New research proposal to Western National Parks Association (WNPA)

The information supplied should be limited to the space provided and submitted on these forms.
A proposal received in any other format will be returned. Additional attachments are not permitted.

Title of project: Is the Causative Agent of White-nose Syndrome, Pseudogymnoascus destructans, Present in Carlsbad Caverns National Park Bats?	Park(s) in which research is to be conducted: Carlsbad Caverns National Park
Name, address, and phone number of principal investigator, (PI)(s): Debbie Buecher, M.S. Buecher Biological Consulting 7050 E. Katchina Court Tucson, AZ 85715 520-722-1287	Payee information - individual name and address or Institution's name and address required: Debbie Buecher Buecher Biological Consulting 7050 E. Katchina Court Tucson, AZ 85715
Is this a multiyear project? No Total amount requested: This year $ 7500 If multiyear project, estimated amount: 2nd year $ _____ 3rd year $ _____	Desired start date: 2/1/19 Note: Not prior to October 1st
Project duration: 1 year Project final completion date: 1/31/20 (see research guidelines)	
Name(s) of research participant(s) who will acquire advanced degree(s) as a result of working on this project, if any: N/A	Product(s) of research (articles, theses, maps, checklists, etc.) in addition to final report to WNPA (see research guidelines): Presentation(s)

Abstract to be provided by PI(s). Do not exceed the half-page space provided below.

Early detection of Pseudogymnoascus destructans (Pd) is an important management tool in reducing spread of the fungus causing white-nose syndrome (WNS). Pd infects bats as they hibernate, creating lesions on their wings and a fungal growth on their nose, ultimately leading to death with more than 99% mortality rate in some bat species (Frank et al., 2014). With either Pd or WNS in two neighboring states, New Mexico is now on the leading edge of WNS advances. New evidence of a very low level presence of Pd in some NM cave deposits adds urgency to this situation. We propose to test (swab) bats in Carlsbad Caverns National Park (CAVE) to determine whether CAVE bats are now infected. We will test up to 100 bats across the park, targeting the most vulnerable Myotis bat species. CAVE personnel will verify hibernacula used by Myotis spp. this coming winter, and identify caves that contain hibernating bat populations. Using these data, and our prior bat netting studies, microclimate data, and guano testing from 2012-2018, we will capture bats in the most vulnerable cave sites at the end of hibernation in spring 2019. We will also net bats at surface water sources within the park. Swab samples will be tested with real-time PCR, a method shown to accurately detect Pd from environmental samples (Muller et al. 2013). Early detection of Pd in NM caves will be a valuable tool for management. Timely closure of caves to visitors where Pd is detected will help limit human transmission.

(1) JUSTIFICATION (to be provided by submitting park): This section should specify the following: 1) Are NPS-appropriated funds available for the project (Yes/No)? 2) Where does this project rank in the submitting park's research priorities for all funding sources? 3) Was this proposal solicited by the park? If not, why is this project important to the park? 4) How will this research enrich visitors' understanding of the park? 5) What are the implications for resource management?

1) No NPS funds are designated for this project, but Carlsbad Caverns National Park (CAVE) will provide in-kind support in the form of fieldwork assistance by two permanent staff and two interns, housing for researchers, and WNS decontamination supplies.

2) White Nose Syndrome (WNS) is a significant management concern of the park and is the foremost concern for natural resources in biological matters. This level of concern is carried throughout the National Park Service. The IMR Regional Director recently elevated the priority of WNS, stating "WNS is one of the top wildlife diseases facing our Region and nationally". This project is an extension of research that has been conducted at Carlsbad Caverns National Park and many other locations within the National Park Service and other federal agencies.

3) This project was solicited by the park.

4) WNS has affected many aspects of the visitor experience at CAVE: from biosecurity walkover mats at the Visitor Center to decontamination procedures for backcountry cave trips to discussions at the Bat Flight Program. Educating visitors about WNS will help them better understand the way the park is managed. Providing visitors with up to date information about WNS surveillance will also highlight its growing threat and the importance of complying with policies to prevent its spread. This project will also provide interpretive staff with opportunities to learn about bats directly from a bat biologist, thus enhancing the quality of their Bat Flight Programs.

5) This project is critical for management of cave resources in the park and will have a wider effect on cave management throughout New Mexico. Results will inform decisions related to cave closures, decontamination requirements, and other actions to prevent the spread of WNS. CAVE is at a critical juncture, with a low level detection of P. destructans in a single cave in 2018. Further testing is needed to guide best management to protect species of bats within the park that have potential to be severely impacted by WNS.

(2) CONCISE STATEMENT OF RESEARCH OBJECTIVES, DESIGN, AND METHODOLOGY. This section should include the facilities and sites to be used. Note: Limit this section to the two pages provided.

A Share with Wildlife (NM Department of Game and Fish) funded project in the spring of 2018, detected very low-level presence of the causative agent of white-nose syndrome, the fungus Pseudogymnoascus destructans in Carlsbad Cavern in CAVE. The detected fungus was found in the Myotis maternity roost guano at Lake of the Clouds, in a remote area of Carlsbad Cavern. None of the other park caves tested showed the presence of P. destructans. The positive results were detected by Jeff Foster's lab at Northern Arizona University, the lab that has detected the positive results in Texas. Duplicate samples were also tested by the USGS National Wildlife Health Center, but they failed to detect the presence of the fungus in the samples. Thus, it is critical that we verify these results in the coming post-hibernation season to better focus management strategies for minimizing the impact of the fungus on CAVE bat populations. Debbie Buecher, a bat biologist who has worked with CAVE bats for many years and who has participated in and led the WNS research conducted by herself and Dr. Diana Northup, will conduct bat netting to obtain target bats for swabbing.

Research Questions

Is Pseudogymnoascus destructans present on CAVE bats emerging from hibernation in the park, or returning to the park from nearby hibernacula?

Objectives

1. Park personnel will survey likely hibernacula sites this winter to determine best sampling sites for spring 2019.
2. Capture bats, especially Myotis bat species, and swab them to obtain any fungal spores present on the bats.
3. Extract DNA from bat swabs.
4. Test extracted DNA for the presence of P. destructans.
5. Share results with CAVE personnel and other agencies managing caves in the area.

Methods

Objective 1: Survey potential hibernacula sites in the winter of 2018-19.

Based on information provided by Dr. Ken Geluso from his previous extensive bat research in CAVE, park personnel will check these and other caves this winter for the presence of hibernating bats, using all caution to limit disturbance to the bats.

Objective 2: Capture bats and swab them.

Debbie Buecher, a bat biologist with decades of experience in netting bats in New Mexico and Arizona, will pluck bats from caves walls or use a hand-held net to capture bats that are higher on cave walls. Other bats will be netted with nets strung across known water sites that bats use within CAVE, based on her previous experience netting at CAVE. All equipment used will be decontaminated between sites, and gloves will be changed between each bat capture to ensure no human-caused transmission of this deadly fungus occurs. Buecher will hold the bats while Northup, an experience cave/bat microbiologist will swab the bats, using a different swab on each half of the

(2) CONCISE STATEMENT OF RESEARCH OBJECTIVES, DESIGN, AND METHODOLOGY (Cont'd):

bats to provide two swabs for testing. Swabs will be stored in RNALater to preserve the DNA and stored at -80oC until DNA extraction.

Objective 3: Extract DNA from bat swabs.

DNA from the swabs of bats will be extracted using the Qiagen DNeasy PowerLyzer PowerSoil kit, 128555-100, using a biological safety cabinet to prevent contamination of the DNA.

Objective 4: Test swabs for the presence of P. destructans.

Dr. Jeff Foster's lab at Northern Arizona University and the USGS National Wildlife Health Center will test extracted DNA for the presence of P. destructans. Testing will be done using real-time PCR following the methods of Muller et al. 2013.

Objective 5: Share results.

Results (positive and negative) will be shared with CAVE personnel immediately and they will make the decision about what other agencies with whom to share results.

Project Timeline

February-March 2019: CAVE personnel identify hibernacula throughout the park. Consult with CAVE personnel to plan sites for bat captures/netting.
March-May 2019: Bat swabbing conducted; time to be determined based on winter conditions and results of hibernacula surveys. Visitor Interpretive Program and Ranger Question Time given by Buecher during research visit.
June-July 2019: DNA extraction and real-time PCR testing conducted.
August-October 2019: Discussions of results with other agencies and CAVE Interpretive staff to incorporate results into programs and management plans.
November 2019-January 2020: Write report and implement management changes deemed necessary.

References

Frank CL, Michalski A, McDonough AA, Rahimian M, Rudd RJ, et al. (2014). The Resistance of a North American Bat Species (Eptesicus fuscus) to White-Nose Syndrome (WNS). PLoS ONE. 9(12): e113958. doi:10.1371/journal.pone.0113958.

Muller, L.K., Lorch, J.M., Lindner, D.L., O'Connor, M., Gargas, A. and Blehert, D.S., 2013. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of Geomyces destructans. Mycologia, 105(2), pp.253-259.

(3) CONCISE STATEMENT OF HOW YOUR RESEARCH CAN ENHANCE THE INTERPRETIVE MISSION OF THE PARK. Also include one paragraph describing the plan for an interpretation-related product of the research. Use this page only.

White-nose syndrome is a newly emerging disease in North America that is rapidly spreading westward and having a devastating effect on North American bats. Although there is limited evidence that humans spread this disease, it is certainly a possibility. Ascertaining whether the causative fungus, P. destructans, is now present in our caves is critical to how we educate park visitors about WNS and their potential role in spreading the fungus that causes the disease. Research by Diana Northup and Jenny Hathaway at the University of New Mexico, in conjunction with Jeff Foster's lab at Northern Arizona University, suggested that very low levels of the causative fungus are present in a remote area of Carlsbad Cavern. Extending the previous research to the testing of bats as they emerge from hibernation in the spring of 2019 in CAVE will allow us to provide up-to-date interpretive programs on WNS and its impact on our bats as we get the results of the testing. Knowing that the threat is indeed on our doorstep at CAVE can increase the impact the interpretive programs will have on park visitors. In turn, these interpretive programs play an important role in encouraging visitor compliance with park policies to prevent the spread of WNS.

An important goal of this project is to increase visitor understanding of bats and WNS. Debbie Buecher will give programs to park visitors during her research at CAVE. During the programs, Buecher will display two of her captive, rehabilitated bats that cannot be released to the wild. Ms. Buecher, with her colleague Dr. Diana Northup, have given numerous successful programs about bats and WNS to park visitors in previous years. The presence of live bats helps engage park visitors and increases both their appreciation for bats and their support for efforts that will help protect bats from the spread of this deadly disease. Buecher will also speak with CAVE's interpretive staff, giving them an opportunity to learn first-hand about bats. This will enhance the quality of the park's Bat Flight Programs.

Northup's husband and professional photographer, Dr. Kenneth Ingham, will take photos of the bat swabbing during the proposed 2019 research. These new images, along with the results of the research will be used to update interpretive displays and enhance interpretive programs.

The PI, Buecher, has a long history of working with the rangers and interpretive staff of CAVE and her bat talks, with her colleague, Northup, have engaged many park visitors and increased their support of the park's bats.

(4) QUALIFICATIONS OF THE PI(S) CONDUCTING THE RESEARCH. Use this page only. List only those qualifications directly related to this grant request. Include a list of other WNPA-funded research conducted by this PI.

Ms. Debbie Buecher, M.S. has been investigating caves since 1976 and specifically studying bats since 1986. She owns Buecher Biological Consulting, a firm that specializes in bat surveys and monitoring bat populations for clients including: AZ Game and Fish Department, State of NM Department of Game & Fish, National Forest Service, Bureau of Land Management, National Park Service, AZ State Parks Department and University of Arizona. Ms. Buecher has extensive field experience with western bat species and uses capture methods approved by the American Society of Mammalogists. She maintains her rabies titre to work safely with wild mammals. Since 2009 she has incorporated the US Fish and Wildlife White Nose Syndrome Protocol during all fieldwork. Ms. Buecher has used temperature/humidity loggers to monitor and characterize the microclimate of both summer maternity roosts and winter hibernacula in New Mexico and Arizona bat caves. This experience gives her an excellent background for evaluating best research sites for the proposed research. Ms. Buecher presents extensive public outreach to schools and community organizations regarding bats. Prior to this project, Buecher was funded for four years by WNPA to monitor bats for signs of WNS and to identify appropriate conditions in caves for WNS.

Buecher will be assisted on a volunteer basis by Dr. Diana E. Northup who has been studying cave life since 1984 and cave microbiology since 1989. She is a Professor Emerita in the University Libraries and a Visiting Associate Professor in Biology at the University of New Mexico. She has published extensively on her research and gives numerous talks to the public and to scientific audiences about the research of the Subsurface Life In Mineral Environments (SLIME) Team (www.caveslime.org). In August 2013 she was awarded the Science Award for her research work in caves by the National Speleological Society and in 2012, she became a fellow of the American Association for the Advancement of Science (AAAS). Northup has extensive field experience with microbial and invertebrate sampling in caves, and extensive laboratory experience with microbiological culturing and molecular characterization of cave microbial communities. She has a strong working relationship with the staff at Carlsbad Caverns National Park and has had collecting permits for most of the last 20 years. Dr. Northup will participate in the bat swabbing. Northup has been funded previously by WNPA (2010) for a study to characterize the microbial communities in visited and non-visited lava caves and as a co-PI (2011 - 2014) for the WNS monitoring project, and in 2017, with Jennifer Hathaway to compare bat microbiota to those present on cave walls. She has given several presentations on the results of the previously funded projects. Buecher and Northup are covered by a UNM and a NPS IACUC Protocol for working with bats.

Selected Relevant and Significant Publications:

Buecher, D.C. and R. Sidner. 2008. Seasonal use and hibernal ecology of Townsend's big-eared bat (Corynorhinus townsendii) and cave myotis (Myotis velifer) at two hibernacula in the Huachuca Mountains of southeastern Arizona, August 2006-April 2008. Report for Arizona Game and Fish Department. 82 pp.

Buecher, D.C. 1993. Low disturbance techniques of bat monitoring. In Proceedings of the National Cave Management Symposium, Carlsbad, New Mexico.

Hamm, P.S., Caimi, N.A., Northup, D.E., Valdez, E.W., Buecher, D.C., Dunlap, C.A., Labeda, D.P., Lueschow, S., Porras-Alfaro, A. 2017. Western bats as a reservoir of novel Streptomyces species with antifungal activity. Applied and Environmental Microbiology 83(5):e03057-16.

Budget for New Research Proposal

Project title and submitting park: Is the Causative Agent of White-nose Syndrome, Pseudogymnoascus destructans, Present in Carlsbad Caverns National Park Bats?

Personnel

PRINCIPAL INVESTIGATOR(S)	Funds requested from WNPA	Cash or in-kind contribution (Please specify which type and source.)
1 Debbie C. Buecher: Cash / In-kind	$1,600	$ 3,000
2 Diana E. Northup: In-kind	$ 0	$ 2,800
3		

OTHER PERSONNEL (Specify number in brackets. Specify duties to be performed to earn funds on next page.)	Funds requested from WNPA	Cash or in-kind contribution (Please specify which type and source.)
1 Technician: Cash	$ 1,288	$ 0
2 Fieldwork Assistants (2): In-kind	$ 0	$ 1,280
3 CAVE Physical Sci.Technician (GS-9): In-kind	$ 0	$ 3,000
4 CAVE Physical Sci. Technician (GS-7): In-kind	$ 0	$ 800
5 CAVE GIP Interns (2): In-kind		$ 1,200
TOTAL PERSONNEL COSTS	$2,888	$12,080

EQUIPMENT COSTS (List item and dollar amounts for those items costing more than $100 each on next page.)		
TRAVEL AND SUBSISTENCE (Itemize on next page.)	$1,258	$ 576 In-kind

OTHER COSTS

1 Supplies and material	$2,354	$ 2,050
2 Consulting services	$1,000	
3 Computer services		
4 Subcontracts (Itemize on next page.)		
TOTAL OTHER COSTS	$3,354	$2,050

TOTAL COSTS If multiyear project, summarize estimated subsequent year(s) budget(s) on next page.	$7,500	$14,706

COSTS (Cont'd). Note: Be sure to explain here the duties that will be performed by any funded individual.

Personnel: PI Debbie Buecher is an unsalaried private consultant. She will handle all bats, identify to species, sex, and assess general physical condition (requested: 40 hr x $40/hr = $1,600, in-kind contribution: 70 hr x $40/hr = $3,000). Dr. Diana Northup will assist with bat swabbing and oversee the technician doing DNA extractions (in-kind: 80 hr x $35/hr = $2,800). The technician will extract DNA from swabs for sending to the Foster lab for testing (requested: 66 hr x ($16/hr + 22% fringe) = $1,288). Fieldwork assistants will assist with netting (in-kind: 2 x 40 hr x $16/hr = $1,280).

Travel: Meals $20/day x 6 days x 4 people = $480; travel $500 (0.555/mile) from Tucson (Buecher) and $278 Albuquerque (Northup). Total $1,258.

Supplies: $745 for new nets as required by national WNS protocol, two DNeasy PowerLyzer PowerSoil kits ($588/each), RNALater ($83.50/50 ml x 2 = $167), gloves for handling bats, pipette tips, pipettors ($2,354 total). In-kind donations by Northup and Buecher include: additional netting supplies (hand nets, bat holding bags, gloves, etc.) additional lab supplies and certifications of biological safety cabinets used in DNA extraction ($2,000 total).

Consulting: Foster lab real-time PCR testing of bats swabs $10/sample x 10 swabs = $1000.

Carlsbad Caverns National Park in-kind contributions: Two Physical Science Technicians (GS-9 & GS7) will identify hibernacula and assist with netting (120 hr x $25/hr + 40 hr x $22/hr = $3,880); two GIP interns will identify hibernacula (2 x 80 hr x $7.5/hr = $1,200); researcher housing ($32 x 6 nights = $576); WNS decontamination supplies $50.

Per the solicitation, no indirect costs are included.

A special note for researchers and the park superintendent:

WNPA is the funder of this grant on behalf of NPS, and WNPA monitors progress, administers the payment schedule, and determines successful completion or default.

All other decisions regarding the conduct of this research grant (e.g., park access, laws, safety, protocols, etc.) and uses of the research, data, and its products (e.g., release of information, publication, intellectual property, etc.) rest in the hands of NPS and are the responsibility of NPS. Researchers and NPS should clarify any questions or assumptions before accepting the grant.

Due to several factors, ALL WNPA grants are for ONE YEAR ONLY (1 year only); however, we welcome and will carefully consider applications for second or third years following a successful first year.

Best wishes and hopes for a successful project. Thank you from WNPA.

I have read and agree to abide by the research guidelines in effect at the time of this application.

Debbie C Buecher — Signature of Principal Investigator(s) — 8-31-2018 — Date

Douglas D. Nelson — Signature of Park Superintendent — 8/31/18 — Date

MICHAEL LARSON — Signature of Chief of Interpretation — 8.31.18 — Date

For WNPA Use Only

WNPA Research Committee Review: Action and Date:

Amount Granted: