Tennessee Winter Bat Population and White-nose Syndrome Monitoring Report for 2014-2015 and 2015-2016

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Region 2

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These surveys could not be conducted with such a high level of effort or as geographically widespread without the assistance of numerous volunteers. Because the majority of caves and winter sites occur on private lands in Tennessee, the number of surveys would be greatly reduced without the support, assistance, and willingness of private landowners. Without the volunteer and landowner support, we would not be able to understand the distribution of winter bat populations and effects of White-nose Syndrome in Tennessee.

Acronyms

AAFB……..Arnold Airforce Base

FORT……...…..Fort Campbell Army Installation

NPS……...……..National Park Service

TDEC…….Tennessee Department of Environment and Conservation

TNC…….The Nature Conservancy of Tennessee

TVA……..……Tennessee Valley Authority

TWRA…….….Tennessee Wildlife Resources Agency

USFWS……..United States Fish and Wildlife Service

UTK……University of Tennessee at Knoxville

Species Codes

CORA……... *Corynorhinus rafinesquii*

EPFU…….. *Eptesicus fuscus*

LANO……..… *Lasionycteris noctivagans*

MYAU……...… *Myotis austroriparius*

MYGR…….. *Myotis grisescens*

MYLE……... *Myotis leibii*

MYLU……...…. *Myotis lucifugus*

MYSE……...…. *Myotis septentrionalis*

MYSO…… *Myotis sodalis*

MYsp……...…..Unknown Myotis

PESU…….. *Perimyotis subflavus*

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Executive Summary

Histologic evidence of White-nose Syndrome (WNS) was confirmed in Coffee, Giles, and Marshall Counties and two other counties, Davidson and Sumner, were confirmed as suspect during the winter of 2014-2015. No new counties were confirmed or determined to be suspect during the 2015-2016 winter field season. Currently, 50 counties have been confirmed WNS positive and 2 counties remain suspect. WNS and its casual fungal pathogen *Pseudogymnoascus destructans* can now be found in 52 of the 78 (66.7%) counties containing caves and is considered widespread in Tennessee.

During the 2015-2016 survey period, surveys were not performed at priority sites and these surveys will continue in 2016-2017 winter as cyclical surveys for endangered bat species resume. No information for these species during this period is reported. Based on counts obtained during the 2014-2015 survey period, *Myotis grisescens* increased at two of three sites and slight declines were observed at the third priority sight. *Myotis sodalis* are trending downward despite an increase in the overall number of observations for this species. Major declines were observed at multiple priority sites during the winter of 2014-2015.

Since beginning intense surveys of non-threatened and endangered bats during the winter in 2009-2010, observations are declining at alarming rates. Although observations of *P. subflavus* increased slightly in 2016, declines between 90-98% have been observed at multiple sites within the state. The lack of observations of *M. lucifugus* and *M. septentrionalis* during 2016, 54 and 13 respectively, is just as alarming as the site declines for *P. subflavus*. Although roost preferences of *M. septentrionalis* may lend it to be under surveyed, the lack of winter observations and summer captures indicate this species is declining at alarming rates.

During the winter of 2015-2016, several observations were made that shed a somewhat optimistic light on potential recovery of bats in Tennessee. A single banded *M. lucifugus* was recovered at a site where it was originally banded four years prior. This bat survived at least 4 years at a site confirmed WNS positive while the population declined significantly during this same time. Also, recoveries made at a site where banding has occurred multiple times since 2009-2010, indicate *P. subflavus* has the ability to survive for multiple years despite the presence of WNS. While survivorship of bats is occurring in the state despite the presence of WNS, the presence of such few individuals will increase the potential difficulty of recovery efforts.

Table of Contents

Acronyms ii
Contributors iii
Executive Summary iv
Introduction 1
Methods 3
WNS Surveillance 3
WNS Mortality Monitoring 4
Bat Population Monitoring 4
2015 Statewide Results 4
Myotis grisescens 5
Myotis sodalis 5
Whiteoak Blowhole 6
2016 Statewide Results 7
Overall 7
Corynorhinus rafinesquii 9
Eptesicus fuscus 9
Myotis leibii 9
Myotis septentrionalis 9
Myotis lucifugus 10
Perimyotis subflavus 10
WNS Mortality / Bat Population Monitoring 10
Camps Gulf Cave 12
Marble Bluff Cave 12
Cooper Creek Cave 12
Great Expectations Cave 12
Morrell/Worley's Cave 13
Norris Dam Cave 13
Whiteside Cave 13
Grindstaff Cave 13

Conclusions 14

Literature Cited 15

Appendix A – Maps detailing the annual progression of WNS through Tennessee since 2009-2010 ..... A-1

Appendix B – A list of all WNS confirmed, suspect, or negative counties in Tennessee B-1

Appendix C – 2014-2015 Winter survey results C-1

Appendix D – 2014-2015 Diagnostic service reports D-1

Appendix E – 2015-2016 Winter survey results E-1

Appendix F – 2015-2016 Diagnostic service report F-1

Appendix G – Trend analysis by species across all survey years G-1

Appendix H – Trend analysis of species at selected sites across all survey years H-1

List of Figures

Figure 1. Documented occurrences of WNS in Tennessee during the 2009-2010 winter. 2
Figure 2. Winter trends of *M. grisescens* in Tennessee. 5
Figure 3. Results of winter monitoring of White Oak Blowhole. 6
Figure 4. Trends of bat non-threatened and endangered bat species and the now threatened *M. septentrionalis* since 2009 – 2010..... 8

List of Tables

Table 1. Trends of *M. sodalis* at selected winter sites in Tennessee. 6
Table 2. Percent increase or decrease for species observed between 2010 and 2016. 7
Table 3. Trends of wintering bats by individual sites monitored a minimum of 4 years. 11

Introduction

This report summarizes data collected by all cooperating agencies in Tennessee during the winters of 2014-2015 and 2015-2016. The results of independent research projects are not included.

Historical survey work within the state of Tennessee was conducted to monitor the success of endangered bats in Tennessee. This was accomplished by state and federal agencies and non-governmental groups conducting winter bat hibernaculum censuses. This work has been either on a bi-annual basis or staggered every three years depending on the species involved and the availability of personnel. At one point, selected sites were monitored annually to establish a dataset that would allow trend analysis of populations. These efforts were disbanded in 2015 because of potential negative impacts as a result of repeated visitation. Historical surveys have generally focused on the two of three endangered species of bat found in Tennessee, *Myotis sodalis* (Indiana bats) and *Myotis grisescens* (gray bats). No winter occurrences of the third species of endangered bat, *Corynorhinus townsendii virginianus* (Virginia big-eared bat), are known from Tennessee.

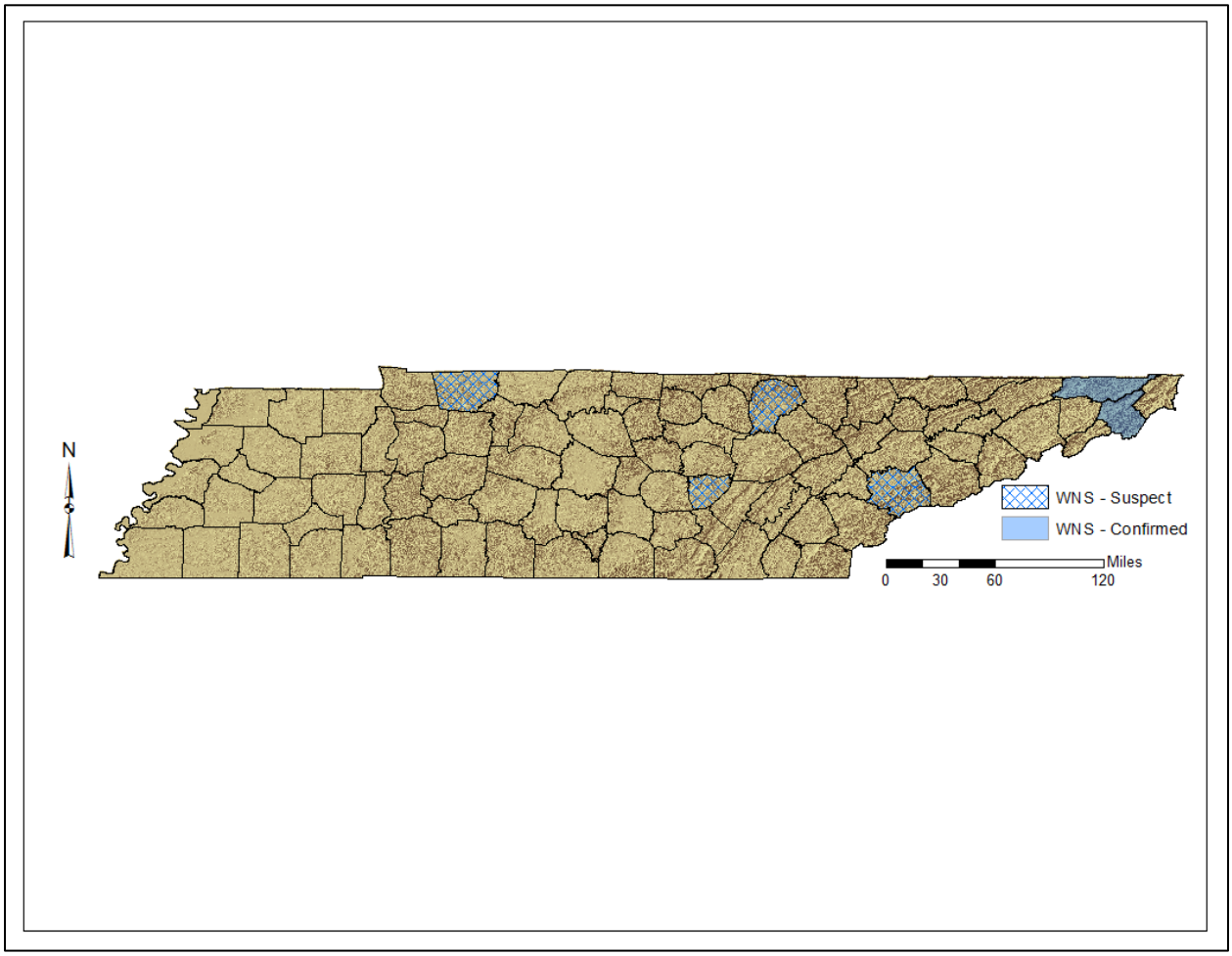
Beginning in 2009 with the concern of bat population decline due to White-nose Syndrome (WNS), there was an increased awareness of the need to not only continue monitoring the status of endangered species, but to also assess the numbers and health of the common species of cave hibernating bats. Prior to the occurrence of WNS, there was very limited information available on bat hibernacula and winter population trends for once common species of cave hibernating bats, that include: *Myotis lucifugus*, (little brown bat), *Myotis septentrionalis* (Northern long-eared bat[1]), *Myotis leibii* (Eastern small-footed bat), *Eptesicus fuscus* (big brown bat), *Perimyotis subflavus* (tri-colored bat), and *Corynorhinus rafinesquii* (Rafinesque's big-eared bat). Because of the paucity of data for these species, assessing trends of winter populations of bats and WNS caused mortality has been difficult.

Initially, a tiered monitoring approach was developed and implemented during initial monitoring efforts with each tier having varying levels of effort for surveys. This approach allowed for survey effort to be adjusted to each cave minimizing potential impacts to hibernating bats, while allowing for the objectives of winter monitoring to be met. A description of the tiered monitoring system can be found in Lamb and Wyckoff (2010) and Flock (2014). As the need to gather data for all species increased, complete surveys of bat populations found within all sites surveyed was implemented in lieu of the tiered monitoring approach.

WNS and its causal pathogen *Pseudogymnoascus destructans* (Pd) fungus were first recorded in Tennessee in the winter of 2010. The fungus was found on three species of bats, *M. lucifugus*, *M. septentrionalis*, and *P. subflavus*, in six different counties (Figure 1). The documented

[1] *Myotis septentrionalis* was listed as threatened by the USFWS April 2, 2015 because of severe declines attributed to WNS (USFWS 2015).

Figure 1. Documented occurrences of WNS in Tennessee during the 2009-2010 winter.



occurrences included two histological confirmations of fungal infections within tissues, and four suspect sites[2]. Since 2010, Pd has been confirmed in 50 counties throughout the state. Over sixty-six percent of the counties having caves (78) in Tennessee have been confirmed WNS positive or suspect. A progression of WNS by county and year can be found in Appendix A. Appendix B lists all confirmed or suspect sites and the species from which samples were collected in Tennessee. A list of all species in which Pd has been diagnostically confirmed or detected can be found at https://www.whitenosesyndrome.org/about/bats-affected-wns.

With over 10,000 caves in Tennessee and 20% of the known caves in the United States (The Nature Conservancy of Tennessee n.d.), conducting annual surveys of all caves or of all winter bat populations in Tennessee is not a realistic and feasible approach, and not one considered by

[2] During monitoring efforts, a site cannot be confirmed positive for the presence of WNS until histologic investigations reveal Pd has infected the tissues of bats. Suspect sites through 2014 are sites which test PCR positive for the presence of Pd and this designation is not removed until histology reports reveal tissue infections. Since 2014, the criteria used to classify WNS suspect sites has changed to minimize the need to euthanize bats and can be found at https://www.whitenosesyndrome.org/resource/revised-case-definitions-white-nose-syndrome-11252014.

the WNS Advisory Council of Tennessee. A significant effort is made each year by all state and federal agencies, non-governmental groups and individuals to perform as many winter surveys as possible. Because of the density of caves throughout the state, less than 1% of the caves are visited each year. As result of this, any conclusions or predictions concerning the spread of WNS across Tennessee and its effect on the bat population should take survey effort into consideration.

The winter of 2014-2015 was a count year for priority caves harboring endangered species. This would be the last year for this type of count until the winter of 2016-2017 as it was agreed among all partners to minimize visitation at these sites and begin the cyclical monitoring once again. Surveys were also conducted at sites monitored in previous years to assess impacts from WNS and efforts were made to locate potentially new winter sites.

During the winter surveys of 2015-2016, no winter counts were performed at priority *M. sodalis* and *M. grisescens* sites. The focus of surveys during this period was to locate new sites harboring winter populations of bats and to revisit sites in which monitoring had previously occurred to assess impacts to these populations. Surveys also occurred at sites previously monitored to continue assessing the impact of WNS.

In all years, surveys are conducted in a manner allowing strict adherence to the USFWS WNS Decontamination protocols (https://www.whitenosesyndrome.org/topics/decontamination). Decontamination has been a high priority in all years to minimize the potential of surveys aiding the spread of Pd across the state. As a result of this priority, the number of caves visited per day is limited based on geography, personnel, and maintaining adequate supplies of decontaminated equipment. Despite the large number of caves in Tennessee and issues surrounding decontamination, efforts have helped to identify new bat hibernacula and to allow changes of winter bat populations to be tracked.

Methods

The 2014-2015 and 2015-2016 winter cave surveys were conducted between December 15, 2015 and April 1, 2016. Extending the survey effort through April 1, 2016, as this is typically later in the season for winter surveys, allows for further development of WNS symptoms as observed during 2009-2010 surveys (Holliday 2012). All surveys performed during this period were designed to monitor the state of WNS in Tennessee and to census non threatened and endangered bats because this was not a census year for listed species. Objectives of all surveys fell into the following three categories with considerable overlap.

WNS Surveillance

Although a majority of the cavernous counties are WNS confirmed or suspect, surveys are still conducted to determine the presence of WNS at all sites. There are countless caves across the state that still appear to be WNS negative despite county WNS designations. Surveys are

implemented to gauge the presence of WNS on a site level because of the lack of uniformity of its progression across the state, and as a result of this lack of uniformity, to monitor impacts of WNS on winter bat populations on a site by site basis.

Because of the need to increase knowledge of wintering populations of bat species not listed, complete censuses of all bats observed in caves were conducted. This approach was different from the tiered monitoring approach used in previous years. In the event cooperators deemed presence within the cave was creating unnecessary disturbance to wintering bats, estimates of large clusters of bats were made to decrease the length of time surveyors were in the cave.

WNS Mortality Monitoring

Selected caves previously confirmed or suspected to be WNS positive were visited to assess the level of mortality that may have occurred since prior visits (Samoray 2011). In order to collect the best data possible under survey conditions, a full census of all bats observed within the caves was conducted. Sites selected for mortality monitoring (Lamb and Wyckoff 2010) were visited again during the 2015-2016 field season to continue these efforts. Two methods have been used at these sites to assess mortality: repeated, annual visits to count all bats or banding of all bats to assess survivorship at sites previously determined to be WNS positive. It should be noted, of the sites previously selected for these efforts in Lamb and Wyckoff (2010), monitoring efforts have been reduced or not occurred annually as a result of manpower concerns, potential impacts from repeated disturbance, eliminating visitation at sites in which severe declines have occurred to the wintering bat populations, or the bat populations declining to critically low levels or levels too low to make these efforts a viable option.

Bat Population Monitoring

Because historic survey efforts were focused on monitoring endangered *M. sodalis* and *M. grisescens*, there is a paucity of data pertaining to other cave hibernating species in Tennessee. A continued goal of the 2014-2015 and 2015-2016 surveys were to identify new sites which serve as hibernacula for non-listed, but WNS affected bats. These species include: *P. subflavus*, *M. septentrionalis*, *M. lucifugus*, and *M. leibii*. Several of the sites visited during this period have been visited during previous survey years. Despite these repeated visits, full censuses of bats observed in the caves were performed. Several sites not previously surveyed, were visited during this period and, again, complete surveys of all bats were performed. Methods detailed by Holliday (2012) were used to select these new sites to determine if they harbor cave hibernating bats.

2015 Statewide Results

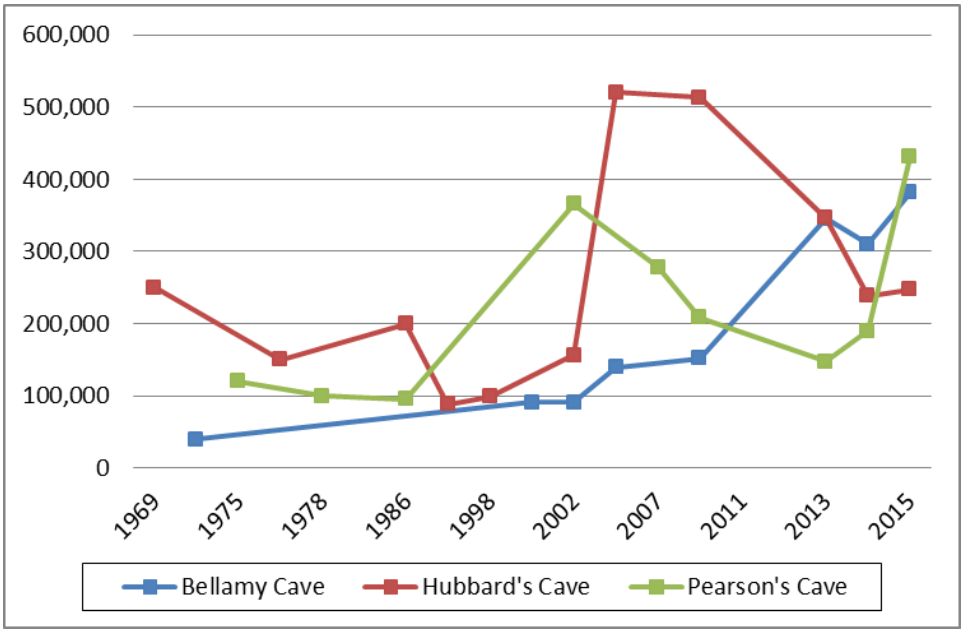
Sixty-one (61) caves were visited across 29 counties during the winter of 2014-2015. WNS was confirmed through histologic analysis of bat tissues at sites in three counties, Coffee, Marshall, and Giles. Suspect sites were also confirmed in Davidson and Sumner Counties. The results of

all caves surveyed can be found in Appendix C. Details of the diagnostic services reports for each of these sites can be found in Appendix D.

Myotis grisescens

During the 2014-2015 survey period, the last of the annual counts performed at priority sites harboring endangered species were conducted. The typical cyclical occurrence of these counts will begin again during the winter of 2016-2017. Observations of *M. grisescens* at the three priority sites increased from 783,715 (2013-2014) to 1,059,524 in 2014-2015. It should be noted, the large increase can be attributed to a section of Pearson's Cave being included in this counting period traditionally not included in previous counts.

Figure 2. Winter trends of *M. grisescens* in Tennessee.



Despite the overall increase of *M. grisescens* statewide, concern should be given to the declining numbers observed at Hubbard's Cave (Figure 2). Although declines attributed to WNS have not been documented for this species, it is unknown the reasons for the declines observed at this site. One possible reason may be the result of repeated annual visitation this site has received since collaborators agreed to the USFWS's requests to perform annual counts at known *M. grisescens* sites.

Myotis sodalis

The number of observed *M. sodalis* increased during this survey period from 4,067 (2013-2014) by more than one thousand to 5,077 in the 2014-2015 survey period. This increase can likely be attributed to the increase in the number of priority *M. sodalis* sites documented as a result of the survey and monitoring efforts. Assessing declines of *M .sodalis* at individual sites is challenging. The majority of sites with documented presence of *M. sodalis* harbor small populations during the winter, outside of White Oak Blowhole discussed below. The majority of sites are experiencing declines ranging from 15.3% to 100% (Table 1). While the observations for this species have increased statewide, widespread and devastating declines were observed for

this species at numerous sites within the state during 2014-2015 winter. Prior to the arrival of WNS, populations of *M. sodalis* were trending upward across much of the eastern portions of its range (Thogmartin et al. 2012), and it is evident WNS is reversing these trends.

Table 1. Trends of *M. sodalis* at selected winter sites in Tennessee.

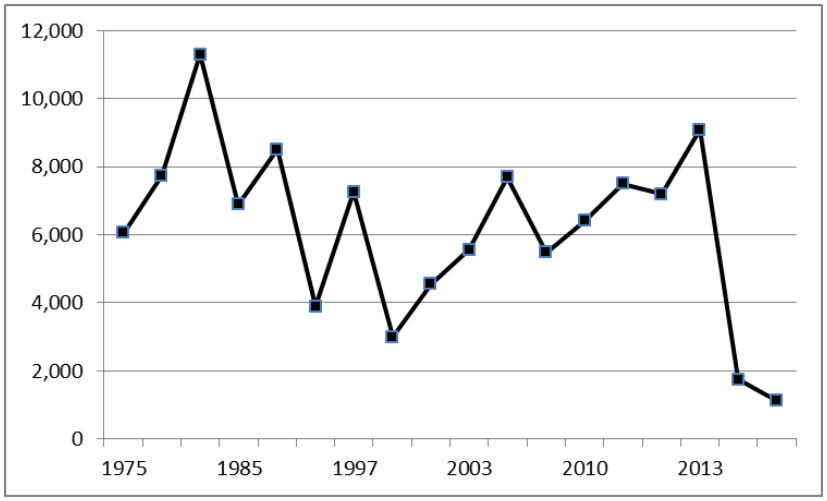
Cave	Year of Observation	Estimate	Year of Observation	Estimate	Trend
Cornstarch Cave	2011	293	2015	13	**-95.56%**
East Fork Saltpeter Cave	2010	248	2015	210	**-15.32%**
Fox Hole Cave	2012	15	2016	0	**-100.00%**
Jaybird Cave	2012	14	2015	8	**-42.86%**
Kelly Ridge Cave	2011	1137	2015	188	**-83.47%**
Lost Creek Cave	2010	51	2015	29	**-43.14%**
New Mammoth Cave	2010	12	2015	76	533.33%
Rice Cave	2010	32	2015	0	**-100.00%**
Rose Cave	2010	75	2015	105	40.00%
Scott Gap Cave	2011	12	2015	0	**-100.00%**
Signature Cave	2011	18	2016	12	**-33.33%**
Wolf River Cave	2011	875	2015	1351	54.40%
Yggdrasil Cave	2013	60	2015	39	**-35.00%**
Zarathustras Cave	2011	53	2015	18	**-66.04%**

Whiteoak Blowhole – A case study

This site exemplifies the impacts of WNS to wintering bats in Tennessee and the devastating impacts to an endangered species. Whiteoak Blowhole is the largest known winter site in Tennessee for *M. sodalis*.

NPS has performed monitoring of Whiteoak Blowhole dating back to 1975 and winter visits have not been made annually, rather occurring cyclical to minimize disturbance from visitation. The site was gated in 1997-1998. Prior to the gating of the cave, the winter population of *M. sodalis* peaked in 1981 at 11,287, but tremendous variation was observed between 1975 and 1999 (Figure 3). Three large declines of this population in excess of 4,000 individuals were observed, reaching its lowest level of 3,000 individuals in 1999. The variation observed prior to the gating

Figure 3. Results of winter monitoring of White Oak Blowhole.



may likely be the result of undesired visitation. With the installation of the cave gate, the population slowly began to rebound.

With the approach of WNS, annual site visits were made between 2010 and 2015 to determine the presence of WNS and its likely impacts. Whiteoak Blowhole was categorized as suspect in 2010 and 2011, and final histologic confirmation of WNS was made in 2012. Despite the presence of WNS at the site, the winter population increased, as 7,495 individuals were observed in 2011, the last year it was deemed suspect, declining to 7,200 in 2012, the first year of confirmation. The population reached its peak in 2013 at 9,076, but a drastic decline has been observed since. During the winter of 2014-2015, the population estimate for this site was 1,117, an 87.7% decline in just two years. This was the lowest recorded winter population for this site. It is evident this site is suffering major impacts as a result of WNS and it is likely these impacts have been more substantial as a result of the roosting behavior of *M. sodalis*. This species is highly gregarious, roosting in tight clusters during the winter, likely facilitating an increase in the transmission of WNS between bats (Langwig et al. 2012). Declines of the magnitude observed at Whiteoak Blowhole increase the difficulties of biologists and conservationists to implement successful recovery conservation actions.

2016 Statewide Results

Eighty-five (85) caves and 1 non-traditional winter site were visited across 33 counties during the 2015-2016 winter survey period. WNS field signs were observed in 29 caves. No new counties were confirmed as WNS positive or suspect. The results of all caves surveyed can be found in Appendix E.

The carcasses of two bats were collected from a civil war bunker in Tipton County, Tennessee and submitted to the Southeastern Cooperative Wildlife Disease Study for WNS testing. Both bats tested negative for the presence of WNS and likely succumbed to injuries as the result of unknown trauma. The complete report can be viewed in Appendix F.

Overall

Because of the lack of historic data for bat species not typically monitored, the 2009-2010 winter survey period was used as base for which comparisons of current bat numbers could be made. Although this is not a preferred method for reasons that include equal survey effort between sites and across years, difficulty in observing cryptic species, addition or discovery of significant bat sites, and movement of bats across sites within and between survey years, it is the best dataset to make comparisons for assessing potential declines of these bats as the result of WNS.

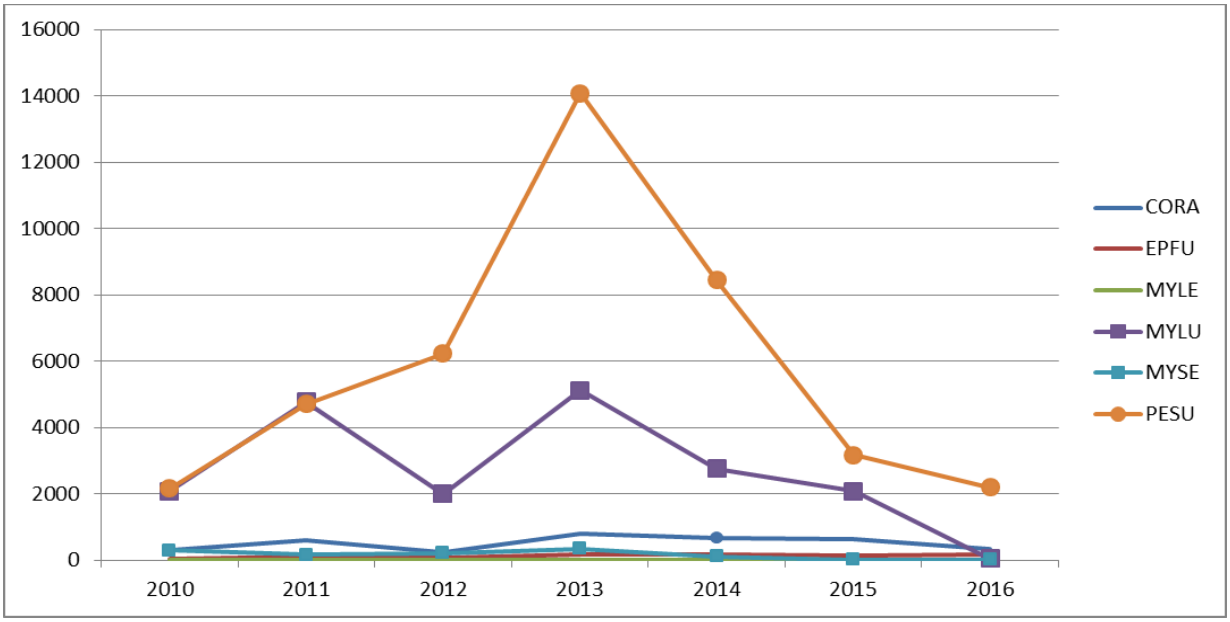
Table 2. Percent increase or decrease for species observed between 2010 and 2016.

	CORA	EPFU	MYLE	MYLU	MYSE	PESU
2010 (n)	313	28	5	2075	292	2159
2016 (n)	331	171	1	54	13	2175
% Decline	**5.80%**	**5.10%**	**-80.00%**	**-97.40%**	**-95.50%**	**0.74%**

Small increases have been observed in the numbers of *C. rafinesquii* and *E. fuscus*, but these increases are too small to draw conclusions from. Conversely, large population declines have been observed for both *M. lucifugus* and *M. septentrionalis*, 97.4% and 95.5%, respectively. And although a very small increase was observed in *P. subflavus*, it appears populations of this species are continuing downward. When combining winter population numbers for *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* with the increased difficulty of capturing these species on the landscape during the summer, the outlook for these three species is highly concerning.

The trends of all non-threatened and endangered species observed during winter surveys since the 2009-2010 field season are shown in Figure 4. Numbers for *M. sodalis* and *M. grisescens* were not included in this analysis because surveys for these species are cyclical. *Myotis austroriparius* (southeastern bat) and *Lasionycteris noctivagans* (silver-haired bat) were also excluded because of the low number of observation each year. It is evident when looking at all winter survey years, non-threatened and endangered bat species are declining across the state and these declining trends become more evident when assessing species trends by site. Observations greatly increased for several of these species in 2013 and have drastically and sharply declined since. A common observation within sites following the presence of WNS is the clustering or presence of higher numbers of bats at or near cave entrances. It is possible the spike observed in 2013 is an artifact of this likely behavioral response to WNS. As a result, bats left roosts traditionally used prior to WNS, typically not surveyed, moving to more open areas of the cave and near cave entrances where they were more easily observed. The high numbers observed in 2013 have never been previously documented and it is unlikely will be observed again.

Figure 4. Trends of bat non-threatened and endangered bat species and the now threatened *M. septentrionalis* since 2009 – 2010.



Corynorhinus rafinesquii

Occurrences of this species which roost in large numbers are limited to just a few localities in Tennessee. Numerous observations of single individuals are made annually at sites other than those harboring larger populations. It appears this species is stable (Appendix G-1), as numbers are slightly above those observed during the 2009-2010 season despite the presence of WNS at numerous winter sites. Presence of Pd has been detected on this species using real-time PCR methods at winter sites in Tennessee (Bernard et al. 2015). Survey effort for this species has not been equal across all years and this is because of the limited number of sites and the sensitivity of the species to repeated visitation.

Eptesicus fuscus

It appears numbers for this species are trending upward during the winter, but due to the low number of observations it is difficult to determine if this is actually the case (Appendix G-2). Observations for this species may be difficult to make because of roost preferences or selection during the winter. Many of the observations made during the winter are in plain sight or open areas of caves; however, if *E. fuscus* select roosts such as rock crevices, as observed by Neubaum et al. (2006), observations within caves may become problematic. Also, in other portions of the species range, the use of man-made structures during the winter (Whitaker Jr. and Gummer 2000) may indicate winter surveys should include nontraditional sites. Diagnostic symptoms of WNS have been documented in this species (Blehert et al. 2009).

Myotis leibii

Observations of this species are extremely limited and have never exceeded 12 in any given year since 2009, making it difficult to ascertain whether populations of this species are stable, increasing or declining (Appendix G-3). Similar to *E. fuscus*, it is likely the roosting preferences of this species lead it to be under surveyed each winter. In contrast with other cave-roosting bats, *M. leibii* chooses roosts on the cave floor, under talus, or in cracks or crevices within the substrate (Erdle and Hobson 2001). Admittedly, these roosts are under surveyed during the winter, as assessing these areas would increase the time of surveys, visitation, and increase disturbance to other roosting bats. Despite the lack of survey effort for this species, there is still concern WNS may impact this species given diagnostic symptoms have been observed in *M. leibii* (https://www.whitenosesyndrome.org/about/bats-affected-wns).

Myotis septentrionalis

This species was listed as threatened by the USFWS on April 2, 2015 because of populations declines attributed to WNS (USFWS 2015). Historically, observations of *M. septentrionalis* have been low as it was recorded anecdotally while conducting surveys for species with more significant designations. Unfortunately, the need to increase data collection efforts for this species was recognized just prior the discovery of WNS in Tennessee. Since 2009-2010, efforts have been made to record each observation of *M. septentrionalis* during all cave visits (Appendix G-4). Similar to other species, observations of *M. septentrionalis* peaked in 2013. It should be noted, this species displays roost preferences similar to those of *E. fuscus* and *M. leibii*, roosting

in cracks and crevices of the cave substrate likely leading to it be under surveyed across all years. Since 2012, winter populations of *M. septentrionalis* have declined precipitously; 11 and 13 individuals were observed in 2015 and 2016, respectively. Although the lack of observations can be attributed to roosting preferences of the species, such a drastic decline in the number of observations the past two winters indicates WNS is having detrimental impacts to *M. septentrionalis*. Given the decrease in observations and known WNS impacts, there is high cause of concern for this species in Tennessee.

Myotis lucifugus

Numbers of *M. lucifugus* have mirrored the cyclical surveys conducted for *M. sodalis*, as these two species are often observed within the same hibernacula; however, there are sites within the state where the two species do not occur together. Numbers for this species peaked in 2013 and declines mirror those for *M. septentrionalis* (Appendix G-5). Only 54 individual *M. lucifugus* were observed at a total of 19 sites during the winter of 2015-2016. Although *M. sodalis* priority sites and sites known to harbor larger winter populations of *M. lucifugus* were not surveyed this past winter, the frequency of observations for this species declined. *M. lucifugus* is often observed roosting in the more open parts of caves, but it is possible it may go under surveyed as surveyors may not have access to all parts of caves where the bats may hibernate. Despite this species once occurring in large numbers at winter sites in northern portions of its range (Davis and Hitchcock 1965) and populations in Tennessee constituting a small portion of the overall population (Kunz and Reichard 2010), and decline of *M. lucifugus* within the state resemble those modeled by Frick et al. (2010), in which a 99% chance of regional extinction of the species was possible. Conservation and recovery efforts for *M. lucifugus* will prove both challenging and difficult given the declines observed in Tennessee.

Perimyotis subflavus

P. subflavus was one of the most commonly encountered solitary roosters within caves during the winter. Sadly, this is species is no longer observed at historic densities and its numbers at sites have declined significantly over the past two years (Appendix G-6). As with other species, numbers peaked in 2013, but have declined at an alarming rate since. The slight increase observed during the winter of 2015-2016 can likely be attributed to the exclusion of priority bat sites, allowing a higher number of *P. subflavus* dominated sites to be surveyed. Although the winter observations are similar to those in 2009-2010, the steep, fast-paced decline has increased concerns surrounding this species. Declines associated with the summer capture rates of *P. subflavus* (D. Thames and J. Lamb pers. comm.) are being observed across the state and appear to be mirroring winter declines, adding to the concern for the species.

WNS Mortality / Bat Population Monitoring

Numerous sites across the state have been visited annually or multiple times since the widespread, multi-species focused survey efforts began in 2009-2010. Seventy-three caves have been visited at least 2 times during this period. Table 3 illustrates the observed declines at sites

visited a minimum of 4 times between 2009-2010 and 2015-2016. Declines of wintering bats at sites vary from minimal to 100 percent, whereas the numbers for some species at sites may be trending upward. Some bat researchers and biologists believe WNS is causing extirpation of species from sites. Although it can be argued roost switching of species between sites impacts observations annually, the drastic decline in species and, now, lack of observations for species at sites illustrates the impacts of WNS to winter cave bats in Tennessee.

Table 3. Trends of wintering bats by individual sites monitored a minimum of 4 years.

Cave Name	No. Years Surveyed	% Decrease or Increase by Species			
		PESU	MYLU	MYSE	EPFU
Camps Gulf Cave	7	95.2%	99.0%	-	-
Marble Bluff Cave	7	54.3%	-	-	-
Copper Creek Cave	6	98.0%	99.2%	100.0%	82.0%
Great Expectations Cave	6	+50.0%	-	-	-
Worley's Cave	6	74.0%	-	100.0%	-
Norris Dam Cave	6	2.0%	-	-	-
Whiteside Cave	6	91.0%	-	-	-
East Fork SLP Cave	5	23.0%	93.0%	100.0%	-
Lost Creek Cave	5	73.0%	-	-	-
New Mammoth Cave	5	83.0%	80.0%	100.0%	-
Red Bud Cave	5	90.0%	100.0%	-	-
Rice Cave	5	54.0%	83.0%	-	-
White Oak Blowhole	5	92.5%	97.0%	100.0%	-
Alexander Cave	4	+1,238.6%	-	-	-
Bridgewater Cave	4	69.0%	+83.0%	-	-
Coleman Cave	4	77.0%	+100.0%	100.0%	-
Cornstarch Cave	4	35.5%	75.8%	-	-
Dunbar Cave	4	34.7%	-	-	-
Eve's Cave	4	61.4%	-	-	-
Grindstaff Cave	4	92.9%	-	100.0%	77.8%
Indian Cave	4	40.5%	-	-	-
Jaybird Cave	4	56.2%	93.7%	100.0%	+100.0%
Measles Gulf Cave	4	+58.0%	-	-	+133.0%
Oaks Cave	4	85.0%	-	-	-
Rose Cave	4	-	99.0%	-	-
Tobaccoport SLP Cave	4	70.0%	-	-	-

Percentages designated with "+" have observed increases when comparing 2010 observations to 2016 observations. All other percentages represent declines at respective sites.

Camps Gulf Cave

Camps Gulf Cave site has been visited each year since 2009-2010. This site was deemed WNS suspect in 2010 and confirmed through histology in 2013. It is a highly diverse winter site as 7 species have been documented in the cave this time. Interestingly, single observations of *L. noctivagans* have been made during the past two winters. The number of *P. subflavus* and *M. lucifugus* has declined significantly, 95.2% and 99.0% respectively (Appendix H-1). The maximum number of observation recorded for *P. subflavus* was 555 in 2013 and 102 for *M. lucifugus* in 2010. Observations have declined significantly as only 8 *P. subflavus* and 1. *M. lucifugus* were observed in 2016.

Marble Bluff Cave

This site has also been visited annually since the winter of 2009-2010. Although a maximum of 4 species have been observed at this site, it is predominately viewed as a *P. subflavus* winter site. Observations of *P. subflavus* peaked at 356 in 2015, but drastically declined in 2016 to 42 (Appendix H-2). Overall the decline of this species at this site is 54.3%.

Cooper Creek Cave

Visited 6 times since the implementation of winter WNS surveys, Cooper Creek Cave was once a diverse winter bat site. Six different species of bat have been observed in the cave during the winter, including one observation of *L. noctivagans*. Numbers of *E. fuscus*, *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* have declined substantially since 2011, the year the highest number of observations was recorded for each species (Appendix H-3). Recorded declines for species observed are as follows; *P. subflavus* – 98.0%, *M. lucifugus* – 99.2%, *M. septentrionalis* – 100%, and *E. fuscus* – 82.0%. *M. septentrionalis* has not been observed at this site since 2012. An optimistic, noteworthy observation was made in 2016. A banded male *M. lucifugus* was recorded during the visit. The band (TNC154) was originally placed on the bat during the winter of 2012 and was observed with field signs typical of WNS. During the 2016 visit, this *M. lucifugus* was not observed with field signs typical of WNS, appearing clean and healthy. This observation indicates some bats have the ability to overcome the negative impacts of WNS, as this bat has survived at least four years after initially being observed with WNS.

Great Expectations Cave

Another highly diverse winter site, 8 species of bat have been observed during the six years surveys have been performed. A single *M. austroriparius* was observed during the 2015 survey. The majority of bats observed at Great Expectations Cave are *P. subflavus,* whose numbers peaked at 529 in 2013, and *C. rafinesquii*, the numbers of which also peaked in 2013 at 485. Because no observations were recorded in 2016, trends for *C. rafinesquii* were not assessed (Appendix H-4). Since 2013, numbers of *P. subflavus* have declined; however, when comparing 2016 numbers to those recorded in 2010, this species has increased by 50% and are likely the result of the inclusion of a room not surveyed during previous years. Despite this increase, observations of *P subflavus* declined to a low of 68 in 2015. It is likely WNS is having impacts

to wintering *P. subflavus* at this site, but the chaotic observations between years make it difficult to determine how significant impacts might be.

Morrell/Worley's Cave

WNS was confirmed at this site during the winter of 2009-2010 and monitoring has been performed in six of the 7 years surveys have taken place. Five species of bat have been observed at this site during this same time, but this cave is predominately used by *P. subflavus* (Appendix H-5). Numbers of other species were too low to infer any trends since WNS invasion. Although any decline of bats is alarming given the state of WNS, *P. subflavus* has only declined at this site 74%, not reaching the 90-100% mortality rates observed at sites within the species range having similar or longer periods of WNS presence.

Norris Dam Cave

Only four species have been observed at this site across six surveys. It is predominately a *P. subflavus* winter site, but small numbers of *E. fuscus* and *M. lucifugus* have been observed throughout the years. Observations of *P. subflavus* peaked in 2015, but were greatly reduced in 2016, 41 (Appendix H-6). When comparing 2016 numbers to those observed in 2010, it appears *P. subflavus* has declined 2%, but further monitoring of Norris Dam Cave will need to continue to determine what impacts WNS may be having on this species.

Whiteside Cave

With the development of the WNS surveillance plan for Tennessee (Lamb and Wyckoff 2010), Whiteside Cave was highlighted as a site where long-term monitoring would occur and banding of bats would be implemented to assess survivorship and mortality associated with WNS. Whiteside cave has been visited 6 times since 2009-2010 and banding of *P. subflavus* took place during 4 of these visits. Attempts were made during each visit to band all bats observed and reachable, but personnel availability, equipment, and time impacted the number of bats banded. *P. subflavus* observations peaked in 2014 at 1,342, one year following the confirmation of WNS, and declined to a low of 42 individuals in 2016 (Appendix H-7). A once common species, *P. subflavus* at Whiteside cave have declined 91% since 2010.

Two-hundred forty-three *P. subflavus* have been banded at this site since 2010. Eighteen individuals (7.4%) have been recaptured at the site during this same span, and only one *P. subflavus* has been recaptured twice. Of all individuals recovered, 72.2% were banded prior to the confirmation of WNS at Whiteside Cave and one individual (AAFB TN 4567) was recaptured 5 years after being given a band. Although the recovery is lower than preferred, these recoveries indicate some *P. subflavus* are surviving multiple years despite the presence of WNS at this site.

Grindstaff Cave

Although this site has only been monitored four of the seven years it has occurred at these levels, it was included here because it was one of the first sites where WNS was documented. Four

species have been documented at this site since 2010 and large declines have been observed for each (Appendix H-8). It is likely *M. lucifugus* and *M. septentrionalis* have declined 100%, but it is difficult to assess trends for these species given observations are restricted single visits. No observations were made for these species in 2016. *E. fuscus* appears to have declined at this site by 78%, but the lack of observations throughout the years decreases confidence with this trend. *P. subflavus* observations at Grindstaff Cave peaked in 2011 at 85 and the overall decline for this species at this site is 92.5%. These declines for these species are similar to those observed in other portions of the species range.

Conclusions

With each year of survey effort, the impact of WNS to winter bats in Tennessee becomes clearer. During the past two years, large declines of *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* have been made, and these declines are even more apparent when assessing WNS impacts at individual winter sites. Unfortunately, the declines are magnified by the increased effort it now takes researchers, biologists and consultants to captures these species on the landscape during summer months. Despite the widespread declines being observed at many winter sites, there are winter bat populations stable or trending upward at some sites. Biologists are cautiously optimistic populations at these sites will maintain as such given similar increases have been observed at sites prior to declines..

Banding at selected sites has indicated some bats do have the ability to survive WNS post invasion and can survive for multiple years following individual infection. However, it should be noted the number of confirmed survivors only constitutes a very small portion of historic populations at these sites. Having only a few survivors will increase the difficulty of recovery efforts over the next decade.

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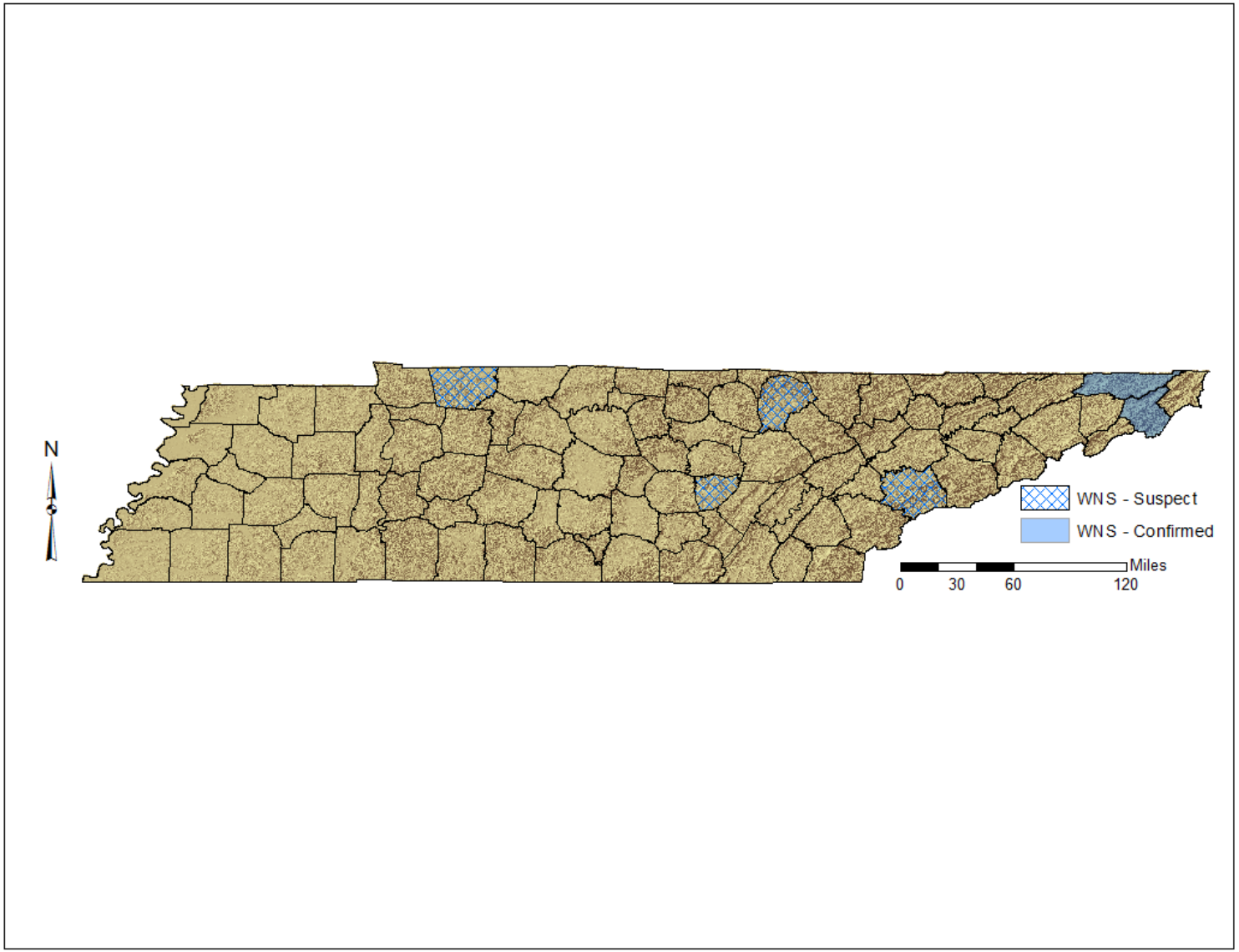
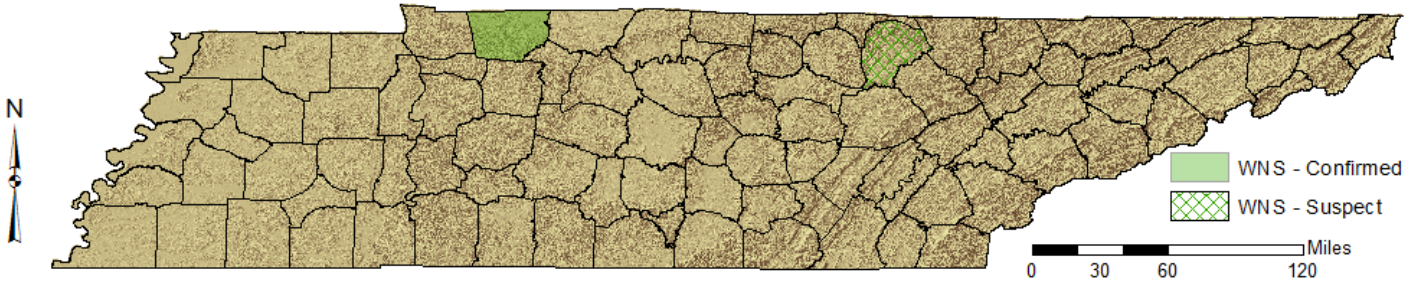
Appendix A

- Maps detailing the annual progression of WNS through Tennessee since 2009-2010.

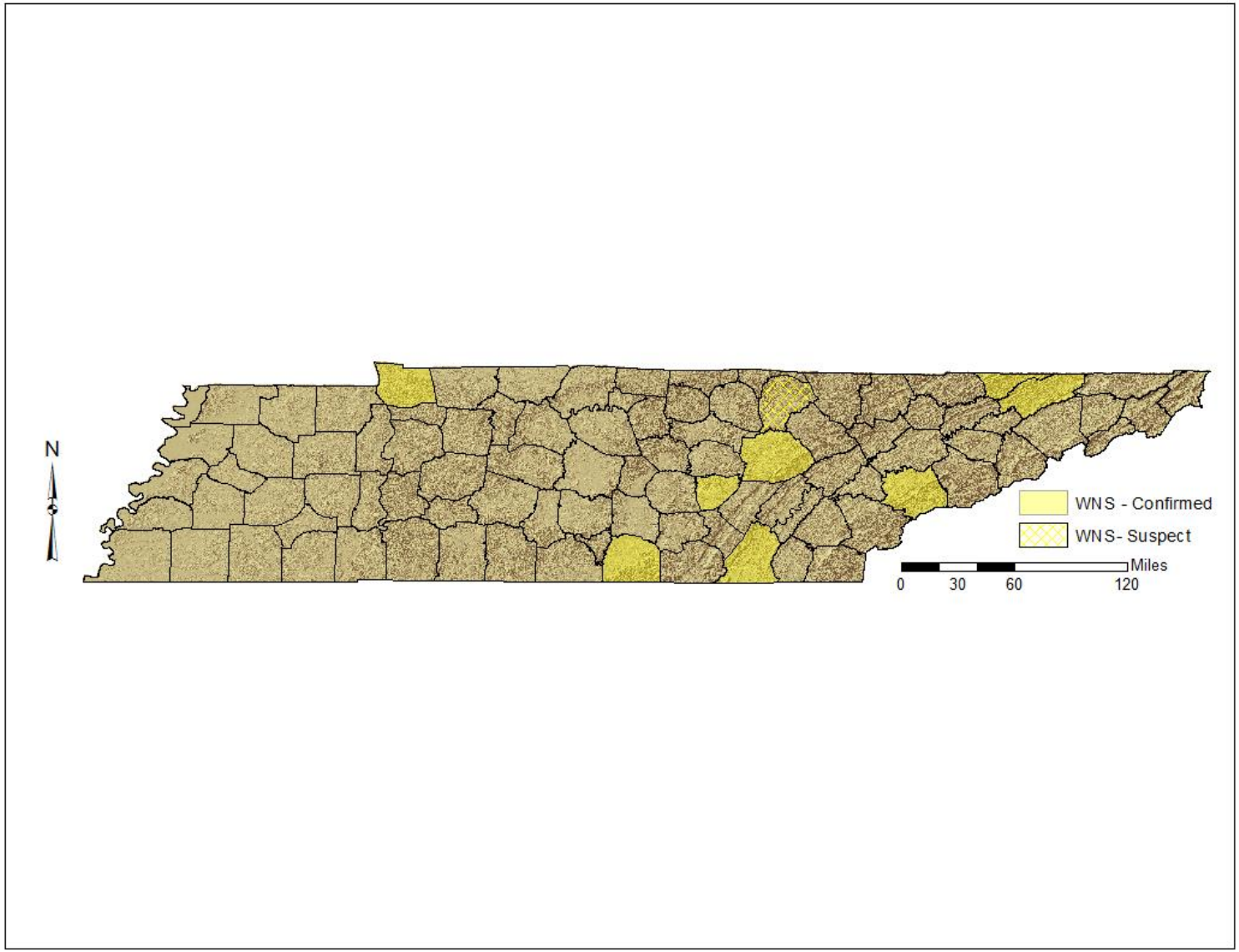
Appendix A-1. WNS confirmations during the winter of 2009-2010.



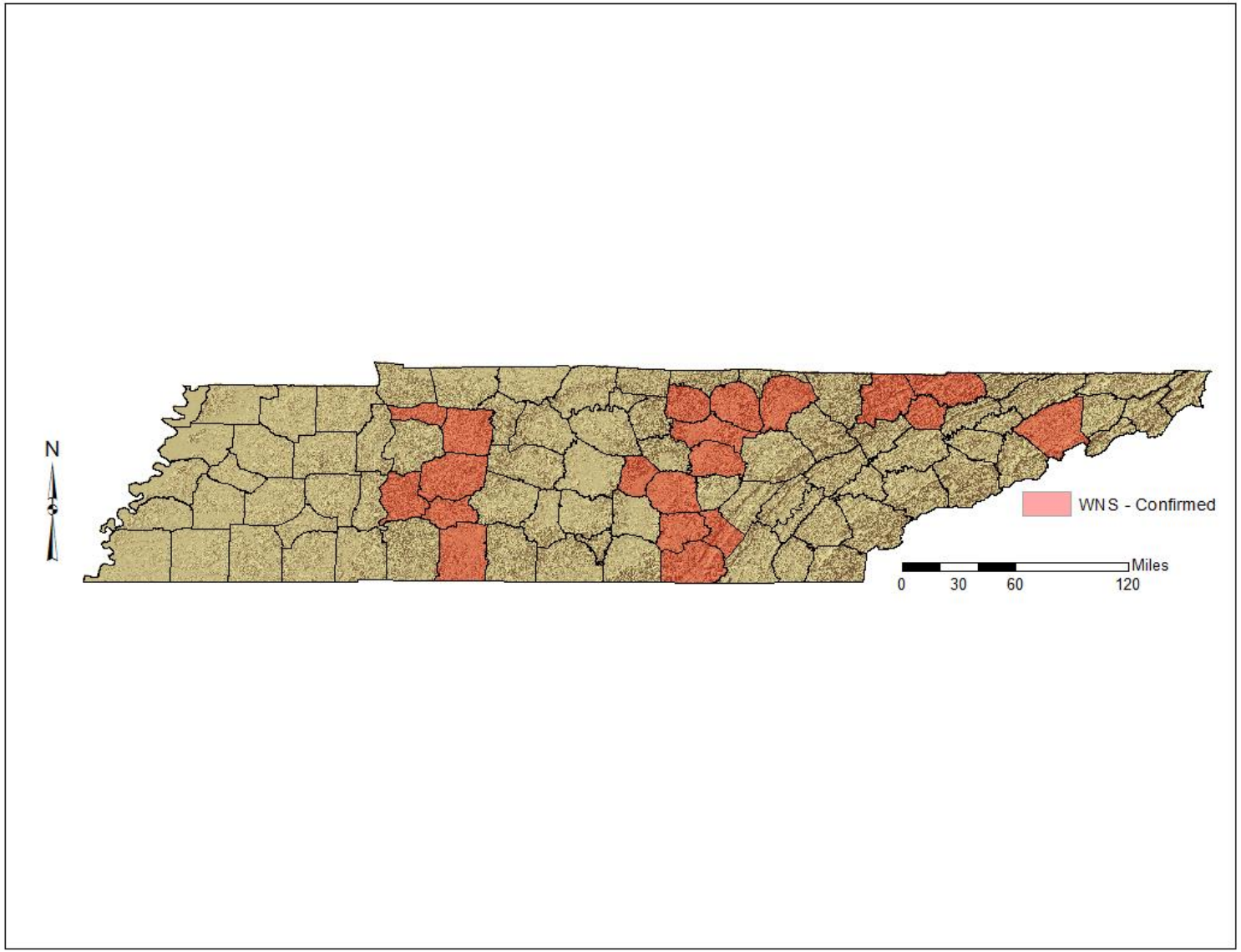
Appendix A-2. WNS confirmations during the winter of 2010-2011.



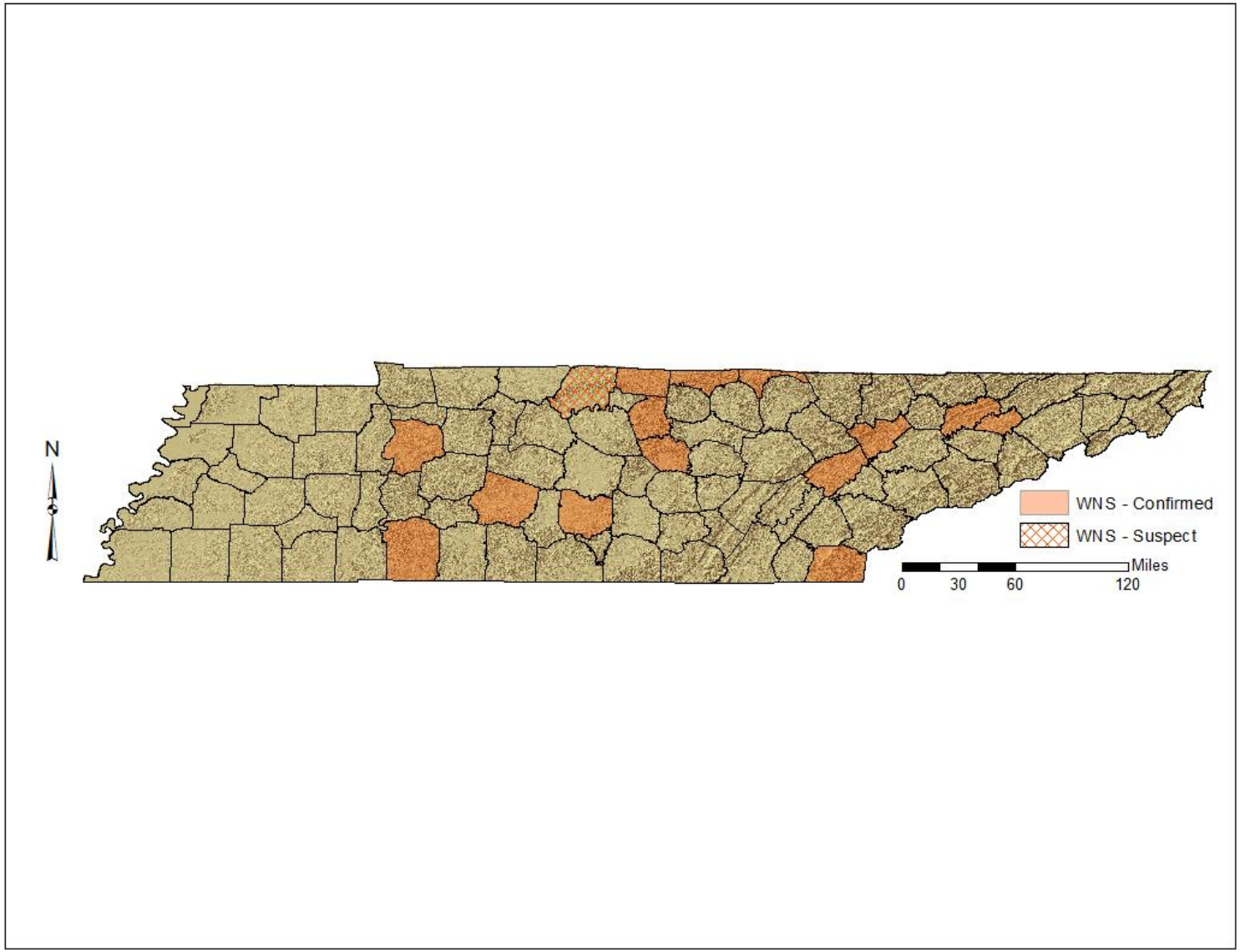
Appendix A-3. WNS confirmations during the winter of 2011-2012.



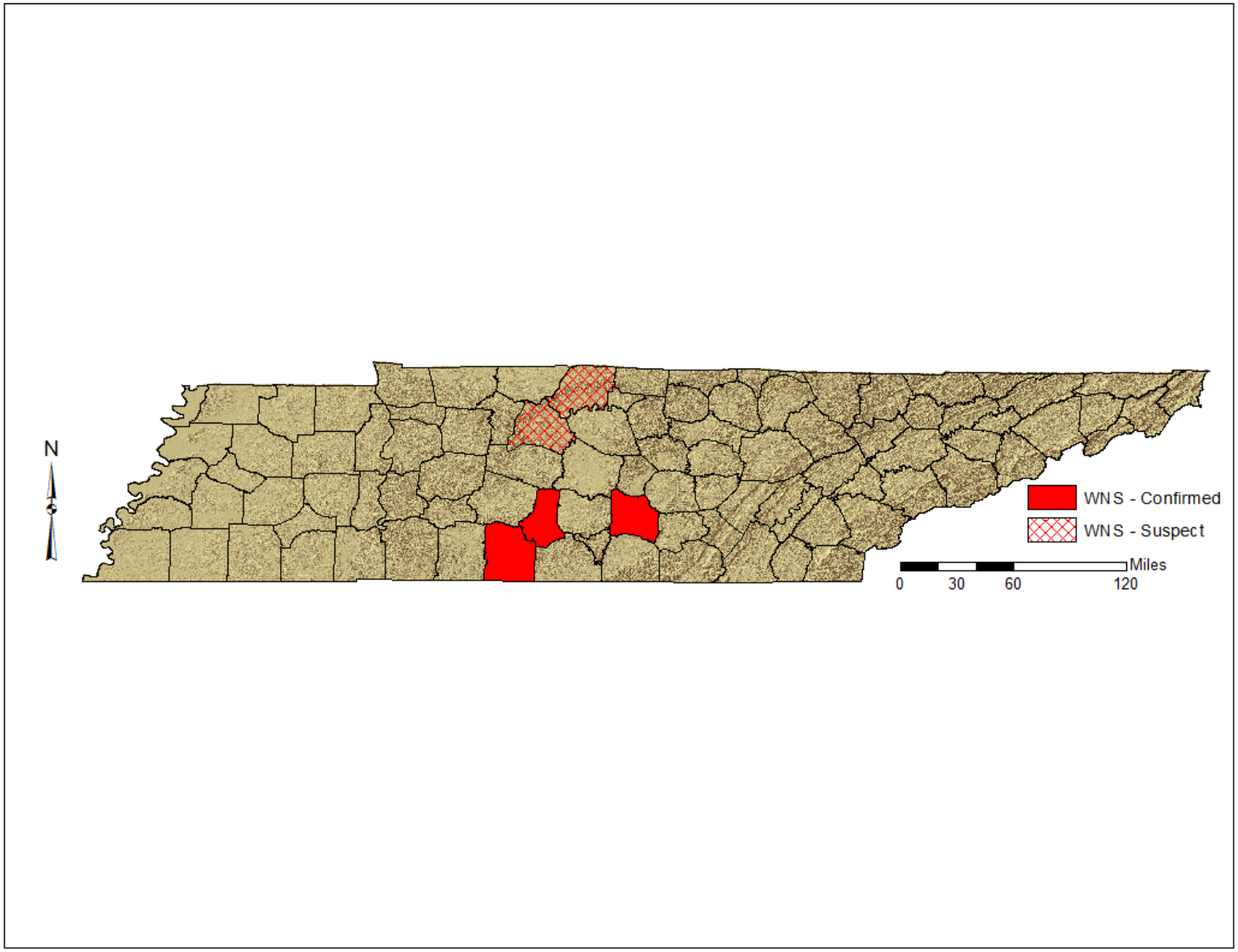
Appendix A-4. WNS confirmations during the winter of 2012-2013.



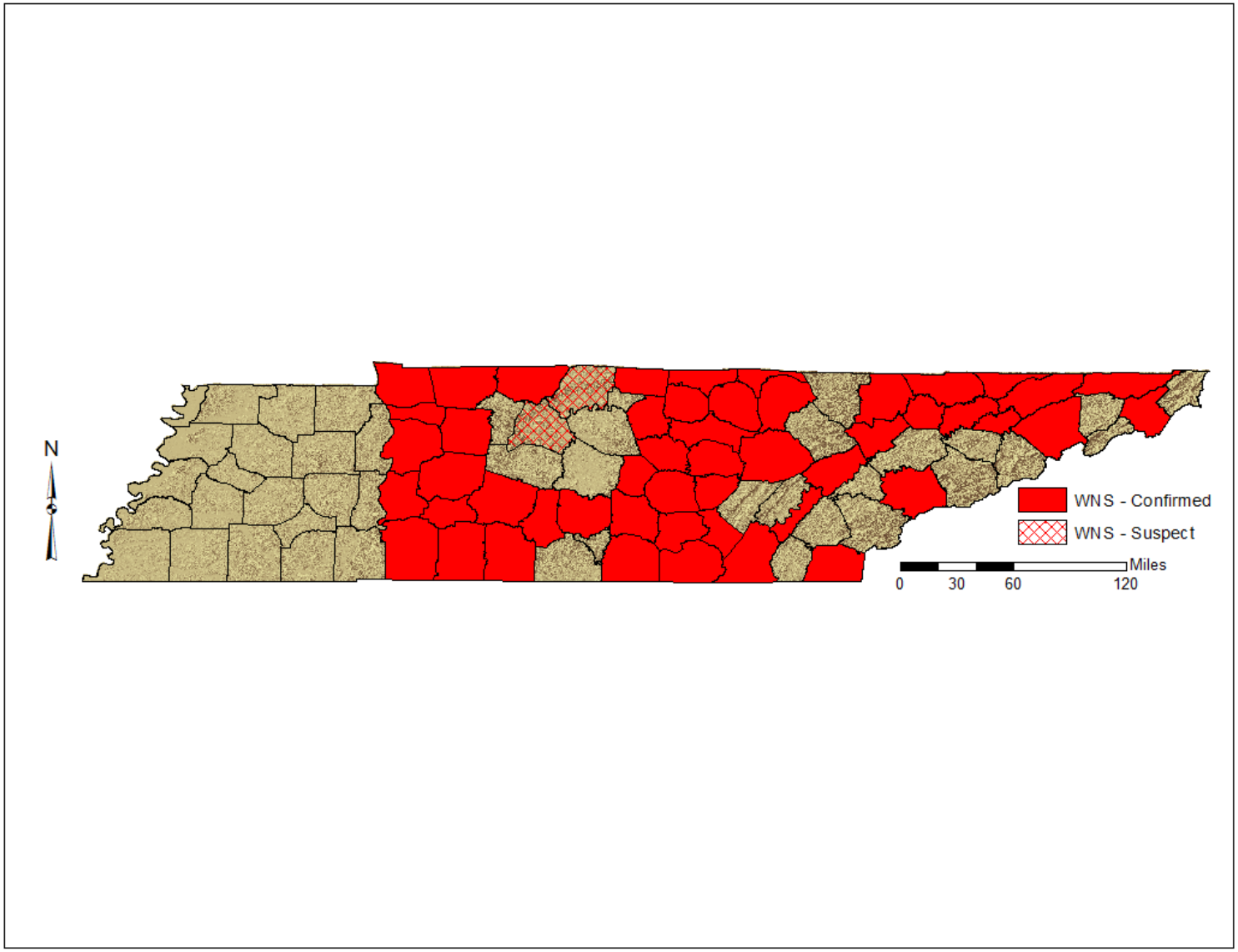
Appendix A-5. WNS confirmations during the winter of 2013-2014.



Appendix A-6. WNS confirmations during the winter of 2014-2015.



Appendix A-7. All WNS confirmed or suspect counties in Tennessee through the 2015-2016 field season.



Appendix B

- A list of all WNS confirmed, suspect, or negative counties in Tennessee

Cave Name or Structure	County	Year	WNS Status	Species	Diagnostic Report Number
Camps Gulf Cave	Van Buren	2010	Suspect	PESU[S], MYSO[1,N]	NWHC-22984
Dunbar Cave	Montgomery	2010	Suspect	MYSE[S]	NWHC Event 15950
East Fork SLP Cave	Fentress	2010	Suspect	MYLU, MYSE[S]	NWHC Event 15979
Grindstaff Cave	Carter	2010	Confirmed	MYSE[C], PESU[C]	NWHC
Hubbards Cave	Warren	2010	Negative	MYGR[N]	NWHC
White Oak Blowhole	Blount	2010	Suspect	N/A	N/A
Worleys Cave	Sullivan	2010	Confirmed	MYSE, PESU	NWHC Event 15948
Bellamy Cave	Montgomery	2011	Negative	MYGR[N]	NWHC-23532
Camps Gulf Cave	Van Buren	2011	Suspect	PESU[S]	NWHC-23481
Cooper Creek Cave	Montgomery	2011	Confirmed	MYLU[C], MYSE[C], PESU[C]	NWHC-23444
East Fork SLP Cave	Fentress	2011	Suspect	MYLU[S]	NWHC-23482
Under a House	Polk	2011	Negative	MYGR[2]	SCWDS CC11-188
White Oak Blowhole	Blount	2011	Suspect	MYLU[N]	NWHC-23466
Austin Peay State University	Montgomery	2012	Suspect	MYLU[S]	SCWDS CC12-235
Bellamy Cave	Montgomery	2012	Confirmed	MYGR, PESU[C]	SCWDS WNS12-54, WNS12-55
Bull Cave	Blount	2012	Negative	PESU[N]	SCWDS WNS12-50
Camps Gulf Cave	Van Buren	2012	Confirmed	N/A	N/A
Cantwell Valley Cave	Hancock	2012	Confirmed	N/A	N/A

[1]Tapelift sample taken and the bat was not euthanized; [2]Bat submitted was found dead at site; [C]WNS confirmed; [S]WNS suspect; [N]WNS Negative [SW]Only a swab sample was taken from the bat tested and was not euthanized; [N/A]Report not available.

Cave Name or Structure	County	Year	WNS Status	Species	Diagnostic Report Number
Carlton Cave	Franklin	2012	Confirmed	PESU[C]	SCWDS WNS12-56
Fort Campbell Nerd Hole	Stewart	2012	Confirmed	PESU[C]	NWHC-23846
Grassy Cove SLP Cave	Cumberland	2012	Confirmed	MYLU[C]	SCWDS WNS12-064 A-B
Gregory Cave	Blount	2012	Negative	PESU[N]	SCWDS WNS12-50
Hubbards Cave	Warren	2012	Negative	MYGR[N]	SCWDS WNS12-067
Hurricane Creek Cave	Humphreys	2012	Negative	PESU[N], MYSO[N]	NWHC-23848
Lookout Mtn. Battlefield Pit #1	Hamilton	2012	Confirmed	PESU[C]	SCWDS WNS12-86
Lost Creek Cave	White	2012	Negative	MYGR[N,SW], MYLU[N, SW], PESU[N,SW]	SCWDS WNS12-41, WNS12-42, WNS12-43
New Mammoth Cave	Campbell	2012	Negative	MYLU[N]	SCWDS WNS12-068
Pearsons Cave	Hawkins	2012	Confirmed	MYGR[C]	SCWDS WNS12-70
Rainbow Cave	Blount	2012	Negative	PESU[N]	SCWDS WNS12-50
Upstream Cave	Hancock	2012	Confirmed	PESU[C]	SCWDS WNS12-072
White Oak Blowhole	Blount	2012	Confirmed	MYLU[C], PESU[C]	SCWDS WNS12-061, WNS12-062
Afton Cave	Greene	2013	Confirmed	PESU[C]	SCWDS WNS13-72 A-C
Big Mouth Cave	Grundy	2013	Confirmed	MYLU[C]	SCWDS WNS13-56

[1]Tapelift sample taken and the bat was not euthanized; [2]Bat submitted was found dead at site; [C]WNS confirmed; [S]WNS suspect; [N]WNS Negative [SW]Only a swab sample was taken from the bat tested and was not euthanized; [N/A]Report not available.

Cave Name or Structure	County	Year	WNS Status	Species	Diagnostic Report Number
Blowing Cave	Hickman	2013	Confirmed	MYLU[C], MYSE[C], PESU[C]	SCWDS WNS13-38, WNS13-39, WNS13-40
Buggytop Cave	Franklin	2013	Confirmed	PESU[C]	SCWDS WNS13-103
Buis SLP Cave	Claiborne	2013	Confirmed	MYLU[C]	SCWDS WNS13-74 A-B
Cornstarch Cave	Fentress	2013	Confirmed	MYLU[C], PESU[C]	SCWDS WNS13-10, WNS13-11
Depriest Branch Cave	Lewis	2013	Confirmed	MYLU[C], MYSE[C], PESU[C]	SCWDS WNS13-46, WNS13-47, WNS48
Dunbar Cave	Montgomery	2013	Confirmed	PESU[C]	SCWDS WNS13-98, WNS13-101
East Fork SLP Cave	Fentress	2013	Confirmed	MYLU[C]	SCWDS WNS13-12
Espey Cave	Cannon	2013	Confirmed	PESU[C]	SCWDS WNS13-95
Eve's cave	Meigs	2013	Confirmed	PESU[C]	SCWDS WNS13-76
Gunter's Cave	Cannon	2013	Negative	PESU[N]	SCWDS WNS13-91
Herd O' Coons Cave	Union	2013	Confirmed	MYLU[C], PESU[C]	SCWDS WNS13-70 A-B, WNS13-71
Hubbards Cave	Warren	2013	Confirmed	PESU[C]	SCWDS WNS13-13
Hunt Cave	Dickson	2013	Confirmed	PESU[C]	SCWDS WNS13-49 A-C
Jaybird Cave	Perry	2013	Confirmed	MYLU[C]	SCWDS WNS13-44
Knob Creek Cave	Lawrence	2013	Confirmed	PESU[C]	SCWDS WNS13-54

[1]Tapelift sample taken and the bat was not euthanized; [2]Bat submitted was found dead at site; [C]WNS confirmed; [S]WNS suspect; [N]WNS Negative [SW]Only a swab sample was taken from the bat tested and was not euthanized; [N/A]Report not available.

Cave Name or Structure	County	Year	WNS Status	Species	Diagnostic Report Number
Lost Creek Cave	White	2013	Confirmed	PESU[C]	SCWDS WNS13-53 A-B
New Mammoth Cave	Campbell	2013	Confirmed	MYSE[C], MYLU[C]	SCWDS WNS13-25 A-B, WNS13-26
North Spivey Cave	Jackson	2013	Confirmed	MYLU[C]	SCWDS WNS13-94
Private Residence	Sequatchie	2013	Confirmed	PESU[C]	SCWDS WNS13-99
Pearsons Cave	Hawkins	2013	Confirmed	MYGR[2,N]	SCWDS WNS13-45
Richardson Cave	Houston	2013	Confirmed	MYLU[C]	SCWDS WNS13-02
Rose Cave	White	2013	Suspect	MYLU[S]	SCWDS WNS13-14
Sour Kraut Cave	Claiborne	2013	Confirmed	PESU[C]	SCWDS WNS13-75
Three Forks Cave	Overton	2013	Confirmed	PESU[C]	SCWDS WNS13-90
Trussell Cave	Grundy	2013	Confirmed	PESU[C]	SCWDS WNS13-55 A-C
Trussell Downstream Cave	Grundy	2013	Confirmed	PESU[C]	SCWDS WNS13-55 A-C
Virgin Falls Cave	White	2013	Confirmed	PESU[C]	SCWDS WNS13-50
Welch-Blowing Cave	Putnam	2013	Confirmed	PESU[C]	SCWDS WNS13-64
Whiteside Cave	Marion	2013	Confirmed	PESU[C]	SCWDS WNS13-63
Wolf River Cave	Fentress	2013	Confirmed	MYLU[C]	SCWDS WNS13-9
Zarathustrus Cave	Fentress	2013	Confirmed	PESU[C]	SCWDS WNS13-27
Aunt Beck Simmons Cave	Macon	2014	Confirmed	N/A	N/A
Biffle Cave	Wayne	2014	Confirmed	PESU[C]	SCWDS WNS14-10 A-C

[1]Tapelift sample taken and the bat was not euthanized; [2]Bat submitted was found dead at site; [C]WNS confirmed; [S]WNS suspect; [N]WNS Negative [SW]Only a swab sample was taken from the bat tested and was not euthanized; [N/A]Report not available.

Cave Name or Structure	County	Year	WNS Status	Species	Diagnostic Report Number
Big Jordan Cave	Pickett	2014	Confirmed	PESU[C], MYLU[C]	SCWDS WNS14-32, WNS14-33
Bridgewater Cave	Smith	2014	Confirmed	PESU[C]	SCWDS WNS14-20 A-B
Cave Creek Cave	Roane	2014	Confirmed	PESU[C]	SCWDS WNS14-31 A-B
Corner Store Cave	Hamblen	2014	Confirmed	PESU[C], MYLU[C]	SCWDS WNS14-29, WNS 14-30
Cripps Mill Cave	Dekalb	2014	Confirmed	PESU[C]	SCWDS WNS14-9
Dunbar Cave area	Montgomery	2014	Confirmed	PESU[C]	SCWDS WNS14-13, WNS14-14, WNS14-16, WNS14-16
Gee Cave	Polk	2014	Confirmed	PESU[C]	SCWDS WNS14-53
Hubbards Cave	Warren	2014	Confirmed	MYGR[2,N]	SCWDS WNS14-7
Hurricane Creek Cave	Humphreys	2014	Confirmed	PESU[C]	SCWDS WNS14-12
Indian Cave	Grainger	2014	Confirmed	PESU[C]	SCWDS WNS14-128, WNS14-129
Leonard Cave	Clay	2014	Confirmed	PESU[C]	SCWDS WNS14-130, WNS14-131, WNS14-132
Mason Cave	Sumner	2014	Suspect	PESU[S]	SCWDS WNS14-52 A-B
Rummage Cave	Maury	2014	Confirmed	PESU[C]	SCWDS WNS14-11 A-C
Springhill SLP Cave	Anderson	2014	Confirmed	MYLU[C]	SCWDS WNS14-8 A
Ward Cave	Bedford	2014	Confirmed	PESU[C]	SCWDS WNS14-51 A-C

[1]Tapelift sample taken and the bat was not euthanized; [2]Bat submitted was found dead at site; [C]WNS confirmed; [S]WNS suspect; [N]WNS Negative [SW]Only a swab sample was taken from the bat tested and was not euthanized; [N/A]Report not available.

Cave Name or Structure	County	Year	WNS Status	Species	Diagnostic Report Number
Crumpton Creek SLP Cave	Coffee	2015	Confirmed	PESU[C]	SCWDS CC15-124
Hardin's Junkyard Cave	Davidson	2015	Suspect	MYLU[S]	Field Signs Observed, UV positive, Photos Taken
Magnussen Cave	Giles	2015	Confirmed	PESU[C]	SCWDS CC15-26
Mason Cave	Sumner	2015	Suspect	N/A	Field Signs Observed, UV positive
Petty Cave	Marshall	2015	Confirmed	PESU[C]	SCWDS CC15-123 A-C
Silvertooth Cave	Moore	2015	Negative	PESU[N]	SCWDS CC15-125
Stark Cave	Robertson	2015	Confirmed	PESU[C]	SCWDS CC15-127
Civil War Bunker	Tipton	2016	Negative	EPFU[N], PESU[N]	SCWDS 16-92 A-B

[1]Tapelift sample taken and the bat was not euthanized; [2]Bat submitted was found dead at site; [C]WNS confirmed; [S]WNS suspect; [N]WNS Negative [SW]Only a swab sample was taken from the bat tested and was not euthanized; [N/A]Report not available.

Appendix C

- 2014-2015 Winter Survey Results

County	Survey Date	Cave Name	CORA	EPFU	LANO	MYAU	MYGR	MYLE	MYLU	MYSE	MYSO	Mysp	PESU	Total Bats	Surveyors
Blount	02/11/15	Gregorys Cave	0	0					0		0		33	33	NPS
Blount	02/20/15	Kelly Ridge Cave	67	1					16		188		17	289	NPS
Blount	02/19/15	Scott Gap Cave	3	0					3		0		18	24	NPS
Blount	02/12/15	Whiteoak Blowhole Cave	0	0					20		1,117		18	1,155	NPS
Blount	02/19/15	WhiteOak Saltpeter Cave	0	0					0		0		6	6	NPS
Campbell	01/21/15	New Mammoth Cave		4			1	3	64		76		13	161	TNC, TWRA
Campbell	01/21/15	Norris Dam Cave		4					1				176	181	TVA
Coffee	03/20/15	Crumpton Creek Saltpeter Cave		2			7						5	14	TNC, TWRA, TVA
Cumberland	03/02/15	Grassy Cove Saltpeter		3					42			2	7	54	TWRA, UTK
Cumberland	01/09/15	Lost Waterfall												0	TWRA, TNC
Cumberland	01/09/15	Oscar Pit											2	2	TWRA, TNC
Cumberland	01/30/15	Run to the Mill Cave		1						1	18		18	38	TNC, TWRA
Cumberland	01/09/15	Spouting Dome		2					2				2	6	TWRA, TNC
Davidson	01/29/15	Hardins Junkyard Cave							7				36	43	TNC
Dekalb	02/05/15	Cripps Mill Cave		6					21		8		263	298	TNC
Fentress	01/23/15	Cornstarch Cave							123	1	13		20	157	TNC, TWRA
Fentress	02/11/15	Dragons Breath Cave							103		40		123	266	TNC, TWRA
Fentress	01/14/15	East Fork Saltpeter Cave	1	6					32		210		49	298	TNC, TWRA
Fentress	01/23/15	Little Jack Creek Cave	29	3	1						8		3	44	TNC, TWRA
Fentress	03/14/15	Mountain Eye System					18		3		92	2	13	128	TNC, TWRA
Fentress	01/23/15	Redbud Cave								1			6	7	TNC, TWRA
Fentress	03/14/15	Smoking Slope?												0	TWRA
Fentress	01/20/15	Wolf River Cave		1			7		796	3	1,351		84	2,242	TNC, TWRA, USFWS
Fentress	02/09/15	Ygdrasils Cave		1							39		7	47	TWRA, UTK
Fentress	02/09/15	Zarathustras Cave	1						6		18		25	50	TWRA, UTK
Franklin	03/31/15	Above Signature Cave												0	TWRA, COHD
Franklin	03/31/15	Siganture Cave	1								1		14	16	TWRA, COHD
Giles	03/24/15	Magnusson Cave											29	29	TNC
Grundy	03/12/15	Trussell Cave					2				16		25	43	TNC, TWRA
Hawkins	01/22/15	Pearsons Cave		5			431,020							431,025	TNC, TWRA
Jackson	03/06/15	North Spivey Cave		6									31	37	TNC

County	Survey Date	Cave Name	CORA	EPFU	LANO	MYAU	MYGR	MYLE	MYLU	MYSE	MYSO	Mysp	PESU	Total Bats	Surveyors
Lewis	02/04/15	Depriest Branch Cave		8									34	42	TNC, TWRA
Marion	03/11/15	Whiteside Cave											138	138	TNC, TVA
Marshall	03/17/15	Petty Cave											118	118	TNC
Meigs	01/28/15	Blythe Ferry Cave											10	10	TVA
Meigs	01/28/15	Eves Cave		2									22	24	TVA
Meigs	03/12/15	Sensabaugh Cave		7									2	9	TVA
Montgomery	01/28/15	Bellamy Cave		11			381,475		1				11	381,498	TNC, TWRA
Montgomery	01/28/15	Cooper Creek Cave		6									6	12	TDEC, TNC, TWRA
Moore	03/16/15	Silvertooth Cave		4									56	60	TNC, TWRA
Overton	01/16/15	Xana 2 Cave	257	2					3	1			1	265	TNC
Perry	02/03/15	Jaybird Cave		2					12		8		134	156	TNC
Perry	02/03/15	Alexander Cave		1			3		3		4		174	185	TNC
Pickett	01/20/15	Big Jordan Cave	2	3					6		29		13	53	TNC, TWRA, USFWS
Roane	01/27/15	Marble Bluff Cave											356	356	TVA
Robertson	03/18/15	Stark Cave					4						137	141	TNC
Stewart	02/02/15	Tobaccoport Saltpeter Cave		15			35		35		160		13	258	TNC, TWRA
Sumner	03/08/15	Mason Cave		6					1	1			204	212	TNC
Tipton	03/04/15	Bluff-side Powder Magazine	1	7									4	12	TWRA
Van buren	02/25/15	Camps Gulf Cave		5	1						10	1	10	27	TDEC, TWRA, UTK
Van buren	02/25/15	Case Brothers Cave E1											3	3	TDEC, TWRA, UTK
Van buren	02/25/15	Case Brothers Cave E2	2	1										3	TDEC, TWRA, UTK
Van Buren	01/29/15	Rice Cave							1				76	77	TNC, USFWS
Warren	01/15/15	Hubbards Cave					210,577				78		4	247,070	AAFB, TNC, TWRA
Wayne	02/04/15	Biffle Cave		2					12	1	12		333	360	TNC
White	02/12/15	Great Expectations Cave Total	282	3		1			7		46		68	407	TNC, TWRA
White	02/12/15	Lost Creek Cave	1	6							29		53	89	TNC, TWRA
White	01/26/15	Rose Cave		2			1,448		3		105		81	1,639	TNC, UTK

Appendix D

- 2014-2015 Diagnostic Services Reports

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC15-124
DATE RECEIVED March 26, 2015
DATE OF REPORT May 27, 2015

STATE TN **COUNTY** Coffee **AREA** Crumpton Creek SLP Cave

SPECIES (NO.) Tri-colored Bat (1) **SEX** Male **AGE** Adult **WEIGHT** 6.8g

CASE HISTORY: An adult, male, tri-colored bat (*Perimyotis subflavus*) was submitted to SCWDS by Mr. Cory Holliday of The Tennessee Nature Conservancy on behalf of the Tennessee Wildlife Resources Agency. This bat was found dead in Crumpton Creek SLP Cave on March 20, 2015. Four other bats were seen dead and 14 additional bats were present at the cave. The carcasses were shipped to SCWDS by Mr. Holliday on March 25, 2015. The carcasses were received on March 26, 2015, and were necropsied the same day.

FINAL DIAGNOSIS: Trauma and white nose syndrome

COMMENTS: The fungal agent that causes white nose syndrome (*Pseudogymnoascus destructans*) was detected in this bat by histology and polymerase chain reaction (PCR). Fungal morphology and patterns of skin invasion were diagnostic for white nose syndrome. This bat also had histologic lesions of acute trauma (pulmonary hemorrhage) so it is likely that this animal was compromised due to the skin lesions and may have been predisposed to traumatic injury.

Intestinal trematodes were also identified in this bat. Trematodes of the genus *Acanthratrium, Ochoterenatrema* and *Paralecithodendrium* have been previously reported in this species, and are not considered clinically significant.

Rabies virus was not detected in the brains by fluorescent antibody testing.

Mr. Holliday was notified of receipt of the carcass by electronic mail on March 26, 2015. Histology and PCR results were provided on May 21, 2015.

WILDLIFE IMPLICATIONS: White nose syndrome, caused by the fungal agent *Pseudogymnoascus destructans*, emerged in New York State in 2006 and has subsequently spread to 25 states and five Canadian provinces. The disease has caused large-scale mortality in hibernating bats in affected caves particularly in northeastern North America.

PUBLIC HEALTH IMPLICATIONS: None reported.

DOMESTIC ANIMAL IMPLICATIONS: None reported.

DIAGNOSTICIAN Betsy Elsmo, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Holiday, Flock, Applegate, Coleman, Reichard, Armstrong

Laboratory Results Begin on Page 2

FINAL REPORT

CASE NUMBER CC15-124
HISTO NUMBER W15-320

GROSS NECROPSY FINDINGS: An adult, male, tri-colored bat that weighs 6.8g and is in good nutritional condition is submitted for necropsy. The degree of postmortem autolysis is mild. White powdery material is present in the fur over the muzzle. The forearm length is 38mm. There are no other significant gross findings.

HISTOLOGIC FINDINGS:

Haired skin (muzzle): Locally extensive areas of the epidermis are eroded to ulcerated and associated with abundant fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Hyphae and arthroconidia occasionally extend into and distend hair follicles. Rare lymphocytes, melanin granules, and cellular debris are associated with the foci of erosion and ulceration.

Skin (patagium): Multiple cupped ulcers are present throughout the patagium, and are associated with fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Rare lymphocytes, melanin granules, cellular debris, and mild interstitial edema are associated with the foci of erosion and ulceration.

Lung: Alveoli are multifocally flooded with hemorrhage.

Small intestine: Several transverse sections through 300µm in diameter trematodes with a parenchymatous body, an oral sucker, and a large uterus containing numerous 20µm in diameter, oval eggs with refractile amber walls surrounding basophilic miracidia, are present within the lumen.

There are no significant histopathologic changes in the brain, heart, trachea, spleen, liver, kidney, adrenal gland, stomach, large intestine, pancreas, or skeletal muscle.

MORPHOLOGIC DIAGNOSIS:

Skin (muzzle and patagium): Multifocal, moderate erosive to ulcerative dermatitis with intralesional fungal hyphae and conidia

Lung: Multifocal to diffuse acute hemorrhage

Small intestine: Intestinal trematodiasis (incidental finding)

MOLECULAR TESTING: A piece of the muzzle and wing membrane were submitted to the SCWDS laboratory for polymerase chain reaction (PCR) testing. The sample was positive by PCR testing. The extracted genetic material from the muzzle was submitted to the Georgia Genomics Facility for genetic sequencing. Sequencing results confirmed the presence of *Pseudogymnoascus destructans* in the submitted samples.

VIROLOGY: A sample of brain was submitted to the Athens Veterinary Diagnostic Laboratory in Athens, Georgia, for rabies virus testing by the fluorescent antibody test. Rabies virus was not detected.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC15-126
DATE RECEIVED March 18, 2015
DATE OF REPORT May 27, 2015

STATE TN **COUNTY** Giles **AREA** Ardmore

SPECIES (NO.) Tri-colored Bat (*Perimyotis subflavus*) (2) **SEX** * **AGE** * **WEIGHT** *

SCWDS ID	SEX	AGE	WEIGHT (g)
CC15-126A	F	Adult	5.2
CC15-126B	F	Adult	2.6

CASE HISTORY: Two bats that have been euthanized by cervical dislocation are submitted from Magnussen Cave. Approximately 8 sick bats were observed out of a total of 32 bats present in the cave. The bats were found with clinical signs suggestive of white nose syndrome on March 24, 2015. The bats were shipped to SCWDS by Mr. Cory Holliday of The Tennessee Nature Conservancy on behalf of the Tennessee Wildlife Resources Agency on March 25, 2015, and were received on March 26, 2015. A necropsy was performed the same day.

FINAL DIAGNOSIS: White nose syndrome (A and B)

COMMENTS: The fungal agent that causes white nose syndrome (*Pseudogymnoascus destructans*) was detected in both bats submitted by histology and polymerase chain reaction (PCR). Fungal morphology and patterns of skin invasion were diagnostic for white nose syndrome.

Intestinal trematodes were also identified in bat "B". Trematodes of the genus *Acanthratrium, Ochoterenatrema* and *Paralecithodendrium* have been previously reported in this species, and are not considered clinically significant.

Rabies was not detected in either bat.

Mr. Holliday was notified of receipt of the carcasses by electronic mail on March 26, 2015. A final update was provided on May 21, 2015.

WILDLIFE IMPLICATIONS: White nose syndrome, caused by the fungal agent *Pseudogymnoascus destructans*, emerged in New York in 2006 and has subsequently spread to 25 states and five Canadian provinces. The disease has caused large-scale mortality in hibernating bats in affected caves particularly in northeastern North America.

PUBLIC HEALTH IMPLICATIONS: None reported.

DOMESTIC ANIMAL IMPLICATIONS: None reported.

DIAGNOSTICIAN Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Holiday, Flock, Applegate, Coleman, Reichard, Armstrong

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS **FINAL REPORT**

CASE NUMBER CC15-126
HISTO NUMBER W15-312

GROSS NECROPSY FINDINGS:

CC15-126A: An adult, female tri-colored bat (*Perimyotis subflavus*) that weighs 5.2g is submitted for necropsy. The forearm length is 33mm. This bat is in poor body condition and moderate carcass condition. Evidence of cervical dislocation is present at necropsy.

CC15-126B: An adult, female tri-colored bat (*Perimyotis subflavus*) that weighs 2.6g is submitted for necropsy. The forearm length is 36mm. This bat is in poor carcass condition and no brain is available for rabies testing. The left ear is missing. Maggots are present in the skull. Evidence of cervical dislocation is present at necropsy.

HISTOLOGIC FINDINGS:

CC15-126A:
Skin; wing membranes and muzzle: Multiple foci of erosion and ulceration are present along the wing membranes often associated with septate fungal hyphae approximately 3-4 microns in diameter and curved arthroconidia that measure approximately 4 by 2 microns. Hyphae and arthroconidia surround hair follicles and are occasionally found deep in the dermis. Fungal hyphae also line the nasal cavity in sections of the muzzle.

No significant findings are present within sections of: brain, lung, intestine, heart, liver, kidney, spleen, and pancreas.

CC15-126B:
Skin; wing membranes and muzzle: Multiple foci of erosion and occasionally ulceration are present along the wing membranes often associated with septate fungal hyphae approximately 2-4 microns in diameter and curved arthroconidia that measure approximately 4 by 2 microns. Hyphae and arthroconidia often surround hair follicles.

Intestine: Multiple trematode eggs are present within the lumen of the intestine.

No significant findings are present within sections of: lung, heart, brain, liver, and kidney.

Marked autolysis is present in sections of visceral organs.

MORPHOLOGIC DIAGNOSIS:

CC15-126A:
Skin, wing membranes and muzzle: Multifocal to coalescing erosive to ulcerative dermatitis and rhinitis with intralesional fungal hyphae and arthroconidia

CC15-126B:
Skin, wing membranes and muzzle: Multifocal to coalescing erosive to ulcerative dermatitis and rhinitis with intralesional fungal hyphae and arthroconidia

Intestinal trematodiasis (incidental finding)

MYCOLOGY: Samples from the muzzle and wings from each bat were submitted to the SCWDS laboratory for fungal culture. Results are pending.

MOLECULAR TESTING: Samples from the muzzle and wing from each bat were submitted to the SCWDS laboratory for polymerase chain reaction (PCR) testing for *Pseudogymnoascus destructans* (Pd). All samples tested positive for Pd by PCR. The extracted genetic material from the muzzle from one bat was submitted to the Georgia Genomics Facility for genetic sequencing. Sequencing results confirmed the presence of *Pseudogymnoascus destructans* in the submitted sample.

VIROLOGY: A sample of brain from CC15-126A is submitted to the Athens Veterinary Diagnostic Laboratory for rabies testing. Rabies was not detected.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC15-123 A-C
DATE RECEIVED March 26, 2015
DATE OF REPORT May 26, 2015

STATE TN **COUNTY** Marshall **AREA** Petty Cave

SPECIES (NO.) Tri-colored Bat (3) **SEX** * **AGE** Adult **WEIGHT** *
***Varies, see table below**

CC15-123	Weight(g)	Sex
A	4.3	Male
B	3.8	Female
C	5.1	Male

CASE HISTORY: Three adult, tri-colored bats (*Perimyotis subflavus*) were submitted to SCWDS by Mr. Cory Holliday of The Tennessee Nature Conservancy on behalf of the Tennessee Wildlife Resources Agency. These bats were found dead in Petty Cave on March 17, 2015. Approximately 50 bats were seen dead and 118 additional bats were present at the cave. The carcasses were shipped to SCWDS by Mr. Holliday on March 25, 2015. The carcasses were received on March 26, 2015, and were necropsied the same day.

FINAL DIAGNOSIS: Trauma and white nose syndrome (A-C)

COMMENTS: The fungal agent that causes white nose syndrome (*Pseudogymnoascus destructans*) was detected in all three bats by histology and polymerase chain reaction (PCR). Fungal morphology and patterns of skin invasion were diagnostic for white nose syndrome. All three bats also had histologic lesions of acute trauma (pulmonary hemorrhage) so it is likely that these animals were compromised due to the skin lesions and may have been predisposed to traumatic injury.

There was significant superficial bacterial overgrowth in the skin bat "B," which likely represents secondary colonization of skin lesions. Some of the bacterial overgrowth may have occurred postmortem.

Intestinal trematodes were identified in bats "A" and "B". Trematodes of the genus *Acanthratrium, Ochoterenatrema* and *Paralecithodendrium* have been previously reported in this species, and are not considered clinically significant. Rabies virus was not detected in any of the brains by fluorescent antibody testing.

Mr. Holliday was notified of receipt of the carcass by electronic mail on March 26, 2015. Histology and PCR results were provided on May 21, 2015.

WILDLIFE IMPLICATIONS: White nose syndrome, caused by the fungal agent *Pseudogymnoascus destructans*, emerged in New York State in 2006 and has subsequently spread to 25 states and five Canadian provinces. The disease has caused large-scale mortality in hibernating bats in affected caves particularly in northeastern North America.

PUBLIC HEALTH IMPLICATIONS: None reported.

DOMESTIC ANIMAL IMPLICATIONS: None reported.

DIAGNOSTICIAN Betsy Elsmo, DVM **SUPERVISOR** Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Holiday, Flock, Applegate, Coleman, Reichard, Armstrong

Laboratory Results Begin on Page 2

FINAL REPORT

CASE NUMBER CC15-123 A-C
HISTO NUMBER W15-319

GROSS NECROPSY FINDINGS:

A: An adult, male tri-colored bat that weighs 4.3g and is in good nutritional condition is submitted for necropsy. Overall, the carcass is mildly decomposed and the head is not attached to the body. Multiple round holes are present within the wing membranes and are most prominent on the left proximal wing. A small amount of clotted blood is present in the thoracic cavity. The lungs are diffusely mottled dark red. The forearm length is 32mm. There are no other significant gross findings.

B: An adult, female tri-colored bat that weighs 3.8g and is in fair body condition is submitted for necropsy. The degree of postmortem autolysis is moderate. The forearm length is 34mm. There are no significant gross findings.

C: An adult, male tri-colored bat that weighs 5.1g and is in fair body condition is submitted for necropsy. The degree of postmortem autolysis is moderate and the head is not attached to the body. The proximal wings are diffusely wrinkled. White powdery material is present in the fur over the muzzle. The lungs are diffusely mottled dark red. The forearm length is 36mm. There are no other significant findings.

HISTOLOGIC FINDINGS:

A:
Haired skin (muzzle): Locally extensive areas of the epidermis are eroded to ulcerated and associated with abundant fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Hyphae and arthroconidia occasionally extend into and distend hair follicles. Rare lymphocytes, melanin granules, and cellular debris are associated with the foci of erosion and ulceration.

Skin (patagium): Multiple cupped ulcers are present throughout the patagium, and are associated with fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Rare lymphocytes, melanin granules, cellular debris, and mild interstitial edema are associated with the foci of erosion and ulceration.

Lung: Alveoli are multifocally flooded with hemorrhage.

Small intestine: A transverse section through a 300µm in diameter trematode with a parenchymatous body, an oral sucker, and a large uterus containing numerous 20µm in diameter, oval eggs with refractile amber walls surrounding basophilic miracidia, is present within the lumen.

There are no significant histopathologic changes in the brain, heart, spleen, liver, kidney, adrenal gland, stomach, large intestine, pancreas, or skeletal muscle.

B:
Haired skin (muzzle): Locally extensive areas of the epidermis are eroded to ulcerated and associated with abundant fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Hyphae and arthroconidia frequently form large aggregates and often extend into and efface the dermis. Rare lymphocytes, melanin granules, and cellular debris are associated with the foci of erosion and ulceration. Large colonies of gram-negative rods are often present superficially in areas of ulceration.

Skin (patagium): Multiple cupped ulcers are present throughout the patagium, and are associated with fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Rare lymphocytes, melanin granules, cellular debris, and mild interstitial edema are associated with the foci of erosion and ulceration. Large colonies of gram-negative rods are often present superficially in areas of ulceration.

Laboratory Results Continue on Page 3

Lung: Alveoli are diffusely flooded with hemorrhage.

Small intestine: Several transverse sections through 300µm in diameter trematodes with a parenchymatous body, an oral sucker, and a large uterus containing numerous 20µm in diameter, oval eggs with refractile amber walls surrounding basophilic miracidia, are present within the lumen.

There are no significant histopathologic changes in the brain, heart, liver, kidney, large intestine, pancreas, or skeletal muscle.

Marked autolysis is present within all sections examined and may mask subtle changes.

C:
Haired skin (muzzle): Locally extensive areas of the epidermis are eroded to ulcerated and associated with abundant fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Hyphae and arthroconidia frequently form large aggregates and often extend into and efface the dermis. Rare lymphocytes, melanin granules, and cellular debris are associated with the foci of erosion and ulceration.

Skin (patagium): Multiple cupped ulcers are present throughout the patagium, and are associated with fungal hyphae with 3-4µm in diameter, parallel, septate walls and numerous approximately 2 by 4µm curved arthroconidia. Rare lymphocytes, melanin granules, cellular debris, and mild interstitial edema are associated with the foci of erosion and ulceration.

Lung: Alveoli are diffusely flooded with hemorrhage.

There are no significant histopathologic changes in the brain, heart, spleen, liver, kidney, stomach, small intestine, large intestine, pancreas, or skeletal muscle.

MORPHOLOGIC DIAGNOSIS:

A, C: Skin (muzzle and patagium): Multifocal, moderate erosive to ulcerative dermatitis with intralesional fungal hyphae and conidia

B: Skin (muzzle and patagium): Multifocal, moderate erosive to ulcerative dermatitis with intralesional fungal hyphae and conidia and superficial gram-negative bacilli

A-C: Lung: Multifocal to diffuse acute hemorrhage

A, B: Small intestine: Intestinal trematodiasis (incidental finding)

MOLECULAR TESTING: A piece of the muzzle and wing membrane from all three bats were submitted to the SCWDS laboratory for polymerase chain reaction (PCR) testing. All samples were positive by PCR testing using primers for *Pseudogymnoascus destructans*. Extracted genetic material from the muzzle of one bat was submitted to the Georgia Genomics Facility for genetic sequencing, which confirmed the presence of *Pseudogymnoascus destructans*.

VIROLOGY: A sample of brain from all three bats was submitted to the Athens Veterinary Diagnostic Laboratory in Athens, Georgia, for rabies virus testing by the fluorescent antibody test. Rabies virus was not detected in any of the samples.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC15-125
DATE RECEIVED March 18, 2015
DATE OF REPORT May 27, 2015

STATE TN **COUNTY** Moore **AREA** Lynchburg

SPECIES (NO.) Tri-colored Bat (*Perimyotis subflavus*) (1) **SEX** F **AGE** Adult **WEIGHT** 2.4g

CASE HISTORY: This bat was found dead near the cave entrance at Silvertooth Cave on Mach 16, 2015, and was subsequently frozen prior to shipment. This bat was the only dead bat found out of a total of 60 bats present in the cave. The bat was shipped to SCWDS by Mr. Cory Holliday of The Tennessee Nature Conservancy on behalf of the Tennessee Wildlife Resources Agency on March 25, 2015, and was received on March 26, 2015. A necropsy was performed the same day.

FINAL DIAGNOSIS: *Pseudogymnoascus destructans* not detected

COMMENTS: The fungal agent that causes white nose syndrome, *Pseudogymnoascus destructans* (Pd), was not detected by polymerase chain reaction (PCR). Autolysis may have interfered with histological examination of the wing membranes, but no characteristic conidia were observed.

Intestinal trematodes were also identified in this bat. Trematodes of the genus *Acanthratrium, Ochoterenatrema* and *Paralecithodendrium* have been previously reported in this species, and are not considered clinically significant.

Rabies was not detected in the brain.

Mr. Holliday was notified of receipt of the carcass by electronic mail on March 26, 2015. A final update was provided on April 15, 2015. A final update was sent on May 21, 2015.

WILDLIFE IMPLICATIONS: White nose syndrome, caused by the fungal agent *Pseudogymnoascus destructans*, emerged in New York in 2006 and has subsequently spread to 25 states and five Canadian provinces. The disease has caused large-scale mortality in hibernating bats in affected caves particularly in northeastern North America.

PUBLIC HEALTH IMPLICATIONS: None reported.

DOMESTIC ANIMAL IMPLICATIONS: None reported.

DIAGNOSTICIAN Heather Fenton
Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Holiday, Flock, Applegate, Coleman, Reichard, Armstrong

Laboratory Results Begin on Page 2

PAGE 2 – SCWDS -- LABORATORY RESULTS

FINAL REPORT

CASE NUMBER CC15-125
HISTO NUMBER W15-311

GROSS NECROPSY FINDINGS: An adult, female tri-colored bat (*Perimyotis subflavus*) that weighs 2.4g is submitted for necropsy. The forearm length is 32mm. The carcass is markedly desiccated and no brain is available for rabies testing. Overall, the bat appears to be in poor body condition.

HISTOLOGIC FINDINGS:

Autolysis hinders meaningful histologic examination.

Skin: Occasional fungal hyphae approximately 7 microns in diameter are present associated with foci of erosion although the morphology is slightly different from that expected with Pd.

Intestine: A large number of adult trematodes and associated eggs are present within the intestine.

MORPHOLOGIC DIAGNOSIS:

Skin: Multifocal erosive to ulcerative dermatitis with intralesional fungal hyphae

Intestinal trematodiasis (incidental finding)

MOLECULAR TESTING: Samples from the muzzle and wing were submitted to the SCWDS laboratory for polymerase chain reaction (PCR) testing for *Pseudogymnoascus destructans* (Pd). Both samples were negative for Pd by PCR.

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC15-127
DATE RECEIVED March 18, 2015
DATE OF REPORT May 27, 2015

STATE TN **COUNTY** Robertson **AREA** Stark Cave

SPECIES (NO.) Tri-colored Bat (*Perimyotis subflavus*) (2) **SEX** * **AGE** * **WEIGHT** *

SCWDS ID	SEX	AGE	WEIGHT (g)
CC15-127A	M	Adult	4.3
CC15-127B	M	Adult	5.2

CASE HISTORY: Two bats that have been euthanized by cervical dislocation are submitted from Stark Cave. Approximately 20 sick bats were observed out of a total of 141 bats present in the cave. The bats were found with clinical signs suggestive of white nose syndrome on March 25, 2015. The bats were shipped to SCWDS by Mr. Cory Holliday of the Tennessee Nature Conservancy on behalf of the Tennessee Wildlife Resources Agency on March 25, 2015, and were received on March 26, 2015. A necropsy was performed the same day.

FINAL DIAGNOSIS: White nose syndrome (A and B)

COMMENTS: The fungal agent that causes white nose syndrome (*Pseudogymnoascus destructans*) was detected in both bats submitted by histology and polymerase chain reaction (PCR). Fungal morphology and patterns of skin invasion were diagnostic for white nose syndrome.

Intestinal trematodes were also identified in this bat. Trematodes of the genus *Acanthratrium, Ochoterenatrema* and *Paralecithodendrium* have been previously reported in this species, and are not considered clinically significant.

Rabies was not detected in either bat.

Mr. Holliday was notified of receipt of the carcasses by electronic mail on March 26, 2015. A final update was provided on May 21, 2015.

WILDLIFE IMPLICATIONS: White nose syndrome, caused by the fungal agent *Pseudogymnoascus destructans*, emerged in New York in 2006 and has subsequently spread to 25 states and five Canadian provinces. The disease has caused large-scale mortality in hibernating bats in affected caves particularly in northeastern North America.

PUBLIC HEALTH IMPLICATIONS: None reported.

DOMESTIC ANIMAL IMPLICATIONS: None reported.

DIAGNOSTICIAN Heather Fenton, DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Holiday, Flock, Applegate, Coleman, Reichard, Armstrong

Laboratory Results Begin on Page 2

FINAL REPORT

CASE NUMBER CC15-127
HISTO NUMBER W15-313

GROSS NECROPSY FINDINGS:

CC15-127A: An adult, male tri-colored bat (*Perimyotis subflavus*) that weighs 4.3g is submitted for necropsy. The forearm length is 32mm. This bat is in moderate body condition and moderate carcass condition. Evidence of cervical dislocation is present at necropsy. White material is present around the nose and muzzle.

CC15-127B: An adult, male tri-colored bat (*Perimyotis subflavus*) that weighs 5.2g is submitted for necropsy. The forearm length is 32mm. This bat is in poor carcass condition and no brain is available for rabies testing. Maggots are present in the skull. Evidence of cervical dislocation is present at necropsy.

HISTOLOGIC FINDINGS:

CC15-127A:
Skin; wing membrane, pinna, and muzzle: Multifocal erosions are present along the wing membranes associated with small numbers of lymphocytes within the dermis, surface bacteria, fungal hyphae approximately 3-4 microns in diameter and curve conidia approximately 2 microns in greatest diameter. Fungal hyphae and conidia are present along the surface of the pinna and muzzle including the nasal cavity. Fungal hyphae and arthroconidia often surround hair follicles.

Intestine: Multiple sections of adult trematodes approximately 250 microns in greatest diameter are present throughout the section.

Lung: Multiple foci of hemorrhage are present throughout the lung (consistent with the euthanasia method).

No significant findings are present within sections of: adrenal, kidney, heart, liver, brain, and pancreas.

CC15-127B:
Skin; wing membrane, pinna, and muzzle: Multifocal erosions are present along the wing membranes associated with small numbers of lymphocytes within the dermis, surface bacterial colonies, fungal hyphae approximately 3-4 microns in diameter and curve conidia approximately 2 microns in greatest diameter. Fungal hyphae and conidia are present along the surface of the pinna and muzzle including the nasal cavity. Fungal hyphae and arthroconidia often surround hair follicles.

Intestine: Multiple sections of adult trematodes approximately 250 microns in greatest diameter are present throughout the section.

Lung: Multiple foci of hemorrhage are present throughout the lung (consistent with the euthanasia method).

No significant findings are present within sections of: heart, skeletal muscle, pancreas, liver, kidney, and brain.

MORPHOLOGIC DIAGNOSIS:

CC15-127A:
Skin; wing membrane, pinna and muzzle: Multifocal to coalescing erosive to ulcerative dermatitis and rhinitis with intralesional fungal hyphae and arthroconidia

Intestinal trematodiasis (incidental finding)

Laboratory Results Continue on Page 3

PAGE 3 – SCWDS -- LABORATORY RESULTS

FINAL REPORT

CASE NUMBER CC15-127
HISTO NUMBER W15-313

CC15-127B:
Skin; wing membrane, pinna and muzzle: Multifocal to coalescing erosive to ulcerative dermatitis and rhinitis with intralesional fungal hyphae and arthroconidia

Intestinal trematodiasis (incidental finding)

MOLECULAR TESTING: Samples from the muzzle and wing from each bat were submitted to the SCWDS laboratory for polymerase chain reaction (PCR) testing for *Pseudogymnoascus destructans* (Pd). All samples tested positive for Pd by PCR. The extracted genetic material from the muzzle of one bat was submitted to the Georgia Genomics Facility for genetic sequencing. Sequencing results confirmed the presence of *Pseudogymnoascus destructans* in the submitted sample.

VIROLOGY: A sample of brain from each bat was submitted to the Athens Veterinary Diagnostic Laboratory for rabies testing. Rabies was not detected from the brain of either bat.

Appendix E

- 2015-2016 Winter Survey Results

County	Survey Date	Cave Name	CORA	EPFU	LANO	MYAU	MYGR	MYLE	MYLU	MYSE	MYSO	Mysp	PESU	Total Bats	Surveyors
Anderson	2/23/2016	Springhill Saltpeter Cave		24					5				1	30	TNC, TWRA
Bedford	2/11/2016	Ward		6					1				96	103	TNC, TWRA
Blount	2/18/2016	Snake Dance (Bull)							2				14	16	NPS
Blount	2/18/2016	Rich Mountain Blowhole Cave											1	1	NPS
Blount	2/11/2016	Rainbow Cave							3				21	24	NPS
Blount	2/13/2016	Bull Cave							3		140		17	160	NPS
Campbell	2/4/2016	Panther Cave A		1										1	TWRA, UTK
Campbell	1/29/2016	Norris Dam Cave		1									41	42	TVA
Cannon	3/4/2016	Pea Ridge Cave											2	2	TWRA
Cannon	2/11/2016	Cane Sink Cave											3	3	TWRA
Carter	3/14/2016	Grindstaff Cave		2									4	5	TWRA, UTK
Fentress	3/8/2016	Xanadu Cave	15	2									46	63	TWRA
Fentress	2/8/2016	Coriolis Cave											23	23	TNC, TWRA
Franklin	2/17/2015	Williams Saltpeter											1	1	TWRA
Franklin	2/4/2016	Signature Cave	1						1		12		26	40	TWRA
Franklin	2/24/2016	Pennington											3	3	TWRA, TVA
Franklin	1/19/2016	Lost Cove Cave	2	3			1			1			85	92	AAFB, TWRA
Franklin	1/28/2016	Keith Cave											12	12	TNC, TWRA
Franklin	2/5/2016	Holy Moly Canyon	2											2	TWRA, UTK
Franklin	2/5/2016	Goats Bluff 5000											11	11	TWRA, UTK
Franklin	2/5/2016	Cave 4998											1	1	TWRA, UTK
Franklin	3/3/2016	Carlton Cave											39	39	TWRA
Franklin	3/10/2016	Caney Hollow Cave		2			32,400						10	32,412	AAFB, TWRA
Greene	2/24/2016	Stillhouse Cave		7			26						17	50	TNC, TWRA
Greene	2/24/2016	Poplar Cave		9					1				10	20	TNC, TWRA
Greene	2/24/2016	Double Mouth Cave		1									2	3	TNC, TWRA
Grundy	2/15/2016	Trussell Cave		1							17		35	53	TWRA
Hickman	3/9/2016	Sugar Creek Cave		7									20	27	TNC, TWRA
Knox	2/22/2016	Tag's Finest Pit											3	3	TWRA, USFWS
Lawrence	2/26/2016	Knob Creek Cave		18			1						41	60	TNC, TWRA
Lincoln	2/21/2016	Lincoln Co. Bat Cave											8	8	TWRA

County	Survey Date	Cave Name	CORA	EPFU	LANO	MYAU	MYGR	MYLE	MYLU	MYSE	MYSO	Mysp	PESU	Total Bats	Surveyors
Macon	1/26/2016	Aunt Beck Simmins Cave		13					1	9			693	716	TNC, TWRA
Marion	2/23/2016	Whiteside Cave											47	47	AAFB, TWRA, USFWS
Meigs	2/11/2016	Eves Cave		2									22	24	TVA
Meigs	2/11/2016	Blythe Ferry											16	16	TVA
Montgomery	2/24/2016	Dunbar Cave								1			49	50	TDEC, TWRA, FORT
Montgomery	2/25/2016	Cooper Creek Cave		3					1				5	9	TWRA, TVA
Overton	2/24/2016	Standing Stone Dam												0	TWRA
Pickett	1/14/2016	Bunkum Cave	1	5									51	57	TWRA
Putnam	2/4/2016	WelchBowling Cave		1									8	9	TNC, TWRA
Putnam	2/4/2016	Johnson Cave	3	3				1					18	25	TNC, TWRA
Putnam	2/3/2016	Bay's Cave											3	3	TWRA, USFWS
Putnam	3/2/2016	Ament Cave					31						21	52	TWRA, USFWS
Roane	1/28/2016	Marble Bluff Cave											42	42	TVA
Robertson	2/1/2016	Whiskey River Cave		2									61	63	TNC, TWRA
Rutherford	2/2/2016	Herron Cave / Herring Cave							1				71	72	TNC, TWRA
Sevier	3/9/2016	Unknown											5	5	TWRA, UTK
Smith	1/25/2016	Piper Cave		3									20	23	TNC, TWRA
Smith	1/25/2016	New Piper											16	16	TNC, TWRA
Smith	1/25/2016	Bridgewater Cave							11				5	16	TNC, TWRA
Sullivan	3/14/2016	Worley's/Morrel Cave											11	11	TWRA, UTK
Sumner	1/26/2016	Mason		4						1			55	60	TNC, TWRA
Union	2/23/2016	Wright		2					3				7	12	TNC, TWRA
Union	2/4/2016	Unknown		1									4	5	TWRA, UTK
Union	3/15/2016	Tickett Boot Cave											1	1	TWRA, UTK
Union	3/15/2016	Oaks Cave					200+		1				9	210	TWRA, UTK
Union	2/4/2016	Lost Creek Cave											2	2	TWRA, UTK
Union	2/3/2016	Herd O Coons Cave		1					12	1	1		14	29	TWRA, UTK
Van Buren	12/28/2015	The Big Wet Spot												0	TWRA
Van Buren	1/28/2016	Run to Big Sink Cave											7	7	TWRA
Van Buren	1/27/2016	Measles Gulf Cave	164	7									19	190	TNC, TWRA

County	Survey Date	Cave Name	CORA	EPFU	LANO	MYAU	MYGR	MYLE	MYLU	MYSE	MYSO	Mysp	PESU	Total Bats	Surveyors
Van Buren	2/3/2016	Foxhole Cave	3	1					2			3	8	17	TWRA, USFWS
Van Buren	3/3/2016	Camps Gulf Cave		3	1				1		12	1	8	26	TDEC, TWRA, USFWS
Van Buren	3/3/2016	Camps Gulf CaveNR2											9	9	TDEC, TWRA, USFWS
Van Buren	2/16/2016	Bone Cave	5	15					2				22	44	TNC, TWRA
Van Buren	2/16/2016	Big Bone Cave	5	15					2				22	44	TDEC, TNC, TWRA
Van Buren	2/16/2016	Near Big Bone Cave											**27**	27	TDEC
Warren	2/16/2016	Little Bat Cave	64										2	68	TNC, TWRA
Warren	2/29/2016	Jaco Sping Cave		2			19		1				44	66	TNC, TWRA
Warren	2/29/2016	Hazel Ward Cave											42	42	TNC, TWRA
White	2/5/2016	Virgin Falls Cave											11	11	TWRA, USFWS
White	3/10/2016	Top of Davis Cave	65											65	TWRA
White	3/10/2016	Sapp Cave											2	2	TWRA
White	3/10/2016	Retriever Well											1	1	TWRA
White	3/10/2016	Muddy Dog Well												0	TWRA
White	1/5/2016	Mill Hole Cave											12	12	TWRA, UTK
White	2/14/2016	Lockwood Cave											35	35	TWRA, UTK
White	3/10/2016	Little Canine		1										1	TWRA
White	1/27/2016	Indian Cave		3									25	28	TNC, TWRA
White	1/7/2016	Great Big Bottom Cave											18	18	TWRA
White	1/11/2016	Ghost River											9	9	TWRA
White	1/15/2016	Gastens Moonshine Cave		2									5	7	TWRA
White	3/10/2016	Davis Cave	1										3	4	TWRA
White	3/10/2016	Big Dog Cave											5	5	TWRA

Appendix F

- 2015-2016 Diagnostic Services Report

DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS)
COLLEGE OF VETERINARY MEDICINE
THE UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602-7393
TELEPHONE: 706-542-1741; FAX: 706-542-5865

CASE NUMBER CC16-92 A and B
DATE RECEIVED February 19, 2016
DATE OF REPORT April 1, 2016

STATE TN **COUNTY** Tipton **AREA** Drummonds

SPECIES (NO.) * **SEX** * **AGE** * **WEIGHT** *
*

A	Big Brown Bat (*Eptesicus fuscus*)	Female	Adult	19.5 g
B	Tri-colored Bat (*Perimyotis subflavus*)	Male	Adult	5.3 g

CASE HISTORY: The refrigerated carcasses of a big brown bat and a tri-colored bat were submitted by Mr. Robert Colvin of the Tennessee Wildlife Resources Agency (TWRA). These bats were two of a group of four under surveillance in a Civil War bunker in Tipton County. The bunker has been used by bats year-round and has been monitored annually. This year (2016) marks the first time that mortalities were recorded. Both bats were found dead on February 16, 2016. Their carcasses were collected and refrigerated by TWRA personnel. They were shipped to SCWDS on February 17, 2016.

The two carcasses were received at SCWDS on February 19, 2016, and necropsies were performed the same day.

FINAL DIAGNOSIS: Bats A and B: Suspect trauma and chronic dermatitis
Bat B: Acariasis
Bats and B: *Pseudogymnoascus destructans* not detected

COMMENTS: Lesions suggestive of a recent traumatic injury were detected in both bats. Although the cause of the traumatic lesions is unknown, attempted predation could be considered. Both bats had evidence of chronic dermatitis of unknown origin. Mites were detected in bat B, but are considered to be an incidental finding. *Pseudogymnoascus destructans* (Pd), the causative agent of white-nose syndrome, was not detected in either bat. Superficial fungal hyphae were detected microscopically on the surface of the skin in bat B, but are not thought to be morphologically consistent with Pd. A common cave fungus in the *Pseudogymnoascus* genus was detected on skin samples from bat A, but was not Pd. Rabies virus was not detected in either bat.

Receipt of the submission and initial results were reported to Mr. Robert Colvin on February 18, 2016. An update was provided on February 19, 2016. Updates via telephone were provided on February 23 and 25, and on March 29, 2016. All reports and updates were provided via electronic mail unless otherwise noted.

WILDLIFE IMPLICATIONS: Trauma is a common cause of morbidity and mortality in free-ranging wildlife.

PUBLIC HEALTH IMPLICATIONS: None reported.

DOMESTIC ANIMAL IMPLICATIONS: None reported.

DIAGNOSTICIAN John A. Bryan, II **SUPERVISOR** Heather Fenton
John A. Bryan, II, DVM, MS — Heather Fenton DVM, MVSc, DACVP

DISTRIBUTION: SCWDS File, Colvin, Applegate, Hatcher, ADD

Laboratory Results Begin on Page 2

FINAL REPORT

CASE NUMBER CC16-92 A and B
HISTO NUMBER W16 520 and 521

GROSS FINDINGS:

Bat A: The submitted carcass of an adult, female big brown bat is in a good plane of nutrition, and is in a moderate state of autolysis. The right and left forearm lengths are each 4.5 cm, the tail length is 3.7 cm, the right and left tarsus lengths are each 0.9 cm, and the right and left tragus lengths are each 0.4 cm. On the right patagium between the body and fifth digit, there are three flat, circular, pale lesions ranging from 0.1 to 2.5 cm in diameter. No other significant lesions are identified.

Bat B: The submitted carcass of an adult, male tricolored bat is in a poor plane of nutrition and is in an advanced state of autolysis. The right and left forearm lengths are each 3 cm, the tail length is 2.5 cm, and the right and left tarsus lengths are each 0.9 cm. The right tragus length is 0.5 cm, and the left tragus is 0.3 cm. The uropatagium, legs, and tail are matted, and coated with copious amounts of dried, crusty, white, brown, and black excreta. The right and left patagia contain a total of five flat, circular, pale lesions ranging from 0.1 cm to 0.5 cm in diameter. No other significant lesions are identified.

MICROSCOPIC FINDINGS:

Bat A:

Haired skin (wing, muzzle, and pinna): Multifocal regions of the dermis are thickened by fibrous connective tissue that is infiltrated by small numbers of lymphocytes, plasma cells, and macrophages. No fungal organisms are detected with PAS staining.

Skeletal muscle: Multiple foci of hemorrhage are present throughout sections of skeletal muscle associated with myocytes with hypereosinophilic sarcoplasm, loss of cross striations, and fragmentation.

Trachea: Large mats of septate fungal hyphae with parallel walls and occasional bulbous dilations approximately 7-10 microns in diameter are present on the mucosal surface of the trachea unassociated with inflammation (interpreted as post mortem overgrowth).

Lung: Multiple foci of hemorrhage are present throughout lung sections.

No significant findings are present in sections of: liver, pancreas, intestine, stomach, kidney, heart, and spleen.

Moderate to marked autolysis is present within all sections examined that may mask subtle changes.

Bat B:

Muzzle: The muzzle is nearly completely covered in mats of variable fungal hyphae ranging in diameter from 7-15 microns with variable staining with PAS and occasional bulbous dilations (interpreted as post mortem overgrowth).

Haired skin (wing and pinna): Occasional cross sections of arthropod mites are present on the surface of the skin (likely *Myobia* spp.). The dermis is multifocally expanded by fibrous connective tissue that is infiltrated by lymphocytes, plasma cells, and macrophages. Multifocal regions of the dermis are thickened by fibrous connective tissue that is infiltrated by small numbers of lymphocytes, plasma cells, and macrophages. Occasional fungal hyphae that range from 10-15 microns in diameter, have non-parallel walls and are occasionally septate and stain with PAS staining are present on the surface of wing membranes.

Lung: Multiple foci of hemorrhage are scattered throughout the lung.

No significant findings are present in sections of: small intestine, liver, kidney, and heart.

Laboratory Results Continue on Page 3

FINAL REPORT

CASE NUMBER CC16-92 A and B
HISTO NUMBER W16 520 and 521

MORPHOLOGIC DIAGNOSIS:

Bat A:

Haired skin (wing, pinna, and muzzle): Multifocal, chronic, lymphoplasmacytic and histiocytic dermatitis

Skeletal muscle: Multifocal, acute hemorrhage and necrosis

Lung: Multifocal, acute hemorrhage

Bat B:

Haired skin (wing, pinna, and muzzle): Multifocal, chronic, lymphoplasmacytic and histiocytic dermatitis with intralesional mites and fungal hyphae

Lung: Multifocal, acute hemorrhage

MYCOLOGY: Samples from the muzzle and patagia from each bat were submitted to the SCWDS laboratory for fungal culture and polymerase chain reaction. No fungi were isolated with cultural morphology consistent with *Pseudogymnoascus destructans* (Pd). Samples from bats A and B both tested negative for Pd by PCR (both conventional and real-time). A common cave fungus in the same genus (*Pseudogymnoascus* spp.) was detected from bat A, but was determined not to be Pd by genetic sequencing.

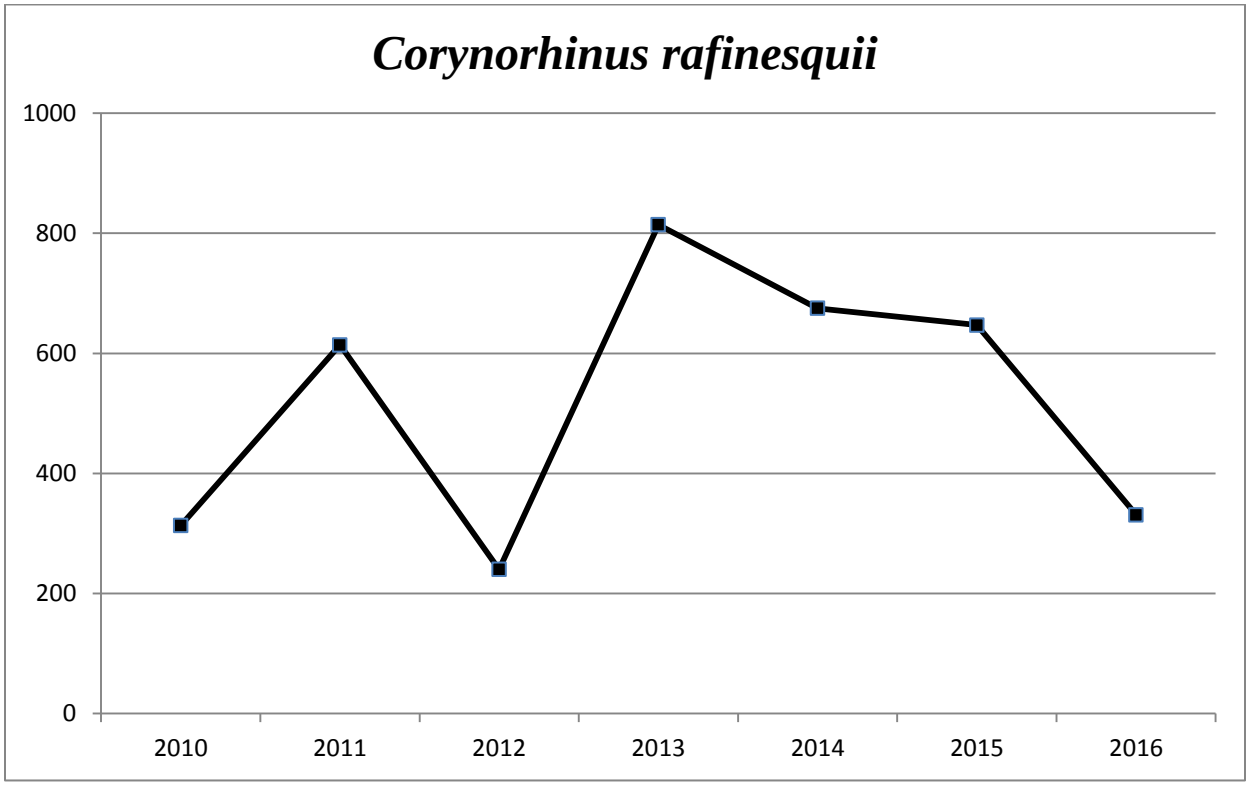
VIROLOGY: Samples of brain tissue from each bat were submitted to the Athens Veterinary Diagnostic Laboratory for rabies testing. No evidence of rabies virus infection was detected in either bat via fluorescent antibody testing.

Appendix G

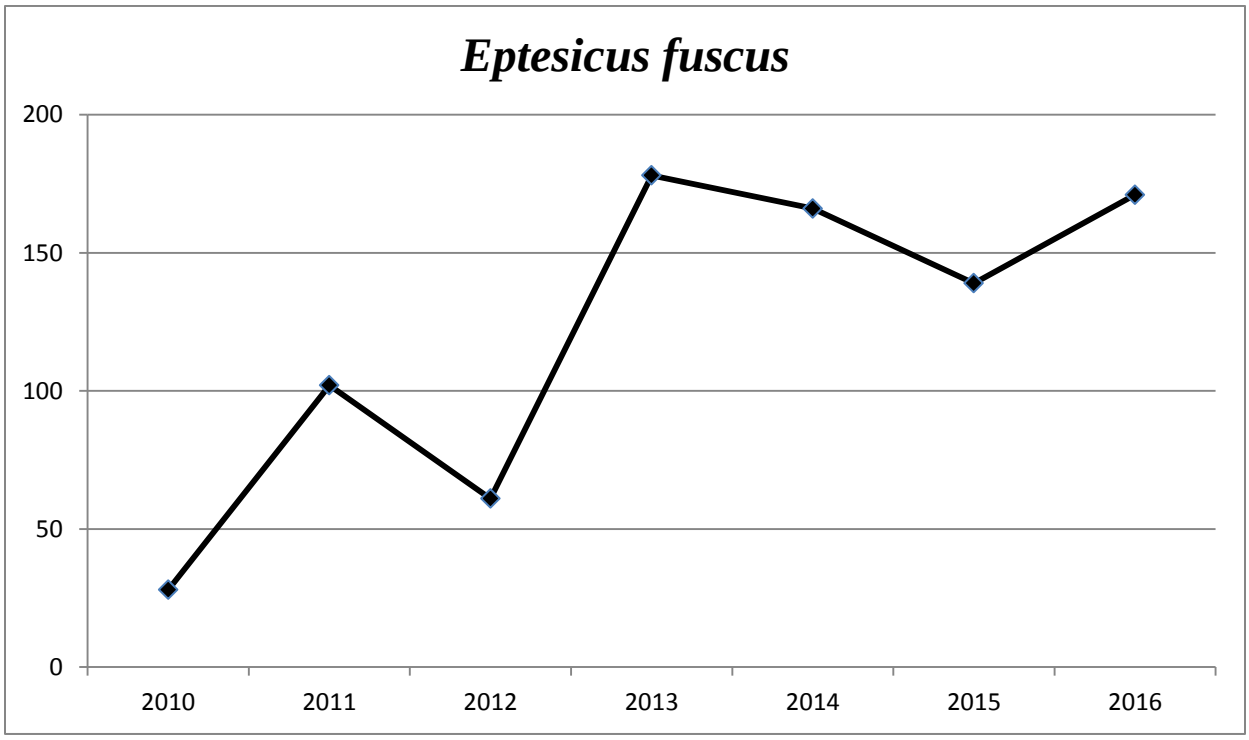
- Trend Analysis by Species Across All Survey Years[3]

[3] It should be noted, trends for species observations across all survey years should consider the effects of survey effort annually and across all years, differences in census techniques, the caves selected for monitoring each year, the discovery of sites dominated by single species not previously surveyed, species roost preferences, etc., all of which impact the number of observations made annually.

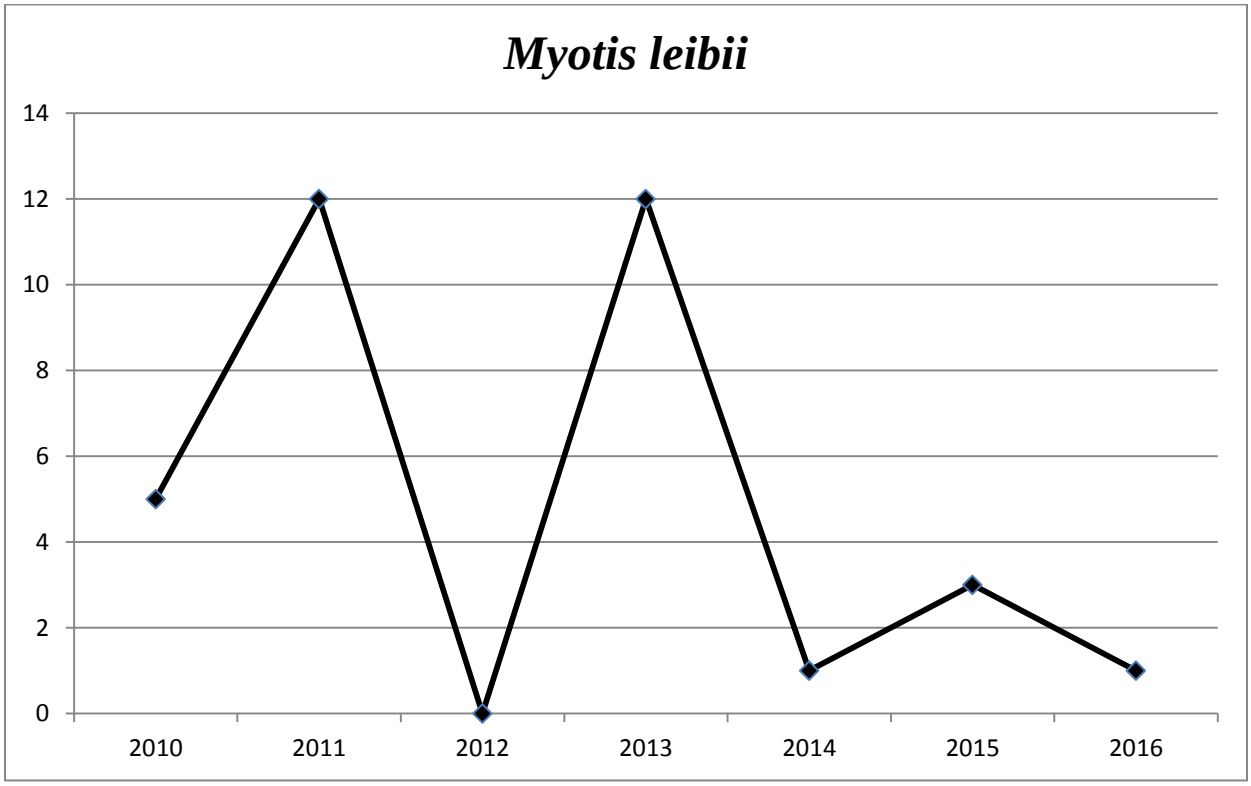
Appendix G - 1. Survey Totals by Year for *Corynorhinus rafinesquii*



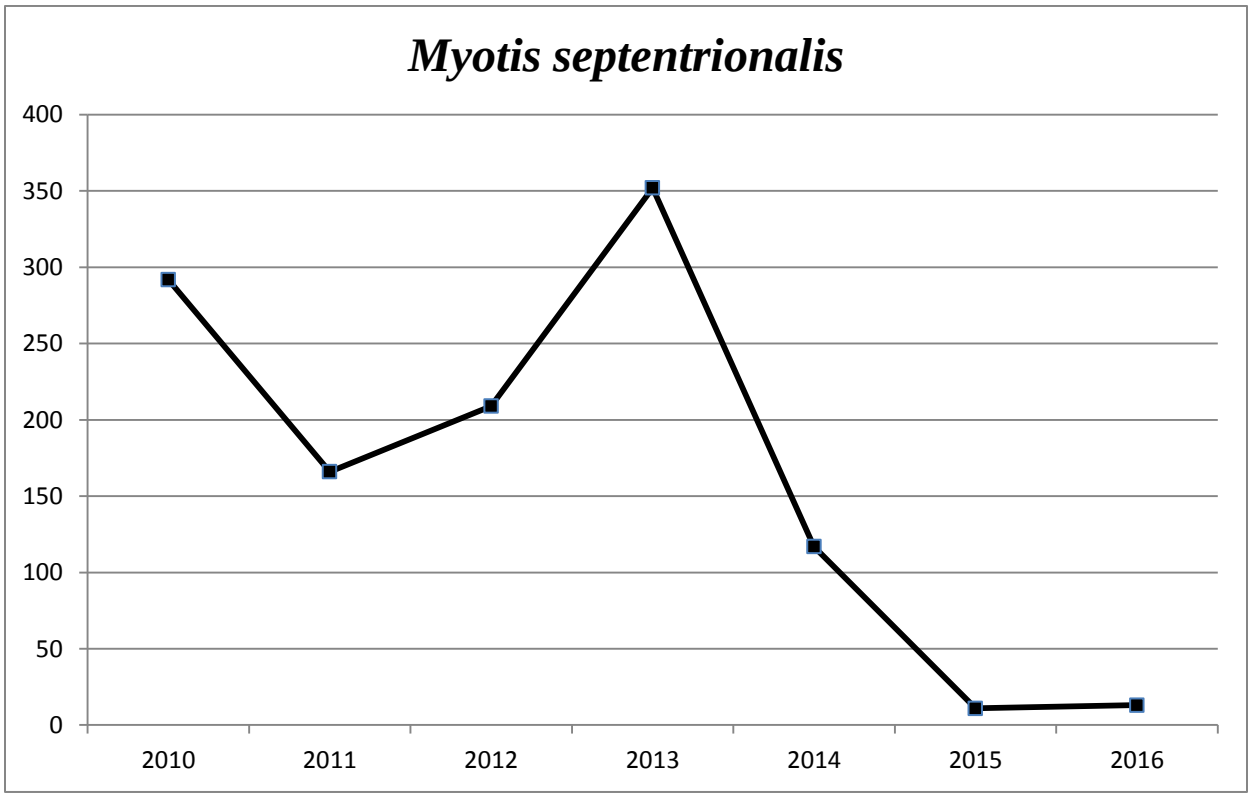
Appendix G - 2. Survey Totals by Year for *Eptesicus fuscus.*



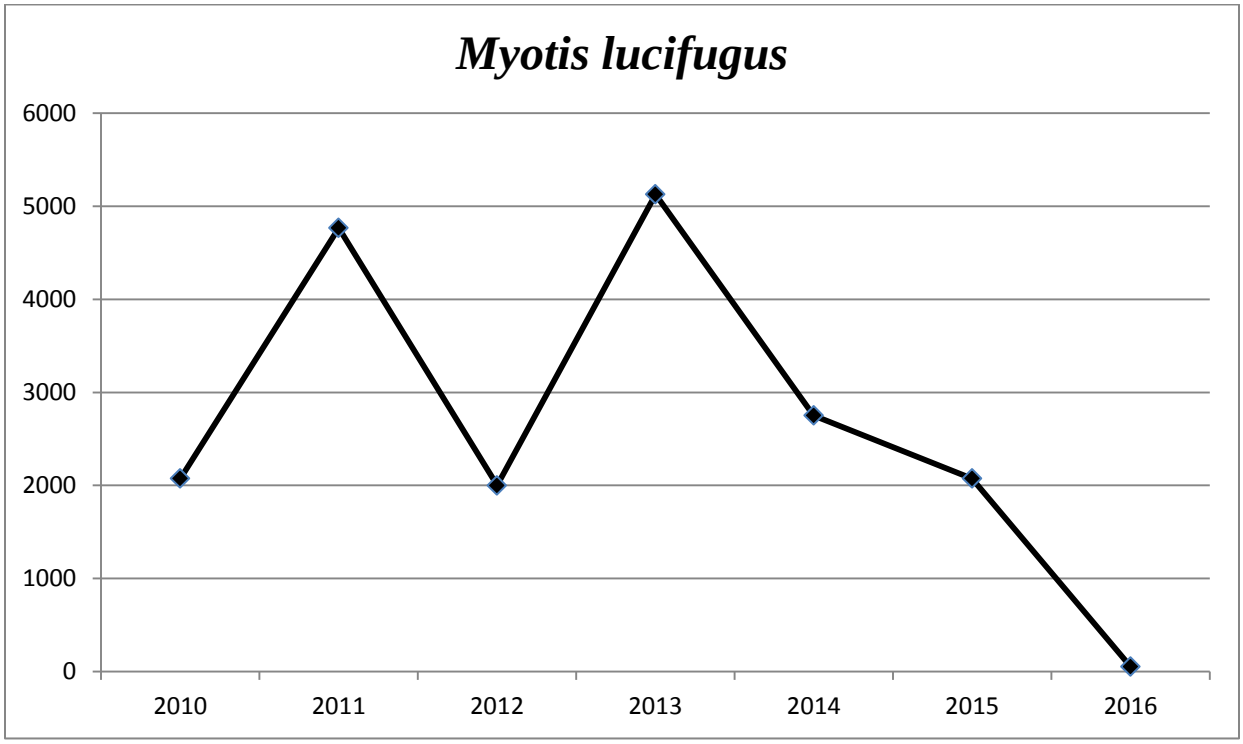
Appendix G - 3. Survey Totals by Year for *Myotis leibii*.



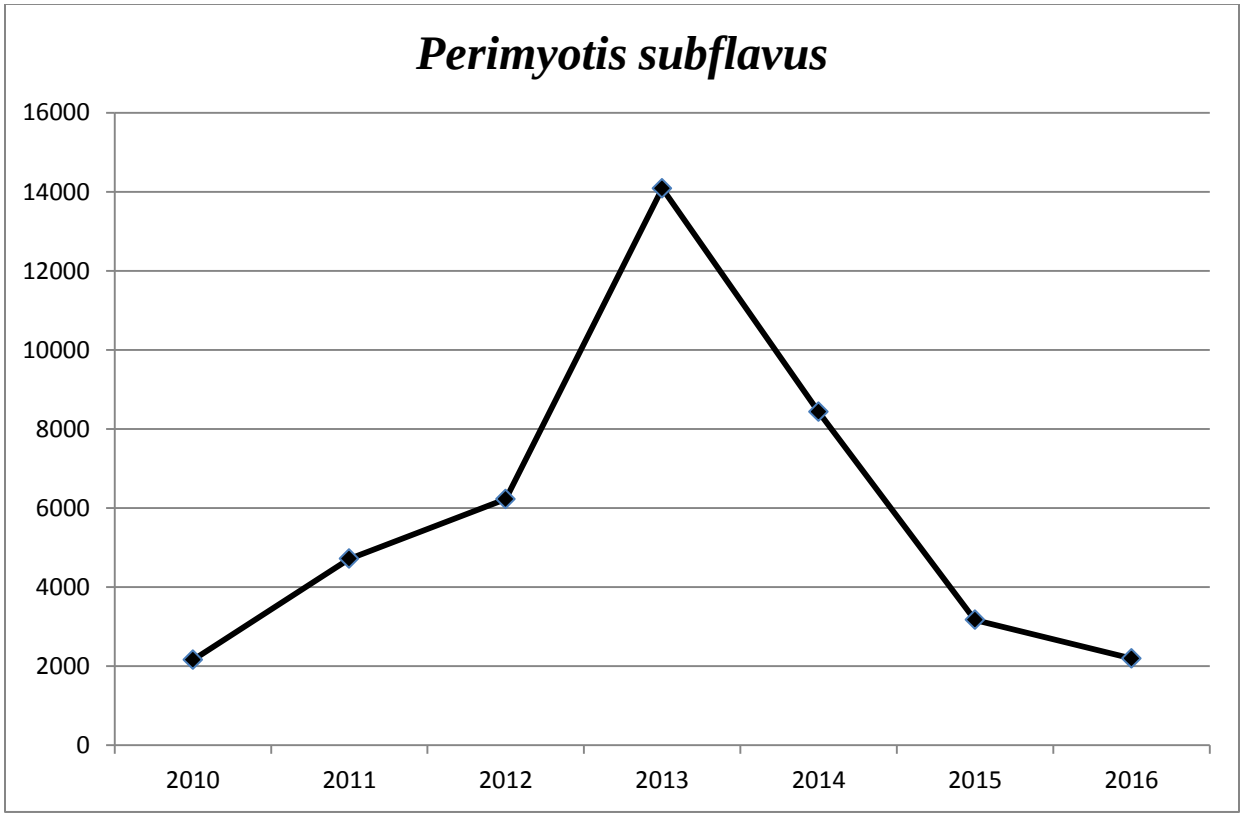
Appendix G - 4. Survey Totals by Year for *Myotis septentrionalis*.



Appendix G - 5. Total Survey Numbers by Year for *Myotis lucifugus*.



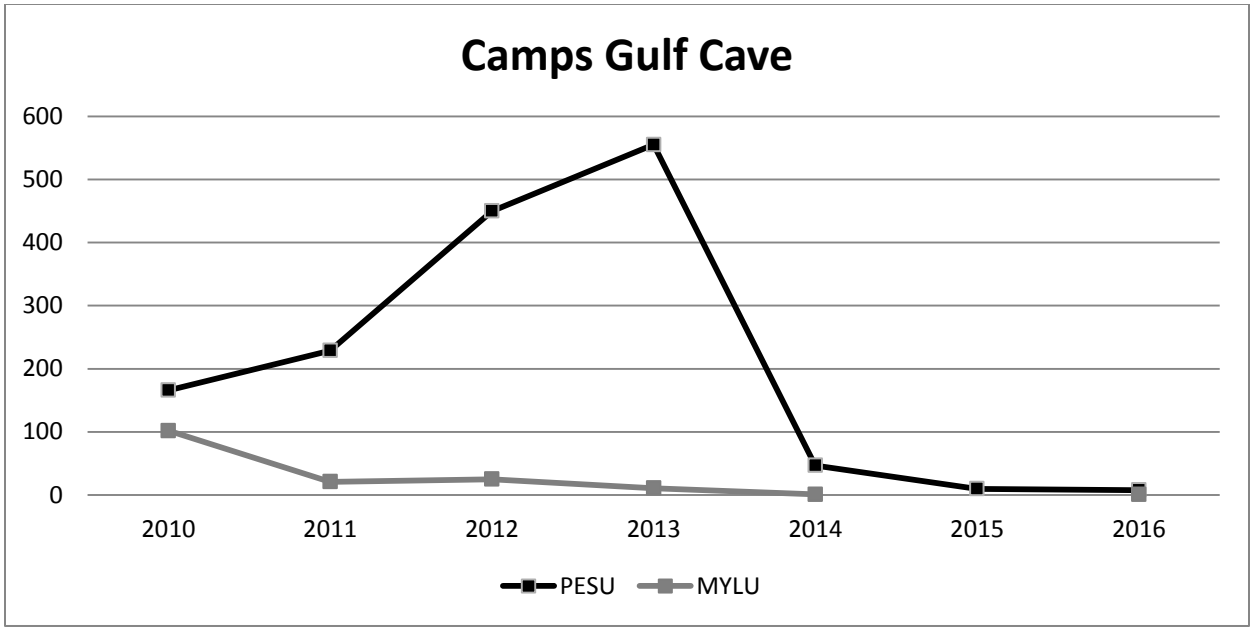
Appendix G - 6. Total Survey Numbers by year for *Perimyotis subflavus*.



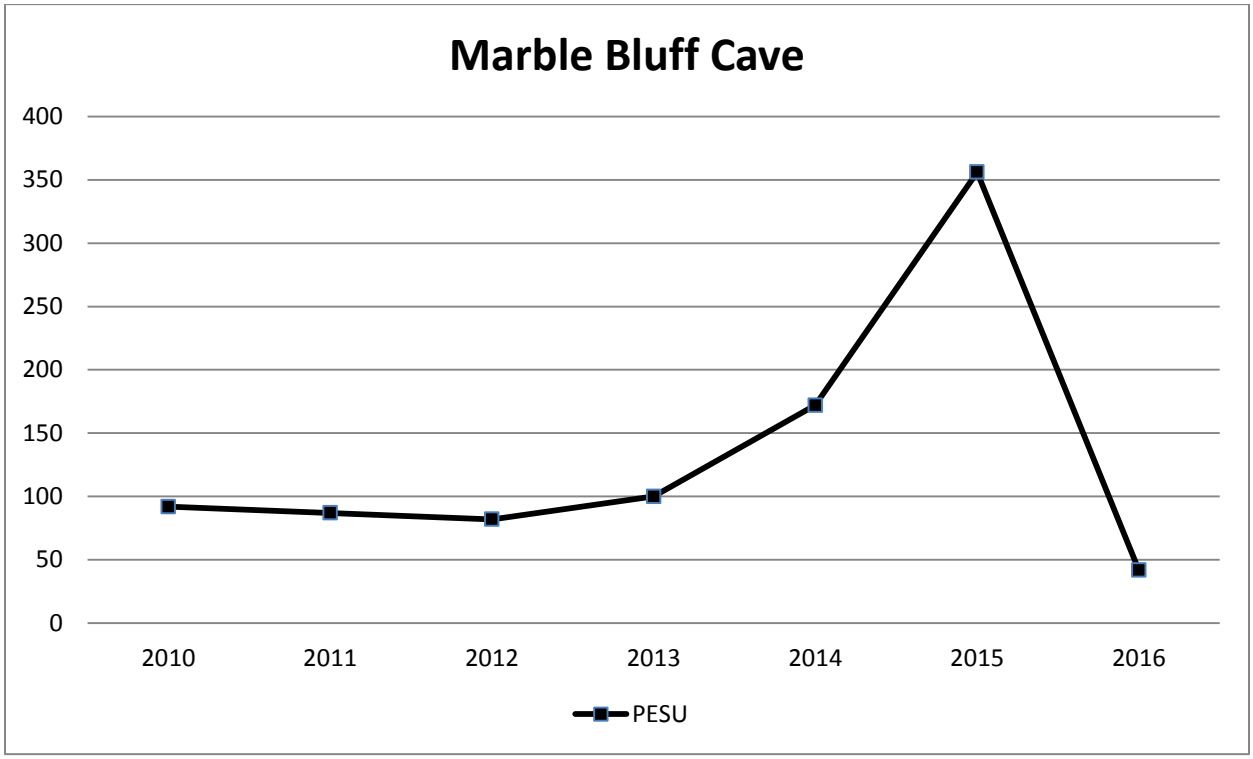
Appendix H

- Trend Analysis of Species at Selected Sites Across All Survey Years

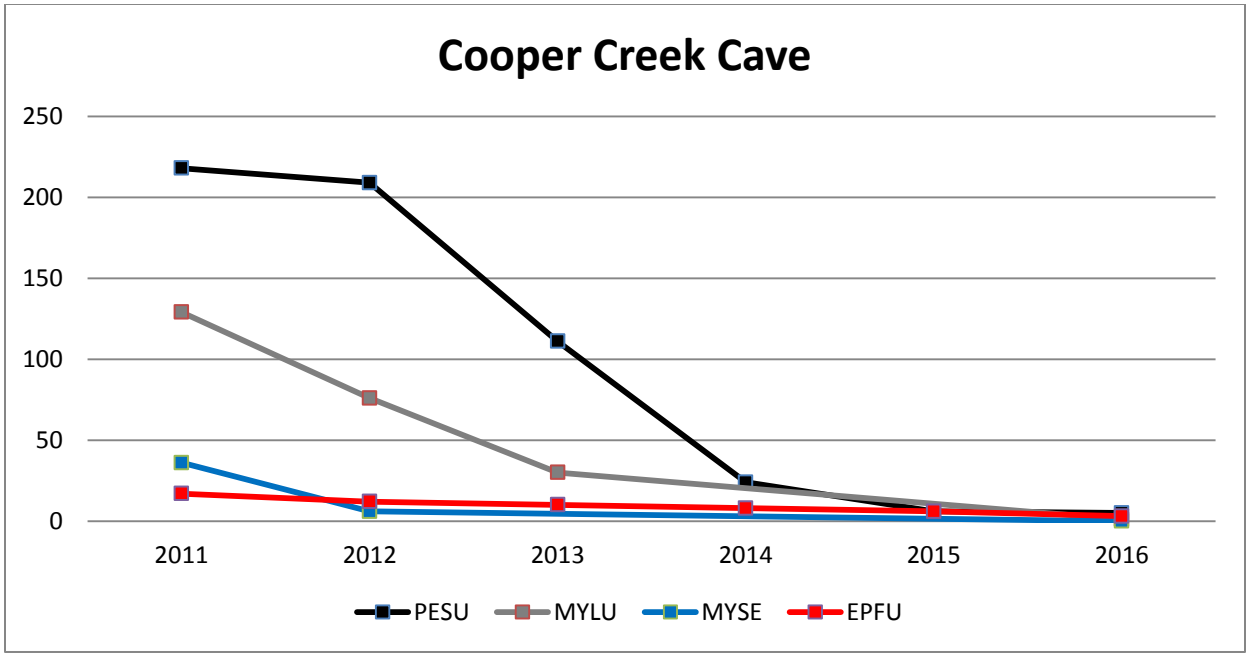
Appendix H – 1. Winter Population Trends at Camps Gulf Cave



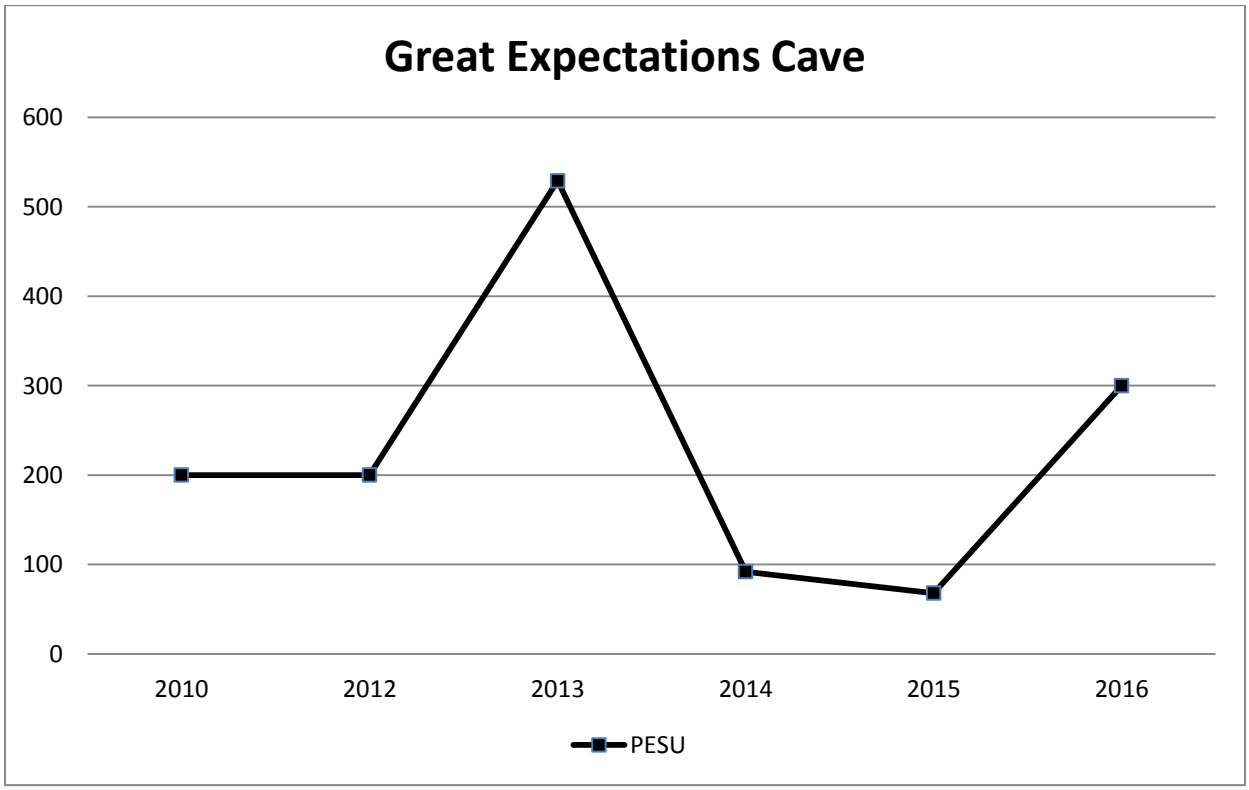
Appendix H – 2. Winter Population Trends at Marble Bluff Cave



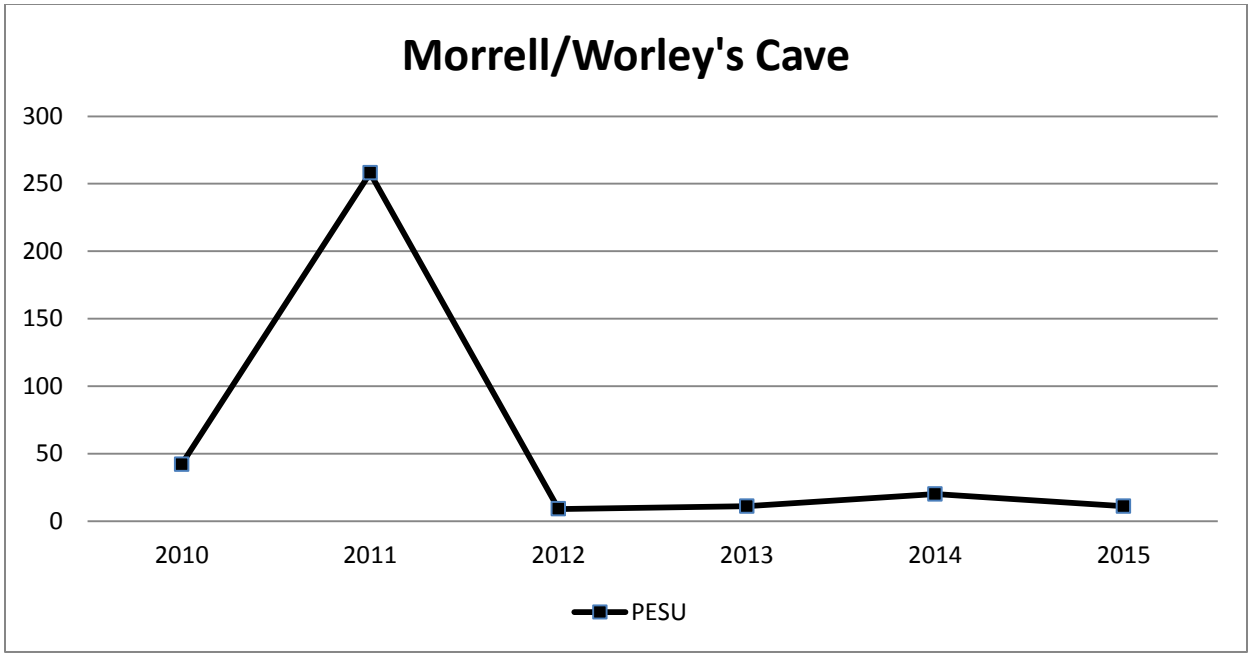
Appendix H – 3. Winter Population Trends at Cooper Creek Cave



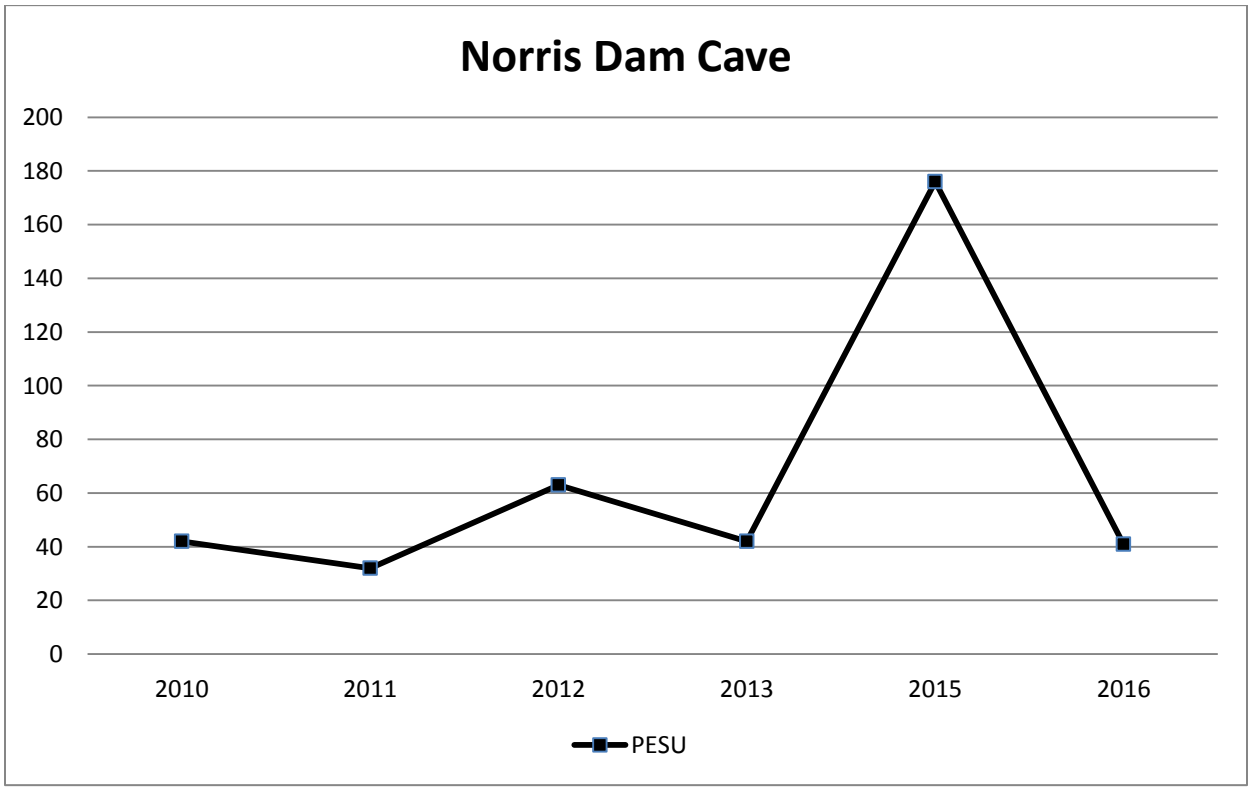
Appendix H – 4. Winter Population Trends at Great Expectations Cave



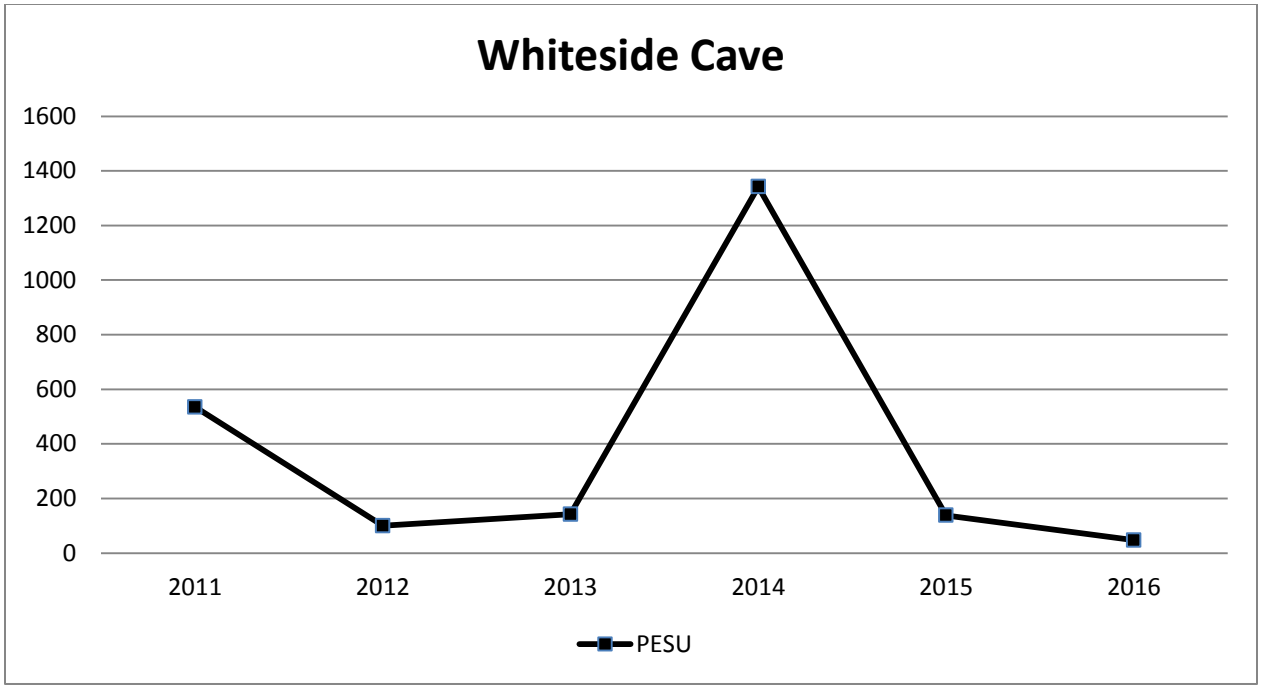
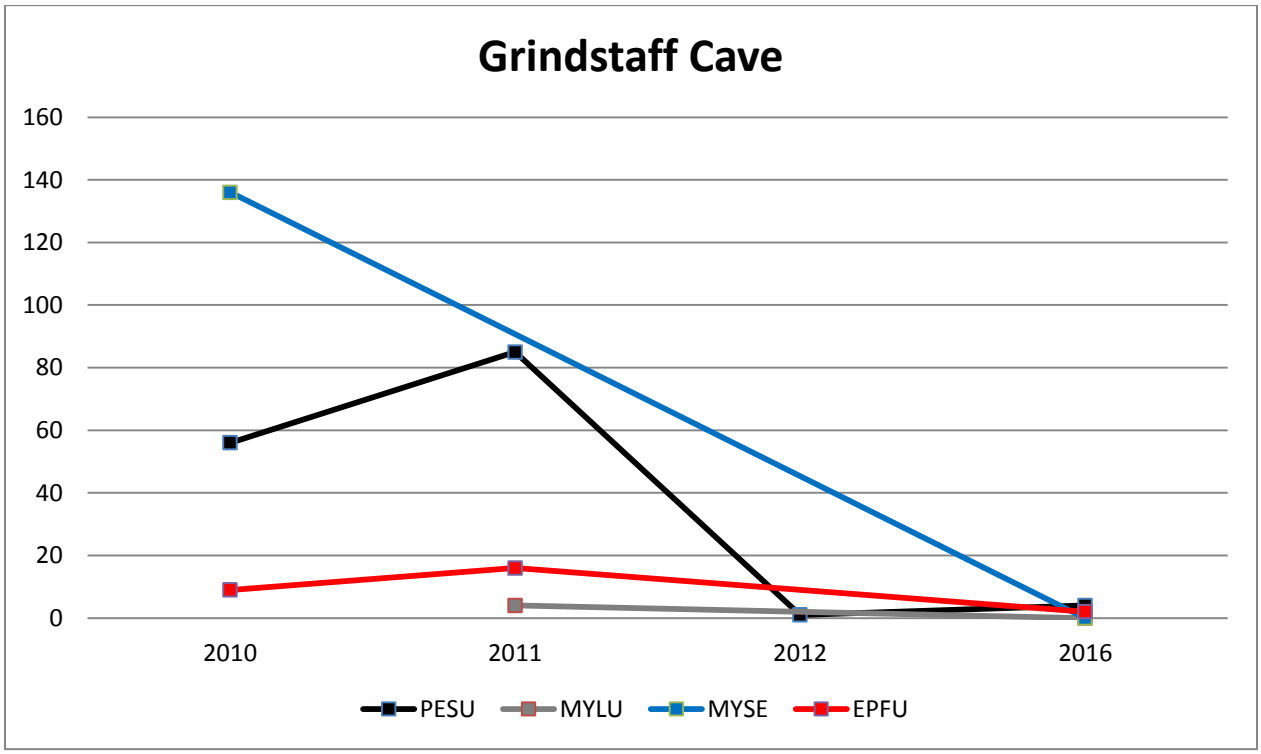
Appendix H – 5. Winter Population Trends at Morrell/Worley's Cave



Appendix H – 6. Winter Population Trends at Norris Dam Cave



Appendix H – 7. Winter Population Trends at Whiteside Cave



Appendix H – 8. Winter Population Trends at Grindstaff Cave