should be visited before the dates listed above under SCHEDULE. This will provide the surveyor the opportunity to evaluate conditions and behavior and develop approach needed to conduct an emergence count during that survey window. If a surveyor is unable to visit the colony prior to conducting the emergence count, the site still can be assessed from May to August (period for summer activity) but will be considered for the following year's counts instead. These initial assessments should determine if the colony is active (bats present), where the primary exits are, the colony's species composition, roost type (e.g., maternity, bachelor, day) and the equipment and staff requirement to implement an emergence count.

Instructions:

- Contact landowner to obtain permission to visit site. When necessary, coordinate with WDFW staff or other partners to obtain landowner permission.
- Print a copy of the Bat Colony Emergence Count Form (Appendix 3.1) to bring to the colony assessment. Be sure to delegate one person as the data recorder and have them fill in the form during the visit to ensure all data are collected.
- Conduct an external survey to locate roosting bats and/or primary exits. Roosting preferences will vary depending on species; some are crevice-dwelling bats that tend to hide out of view (e.g., behind siding, shakes or shingles, beams), while others roost in the open. A surveyor should be aware of these differences and take the time to search the roost structure thoroughly. Primary exits can sometimes be determined based on concentrations of guano or discolored areas such as in and around chimneys and eaves (Fig. 2).
- An internal survey can be conducted if the colony status is unknown after an external survey and it is appropriate to do so. Surveyors should be aware that entering roosts and/or coming in close proximity to roosting bats will cause a disturbance during a period that already is energetically demanding; therefore, surveyors should be as quiet and efficient as possible when searching. Limit group size to 2-3 people, avoid shining lights directly on bats for more than a few seconds, use a low soft tone when speaking, and avoid wearing clothing that will generate noise (e.g., synthetic material like nylon jackets or pants can make 'swishing' sounds when worn).
- Some species can be visually identified by certain physiological characteristics (e.g., ear length, size, tragus shape). Those surveyors with experience should determine species during external and/or internal surveys. Species in Washington that have distinguishing characteristics include Townsend's big-eared bat (*Corynorhinus townsendii*), big brown bat (*Eptesicus fuscus*), silver-haired bat (*Lasionycteris noctivagans*), hoary bat (*Lasiurus cinereus*), canyon bat (*Parastrellus hesperus*), pallid bat (*Antrozous pallidus*), and spotted bat (*Euderma maculatum*). Most *Myotis* cannot be identified to species visually. With experience, a *Myotis* species can be narrowed down into 2 groups based on ear length; for example, the western long-eared myotis (*Myotis evotis*), fringed myotis (*Myotis thysanodes*) and Keen's myotis (*Myotis Keenii*) are the *Myotis* species with longer ears (16-22mm).
- Once occupancy at the site is confirmed, surveyors should prepare for an emergence count that evening or soon thereafter.
- Each surveyor should be in position at one exit at least 30 minutes before sunset. If primary exits were not located during external or internal surveys, position surveyors so that all sides and angles of the structure can be observed. Try to position surveyors at the best vantage point to observe emerging bats. For example, having the open sky as a backdrop provides good contrast

to observe emerging bats. A backdrop of trees or other vegetation can make counting difficult and produce results that are less reliable.

- Surveyors do not need to stay for the entire emergence, only enough time to confirm primary exits, the best vantage points to observe bats, and to determine the number of staff and type of equipment needed to implement an emergence count.
- If time permits, a colony size can be estimated by having each surveyor count the number of bats exiting ('outs') and entering ('ins') the roost until it's either, 1) too dark to see, 2) when there is no activity at exits for 5 minutes, or 3) more bats are entering the roost than exiting. When necessary, the colony size can be categorized as *small* (5-25 bats), *medium* (25-100 bats), *large* (100-300 bats) or *very large* (>300 bats) instead of reporting an actual number. This information will help prioritize the site in future monitoring or surveillance work, but not be included in population trend analysis.
- Species composition of colony should be determined during emergence count if species was not determined during external or internal surveys. Those surveyors with experience and necessary equipment should deploy acoustic detectors before sunset along either the bat's presumed flight path or at least 30 feet from one of the primary exits. The detector should be placed away from water surfaces or dense vegetation and elevated at least 5 feet off the ground to minimize noise and/or echoes that can cause poor-quality calls (Fig. 3). If a surveyor needs assistance with detector settings (Appendix 3.2) and deployment or with data analysis, please contact WDFW's WNS Coordinator.

A.
B.
C.
D.

Figure 2: (A) discrete piles of guano are usually indicative of the presence of a maternity, day or transitional roost. Photo: D. Youkey. (B) Close-up of bat guano. Mice, wood rat and chipmunk droppings can look similar to bat guano. To distinguish bat guano from these others, squash a piece between your fingers, if there are shiny pieces present then this would be bat guano (shiny pieces are the exoskeleton of digested insects). Photo: S. Thomas. (C) Insect parts are indicative of a night roost. Photo: A. Hyde. (D) Roost staining and droppings at an exit to a bat roost. Photo: Center for Wildlife Damage Management.

Figure 3: Examples of acoustic detectors deployed at bat roosts. The detector microphone should be elevated off the ground using a tripod or pole and placed away from dense vegetation, structures or water surfaces, but within the colony's flight path or near primary exits.

Emergence Count

Colonies that are monitored using this protocol will be included in population trend analysis. Please read the instructions below beforehand to ensure all surveyors understand their responsibilities and all necessary equipment is obtained for the count.

Instructions:

- Contact landowner to obtain permission to visit site during proposed dates. When necessary, coordinate with WDFW staff or other partners to obtain landowner permission.
- It is recommended that immediately prior to the scheduled counts the surveyors conduct a colony assessment to determine emergence behaviors, optimal observation positions, flight patterns and other factors that may affect counting emerging bats.
- These counts require a **double-surveyor effort**, meaning two surveyors will be counting the same bats at the same exit. Make sure there are enough surveyors available to have two cover each primary exit[1, 2]. A double-surveyor effort will provide a robust data set that will minimize surveyor bias.
- Identify surveyors that can commit to the proposed dates. All surveyors should be able to sit or stand quietly for 1 to 2 hours.
- Print a copy of the Bat Colony Emergence Count Form (Appendix 3.1) to bring to the count. Be sure to delegate one person as the data recorder and have them fill in the form before and after the count to ensure all data are collected.
- Arrive to the site at least 30 minutes before sunset. Sunset times can be found at: *http://www.timeanddate.com/sun/usa/.*
- Be as quiet as possible and avoid shining lights on bats when within 100 feet of the roost. Loud sounds and other disturbances can influence the emergence behavior of bats, so all discussions about surveyor responsibilities and roles should occur away from the roost.
- Be in position to count emerging bats with all needed equipment before the official sunset time. The weather and/or stage of development of pups can influence when bats start flying out of the roost.
- Try to position surveyors in the best vantage point to observe emerging bats. For example, having the open sky as a backdrop provides good contrast to observe emerging bats. A backdrop of trees or other vegetation can make counting difficult.

- Do not continue with emergence count if:
  - Temperature is below 32°F
  - Heavy precipitation or fog is present
  - Strong steady breeze present (leaves and twigs in constant motion)
- Each surveyor should count bats as they exit ('outs') and count any bats that may re-enter ('ins') the roost using a tally counter. For sites where this behavior is common, two tally counters can be used to keep track of the number of 'outs' and 'ins'.
- Surveyors should be aware that re-entering, circling, and/or fly retreating behaviors may be common depending on the developmental stage of pups. Surveyors should count those bats that re-enter, but careful not to count those that are just circling around the roost. For those surveyors with limited experience observing such behaviors, it recommended that they observe an emergence flight or two before participating in counts.
- Surveyors should not share their observations of bats with other surveyors during the emergence, as the counts need to be independent.
- Surveyors counting at the same exit do not necessarily have to be in the same position; they only need agree upon the area they are observing and that they are observing the same bats.
- Continue with the count until one of these scenarios occur:
  - It is too dark to observe bats.
  - There is no longer any activity at exits after 5 minutes.
  - There are more than or equal to number of bats re-entering roost than exiting over a 5-minute period.

[1] Under special circumstances, a slight modification can be used at roosts where it is difficult to adhere to the double-surveyor approach due to there being multiple exits. In these cases, surveyors can focus counts at those exits that receive ≥25% of activity instead. This will help reduce the number surveyors needed to conduct the count. If this approach is used, subsequent counts should occur at these same exits to maintain consistency throughout the monitoring effort. All instructions under *Emergence Count* still apply when using this approach. Prior to implementing this approach at a site please contact WDFW.

[2] Under special circumstances, another modification can be made to the approach when a roost has multiple exits used by bats, and none receive ≥25% of the activity. In those cases, surveyors can block exits to force bats to emerge through a few exits. This will help reduce the number of surveyors needed to conduct the count. This approach is best used on those openings that are large and easy to block (e.g., window). Smaller openings may be hard to locate and thus not easy to successfully block. This approach is not preferred and should only be considered if there are no other options due to the level of disturbance this may cause during the actual emergence. All instructions under *Emergence Count* still apply when using this approach. Prior to implementing this approach at a site please contact WDFW.

Internal Count

In some special circumstances, monitoring counts for a colony can be based on internal surveys if conditions allow. Although conditions at many roosts are not conducive to this approach, in some cases, this may be the best and only option to obtain a colony count. The most important condition to consider when evaluating the feasibility of this approach, is the visibility of the colony. The colony must be visible and not obscured behind siding, walls, or other structures. A colony reconnaissance survey will be required to determine if this approach is feasible. It is important to note that this approach will cause some level of disturbance to the colony, so it is recommended to limit the number of surveyors (2 minimum, 3 maximum), minimize the amount of time inside the roost and avoid making loud noises while inside.

Instructions:

- Contact landowner to obtain permission to visit site during the proposed dates. When necessary, coordinate with WDFW staff or other partners to obtain landowner permission.
- Attempt to schedule internal surveys for the first part of the survey window (closer to June 15). It is preferable to conduct internal surveys earlier in this window so surveyors are more likely to encounter only pregnant females and not neonates.
- Identify all surveyors well in advance.
- Print a copy of the Bat Colony Emergence Count Form (Appendix 3.1) to bring to the count. Be sure to delegate one person as the data recorder and have them fill in the form before and after the count to ensure all data are collected.
- Counts should be conducted in the early to mid-afternoon. Bats should be settled at this point after their morning activities but not yet active for evening emergence.
- Each surveyor should thoroughly search inside the roost while counting bats. The surveyor(s) should be careful not to double count any bats. Looking for bat guano or roost staining can cue a surveyor to where bats may be roosting (Fig. 2). This can be especially helpful when there are areas that bats hide or become obscured by roost material. Surveyors should include in the detailed notes a description of areas where bats are most frequently found to enhance efficiency (and reduce disturbance) during future surveys.
- During the survey, surveyors should avoid shining lights on bats for more than a few seconds, not make any loud noises and avoid wearing clothing that can generate noise (e.g., synthetic materials).
- Surveyors should not share their observations of bats with other surveyors during the count, as the counts need to be independent.
- When large clusters of bats are present, using a laser pointer to keep track of count progress can be useful. The laser pointer can help surveyors move systemically through the cluster while not losing track of which bats have been counted. In addition, using defining features in the roost to count bats systemically can help avoid double counting bats, e.g., use cross beams to define an area to count bats within. The technique surveyor uses to counts bats should be noted on the data sheet.
- It can sometimes be helpful for surveyors to focus on counting noses or pairs of ears when bats are tightly clustered.
- Surveyors should also note the size of each cluster (e.g., noting the specific size of the occupied area). In some cases, the area occupied by bats can be correlated to colony size. For example, in Tuttle 2015, clustering density for some species in warm temperatures (about 50°F) is 25-50 bats. Clustering density, however, will vary among species, roost type (e.g., maternity, hibernacula), and roost conditions (e.g., temperature, surface roughness). Alternatively, when bats are tightly clustered using a high-resolution photograph to conduct the count back at the office can be most effective (see below).
- An alternative approach to conducting visual counts is to have 1 or 2 surveyors enter the roost to take high-resolution photographs of the colony (minimum of eight megapixels). This is especially effective approach when a colony is tightly clustered. The entire cluster of bats needs to be in the same frame of the photograph or some defining feature in the roost or laser pointer needs to be in frame to avoid or minimize double counting bats. Camera type and settings will vary depending on site conditions, but we recommend using a single lens reflex (SLR) digital camera with a zoom lens that has adequate flash capabilities. Although at some sites, a point-and-shoot digital camera may be adequate especially when lighting is good and bats are close to surveyors. Surveyors should take a few test photographs in similar roost conditions to ensure settings are appropriate before conducting survey. This will help reduce the amount of time and

number of photographs a surveyor needs to take once near the bats. Flash photography is permitted but should not be used excessively; a surveyor should only need to take 1 or 2 photographs. Back at an office, two people can conduct independent counts of the number of bats shown in the photographs. Surveyors should focus on counting noses or pairs of ears. Software programs such as ArcGIS can help surveyors count bats by using the editor tool to place points on each bat. This approach can be less invasive, if the surveyor has experience using camera equipment, by limiting the number of surveyors and the amount of time spent inside the roost.

DATA SUBMISSION

- Data forms and pictures should be submitted electronically either, 1) using the *Bat Emergent Colony Count* Survey form in the Survey123 app*, or 2) by emailing Lori Salzer (Lori.Salzer@dfw.wa.gov) and Abby Tobin (Abigail.Tobin@dfw.wa.gov).
- Acoustic data files should be sent to Abby Tobin (Abigail.Tobin@dfw.wa.gov) if the surveyor does not have expertise and/or software to conduct data analysis.

**Contact Bill Simper If you need assistance setting up the Survey123 app; William.Simper@dfw.wa.gov*

WNS DECONTAMINATION

Surveyors that become exposed to the bat's roosting environment (e.g., guano) during internal and/or external surveys should follow WNS decontamination procedures to reduce the potential for human-assisted spread. Please refer to the National WNS Decontamination Protocol (https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf), or contact WDFW's WNS Coordinator for a step-by-step guideline for implementing decontamination in Washington.

Appendix 3.1: Colony count data form

Bat Colony Emergence Count Data Form

Site name: _____ **Survey date:** _____ **Arrival time to site:** _____
Lead surveyor name: _____ **Contact information:** _____
Other surveyor names: _____
_____

Landowner name: _____ **Landowner contact information:** _____

Survey method[1] (check one)**:** Colony reconnaissance ❑ Emergence count including all exits ❑ Internal Count ❑
Emergence count focused at subset of exits ❑ Total number of exits: _____ Number of exits counted: _____
Emergence count with exits blocked ❑ Total number of exits: _____ Number of exits blocked: _____
[1]Surveyors should fill out all applicable information regardless of survey method

Sunset time: _____ **Temperature at sunset:** ____ °C or ____ °F ***Do not continue with count if temperature below 32°F***
Wind conditions (check one)**:** Calm ❑ Light breeze ❑ Steady breeze ❑ Windy - over 10 mph[2] ❑
Precipitation (check one)**:** None ❑ Light rain ❑ Heavy rain[2] ❑ Fog[2] ❑
[2]Do not continue with counts in these conditions

Location (check at least one): ❑ Street Address: _____ City: _____ County: _____
❑ GPS location - prefer decimal degrees (hddd.ddddd°) and datum WGS84
Latitude: _____ (N) Longitude: _____ (W)
Datum (check one): WGS84 ❑ NAD83 ❑ NAD27 ❑ Unknown ❑

Roost structure (check one): Barn ❑ Bunker ❑ Occupied house ❑ Unoccupied house ❑ Utility building ❑
Bat house ❑ Bridge ❑ Tree ❑ Cave ❑ Abandoned mine ❑ Other (describe):_____

Species within roost (see list below): ANPA ❑ COTO ❑ EPFU ❑ LANO ❑ MYCA ❑ MYCI ❑ MYEV ❑ MYLU ❑ MYTH ❑ MYVO ❑ MYYU ❑ PAHE ❑ Unknown ❑ Other: _____
Method used to identify species (check all that apply)**:** Acoustic detector ❑ Brand, model: _____ Visual ❑
Other (describe): _____

Internal Count (fill out this section if method used)

	Number of bats roosting
Surveyor 1	
Surveyor 2	
Surveyor 3	
TOTAL colony count (average surveyor counts)	

All areas of roost surveyed? Yes ❑ No ❑
Method for counting: Photography ❑ Brand, model: _____ Visual ❑

Emergence Count (fill out this section if method used)
Roost counted before? Yes ❑ No ❑ *If Yes, date of prior count if known:* _____
Time *first* bat exited roost: _____ **Time *last* bat exited roost:** _____ **Time count ended**: _____
Surveyor distances from exit point(s) (ft): Exit 1: _____ Exit 2: _____ Exit 3: _____ Exit 4: _____

		Number of bats exiting	Number of bats entering	Total number of bats (# of exiting minus # of entering)	Average number of bats (average 2 surveyors total)
Exit #1	Surveyor 1				----
	Surveyor 2				
Exit #2	Surveyor 3				----
	Surveyor 4				
Exit #3	Surveyor 5				----
	Surveyor 6				
Exit #4	Surveyor 7				----
	Surveyor 8				
	TOTAL colony count (sum average number)	----	----	----	

Comments[3]: _____
_____
_____
_____
_____
_____

[3]*Include comments such as bat behavior during count, descriptions of surveyor positioning, whether all exits or areas of roost were counted, equipment used during count or other recommendations for future counts.*

Sketch of roost and surveyor(s) location (attach photos when entering data)**:**

Bat species list:
ANPA = Pallid Bat
COTO = Townsend's big-eared bat
EUMA = Spotted bat
EPFU = Big brown bat
LACI = Hoary bat
LANO = Silver-haired bat
MYCA = California myotis
MYCI = Western small-footed myotis
MYEV = Western long-eared myotis
MYKE = Keen's myoits
MYLU – Little brown myotis
MYTH = Fringed myotis
MYVO = Long-legged myotis
MYYU = Yuma myotis
PAHE = Canyon bat
Unknown

Appendix 3.2: Settings for Wildlife Acoustic Song Meter SM2BAT+ and SM4BAT FS detectors. These settings may need to be adjusted depending on specific site conditions. Please contact WDFW's WNS Coordinator with questions about settings and deployment.

SM2BAT+

Select "**Schedule**" →"Advanced"

01 AT SSET-00:00:00
02 DO
03 RECORD 00:30:00
04 GOTO LINE 03 00x
05 UNT SRIS+00:00:00
06 GOTO LINE 01 00x

Select "**Settings**" →"Time and Date" → *Check for accuracy*

"Location" → "Prefix": *site name*
"Lat": *add location*
"Long": *add location*
"Time zone UTC": -7:00[1]

"Audio"→"Sample rate": 192000
"Channels": MONO-L *(microphone should be connected to left port)*
"File Format": WAV
"Gain Left": 12.0 dB
"Gain Right": 12.0 dB
"Advanced settings"→ "Dig HPF left": fs/12 (16000)
"Dig HPF right": off
"Dig LPF left": off
"Dig LPF right": off
"Trg lvl left": 18 SNR
"Trg lvl right": off
"Trg win left": 2.0 s
"Trg win right": 2.0 s
"Trg max length": 8s
"Bits": 16
"Div ratio": 16
"Nap trg level": off

Notes:

- Turn switch to "internal" battery if using D batteries to power detector.
- Hit "wake/exit" button to turn detector on. Hit this button again when ready to deploy the detector. Make sure the window reads the correct time and date for the schedule start.
- Leave settings as default unless differences indicated above.
- SMX-U1 (new) microphone has an internal gain so audio gain should be set to 12 dB. If using the older model microphone (SMX-UT), the gain should be set to 0 dB.
- Board switches should be as follows: Number **6, 7, 8, 9** on both sides (left and right) should be **on, off, off, on**, respectively.
- [1]Change UTC when daylight savings occurs (Nov 5 to March 4 UTC = -8, March 5 to Nov 4 UTC = -7)
- Contact WDFW's WNS Coordinator if assistance is needed to analyze data.

<u>SM4BAT FS</u>

Select "**Quick start**"→"Sunset to Sunrise"
"record always"

Select "**Settings**"→"Audio"→"Gain": 12dB
"16k high filter": on
"Sample rate": 256 kHz
"Min duration": 1.5 s
"Max duration": none
"Min trig freq":16 kHz
"Trigger level":12 dB
"Trigger window": 3 s
"Max length": 00m:15s
"Date and Time"→ *check for accuracy*
"Location"→"Prefix": *site name*
"Lat": *add location*
"Long": *add location*
"UTC": -7:00[1]
"LED indicator": 5 min only
"Schedule": At sunset 00:00
Always
At sunrise 00:00

Notes:

- Turn switch to internal power to turn on detector.
- Leave settings as default unless differences indicated above.
- If need to format SD cards select "Utilities"→"Format".
- Press "schedule start" when ready to deploy. Make sure there are no error messages and the start recording date and time are correct.
- Contact WDFW's WNS Coordinator if assistance is needed to analyze data.

Appendix 4: White-nose syndrome decontamination guideline for Washington – Draft.

Washington Department of Fish & Wildlife

DRAFT Pacific Northwest White-nose Syndrome Decontamination Guideline

OVERVIEW

White-nose Syndrome (WNS) is a deadly fungal disease, caused by *Pseudogymnoascus destructans* (Pd) that has killed millions of hibernating bats in eastern North America. The fungus grows on the hairless areas of bats (e.g., wings, muzzle) and disrupts physiological processes (e.g., thermoregulation) causing the bat to rouse from hibernation, burning critical fat reserves that are required to survive through the winter. In March 2016, the first western case of WNS was confirmed in Washington State on a little brown bat (*Myotis lucifugus*). It is not known how WNS reached Washington; the disease is primarily transmitted by bat-to-bat or bat-to-environment contact, but it has been demonstrated that it is possible for humans to inadvertently carry Pd on clothing or equipment.

Washington Department of Fish & Wildlife (WDFW) is collaborating with state, federal, and tribal agencies and non-governmental organizations to determine how widespread WNS is in the state and the effects the disease will have on bat populations. To achieve this, we will monitor bat populations to elucidate roosting ecology and population trends, and conduct WNS surveillance at hibernacula and maternity roosts. To limit any inadvertent human-assisted spread of WNS/Pd, any field work that involves handling bats and/or entering their roosts are required to follow decontamination.

The National WNS Decontamination Protocol is currently being followed at caves and mines, but there has been questions about the necessity of this national protocol at non-subterranean roosts that many of Washington's bat species utilize (e.g., bridges, talus slopes, cliffs). These questions initiated the development of a modified version that encourages participation by having easily achievable steps, while still limiting the human-assisted spread of WNS.

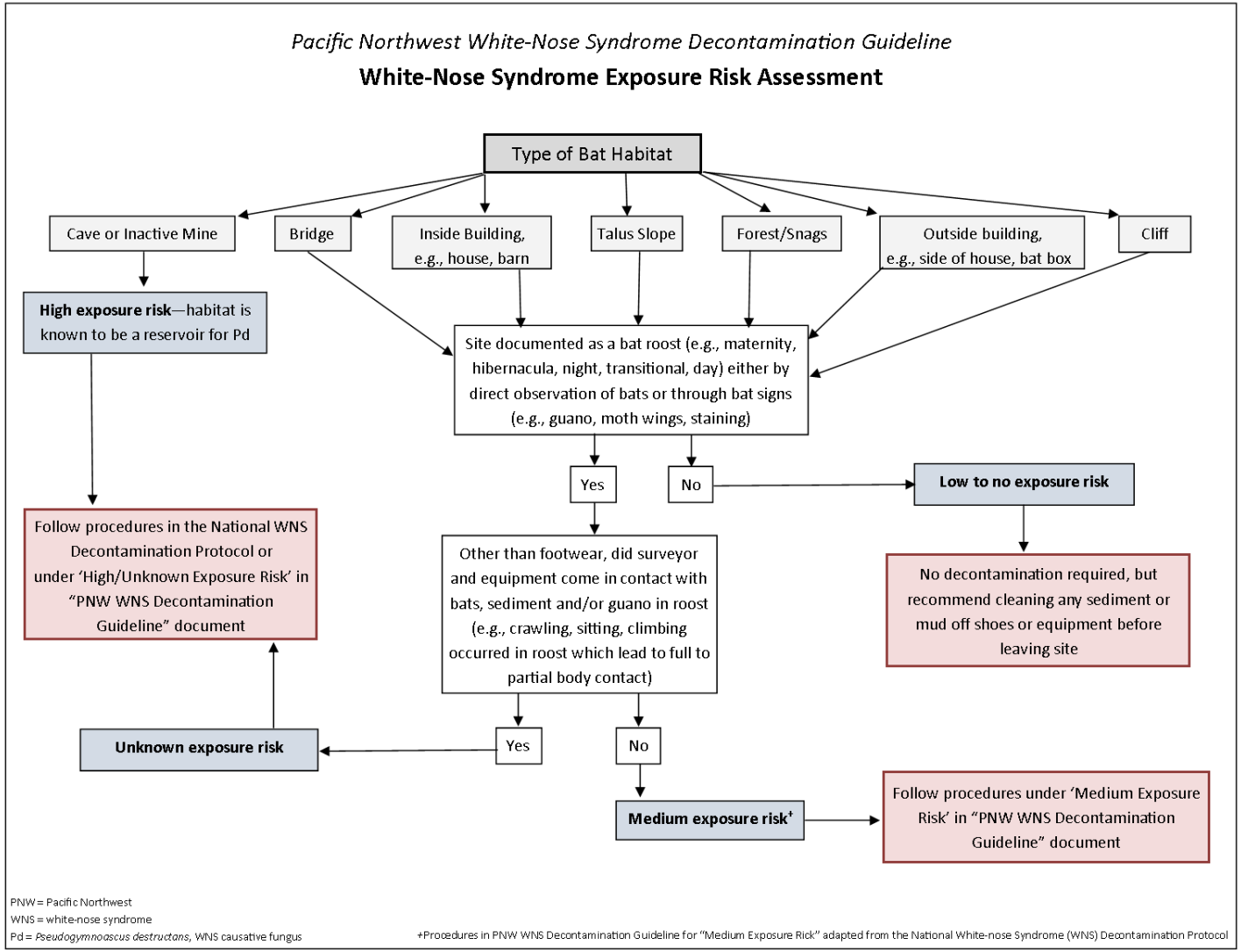
PURPOSE OF THIS DOCUMENT

An essential component to a successful WNS management plan is limiting any human-assisted spread by implementing decontamination after working with bats or in their roosts. The purpose of this document is to help facilitate decontamination practices for staff, partners and researchers conducting surveillance and/or bat monitoring in Washington State. This document includes a WNS exposure risk assessment (Fig.1) that helps determine whether the national protocol or the modified version should be implemented and step-by-step instructions on how to implement both methods of decontamination. This guideline is focused on equipment decontamination; for more information on decontamination steps for capturing and handling bats please refer to Appendix 4.1. The winter roosting ecology of bats and the habitat requirements for WNS is not well understood in Pacific Northwest, therefore this guidance document will be modified according to the latest research.

HOW TO USE THIS DOCUMENT

Follow the exposure risk assessment decision tree (Fig.1) to determine what WNS/Pd exposure risk may occur as a result of the intended field work; 1) low to no exposure risk, 2) medium exposure risk, 3) high exposure risk, or 4) unknown exposure risk. Once the exposure risk is determined, locate that heading under the PROCEDURES FOR WNS DECONTAMINATION and follow the steps for decontamination. ***If there is any uncertainty on what the exposure risk is at a site, we recommend following instructions under "high exposure risk"***.

Figure 1: DECISION TREE TO ASSESS WNS EXPOSURE RISKS AND DECONTAMINATION NEEDS



TRIP PLANNING

1. Contact WDFW, or local state/federal or other land management agencies to determine if there are any special requirements for site visits.
    a. Currently, research is being conducted to identify WNS/Pd reservoirs in Washington, and so in order to avoid any human inadvertently spreading WNS, *decontamination is required between all sites*.
    b. A site is defined as an area that is interconnected biologically (e.g., natural bat movement) and/or geologically (e.g., hydrology). For example, a bridge, cave, mine, or building is each considered a site since bats naturally move within those sites. A continuously connected talus slope, or cliff is also each considered a site since they are biologically and geologically connected.
    c. Exceptions will exist for those sites that have a high/unknown exposure risk, but are remote and difficult to access. For logistical and biological reasons (e.g., bat movement), these sites can be grouped into a zone, where those in close proximity (within 10km) or in the same watershed, can follow the decontamination procedures under the 'Medium Exposure Risk' between sites and 'High/Unknown Exposure Risk' between zones. The zones will be created on a case-by-case basis and will be determined and approved by WDFW or other land management agency.
2. Manage your equipment use.
    a. Equipment from eastern North America and endemic/intermediate areas in the mid-west are not permitted to be used in Washington.
    b. Equipment used on bats in King County, Washington should not be used in other Washington counties that are at-risk (not confirmed for WNS).
    c. Choose equipment that can most effectively be decontaminated, keeping in mind those items that may be damaged through the decontamination process, such as submerging in liquid.
    d. Only bring essential equipment into a site in order to avoid unnecessary contamination.
    e. Equipment that cannot be safely treated in accordance with manufacturer's recommendations and product label instructions should be dedicated to an individual site or WNS endemic area, such as vertical caving equipment (e.g., ropes, webbing, harnesses).
3. Ensure all necessary equipment and decontamination supplies are prepared and organized prior to site visits.
    a. Be prepared to properly isolate and contain potentially contaminated equipment in a bag, storage container or sealable bucket before entering a vehicle or building.
    b. If a site is in a remote location and not close to a vehicle, surveyors can carry all the decontamination supplies in a small container or bag and complete the cleaning at the site upon exiting. Items such as wipes can be placed inside labeled resealable bags to reduce the amount of bulk carried to the field site.

PROCEDURES FOR WNS DECONTAMINATION

Designate a storage container for decontamination supplies. The container should contain the following:

- Hand brushes
- Spray bottle
- Container of disinfectant wipes (see National Guidelines [Appendix 4.2])
- Hand/face wipes or soap
- Trash bags
- Disinfectant solution (Appendix 4.2)
- Tyvek® disposable suit (recommend suit with hood)
- Tyvek® or similar boot covers
- Latex or nitrile gloves
- Duct tape

- Knife
- Resealable bags (e.g., Ziplocs®)
- Household gloves
- Small tarp

Low to No Exposure Risk

No decontamination required, but recommend cleaning sediment or mud from footwear or equipment before leaving site. A small hand brush and spray bottle can be used for cleaning.

Medium Exposure Risk

Note: This procedure is being discussed within the regional and national decontamination working groups for effectiveness and appropriateness as a modified approach to the national protocol.

Supplies

- ☐ 1 hand brush
- ☐ 1 spray bottle with approved product solution (see Appendix 4.2 for concentrations; e.g., ethanol, Clorox® Bleach, Lysol® IC Quaternary Disinfectant Cleaner)
- ☐ 1 container of disinfectant wipes (Appendix 4.2; e.g., isopropyl, hydrogen peroxide)
- ☐ 1 container of hand/face wipes or soap (e.g., Wet Ones®)
- ☐ 1 trash bag
- ☐ 1 box of latex or nitrile gloves
- ☐ 1 Tyvek® coverall or similar, or extra clothes

Optional

- ☐ 1 pair of boot covers (e.g., Tyvek®)
- ☐ Rubber or synthetic boots (e.g., XtraTuf® boots, Wellington boots)

Decontamination Procedure (Fig. 2)

Before entering site

1. Place container with decontamination supplies in a staging area that is relatively close to site.
2. Remove hand brush, spray bottle, a pair of latex or nitrile gloves and trash bag and place next to container.
3. Put on a Tyvek® coverall if using one. This protects clothing from potential contamination and prevents the need to change clothing afterwards.
4. If using boot covers, secure them over shoes. Boot covers prevent the sole of the boot from coming in contact with sediment/soil and so help reduce contamination.

After leaving site

5. Go to staging area where decontamination supplies were placed.
6. Put on latex or nitrile gloves to protect hands from the disinfectant solution and wipes.
7. If Tyvek® coverall was worn, remove and dispose of in trash bag. If Tyvek® was not worn, change into clean clothes.
8. If boot covers were used, remove and throw disposable covers into trash bag. Skip to step 13 if a breach did not occur. If a breach occurred in covers then continue step 11.
9. Away from the supplies, brush boots thoroughly with hand brush to remove all sediment/dirt.
10. Thoroughly spray hand brush with approved solution and allow appropriate drying time (Appendix 4.2).
11. Thoroughly spray the outside of the boot with approved solution and allow appropriate drying time (Appendix 4.2). These boots are acceptable to wear when returning to vehicle now. Note: Avoid spraying this solution directly on the soil.

12. Place hand brush and spray bottle back into supply container.
13. Remove disinfectant wipes from supply container.
14. Wipe all non-submersible equipment that entered site with a disinfectant wipe and allow appropriate drying time (Appendix 4.2).
15. Remove latex or nitrile gloves and throw in trash bag.
16. Use face/hand wipes or soap to clean any exposed skin.
17. Place face/hand wipes or soap back into supply container.
18. Wipe the side of the supply container with a disinfectant wipe.
19. Place disinfectant wipes back into supply container and close lid.
20. Place all used wipes and other trash into trash bag and tie shut.
21. Return to vehicle.

High/Unknown Exposure Risk

Note: These procedures follow the national WNS decontamination protocol.

Supplies

On site

- ☐ 1 hand brush
- ☐ 1 container of disinfectant wipes (Appendix 4.2; e.g., isopropyl, hydrogen peroxide)
- ☐ 1 container of hand/face wipes or soap (e.g., Wet Ones®)
- ☐ 2 trash bags
- ☐ Extra clean clothes and pair of shoes
- ☐ Resealable bag (e.g., Ziplocs®)
- ☐ Large container, labeled as "dirty equipment"
- ☐ Small tarp

Optional supplies

- ☐ Tyvek® disposable suit
- ☐ Nitrile or latex gloves
- ☐ Duct tape
- ☐ Rubber or synthetic boots (e.g., XtraTuf® boots, Wellington boots)

Off site

Option 1 (disinfectant bath)

- ☐ Disinfectant solution (Appendix 4.2; e.g., Lysol® IC Quaternary Disinfectant Cleaner)
- ☐ Two large containers; one labeled as "disinfectant" and the other as "water"
- ☐ Household gloves

Option 2 (hot water bath)

- ☐ Candy thermometer
- ☐ Propane burner
- ☐ Propane tank
- ☐ Large cooking pot (e.g., 32 qt)
- ☐ Household gloves

Optional supply

- ☐ Marine cooler

Decontamination Procedure (Fig. 3)

Before entering site

1. Place “dirty equipment” container and container with decontamination supplies in an area that is easily accessible upon exiting site, e.g., outside vehicle.
2. Remove 2 trash bags, hand brush, small tarp and hand/face wipes from container and take to a staging area near the site.
3. Open the tarp and place wipes and trash bags on top. Make sure to secure the tarp and bags in place to prevent them from blowing away.
4. Place extra pair of shoes on tarp. Recommend storing shoes in a sealed plastic bag to keep them dry and clean while in site.
5. If not using a Tyvek® suit, place extra clean clothing on tarp. Recommend storing clothing in a sealed plastic bag to keep clothes dry and clean while in site.
6. Place any non-submersible gear that will enter site but may not be used into a resealable bag (e.g., Ziploc®), such as a watch.
7. Pack any essential personal items (e.g., food, water) into a resealable bag (avoids contamination if not removed from bag) and into a sealed bag or backpack.
8. If using a Tyvek® suit, put on rubber or synthetic boots and suit and tape the leg cuffs to the boot using duct tape to prevent the inside of the boot being exposed to the environment.
9. Wear any personal protective equipment that is required to enter site (e.g., helmet).

After leaving site

10. Return to the tarp in the staging area.
11. Thoroughly remove sediment/dirt with hand brush from equipment immediately upon exiting site.
12. Use a hand brush to thoroughly remove sediment/dirt from boots.
13. Remove any items from inside backpack that are contained in a resealable bag and were not used in site. Remove items from bag and throw bag away into trash bag. Place items on ground off the tarp in a clean area.
14. While standing on the tarp, place boots, other potentially contaminated equipment (e.g., backpack or gear bag) and hand brush into a trash bag – designate this bag for potentially contaminated equipment.
15. If using a Tyvek® suit, remove tape around foot cuffs and place tape and suit into a second trash bag – designate this one as the bag for trash.
16. If not using a Tyvek® suit, remove clothing and place inside trash bag with other potentially contaminated equipment (e.g., boots, backpack).
17. Clean hands, forearms, face and other exposed skin using hand/face wipes.
18. Change into clean shoes and clothing, if applicable and step off the tarp. The inner clothing layer can be left on but should be washed as soon as possible.
19. Fold up tarp and place inside bag designated for potentially contaminated equipment.
20. Tie both trash bags closed to prevent any loss of gear or trash.
21. Carry trash bags and other equipment/items back to vehicle.
22. Place both trash bags inside “dirty equipment” container.
23. Use disinfectant wipes to clean outside of “dirty equipment” container and place it into vehicle.
24. Place all decontamination supplies back into container and put container in vehicle.

Off site – select one of the options below

Option 1 – disinfectant bath

25. Remove “dirty equipment” container from vehicle and take to your designated cleaning area.
26. Remove both trash bags from container.
27. Throw the bag designated for trash into a dumpster.

28. If applicable, remove clothing from bag designated for contaminated equipment and wash in your home washing machine on a hot temperature setting using conventional detergents to aid in removing sediment/dirt. Woolite® detergent has been demonstrated to be an effective cleaning product.
29. Wear household gloves to protect your hands from disinfectant products.
30. Fill the "disinfectant" container with approved product solution (Appendix 4.2; e.g., 1-2 oz. of Lysol IC Quaternary Disinfectant Cleaner for every gallon of water).
31. Fill the "water' container with water.
32. Remove boots, hand brush, and other submersible equipment from bag and if sediment/dirt is still visible use a hose outside to remove any remaining sediment/dirt.
33. Place boots, hand brush, and other submersible equipment into disinfectant bath for the specified time outlined in national protocol or the product label (Appendix 4.2), e.g., gear submersed in Lysol® IC Quaternary should soak for 10 minutes.
34. Clean non-submersible equipment (equipment that may be damaged by liquid submersion) using disinfectant wipes (Appendix 4.2).
35. Once the clothing in the home washing machine are finished, place them into the disinfectant bath and submerge for the specified time outlined in national guidelines or the product label (Appendix 4.2).
36. After appropriate time, remove equipment and clothes from disinfectant container and place into "water" container to be rinsed.
37. Allow equipment to completely dry before next use.
38. Decontaminate household gloves in disinfectant bath and rinse.
39. Clean any other areas that may have come in contact with contaminated equipment, such as "dirty equipment" container or countertops, using a spray bottle with appropriate product solution.

Option 2 – hot water bath

40. Remove "dirty equipment" container from vehicle and take to your designated cleaning area.
41. Fill large pot with water and place on propane stove.
42. Turn on propane stove.
43. Place candy thermometer on side of large pot.
44. The hot water bath will be ready when it is at least 55°C (151°F). Ensure that the bath remains at least this temperature throughout the treatment period. Alternatively, if large sized equipment needs to be decontaminated and does not fit into the large pot, a marine cooler can be used. Water heated up to 55°C (151°F) can be poured carefully into the cooler and used to decontaminate equipment. The water in the cooler has been demonstrated to stay at 55°C (151°F) for more than 20 minutes.
45. Remove both trash bags from container.
46. Throw the bag designated for trash into a dumpster.
47. If applicable, remove clothing from bag designated for contaminated equipment and wash in your home washing machine on a hot temperature setting using conventional detergents to aid in removing sediment/dirt. Woolite® detergent has been demonstrated to be an effective cleaning product.
48. Wear household gloves to protect your hands when placing and removing equipment from hot water bath.
49. Remove boots, hand brush, and other submersible equipment from bag and if sediment/dirt is still visible use a hose outside to remove any remaining sediment/dirt.

50. Place boots, hand brush, and other submersible equipment into hot water bath for a minimum of 20 minutes. Ensure that all equipment surfaces remain in direct contact with the hot water during the treatment period.
51. Once the clothing in the home washing machine are finished, place them and household gloves used to handle equipment into the hot water bath for a minimum of 20 minutes.
52. Allow equipment to completely dry before next use.
53. Clean any other areas that may have come in contact with contaminated equipment, such as the "dirty equipment" container or countertops, using a spray bottle with appropriate product solution.

Figure 2: Photo guide for medium exposure risk decontamination procedures; A. decontamination supplies container, B. spray bottle, hand brushes, and trash bag removed from supply container, C. removing boot covers (optional), D. brushing boots with hand brushes, E. spraying boots with product solution, F. spraying hand brush with product solution, G. wiping equipment with disinfectant wipe, H. wiping exposed skin with hand/face wipe, I. closed supply container and sealed trash bag.

Figure 3: Photo guide for high/unknown exposure risk decontamination procedures; A. designate a dirty and a transition zone when arriving at site. Prepare tarp, trash bags and other decontamination supplies; B. Upon exiting site, remove dirty clothing and other equipment and place into trash bag; C. Place equipment into trash bag for decontamination; D. Change into clean footwear and place dirty footwear into trash bag; E. Seal trash bag and place in dirty container; F. At off site location, remove trash bags from container and wash any clothing or submersible equipment on hot temperature cycle in washer and dryer to help remove any excess sediment; G. If using the disinfectant bath method, place submersible equipment (e.g., boots, helmet) into disinfectant bath for appropriate time and then into the water bath to rinse; H. If using the hot water bath method, place submersible equipment (e.g., boots, helmet) into bath for 20 minutes; I. Dry equipment before using again.

Appendix 4.1: White-nose syndrome guidelines for capturing and handling bats in Washington

Washington Department of Fish and Wildlife

White-nose Syndrome Guidelines for Capturing and Handling Bats in Washington

OVERVIEW

White-nose Syndrome (WNS) is a deadly fungal disease, caused by the fungus *Pseudogymnoascus destructans* (Pd) that has killed millions of hibernating bats in eastern North America. The fungus grows on the wings, muzzle and ears of hibernating bats and disrupts physiological processes (e.g., thermoregulation) causing the bat to rouse from hibernation, burning critical fat reserves that are required to survive through the winter. In March 2016, the first western detection of WNS occurred in Washington State on a little brown bat (*Myotis lucifugus*). It is not known how WNS/Pd reached Washington, but we know that the disease is primarily transmitted by bat-to-bat or bat-to-environment contact. It has, however, been demonstrated that humans can inadvertently carry Pd on clothing or equipment. Given the substantial risk of WNS to bats and the possibility of inadvertent transmission of Pd by humans, this document was produced to provide guidance regarding the spatial and temporal conditions that WDFW will include in scientific collection permits that are required to capture and handle bats in Washington.

People planning to conduct bat-related research and monitoring projects should coordinate with Washington Department of Fish and Wildlife (WDFW; the lead Agency in responding to WNS in Washington) to receive a scientific collecting permit and the most current WNS information and guidance. Currently, capturing bats at known aggregation sites is allowed year-round with a scientific collecting permit; this activity includes capture and handling of bats at maternity roosts, night roosts, hibernacula and other aggregation sites. WDFW will permit landscape trapping between July 1 and September 30, and outside of those dates, permit requests will be evaluated on a case-by-case basis due to the increased risk that bats could be exposed to WNS or Pd through interactions with infected individuals or environments. Landscape trapping is defined as any trapping effort that is conducted away from aggregation sites (e.g. at foraging sites or at travel corridors) such that encountered bats may be traveling to or from multiple aggregation sites. All bats that are captured will be assessed for clinical signs of WNS (e.g., wing damage, orange UV fluorescence) and any with questionable signs will be reported to WDFW immediately. To limit any inadvertent human-assisted spread of WNS/Pd, any field work that involves handling bats and/or entering their roosts are required to follow the decontamination protocol. Below is guidance for facilitating decontamination while capturing and handling bats.

DECONTAMINATION GUIDELINES

Capturing and Handling Bats

- Use one breathable holding bag per bat; after use each holding bag should be placed in garbage bag designated for equipment that needs to be decontaminated. Do not reuse any of the holding bags until they are properly decontaminated following the National WNS Decontamination Protocol and/or the steps outlined under the high/unknown exposure risk for offsite decontamination (e.g., hot water bath). Holding bags used in WNS infected areas (e.g., King County) should not be used in other unaffected areas.
- Disposable gloves, such as nitrile or latex, can be worn when handling bats and should/must be disposed of after handling each bat. Alternatively, smooth leather handling gloves can be worn and wiped down with a disinfectant wipe between bats (e.g., isopropyl; Appendix 4.2). If bare

hand is needed to handle bat, e.g., when attaching transmitter, apply hand sanitizer with alcohol after handling bat (e.g., Purell®).

- Dead bats that are collected should be placed in a sealed bag and then put into another container or sealed bag using a clean pair of gloves. Used gloves should then be disposed of in a garbage bag designated for trash.
- All handlers should wear either a Tyvek® coverall (or similar) or change clothes after netting and before entering vehicle. Clothing should be placed in bag with equipment needed to be decontaminated.

Mist Nets

- Nets should be placed in a bag with other equipment after the trapping session so they can be decontaminated off site following the National WNS Decontamination Protocol and/or the steps outlined under the high/unknown exposure risk for offsite decontamination (e.g., hot water bath).
- Mist nets used in WNS infected areas (e.g., King County) should not be used in other unaffected areas.
- Mist net poles should be wiped down on site after the trapping session with approved disinfectant solution or wipe (Appendix 4.2) before placing back into storage bag or vehicle.

Harp Traps

- The entire trap should be sprayed and wiped down with an approved disinfectant solution or wipe (Appendix 4.2) at the end of the trapping session and before placed back into storage bag or vehicle.
- At sites where bat-to-bat contact occurs regularly (e.g., maternity colonies), the catch bag should be checked frequently to reduce the amount of time bats come into contact with each other and with the catch bag. The catch bag should be decontaminated periodically during the trapping session as well using a disinfectant solution or wipe (Appendix 4.2).
- Harp traps used in WNS infected areas (e.g., King County) should not be used in other unaffected areas.

Other Trapping Equipment

- Any equipment used to capture bats (e.g., buckets, butterfly nets, funnel traps) should be decontaminated after each trapping session following the National WNS Decontamination Protocol and/or the steps outlined under the high/unknown exposure risk for offsite decontamination (e.g., chemical bath).
- Trapping equipment used in WNS infected areas (e.g., King County) should not be used in other unaffected areas.

Processing Bats

- Do not process bats in vehicle instead all processing should occur on a table to prevent contaminating the inside of the vehicle.
- Always use new sterile wing biopsy punches, swabs or other sampling equipment when collecting samples from bats.
  Any object that comes into contact with a bat should be wiped down with a disinfectant wipe (Appendix 4.2) before the next bat is processed and after the trapping session, this includes but is not limited to, ruler, caliper, weighing tube, wing punch cutting board, banding equipment, scissors, tweezers, processing table.

Appendix 4.2: Applications and products with demonstrated efficacy against Pd from the National WNS Decontamination Protocol – Version 04.12.2016 (https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf).

Table 1. Applications and products with demonstrated efficacy against Pd [3, 4, 5, 6, & 7]. Remember to consult equipment labels, registered product labels, and the appropriate SDS for regulations on safe and acceptable use.

	Tested Applications & Products [3, 4, 5, 6, & 7]	Federal Reg No.:	Laboratory Results
Preferred Applications	Equipment Dedication	N/A	Clean according to manufacturer standards and dedicated to a site
	Submersion in Hot Water[4, 6, & 7]	N/A	Laboratory effectiveness demonstrated upon submersion in water with sustained temperature ≥ 55°C *(131°F)* for 20 minutes.
Other Products	Ethanol (60% or greater)[4, 6, & 7]	CAS - 64-17-5	Laboratory effectiveness demonstrated upon exposure in solution for at least 1 minute.
	Isopropanol (60% or greater)[4, 6, & 7]	CAS - 67-63-0	
	Isopropyl Alcohol Wipes (70%)[4, 6, & 7]	CAS - 67-63-0	Laboratory effectiveness demonstrated immediately following contact and associated drying time.
	Hydrogen Peroxide Wipes (3%)[4, 6, & 7]	CAS - 7722-84-1	
	Accel®[4, 5, 6, & 7]	EPA - 74559-4	Laboratory effectiveness demonstrated when used in accordance with product label.
	Clorox® Bleach[3, 4, 5, 6, & 7]	EPA - 5813-100	
	Clorox® Wipes[4, 5, 6, & 7]	EPA - 5813-79	
	Clorox® Clean-Up Cleaner + Bleach[4, 5, 6, & 7]	EPA - 5813-21	
	Hibiclens®[4, 5, 6, & 7]	NDA - 017768	
	Lysol® IC Quaternary Disinfectant Cleaner [3, 4, 5, 6, & 7]	EPA - 47371-129	

Other effective treatments with similar water based applications or chemical formulas (e.g., a minimum of 0.3% quaternary ammonium compound) may exist but remain untested at this time. Find more information on the EPA or FDA registered product labels by accessing the individual hyperlink or searching EPA or FDA Registration Numbers at: http://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1 or http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.

Products with USEPA registration numbers mitigate persistence of living organisms on surfaces and are regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 USC 136, et seq.). FIFRA provides for federal regulation of pesticide distribution, sale, and use. Within FIFRA, pesticides are defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. FIFRA further defines pests as any insect, rodent, nematode, fungus, weed, or any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 25(c)(1). Find more information on FIFRA at: http://www.epa.gov/oecaagct/lfra.html.

Appendix 5: Additional WNS information

Washington Department of Fish and Wildlife bat reporting website
http://wdfw.wa.gov/conservation/health/wns/

Washington Department of Fish and Wildlife Living with Wildlife (Bats)
https://wdfw.wa.gov/living/bats.html

White-nose syndrome information and resources
https://www.whitenosesyndrome.org/

USGS National Wildlife Health Center
https://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/