Is the Causative Agent of White-nose Syndrome, *Pseudogymnoascus destructans*, Present in Carlsbad Caverns National Park (CAVE) Bats?

Final Report to the Western National Park Association

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Debbie C. Buecher, Buecher Biological Consulting
Diana E. Northup, University of New Mexico

Executive Summary

The most critical threat to bats today is the rapid spread of white-nose syndrome (WNS) and the causative fungus, *Pseudogymnoascus destructans*, across the United States. Because the fungus has been detected in neighboring states, we assessed the WNS threat for CAVE bats by: 1) surveying bat winter hibernacula within the park, 2) netting and swabbing bats within Carlsbad Cavern and at surface water sites during May 2019, and 3) testing for the presence of the WNS fungus on our swabs. CAVE NPS personnel surveyed for winter bat hibernacula in known caves on the Park. They found only low numbers of hibernating bats in Park caves. The exceptions were Deep Cave, which had 15 hibernating bats but conditions too dry to support *P. destructans*, and Ogle Cave, where the bats were roosting too high up on the ceiling for accurate identification. Buecher conducted mist netting for bats over five consecutive nights between 27 May and 31 May 2019. Netting was conducted two nights along Left Hand Tunnel, which is off the Lunch Room of Carlsbad Cavern, and three nights at surface waters. We focused two nights along Left Hand Tunnel to capture cave myotis and fringed myotis day-roosting above Lake of the Clouds or in the Right Fork of Left Hand Tunnel. We also set nets on three nights at surface water sources in or near the Park. Little is known regarding where Park bat species hibernate, so swabbing bats shortly after emergence from wherever they are hibernating allows us to determine if *P. destructans* has been introduced onto Park bats. We captured a total of 51 bats from eight species (Table 1) including: Brazilian free-tailed bats (8), big brown bat (1), silver-haired bats (2), western canyon bats (3), pallid bats (10), cave myotis (22), fringed myotis (2), and hoary bats (3). Northup swabbed 32 bats to test for the presence of *P. destructans* DNA by Dr. Jeff Foster's lab at Northern Arizona University. She also swabbed 13 bats for culturing studies targeting the WNS fungus in Northup's lab at University of New Mexico. All bat swab samples were negative for the presence of *P. destructans.* A public presentation was given by Buecher and Northup during their May work at CAVE and the stars of the show were Buecher's rehabilitated bats. The visitors loved hearing about the bats and seeing live bats. **All DNA extracted from the bat swab samples were negative for the presence of *P. destructans* and all CAVE cultures were also negative for *P. destructans.*** Because WNS is moving ever westward, it is critical that CAVE scientists continue to monitor their bats for the appearance of any tissue damage (necrosis), lesions, scarring or skin mottling currently attributed to *P. destructans* (Reichard and Kunz 2009, Cryan et al. 2010).

Research Objectives:

To answer the question: Is *Pseudogymnoascus destructans* present on CAVE bats emerging from hibernation in the park, or returning to the park from nearby hibernacula?, we addressed these objectives with the following steps: (1) Park personnel surveyed likely hibernation sites

during winter to determine best sampling sites. (2) Mist netting bats, especially *Myotis* bat species, to swab them for the presence of any fungal spores. (3) Extracting DNA from bat swabs and from target cultures. (4) Testing extracted DNA for the presence of *P. destructans.* (5) Sharing results with CAVE resource managers.

Objective 1 – Survey likely hibernacula sites during winter.
Based on information provided by Dr. Ken Geluso from his previous extensive bat research in CAVE, park personnel checked caves in which he found hibernating bats, and other caves during winter for the presence of hibernating bats, using special caution to limit disturbance to the bats. The Cave Resources staff found 15 hibernating bats in Deep Cave, but environmental conditions were too dry for the WNS fungus. Bats were detected in the high ceilings of Ogle Cave during the winter. Other caves showed limited evidence of hibernating bats.

Objective 2 – Mist net bats, especially *Myotis* species, to swab for fungal spores.
Prior to mist netting we reviewed Geluso and Geluso (2004) for previously successful netting sites. Netting was conducted two nights along Left Hand Tunnel, located off Carlsbad Cavern's Lunch Room (Figure 1). The goal was to capture cave myotis and fringed myotis day-roosting either above Lake of the Clouds or in the Right Fork of Left Hand Tunnel. We also mist netted three nights over surface waters (Figure 2). We captured a total of 51 bats from eight species (Table 1) including: Brazilian free-tailed bats (8), big brown bat (1), silver-haired bats (2), western canyon bats (3), pallid bats (10), cave myotis (22), fringed myotis (2), and hoary bats (3).

Table 1. Bat species captured during mist netting at CAVE - 27 May to 31 May 2019.

Common Name	Scientific Name	Males	Females	Totals
Big brown bat	*Eptesicus fuscus*	0	1	1
Cave myotis	*Myotis velifer*	12	10	22
Fringed myotis	*Myotis thysanodes*	0	2	2
Hoary bat	*Lasiurus cinereus*	2	1	3
Pallid bat	*Antrozous pallidus*	10	0	10
Silver-haired bat	*Lasionycteris noctivagans*	2	0	2
Western canyon bat	*Parastrellus hesperus*	3	0	3
*Brazilian free-tailed bat	*Tadarida brasiliensis*	6	2	8
	Totals	**35**	**16**	**51**

* Brazilian free-tailed bats are in the Family Molossidae. All other bats captured are in the Family Vespertilionidae.

Netting along Left Hand Tunnel at Iron Pool and after the turn to Lake of the Clouds, resulted in the capture of 17 bats including fringed myotis (0 M/1 F) and cave myotis (5 M/1 F). Although it was early in the reproductive season, six were pregnant females, showing minor belly swelling. We deployed an Anabat ultrasonic bat detector both nights we netted in Left Hand Tunnel. Most of the calls were from cave myotis with only a few fringed myotis. Geluso and Best (2017) and Baker (1962) had reported a fringed myotis maternity colony on the Balcony above Lake of

the Clouds, so we were surprised to capture so few female fringed myotis. Through the years these researchers have also documented what appears to be a bachelor colony of cave myotis also using the Balcony. It may be that the reproductive fringed myotis do not move into the maternity roost until just before they give birth. This has been observed at Kartchner Caverns State Park (NSS 1996) where the cave myotis only move into the maternity dome just prior to birth and to rear the pups. Five Park volunteers helped us with note-taking and other tasks at these sites.

Figure 1 Left: male cave myotis captured at Iron Pools. Right: female fringed myotis captured at Iron Pool along Left Hand Tunnel. She had obviously reared a pup before and was showing evidence of early pregnancy.

Figure 2: Left: View of a long shallow pool along the Black River where we deployed mist nets. Right: View of the pool at Indian Shelter along Walnut Canyon.

We captured 17 bats at Black River (Figure 2), from six species including: Brazilian free-tailed bats (8), hoary bats (2), a pallid bat (1), a silver-haired bat (1), a big brown bat (1), and cave myotis (4) (Figure 3). Besides the bats that we captured, there was tremendous foraging by bats during the evening, no doubt on all the insects drawn to the water. An Anabat SD2 ultrasonic bat detector recorded over 800 call files during the period of netting (Figure 4).

Because a Brazilian free-tailed bat was found with *P. destructans* spores in Texas, we swabbed three of that species for *P. destructans* DNA testing. We also swabbed the big brown bat, the silver-haired bat and a cave myotis for *P. destructans*. Six Park volunteers helped us with note-taking and other tasks. At Indian Shelter in Walnut Canyon, we captured 15 bats from four species including: western canyon bats (3), pallid bats (9), a hoary bat (1), and cave myotis (2). We only caught male bats this night and the pallid bats all had moderately heavy ectoparasitic loads. Four Park volunteers helped us with note-taking and other tasks. At Rattlesnake Springs, we captured two bats, a cave myotis and a silver-haired bat (Figure 3). The Anabat SD2 bat detector recorded approximately 700 call files over 4.5 hours including calls from: Brazilian free-tailed bats, western canyon bats, big brown bats, silver-haired bats, and cave myotis. Two Park volunteers helped us with note-taking and other tasks.

Figure 3: Bats caught at surface sites. Left: a male silver-haired bat. Middle: Weighing a male hoary bat using a spring Pesola. Right: A Brazilian free-tailed bat, the species that roosts in Bat Cave in large numbers.

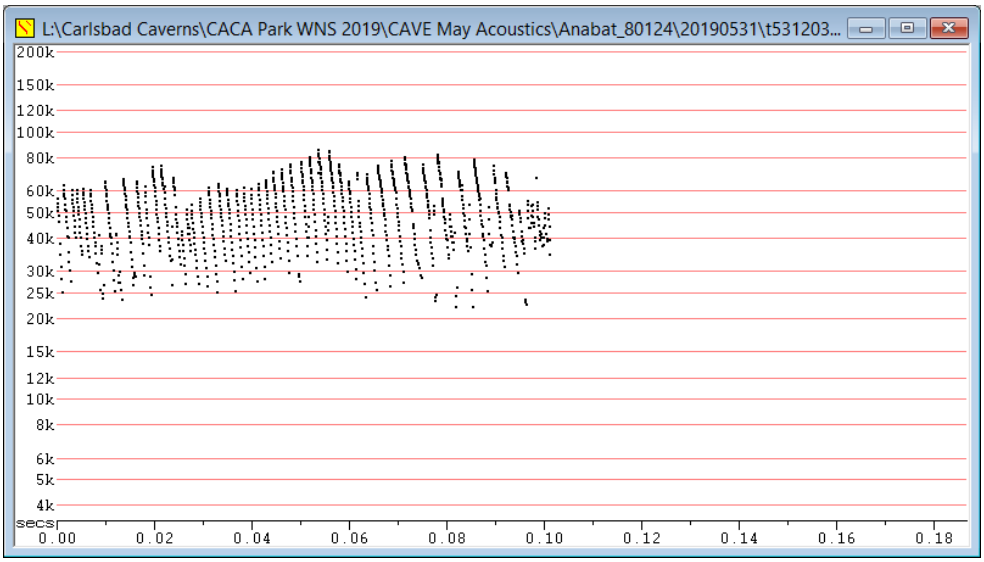

Figure 4. Ultrasonic bat call recorded along Left Hand Tunnel that was acoustically identified as from a fringed myotis.

In total Northup swabbed 32 bats to test for the presence of *P. destructans* DNA by Dr. Jeff Foster's lab at Northern Arizona University. All swabs were immediately stored in sucrose lysis buffer to preserve the DNA and kept cool. She also swabbed 13 bats for culturing studies targeting the WNS fungus in Northup's lab at the University of New Mexico (UNM).

Objective 3: Extract DNA from bat swabs and cultures targeting *P. destructans*.
All DNA bat swabs were stored on dry ice and transported to the Northup Lab, where J. Hathaway extracted the DNA using current WNS protocols. The extracted DNA was immediately sent to the Pathogen and Microbiome Institute lab of Dr. Jeff Foster at Northern Arizona University for testing. Dr. Foster's lab has tested more than 72,000 samples for the presence of *P. destructans.* Liquid culture medium with dextrose-peptone-yeast extract broth, that targets *P. destructans* (Vanderwolf et al. 2016), was inoculated with swabs immediately after bats were swabbed. Inoculated cultures were stored in a cooler in the field and then at 4^{o} C in a walk-in refrigerator at UNM, where they were incubated for six-10 months, until they were DNA-extracted with the Qiagen culture isolation kit, and tested with general fungal and *Pseudogymnoascus* primers. Those that tested positive on these primers were tested with IGS primers specific for *P. destructans* (Muller et al. 2013).

All CAVE cultures were negative for *P. destructans*.

Objective 4: Test extracted DNA for the presence of *P. destructans.*
The Foster lab tested all DNA extracted from the bat swabs using the primers from the Muller et al. 2013 Mycologia paper, which are also the primers and techniques used by the USGS National Wildlife Health Center.

All DNA from bat swab samples were negative for the presence of *P. destructans*.

Figure 5. One of the cave myotis in LHT had a number of light colored wing spots. None of them appeared to be lesions but rather variation in the wings' melanin.

Objective 5: Share results with CAVE personnel.
A trip report entitled: Is the Causative Agent of White-nose Syndrome, *Pseudogymnoascus destructans*, Present in Carlsbad Caverns National Park Bats?, was prepared and submitted to the Park's Cave Resources Office and covered our work of 27-31 May 2019, and follow-up lab work and testing. Buecher and Northup also gave a public talk to visitors and Park staff entitled:

> *Natural History of Bats and the Threat of White-Nose Syndrome*
> *Learn fun and interesting information about the amazing bats of Carlsbad Cavern and other southwestern bats. Debbie Buecher, a bat biologist who rehabilitates bats, will introduce you to Maggie, a western mastiff, and Archie, a big brown bat. Diana Northup, a cave microbiologist, will introduce you to the world of who lives on a bat and how they might help bats survive the threat of white-nose syndrome.*

Management Implications:
Our results suggest that the previous finding of low-level detection of *P. destructans* in the guano at Lake of the Clouds in 2018 samples is not yet being documented on CAVE bats. Because of these findings, we recommend the following be considered by Park management:

1. Continue to educate park visitors about the nature and value of bats and how best to protect them from the potential introduction of this deadly fungus into CAVE caves.
2. Continue and expand efforts to determine which caves are used as hibernacula by bats in CAVE.
3. Continue and expand testing of bats and guano for the presence of *P. destructans*.

Plan for Interpretation-related Product of the Research
We shared our research in the following ways:

1. Inclusion of interpretive rangers in our fieldwork on each of the netting nights: This opportunity allowed rangers to ask us questions, see the bats up close, and provided opportunities for rangers and researchers to bond in a way that encouraged follow-up questions.
2. Our public talk was a big hit with park visitors who loved seeing live bats.
3. The research provided opportunities for discussions across Resources and Interpretation and for the researchers to work with both departments.

Acknowledgements
We wish to thank the WNPA for their support of this project, which has enhanced our knowledge of the bats at Carlsbad Caverns National Park and the danger posed by white-nose syndrome. This has been a great opportunity to share our research and knowledge of bats and microbes with the CAVE interpretive rangers who assisted us, as well as visitors during our public talk. We gratefully acknowledge the wonderful support that the staff of CAVE has provided for this research and the sharing of our results, especially Erin Lynch, Ellen Trautner, Pam Cox, Michael Larson, and Rod Horrocks.

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