Experimental Infection of *Tadarida brasiliensis* with *Pseudogymnoascus destructans*, the Fungus That Causes White-Nose Syndrome

Michelle L. Verant,[a,b]* Carol U. Meteyer,[c] Benjamin Stading,[a,b]* David S. Blehert[b]

[a]Department of Pathobiological Sciences, University of Wisconsin—Madison, School of Veterinary Medicine, Madison, Wisconsin, USA
[b]U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA
[c]U.S. Geological Survey, National Center, Reston, Virginia, USA

ABSTRACT White-nose syndrome (WNS) is causing significant declines in populations of North American hibernating bats, and recent western and southern expansions of the disease have placed additional species at risk. Understanding differences in species susceptibility and identifying management actions to reduce mortality of bats from WNS are top research priorities. However, the use of wild-caught susceptible bats, such as *Myotis lucifugus*, as model species for WNS research is problematic and places additional pressure on remnant populations. We investigated the feasibility of using *Tadarida brasiliensis*, a highly abundant species of bat that tolerates captivity, as the basis for an experimental animal model for WNS. Using methods previously established to confirm the etiology of WNS in *M. lucifugus*, we experimentally infected 11 *T. brasiliensis* bats with *Pseudogymnoascus destructans* in the laboratory under conditions that induced hibernation. We detected *P. destructans* on all 11 experimentally infected bats, 7 of which exhibited localized proliferation of hyphae within the epidermis, dermis, and subcutaneous tissue, similar to invasive cutaneous ascomycosis observed in *M. lucifugus* bats with WNS. However, the distribution of lesions across wing membranes of *T. brasiliensis* bats was limited, and only one discrete "cupping erosion," diagnostic for WNS, was identified. Thus, the rarity of lesions definitive for WNS suggests that *T. brasiliensis* does not likely represent an appropriate model for studying the pathophysiology of this disease. Nonetheless, the results of this study prompt questions concerning the potential for free-ranging, migratory *T. brasiliensis* bats to become infected with *P. destructans* and move the fungal pathogen between roost sites used by species susceptible to WNS.

IMPORTANCE White-nose syndrome (WNS) is a fungal disease that is causing severe declines of bat populations in North America. Identifying ways to reduce the impacts of this disease is a priority but is inhibited by the lack of an experimental animal model that does not require the use of wild-caught bat species already impacted by WNS. We tested whether *Tadarida brasiliensis*, one of the most abundant species of bats in the Americas, could serve as a suitable animal model for WNS research. While *T. brasiliensis* bats were susceptible to experimental infection with the fungus under conditions that induced hibernation, the species exhibited limited pathology diagnostic for WNS. These results indicate that *T. brasiliensis* is not likely a suitable experimental model for WNS research. However, the recovery of viable WNS-causing fungus from experimentally infected bats indicates a potential for this species to contribute to the spread of the pathogen where it coexists with other species of bats affected by WNS.

KEYWORDS *Pseudogymnoascus destructans*, *Tadarida brasiliensis*, WNS, bat, cutaneous invasive ascomycosis, experimental disease model, white-nose syndrome

Since they were first observed in 2006 (1), white-nose syndrome (WNS) and the causative fungal pathogen, *Pseudogymnoascus destructans*, continue to spread and threaten the conservation status of bats across North America (2). This emergent disease has caused populations of severely impacted species to decline significantly, and some are consequently at risk for extinction (3). Bat species that have exhibited the highest rates of mortality, such as the little brown bat (*Myotis lucifugus*), the tri-colored bat (*Perimyotis subflavus*), and the northern long-eared bat (*Myotis septentrionalis*), are those which obligately hibernate during winter.

Wing skin is the primary tissue colonized by *P. destructans*, and the cold, torpid state of hibernating bats provides ideal conditions for a psychrophilic fungal pathogen, such as *P. destructans*, to colonize and invade the epidermis of the wing membrane (4–6). Destruction of the epidermal barrier of the wing by *P. destructans* induces physiologic disruptions (7–9) and altered hibernation behaviors (10–12) that collectively contribute to the high rates of mortality characteristic of WNS.

Characterizing disease processes and identifying potential management strategies are top research priorities for mitigating impacts of WNS on bats. However, remnant populations of susceptible species of bats, such as *M. lucifugus*, likely cannot sustain continued removal or "take" of individual animals for research purposes. These colonies of bats surviving with WNS additionally include resistant individuals (13), and further pressures on these remnant populations could impact the potential for long-term recovery (14). Some species of hibernating bats, such as *M. lucifugus*, are also difficult to maintain in captivity. Thus, an experimental animal model for WNS is needed.

Experimental models are widely used in human and veterinary medicine to investigate disease processes and to assess the safety and efficacy of potential treatments or vaccines. For human diseases, these models often use animals that naturally or experimentally experience similar disease processes. Additionally, model animal species are ideally those which can be easily maintained and propagated in captivity or are abundant in the wild. For example, the mouse is a widely used animal model for inflammatory disorders in humans due to striking similarities in gene expression (15), and invertebrates have demonstrated utility for studying fungal virulence factors and for evaluating compounds for antifungal properties (16).

In this study, we investigated the feasibility of using *Tadarida brasiliensis* (Mexican or Brazilian free-tailed bat) as an experimental animal model for WNS. *Tadarida brasiliensis* is one of the most abundant species of insectivorous bats in the Americas and ranges throughout the southern United States, Mexico, Central America, and southwestern South America (17). Additionally, small colonies of *T. brasiliensis* bats have been successfully maintained in captivity (18). The species is primarily considered to be migratory (19), but some populations and individuals forgo seasonal migrations and overwinter, potentially using short-term torpor or hibernation during adverse environmental conditions as an alternate survival strategy (20–22). In the United States, the largest populations of *T. brasiliensis* bats, up to 15 million animals, congregate in caves and under bridges (23). However, *T. brasiliensis* bats may also roost in smaller aggregations within caves, trees, and attics (24), and the species has been found to share roost sites with hibernating species of bats (25). Given these attributes, wild *T. brasiliensis* bats could be exposed to *P. destructans* either through direct contact with infected bats or by contact with environmental reservoirs of *P. destructans* in roost sites. If *T. brasiliensis* bats exposed to *P. destructans* were to subsequently enter prolonged torpor during an adverse weather event, they might be further susceptible to colonization and epidermal invasion by *P. destructans*. Together, these attributes suggest that *T. brasiliensis* could be a suitable experimental model for WNS.

We tested whether *T. brasiliensis* bats could become infected with *P. destructans* and develop cutaneous invasive ascomycosis, pathology of the skin characteristic of WNS, following experimental infection and induction of hibernation in the laboratory. Although prolonged periods of torpor may not reflect typical behavior of this species in the wild, these conditions were chosen to replicate previous laboratory experiments that demonstrated the development of WNS following experimental infection of

TABLE 1 Summary of results for *Tadarida brasiliensis* bats, including experimentally infected bats and sham-treated negative controls

Group	Bat	Survival postinfection (days)	Fluorescence observed under ultraviolet light	*P. destructans* detected by PCR	No. of wing sections: Examined (no. of UV-targeted sections)	No. of wing sections: With localized dermal invasion (severity)
Negative control	C-1	49	No	No	8	0
	C-2	82	No	No	6	0
	C-3	61	No	No	8	0
	C-4	84	No	No	8 (2)	0
Infected	I-1	51	No	Yes	6	0
	I-2	35	No	Yes	8	0
	I-3	75	Yes	Yes	8 (2)	1 (severe)[b]
	I-4	61	Yes	Yes	8	1 (severe)
	I-5	51	No	Yes	9	0
	I-6	84	Yes	Yes	9	4 (moderate)
	I-7	51	Yes	Yes	9	2 (moderate)
	I-8	35	No	Yes	8	0
	I-9	84 (E)[a]	Yes	Yes	9 (2)	1 (severe), 1 (moderate)[b,c]
	I-10	35 (E)	No	Yes	7	1 (severe)
	I-11	44	Yes	Yes	9	1 (moderate)

[a]E, bat was euthanized.
[b]A section was targeted for sampling based on fluorescence observed under UV light.
[c]A single area of severe fungal invasion of skin from muzzle was also observed.

M. lucifugus bats with *P. destructans* (7, 26). Altogether, the experiment described herein assessed the susceptibility of *T. brasiliensis* bats to infection by *P. destructans*, the suitability of this species as a model for WNS research, and the potential for this migratory species of bat to contribute to the spread of *P. destructans*.

RESULTS

We identified the presence of DNA from *P. destructans* by real-time PCR of tissue specimens from all experimentally infected bats at the time of death (35 to 84 days following the initiation of the experiment) (Table 1). While *P. destructans* was isolated in pure culture from one experimentally infected bat, culture attempts from wing skin samples of other infected bats were overgrown by yeast identified as *Debaryomyces* sp. prior to observing fungal colonies with morphology suggestive of *P. destructans*. Of the experimentally infected bats, four had localized areas with visible white hyphae on the wing surface. The presence of visible fungal hyphae was associated with localized areas of tissue contraction and bright green-blue fluorescence in two bats (Fig. 1 shows representative images from one bat). These sections were specifically trimmed for histopathology (Table 1, I-3 and I-9). Only one of the experimentally infected bats (I-11) had UV fluorescence characteristic of WNS, although this fluorescence was restricted to a small area in the plagiopatagium and there were no visible white hyphae on the wing surface (Fig. 2). All noninfected control bats were negative for *P. destructans* by
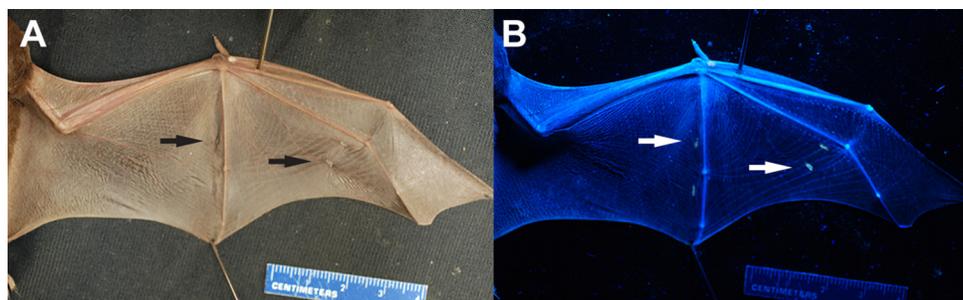

FIG 1 Images of one wing membrane from a *Tadarida brasiliensis* bat experimentally infected with *Pseudogymnoascus destructans*. The wing shown is from bat I-9 (Table 1). Areas of contracted tissue with white hyphae (black arrows) are seen under illumination by visible light (A) and are associated with areas of fluorescence (white arrows) apparent under illumination by UV light at 365 nm (B).
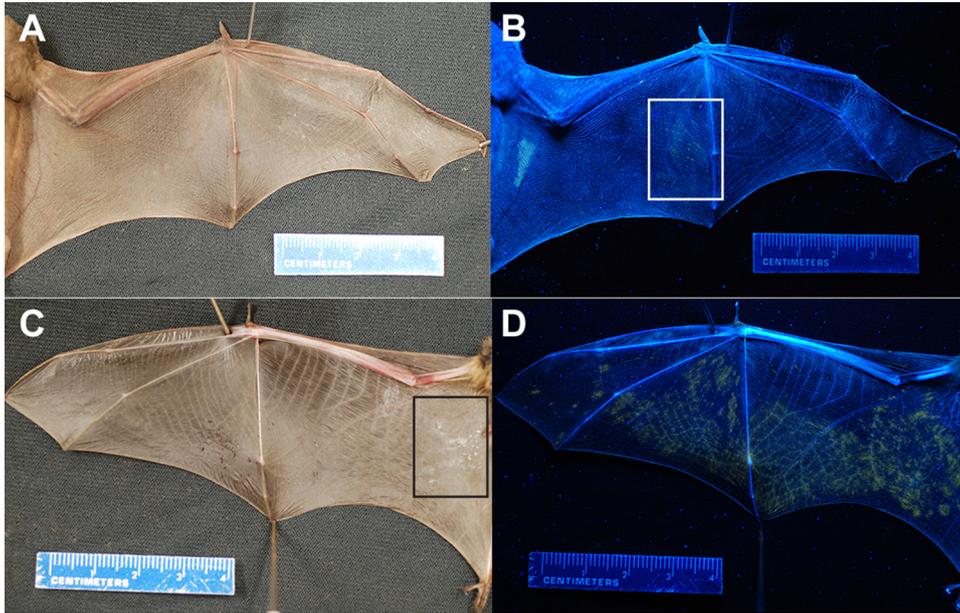
FIG 2 Images of wing membranes from *Tadarida brasiliensis* and *Myotis lucifugus* bats that were experimentally infected with *Pseudogymnoascus destructans*. (A, B) The wing shown is from *T. brasiliensis* bat I-11 from this study (Table 1). The wing appears normal under visible light (A), but areas of fluorescence characteristic of white-nose syndrome (white-boxed area) are apparent under illumination by UV light at 365 nm (B). (C, D) The wing shown is from an *M. lucifugus* bat from a previous experiment (7). White hyphal growth (black-boxed area) can be seen on the surface of the wing under visible light (C), and areas of fluorescence characteristic of white-nose syndrome are visible across the majority of the wing surface under illumination by UV light at 365 nm (D).

real-time PCR at the time of death (49 to 84 days following initiation of the experiment), and they did not exhibit fluorescence of wing membranes characteristic of WNS (Table 1).

On histologic examination, epidermal sections from noninfected *T. brasiliensis* bats were unremarkable, and no conidia with morphology typical of *P. destructans* were present (Fig. 3A, inset). We observed evidence of cutaneous invasive ascomycosis as described for WNS (27) in 7 of the 11 *T. brasiliensis* bats that were experimentally infected with *P. destructans*, including within wing sections that were targeted for histopathology by screening under UV light (Table 1). Additionally, three experimentally infected bats had multiple curved conidia characteristic of *P. destructans* on or near the epidermal surface, one of which had clusters of germinating conidia with emerging hyphae located superficial to the epidermis (Fig. 3A).

The first case of cutaneous invasive ascomycosis detected involved limited infection (e.g., one affected section of wing tissue with one region of infection) on a *T. brasiliensis* bat that was moribund and euthanized on day 35 following initiation of the experiment. Despite the presence of cutaneous invasive ascomycosis in 7 of the experimentally infected *T. brasiliensis* bats, the distribution of these lesions in histologic sections of wing membrane was similarly limited (Table 1). Of the affected sections, generally only one or two regions in the section contained invading hyphae. A single section from one of the 7 bats examined had a discrete "cupping erosion" of the epidermis (Fig. 3B), as typical for WNS in obligately hibernating species like *M. lucifugus* (4). Most other lesions had focally extensive invasion of the epidermis, dermis, and subcutaneous tissue with dense proliferations of irregular fungal hyphae, consistent with morphology described for *P. destructans* (Fig. 3C). Nearly complete obliteration of the epidermal and dermal boundaries by dense aggregates of hyphae was also common in these localized areas of infection (Fig. 3D). Only one experimentally infected bat had hyphal invasion of the muzzle, and no histologic changes were observed in ear sections.

When held under conditions intended to induce hibernation, 7.7°C (standard deviation [SD] = 0.9°C) and 91.8% (SD = 0.8%) relative humidity, *T. brasiliensis* bats
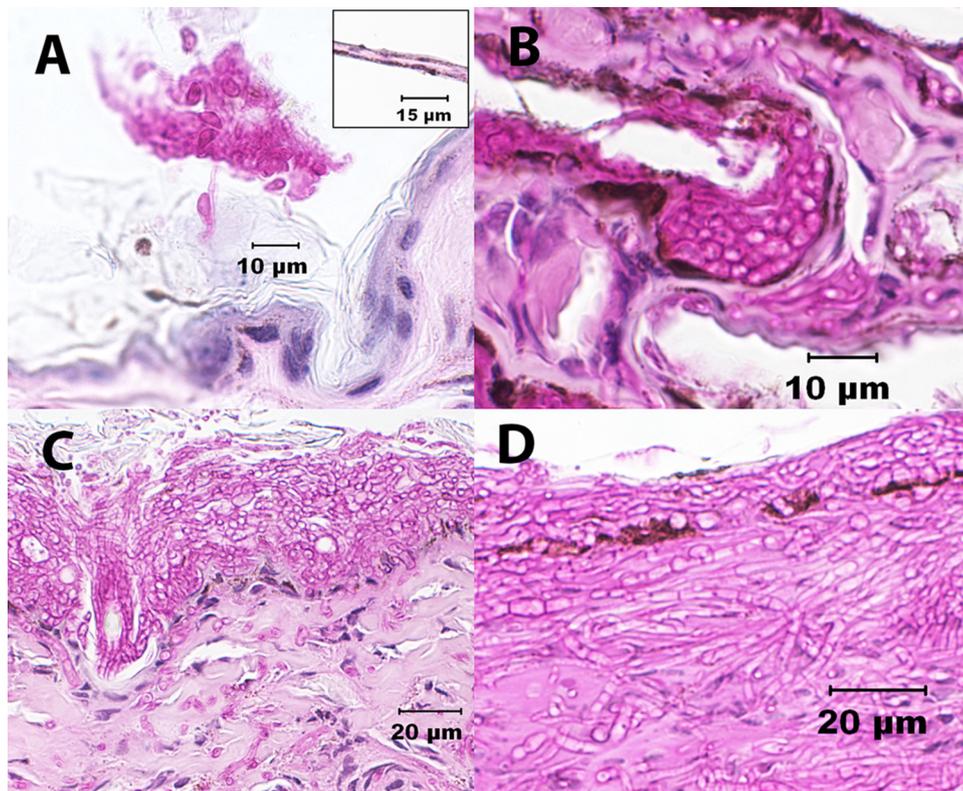
FIG 3 Cutaneous invasive ascomycosis in representative histologic sections of wing membranes from *Tadarida brasiliensis* bats experimentally infected with *Pseudogymnoascus destructans*. (A) Characteristic curved conidia of *P. destructans* are evident superficial to the epidermis. A cross section of normal wing tissue from a noninfected control bat is provided for comparison (inset). (B) One section of wing membrane contained a discrete cupping erosion of the epidermis filled with hyphae, a diagnostic characteristic of white-nose syndrome. (C) Focal proliferation of irregular hyphae on the skin surface with invasion of the epidermal, dermal, and subcutaneous tissue. (D) Dense aggregates of hyphae obscuring the epidermal and dermal boundaries of focal lesions.

demonstrated the use of torpor-arousal cycles (Table 2). All bats for which temperature data were available ($n = 11$) spent a median of 6.8 days (range, 5.2 to 10.7 days) per torpor bout and 6 h (range, 3.6 to 7.6 h) per arousal. Experimentally infected bats had shorter torpor bouts and longer arousals than noninfected control animals, but these differences were not statistically significant (torpor, $U = 19$, $P = 0.19$, and $r = 5.7$; arousal, $U = 3$, $P = 0.08$, and $r = 0.9$ [Mann-Whitney U test]). The rates of mortality were similar between groups and were positively associated with the body mass index at the start of the experiment [$r_{(13)} = 0.89$, $P < 0.001$].

DISCUSSION

In this controlled laboratory experiment, we demonstrated that experimental infection of *T. brasiliensis* bats with *P. destructans* under conditions of induced hibernation caused cutaneous invasive ascomycosis with epidermal lesions characteristic of WNS (4). The lesions we characterized in experimentally infected *T. brasiliensis* bats were similar to those observed in bats of the obligate hibernator species *M. lucifugus* infected with *P. destructans* in the wild or under equivalent experimental conditions (10, 26).

TABLE 2 Duration of torpor and arousal periods for *Tadarida brasiliensis* bats experimentally infected with *Pseudogymnoascus destructans* or sham treated

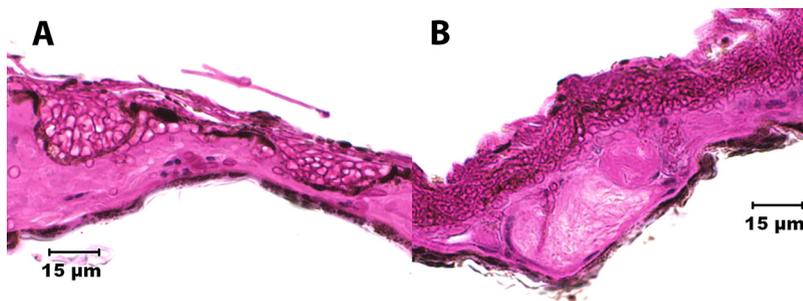| Group	No. of bats	Torpor duration (days) [median (range)]	Arousal duration (h) [median (range)]
Negative control	3	8.1 (6.8–9.9)	4.5 (3.6–5.7)
Infected	8	6.1 (5.3–10.7)	6.4 (4.3–7.6)

FIG 4 Histologic sections of wing membranes from *Myotis lucifugus* bats experimentally infected with *Pseudogymnoascus destructans* in a previous experiment (7). (A) Epidermal cupping erosions diagnostic for white-nose syndrome (WNS). (B) Dense proliferation of hyphae and invasion of epidermal, dermal, and subcutaneous tissues of wing membrane from a bat with severe WNS.

However, the distribution of pathology over wing membranes of *T. brasiliensis* bats was much more limited than in *M. lucifugus* bats. We observed minimal UV fluorescence of wing membranes in *T. brasiliensis* bats that could be considered characteristic of WNS, and rare epidermal lesions were identified through extensive histopathologic examination of multiple sections of wing from each bat.

In *M. lucifugus* and other species of hibernating bats, WNS is confirmed by the presence of lesions described as "cupping erosions," which are characterized by bundles of irregular fungal hyphae forming a discrete interface with host tissue (Fig. 4A) (4). These lesions are generally readily observed across the wing membrane. In contrast, among experimentally infected *T. brasiliensis* bats, cupping erosions were identified in only one wing section from a single bat. When present, lesions in *T. brasiliensis* bats primarily consisted of extensive hyphal invasion and proliferation in the focal area of affected wing skin. This deep dermal invasion is typically associated with severe WNS in *M. lucifugus* bats (Fig. 4B) (4). The rare occurrence of histologic lesions in *T. brasiliensis* bats infected with *P. destructans* and the relative lack of cupping erosions may reduce the sensitivity of histologic assessment for WNS in this species.

The case definition for WNS states that both histologic lesions (i.e., epidermal cupping erosions) and *P. destructans* must be present to confirm a diagnosis for WNS (28). Based on this definition, 1 of 11 *T. brasiliensis* bats experimentally infected with *P. destructans* developed WNS. However, WNS in obligately hibernating bats in North America is a multistage disease process that is associated with aberrant behaviors, systemic physiologic effects, and mortality (7–9, 11, 12, 29). In this study, the limited distribution of pathology and lack of differences in torpor-arousal profiles or survival in experimentally infected *T. brasiliensis* bats compared to controls suggest that the pathogenesis of WNS in this species likely differs from what has been described in obligately hibernating species of bats.

We detected the first evidence of cutaneous invasive ascomycosis in a *T. brasiliensis* bat that died 35 days postinfection, a shorter time frame than described for the development of pathology in *M. lucifugus* bats (26). Furthermore, epidermal lesions in *T. brasiliensis* bats were generally observed in only one of several sections of wing membrane examined from each bat and included only one or two focal areas of invading hyphae per affected section. This is distinct from the often diffuse distribution of epidermal lesions and hyphal proliferation observed in wing membranes of obligately hibernating species of bats with WNS. Additionally, the median torpor bout durations for *T. brasiliensis* bats in this study (6.1 to 8.1 days) (Table 2) were shorter than what has been described for *M. lucifugus* bats (approximately 9 to 16 days) in similar published experiments (7, 29). While we observed mortality of bats during this study, the rates were similar in the infected and control groups and started 35 days following initiation of the experiment. In contrast, previous reports indicated that mortality from WNS in *M. lucifugus* bats under experimental and natural conditions does not occur until approximately 120 days following infection (26, 29). Thus, the mortality observed

in *T. brasiliensis* bats experimentally infected with *P. destructans* likely resulted from causes other than WNS, such as inefficient use of energy during induced hibernation compared to the energy use of an obligately hibernating species. The body mass index of a bat at the start of this experiment was positively correlated with the number of days a bat remained alive posttreatment, suggesting that survival time was related, at least in part, to energy reserves.

Experimental animal models are a valuable tool for investigating disease processes and potential treatments. To date, research on WNS has primarily relied upon the use of wild-caught members of bat species that are naturally susceptible to the disease but are not readily amenable to long-term maintenance in captivity. Alternatively, tissue explants from bat wings have shown some utility for testing potential inhibitors of *P. destructans* (30), but it remains necessary to collect explants from wild-caught bats. Based on the results of this study, *T. brasiliensis* may have potential as an experimental model for investigating aspects of *P. destructans* persistence and proliferation on bats or for testing hypotheses on variations in species susceptibility to *P. destructans* and WNS. However, given the observed atypical manifestation of *P. destructans* infection in *T. brasiliensis* and shorter duration of survival under conditions of induced hibernation, the use of this species is not likely appropriate to study the physiologic effects and pathogenesis of WNS or to test potential treatments designed to reduce mortality.

This experiment leaves unanswered questions regarding the susceptibility of wild *T. brasiliensis* bats to infection with *P. destructans* under natural conditions. Our results reflect experimentally induced conditions that were intended to provide the highest likelihood of infection by replicating prior experiments with *M. lucifugus* bats (e.g., see references 26 and 29). In contrast, *T. brasiliensis* bats are not obligate hibernators, but individuals of this species have been shown to use torpor to conserve energy over short periods and may hibernate in some locations (22, 31). Different environmental conditions (e.g., temperature or humidity) within roosts may influence susceptibility to WNS, as has been described for other species (32). If wild *T. brasiliensis* bats are susceptible to infection with *P. destructans*, it may be challenging to definitively diagnose WNS in this species in accordance with the current case definition, if epidermal pathology in naturally infected bats is limited similarly to that described for the bats in this study.

The recovery of viable *P. destructans* from an experimentally infected bat suggests that *T. brasiliensis* bats have the potential to harbor the fungus under certain conditions. The low recovery rate of viable *P. destructans* from infected bats in this study may have resulted from the limited extent of epidermal fungal infections; recovery was also hampered by overgrowth of culture plates by more rapidly growing yeast (e.g., *Debaromyces* sp.) present on the wing membranes. Regardless, our results indicate a potential for *T. brasiliensis* bats to contribute to movement of *P. destructans* between roost sites that may also harbor other bat species susceptible to WNS. This risk is highlighted by a recent report of PCR-based detection of the presence of *P. destructans* on *T. brasiliensis* bats at a roost in central Texas that is occupied by approximately 3 million *T. brasiliensis* bats from May through October of each year (33). Such dense aggregations of bats, typical for colonies of this species, have the potential to facilitate high pathogen transmission rates (34) under suitable environmental conditions. Additionally, *T. brasiliensis* bats have been shown to migrate up to 1,500 km seasonally (35) and travel up to 50 km during daily movements (36), which could facilitate pathogen dispersal. However, fluctuating environmental temperatures and activity patterns are likely to influence the persistence and viability of *P. destructans* on *T. brasiliensis* bats in nature.

Understanding the potential for *T. brasiliensis* bats to become infected with *P. destructans* or contribute to the spread of WNS is timely, given the continued spread of this disease and the pathogen within the range of this bat species in the southern United States (37). Additional surveillance for *P. destructans* and WNS in wild *T. brasiliensis* bats along the leading edge of WNS will help to elucidate the role this abundant and far-ranging species may have in the movement of this fungal pathogen across western and southern North America.

MATERIALS AND METHODS

Bats, husbandry, and permissions. This experiment was conducted in accordance with U.S. Geological Survey National Wildlife Health Center (NWHC, Madison, WI) Institutional Animal Care and Use Committee (IACUC) experimental protocol 121025. Apparently healthy, adult male *T. brasiliensis mexicana* bats ($n = 15$) were caught in Brazos County, Texas, in 2013 under Texas Parks and Wildlife Department permit number SPR-1104-610 and Texas A&M University IACUC approval number 2012-130. After acclimating to captivity in Texas, the bats were transferred to and held at the U.S. Geological Survey National Wildlife Health Center (NWHC, Madison, WI). At NWHC, the bats were maintained under animal biosafety level 3 conditions in flight cages for a 30-day quarantine period, during which blood samples were collected and the bats were topically treated for parasites with selamectin (Revolution; Zoetis, Inc., Parsippany-Troy Hills, NJ). A unique electronic microchip identification unit (Avid Identification Systems, Inc., Folsom, LA) was subcutaneously injected between the scapulae of each animal. Bats were maintained on mealworms (*Tenebrio molitor*) supplemented with vitamins and an omega fatty acid mixture, and water was provided *ad libitum*. The light cycle was set to 12 h of light per day, inverted from the natural cycle to allow monitoring of bat activities during daytime hours. One month prior to initiation of the *P. destructans* infection trial described herein, a poxvirus infection study was completed at NWHC (38), involving all bats subsequently used in this study. At the start of this infection trial, no poxviral activity was evident in any bat, based on lack of activity from the luciferase marker gene inserted into the poxvirus, and no bats showed signs of clinical illness. Additionally, bats were assumed to be negative for *P. destructans* prior to the start of this infection trial due to their origin and time spent active in captivity.

Infection trial. An iBBat temperature logger (Alpha Mach, Sainte-Julie, Quebec, Canada) was attached to the dorsal surface of each bat to assess torpor-arousal patterns as previously described (7). Arousal thresholds for each individual were defined as 10% of maximum skin temperature (10). The length of the right forearm and mass of each bat was measured and used to calculate the body mass index (forearm length/mass) at the start of the study. Bats were randomly assigned to infected ($n = 11$) and control ($n = 4$) groups. Conidia of *P. destructans* (5×10^5, suspended in 20 μl phosphate-buffered saline solution containing 0.5% Tween 20 [PBST]) were applied to the muzzle and the dorsal surface of the wings of each bat in the infected group as previously described (26). Bats in the control group were treated similarly but with vehicle solution (PBST) lacking conidia. Infected and control groups were then placed in separate mesh enclosures (25 inches high by 14.5 inches wide by 14.5 inches deep; Apogee Reptaria, Dallas, TX) separated by a plastic divider within an environmental chamber (model number I-36NL; Percival Scientific, Perry, IA) maintained at 7.7°C (SD = 0.9°C) and 91.8% (SD = 0.8%) relative humidity to induce and support extended hibernation. Water was provided *ad libitum* using a gravity-fed water bowl on the floor of each enclosure. Bats were monitored twice daily through a window in the door of the chamber, with the interior illuminated by red light. Any bat observed on the floor of an enclosure for two consecutive monitoring checks was removed from the chamber for assessment. Moribund bats were euthanized, and diagnostic samples were promptly collected from carcasses as described below.

Evaluation for *P. destructans* and WNS. The presence of *P. destructans* on each bat at the time of death was determined by real-time PCR analysis of wing skin (39) and culture-based assessment of a section of skin (approximately 3 cm by 3 cm) from the left wing placed onto Sabouraud dextrose agar containing chloramphenicol and gentamicin and incubated at 10°C for up to 1 month. The entire membrane of the right wing was examined using a handheld UV lamp (model number UVL-56, 365 nm; UVP, Inc., Upland, CA) to identify areas of orange-yellow fluorescence under UV illumination as previously described for WNS (40).

Following examination under UV light, the entire membrane from the right wing of each bat was removed and processed for histopathology analysis as previously described (4). Briefly, the wing membrane was cut into 1-cm strips and rolled in overlapping spirals around dowels (approximately 2 cm by 0.25 cm) of colorless dental orthodontic paraffin. The paraffin dowels with wing tissue were then placed in numerically coded cassettes without notation of treatment group and submerged in 10% neutral buffered formalin for at least 24 h. Each paraffin dowel with tissue was then trimmed to produce 0.5-cm cross sections, yielding approximately eight whorls of wing tissue from each bat. These cross sections were then placed cut-side down in the coded cassette for processing and embedding in paraffin; 4-μm sections were then placed on similarly coded glass slides and stained using the periodic acid-Schiff (PAS) method. Sections of muzzle and ear were also sampled for histopathology and processed together with the wing membrane.

Histologic sections were examined for epidermal lesions (cutaneous invasive ascomycosis [27]) considered characteristic of WNS, which include cupping erosion of the epidermis by dense irregular fungal hyphae that form a discrete interface with the host tissue (4). The degree of erosion and ulceration and the extent of distribution of lesions over the surface area of the wing sections examined were used to determine the severity of infection (10).

Data availability. Data supporting the results of this study and data from iBBat temperature loggers are available from the U.S. Geological Survey ScienceBase Catalog at https://doi.org/10.5066/P93WAKH3 (41).

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