



Effects of white-nose syndrome on regional population patterns of 3 hibernating bat species

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Abstract: *Hibernating bats have undergone severe recent declines across the eastern United States, but the cause of these regional-scale declines has not been systematically evaluated. We assessed the influence of white-nose syndrome (an emerging bat disease caused by the fungus *Pseudogymnoascus destructans*, formerly *Geomyces destructans*) on large-scale, long-term population patterns in the little brown myotis (*Myotis lucifugus*), the northern myotis (*Myotis septentrionalis*), and the tricolored bat (*Perimyotis subflavus*). We modeled population trajectories for each species on the basis of an extensive data set of winter hibernacula counts of more than 1 million individual bats from a 4-state region over 13 years and with data on locations of hibernacula and first detections of white-nose syndrome at each hibernaculum. We used generalized additive mixed models to determine population change relative to expectations, that is, how population trajectories differed with a colony's infection status, how trajectories differed with distance from the point of introduction of white-nose syndrome, and whether declines were concordant with first local observation of the disease. Population trajectories in all species met at least one of the 3 expectations, but none met all 3. Our results suggest, therefore, that white-nose syndrome has affected regional populations differently than was previously understood and has not been the sole cause of declines. Specifically, our results suggest that in some areas and species, threats other than white-nose syndrome are also contributing to population declines, declines linked to white-nose syndrome have spread across large geographic areas with unexpected speed, and the disease or other threats led to declines in bat populations for years prior to disease detection. Effective conservation will require further research to mitigate impacts of white-nose syndrome, renewed attention to other threats to bats, and improved surveillance efforts to ensure early detection of white-nose syndrome.*

Keywords: emerging infectious disease of wildlife, generalized additive mixed models, *Myotis lucifugus*, *Myotis septentrionalis*, *Perimyotis subflavus*, population monitoring, *Pseudogymnoascus destructans*

Los Efectos del Síndrome de Nariz Blanca sobre los Patrones Regionales de Población de Tres Especies de Murciélagos que Hibernan

Resumen: *Los murciélagos que hibernan han experimentado declinaciones severas recientes a lo largo del este de los Estados Unidos pero la causa de estas declinaciones a escala regional no ha sido evaluada sistemáticamente. Valoramos la influencia del síndrome de nariz blanca (una enfermedad emergente de los murciélagos causada por el hongo *Pseudogymnoascus destructans*, antes *Geomyces destructans*) en el myotis café (*Myotis lucifugus*), el myotis del norte (*M. septentrionalis*) y el murciélago tricolor (*Perimyotis subflavus*). Modelamos las trayectorias poblacionales para cada especie con base en un conjunto extenso de datos de conteos de sitios de hibernación de más de un millón de murciélagos individuales en una región de cuatro estados durante más de 13 años y con datos sobre las localidades de los sitios de hibernación y las primeras detecciones del síndrome de nariz blanca en cada sitio de hibernación. Usamos modelos aditivos mixtos generalizados para determinar el cambio poblacional en relación a las expectativas, es decir, cómo las trayectorias poblacionales difirieron con el estado de infección de una colonia, cómo difirieron con la distancia*

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desde el punto de introducción del síndrome de nariz blanca y si las declinaciones fueron concordantes con la primera observación local de la enfermedad. Las trayectorias poblacionales de todas las especies cumplieron con al menos una de las tres expectativas, pero ninguna cumplió con las tres. Nuestros resultados sugieren que el síndrome de nariz blanca ha afectado a las poblaciones regionales de maneras diferentes a como se entendía previamente y no ha sido la causa única de las declinaciones. Específicamente, nuestros resultados sugieren que en algunas áreas y en algunas especies, otras amenazas además del síndrome de nariz blanca también están contribuyendo a las declinaciones poblacionales, que las declinaciones conectadas al síndrome de nariz blanca se han esparcido en extensas áreas geográficas con una velocidad inesperada, y que la enfermedad y otras amenazas resultaron en declinaciones de la población de murciélagos durante años previos a la detección. La conservación efectiva requerirá de una investigación más profunda para mitigar los impactos del síndrome de nariz blanca, de una atención renovada a las otras amenazas y de esfuerzos mejorados de vigilancia para asegurar la detección temprana del síndrome de nariz blanca.

Palabras Clave: enfermedades infecciosas emergentes de la vida silvestre, modelos aditivos mixtos generalizados, monitoreo de poblaciones, *Myotis lucifugus*, *Myotis septentrionalis*, *Perimyotis subflavus*, *Pseudogymnoascus destructans*

Introduction

Bats are highly diverse (Wilson & Reeder 2005), crucial to ecosystem function (Kunz et al. 2011), and valuable to people (Boyles et al. 2011), but they also face a range of severe threats globally (Mickleburgh et al. 2002). Hibernating bats of the eastern United States are particularly threatened. Recent, regional-scale population declines have reached or exceeded 70% in the little brown myotis (*Myotis lucifugus*) (Frick et al. 2010a; Turner et al. 2011; Ingersoll et al. 2013) and 30% in the northern myotis (*Myotis septentrionalis*), the Indiana myotis (*Myotis sodalis*), and the tricolored bat (*Perimyotis subflavus*) (Turner et al. 2011; Ingersoll et al. 2013).

These recent declines have typically been attributed to white-nose syndrome (WNS) (e.g., Turner et al. 2011; Langwig et al. 2012; USFWS 2012b), an emerging infectious disease of hibernating bats caused by a newly discovered fungal pathogen (*Pseudogymnoascus destructans* [Pd], previously known as *Geomyces destructans*) (Lorch et al. 2011). This disease is thought to have had a single introduction in North America near Howe Caverns, New York (hereafter, the point of introduction), where it was first recorded in 2006 (Blehert et al. 2009). In 9 years, the disease has spread to hundreds of caves and mines in 27 eastern, southeastern, and Midwestern states and 5 eastern Canadian provinces (Heffernan 2015). Several lines of evidence suggest WNS is a severe threat to bats, including experimental confirmation that Pd causes WNS and leads to bat mortality (Lorch et al. 2011; Warnecke et al. 2012), clinical signs of WNS on dead and dying bats (Blehert et al. 2009), observations of mass mortality events at hibernacula where WNS has been detected (Blehert et al. 2009; Frick et al. 2010a), and comparisons of bat counts conducted prior to and after the initial detection of WNS (Turner et al. 2011).

Local WNS effects have been so dramatic it might be presumed that WNS is the only significant driver of recent declines in hibernating bat populations in the

eastern United States (e.g., Frick et al. 2010a; Turner et al. 2011; USFWS 2012a, b). However, convincing evidence of local-scale impacts of WNS does not necessarily indicate WNS is the principal cause of the recent regional-scale declines. This is because local mortality events alone may not be large or frequent enough to account for the magnitude of observed changes in regional populations (Levin 1992) because populations may compensate for mortality with increases in reproduction or immigration (Gotelli 1998), population declines in one locality may be offset by increases in other localities (Hanski 1998), and pathogens may interact synergistically with other factors to precipitate declines (de Castro & Bolker 2005). Threats other than WNS could also contribute substantially to observed declines (reviewed in Ingersoll et al. 2013), but, except for endangered Indiana myotis (Thogmartin et al. 2012a), the influence of WNS on regional declines in bat populations has yet to be evaluated systematically. The incomplete understanding of the key cause or causes of regional declines is troublesome because it could lead to misallocation of conservation effort and limit the effectiveness of conservation action.

Our broad aim was to evaluate the role of WNS in the long-term, regional-scale declines in bat populations of the eastern United States. Specifically, we evaluated the idea that recent regional-scale declines in bat populations have been driven solely or principally by the impacts of WNS (e.g., Frick et al. 2010a; Turner et al. 2011; USFWS 2012b) and that declines will follow predictable patterns resulting from time-dependent spatial spread of the disease (Wilder et al. 2011; Thogmartin et al. 2012b). We refer to this idea hereafter as the WNS-driver model. To inform this model, we evaluated the extent to which temporal trajectories of bat populations differed with the observed infection status of a colony, exhibited changes with distance from the WNS point of introduction in a manner that suggested spatial spread over time, and revealed declines that were concordant with first local observation of the disease. Specifically, the WNS-driver

model predicts that declines should occur in hibernacula where WNS is present but not in hibernacula where WNS is not observed, begin later at distances farther from the point of introduction (Wilder et al. 2011; Thogmartin et al. 2012b), and initiate soon after disease introduction in each area.

We tested these 3 predictions on the 3 hibernating bat species for which we could obtain data sufficient to model spatially explicit regional changes over time: *M. lucifugus*, *M. septentrionalis*, and *P. subflavus*. Data were winter counts in 4 U.S. states over 13 years. We used generalized additive mixed models (GAMMs) (Link & Sauer 1997; Fewster et al. 2000; Ingersoll et al. 2013), which address several of the well-known challenges complicating inference of count data for wildlife management (Thomas 1996; Williams et al. 2002; Ingersoll et al. 2013). A GAMM also provides a means to determine if monitoring data are consistent with theoretical predictions of the timing and spatial distribution of population changes and their putative causal factors (Fewster et al. 2000).

Methods

Data

The most consistently sampled, long-term, and regional-scale count data for bats in North America are from surveys of bat hibernacula by state wildlife agencies (Turner et al. 2011; Ingersoll et al. 2013). We obtained data from systematic winter surveys for bats conducted by trained state biologists along standardized routes through major, accessible hibernation areas in caves and mines in New York, Pennsylvania, West Virginia, and Tennessee. Winter surveys were typically completed every 2 years (median between-survey interval was 2.00) January to March from 1999 to 2011. Further details on data collection and the resulting data set are in Ingersoll et al. (2013). Survey data for this study also included spatial coordinates and records of state-confirmed WNS occurrence at each hibernaculum since survey teams began looking for the disease in 2007. Disease presence was confirmed via laboratory detection of characteristic skin lesions on bat carcasses or skin samples (Blehert et al. 2009) typically collected during mortality events at hibernacula. We examined data for each species separately, excluding all surveys that were incomplete or otherwise inconsistent. We also excluded all routes in which a bat species was never observed or for which only a single survey remained in the data set. This rendered a total of 577 surveys along 145 routes and yielded 982,974 *M. lucifugus* individuals, 460 surveys along 109 routes with 5,206 *M. septentrionalis* individuals, and 576 surveys along 145 routes with 68,148 *P. subflavus* individuals. The disease was present by 2011 on 44.1–47.6% of routes for all 3 species.

We identified the WNS category (i.e., infection status) and distance from WNS point of introduction for bat colonies on each survey route. We categorized a colony as WNS present if WNS was detected at any time prior to the end of the study and as WNS not observed otherwise. Because Pd is highly persistent (Lorch et al. 2013), we assumed a colony remained infected once WNS symptoms were observed there. We used spatial coordinates to estimate distance from the presumed point of introduction as a continuous variable and compared this variable to a categorical spatial term: state (New York closest, Tennessee farthest from the point of introduction). The WNS and state categories allowed us to use interactions with time with smoothed terms to address nonlinearity and erratic variation in count data, to compare separate bat population trajectories by infection status and distance, and to note divergences among these trajectories concurrent with first local observation of the disease.

Analyses

We used a 6-stage modeling process to select and evaluate GAMMs: comparison of spatial terms with Akaike's information criterion (AIC) (Akaike 1973; Burnham & Anderson 2002) to determine the overall structure of spatial variation; examination of WNS association terms via AIC (Zuur et al. 2009) to evaluate the infection status prediction of the WNS-driver hypothesis; examination of spatiotemporal interaction terms via AIC (Wilder et al. 2011) to evaluate the spatial spread prediction of the WNS-driver hypothesis; reduction of the selected spatial models to determine the most appropriate model for graphing; control of observation heterogeneity associated with survey date (Ingersoll et al. 2013); and graphical analysis of the selected bat-count trajectories over time and associated confidence intervals to further evaluate all predictions of the WNS-driver model (Wood 2006; Zuur et al. 2009).

The most inclusive models included the response of bat counts to WNS category (WNS), distance from the presumed point of introduction (distance or state), and the interactions with smoothed year (year). We included a term for day of winter (day), which represented the date at which hibernacula were surveyed to address a documented source of heterogeneity in detection of bats (Ingersoll et al. 2013). We also included random terms for survey route in all models, to adjust for nonindependence and local detection effects (Ingersoll et al. 2013). For each species, we first compared 3 alternative spatial models to determine overall spatial structure:

$$E[y_{it}] = g \left(\begin{array}{l} u_i + s_1(\text{WNS} \times \text{year}) + s_2(\text{state} \times \text{year}) \\ + s_3(\text{day}) + v_{ijt} \end{array} \right), \quad (1)$$

$$E[y_{it}] = g \left(\begin{array}{l} u_i + s_1(\text{WNS} \times \text{year}) + s_2(\text{distance}) \\ + s_3(\text{day}) + v_{ijt} \end{array} \right), \quad (2)$$

$$E[y_{it}] = g \left(\begin{array}{l} u_i + s_1(\text{WNS} \times \text{year}) + \beta_1(\text{distance}) \\ \times \text{year} + s_2(\text{day}) + v_{ijt} \end{array} \right), \quad (3)$$

where $y_{it} \sim \text{NegBin}(E[y_{it}], k)$; $E[y_{it}]$ is the expected count for species i at time t ; k is a dispersion parameter, g is the inverse of the selected link function (in our case the inverse of the natural logarithm \ln); u_i was the mean count for a species; v_{ijt} is a random effect for species i on survey route j at time t ; s_1 , s_2 , and s_3 are smoothing functions (here, cubic regression splines) for the interaction and main effects between WNS and year, the interaction and main effects between state (categorical distance) and year or main effects of distance, and main effects of day; and β_1 is a vector of linear coefficients for the distance \times year interaction and main effects. The models assumed negative-binomial distributed counts and were compared on the basis of AIC (Zuur et al. 2009).

To evaluate the first 2 predictions of the WNS-driver hypothesis, we then compared selected models with models with reduced WNS interaction terms and with models with reduced spatiotemporal interaction terms on the basis of AIC. Selection of the model with an interaction term between WNS and smoothed year would indicate that trajectories differ with observed infection status, concordant with the WNS-driver model. Selection of models with spatiotemporal interactions would indicate trajectories differ with categorical (state) or continuous distance from the point of introduction, concordant with the WNS-driver model.

For further inference and for selection of a model for graphing, we then used stepwise model reduction from the selected spatiotemporal model, first reducing the most complex interaction terms, then reducing the smoothed terms, and finally reducing the simpler linear terms on the basis of AIC (Zuur et al. 2009). In all cases, selection of model terms was clear (cumulative Akaike weights, $w_i > 0.9$ for the set of models that only varied in the day term). The models with the highest w_i represented the best predictive model, but smoothed day terms were retained in models for graphic comparison to ensure that the effect of unmodeled day did not bias results for this particular data set. Modeling of day entailed using GAMMs to determine within-season response of relative abundance to day, fixing the value for day at its median, and then calculating trajectories as if they had all been sampled on the same day (Ingersoll et al. 2013).

Trajectories were rendered separately by WNS category and state or distance category, resulting in 8 separate trajectories per species. Predicted counts were presented on a natural-logarithmic scale and normalized by dividing all estimated values within a WNS and state category by the largest estimate within that category. This provided a standardized value for relative abundance, which was in line with inference appropriate to GAMMs (Royle & Dorazio 2008) and reflected our emphasis on comparing

changes in trend rather than absolute abundances. The 95% CIs were estimated at plus and minus twice the standard error. Graphical rendering enabled further inference about the WNS-driver model. In particular, observation of declines that begin with first local detection of WNS would indicate temporal concordance between putative cause (WNS) and effect (population change), supporting the third prediction of the WNS-driver model.

We examined count data, compared non-normalized models to data, and examined diagnostic graphs, residual plots, and goodness-of-fit measures to validate assumptions associated with the negative binomial distribution and evaluate model fit (Supporting Information). All analyses were completed with *R* version 3.01 statistical software (R Core Team 2013) with the *mgcv* package (Wood 2004, 2011).

Results

Temporal variation in bat populations interacted with categorical distance (state) in *M. lucifugus* (Table 1), interacted with continuous distance in *M. septentrionalis* (Table 2), and did not interact with distance in *P. subflavus* (Table 3). In model comparisons examining WNS and spatiotemporal interaction terms, selected models were strongly supported in *M. lucifugus* and *M. septentrionalis* (all $w_i \leq 0.02$ for alternate models; Tables 1 & 2), which enabled clear inferences, but in *P. subflavus* there was some limited support ($w_i > 0.07$) for alternate models (Table 3). For overall within-species comparisons, the top 3 models for each species differed only in the nuisance term day (Tables 1–3), which was included to reduce bias, not to inform the WNS driver model. Other, substantively different models had almost no support (all $w_i \leq 0.01$). A detailed exploration of model fit to data and to assumptions is in the Supporting Information.

As expected under the WNS-driver model, models with temporal interactions with infection status were supported in *M. lucifugus* and *P. subflavus* (smoothed year \times WNS interactions, Tables 1 & 3). Differences in trajectory with infection status were conspicuous in *P. subflavus*; WNS-present colonies exhibited a much more negative trend late in the study period than WNS-not-observed colonies (blue versus red traces, Fig. 3). In contrast, trajectories of WNS-present and WNS-not-observed colonies in *M. lucifugus* were nearly coincident throughout (Fig. 1). In contrast to expectations, models with year \times WNS interactions were not supported in *M. septentrionalis* (Table 2), resulting in fully coincident trajectories for affected and unaffected colonies (Fig. 2).

As expected under the WNS-driver model, models with spatiotemporal interactions had strong support in *M. lucifugus* (smoothed interaction between year and state [Table 1]) and *M. septentrionalis* (interaction between year and continuous distance [Table 2]). Population

Table 1. Information criteria for comparison of candidate models of population trajectories for *M. lucifugus*.

Focus of model selection	Fixed terms ^a	<i>K</i> ^b	Log lik ^c	AIC ^d	Δ_i ^e	w_i ^f
Comparison of spatial terms						
	$s(\text{year} \times \text{WNS}) + s(\text{year} \times \text{state}) + s(\text{day})$	16	-970.90	1973.79	0.00	1.00
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-988.40	1996.80	23.01	0.00
	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-995.62	2011.23	37.44	0.00
Reduction of WNS association terms						
	$s(\text{year} \times \text{WNS}) + s(\text{state} \times \text{year}) + s(\text{day})$	16	-970.90	1973.79	0.00	0.98
	$s(\text{state} \times \text{year}) + s(\text{day})$	13	-977.68	1981.36	7.57	0.02
Reduction of spatiotemporal interaction terms						
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-988.40	1996.80	0.00	1.00
	$s(\text{year} \times \text{WNS}) + \text{dist} + s(\text{day})$	9	-996.51	2013.77	16.97	0.00
Overall within-species comparison						
	$s(\text{year} \times \text{WNS}) + s(\text{year} \times \text{state})$	14	-970.85	1969.70	0.00	0.67
	$s(\text{year} \times \text{WNS}) + s(\text{year} \times \text{state}) + \text{day}$	15	-970.89	1971.78	2.08	0.24
	$s(\text{year} \times \text{WNS}) + s(\text{year} \times \text{state}) + s(\text{day})$	16	-970.90	1973.79	4.09	0.09
	$s(\text{year} \times \text{state}) + s(\text{day})$	13	-977.68	1981.36	11.66	0.00
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-988.40	1996.80	27.10	0.00
	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-995.62	2011.23	41.53	0.00
	$s(\text{year} \times \text{WNS}) + \text{dist} + s(\text{day})$	9	-997.88	2013.77	44.07	0.00

^aAbbreviations: WNS, presence or absence of detection of white-nose syndrome in a colony by 2011; state, a categorical measure of the distance of a hibernaculum from the point of introduction; distance, a continuous measure of the distance of a hibernaculum from the point of introduction; day, day of winter, the sequential date of the hibernaculum survey; year, year of the hibernation survey. Variables with *s()* notation are smoothed, otherwise they are linear. Interaction terms indicated by \times (e.g., year \times WNS) imply inclusion of main effects as well. The random-effect survey route is not shown in the table but was included in all models.

^bThe number of linear terms included in the model.

^cThe negative model log likelihood.

^dAkaike's information criterion.

^eDifference in AIC between the top-ranked model (i.e., model with lowest AIC) and listed model.

^fAkaike weight (i.e., the weight of evidence for each model in the set given the data, where 1.00 is the highest likelihood of the model relative to other models).

Table 2. Information criteria for comparison of candidate models of population trajectories for *M. septentrionalis*.

Focus of model selection	Fixed terms	<i>K</i>	Log lik	AIC	Δ_i	w_i
Comparison of spatial terms						
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-871.11	1762.22	0.00	0.98
	$s(\text{year} \times \text{WNS}) + s(\text{year} \times \text{state}) + s(\text{day})$	17	-868.43	1770.87	8.65	0.01
	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-876.81	1773.63	11.41	0.00
Reduction of WNS association terms						
	$s(\text{year}) + \text{year} \times \text{dist} + s(\text{day})$	9	-865.19	1748.38	0.00	1.00
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-871.11	1762.22	13.84	0.00
Reduction of spatiotemporal interaction terms						
	$s(\text{year}) + \text{year} \times \text{dist} + s(\text{day})$	9	-865.19	1748.38	0.00	1.00
	$s(\text{year}) + \text{dist} + s(\text{day})$	8	-875.73	1767.46	19.08	0.00
Overall within-species comparison						
	$s(\text{year}) + \text{year} \times \text{dist}$	7	-864.89	1743.77	0.00	0.74
	$s(\text{year}) + \text{year} \times \text{dist} + \text{day}$	8	-865.24	1746.49	2.72	0.19
	$s(\text{year}) + \text{year} \times \text{dist} + s(\text{day})$	9	-865.19	1748.38	4.61	0.07
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-871.11	1762.22	18.45	0.00
	$s(\text{year}) + \text{dist} + s(\text{day})$	8	-875.73	1767.46	23.69	0.00
	$s(\text{year} \times \text{WNS}) + s(\text{year} \times \text{state}) + s(\text{day})$	17	-868.43	1770.87	27.10	0.00
	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-876.81	1773.63	29.86	0.00

Note: All notations are as described in footnotes to Table 1. A random effect for survey route is not shown in the table but is included in all models.

trajectories were highly variable among regions in *M. lucifugus*, although declines were evident in most areas late in the study period (compare trajectories among panels in Fig. 1). Spatial variation in *M. septentrionalis* was more gradual and less negative farther from the point

-of-WNS-introduction (Fig. 2). In contrast to expectations, models with spatiotemporal interactions received only limited support in *P. subflavus* (Table 3), and trajectories varied little with distance from the point of introduction (Fig. 3).

Table 3. Information criteria for comparison of candidate models of population trajectories for *P. subflavus*.

Focus of model selection	Fixed terms ^a	K	Log lik	AIC	Δ_i	w_i
Comparison of spatial terms	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-851.58	1723.15	0.00	0.99
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-856.12	1732.23	9.08	0.01
Reduction of WNS association terms	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-851.58	1723.15	0.00	0.93
	$s(\text{year}) + \text{WNS} + s(\text{dist}) + s(\text{day})$	10	-854.16	1728.32	5.16	0.07
Reduction of spatiotemporal interaction terms	$s(\text{year} \times \text{WNS}) + \text{dist} + s(\text{day})$	9	-855.90	1729.81	0.00	0.77
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-856.12	1732.23	2.42	0.23
Overall within-species comparison	$s(\text{year} \times \text{WNS}) + s(\text{dist})$	8	-851.53	1719.06	0.00	0.67
	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + \text{day}$	9	-851.58	1721.15	2.09	0.24
	$s(\text{year} \times \text{WNS}) + s(\text{dist}) + s(\text{day})$	10	-851.58	1723.15	4.09	0.09
	$s(\text{year}) + \text{WNS} + s(\text{dist}) + s(\text{day})$	10	-854.16	1728.32	9.25	0.01
	$s(\text{year} \times \text{WNS}) + \text{dist} + s(\text{day})$	9	-855.90	1729.81	10.75	0.00
	$s(\text{year} \times \text{WNS}) + \text{year} \times \text{dist} + s(\text{day})$	10	-856.12	1732.23	13.17	0.00

Note: All notations are as described in footnotes to Table 1. A random effect for survey route is not shown in the table but is included in all models.

^aFor the comparison of spatial terms, a full model including a $s(\text{year} \times \text{state})$ term could not be included for *P. subflavus* due to convergence failure.

As expected under the WNS-driver model, population trajectories often exhibited changes in trend medial to the study period (change in blue traces, Figs. 1a-c & 3). However, only in *M. lucifugus* did the onset of declines match the timing of local detection of WNS within one year (blue trace versus vertical dotted line, Figs. 1a-c). Otherwise, either the onset of recent declines preceded the first local detection of WNS (Fig. 3) or steady declines (Figs. 2a & 2b), stability (Figs. 1d & 2c), or positive growth (Fig. 2d) occurred in WNS-present colonies throughout the study.

Discussion

Evaluation of the WNS-Driver Model

It is often assumed that WNS is the sole or principal driver of recent population declines in hibernating bats of the eastern United States (e.g., Frick et al. 2010a; Turner et al. 2011; USFWS 2012b) and that these declines will follow predictable patterns resulting from time-dependent spatial spread of the disease (Wilder et al. 2011; Thogmartin et al. 2012b), with timing of declines following soon after local disease detection. Our systematic evaluation of the role of WNS in regional bat declines partially supports these ideas. First, population trajectories in *P. subflavus* varied with infection status (smoothed $\text{year} \times \text{WNS}$ interactions; Table 3) in the expected manner: WNS-present and WNS-not-observed colonies first exhibited similar stable or somewhat increasing trajectories and then diverged increasingly as WNS-present colonies declined (blue versus red traces, Fig. 3). Second, temporal variation in population trajectories often varied with distance (spatiotemporal interactions [Tables

1 & 2]). In particular, patterns in *M. septentrionalis* were consistent with expected wave-like spread of disease impacts; trajectories of affected colonies were more negative closer to the point of introduction (compare panels in Fig. 2). Third, timing of declines sometimes coincided with local detection of WNS. In particular, the onset of declines in affected colonies of *M. lucifugus* roughly coincided with first local detection of WNS in most regions (blue trace versus vertical line, Figs. 1a-c). These results—suggesting poorer outcomes for colonies affected by WNS, evidence for time-dependent spatial spread of declines in bat populations over time, and temporal coincidence of the onset of bat population declines with first local detection of WNS—together provide support for expected correlations between WNS and regional-scale trajectories of hibernating bat species.

Nonetheless, several results diverged in important ways from expectations relating to infection status, distance, and timing. First, we did not detect a divergence in trend with infection status in *M. septentrionalis* (smoothed $\text{year} \times \text{WNS}$ interaction [Table 2; blue and red traces, Fig. 2]), and divergences by infection status in *M. lucifugus* trajectories (Table 1) were small (Fig. 1). Second, population declines did not always initiate later at locations more distant from the point of introduction. In particular, declines in *P. subflavus* were simultaneous over space (spatiotemporal interaction [Table 3; compare panels in Fig. 3]). Third, the timing of declines was generally earlier than expected. For instance, the onset of local declines of WNS-present colonies always preceded first local detection of WNS by 3–7 years in *P. subflavus* (onset of declines in blue traces versus vertical lines, Fig. 3) and by as much as 10 years in *M. septentrionalis* (Fig. 2b).

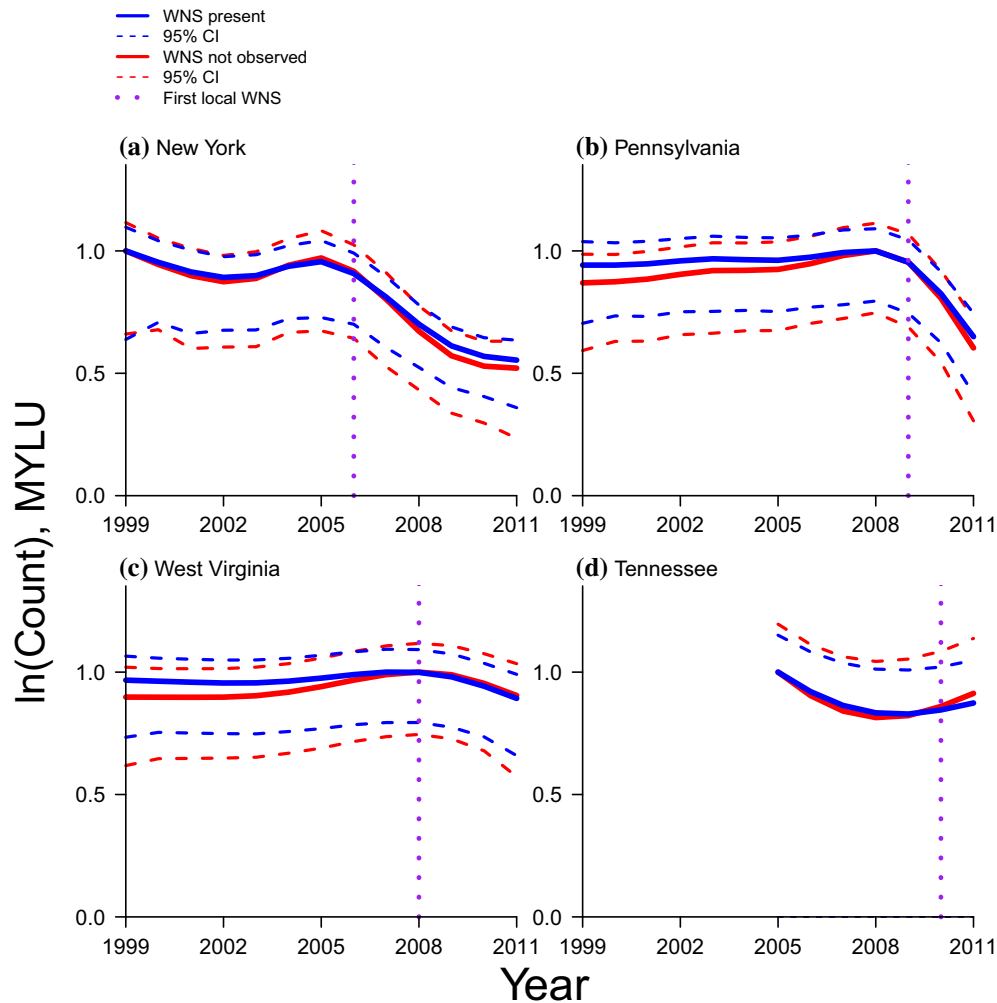


Figure 1. Population trajectories and 95% CIs for *M. lucifugus* (MYLU) along survey routes where white-nose syndrome was (WNS present) and was not observed (WNS not observed) in 4 U.S. states, representing the range of distance categories relative to the point of introduction: (a) New York, closest to the point of introduction; (b) Pennsylvania; (c) West Virginia; and (d) Tennessee, farthest from the point of introduction. Vertical dotted lines indicate the year of first detection of WNS at any site within a state.

Potential Causes of the Observed Trajectories

Together, these results suggest important yet incomplete support for the WNS-driver model. We interpret our results as evidence that WNS is severely affecting bat populations at a regional scale but that its effects are inconsistent with previous understanding. Our results also suggest WNS is not the sole cause of regional declines. More broadly, the unexpected patterns we observed raise important questions about processes underlying bat responses to WNS.

First, similar trajectories in affected and unaffected colonies of *M. lucifugus* and *M. septentrionalis* could result if colonies are strongly linked by metapopulation effects (Hanski 1998) resulting from substantial among-year or within-winter movements of bats among hibernacula (e.g., Boyles et al. 2006). Such movement and heavy

mortality could together cause affected colonies to become population sinks, draining individuals from unaffected colonies via immigration without compensatory emigration. Alternatively, similar trajectories could result from important threats other than WNS that affect bat colonies regardless of infection status (Ingersoll et al. 2013) or from incomplete detection of WNS at some hibernacula leading to mischaracterization of colony infection status. In addition, inherent features of the data set (counts that varied considerably from site to site and year to year and sample sizes that varied over time and were sometimes unbalanced over year, by region, and by WNS category) (Supporting Information) and well-known complications of modeling count data (Ingersoll et al. 2013) resulted in uncertainties that decreased power to detect the regional-scale signal of WNS infection.

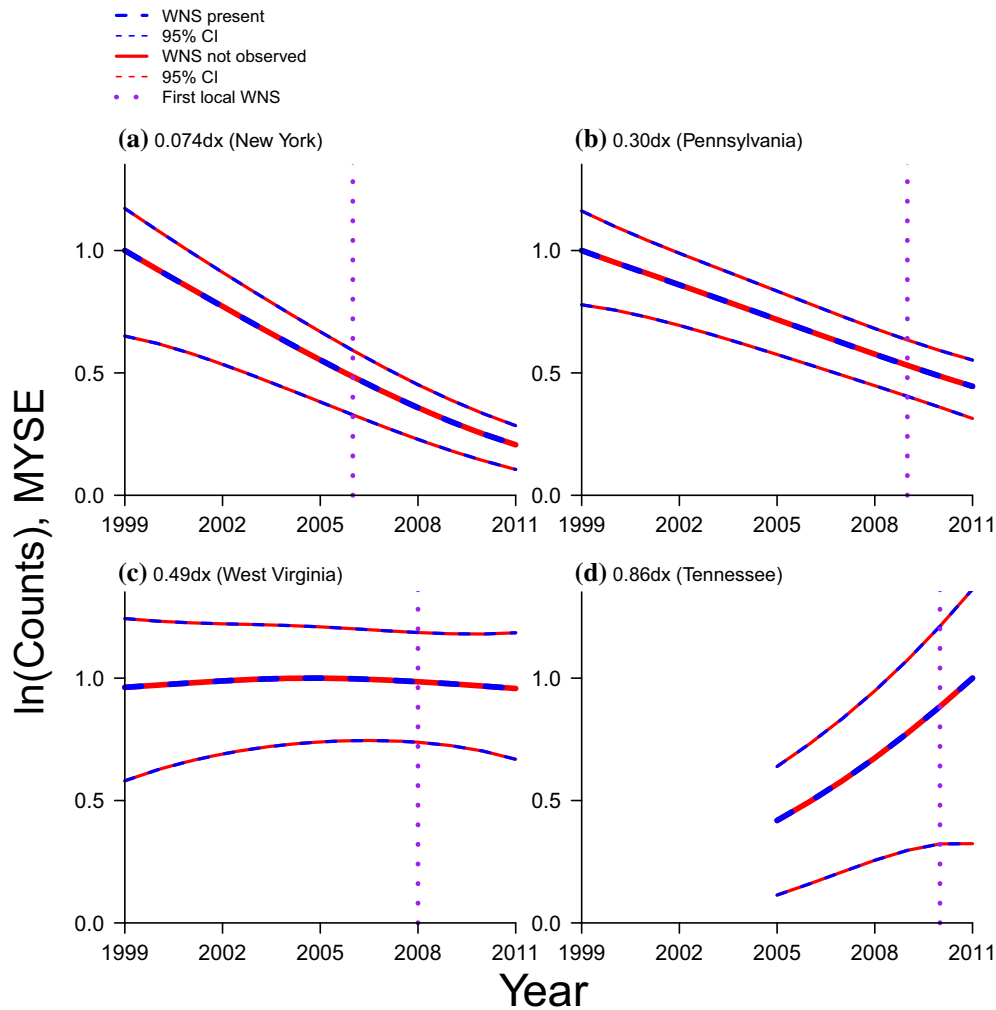


Figure 2. Population trajectories and 95% CIs for *M. septentrionalis* (MYSE) along survey routes where white-nose syndrome was (WNS present) and was not observed (WNS not observed) at 4 distances from the point of introduction. Because the selected MYSE model had continuous distance rather than categorical distance (state), trajectories represent plots extracted from the model at different points along the distance continuum. To facilitate comparisons with the models shown in the other figures, the points correspond with mean distances in the MYSE sample from the point of introduction for each state. Specifically, trajectories are represented at the following locations: (a) 0.074 of the distance from the point of introduction to the farthest site (i.e., at the mean distance from the point of introduction for New York); (b) 0.30 of the distance from the point of introduction to the farthest site, the mean distance for Pennsylvania; (c) 0.49 of the distance from the point of introduction to the farthest site, the mean distance for West Virginia; and (d) 0.86 of the distance from the point of introduction to the farthest site, the mean distance for Tennessee (vertical dotted lines, year of first detection of WNS at any site within a state).

Second, the limited support for wave-like spatiotemporal spread in *P. subflavus* suggests population-level changes may occur quickly over space, perhaps as a consequence of rapid WNS spread via both bat movement and human-mediated pathways (USGS 2011). Although temporal congruence over space was unexpected, results are consistent with evidence that WNS spread accelerates in areas with high densities of caves (Maher et al. 2012); such as in the Appalachian region studied here. Temporal congruence over space

may also result from the influence of factors other than proximity to contaminated sites in promoting disease transmission. For instance, topography (Flory et al. 2012; Miller-Butterworth et al. 2014), climate (Flory et al. 2012; Hallam & Federico 2012; Maher et al. 2012), geographic variation in hibernaculum density (Maher et al. 2012), hibernaculum type (Wilder et al. 2011), roost microclimates (Hallam & Federico 2012; Langwig et al. 2012), bat species composition (Wilder et al. 2011), bat colony size (Wilder et al. 2011; Langwig et al. 2012;

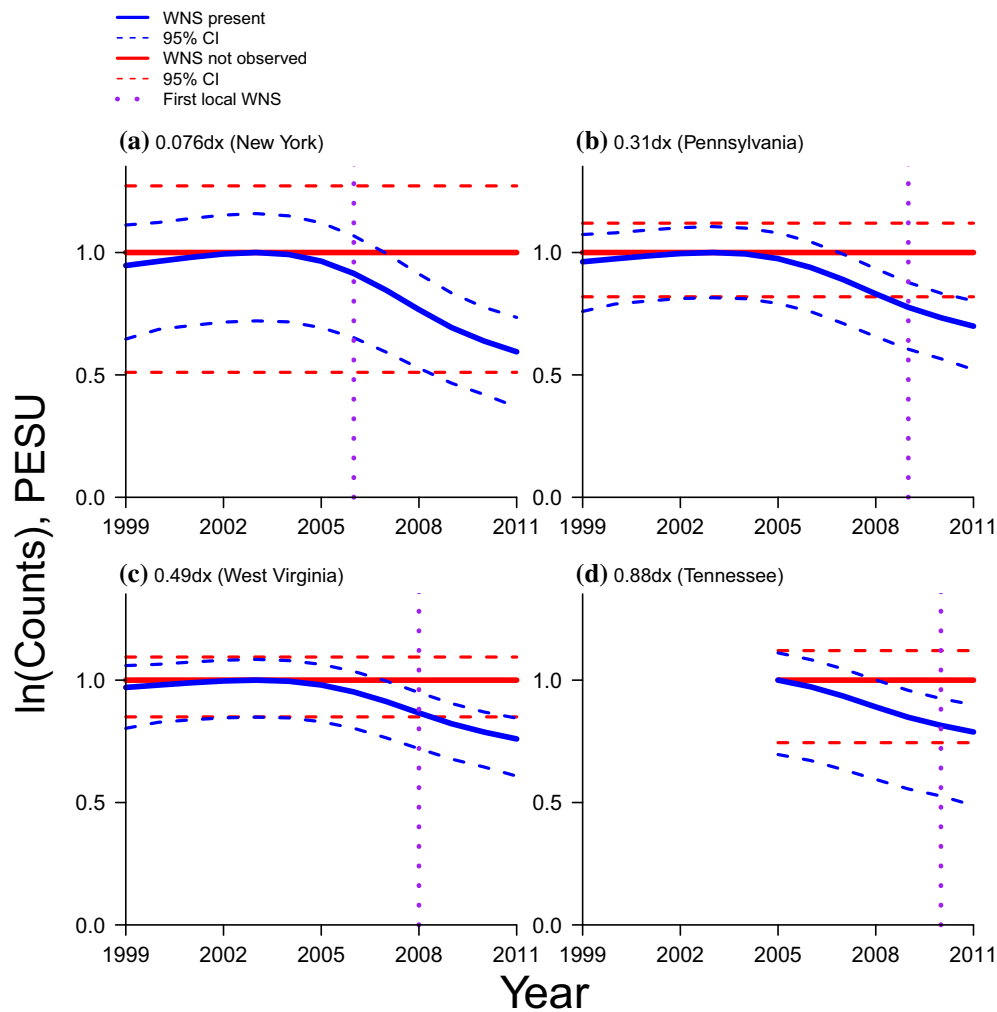


Figure 3. Population trajectories and 95% CIs for *P. subflavus* (PESU) along survey routes where white-nose syndrome was (WNS present) and was not observed (WNS not observed) at 4 distances from the point of introduction. Because the selected *P. subflavus* model had continuous distance rather than categorical distance (state), trajectories represent plots extracted from the model at different points along the distance continuum. To facilitate comparisons with the models shown in the other figures, the points correspond with mean distances in the PESU sample from the point of introduction for each state. Specifically, trajectories are represented at the following locations: (a) 0.076 of the distance from the point of introduction to the farthest site (i.e., at the mean distance from the point of introduction for New York); (b) 0.31 of the distance from the point of introduction to the farthest site, the mean distance for Pennsylvania; (c) 0.49 of the distance from the point of introduction to the farthest site, the mean distance for West Virginia; and (d) 0.88 of the distance from the point of introduction to the farthest site, the mean distance for Tennessee (vertical dotted lines, year of first detection of WNS at any site within a state).

Thogmartin et al. 2012b), and location in relation to bat migration patterns (Miller-Butterworth et al. 2014) have all been correlated with risk to bat colonies from WNS.

Third, while irregular spacing of surveys could have hindered our ability to pinpoint the exact timing of population changes, the early onset of declines in WNS-present colonies (e.g., Figs. 2a, 2b, & 3d) may suggest WNS affected some areas for years without detection. This would be surprising because dead and dying bats at hibernacula are such obvious indicators that surveillance efforts have

often been assumed to reliably detect WNS (e.g., Frick et al. 2010a; Wilder et al. 2011; Flory et al. 2012; but see Maher et al. 2012). Definitive confirmation of WNS, however, is often difficult and effects of the disease are not always conspicuous in the field (Turner et al. 2011; Turner et al. 2014; Janicki et al. 2015), especially if surveys do not coincide with mass mortality events (Langwig et al. 2012). Alternatively, mortality caused by WNS may have been subtle at first, prior to the mass-mortality events that alarmed managers and led to confirmation of WNS

in hibernacula. Mortality could also have occurred in inaccessible areas outside survey routes within hibernacula or on the landscape outside of hibernacula due to bats' behavioral responses to infection (Turner et al. 2011). Another possibility is that WNS more successfully invaded colonies (Fig. 3) or regional populations (Fig. 2) already compromised by other, as-yet-unidentified stressors, reinforcing ongoing declines (de Castro & Bolker 2005). Further research on bat responses to WNS is needed to evaluate each of these possibilities.

Conservation Implications

Our results, while consistent with the idea that WNS contributed to ongoing bat population declines (Turner et al. 2011; Ingersoll et al. 2013), do not implicate WNS as the sole cause of these declines. Further, because existing methods to prevent or cure WNS infection in natural settings are untested or of limited efficacy, management efforts have been limited to reducing human-mediated spread of Pd among hibernacula (USFWS 2010, 2012a). Such efforts by themselves will not be sufficient to stop WNS spread because Pd is readily transmitted via bat-substrate-bat and bat-to-bat contact (Foley et al. 2011; Hallam & McCracken 2011; Lorch et al. 2011). Thus, although research on bat responses to WNS must proceed apace in hopes of mitigating the most severe effects of this disease, renewed management attention to other threats may hold more immediate promise for reducing further declines. Although determination of which threats other than WNS may have contributed to declines was beyond the scope of this study, other threats to bats are well-known and include loss and disturbance of critical roost (Thomas 1995) and foraging sites (Jones et al. 2009); toxicity from agricultural pesticides and other chemical compounds (Clark 1988); altered roost microclimates, foraging habitats, and prey communities from climate change (Rodenhouse et al. 2009; Frick et al. 2010b); and heightened mortality from inflight collisions with vehicles, buildings, and wind turbines (Arnett et al. 2008; Russell et al. 2009). Reducing such threats could alleviate synergistic or interacting effects that may be compounding threats to bats (Harvell et al. 2002; Kannan et al. 2010), ameliorate other stressors to make bats more resilient to WNS, and enable immediate intervention on threats more amenable to management than WNS.

Our results indicating correlated declines in affected and unaffected colonies, rapid spread of population declines, and earlier-than-expected declines also suggest a need to improve WNS surveillance. Past surveillance efforts—which confirmed WNS via laboratory analysis of samples from dead or dying bats or from bats with visual evidence of fungal growth (Turner et al. 2011)—have been considered comprehensive but appear from our results to have often missed initial stages of WNS

infection and bat decline. Further, the correlated declines and rapid long-distance spread of population declines suggest that even hibernacula far from currently infected sites—where managers may not yet be engaged in surveillance or fully prepared to respond to the disease—may be vulnerable to direct or indirect effects of WNS. Surveillance could be improved through wide application of new highly sensitive methods for detection of Pd or WNS lesions (e.g., Muller et al. 2013; Turner et al. 2014), even in areas far from known infected colonies. Improved WNS surveillance could facilitate tracking of disease progression, increase understanding of behavioral or environmental factors influencing bat susceptibility, and provide time to conserve critical or vulnerable bat colonies after initial infection but prior to mass mortality. Such improved surveillance would be particularly useful in western states where WNS has not yet been detected but that are home to several vulnerable or potentially vulnerable bat species (Foley et al. 2011).

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Supporting Information

Examination of model fit (Appendix S1) and count data, modeled population trajectories with count data, diagnostic plots, and residuals plots (Appendix S2) are available as part of the on-line article. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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