


Fig. 3. Geomyces destructans. A. Colony on CMA after 16 days at 7°C. B. Conidiophores with conidia in short chains. C. Conidia with separating cell. D. Curved conidia. E. Conidiophores showing acute branching.

Scale bars: A = 1 cm, B-E = 10 μm.
Figure 2 | Survival curves. a, Survival curves for the treated ($n = 29$), contact exposure ($n = 18$), airborne exposure ($n = 36$), negative control ($n = 34$) and
Fig. 1. Representative traces of skin temperature ($T_{\text{skin}}$) for six *M. lucifugus*, two each from the following groups: (A) inoculated with NAGd; (B) inoculated with EUGd; (C) sham-inoculated control. The x-axis shows the day of study, where day 1 is November 27, 2010; the bars at the bottom indicate the division of the study period into 26-d intervals, and months.
Fig. 2. Changes in torpor patterns in *M. lucifugus* following inoculation with NAGd, EUGd, or CO. Frequency of arousals based on skin temperature (A), total count of arousals based on video observations (B), and mean arousal duration (C). Within intervals, different letters above bars indicate significant differences between groups (SNK post hoc tests following significant ANOVA in Table 1).
Fig. 4. Survival of individual *M. lucifugus* over the course of the study for group *NAGd* (dashed line), *EUGd* (dotted line), and *CO* (solid line). The closed circle at the end of each line indicates the day when the group was terminated, day 1 is November 27, 2010.
The wing damage index, described below, is a four-point scale ranging from 0 (no/minimal damage) to 3 (severe damage) for recording the occurrence of these symptoms. After examining both wings and the uropatagium, each but was assigned a single WDI corresponding to the highest score for which it exhibited one or more types of damage for that level (Table 2). Thus, the WDI is a composite assessment for the wing membranes and uropatagium. Because the severity of forearm flaking, when present, was fairly consistent, other categories of damage characteristic of WDI = 2 and WDI = 3 were considered for assigning these scores.

WDI scores were determined based on the physical conditions of the wings, without consideration of the causes of observed damage. When a cause could be hypothesized (e.g., bites from ectoparasites or tears from assorted environmental hazards) these notes were recorded in addition to WDI.

**Analytical Methods**

Separate contingency tables were created for adult females and juveniles to test for changes in the relative abundance of

**Table 1.** Wing conditions observed in *M. lucifugus* used for developing the wing damage index (WDI) for assessing the physical condition of flight membranes

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Description</th>
<th>Example</th>
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<tbody>
<tr>
<td>Spoting, splotching and depigmented membrane</td>
<td>Light spots appear on the darker wing and tail membranes. These spots are often more visible when the membrane is backlit</td>
<td>Fig. 1</td>
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<tr>
<td>Flaking and depigmented forearm</td>
<td>Dry skin appears along the forearm. Some spots appear lighter brown or pink where skin appears to have flaked off</td>
<td>Fig. 2</td>
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<td>Necrotic tissue</td>
<td>Membranes may have visible scars, open wounds, or infections. In more severe cases, large sections of membrane are sloughing from the wing</td>
<td>Fig. 3</td>
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<td>Holes</td>
<td>Some very small pin-holes appear to be associated with ectoparasite wounds. Other holes are larger and often surrounded by depigmented or necrotic tissue. The appearance of the edges of holes may be likened to singed nylon</td>
<td>Fig. 4</td>
</tr>
<tr>
<td>Membrane loss</td>
<td>Wing areas are notably reduced along edges. Most commonly, the trailing edge of the plagiopatagium is receded in an arc from the leg to the fifth digit. Such damage may be severe, greatly reducing the overall surface area of the wings</td>
<td>Fig. 5</td>
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Figure 2. Wing membranes of *Myotis lucifugus* bats infected with white-nose syndrome (WNS). A, histologic section of wing membrane from the same bat as in Figure 1B. Invasive fungus (arrow) stains poorly with hematoxylin and eosin stain, and inflammatory infiltrates are not present. Bar = 15 μm. B, periodic acid–Schiff (PAS) stain of serial section from same tissue as in panel A. Fungal hyphae stain bright magenta. Hyphae are associated with cup-shaped epidermal erosions (arrowhead) and ulcers (arrow) with invasion of the underlying connective tissue. Bar = 15 μm. C, section of wing membrane, collected while inside the cave, from a little brown bat immediately after euthanasia. Exuberant fungal growth is present on the surface of the skin (arrow) and penetrates the wing membrane (arrowheads) without associated inflammation. PAS stain. Bar = 15 μm. D, conidia on the surface of the wing membrane of a cave-dwelling little brown bat fixed immediately after euthanasia in the cave. The characteristic curved conidia measure approximately 2.5 μm in diameter and 7.5 μm in curved length, have one or two blunt ends, and have a deeply basophilic central region (arrowheads). These conidia are identical to those of *Geomyces* sp. fungus isolated from bats with WNS. A focal cluster of fungal hyphae is present within the epithelium on opposite wing margin. PAS stain. Bar = 15 μm.
Figure 3. *Myotis lucifugus* with white-nose syndrome (WNS) submitted to the National Wildlife Health Center, Madison, Wisconsin, from Connecticut and Vermont. A, histologic section of bat muzzle with fungal hyphae filling the hair follicle (arrowhead), obliterating the epidermal sheath, and invading the regional connective tissue (arrow). No inflammatory response is present and bacteria do not colonize the surface. Periodic acid–Schiff (PAS) stain. Bar = 15 μm. B, fungal hyphae obscure the follicular epithelium (white arrow) and associated sebaceous gland (long arrow) of a bat’s muzzle. The fungal hyphae are branching, septate (arrowhead), and of variable morphology ranging from parallel walls measuring 2 μm diameter to bulging or globose walls measuring 3–5 μm in diameter. There is no associated inflammation. PAS stain. Bar = 15 μm. C, wing membrane from a bat collected in May after emergence from hibernation but before flights. Inflammatory cells (long arrow) surround fungal hyphae (white arrow) forming a cellular crust overlying intact epidermis (arrowheads). PAS stain. Bar = 15 μm. D, different bat with similar history to that in panel C. Quiescent nests of fungus are surrounded by a thin layer of amorphous material within the epidermis of the wing (arrows). PAS stain. Bar = 15 μm.
Figure 2. Photomicrographs of periodic acid Schiff-stained 4-μm sections of wing membrane prepared as previously described [7] from a little brown bat (Myotis lucifugus) infected by Geomyces destructans. (a) Fungal hyphae penetrate and replace apocrine gland (white arrow), hair follicle (black arrow pointing to hair shaft), and sebaceous gland (arrowhead). (b) Normal pilosebaceous unit including the apocrine gland (white arrow), hair follicle (black arrow pointing to hair shaft) and sebaceous gland (arrowhead). (c) Infarcted region of wing membrane showing loss of all identifiable vital structures in the dermis, including blood vessels, connective tissue, muscle, elastin fibers and the large bands of connective tissue that traverse and stabilize wing membrane (arrow). No discernable cell structures or nuclei remain, the wing membrane is contracted and hypereosinophilic (intense red staining), and only residual pigment is present on the membrane surface (arrowhead). (d) Microscopic section of normal wing membrane with identifiable blood vessel containing circulating red blood cells (arrow) and nuclei of connective tissue cells (arrowheads).
Figure 2. Average activity budget for a (A) white-nose syndrome (WNS)-affected (n = 16) and (B) WNS-affected (n = 15) little brown myotis (Myotis lucifugus), as portions of time aroused.
Bat white-nose syndrome:
multi-stage disease progression model

<table>
<thead>
<tr>
<th>PATHOLOGY</th>
<th>PHYSIOLOGIC EFFECTS</th>
<th>OUTCOMES</th>
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<tbody>
<tr>
<td>Colonization of wing by <em>Pd</em></td>
<td>Increase metabolic rate of <em>CO₂</em></td>
<td>Respiratory acidosis</td>
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<tr>
<td>Epidermal invasion</td>
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<td>Hyperkalemia</td>
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<tr>
<td>Erosion and ulceration</td>
<td></td>
<td>Fat depletion</td>
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<tr>
<td>Connective tissue invasion and Disruption of wing functions</td>
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<td>Increased arousals</td>
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<td>Failure of physiologic and behavioral compensation</td>
<td></td>
<td>Normal blood pH</td>
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<tr>
<td></td>
<td></td>
<td>(Hypotonic)</td>
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<tr>
<td></td>
<td></td>
<td>Dehydration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Emaciation</td>
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<tr>
<td></td>
<td></td>
<td>Death or spring emergence and recovery</td>
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Metabolic rate: Increase in metabolic rate leads to increased excretion of *CO₂*

Arousal:

Hyperventilation: Increased *CO₂* leads to hyperventilation

Energy use: Increased *CO₂* leads to increased energy use

Water loss: Increased energy use leads to increased water loss

Electrolyte loss: Increased water loss leads to increased electrolyte loss

Mortality: Increased electrolyte loss leads to mortality
Figure 1. Comparison between *Trichophyton rodei* infection (top panels) and *Pseudogymnoascus destructans* infection (i.e., white-nose syndrome [WNS]; bottom panels) in bats. Bats infected with *T. rodei* have visible white fungal growth on the ears, legs, wings, tail, or uropatagium (A); lesions may manifest as a distinct ring (arrow); the muzzle often lacks clinical signs of infection. Bats with WNS generally have visible fungus on the muzzle in addition to other areas of unfurred skin (B). Fungal tape impressions collected from bats with clinical signs of *T. rodei* infection display radially symmetric oblate to pyriform macroconidia (C), as opposed to the asymmetrical curved conidia typical of *P. destructans* (D). In histologic sections (prepared with periodic acid–Schiff staining), the wing skin of bats with *T. rodei* infections (E) generally have superficial colonization and invasion of the keratin layers by the dermatophyte (arrow); aerial hyphae and fertile structures in the form of conidiophores and microconidia may also be present (arrowhead). In contrast, histologic cross sections of wing skin from bats with WNS (F) typically display cup-like aggregations of fungal hyphae (arrows), erosion of the epidermis, and occasional curved conidia (arrowheads). Scale bars=20 μm.
White-nose Syndrome in bats.
1st documented in photograph in cave in Albany, NY in 2006.
-see pics from Blehert (WNSpic_1).
In 2 years, documented throughout mid- and upper-Atlantic states.
Profuse, delicate fungal hyphae and conidia.
- muzzles.
- wing membranes.
- ears.
Hyphae fill hair follicles and sebaceous glands.
- no inflammation.
- no obvious immune response.

Gargas et al., 2009.
Isolated fungus from 8 bats of 2 species from NY, VT, MA, CT.
Infected wing tissue cultured on Sabouraud agar.
Growth characteristics on cornmeal agar at 7, 14, and 24 C.
DNA extracted and ITS1-5.8s-ITS2 and SSU (18s) regions amplified by PCR.
- sequenced with BigDye.
- compared to blast searches from GenBank.
Phylogenetic analysis places with other Geomyces and Pseudogymnoascus.
- see diagram from Blehert (WNSpic_2).
Describes species as Geomyces destructans Blehert and Gargas, 2009.
- see pics from Blehert (WNSpic_3).
- note no growth at 24 C.
- other Geomyces are soil inhabitants from mostly cooler climes.

Lorch et al., 2011.
G. destructans occurs in Europe, but bats don’t die.
Does G. destructans cause WNS or is it an opportunistic infection resulting from some other condition or pathology in bats?
Myotis lucifugus (little brown bat) collected from Wisconsin site lacking WNS.
Control group: 34.
Treatment: 29.
Direct contact (n=18): spores from American Type Culture Collection pipetted onto wing and fur near ears.
Airborne exposure (N=36): held in enclosures near to infected bats.
102 days at 6.5 C, 82% RH.
Diagnosis by:
Histology.
Isolation and culture.
PCR and sequencing to verify.
WNS 1st detected at 83 days; 100% showing WNS by 102 days.
Co-housing bats led to 89% infected by 102 days.
Airborne trails, 0% infected.
Control bats: 0% infected.
No GD in internal organs.
See Figs. (WNSpic_4).

Warneke et al., 2012.
Does EUGD cause WNS as does NAGD?
Inoculated bats with strains of GD from Europe and N. America.
Monitored skin temperatures during hibernation to evaluate frequency of warming.
Monitored mortality.
Histology.
Arousal frequency elevated in both EUGD and NAGD bats (WNSpic_5).
Arousal duration not affected; but total arousal counts increased (WNSpic_6).
Survival significantly reduced in both EUGD and NAGD bats (WNSpic_7).
Control bats retained sub-cutaneous fat reserves.
EU and NA bats without fat reserves.
Histology confirmed WNS.
Suggests a mechanisms of mortality.
- emaciation due to increased arousals.
- each arousal uses about 5% of fat reserves.
  -shortens hibernation time by about 9 days.
  -most bat in NA need to hibernate 190 or more days.
Demonstates GD is a novel pathogen to N. America, i.e., it was introduced.
  -if EUGD did not cause WNS, then NAGD would have to be different.

Minnis and Lindner, 2013
Phylogenetic analysis of GD and putative relatives.
Mitochondrial ITS region, and nuclear LSU and 3 other nuclear genes.
Found “GD” inside a large clade of Pseudogymnoascus (WNSpic_8).
Pseudogymnoascus destructans (Blehert and Gargas, 2009) Minnis and Linder, 2013
Two obvious things happen to bats infected with Pd compared to uninfected bats.

1. The fungus spreads over the wings, muzzle and around the ears.
   Reichard and Kunz, 2009.
   5 types of wing damage (**WNSpic_9**).
   - Splotching.
   - Flaking.
   - Necrosis.
   - Holes.
   - Membrane loss.
   Meteyer et al., 2009.
   Normal wing:
   - 2 layers of epidermis.
   - Separated by layer of connective tissue with elastin.
   - Includes nerves, muscles, lymph vessels.
   **WNSpic_10**.
   - 2A and 2B→demonstrates PAS superior stain.
   - 2B→cup like erosions and ulcers (could span full thickness of membrane).
   - 2C→fungal growth on skin surface and pts of penetration (no inflammation).
   - 2D→typical comma shaped conidia.
   **WNSpic_11**.
   - 3A→hyphae filling hair follicle and invading connective tissue.
   - 3B→in sebaceous gland; no inflammation.
   - 3C→inflammatory cells forming cellular crust over epidermis.
     - in bat after emergence.
   - 3D→quiescent nests of fungi in epidermis, surrounded by amorphous material.
   Very little inflammation in hibernating bats.
   - When present, mild edema and neutrophils present, occasionally with abscesses.
   Lots of inflammation in emerged bats.
   - Suppurative dermatitis.
   - Folliculitis.
   - Edema.
   - Infiltrates with macrophages.
   - Serocellular inflammatory crusts.
   - Packets of hyphae within dermis.
   Cryan et al., 2010.
   - Normal wing→supple and elastic and strong (good tone).
   - Affected wing.
     - Folded surfaces adhere to each other.
     - Tone, tensile strength, and elasticity lost.
Tear easily.
Resemble crumpled tissue paper.
Invasion of glands (WNSpic_12→comparison to normal).
Infarction.
WNSpic_12→comparison to normal.
Loss of structures.
Hypereosinophilia.

Healthy wing membranes.
Critical for water balance.
Large lungs and exposed wings make them susceptible to dehydration.
99% of water loss in healthy bats is through skin.

Evap. Water loss related to RH.
Bats select high humidity sites.
Bats most susceptible to WNS select high humidity sites.

WNS and EWL.
Evidence→bat muscles adhere to a gloved muscle (to the point you can pick the bat up this way), which is indicative of pre-death dehydration.

Mechanism.
Direct physical destruction.
Disruption of glands that secrete moisturizing and waterproofing compounds.
We don’t know for sure why bats arouse during hibernation.
-probably related to metabolic waste, muscle function, and/or water.

WNS bats have been observed eating snow.

Other potential effects.
Circulatory.
Pd does not invade blood vessels.
But, degradation of wing could alter blood function.
Wing vessels of bats do tons of things.
Peristaltic contractions to push blood to heart.
-when flying and roosting upside down.
Precapillary sphincters and venous anastomoses.
-shunt blood away from capillary beds.
Regulate blood pressure.
-allows transition from upside down to flight.

Respiratory.
Bat wings can account for up to 10% of oxygen and c-oxide exchange.

Heat flux.
During arousals, damaged wings likely retain less heat.
Impairment of flight.
2. Infected bats arouse from torpor more frequently (WNSpic_5).
   
   Skin irritation hypothesis.
   Bats are intensifying grooming because the fungus is spreading. 
   Grooming is expensive behavior, especially for small animals like bats.
   Would predict increased time spent out of torpor.
   -No evidence of this.
   However, Brownlee and Reeder, 2013 (WNSpic_13) found increased grooming.

   Immune response hypothesis.
   Bats are raising temps to mount an immune response to fungus.
   Would also predict increased time spent out of torpor.
   -No evidence of this.

   Dehydration.
   Damage to bat wings causes increased evaporative water loss.
   Bats come out of torpor to drink.
   Would explain increased activity outside hibernacula.
   Cryan et al., 2013→Na and Cl decline in blood of bats as pathology increases.

   Wilcox et al., 2014.
   Experiment: infected vs. uninfected.
   No change in grooming.
   No change in visits to water.
   No change in locomotion.

   Trying to put it all together.
   Verant et al., 2014.
   See WNSpic_14.

Diagnosis.
Lorch et al., 2015.
   Trichophyton redelii causes similar lesions on bats.
   See WNSpic_15.
WNS Control

As of 2014, >7 million bats dead.

It’s rare for a superficial mycosis to cause mortality in otherwise healthy bats.
- some thought that immunocompetence was being compromised by some other agent.
- and, the WNS was secondary.
- this remains possible, but hasn’t been proven.

Initial molecular work suggested a single source, followed by epidemic spread.
- the EU vs NA GD work further demonstrated the source was European.
- this is important b/c strategies for controlling a new pathogen in a naïve set of populations are different than for controlling an endemic disease.
  1. Endemic ➔ manage the mortality.
  2. Novel ➔ manage the spread.
- the problem here is that the bats are what probably do most of the spreading.

National Plan: USFWS in May 2011.

Whitenosesyndrom.org is the face of a coordination effort.

Limiting human-mediated transport.
  - Caves and mines closed to public.
  - Decontamination protocols established for scientists and managers.
    - 10% bleach and things like Formula 409 work.
    - 20 minutes in 50 C water.
    - Hot air, >70 C, also works, but impractical for non-scientist.

Vaccines are unlikely to work b/c there is little immune response to the infection itself.

Most anti-fungals are highly toxic to bats.
- any treatment would need to be long-lasting.
  - bats would remove treatment during grooming.
  - difficult to administer to hibernating bats.
Cornelison et al., 2014.
  - tested 6 bacterially-produced volatile organic compounds (VOCs).
  - all inhibited growth to varying degrees.
  - combinations of VOCs worked better.
  - inhibition was best at 4 C, which would be good.
Boire et al., 2016.
  - tested cold-pressed terpeneless orange oil (CPT).
  - compared to amphotericin, caspofungin, fluconazole, voriconazole.
  - CPT also tested against various species of fungi and bacteria.
  - New CPT worked down to 6.25% dilution; complete inhibition at 100%.
  - Old CPT did not work below 100%
-CPT did not inhibit other bacteria or fungi tested.
-Amphotericin was only other that worked on WNS.

Cheng et al., 2016.
-tested *Pseudomonas fluorescens*.
-occurs on bats naturally.
-known to compete with fungi.
-only worked when applied simultaneous with the WNS itself.
-pre-treatment and post-infection treatment did not work.

Culling has been proposed, especially for initial control in new loci.
-modelling studies suggest it probably wouldn’t work.
-it’s never worked before.

Ark populations being established—see similarity to plague and distemper in black footed ferrets.
-will Pd still be there in the caves at reintroduction?
-difficult to maintain bat populations in captivity.

Disinfectant use in caves themselves.
-roughly the equivalent of spraying for mosquitoes.
-itself, highly effective.
-goal is to break the transmission cycle.
-problems.
-unintended consequences—mortality of other cave-dwelling organisms.
-we don’t know what those organisms would be, nor do we know what they do, nor do we know what would change if they died.
-we do know that cave-dwelling organisms are rare and unique, so losing them is to lose an entire evolutionary lineage.
-example Lascaux Cave, France.
-biocides used, including quaternary ammonium compounds.
-shifted the balance of microbiota.
-melanin-forming fungi now present and spreading.

See epidemic spread figures.