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Post-White-nose syndrome trends in Virginia's cave bats, 2008-2013

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Since its 2009 detection in Virginia hibernacula, the fungal pathogen *Pseudogymnoascus destructans* causing White-nose Syndrome (WNS) has had a marked impact on cave bats locally. From 2008-2013, we documented numeric and physiologic changes in cave bats through fall swarm (FS), early hibernation (EH), and late hibernation (LH) capture and banding surveys at 18 hibernacula in western Virginia. We coupled active surveys with passive biennial winter counts in 2009, 2011, and 2013. We compared individual body mass index (BMI) across years for FS, EH, and LH hibernation to determine if WNS impacts on extant bats would be manifested by changes in body condition (as anecdotally observed elsewhere for WNS-impacted bats) as well as a population reduction. To estimate percent declines in bat presence or relative activity, we used FS capture per-unit-effort data, and the winter hibernacula absolute counts. We captured 4,524 bats of eight species, with species-specific capture success declining by 75-100% post-WNS. Little brown bats (*Myotis lucifugus*) exhibited the greatest declines in winter hibernacula counts (AVG. = 99.0% decline), followed by tri-colored bats (*Perimyotis subflavus*; 89.5% decline) and Indiana bats (*M. sodalis*; 33.5% decline). Graphical analyses of captures-per-trap-hour in FS showed declines for little brown bats, tri-colored bats, and northern long-eared bats (*M. septentrionalis*), but suggest a modest rebound of Indiana bat numbers. Fall swarm trends in BMI suggested some drops post-WNS exposure, but these trends were not consistent across sexes or seasonal time blocks. Our inconclusive BMI metrics and little brown bat band recapture data suggest little competitive advantage or selection for surviving bats. Lesser (but apparent) declines in Indiana bat numbers mirrors trends seen elsewhere regionally, and band recoveries do show that some individuals are persisting. Additional surveys will determine if bats in Virginia will persist or face extirpation due to presumed low recruitment and survivorship.

Key words: Bat, hibernaculum, *Pseudogymnoascus destructans*, Virginia, white-nose syndrome.

INTRODUCTION

White-nose Syndrome (WNS), a disease caused by the fungal pathogen, *Pseudogymnoascus destructans,* has caused the collapse of some bat populations in the eastern United States (Blehert et al., 2009; Frick et al., 2010; Lorch et al., 2011; Turner et al., 2011). Since its detection in New York in 2006, total bat mortaliy associated with this pathogen is now estimated at approximately 6 million individuals (USFWS, 2013). In Virginia, WNS was first documented in three hibernacula in the Ridge and Valley province (Bath and Giles counties) in February 2009, thereby prompting increased monitoring statewide of hibernating bats. In Virginia hibernacula, observed abundances of myotid bats have historically been lower than those in the northeastern United States and surroundding states in the central Appalachians (hundreds of thousands of little brown bats [*Myotis lucifugus*]). In Virginia, single-cave historical bat counts rarely exceeded 2000 across all species combined, with little brown bats dominating the counts in most locales pre-WNS (Appendix 1, in part; VDGIF, unpublished data).

Despite the catastrophic declines in populations of multiple species across the eastern United States, reports of smaller populations of bats persisting in hibernacula through the winter (Turner et al., 2011) and into summer (Dobony et al., 2011; Dzal et al., 2011; Francl et al., 2012) raises the question about differences in survivorship among colonies. Moreover, for Virginia with its lower pre-WNS bat populations, are WNS impacts similar in relative magnitude to those observed in other states?

To ascertain the effects of WNS at the individual level, indirect measures of health, such as body mass index, can readily be examined (Jonasson and Willis, 2011). An individual bat's body mass index (BMI, weight [g]/ forearm length [mm] (Chappell and Titman, 1983) changes seasonally, as bats attempt to gain mass in the month prior to entering hibernation (Kunz et al., 1998). Differences in weight gain also are documented between sexes (females are generally heavier than males of comparable size, due to reproductive demands (Gerell and Lundberg, 1990) and between age categories (juveniles [young-of-the-year] gain proportionally less weight than adults (Kunz et al., 1998). Therefore, year-to-year comparisons must take into account date, sex, and species. However, because young-of-the-year could not be distinguished from older adults by the time our surveys began in fall (epiphyseal fusion complete; Haarsma, 2008), we could not examine age as a factor.

Prior to WNS establishment in North America, Boyles et al. (2007) found that little brown bat body mass was a predictor of roost selection within a hibernaculum; specifically, bats with a lower body mass (and presumably, lower BMI) that could be considered at energetic risk of surviving hibernation chose cooler microclimates within a hibernaculum. The cooler temperatures possibly allowed individuals to conserve energy more efficiently upon arousal, and have a greater chance of survival through the winter months. Similarly, Reeder et al. (2012) tested the relationships amongst little brown bat BMI, winter arousal behavior, and hibernacula survivorship after WNS emergence. They hypothesized that a higher BMI might enable *P. destructans*-infected bats to arouse more often, yet survive the hibernation period (Reeder et al., 2012). We, therefore, followed up by examining BMI immediately preceding and immediately after the hibernation period. For example, if surviving bats show higher BMI values post-WNS, this might provide insights into possible genetic and physiological traits that help explain why some bats do survive from year to year despite exposure to *P. destructans*.

To investigate trends at the population level, our multi-year study sought to document changes due to the presence of *P. destructans* in examining: 1) absolute counts of bats in known hibernacula in biennial winter surveys, 2) captures per unit effort across species and across years during fall swarm periods, and 3) rates of band recapture across years. We examined population-level changes that may serve as a proxy for individual condition (BMI) across three seasonal periods: fall swarm, early hibernation, and late hibernation (spring staging). We expected that absolute counts for cave bats in Virginia would decline precipitously, as compared to pre-WNS surveys. We further hypothesized that fall swarm capture rates would decline across years following the onset of WNS when comparing fall surveys among years. As a potential metric to indicate competitive advantages of survivors, we expected to find higher BMIs in pre- and post-hibernaculum surveys several years after the emergence of WNS. Relatedly, we expected to find some longevity in band returns, indicating that bats who had survived multiple years of exposure to *P. destructans* would continue to persist.

MATERIALS AND METHODS

Field methods

Between fall 2008 and fall 2013, we conducted surveys of fall swarming, early-hibernating, and late hibernating (spring staging, emerging) bats across 18 hibernacula in 8 counties (Bath, Bland, Craig, Giles, Highland, Lee, Tazewell, and Wise) in western Virginia. Due to logistics and landowners declining our request to access some sites, not all hibernacula were surveyed every year or during every time period. Survey techniques included harp trapping and mist netting at cave entrances during the pre-hibernation or fall swarm (September-October; FS; no precipitation, temperatures above 10°C) and in the early hibernation period (November, EH) when bats were active. We also hand-collected bats from inside the hibernacula in EH and late hibernation/spring staging (LH, early April). Because netting and harp trapping were not the primary capture techniques for EH and LH, we performed no statistical analyses on captures per unit effort for these two time blocks.

We initially selected caves that were known hibernacula of the little brown bat and the Indiana bat (*Myotis sodalis*), a federally endangered species (Ford and Chapman, 2007). However, at least

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five additional species are known to hibernate in these same caves, and some of these species were also assessed: the endangered Virginia big-eared bat (*Corynorhinus townsendii virginianus*; currently unaffected by *P. destructans*), big brown bat (*Eptesicus fuscus*), eastern small-footed bat (*M. leibii*), northern long-eared bat (*M. septentrionalis*), and tri-colored bat (*Perimyotis subflavus*; Brack et al., 2005, personal observation). We followed standard WNS decontamination protocol (USFWS, 2015).

For all survey periods in which bats were captured, we identified the individual bats to species and recorded sex, right forearm length (±0.1 mm), and weight (±0.1 g).All cave bats were banded with 2.9 mm (all myotines, tri-colored bats) or 4.2 mm (big brown, Virginia big-eared bats; Porzana Ltd., East Sussex, UK) bands to document recaptures. At each location, we attempted to place the same number of nets and harp traps in the same locations, so that captures per unit effort (statistically analyzed for fall swarm) could be standardized and comparable across years.

In January 2009, 2011, and 2013, we conducted visual counts inside 13 of the 18 hibernacula. In doing so, we could compare percent declines in species as estimated from mistnetting efforts to declines in absolute winter counts at the same localities. Because WNS infections appeared at hibernacula at different times over the course of our study, from the first infections in 2009 to the last major hibernacula infected in 2013, we present averaged historical (2007 or earlier, pre-WNS) hibernacula counts for baseline comparisons. Average pre-WNS hibernacula counts were calculated using counts from the same passages with author Reynolds present for the majority of the counts. Because of small cluster size, bats were identified and counted individually in both pre- and post-WNS counts (VDGIF, unpublished data).

Analytical methods and statistics

For winter visual counts, we calculated the average number of bats per species in pre-WNS surveys conducted from 1986-2007. Sample size for averages varied based on number of reliable counts completed at each hibernaculum. Due to yearly variation, this average calculation reasonably reflected actual counts documented prior to the arrival of *P. destructans*.

To examine band recapture trends, we assessed if absolute recapture rate(percent; based on the number of bats banded annually) changed over time. We also examined the change in potential recaptures (based on the running total of all bats previously banded) over time in a similar approach. Therefore, our goal was not to estimate population size from mark-recaptures, but instead to document changes in recapture rates over time.

When analyzing our winter hibernaculum counts, our recapture rates from bands, and our captures per trap-hour by species, we employed localized regression and associated 99% confidence interval bands (PROC LOESS; SAS Institute Inc., Cary, NC) separately for each species. Localized regression is a nonparametric line-fitting procedure that is appropriate in visualizing trends without forcing a fit to standard linear, exponential, or other parametric forms (Cleveland et al., 1988). Because we did not want to assume patterns in these data, this regression with few assumptions and limited statistical metrics was an appropriate exploratory choice (Littell et al. 2006).

We calculated the BMI (Chappell and Titman, 1983) for each individual, but given the acknowledged differences in BMI between sexes (Gerell and Lundberg, 1990), all analyses involving this metric were completed separately for males and females. Because of unbalanced and low sample sizes for some year and within year seasons, we compared BMI measures among species by season (fall swarm, early hibernation, and late hibernation) across years using a 1-way non-parametric ANOVA on ranked data (PROC GLM; SAS Institute Inc.). When significant year effects were observed ($\alpha = 0.05$), we used Tukey's *post-hoc* analyses to examine year-to-year differences.

RESULTS

Winter hibernaculum counts

Biennial winter visual counts at 13 hibernacula depict declines for little brown, Indiana, and tri-colored bats, the three most commonly encountered species (Appendix 1). In comparing 2013 counts to average pre-WNS counts, little brown bats declined by 99.0%, tri-colored bats by 89.5%, and Indiana bats by 33.5%. Total counts for each cave (which included the detection of big brown bats, small-footed bats, and Virginia big-eared bats) showed the same declining patterns with drops of 94.6% after the discovery of WNS (Appendix 1).

When we examined hibernaculum counts using localized regression, declines in little brown bats were evident from 2009 to 2013, and absolute declines were noted in the tri-colored bat and the Indiana bat (Figure 1). For these latter two species, the magnitude of decline was less than that of the little brown bat, and year-to-year counts were more variable (Figure 1).

Fall swarm captures

From 2008 – 2013, we captured 1452 individuals of 8 species during the fall swarm (15 sites): little brown bat (N=617), tri-colored bat (349), Indiana bat (162), northern long-eared bat (257), small-footed bat (27), big brown bat (18), Virginia big-eared bat (18), and eastern red bat (*Lasiurus borealis*, 4). Captures-per-hour peaked in 2009 (15.0 ±3.3SE) and declined to 1.3/h (±0.8) by 2013 (Table 1).

When examining fall capture success on a by-species basis, declines over time were evident for northern long-eared bats and little brown bats, and negligible for tri-colored bats (Figure 2). Captures-per-hour appeared to increase for Indiana bats over survey years (Figure 2). However, due to the overlapping confidence intervals, there is considerable variability in the data and absolute declines should be viewed more as trends.

Early and late hibernation captures

In early hibernation, 2008 – 2012, we netted, harp-trapped, and hand-captured 1502 individuals of 7 species (14 sites): little brown bat (N=1234), tri-colored bat (127), Indiana bat (124), northern long-eared bat (7), big brown bat (5), eastern small-footed bat (4), and Virginia big-eared bat (1). Males of all species (63.8%) were captured more often than females. During late hibernation/spring staging, 2009 – 2013, we hand-collected 1570 individuals of 6 species (14 sites): little brown bat (N=1023), tri-colored bat (423), Indiana bat (96), northern long-eared

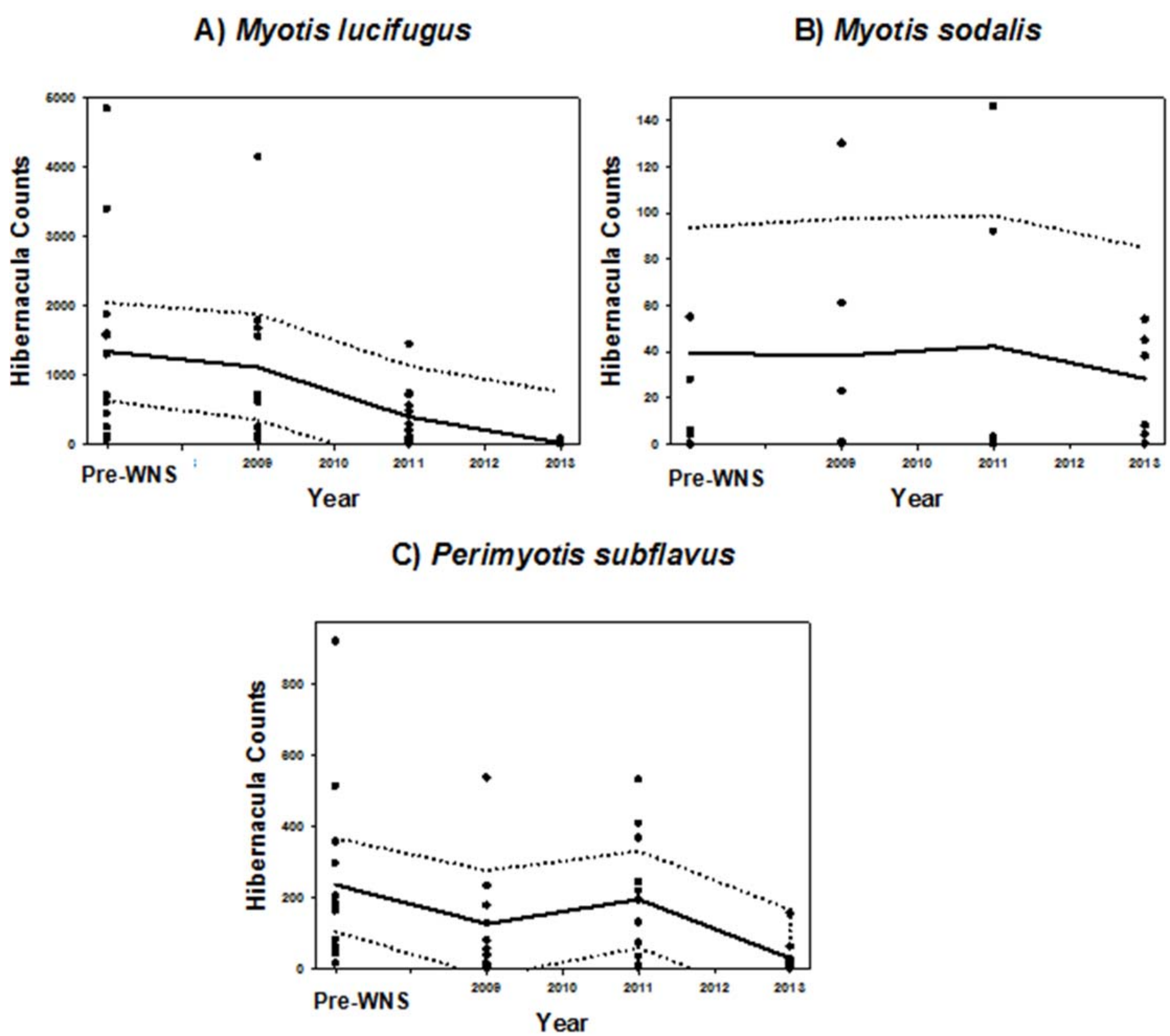

Figure 1. A localized regression examining hibernaculum counts for A) *Myotis lucifugus,* B) *M. sodalis*, and C) *Perimyotis subflavus* as surveyed in winter (January-February) 2009, 2011, and 2013 in western Virginia. Average pre-WNS counts also presented.

Table 1. Summary results from 2008-2013 surveys of 15 western Virginia hibernaculum entrances during fall swarm (September-October). Presented are number of hibernacula visited, number of nights netted or harp-trapped, cumulative netting effort (hours), and average captures per trap-hour (CTH ± SE).

Year	Number of Sites	Number of Nights	Number of Net Hours	Avg. CTH (±SE)
2008	4	4	13.7	7.3 (±1.6)
2009	8	10	33.5	15.0 (±3.3)
2010	9	10	26.8*	11.8 (±3.0)
2011	10	13	40.8	4.1 (±1.0)
2012	11	12	27.4*	3.6 (±1.2)
2013	2	4	15.5	1.3 (±0.8)

*2010: 2 nights untimed, 2012: 1 night untimed; not part of CTH calculations.

bat (19), eastern small-footed bat (6), and big brown bat (3). Across all species, males (70.5%) were captured more often than females.

When examining band recapture data across all sampling periods, we had sufficient captures of two species to warrant further investigation: little brown bats

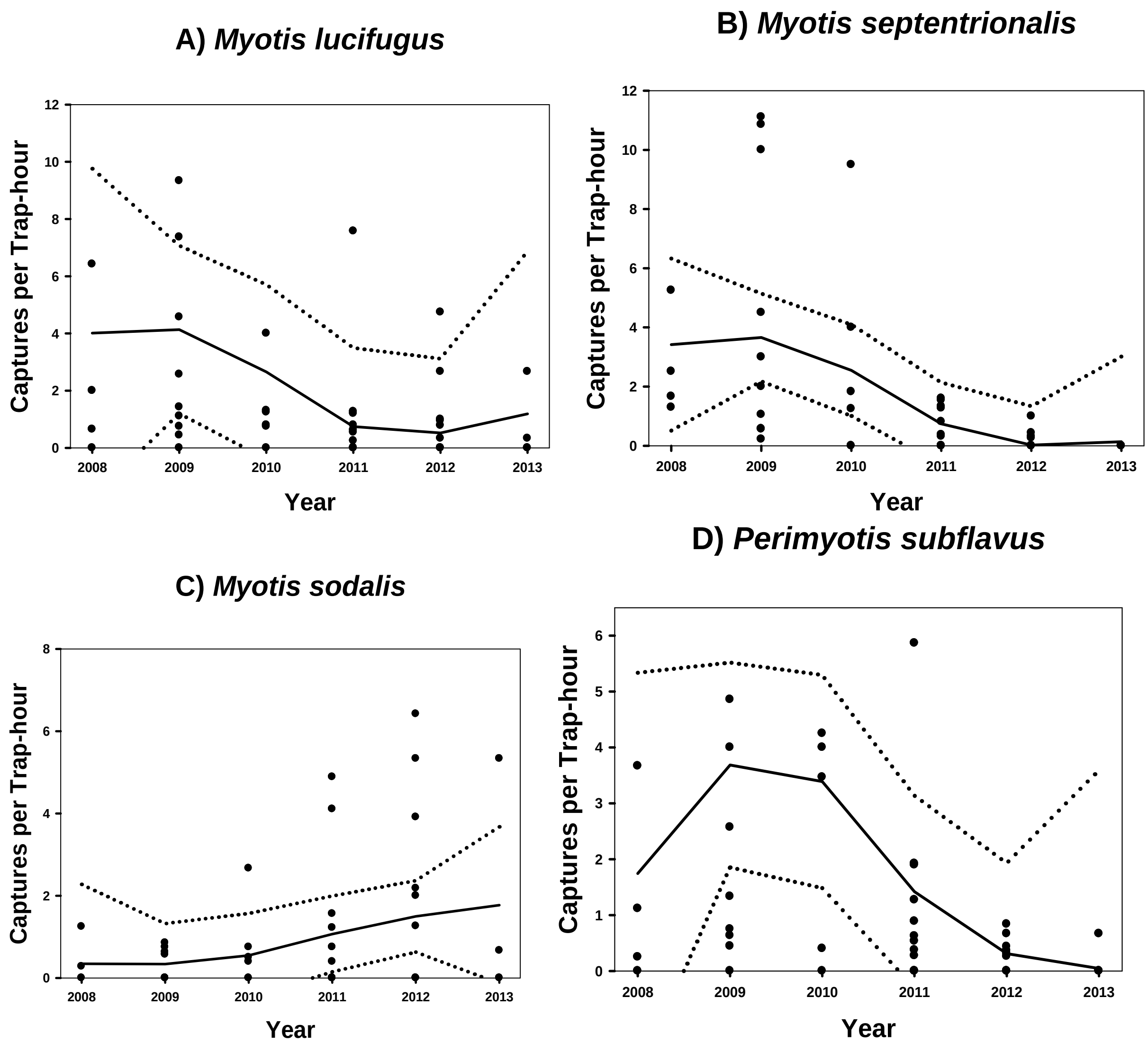

Figure 2. A localized regression comparing captures-per-trap hour by year for four species captured in fall swarm netting efforts from 2008-2013 in western Virginia: A) *Myotis lucifugus*, B) *Myotis septentrionalis,* C) *Myotis sodalis,* and D) *Perimyotis subflavus*.

and Indiana bats. When we examined if absolute recaptures based on the annual number of bats observed changed, we found that recapture rate slightly increased over time for little brown bats (Figure 3). Indiana bats showed a more pronounced increase in proportional capture rate. These increases suggest that banded individuals comprised a markedly greater proportion of remaining bats in the population for both species.

Trends in bat health

For fall swarm data, sufficient sample sizes allowed for statistical analyses of BMI for four species: little brown bat, northern long-eared bat, Indiana bat (males only), and tri-colored bat (Figure 4). We found that males of little brown and Indiana bats exhibited significantly greater BMI values in 2008 (Figure 4), the year before the first documentation of WNS in Virginia, than in subsequent years. Although BMI values were significantly higher for male little brown bats in 2011 and 2012, compared to their lowest level in 2010, no positive gains were seen. Male northern long-eared bats exhibited a significant decline only in 2011, but differences in values were nominal. Declines in male or female tri-colored bats were not apparent until 2011 and 2012. Among myotine females there were no changes in BMI across years in the fall survey (Figure 4).

Results from early hibernation BMI analyses were limited to just three species, and not for all years: little

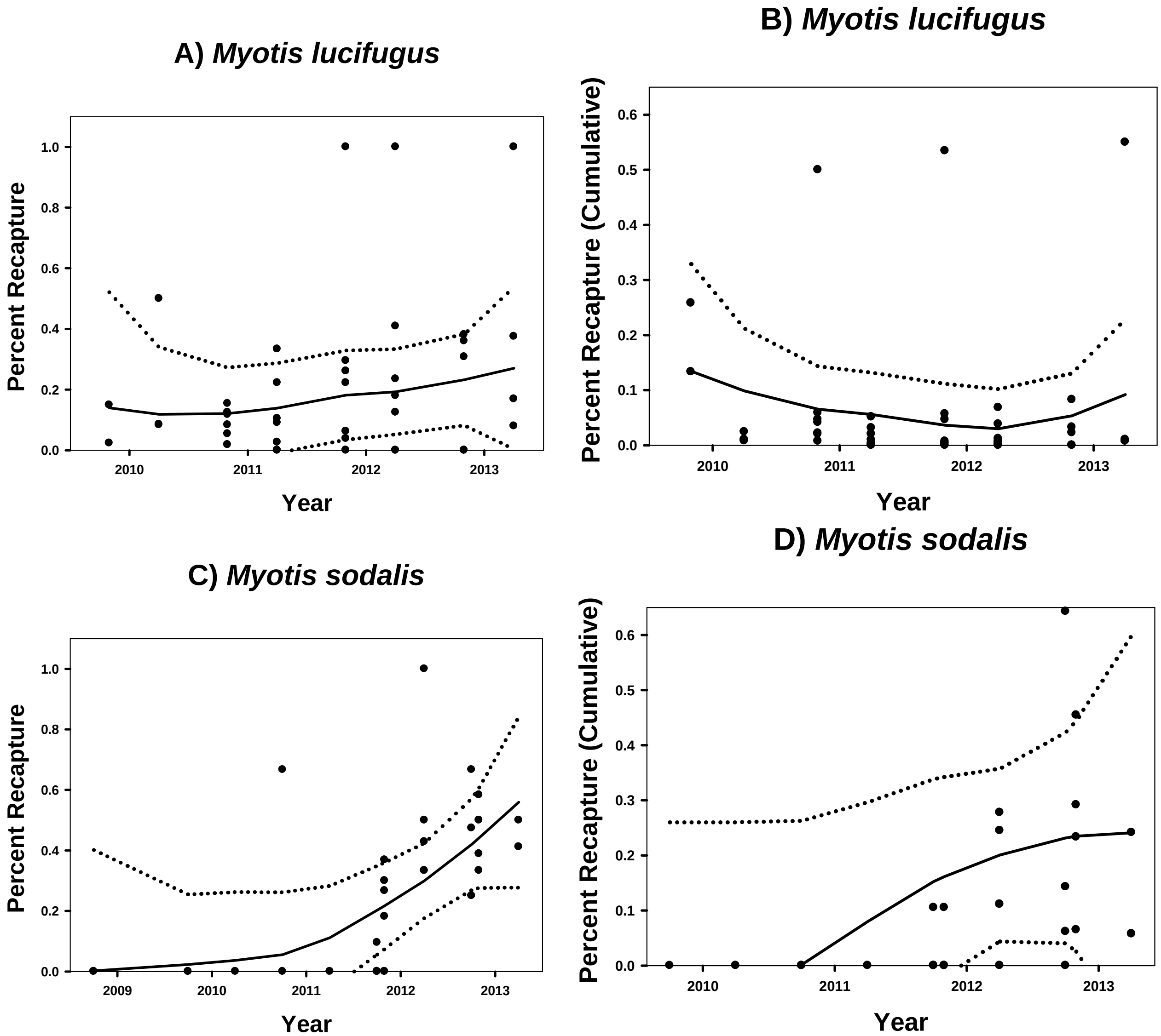

Figure 3. A localized regression examining recapture rates (percent recapture and percent cumulative recapture) from banded *Myotis lucifugus*(A, B) and *Myotis sodalis* (C,D) from 2008-2013 in western Virginia.

brown, Indiana, and tri-colored bats (Figure 5). All data were collected after the introduction of WNS, and no BMI differences were discovered for males or females of both Indiana and tri-colored bats. For both sexes of little brown bats, declines in BMI were only observed in 2010.

In late hibernation surveys, all BMI data were collected after the introduction of WNS, and we found no BMI differences for females of three species: little brown bats, Indiana bats, and tri-colored bats (Figure 6). In males, little brown bats showed declining trends in BMI in 2010 and 2011 relative to the previous years, with a rebound in surviving bats in 2012 and 2013. Male Indiana bats showed declining trends in BMI over time, and tri-colored bats showed no trends in BMI but lacked sufficient captures in 2013 to assess the final year (Figure 6).

DISCUSSION

In an examination of our multiple metrics in assessing population status and relative health for bat species, it is apparent that Virginia populations of little brown, northern long-eared, and tri-colored bats have declined precipitously in the hibernacula we surveyed, and Indiana bats are showing substantial declines, as well. Raw counts from passive, biennial hibernacula surveys provide the strongest evidence for population declines on a site-by-site basis (Appendix 1), and more conservative, descriptive statistical analyses of captures-per-hour in fall swarm show evident declines for all but the Indiana bat. The fall trend for Indiana bats reflects proportionally higher capture rates as total populations of other myotines decreased at a

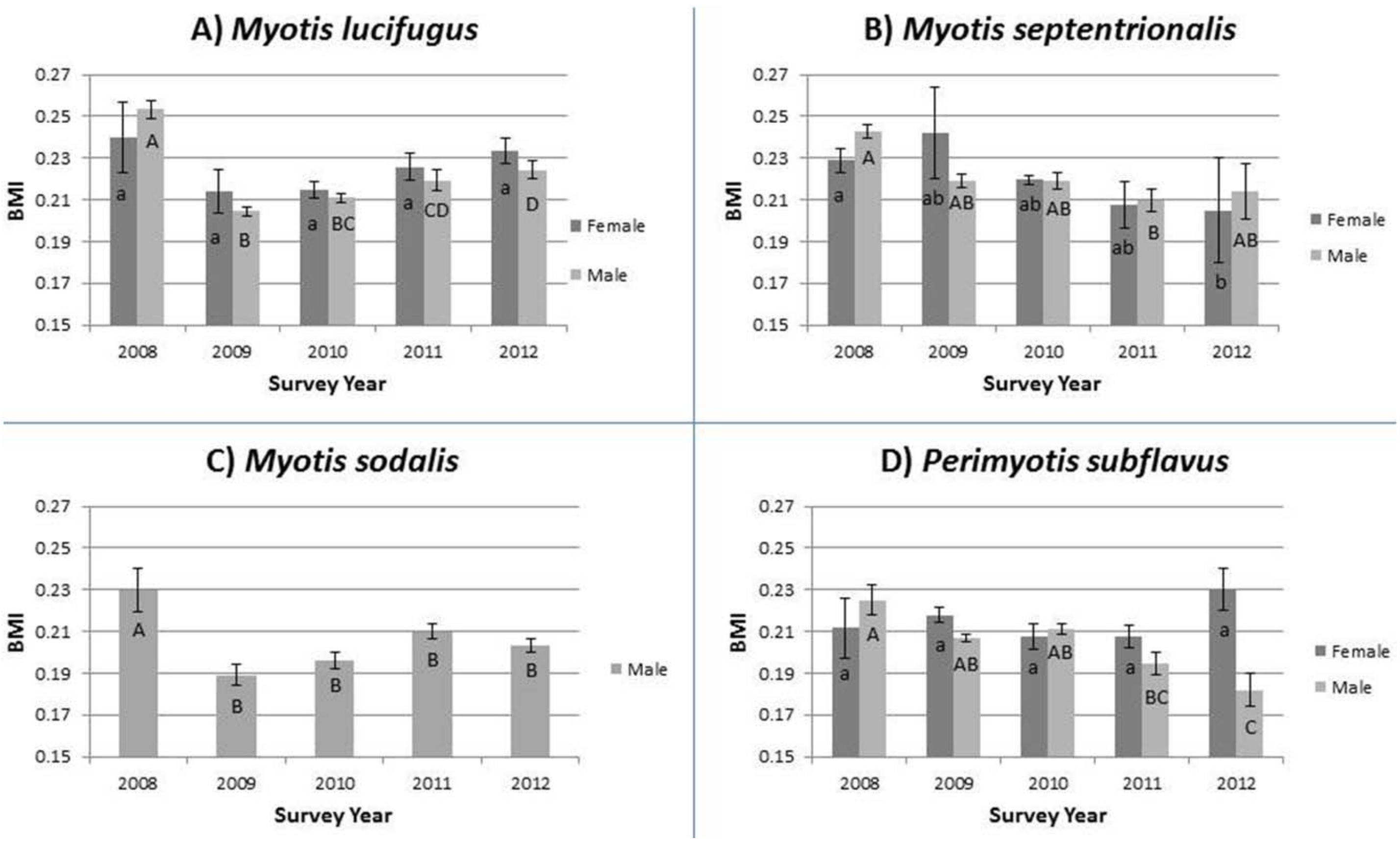

Figure 4. Body Mass Index (BMI) scores for bats captured during fall swarm, 2008-2012, at 15 hibernacula in western Virginia. BMI (±SE) from four species are presented, separately for males and females: A) *Myotis lucifugus*, B) *M. septentrionalis*, C) *M. sodalis*, and D) *Perimyotis subflavus*. BMI averages significantly different from one another in a *post-hoc* Tukey's test are denoted by differing letters (lower case for females, uppercase for males).

faster rate. The appearance of 38 Indiana bats at one cave in which they were historically not found (Appendix 1) was unexpected. However, it is unclear whether the bats arrived from known historical hibernacula (three Indiana bat sites are 10.5 – 14.4 km from this new site) or if the Indiana bats have simply moved from an unreachable, undocumented portion of the cave into areas previously dominated by clusters of little brown bat. Jachowski et al. (2014) suggested that indirect effects of *P. destructans*, such as changes in niche partitioning at both spatial and temporal scales, are already evident in summer bat communities in the northeastern United States. It is possible that these effects carry into the hibernation period, and we are just beginning to see them in Virginia. Continued monitoring may provide more clarity.

We also saw substantial movement patterns with tri-colored bats in 2011; winter counts spiked at sites that were recently suspect or confirmed positive for *P. destructans*. We suggest that this movement was due to a reaction to the effects of the fungus on the bats. In frequently arousing and attempting to forage outside the cave, individuals moved nearer cave entrances and into passageways that were easily countable. Turner et al. (2011) described similar scenarios for hibernating bats in a Pennsylvania cave – spikes in the first year of *P. destructans* infection, followed by historically low counts the following year. Our ideas were supported when our tri-colored bat counts plummeted in 2012, presumably because these bats were deceased.

We acknowledge that the dates of initial WNS detection in bats at different Virginia hibernacula ranged from 2009-2013 (Appendix 1), and that some of our figures do not take this into account. Ingersoll et al. (2013) examined long-term (1999-2011) trends for these same cave bat species in West Virginia, New York, Pennsylvania, and Tennessee, grouping *P. destructans*-infected hibernaculum with those that were not yet infected. Still, they documented similar dramatic (>30%) declines for all species. This corroborative evidence only emphasizes the documented declines in capture rates were sufficiently dramatic by 2011 to show population declines.

Based on our winter hibernaculum count sit appears that, post-emergence of WNS, some bats may persist in hibernacula in extremely low numbers. Whether or not these numbers persist over time (often less than 10 ndividuals per species per hibernaculum in 2013) and/or

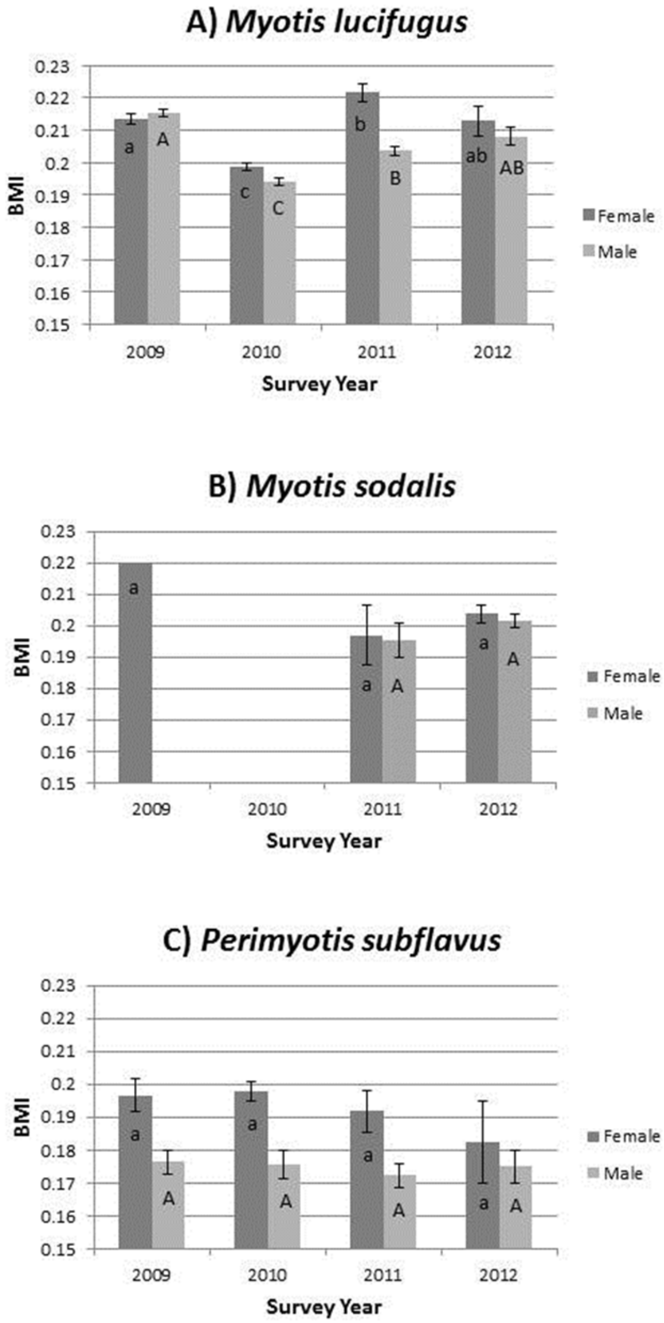

Figure 5. Body Mass Index (BMI) scores for bats captured during early hibernation, 2009-2012, at 14 hibernacula in western Virginia. BMI (±SE) from three species are presented separately for males and females: A) *Myotis lucifugus*, B) *M. sodalis*, and C) *Perimyotis subflavus*. BMI averages significantly different from one another in a *post-hoc* Tukey's test are denoted by differing letters (lower case for females, uppercase for males).

i at levels suggesting continued viability is unknown. At the advent of WNS, Francl et al. (2012) had already documented a substantial decline in reproductive rates in summer bats in West Virginia-juveniles present on the

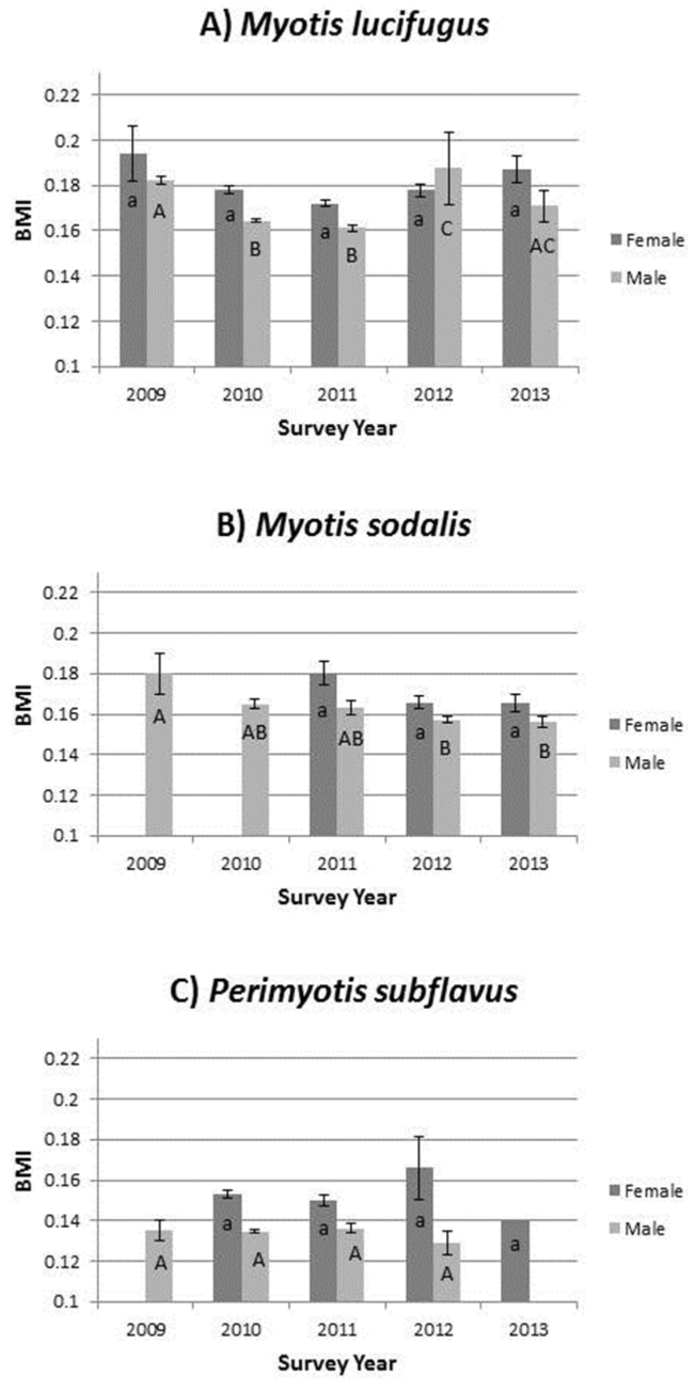

Figure 6. Body Mass Index (BMI) scores for bats captured during late hibernation/spring staging, 2009-2013, at 14 hibernacula in western Virginia. BMI (±SE) from three species are presented, separately for males and females. **A)** *Myotis lucifugus*. **B)** *M. sodalist*. **C)** *Perimyotis subflavus*. BMI averages significantly different from one another in a *post-hoc* Tukey's test are denoted by differing letters (lower case for females, uppercase for males).

landscape had fallen to 10% of historical numbers. Indeed, summer maternity colony data from Kentucky and West Virginia suggest substantial maternity colony collapse and a general lack of recruitment success in large parts of the landscape for northern long-eared bats (Silvis et al., 2014; J. Rodrigue, U.S. Forest Service, un published data). Further, band recapture data from little brown bats and Indiana bats in our study suggest that banded bats are becoming an increasingly larger component of the remaining, smaller population. This potentially provides indirect evidence for lower recruitment rates over time. In combination with Virginia hibernacula counts and fall swarm declines, these findings suggest that fecundity is not keeping pace with mortality rates due to WNS. Frick et al. (2010) predicted that with similar trends such as these, the little brown bat may be regionally extirpated < 20 years from the onset of WNS.

Our BMI analyses suggest that its use as a metric to predict effects of *P. destructans* is limited. Early and late hibernation data are difficult to interpret, primarily due to the lack of pre-WNS data and smaller sample sizes in later survey years. In the period for which we have pre-WNS data for all hibernacula (fall swarm), declines in BMI were documented in several cave bat species. However, females, with markedly lower capture rates, showed no such differences in BMI. Despite our small sample sizes pre-WNS, we believe the smaller sample of females showing no BMI change indicates no ongoing or observable selective pressure. Similarly, Reeder et al. (2012), found just a weak predictive relationship of BMI for survivorship and arousal rates, suggesting that BMI might not be an appropriate measure that provides meaningful context in *P. destructans* infection, hibernation, and survival rate. We concur with this assessment. If selection pressure by BMI is occurring, it is manifesting itself far slower than *P. destructans* progression. At the rapid rate at which these species are declining, selective pressure by BMI may be a moot point.

Although hibernacula surveys in New York (NYDEC, 2012) suggest stabilization of hibernating little brown bat numbers may be rebounding six years after initial detection of *P. destructans*; no such trends were obvious in our early counts (ca. 1-4 years post-*P. destructans* detection). Additional monitoring in subsequent years will be necessary to ascertain if long-term survival is possible for bat species associated with Virginia hibernacula. Undoubtedly, either some level of persistence or local extirpation will have some spatial (landscape-level), physical (local), epidemiological (probability of *P. destructans* contraction), or environmental (within-cave) correlate. Therefore, continued surveys –at minimum, continuing winter visual surveys when absolute counts can be made—and an examination of inter-cave differentials are logical next steps, if we hope to understand the long-term impacts of *P. destructans* in Virginia.

Conflict of interests

The authors did not declare any conflict of interest.

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Appendix 1. Winter counts for 13 hibernacula (named by county; formal names omitted due to sensitivity in endangered species locales) in western Virginia surveyed for bats. Totals from "Pre" time block indicate average counts (1986-2007) to best reflect representative pre-WNS counts. Counts presented for three most common species detected in the hibernacula, *Myotis lucifugus*, *Perimyotis subflavus,* and *M. sodalis* – and for all bats (inclusive of the three species plus additional finds). "N/A" indicates surveys were not completed in that particular year. "% Decline" presents the percent decline in pre-WNS counts to 2013 counts (when all surveyed hibernacula were P.d-positive) across all sites.

Parameter	*Myotis lucifugus*	*Perimyotis subflavus*	*Myotis sodalis*	All bats
Bath 1 - 2011 [suspect]				
Pre	N/A	N/A	N/A	N/A
2009	63	178	0	241
2011	94	532	0	628
2013	3	62	0	65
Bath 2 - 2010 [suspect]				
Pre	1593	163.2	54.9	1847.2
2009	1672	128	61	1897
2011	18	10	92	133
2013	12	14	54	111
Bath 3 - 2011 [suspect]				
Pre	1564.7	296.3	0	1933.5
2009	1554	79	0	1676
2011	1444	368	0	1862
2013	2	22	0	54
Bland 1 - 2009 [confirmed]				
Pre	4838.5	185	221.5	5260
2009	4143	233	208	4596
2011	557	219	146	927
2013	34	5	45	91
Bland 2 - 2010 [suspect]				
Pre	1872.8	81.3	0	1967.8
2009	1784	56	0	2732
2011	N/A	N/A	N/A	N/A
2013	5	0	0	9
Bland 3 - 2009 [suspect]				
Pre	1296	920	0	2219
2009	N/A	N/A	N/A	N/A
2011	89	131	0	220
2013	2	17	0	19
Craig 1 - 2010 [confirmed]				
Pre	601.3	61	27.8	690.1
2009	715	15	23	754
2011	282	130	1	421
2013	5	17	4	28
Giles 1 - 2010 [suspect]				
Pre	118.3	357.3	0	482.7
2009	138	536	0	678
2011	34	193	0	231
2013	0	17	0	22
Highland 1 - 2009 [confirmed]				
Pre	701	513	0	1233
2009	684*	0	0	684*
2011	475	408	0	923

Appendix 1. Contd

2013	7	28	38	79
Highland 2 - 2010 [suspect]				
Pre	3391	205	6	3778
2009	N/A	N/A	N/A	N/A
2011	732	73	3	822
2013	54	9	0	93
Tazewell 1 - 2010 [suspect]				
Pre	684	58.7	0	745.3
2009	600	1	0	601
2011	103	244	0	349
2013	N/A	N/A	N/A	N/A
Wise 1 - 2012 [suspect]				
Pre	244.2	42.8	4	293.4
2009	243	39	1	292
2011	198	5	1	205
2013	82	156	8	253
Wise 2 - 2011 [confirmed]				
Pre	438	15.4	198.6	672.7
2009	655	7	130	807
2011	711	34	266	1024
2013	67	13	192	346