White Nose Syndrome
March, 2009 Research Status Report
By Peter Youngbaer, NSS WNS Liaison

Introduction:

Many people outside the White Nose Syndrome (WNS) research community are hungry for detailed information. They want to know what research is in progress and what results have been obtained. This report is an attempt to summarize what is known to date, drawing from presentations made by the researchers to each other during a Feb. 20, 2009 national webinar. It also reflects direct communications and observations, particularly with the NSS-funded projects.

This paper will not reiterate earlier reports from the WNS Science Strategy meeting in Albany, NY, in June of 2008. Some of the findings reported at that time indicated little or no evidence of the bats being affected by viruses, parasites, or environmental toxins. While these were not eliminated as possible causes, the scientific consensus was that these were low priority for investigation. For context, please refer to the Science Strategy Proceedings link on the main WNS webpage.

To date, despite literally hundreds of media interviews that often contain tidbits of new information, only two studies have been published. The first, in October, was by Dr. David Blehert, et al, in which the new psychrophilic (cold-loving) fungus of the *Geomyces spp*. was isolated and described. It is the presence of this specific fungus that has been identified in most of the bats' bodies that have been analyzed by Dr. Blehert and his team at the United States Geological Survey's National Wildlife Health Center (NWHC) laboratory, in Madison, Wisconsin. While there is no test, per se, for WNS, when state wildlife officials "confirm" WNS in their bats, what they are really saying is that the NWHC lab has confirmed the presence of the *Geomyces spp.* fungus.

The second publication is very recent, and pertains to the work of Justin Boyles, et al, regarding a potential mitigation strategy involving thermal refugia. The newspapers have had a field day with their headlines, such as "Heaters seen as cure for WNS, "etc. While the publication only announces the completion of their simulation, the strategy is currently being field tested in a cave in Manitoba, Canada. This project models what bats have been observed doing in nature, which is, upon arousal, seeking a warm spot in the cave in order to conserve energy while awake. Practical application on a large scale remains an open question.

Both of these published results have been linked on the WNS Liaison website, directly and through the media digest, so we will not repeat them here. The majority of what follows is a summary of presentations made during the February 20 webinar. Additional comments are drawn from direct communications with the researchers, and direct work with the NSS-funded projects.

Al Hicks, NYDEC Mammologist, et al:

The Feb. 20 WNS Web Conference began with an update on bat population survey trends so far this winter. States differ in the number and kinds of bat hibernacula they survey, and baseline data varies. 2008-2009 is also the year of the biennial Indiana bat survey, as part of the endangered species management plan, so field staff had funding to check hibernacula.

With data from many states throughout the northeast, Hicks reported that the numbers for *Myotis lucifugus* (little brown) continue to decline. Some comparative NY sites (up through Feb. 17) are:

Site Name (WNS year)	Date	Myluc	Myle	Myse	Pesu	Epfu	Total
Gages (2)	03/01/08	93	0	0	2	0	95
Gages (3)	02/17/09	56	0	0	0	0	56
Barytes (3)	01/06/08	3	0	0	1	7	11
Barytes (4)	02/13/09	1	2	0	1	13	17
Howe (3)	01/06/08	63	5	1	6	2	77
Howe (4)	02/13/09	27	11	1	4	8	51
Knox (2)	03/12/08	363	3	0	0	0	366
Knox (3)	02/17/09	179	0	0	0	3	182

(Key: Myluc= *Myotis lucifugus*; Myle= *Myotis leibii*; Myse= *Myotis septentrionalis*; Pesu= *Perimyotis subflavus*; Epfu= *Eptesicus fuscus*)

(Note: Gage's Cave had 968 bats in 1985; Barytes 66 in 1986; Howe 96 in 1995; and Knox 571 in 1986)

For *Myotis Sodalis* (Indiana):	2007	2008	2009
Williams Preserve	13,014	124	apparently unchanged
Graphite Mine	109	158	20
Williams Hotel	24,317	19,996	11,500
Barton Hill Mine	9,393	9,564	apparently unchanged

(note on Graphite – parts of the mine are unstable and not visitable – Hicks believes bats may be moving within the mine).

Conclusions: Populations of *Myotis lucifugus* continue to decline in years 3 and 4 of WNS. Small samples of WNS continue in *Myotis septentrionalis* and *Perimyotis subflavus. Myotis leibii* uncertain. No confirmation yet of WNS in *Eptesicus fuscus*. Drier hibernacula appear to only slow down WNS in *Myotis sodalis*, but not stop it.

Hicks finished his presentation with short descriptions of the chronology of WNS appearance in new states: NH, NJ, PA, WVA, plus new counties in VT and NY. One detailed chronology of Shindle Iron Mine, PA, showed the initial appearance of WNS to full blown to be a period of about 8 weeks. During that time, the number of bats showing fungus grew rapidly, and the colony became much more active and moved to the entrance of the mine.

Dr. David Blehert, et al, USGS National Wildlife Health Center:

The NWHC is involved in numerous activities related to WNS. Since last winter, 69 of 109 bats sent from 16 states have had postmortem workups and mycology performed on them. In addition, field personnel have taken many tape samples of fungal appearances on bat muzzles and sent them for analysis. It is through this process that the presence of the *Geomyces spp*. is confirmed and states announce that WNS has been found in their jurisdiction. In addition, a data base is growing of various bat species that have been tested for the fungus.

A major winter research project this year has been a series of infection trials, in climate controlled laboratory bat chambers. Eight separate trials are underway with three groups of bats: A control group of healthy Wisconsin *Myotis lucifugus* (little brown bats), in two different settings; a second group of healthy WI *Myotis lucifugus*, which have been exposed to the *Geomyces* fungal strain in three different manners; a third group of healthy WI *Myotis lucifugus* that have been exposed to WNS-affected NY *Myotis lucifugus*.

While this project is still underway, Dr. Blehert reported some interim findings. Many of the NY bats, as might be expected, have now died. However, their arousal bouts were observed to be every three days (compared to a typically healthy bout interval of 13-15 days). WI bats became infected with the fungus – both from exposure to the fungus in the second group, and exposure to the sick NY bats. A few of them have been euthanized and necropsied, and they show early stage cutaneous fungal infection, a 10-day arousal bout, and gross wing damage as was observed in WNS-affected bats last spring upon emergence. Thus, it is apparent that bats can get the fungus from other bats, and from environmental exposure.

Dr. Blehert is also one of the Principle Investigators of the so-called Soil Sampling Project (along with Kunz, Hicks, and Youngbaer), funded by the NSS. At this writing, most of the sampling (sediment with organic material taken from nearly 200 caves and mines with hibernating bats in nearly 30 states) has been completed and the samples sent to the NWHC. Over the spring and summer, the nearly 1000 samples will be analyzed by PCR (Polymerase Chain Reaction), which amplifies the fungal DNA. Results are not expected until the fall. The goal is to determine how widespread, or ubiquitous, this fungus is in the background environment. This is envisioned as a three-year project, with the sites and methodology of the out years to be determined by this year's results.

Finally, the NWHC lab has also done some examining of the decontamination protocols, and has determined that the product Pure Green 24 is ineffective on this fungus, that UV light in a laboratory is unsatisfactory, and that 10% bleach works fine. Of course, this is bad news for rope, so alternatives are being explored. Dr. Hazel Barton stepped forward to assist in the analysis of ropes and other caving textiles, which has been welcomed by Dr. Blehert. Samples from various manufacturers have been sent to Dr. Barton, and we are working to provide funding for that effort.

Dr. DeeAnn Reeder, Bucknell University:

Dr. Reeder is recognized as one of the leading researchers on bat energetics. She and her partners at Fordham University, the US Army Corps of Engineers, and the PA Game Commission, are in the midst of a project studying the arousal patterns of bats. Working at thirteen sites in NY, VT, PA, MI, WV, and KY, they are comparing affected and unaffected bats.

One of the major hypotheses about why WNS bats are starving to death is that something is causing them to arouse more frequently, and perhaps to stay aroused (euthermic) longer, thus consuming precious stored body fat, deteriorating and ultimately dying. Are the bats being aroused by some immune system function, being bothered by the fungus, or something else?

Preliminary findings showed that the WNS-affected bats aroused much more frequently than normal, but that, by and large, they remained aroused for a normal amount of time. Vermont (Greeley Mine) bats and PA (Shindle Iron Mine) bats aroused about every four days. Williams Hotel and Williams Preserve (NY) were about every eight days, compared to a normal 13-17 days.

While there were more frequent arousals at all sites early in the experiment, this was attributed to the initial handling of the bats and placement of the data loggers on them. Bats remained euthermic generally for 1.5 – 2 hours, not unusual, except near the end of their lives. Some of the Shindle Mine bats had permanent arousal – over four days – and then presumably died. There were high temperatures recorded at that time, and speculation is that the bats were flying, perhaps trying to get out of the sites (some mines were sealed with wire to trap carcasses), but Reeder noted there is no baseline data with which to compare.

Dr. Thomas Kunz, Jonathan Reichard, Boston University:

In a multifaceted study, funded in part by the NSS, this project is looking at the annual patterns of body condition and fat reserves in *Myotis lucifugus* (little brown bat) in several different ways and in several different subprojects.

Low body fat (or emaciation) during the latter stages of hibernation has been correlated to WNS. But is low fat a cause or a symptom? Are bats entering hibernation without enough stored fat to get through the winter (is something going on outside?), or is the fatal loss occurring during hibernation?

For this component, biweekly sampling took place during the summer at a maternity colony, plus biweekly sampling during fall swarming, plus periodic sampling during winter hibernation (still ongoing), at Mt. Aeolus Bat Cave in Vermont. Aeolus has been studied for decades, and comparative baseline information was available. Similarly, comparative information was available for maternity colonies in MA and NH. Other study sites were in NY, NJ, PA, KY, and OH to include both known WNS affected bats, bats from well outside the region, and others in between.

Nondestructive body analysis (Body Mass Index, or BMI, and Total Body Electrical Conductivity, or TOBEC), and destructive body composition analysis are both being used in the study.

Results show that WNS-affected bats in the maternity colonies clearly weighed less and showed significant wing damage. The data also suggest that the wing damage tended to heal over the summer. The comparative gap in body condition (TOBEC) tended to catch up over the same time period. However, a comparison of body mass between 1975 and 2008 showed the 2008 bats consistently smaller throughout the entire period of early pregnancy, late pregnancy, lactation, and post lactation.

In another component of the study, the results showed no difference between bats of the same species at the same site with and without fungus. This suggests that the absence of fungus does not equal a healthy bat.

Interestingly, climate data from 1975 through 2008 show that there has been a mean warming of one degree over the past 33 years. This study's data showed that while the BMI of 2008 bats from mid-August through early October were equal to or slightly greater than those from 1975, they fell notably below by mid-October. Could this climate change require bats to burn more fat prior to hibernation? This will require more study.

This project is mid-stream, but Reichard/Kunz' preliminary conclusions are:

> Poor body condition entering hibernation *and* rapid fat depletion during hibernation likely *both* contribute to winter emaciation.

Monitoring body composition during active months (at maternity roosts) will be important to determine full impact of WNS on populations.

H. Kathleen Dannelly, et al, Center for North American Bat Research and Conservation at Indiana State University:

Dannelly and her colleagues brought some of the newest information and perhaps most intriguing line of inquiry to the webinar. Referring back to a 2004 study of healthy bats from Indiana (not I bats), she hypothesized that chitin (pron. kite in), derived from the exoskeletons of insects, could be a source of carbon, nitrogen, and energy for bats throughout the year.

The 2004 study isolated chitinase-producing bacteria in the intestinal tract of the bats. The numbers of the bacteria were as high as 10 to the 7^{th} power, and 4-9 different bacteria were present. However, comparing these to 18 WNS-affected bats from Rosendale, NY, 11 showed no chintase-producing bacteria in any of the four digestive tract sites tested. In the remaining 7 bats, the numbers and diversity were extremely low.

Her initial conclusion is that these bacteria are greatly reduced or non-existent in WNS-affected bats, and that this could reduce the potential energy and ability of bats to survive the winter. They also observed other differences in the intestinal flora between healthy and affected bats, but did not have healthy bats from the WNS area to examine. This will need to be done this summer.

Potential causes of change in the intestinal flora are wide-ranging, from pesticides to other environmental toxins, to changes in the metabolism of the bats themselves. They also plan to give radioactive chitin to bats and then measure energy uptake by carbon dioxide production.

Dr. Elizabeth Buckles, Cornell University:

This project analyzes bats post mortem to document wing membrane changes over the course of a year, including skin and body condition. The lab checks for fungus, bacteria, and inflammation. Bats are sent from both affected and unaffected sites. Wing lesions are similar to those observed last year. Tissues are scored for the amount of fungus, the amount of inflammation, and any reactions to fungus and other pathogens.

A second project is running PHA tests. (Note: Polyhydroxyalkanoates or PHAs are linear polyesters produced in nature by bacterial fermentation of sugar or lipids. They are produced by the bacteria to store carbon and energy). The purpose is to document the timing of the inflammatory response bats to an injected mitogen. This is just getting started.

Buckles' work last year on viruses, parasites, and bacteria eliminated most of those as potential causes of WNS.

Marianne Moore, Jonathan Reichard, Thomas Kunz, Boston University:

This is a project funded by the NSS and Bat Conservation International. It seeks to determine whether bats affected with WNS are immunocompromised.

Several hypotheses are being tested, base on preliminary research in 2008: Bats with visible signs of the fungus will have different immune competence than those without; bats at unaffected sites will have significantly different immune responses than those at affected sites; arousal from torpor is necessary to mount an effective immune response.

2008 research involved drawing blood from bats at sites in MA, VT, and NY at different times during the winter, and at different stages of arousal – encouraged by artificial warming. E. coli was then introduced to the blood samples to observe how the blood fought it off.

2009 involves significantly more sites and samples, plus the introduction of a variety of immune assays. Initial control sites in NY and PA became WNS sites, so a new control was added in Michigan.

This project is very much still in progress, but one set of data shows a significant negative association between immune response and body composition. There is also a difference between sites, from preliminary data.

There are no conclusions yet. The project timeline runs through September.

Tom Tomasi and Amanda Janicki, Missouri State University:

This is another project funded by both the NSS and BCI. It is looking at another potential factor in WNS-affected bats dying of starvation: the bats may have a higher metabolic rate during torpor, thus leading to premature burning of energy reserves.

20 – 30 *Myotis lucifugus* (little browns) from each of the Williams Lake Mine, NY, and Woodward Cave, PA, are being compared with bats from a control cave in MO. Bats were/will be sampled in October, January and March. Data loggers for monitoring body temperature (iBBats) were attached, the bats reinduced into torpor, and then monitored for metabolic rates.

Although the Williams and Woodward sites differ in temperature, there appeared to be no difference in torpid metabolic rate. However, in January, when the bats in WNS-affected Williams Mine were almost dead, their torpid metabolic rate was much higher than those in Woodward Cave, which were similar to bats previously analyzed in their lab. MO data have not yet been analyzed.

Summer data indicate that bats with elevated metabolic rates were in a more shallow torpor. This project also has a September end date, so conclusions are premature.

Craig Frank, Fordham University:

Funded by the National Science Foundation, this project looked at polyunsaturated fatty acids (PUFAS) in *Myotis lucifugus* (little browns) and compared them to *Eptesicus fuscus* (big browns). The hypothesis was that Species/Populations of bats with WNS will have significantly different levels of: Linoleic acid (18:2), and/or Alpha-Linolenic (18:3) acid in their diets and tissues than those without WNS.

PUFAs are not synthesized by mammals. They are produced by plants and are incorporated into insect and mammal adipose (fat) tissue through diet.

Prior to hibernation, body mass doubles and body fat increases from 6% to 36%. During hibernation, bats don't eat, and they have periodic torpor bouts. From hibernation studies of other animals (such as chipmunks, ground squirrels, and other mammals), it is known that a high 18:2 diet enhances hibernation by lowering body temperature and metabolic rate, and increasing torpor bout length and propensity.

Bats of both species were taken from the same NY sites, and white adipose tissue (WAT) assayed from all. Both species showed the same levels of Linoleic acid, but the WNS-affected little browns had significantly greater (@ 4x) alpha-linolenic acid levels than the big browns at the same sites. This elevated level put the bats under stress and make torpor more difficult. More investigation is needed, particularly completion of the analysis of comparative bats from unaffected sites.

Paul Cryan, USGS Fort Collins Science Center:

Cryan reported on a special WNS pre-conference session at the January, 2009 Bat Migration Conference, Berlin, Germany. This presentation focused on WNS research in Europe.

There have been reports of bats with fungi from the past several years, but also back in historical literature to 1983 in Germany. Importantly, no mass mortality has been reported. Most bats are *Myotis* species (e.g. *Myotis dasycneme* (Pond Bat) and *Myotis myotis* (Greater Mouse-Eared Bat)). Hibernacula are very moist. White growth is most apparent at the end of winter, and when the fuzz disappears when the bats are taken out of the hibernacula, and aroused bats quickly groom it off.

In January of this year, bats with fungi were reported in the Netherlands, Germany, and Romania. In February, reports came from those three countries, plus the Czech Republic and France.

A mycologist from the University of Erlangen, Carsten Dense showed electron microscope pictures of fungal samples taken from the faces of bats in Germany. They showed branched conidiophores with curved conidia, similar to those described by Blehert, et al in their 2008 published research.

A warning and call for help was issued to bat researchers in Europe. If this new fungus is an invasive species encountering new ecosystems, something in its native ecosystem may inhibit its growth or contain its invasive characteristics.

If the fungus is endemic to Europe, then how local bats survived may hold lessons for the survivability of bats in the U.S. And, if the fungus isn't in Europe, they'd like to keep it that way.

Protocols for field identification, laboratory analysis, and decontamination were quickly developed (based in part on US experience), and international contacts were firmed among researchers.

Eric Britzke, Cal Buchkowski, Al Hicks:

The webinar concluded with three position statements (they drew straws, so they don't necessarily represent personal positions) to instigate a discussion on the topic: ***Is now the time to undertake drastic measures in an attempt to control WNS?***

There were two assumptions put forth:

> Based on the rate of spread of WNS, a biological agent is involved.
>
> Spread of WNS is most easily explained by bat to bat transmission, with some transmission occurring at maternity sites.

Britzke defended the position that drastic control measures, such as colony destruction, were needed at affected sites. Most bats at a WNS site will die anyway. Those who survive will go contaminate others. WNS is caused by an infectious agent. As bat biologists concerned about all bat species, don't we have an obligation to collect all bats at affected sites?

Buchkowski defended the position that suspending hibernacula surveys was the appropriate tack. He argued that WNS has an unknown causal agent, that decontamination protocols are inefficient, that surveys themselves could cause transmission, that surveys cause stress on the bats, that knowing the numbers of bats hasn't contributed to slowing the spread, and that manpower could be more efficiently used monitoring site entrances for WNS. Focusing on how WNS develops at a few sites is a better use of time and resources. The management goal is to slow the progression and to buy time for the science to find solutions. Continuing surveys doesn't contribute to this goal.

Hicks argued for continuing surveys. What would we know about WNS without them? Are there resistant species, sites, and individuals? Where would we get lab specimens? Affected bats are going to die anyway, and many don't respond to human activity anyway. Unaffected bats aren't harmed by surveys. However, transmission to clean sites is a real concern. Automated bat counters could be used; absolutely clean teams/gear must be used for unaffected sites.

These presentations generated a rather vigorous discussion, much the same as the thread on the NSS Chat forum. Opinions ranged from quarantine and destruction to that being totally pointless. Aren't other options and causes being ignored? Maybe it's the habitat that's infected, and then the bats get it. What about environmental manipulation, such as heat? What about chemicals? What about biological controls (discussion of a trichoderma that acts as a parasite on other fungi)? What about the other life forms in a cave ecosystem? Pretty wild and woolly, but no conclusions.

The webinar ended with discussion of putting together a committee to work on an action plan for the coming year – much as the Science Strategy Conference in Albany laid the groundwork for this year's work.

(Note: this next section was not a part of the webinar, but is another WNS project currently underway)

Nancy Simmons, American Museum of Natural History, et al:

(from their website): **"**Researchers are using molecular methods to collect baseline genetic data on little brown myotis populations across the Northeast. To do this, we need large numbers of samples from a broad geographic range, including from colonies not yet affected by WNS. We are using genetic tools to determine current levels of genetic variation, whether gene flow among colonies is sex-biased, and how the genetic diversity is distributed geographically among populations. In other words, how healthy are *M. lucifugus* colonies (pre-WNS) in genetics-terms, how are they related to one another, and does one sex play a larger role in mediating gene flow? These data will provide invaluable information on the current status and estimated size of the little brown myotis population, and will enable us to track changes in population size and loss of genetic diversity over time."

Mitochondrial and nuclear gene sequencing is being done at laboratories at Penn State University, Grand Valley State University, and the University of Western Michigan, with sample collection being coordinated by the PA Game Commission and NYDEC. There are no published reports at this time.

Conclusion:

There are many other individuals working on WNS across the country, and this summary is not intended to exclude any of their contributions. Tests of various kinds in many state laboratories, the work of bat rehabilitators, cavers who have assisted with surveys and sampling, and many others continue in an attempt to help solve the WNS mystery.

Due to the seasonal nature of research on hibernating bats, activities not undertaken during a specific season must wait a full year before taking them up again. The availability of funding has limited what can be done, and has clearly left some investigations undone.

It is my hope that this summary has provided a comprehensive look at where the primary research on WNS stands at this time. While I'm sure it answers many questions, I'm equally sure that it raises many more. Such is the nature of scientific inquiry.

Questions may be directed to wnsliaison@caves.org. Thank you.

Peter Youngbaer
NSS 16161
WNS Liaison