Welcome to the Conservation Lecture Series



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White-nose Syndrome: A Review of the Progression of the Disease and the Detection Challenges Facing the Western States

David T. Wyatt Sacramento City College, Biology Department California Department of Fish and Wildlife, Conservation Lecture Series – April 14, 2015

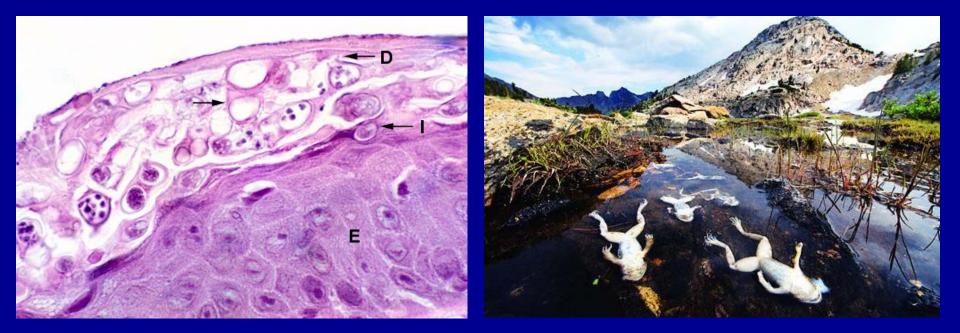
Emerging Infectious Diseases in Wildlife

- A number of highly infectious wildlife diseases with high mortality rates have emerged over the past few decades. These include:
 - <u>Chytridiomycosis</u> affecting amphibians worldwide.
 - <u>Myxomatosis</u> affecting rabbits in Great Britain.
 - <u>Devil Facial Tumor Disease</u> affecting Tasmanian Devils.
 - <u>West Nile Virus</u> affecting birds in North America.
 - <u>White-nose Syndrome</u> affecting bats in North America and Europe.



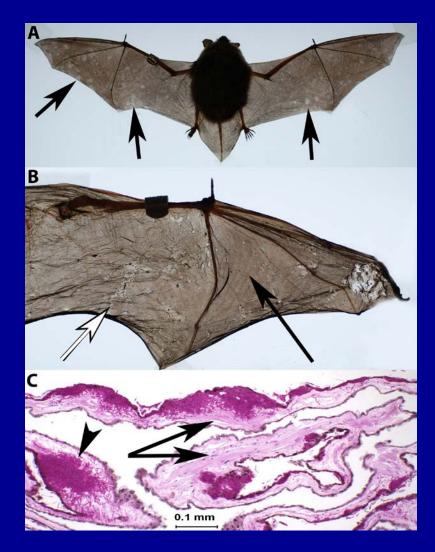
Batrachochytrium dendrobatidis (Bd)

• Bd is the causative organism for an fungal disease called Chytridiomycosis that is affecting amphibians worldwide.



Pseudogymnoascus destructans (Pd)

• Pd is the causative organism for an fungal disease called White-nose Syndrome that is affecting bats in North America and Europe.



Chytridiomycosis & WNS

- Both of these wildlife diseases have caused (and continue to cause) dramatic population declines in amphibians and bats.
- Chytrid has been found on all continents except Antarctica.
- White-nose Syndrome has been found in North America and Europe.



Chytridiomycosis

- The disease was first discovered in Queensland, Australia in 1993.
- Waterborne pathogen.
- The genus was first documented in an African Clawed Frog collected in 1938.
- Some frogs are unaffected others have 100% mortality.
- Unknown if current virulence is due to a new emergent pathogen or to recently increased virulence.

White-nose Syndrome

- The disease was first discovered in New York in 2006.
- Now recognized that it has been present in Europe for potentially an eon or more.
- Soil-borne pathogen.
- This is a cold-associated fungus.
- Bats that hibernate are susceptible to WNS and can have mortality rates up to 99% in some species.

Both Diseases...

- Prefer cool temperatures
- Penetrate skin and affect underlying tissues
- Affected individuals become anorexic
- Currently, no treatment is available in wild populations
- Recent research to affect the fungi has focused on bacteria species that could inhibit the growth of fungus

Fungal Disease

- Notoriously difficult to treat
- In humans, we have been unsuccessful in developing any vaccines against fungal illnesses
- Some topical treatments exist, but short term results for the most part





White-nose Syndrome

First noticed in Feb 2006. Caused by a fungus that was given the scientific name *Pseudogymnoascus destructans.*

White-nose Syndrome

- First detected in North America on February 17, 2006 in Howes Cave, New York.
- Now found in 25 U.S. States and 5 Canadian Provinces (2015).
- WNS has also been found in Europe.
- The cold-loving fungus *Pseudogymnoascus destructans* is the causative agent.
- Europe has many genetic variants of *P.d.* while North America has a single genetic variant.
 - This indicates that *P.d.* has been present in Europe for a greater length of time.
 - European bats appear to exhibit lower mortality rates.
- Bat mortality can be as high as 99% annually in the Northeast, the regional <u>mean</u> mortality rate is approximately 75%.
- In the Northeast, large hibernacula sites became infected within two years of the arrival of WNS in that region.

Hibernation

Benefits:

- Significantly reduces energy (food) needs when these resources are not available.
- Allows organisms to survive in areas that undergo severe cold and adverse climate conditions.

Challenges:

- Organism needs to store enough energy to survive hibernation.
- Requires that many metabolic functions be reduced or only periodically being turned on.





WNS and Hibernating Bats

Pseudogymnoascus destructans:

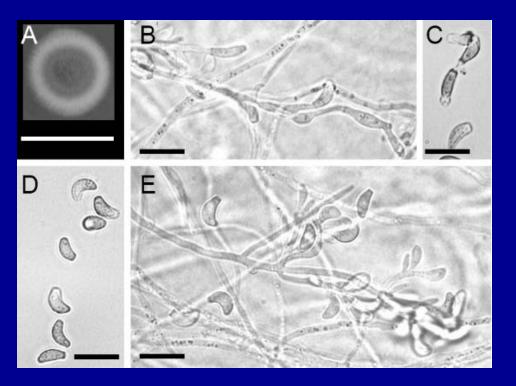
- Cold-adapted fungus that grows well at 40⁰F.
- Prefers high humidity conditions.

Bats:

- Hibernate at temperatures around 40⁰F.
- Hibernacula tend to have high humidity conditions.
- To conserve energy, bats "turn off" many metabolic processes (including the immune system) for long periods of time.
- When infected with WNS, bats "wake up" their immune systems more frequently and simply run out of energy before winter is finished. They literally starve to death.

Patterns of Disease Progression in Hibernacula

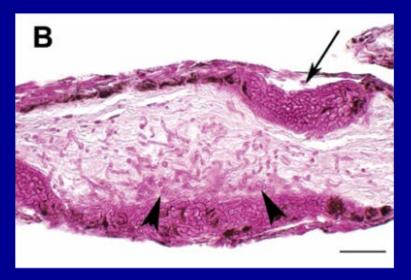
- <u>Pre-WNS Detection</u>
 - Some studies have reported an increased population count in winter preceding initial detection
- First Detection
 - Increased day flight activity of bats and changes in roosting areas.
 - One to several live bats detected with visible fungus on skin.
 - Little to no mortality is observed, population count remains stable.
- <u>Subsequent Winters</u>
 - Increasing prevalence of visibly affected bats (earlier onset of fungal growth).
 - Increased mortality of bats.
 - Decreasing population counts (early emergence of bats from hibernation) (off-site mortality is another possibility).

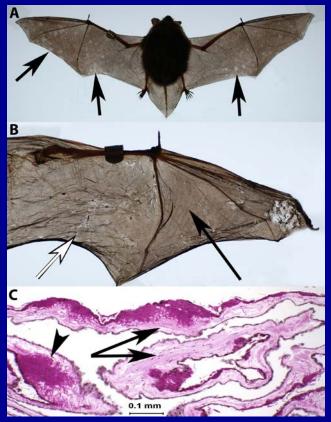




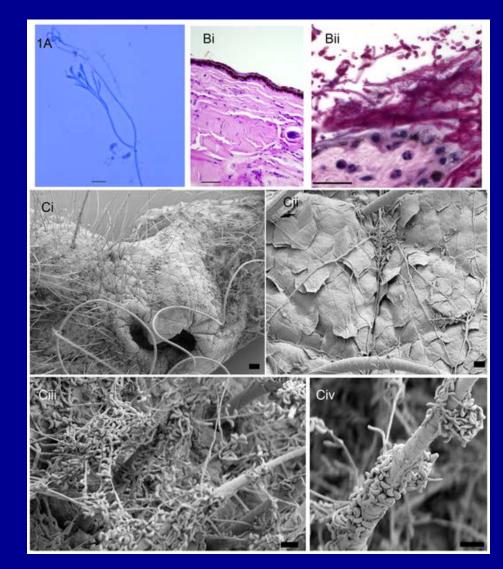
Pseudogymnoascus destructans

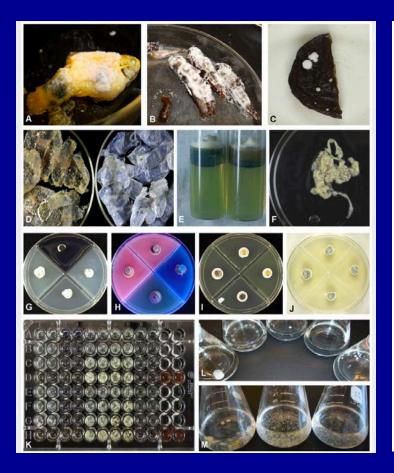






Tissue Damage from Pd





Nutritional Capability of and Substrate Suitability for *Pseudogymnoascus destructans*, the Causal Agent of Bat White-Nose Syndrome

Daniel B. Raudabaugh^{1,2}*, Andrew N. Miller²

1 Department of Plant Biology, University of Illinois, Urbana, Illinois, United States of America, 2 Illinois Natural History Survey, University of Illinois, Champaign, Illinois, United States of America

Abstract

Pseudogymnoascus destructans, the causal agent of bat white-nose syndrome, has caused nearly six million deaths in North American bats since its introduction into the United States in 2006. Current research has shown that caves can harbor P. destructans even after the infected bats are removed and bats no longer visit or inhabit previously infected caves. Our research focuses on elucidating reservoir requirements by investigating the nutritional capabilities of and substrate suitability requirements for six different P. destructans isolates from various localities including Illinois, Indiana, New York (Type specimen), and Pennsylvania. Enzyme assays implicate that both urease and b-glucosidase appear to be constitutive, lipase and esterase activity were more rapid than proteinase activity on 6% gelatin, gelatin degradation was accompanied by medium alkalinization, the reduction of thiosulfate generated hydrogen sulfide gas, chitinase and manganese dependent peroxidase activity were not visually demonstrated within eight weeks, and keratinase activity was not evident at pH 8 within eight weeks. We demonstrate that all P. destructans isolates are capable of growth and sporulation on dead fish, insect, and mushroom tissues. Sole nitrogen source assays demonstrated that all P. destructans isolates exhibit Class 2 nitrogen utilization and that growth-dependent interactions occur among different pH and nitrogen sources. Substrate suitability assays demonstrated that all isolates could grow and sporulate on media ranging from pH 5-11 and tolerated media supplemented with 2000 mg/L of calcium and 700 mg/L of three separated sulfur compounds: thiosulfate L-cysteine. and sulfite. All isolates were intolerant to PEG-induced matric potential with delayed germination and growth at -2.5 MPa with no visible germination at -5 MPa. Interestingly, decreasing the surface tension with Tween 80 permitted germination and growth of P. destructans in -5 MPa PEG medium within 14 days suggesting a link between substrate suitability and aqueous surface tension altering substances.

Citation: Raudabaugh DB, Miller AN (2013) Nutritional Capability of and Substrate Suitability for Pseudogymnoascus destructans, the Causal Agent of Bat White-Nose Syndrome. PLoS ONE 8(10): e78300. doi:10.1371/journal.pone.0078300

Editor: Vishnu Chaturvedi, California Department of Public Health, United States of America

Received June 25, 2013; Accepted September 18, 2013; Published October 21, 2013

In summary, Pd can break down pretty much all complex carbon sources and obtain energy and can grow at a wide range of pH values (only inhibition observed was with very acidic conditions). Pd produces a very alkaline material that essentially chemically "burns" the skin tissues.

But the study also found that Pd is very temperature dependent (needing cold temperatures – it stops growing above 68⁰F) and it has difficulty absorbing water.



Frequent Arousal from Hibernation Linked to Severity of Infection and Mortality in Bats with White-Nose Syndrome

DeeAnn M. Reeder¹*, Craig L. Frank², Gregory G. Turner³, Carol U. Meteyer⁴, Allen Kurta⁵, Eric R. Britzke⁶, Megan E. Vodzak¹, Scott R. Darling⁷, Craig W. Stihler⁸, Alan C. Hicks⁹, Roymon Jacob¹, Laura E. Grieneisen¹, Sarah A. Brownlee¹, Laura K. Muller⁴, David S. Blehert⁴

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Abstract

White-nose syndrome (WNS), an emerging infectious disease that has killed over 5.5 million hibernating bats, is named for the causative agent, a white fungus (*Geomyces destructans* (Gd)) that invades the skin of torpid bats. During hibernation, arousals to warm (euthermic) body temperatures are normal but deplete fat stores. Temperature-sensitive dataloggers were attached to the backs of 504 free-ranging little brown bats (*Myotis lucifugus*) in hibernacula located throughout the northeastern USA. Dataloggers were retrieved at the end of the hibernation season and complete profiles of skin temperature data were available from 83 bats, which were categorized as: (1) unaffected, (2) WNS-affected but alive at time of datalogger removal, or (3) WNS-affected but found dead at time of datalogger removal. Histological confirmation of WNS severity (as indexed by degree of fungal infection) as well as confirmation of presence/absence of DNA from Gd by PCR was determined for 26 animals. We demonstrated that WNS-affected bats aroused to euthermic body temperatures more frequently than unaffected bats, likely contributing to subsequent mortality. Within the subset of WNS-affected bats that were found dead at the time of datalogger removal, the number of arousal bouts since datalogger attachment significantly predicted date of death. Additionally, the severity of cutaneous Gd infection correlated with the number of arousal episodes from torpor during hibernation. Thus, increased frequency of arousal from torpor likely contributes to WNS-associated mortality, but the question of how Gd infection induces increased arousals remains unanswered.

Citation: Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A, et al. (2012) Frequent Arousal from Hibernation Linked to Severity of Infection and Mortality in Bats with White-Nose Syndrome. PLoS ONE 7(6): e38920. doi:10.1371/journal.pone.0038920

Editor: Raphaël Arlettaz, University of Bern, Switzerland

Received September 20, 2011; Accepted May 16, 2012; Published June 20, 2012

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Epidemiology of WNS

- Earliest known case of WNS was September 30 (Histological Only).
- Latest known case of WNS was June 1 in Canada (Histological Only).
- The incubation period for fungal growth appears to be 7 to 12 weeks.
- Mortality can occur as soon as 8 weeks.
- Visibly affected bats have been observed throughout a hibernacula.
- Juveniles are susceptible in their first year of hibernation.
- No apparent sex/gender selection.
- Scar tissue is often depigmented but this is <u>not</u> diagnostic.
- Bats can recover from tissue damage if they survive hibernation with WNS supportive care increases survival rates.





Immune Reconstitution Inflammatory Syndrome

- Immune Reconstitution Inflammatory Syndrome
 - Bat immune systems are suppressed during hibernation.
 - Some bats, after surviving WNS over the winter, emerge from hibernation and their immune systems over-react and it begins to aggressively attack healthy tissue as well as infected tissue.
 - "It's cellular suicide. [The immune system] comes out in a huge wave, going out to those areas of infection and kills everything."
 - Carol Meteyer (USGS)
 - Never before seen in wildlife.
 - Only seen in one other species humans with AIDS.

From: Meteyer, et. al. - Virulence, Vol 3, Issue 7 (November 2012).

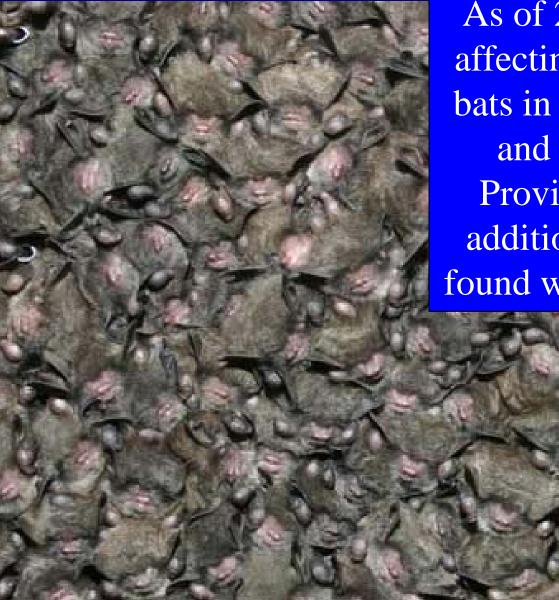


A scientific paper published on April 8, 2012 is suggestive that this fungus was brought over from Europe – possibly on shoes or clothing of someone. In the U.S. the first outbreak occurred in a cave frequented by visitors.

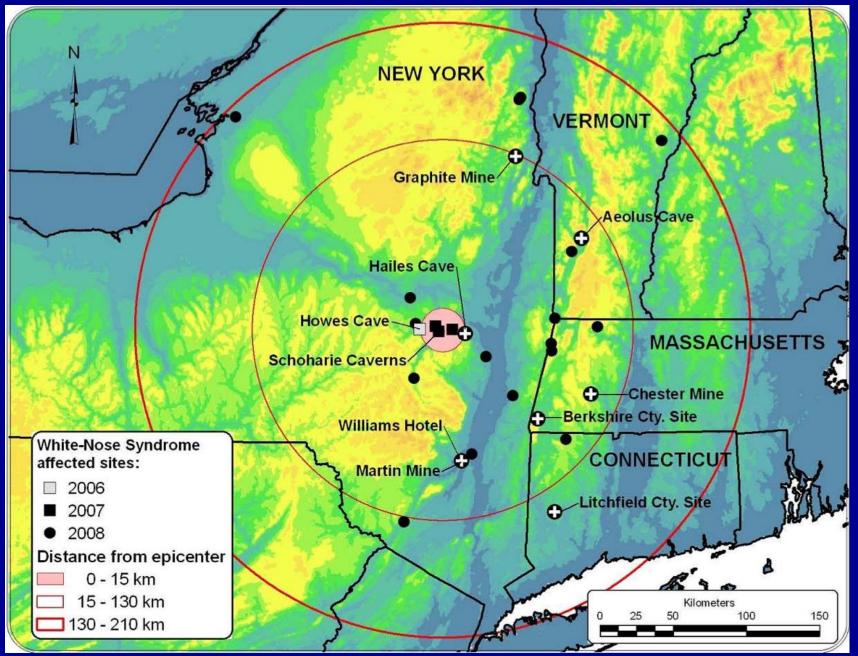


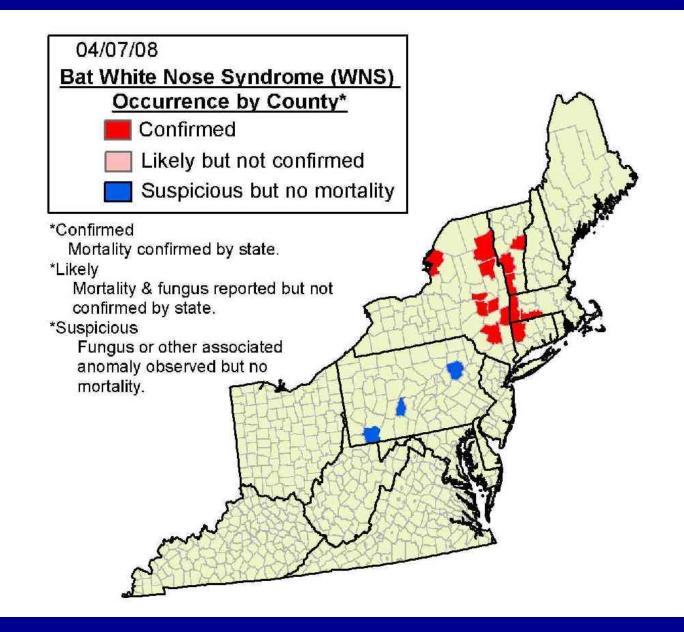


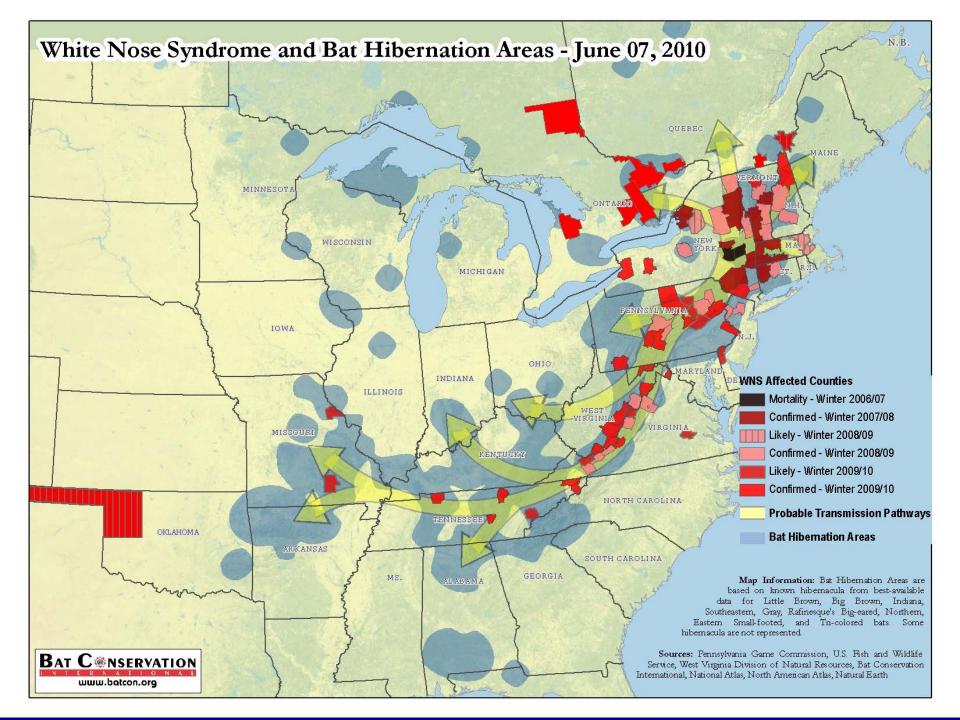


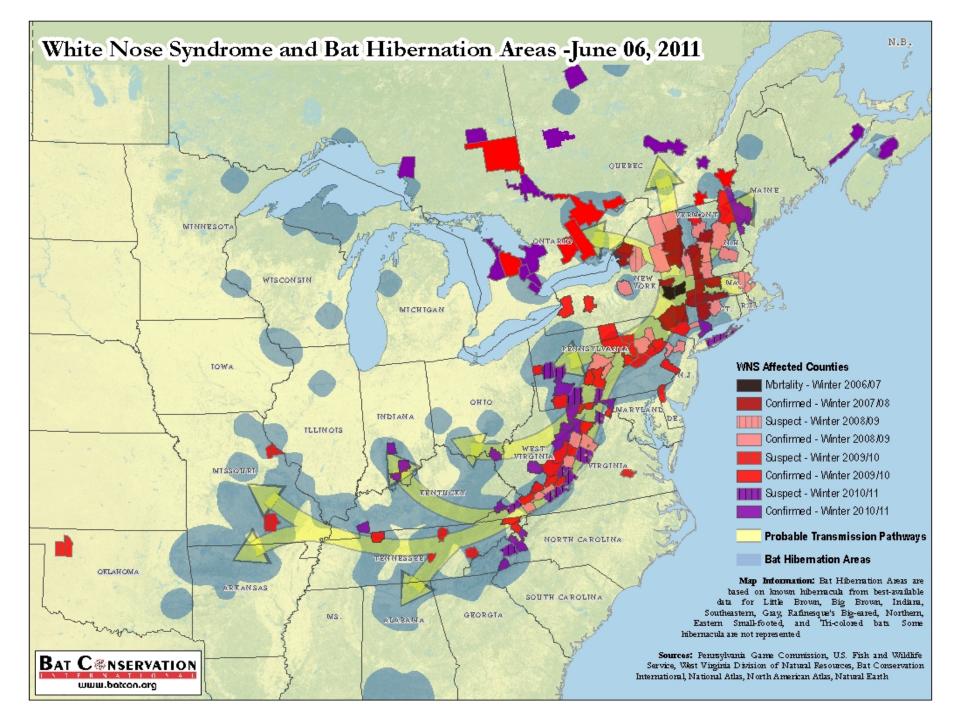


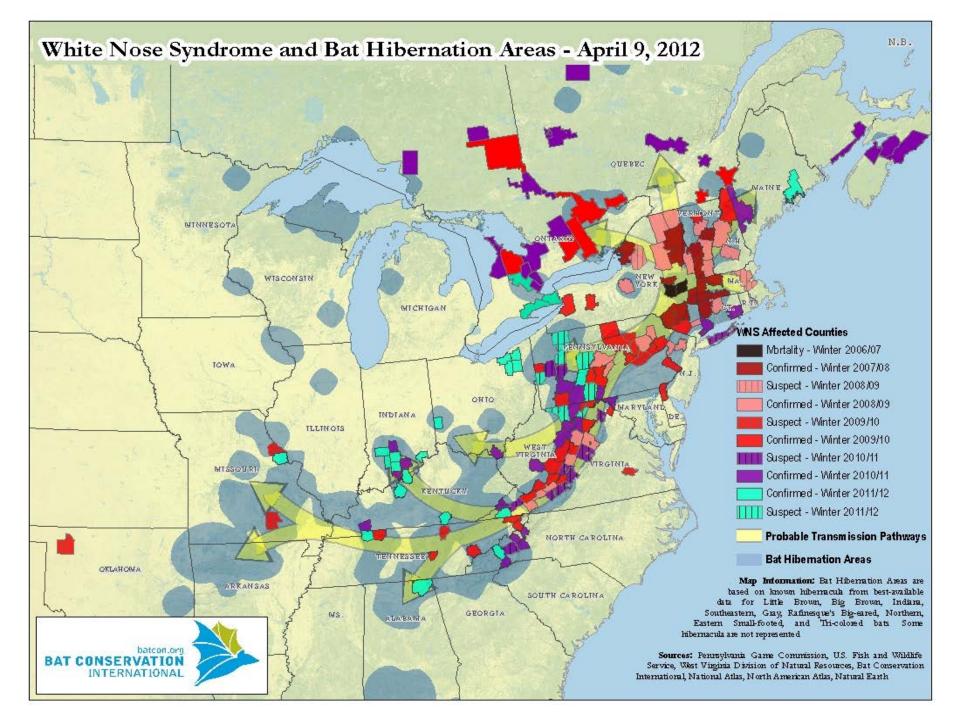
As of 2015, WNS is affecting 7 species of bats in 25 U.S. States and 5 Canadian Provinces with an additional 5 species found with the fungus. Blehert, et. al. (2008) – Fungal isolates were cultured from caves ID' d with plus signs.

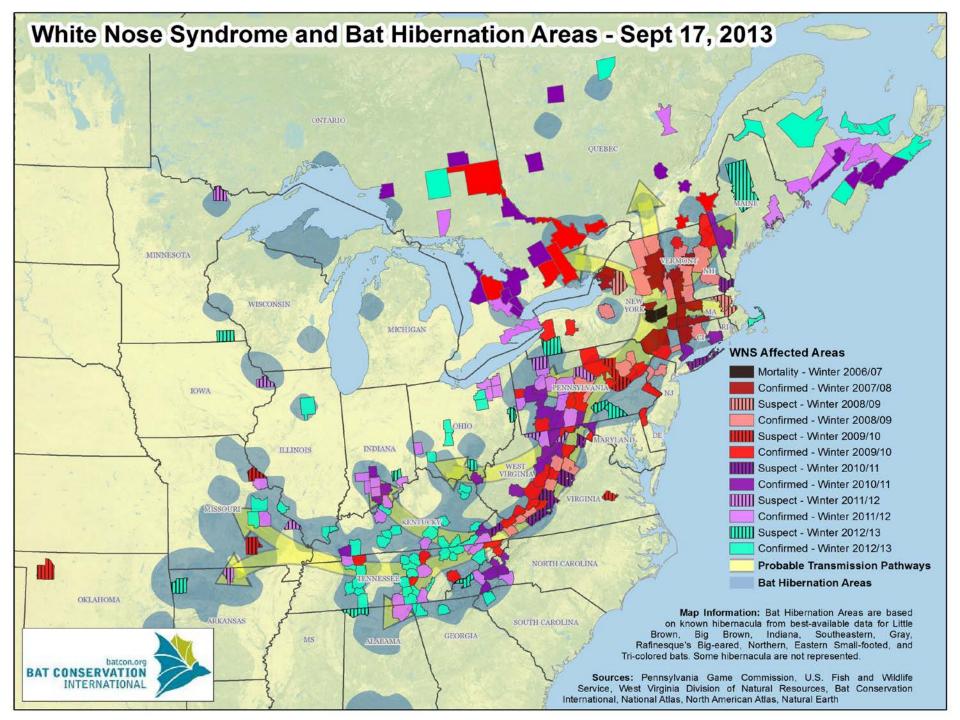


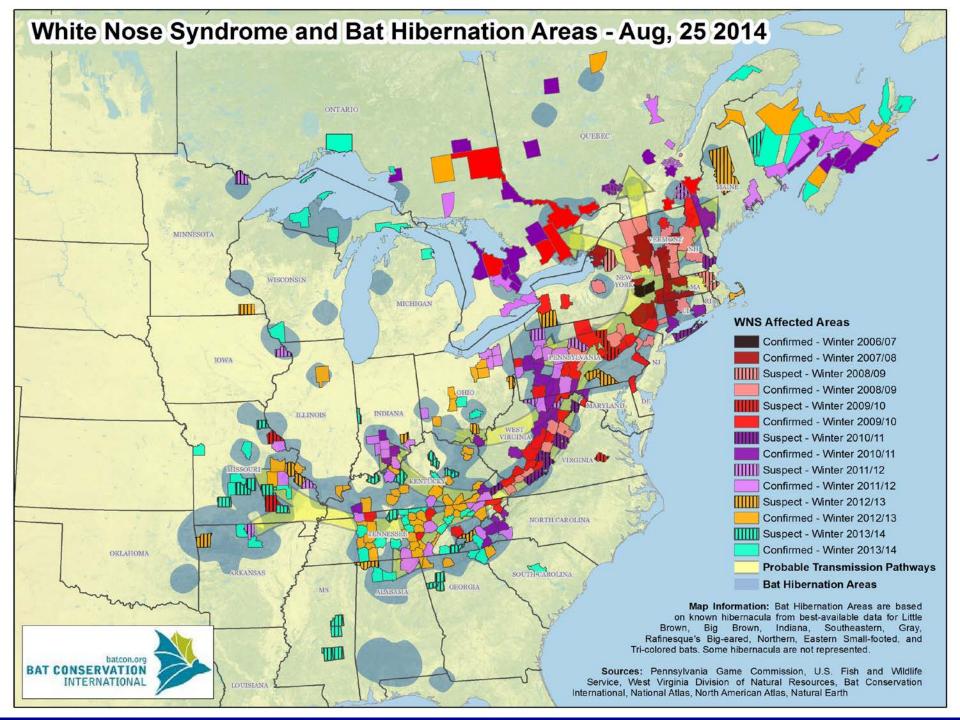


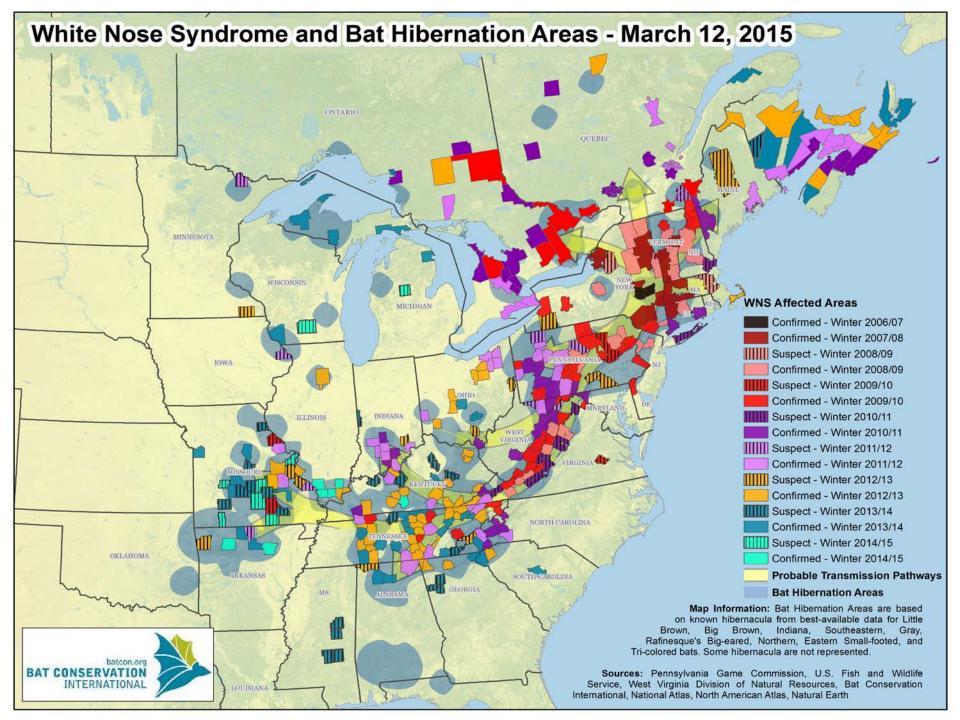


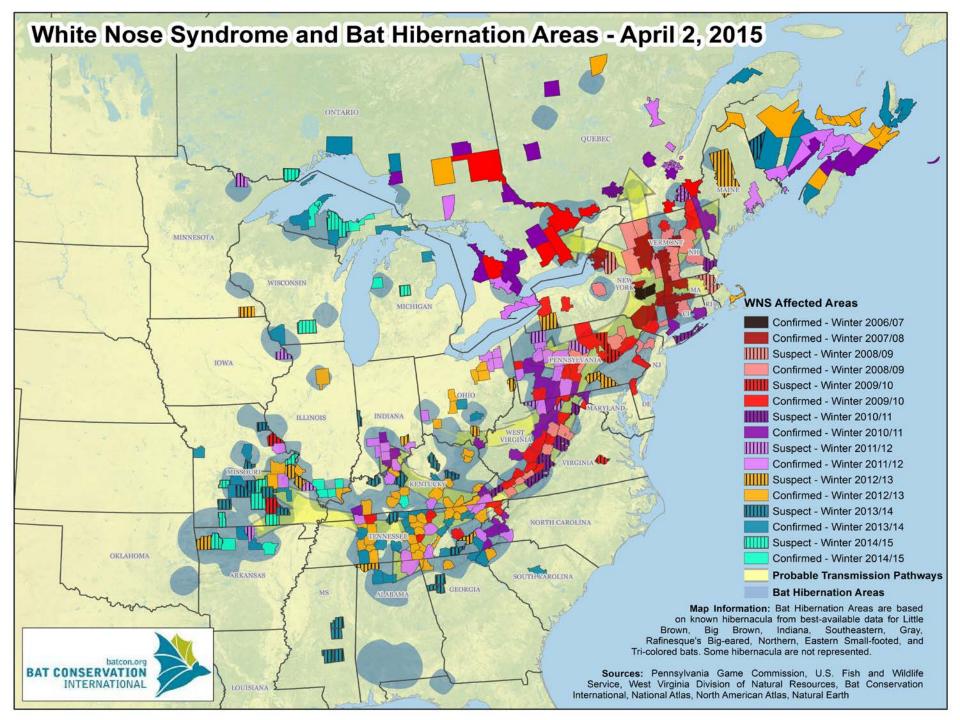












Hibernating Bat Species and WNS

Affected Bat Species (2015)

Eptesicus fuscus – Big brown bat *Myotis grisescens* – Gray bat (E) *Myotis leibii* – Eastern small-footed bat *Myotis lucifugus* – Little brown bat *Myotis septentrionalis* – Northern bat (T) *Myotis sodalis* – Indiana bat (E) *Perimyotis subflavus* – Tricolored bat

Corynorhinus townsendii virginianus – Virginia Big-eared Bat Corynorhinus rafinesquii – Rafinesque's big-eared bat Myotis austroriparius – Southeastern bat Lasionycteris noctivagans – Silverhaired bat Lasiurus borealis – Eastern red bat **Yellow highlight = Pd positive**

N. California Bat Species

Antrozous pallidus – Pallid bat (SC) Corynorhinus townsendii – Townsend's big-eared bat (T) Eptesicus fuscus – Big brown bat Euderma maculatum – Spotted bat (SC) Lasionycteris noctivagans – Silverhaired bat

Myotis californicus – California bat Myotis ciliolabrum –

Western small-footed bat Myotis evotis – Western long-eared bat Myotis lucifugus – Little brown bat Myotis thysanodes – Fringed bat (SC) Myotis velifer – Cave myotis Myotis volans – Long-legged bat (SC) Myotis yumanensis – Yuma bat Parastrellus hesperus – Canyon bat

Size Comparisons



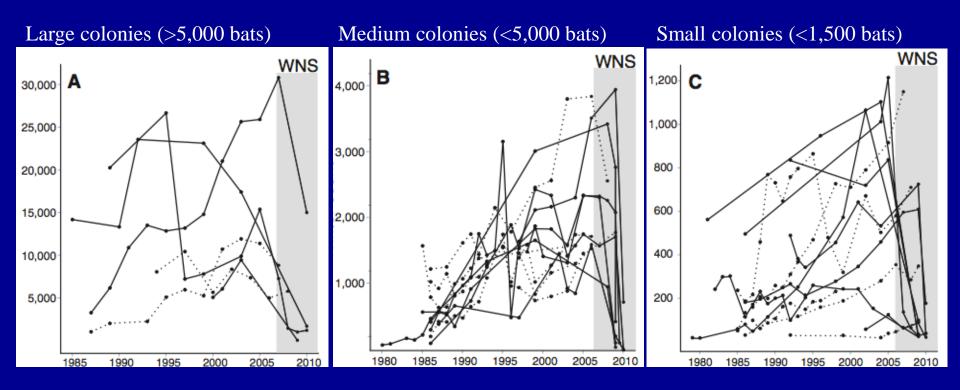








Population Trends of Myotis lucifugus in the Northeastern U.S.



Solid Lines = sites with bats infected by White-nose Syndrome. Dotted lines = sites without bats infected by White-nose Syndrome.

Some uninfected sites had large declines in the mid-1990's due to winter flooding.

Mortality from 42 Hibernacula in NY, PA, VT, VA, WV

The below table summarizes survival at 42 bat hibernacula with 2+ years of mortality from White-nose Syndrome

Species	Sum Pre-WNS	Sum Post-WNS	Total Decline
Little Brown Bat	350,000+	30,280	91%
Northern Long-eared Bat	1,700+	31	98%
Tricolored Bat	3,107	783	75%
Indiana Bat	55,028	15,650	72%
Eastern Small-footed Bat	1,303	1,142	12%
Big Brown Bat	2,919	1,713	41%
ALL BATS	412,340	49,579	88%

Economic Impacts of WNS:

Between 2006 and 2012, WNS has killed between 5.7-6.7 million bats.

A study published in April 2011 examined the economic impact of WNS to Agriculture:

• "Value of pest-control services to agriculture provided by bats in the United States range from a low of \$3.7 billion to a high of \$53 billion per year." - 2011

• "Noticeable economic losses to North American agriculture could occur in the next 4-5 years as a result of emerging threats to bat populations." -2011

• In California – for 2010 – nearly \$40 Billion in revenue was generated by agriculture.

Economic Impacts of WNS:

"A single little brown bat, which as a body no bigger than an adult' s thumb, can eat 4-8 grams (the weight of about a grape or two) of insects each night...although this may not sound like much, it adds up...the loss of the one million bats in the Northeast has probably resulted in between 660-1320 metric tons of insects no longer being eaten each year by bats in the region." – 2011 - (As of 2012, this estimate has gone up to 3,762-7,524 metric tons = 8.3-16.6 Million Pounds of insects)

"Bats eat tremendous quantities of flying pest insects, so the loss of bats is likely to have long-term effects on agriculture and ecological systems."

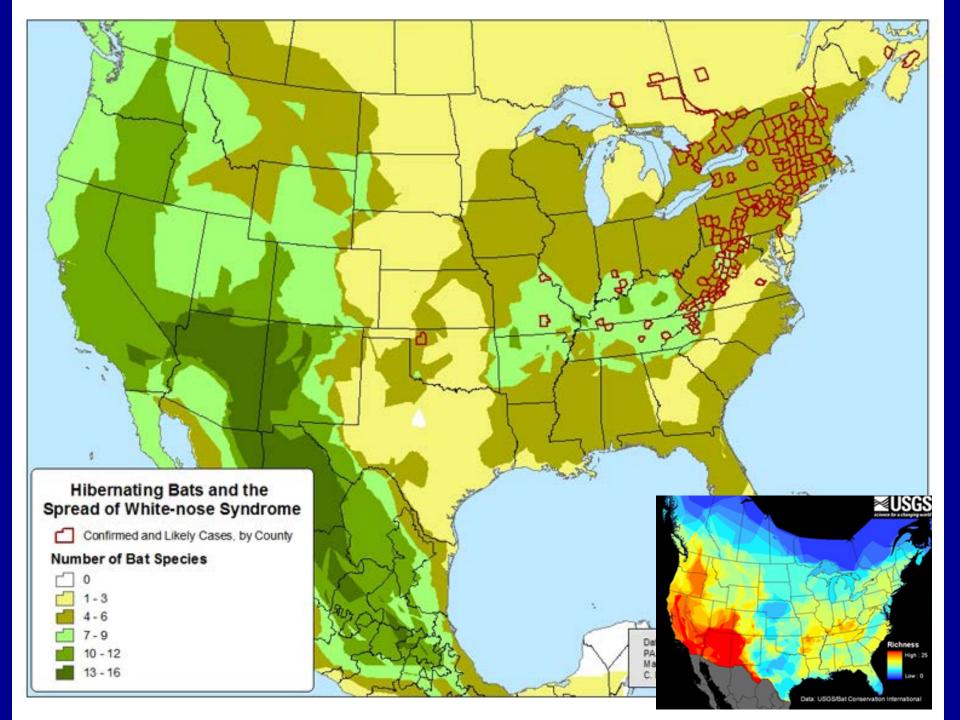


"This is one of those situations where humans are definitely going to see the effects of it. It's like your health; you don't think about it or notice it until it's gone. Bats pull tremendous numbers of insects out of the air every night and we don't thank them for that often enough. When they disappear, people are really going to start noticing."

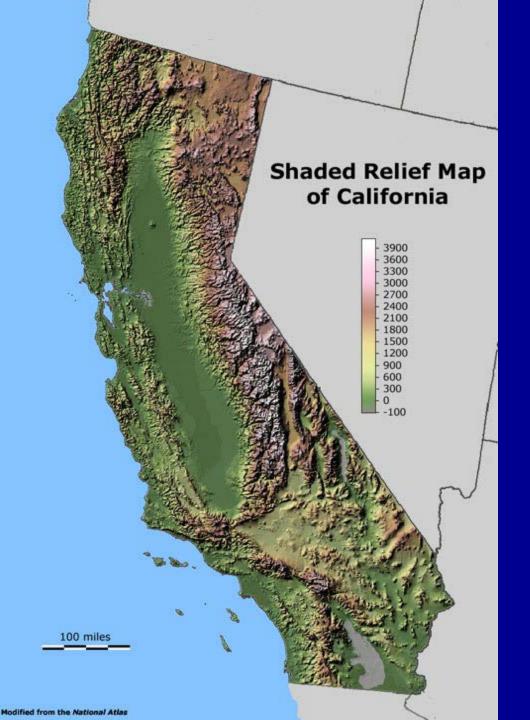
- Rick Adams, University of Northern Colorado

"If it gets into the West, it's really going to accelerate even further and affect so many more species of bats. Ecologically, it would be an Armageddon type of scenario."

- Rick Adams, University of Northern Colorado







Challenges out West:

- Hibernating Western bats do not aggregate in very large colonies.
- Western bats are spread more diffusely through day-roosting locations.
- The West has exponentially more caves and mines than the East.
- Small colonies of WNS susceptible species make detection of impacts much harder.







Bats with Dataloggers



Bats with Radiotransmitters





Documenting Scar Tissue on Wings and Unusual Behaviors



So, What Else Are We Doing?



Bacteria as a Probiotic?

- Paper published April 8, 2015.
- Describes results of studies of bacteria species affecting Pd.
- Found six bacterial isolates in the genus *Pseudomonas* that significantly inhibited growth of Pd.
- These *Pseudomonas* isolates naturally occur on bats.

RESEARCH ARTICLE

Bacteria Isolated from Bats Inhibit the Growth of *Pseudogymnoascus destructans*, the Causative Agent of White-Nose Syndrome

Joseph R. Hoyt*, Tina L. Cheng, Kate E. Langwig, Mallory M. Hee, Winifred F. Frick, A. Marm Kilpatrick

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Abstract

Emerging infectious diseases are a have recently emerged and caused in cluding amphibians, bats, rattlesnak fungal skin pathogen *Pseudogymno* species with extinction and there are biome is increasingly understood to p isolated bacteria from the skin of fou

OPEN ACCESS

Citation: Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF, Kilpatrick AM (2015) Bacteria Isolated from Bats Inhibit the Growth of *Pseudogymnoascus destructans*, the Causative Agent of White-Nose Syndrome. PLoS ONE 10(4): e0121329. doi:10.1371/ journal.pone.0121329

Academic Editor: R. Mark Brigham, University of Regina, CANADA

Received: August 26, 2014

Accepted: January 30, 2015

Published: April 8, 2015

Bacteria as a Probiotic?

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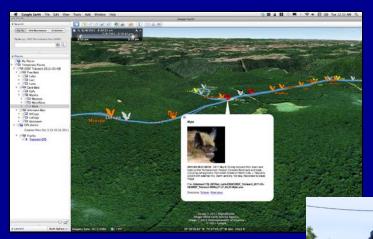
Emerging infectious diseases are a have recently emerged and caused cluding amphibians, bats, rattlesnak fungal skin pathogen *Pseudogymno* species with extinction and there are biome is increasingly understood to isolated bacteria from the skin of fou



- *Pseudomonas* were cultured from four species of bats from eastern North America.
- Can these species of bacteria provide a means to partially inhibit Pd growth and thereby allow bats to survive hibernation?

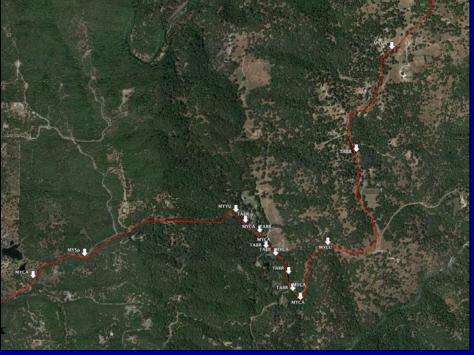
Current few management options exist that reduce mortality of bats in regions affected by WNS. Antifungal drugs resulted in higher mortality of treated groups than in control groups and other chemical treatments have not been applied *in situ* therefore remain an unknown in terms of effectiveness.

Acoustic Monitoring:



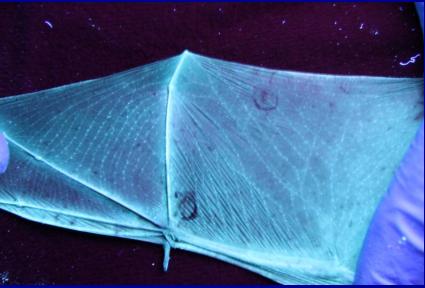








Pre-WNS infection under UV light

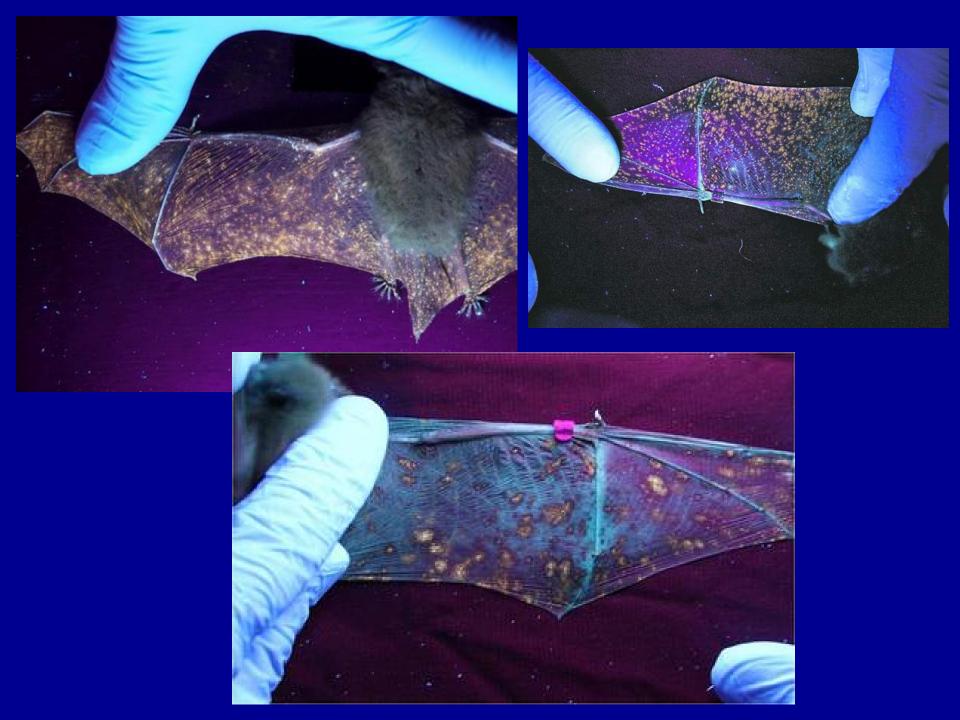


Post-WNS infection under UV light

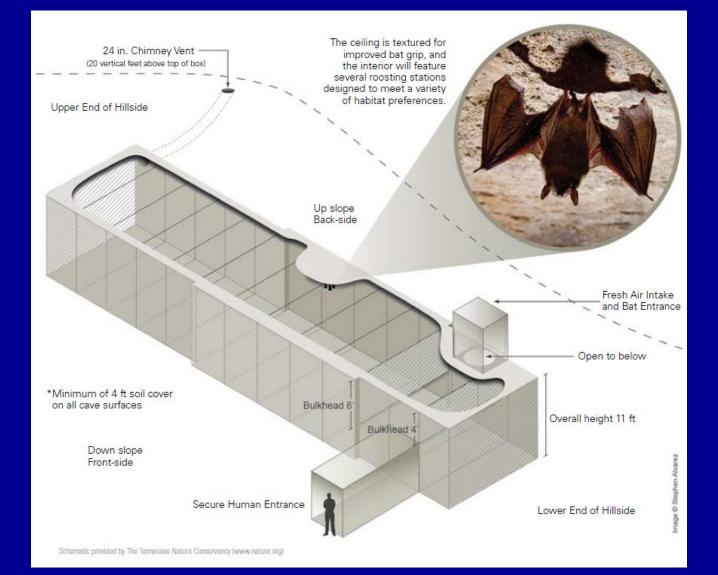
Use of Ultra-violet Light: Pd glows yellowish to yellow-green under UV light (Blacklights)



Photos on the left are courtesy of John Gumbs who recently studied this aspect of Pd in a project funded through Experiment.com (Science Crowdfunding Site).



Artificial Hibernacula:



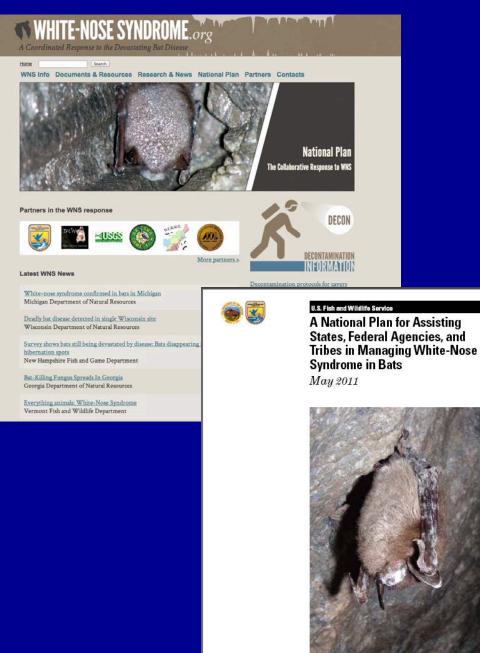


Alvarez Photography Stephen Alvarez



WNS National Plan

- A National Plan to address WNS was developed in May 2011.
- Federal, State, Tribal, and Private entities were involved in preparation of the National Plan.
- Three major objectives:
 - Tracking the geographic spread of WNS.
 - Identifying new susceptible species.
 - Minimizing transmission risks to bats.
- National WNS Tracking Database:
 - Being developed and maintained by USGS Ft. Collins.
 - Federal, State, Tribal Agency users.
 - Centralized repository for population monitoring and surveillance.
 - Will be a secure database with the ability to map, search, and report.
- Primary Focus by Agencies:
 - Decontamination procedures, cave closures/advisories, education (both public and government).



Bat affected by white-nose syndro



WNS Surveillance and Epidemiology

- Active Surveillance
 - Hibernacula surveys, maternity colony surveys, capture studies, acoustical surveys.
 - •Advantages: Earlier detection, targeted sampling, population/ environmental information.
 - Disadvantages: Expensive, time consuming, typically more invasive and has the potential to introduce *G.d.* to new areas.
- Passive Surveillance
 - Citizen reporting, wildlife rehabilitators, animal control agents, County health departments.
 - Advantages: Non-invasive, inexpensive, may identify new areas of bat activity.
 - Disadvantages: Biased samples, unknown points of exposure.

North American Bat Monitoring Program

- North American Bat Monitoring Program establishment of a national program to monitor and track bat populations in North America.
- Listed as a high priority in the National WNS Response Plan.
- NaBAT establishes 10x10km grid cells throughout a state for evaluation to determine feasibility for stationary and/or mobile acoustic monitoring.
- Pilot program recently established in CDFW (Scott Osborn and D. Scott Newton).
- Susan Loeb (USFS), Jeremy Coleman (USFWS), Laura Ellison (USGS), Tom Rodhouse (NPS), Tom Ingersoll (DoD), Cori Lausen (WCS Canada), Wayne Thogmartin (USGS), Kathi Irvine (USGS), John Sauer (USGS), and Jonathan Reichard (USFWS)

WNS Surveillance – Western North America

- Western hibernacula are typically less than 25 bats.
- Difficult access due to topography surveys are difficult.
- Significant extent of mountainous terrain.
- The west has many abandoned mines.
- Many western bat species will hibernate in non-cave structures.
- Not all normally hibernating species hibernate out here.
- Not much is known about wintering behavior.





Questions in the West

- Lots of questions because western bats have different behavior, different environmental conditions, and more varied roost sites than eastern bats.
 - Could small, dispersed winter colonies with varied roost options be less susceptible to WNS?
 - If small colonies arouse from torpor more often in some areas, will this result in resistance to WNS?
 - Will *P.d.* persist in non-cave habitat such as cracks and crevices?
 - Are there geographical barriers or hostile ecological checks that might inhibit the spread of *P.d.*?
 - Will some western bat species show resistance to WNS?
 - How will a drying and warming climate in the west affect bat behaviors involving hibernation, migration, etc?

Some Recently Funded Research (Partial List)

- Growth and survival of *Pseudogymnoascus destructans* under various conditions.
- Immune response of hibernating bats and postexposure response.
- Behavior and physiology of hibernation.
- Population-level impacts (local and range-wide).
- Genetics predisposition, post-exposure response, and fungal.
- Disinfection/Control of *P.d.* including in the environment.
- Dynamics of transmission.
- Epidemiological modeling.
- Improving detection of *P.d.* on bats and in the environment.
- Susceptibility and potential for resistance.







So, What Can Biologists Do?

- Get the National WNS Plan (www.whitenosesyndrome.org).
- Educate professional & amateur biologists, wildlife rehabbers, cavers.
- Take pictures of suspicious fungal growths or scars on bats.
- When handling bats, use appropriate techniques to prevent contamination.
- Disinfect all equipment Clorox (10% Solution), Formula 409 Antibacterial Cleaner, Lysol Quaternary Disinfectant Cleaner, Lysol Disinfecting Wipes, or submerse equipment for a minimum of 20 mins in water >122 F.
- Report unusual observations to the California Department of Fish and Wildlife and to the U.S. Fish and Wildlife Service.

How The Public Can Help:

- Encourage your state and federal legislators to allocate funding towards the effort to understand and fight White-nose Syndrome.
- Report unusual late-winter bat behavior (such as bats flying during the daytime, bats crawling around the ground during winter, etc) and reporting unexplained bat deaths to your state wildlife agency.
- Adhere to state, federal, and local cave advisories and closures to help prevent the transmission of WNS.
- Educate your friends and families about the benefits of bats and the WNS crisis.
- Follow established decontamination guidelines when caving or working with bats never bring equipment from a WNS-positive State to a WNS-negative State.

Thank you to:

- California Department of Fish and Wildlife for the Conservation Lecture Series and for joining the NaBAT effort.
- The many agencies and organizations that have joined to understand and address WNS and to coordinate efforts to address this disease.
- Bat Conservation International for actively pursuing public education about WNS.
- Dr. Merlin Tuttle for being a public voice educating about the importance of bats and teaching about the threat of WNS...and for introducing me to the fantastic world of bat research.
- All of the biologists working to study this disease.
- And a toast to our furry, flying subjects.

Pertinent Websites:

- http://whitenosesyndrome.org
- http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/
- http://www.batcon.org/

White-nose Syndrome: A Review of the Progression of the Disease and the Detection Challenges Facing the Western States

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