2023 CDFW Spring White-nose Syndrome Surveillance Summary Report

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Introduction

In spring 2023, the California Department of Fish and Wildlife (CDFW) led active surveillance efforts for *Pseudogymnoascus destructans* (Pd), the fungus that causes White-nose Syndrome (WNS) in bats, at 11 sites in California (see map at end of report). This effort was part of the larger national WNS surveillance program conducted each year throughout North America by numerous state wildlife agencies, the National Park Service (NPS), and others. CDFW assisted with surveillance work in California starting in 2018 and began leading its own surveillance program in 2021. Much of the work is supported and coordinated by U.S. Geological Survey National Wildlife Health Center (NWHC), National Park Service (NPS), and US Fish and Wildlife Service (USFWS). In California, CDFW's work is supported by grants from the US Fish and Wildlife Service and by State funds. Numerous volunteers from agencies, academia, and the private sector contributed to CDFW's surveillance effort.

Methods

Our efforts were focused on collecting swab samples from known or presumed WNS-susceptible bat species, especially those in the genus *Myotis*. We monitored bat activity at capture sites in early spring so we could collect samples as early as possible in the active season. Once the number of bats at a site was deemed adequate to meet our target sample size, we initiated capture and sampling. At sites where an insufficient number of the targeted bat species were captured, we also swabbed other species, if available, with the goal of obtaining 25 swab samples at each site. If fewer than 25 bats were captured on the initial attempt, we sometimes revisited the site later to collect more samples. We used standard techniques for capturing bats in or near spring (active season) roosts, including mist-netting, funnel traps, hand-netting, and hand capture.

We collected swab samples using the methods outlined in the USGS National Wildlife Health Center's (NWHC) Pd Surveillance Guidelines. In addition to collecting swab samples, we took standard morphological measurements of the bats, identified them to species or species group, and assessed wing damage according to the Reichard Wing Damage Index. Where dark ambient light levels allowed, we used a 385-nm wavelength ultraviolet lamp with a visible light filter to examine bat wings for fluorescence suggestive of WNS infection. At many of the sites, staff from the CDFW Wildlife Genetics Research Unit (GRU) collected wing punch biopsies for species identification and archiving of samples. Species were identified using a combination of morphological measurements, inspection of recorded echolocation calls upon release, and genetic sequences.

The Pathogen and Microbiome Institute at Northern Arizona University (NAU) and the NWHC tested swab samples for the presence of Pd fungal DNA using quantitative Polymerase Chain Reaction (qPCR). We collected swabs for NAU at all 11 sites. At five of the sites, a second set of swabs from the same bats sampled for NAU was collected for submission to the NWHC. At sites where bats were double-swabbed, NWHC swabs were collected from the bat's right forearm and muzzle, while the NAU swabs were collected from the left forearm and muzzle.

Results

We conducted Pd surveillance 12 April through 1 June, 2023, at 11 sites, including two sites (DR Ponds and MLLT Barn) where a second night of captures was conducted in an attempt to obtain the target sample size of 25 bats (see Table 1). We achieved our target sample size of 25 bats at 9 of the sites. At the other two sites, we captured 17 and 16 bats, for a total of 258 bats captured and swabbed.

We mostly sampled at or near a variety of roost types, but also at one pond site, and from San Diego County in the south to Humboldt and Trinity counties in the north (Table 1 and Figure). Species and numbers sampled included:

Myotis yumanensis (MYYU) – 154
Myotis californicus (MYCA) – 1
Myotis californicus/*ciliolabrum* (CA/CI) – not distinguished – 1
Myotis volans (MYVO) – 17
Myotis spp. – not distinguished (MYSP) – 30
Parastrellus hesperus (PAHE) – 1
Eptesicus fuscus (EPFU) – 28
Antrozous pallidus (ANPA) – 3
Lasiurus frantzii (LAFR)(aka *L. blossevillii*) – 1
Tadarida brasiliensis (TABR) – 22

We did not observe UV fluorescence suggestive of WNS infection on any bat. We observed little or no wing damage in most of the bats, with 245 bats recorded as having a wing damage index (WDI) score of 0 and 13 bats with a WDI of 1. Although we did not quantify all observations, we observed bats at several sites with subtle stippling on portions of the wing membrane. Anecdotally, we believe these observations were more common in 2023 than in previous years.

Of the 258 bats sampled, all but 13 had samples that did not amplify in the PCR tests for Pd. All the NWHC samples, collected from 96 bats at five sites, were Negative, as were 245 of the 258 NAU samples.

Two NAU samples were Positive, and eleven samples were classified as 'Ambiguous Negative[1]'. These non-Negative results occurred at seven sites in seven counties from Trinity County in the north to central San Diego County in the south (see Summary Results table and map). We sampled most of these same sites in 2022 and had only one non-Negative result that year, an Ambiguous Negative sample from the DR Ponds site in Sutter County. The C Mine site in Inyo County was Negative in 2022 but had one Ambiguous Negative result in 2023. This adds to a 2021 low-level Pd detection from another site in Inyo County sampled by the NPS Mojave Network team.

[1] The NAU lab considers a sample to be Ambiguous Negative when "Ct [cycle threshold] results [are] greater than 40 for one or more replicate. Either one replicate is greater than 40 while the other is negative, or both replicates are greater than 40. Result is ambiguous." NAU suggests that ambiguous "may mean that this result could equally indicate either a low-level Positive at the limits of our detection range or a true Negative with false amplification, and are therefore undetermined results that are open to interpretation based on the information given. These results may require other biological information, observations, or additional analyses to confirm or interpret." The NWHC uses the term "Inconclusive" to describe high Ct value qPCR results.

Table 1. Dates, site names, and general results of CDFW Pd surveillance in 2023. Sites are listed roughly in order from south to north.

Sample Date(s)	Site	County	Type	Species	Bats Sampled	NAU Results	NWHC Results	Sample IDs with non-Negative results
4/18/2023	E Bridge	San Diego	Bridge Roost	MYYU, TABR	25	Negative	n/a	
4/19/2023	B Culvert	San Diego	Culvert Roost	MYYU	25	Negative	n/a	
4/17/2023	SV Culvert	San Diego	Culvert Roost	MYYU	25	Negative, Ambiguous Negative	n/a	MYYU 07 Ambiguous Negative
5/25/2023	RP House	Placer	Bat House, Building Roost	MYYU	25	Negative, Ambiguous Negative	Negative	MYYU 01 Ambiguous Negative
5/24/2023, 6/1/2023	DR Ponds	Sutter	Landscape	MYCA, LAFR, EPFU, PAHE, TABR, ANPA	16	Negative, Ambiguous Negative	Negative	MYCA 317 Ambiguous Negative
4/25/2023, 5/10/2023	MLLT Barn	Amador	Building Roost/Landscape	EPFU, TABR, MYSP	25	Negative	n/a	
5/1/2023	IC Cmpgrd	Alpine	Bat Condo	MYYU/MYLU	25	Negative, Ambiguous Negative	Negative	MYSP 210 Ambiguous Negative
5/2/2023	C Mine	Inyo	Mine Roost	MYVO	17	Negative, Ambiguous Negative	Negative	MYVO 228, MYVO 232 Ambiguous Negative
4/12/2023	BL House	Humboldt	Building Roost	MYYU/MYLU	25	Positive, Ambiguous Negative, Negative	n/a	MYYU 89, MYYU 98 Positive; YULU 100, YULU 101 Ambiguous Negative
5/21/2023	D Bridge	Trinity	Bridge Roost	MYSP	25	Negative	Negative	
5/22/2023	G Cabin	Trinity	Building Roost	EPFU, MYSP	25	Negative, Ambiguous Negative	n/a	EPFU 265, MYYU 287, EPFU 289 Ambiguous Negative

Discussion

Overall, the lack of sign of previous WNS infection and the great preponderance of Negative qPCR results at the sites we sampled indicate Pd prevalence is low and suggest that WNS may not be endemic in these bat populations. However, compared to our results in 2022, in which only one sample at one site had an Ambiguous Negative result, we collected many more Ambiguous Negative samples and two true Positives in 2023. The active surveillance conducted by CDFW and by our NPS partners in California has revealed an inconsistent but general progression of increasing numbers of samples and sites with low-level type detections of Pd since the first Inconclusive detection of Pd in Plumas County in 2018 (see Table 2).

Table 2. Summary of first Pd detections in California by county and year.

Year	County	Status[2]	Surveillance Team	Notes
2018	Plumas	Pd Presumed	NPS-KLMN	
2021	Shasta	Pd Presumed	NPS-KLMN	
2021	Inyo	Pd Presumed	NPS-MOJN	
2021	San Bernardino	Pd Presumed	NPS-MOJN	
2022	Sutter	Pd Presumed	CDFW	Not reported to WNS National Map
2023	Siskiyou	Pd Presumed	NPS-KLMN	
2023	Sutter	Pd Presumed	CDFW	Second year of Pd Presumed at capture site
2023	Trinity	Pd Presumed	CDFW	
2023	Alpine	Pd Presumed	CDFW	
2023	San Diego	Pd Presumed	CDFW	
2023	Inyo	Pd Presumed	CDFW	Additional Inyo Co. site; mine west of Big Pine
2023	Humboldt	Pd Positive	CDFW	

Several factors may have contributed to the inconsistent trend in increasing Pd detections in California since 2018. Although considerable resources have been directed to Pd surveillance in California since 2016 by CDFW and partners, inconsistencies in effort have occurred due to logistical challenges, including the Covid-19 pandemic, vagaries in spring weather patterns, and the variable start of the active season for bats in different parts of the state. It is also possible that WNS/Pd may not always increase in prevalence in an area, even after being detected by our surveillance. This may be especially likely in areas with relatively mild winter conditions. It

[2] In this table, we consider Status to be the county designation used on the National WNS Map. Note that these designations are from the first (or in some cases, second) "Inconclusive" or "Ambiguous Negative" result in the listed counties. In most counties, additional low-level detections of Pd have been made at the same and/or nearby sites in subsequent years. NPS-KLMN is the National Park Service Klamath Network. NPS-MOJN is the NPS Mojave Network.

may also be that that endemism takes longer to develop in western bat populations, where bats of susceptible species hibernate singly or in small groups, compared to eastern North American populations, where large hibernating colonies are the norm.

Given the pattern of progression of Pd detections and WNS in other western states, CDFW has concluded that the "Inconclusive" and "Ambiguous Negative" PCR results should be designated as "Pd Presumed" on the National WNS Map. Our 2023 results, combined with results from the NPS surveillance efforts in northern and southern California, suggest that Pd is likely to be present at low levels in bat populations spanning the state from north to south.

This conclusion is bolstered by the true Positive result obtained at the BL House site in Humboldt County, northwestern California. The NAU lab considers a sample to be a true Positive for Pd when "Ct results [are] less than 40 with two amplifying replicates. One replicate must be less than 40, the second replicate may either be greater or less than 40 but cannot be Negative." With swabs from two *Myotis yumanensis* bats at the site testing Positive for Pd, and two others yielding an Ambiguous Negative result, there is strong support for Pd being present in that bat population.

The Humboldt County site is a low elevation maternity roost in a cluster of several abandoned buildings within a coastal redwood forest area. Although all bats sampled at the site in 2023 were genetically determined to be *Myotis yumanensis*, previous genetic tests indicated that there are also *M. lucifugus* at the roost. The site is less than 3 km from the Pacific Ocean and experiences abundant winter rainfall, is typically rain-free but moist the rest of the year and has mild temperatures year-round – air temperature rarely falls below 0 C and rarely exceeds 20 C. We do not know where and under what conditions these bats spend late fall, winter, and early spring.

2024 General Surveillance and Management Recommendations

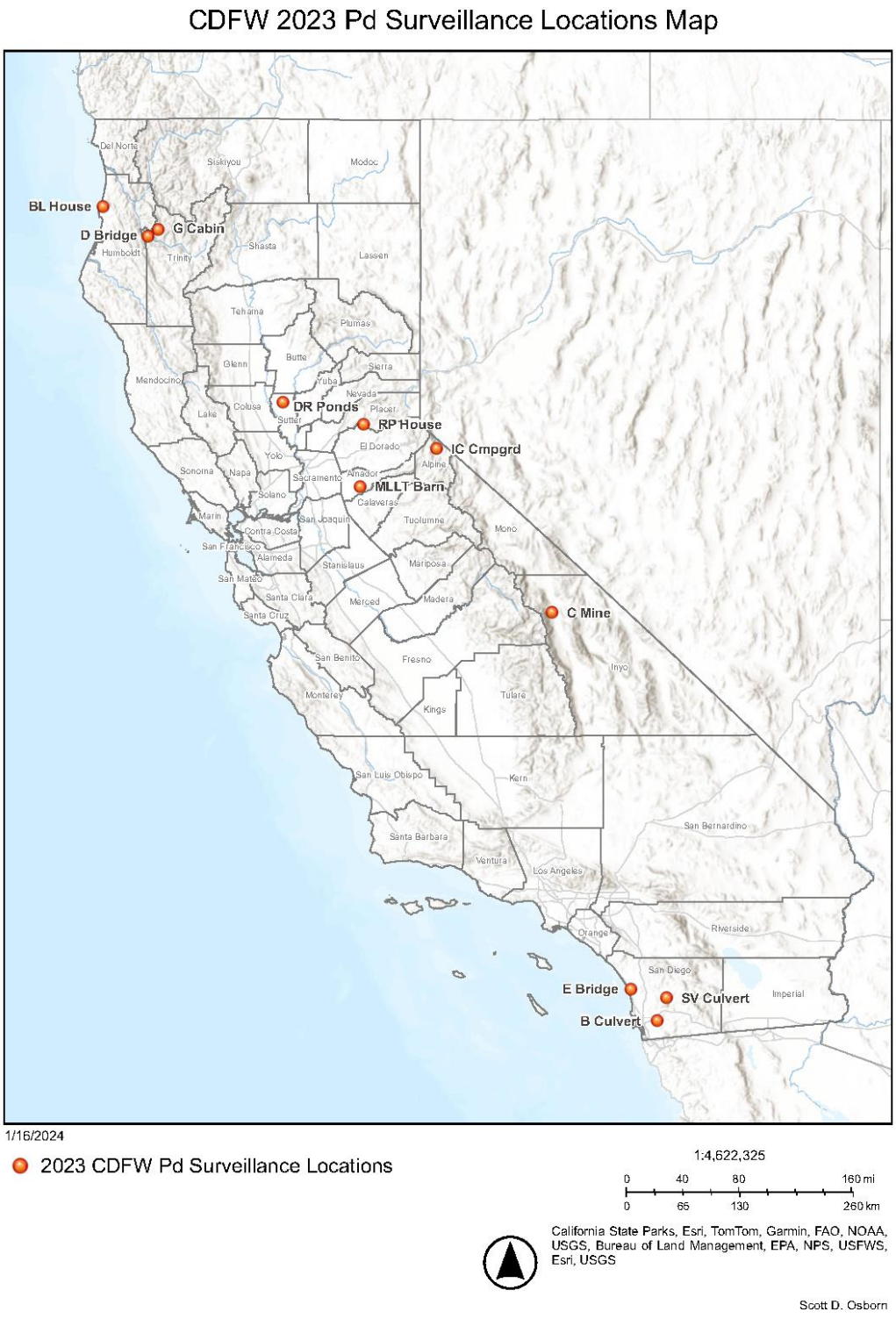
1. Continue to conduct Pd surveillance at the 11 existing spring surveillance sites using the NAU lab for diagnostics. Where possible, identify and conduct surveillance at additional sites near existing Ambiguous Negative sites. At existing sites with Ambiguous Negative results that may support additional captures above 25 bats, consider capturing an additional set of 25 bats and taking swab samples from the additional bats for the NWHC diagnostics lab.
2. Record observations and collect photographs of wing membrane stippling, if observed.
3. Continue all existing passive surveillance activities (e.g., screen bats submitted for rabies testing, monitor CDFW's "Report a Sick or Dead Bat" and "Report a Bat Colony" website reports, monitor reports from wildlife rehabilitators).
4. Update the National WNS Map to indicate "Pd Positive" for Humboldt County and "Pd Presumed" for the counties in which new low-level detections of Pd were made in 2023.

5. Conduct media and/or social media outreach in collaboration with CDFW's Office of Communication, Education and Outreach about the 2023 results to reinforce public knowledge of WNS and strengthen the passive surveillance network.

Recommendations for the Humboldt County Pd Positive Site

1. Monitor seasonal activity of *Myotis* at the roost site, which contains several abandoned buildings used by the bats, to identify the start and end of the spring-summer activity season. This would allow us to conduct Pd/WNS surveillance as early as possible in the active season, which increases the chance of detecting bats that have been exposed to Pd and may possibly still show signs of infection from the hibernation season. Identifying the date of transition from summer active season to winter season would also inform the scheduling of radio-telemetry studies of bats moving to their winter roosts (see below). We would accomplish activity monitoring by installing an ultrasonic bat detector at the site in March 2024. We would access it weekly starting around March 15 to assess bat activity at the site. We would screen the recorded calls for upticks in *Myotis* activity, which would then prompt an inspection of the roosts for presence of bats. The detector would not be used during most of the summer, but around the end of August or in September it could be reactivated to identify a drop off in activity, signaling the transition of the bats to their winter roosts.
2. Continue annual WNS/Pd surveillance. We will continue doing standard qPCR and UV light surveillance for Pd and WNS at this site by capturing up to 50 bats from the site in spring 2024, conducting visual screening for signs of recent Pd infection and UV screening for fluorescence of Pd hyphae in wing membranes, and collecting swab samples from the bats for qPCR testing (25 bats each for the two labs that are participating in national WNS surveillance).
3. Initiate population monitoring at the site. The goal of population monitoring at this site is to look for a trend in maternity colony size over time, especially if Pd/WNS continues to be detected there. We will conduct more precise visual counts of individuals in the multiple roost buildings at the site prior to pupping. Given roost-switching is common, we would conduct multiple visual counts over the course of two or three weeks in May. We will PIT tag 10% to 50% of the adult bats in the roost houses in 2024. We will read the PIT tags in subsequent years (and tag bats not previously PIT-tagged) for longer term monitoring of population size and survival using the Cormack-Jolly-Seber model and Maximum Likelihood Estimation methods.
4. Conduct winter radio telemetry studies of the colony bats. Little is known about where *Myotis* bats overwinter, especially on the coast, other than that they typically depart their summer roosts. Because WNS affects bats during hibernation, and because treatments to manage WNS may be most effective at that time, it is essential to learn more about where and under what circumstances these bats hibernate. In particular, the duration and depth of hibernation are critically important to understanding how WNS may affect these bats. To learn more about the winter ecology of these bats, we

will conduct a radio telemetry study of the bats as they transition from their summer roosts in the fall. This would likely be done in partnership with NPS, USFS, and/or USGS bat researchers with experience tagging and tracking bats. Based on 2023 observations, capture efforts would likely begin in October 2024 and continue for several weeks, with the objective of capturing and tracking 6-10 bats at a time. Any discovered winter roosts would be characterized to enhance understanding of suitable roost characteristics and to assist with searches for additional roosts. At a minimum, location, elevation, slope, aspect, canopy cover, and type of structure would be described. Data loggers to record temperature and relative humidity would be installed to determine the conditions in which the bats spend their winters.



Map depicting 2023 CDFW-led WNS/Pd active surveillance sites (red dots) in California. These eleven sites were sampled in the spring field season. Please note that our National Park Service partners sampled at several additional sites in northeastern and southeastern California.