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Environmental conditions associated with bat white-nose syndrome mortality in the north-eastern United States

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Summary

1. White-nose syndrome (WNS) is an emerging disease of hibernating North American bats that is caused by the cold-growing fungus *Geomyces destructans*. Since first observed in the winter of 2007, WNS has led to unprecedented mortality in several species of bats and may threaten more than 15 additional hibernating bat species if it continues across the continent. Although the exact means by which fungal infection causes mortality are undetermined, available evidence suggests a strong role of winter environmental conditions in disease mortality.

2. By 2010, the fungus *G. destructans* was detected in new areas of North America far from the area it was first observed, as well as in eight European bat species in different countries, yet mortality was not observed in many of these new areas of North America or in any part of Europe. This could be because of the differences in the fungus, rates of disease progression and/or in life-history or physiological traits of the affected bat species between different regions. Infection of bats by *G. destructans* without associated mortality might also suggest that certain environmental conditions might have to co-occur with fungal infection to cause mortality.

3. We tested the environmental conditions hypothesis using Maxent to map and model landscape surface conditions associated with WNS mortality. This approach was unique in that we modelled possible requisite environmental conditions for disease mortality and not simply the presence of the causative agent.

4. The top predictors of WNS mortality were land use/land cover types, mean air temperature of wettest quarter, elevation, frequency of precipitation and annual temperature range. Model results suggest that WNS mortality is most likely to occur in landscapes that are higher in elevation and topographically heterogeneous, drier and colder during winter, and more seasonally variable than surrounding landscapes.

5. *Synthesis and applications*. This study mapped the most likely environmental surface conditions associated with bat mortality owing to WNS in the north-eastern United Sates; maps can be used for selection of priority monitoring sites. Our results provide a starting point from which to investigate and predict the potential spread and population impacts of this catastrophic emerging disease.

Key-words: bioclimatic modelling, Chiroptera, disease modelling, ecological niche models, fungus, *Geomyces destructans*, Maxent, pathogen risk assessment

Introduction

White-nose syndrome (WNS) is an emerging disease of hibernating bats in North America (Blehert *et al.* 2009). The fungus that causes WNS in North America (Lorch *et al.* 2011) also occurs on hibernating bats in Europe without causing WNS and mass mortality (Martínková *et al.* 2010; Puechmaille *et al.* 2010, 2011a,b; Wibbelt *et al.* 2010). In North America, mass mortality of bats affected by WNS was first observed at a few caves where bats hibernate (hereafter 'hibernacula') in New York state during the late winter of 2007 (Blehert *et al.* 2009). In the four subsequent winters, WNS rapidly spread throughout the Appalachian region of eastern North America and is now affecting at least six species of hibernating bats that occur

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in that region (Turner, Reeder & Coleman 2011). Species of bats diagnosed with WNS include *Eptesicus fuscus* (big brown bats), *Myotis leibii* (eastern small-footed bats), *Myotis lucifugus* (little brown bats), *Myotis septentrionalis* (northern long-eared bats), *Myotis sodalis* (Indiana bats) and *Perimyotis subflavus* (tricoloured bats) (Foley *et al.* 2011). Previously, common species (e.g. *M. lucifugus*) in the north-eastern United States are now at risk of regional extirpation or extinction owing to WNS (Frick *et al.* 2010).

A recently discovered cold-loving fungus (*Geomyces destructans*; Blehert *et al.* 2009; Gargas *et al.* 2009) causes serious skin infection in bats affected by WNS while they hibernate (Meteyer *et al.* 2009; Lorch *et al.* 2011). In North America, this fungus or its DNA has only been found on the bodies of bats showing clinical signs of WNS or in the soils of hibernacula where WNS mortality occurred (Lindner *et al.* 2011). Patterns of WNS spread indicate that *G. destructans* may be an exotic-invasive species that was recently introduced to the United States (Wibbelt *et al.* 2010) or that the fungus is native and only recently became pathogenic to bats (Puechmaille *et al.* 2011a). Infection of bat wings by *G. destructans* is presumed to be a primary cause of WNS and subsequent mortality, most likely through physical or behavioural disruption of homoeostasis during hibernation (Blehert *et al.* 2009; Boyles & Willis 2010; Cryan *et al.* 2010; Lorch *et al.* 2011). However, skin infection by *G. destructans* may not always coincide with mortality. During spring of 2010, DNA of *G. destructans* was detected in three additional species of hibernating bats (*Myotis austroriparius*, *Myotis grisescens*, *Myotis velifer*) west of the Appalachian Region (e.g. Missouri and Oklahoma), yet mortality was not observed (USGS, 2010). Although observations of fungal presence without mortality or additional clinical signs of disease (e.g. bats flying during daytime in winter) may simply reflect detection of the disease in its earliest stages, it is possible that environmental limits on the disease might help explain the more restricted distribution of observed mortality relative to the overall pattern of infection.

A model of infectious disease causation, the so-called epidemiologic triad, posits that three factors are necessary for a disease to occur: (i) a susceptible host, (ii) a pathogen capable of infecting the host and (iii) environmental conditions favourable to the existence of both host and pathogen that bring them together in a way that causes disease (Thisted 2003). With WNS, hibernating bats are accommodating hosts for *G. destructans* – their body temperatures are within the optimal growth range of the fungus, their immune function is likely to be limited, and they inhabit cold, dark, damp places where the fungus can easily persist (Meteyer *et al.* 2009; Cryan *et al.* 2010). The unique ability of *G. destructans* to infect the living skin tissues of North American hibernating bats also makes it a potentially lethal pathogen (Meteyer *et al.* 2009; Cryan *et al.* 2010; Lorch *et al.* 2011). However, little is known about how environmental conditions influence the chain of events leading from exposure of bat skin to *G. destructans* through infection to WNS mortality.

The fungus *G. destructans* only grows at cold temperatures (< 20 °C; Gargas *et al.* 2009), and thus, WNS is a disease of hibernating bats. All hibernating bats in North America are insectivorous and survive winter insect shortage by moving to hibernacula that are consistently cold and humid. During hibernation, bats suppress their metabolism and body temperature to save energy and thus survive for 6–8 months on stored body fat (Speakman & Thomas 2003). Current evidence suggests that *G. destructans* infects bats and causes the most physiological damage while their body temperatures are suppressed during hibernation (Meteyer *et al.* 2009). However, bats infected by *G. destructans* might be capable of surviving fungal infection by arousing from hibernation [metabolically warming their bodies to euthermic temperatures (*c.* 35 °C)] and seeking warm conditions and/or insect prey to offset metabolic costs of remaining euthermic (Boyles & Willis 2010; Dobony *et al.* 2011; Meteyer *et al.* 2011; Storm & Boyles 2011). Therefore, environmental conditions both inside and outside hibernacula are likely to influence winter survival.

Microclimate conditions inside bat hibernacula can vary considerably among regions and sites (McNab 1974; Humphries, Thomas & Speakman 2002; Brack 2007), and different species of bats select different conditions for hibernation (Davis 1970). However, bats in North America tend to hibernate in sites with high humidity and temperatures that generally range between about 3 and 15 °C (Davis 1970; McNab 1974), whereas environmental surface conditions outside hibernacula fluctuate much more dramatically and can be much more variable during winter. Few data on underground conditions within bat hibernacula are available, yet data on surface conditions outside hibernacula are readily available. Environmental conditions outside hibernacula during winter probably have a strong influence on bat survival (Ransome 1990), because such conditions govern the range of survival options bats have when disturbed from hibernation. For example, a common indication that WNS is affecting a hibernaculum is the flight of affected bats outside during the daytime (Blehert *et al.* 2009). Whether bats leaving affected hibernacula are escaping something inside, such as fungal infestation of the site, or seeking more favourable conditions outside remains to be determined. Underground conditions within bat hibernacula also correspond to certain surface conditions, such as temperature (Humphries, Thomas & Speakman 2002). In general, winter conditions outside hibernacula in the Appalachian Region where WNS mortality has been observed thus far are generally harsher than winter conditions in areas farther south and west where *G. destructans* has been detected in bats, but without associated mortality in the first years of detection (e.g. western Tennessee, Missouri and Oklahoma). Furthermore, *G. destructans* has been observed on several species of bats across Europe, but without associated mortality (Martínková *et al.* 2010; Puechmaille *et al.* 2010; Wibbelt *et al.* 2010). Although the European fungus may be less virulent or European bats may have evolved immunity to *G. destructans* (Wibbelt *et al.* 2010; Puechmaille *et al.* 2011a), another hypothesis for the disparity in mortality between North America and Europe is differences in winter conditions outside hibernacula. Certain environmental conditions outside hibernacula (e.g. sustained subfreezing

temperatures) might prevent bats from surviving infection by *G. destructans* and thus be an important co-factor in the virulence of WNS.

Species–environmental matching (SEM) models statistically relate species presence and/or absence locations to environmental predictors (Phillips, Anderson & Schapire 2006). In the case of WNS, models using presence-only data may be more appropriate than models using presence and absence data (e.g. logistic regression), because it is currently impossible to determine true 'absence' [i.e. difficulty of detecting fungus in the environment (Lindner *et al.* 2011) or differentiating suitable from unsuitable habitat]. Furthermore, fungal infection by *G. destructans* may not always cause WNS and mortality, as seems to be the case in Europe. Presence-only SEM modelling was recently used to map the environmental suitability for infection with the pathogen *Batrachochytrium dendrobatidis*, which causes chytridiomycosis disease in multiple species of amphibians (Murray *et al.* 2011). Building upon the concept of using SEM models to investigate disease processes in wildlife, we modelled and mapped potentially suitable habitat for mortality associated with infection by an organism, rather than the traditional approach of modelling the distribution of the organism itself.

In this study, we investigated relationships between environmental surface conditions and landscape-scale patterns of WNS mortality. Our objective was to test the hypothesis that environmental conditions might restrict the distribution of WNS mortality relative to the overall pattern of infection by *G. destructans*.

Materials and methods

OCCURRENCE RECORDS AND ENVIRONMENTAL LAYERS

We obtained geographic coordinates from the US Fish and Wildlife Service for all known records of WNS-infected hibernacula, as of 1 May 2010, comprising 81 WNS-positive records and 31 WNS negative (Table 1). WNS-positive records were sites where bats had been diagnosed with disease by histopathology (*sensu* Meteyer *et al.* 2009) or mortality, and clinical signs of disease were observed. WNS-negative records were sites where no WNS diagnosis or clinical signs and mortality had been recorded. Although the environmental conditions inside bat hibernacula undoubtedly influence the way *G. destructans* is transmitted and affects bats, the lack of existing information on such underground conditions, coupled with the urgency of the situation, warrants a focus on modelling how survival of hibernating bats exposed to *G. destructans* relates to surface conditions. We gathered a set of relevant bioclimatic and environmental variables relating to surface conditions originating from 61 available Geographic Information System (GIS) layers at different spatial resolutions. These included 24 bioclimatic variables (climate data from Daymet; http://www.daymet.org/), 28 MODIS (MODerate resolution Imaging Spectroradiometer) phenology layers (Tan *et al.* 2008), and nine topographic, land use/land cover type and other environmental variables (Table S1, Supporting information), all of which spanned the north-eastern US where *G. destructans* was known to occur at the time of analysis. These variables were considered as potential factors that might represent the environmental conditions associated with WNS mortality and were chosen based on previous spatial modelling studies on other pathogens (Reed *et al.* 2008; Puschendorf *et al.* 2009; Murray *et al.* 2011). All of these variables were resampled (using 'Aggregate' function in GIS) to 1-km spatial resolution to match with the bioclimatic layers. Mean values for MODIS phenology layers (250-m original resolution) and mean and standard deviation for 'Elevation' and 'Slope' variables (30-m original resolution) were calculated to capture heterogeneity in vegetation phenology and topography within 1-km cells.

SPATIAL MODELLING AND STATISTICAL ANALYSES

We believe the SEM approach for investigating mortality associated with WNS is justified for two reasons. First, because *G. destructans* is known to grow under conditions of high humidity and temperature between about 3 and 15 °C (Gargas *et al.* 2009; Chaturvedi *et al.* 2010), which is generally wider than the range of temperatures used by hibernating bats (Webb, Speakman & Racey 1996; Humphries, Thomas & Speakman 2002). Although hibernacula conditions or bat behaviour (e.g. grooming) are likely to lead to variation in the ability of the fungus to infect bats, there is not yet evidence suggesting that *G. destructans* will be limited by conditions within bat hibernacula. Secondly, it is possible that *G. destructans* may already be present in hibernacula across North America and has gone undetected. By modelling environmental conditions associated with disease occurrences, we believe that SEM modelling has the potential to help inform understanding of cryptic disease processes.

Maximum entropy modelling or Maxent (Phillips, Anderson & Schapire 2006) shows great promise for predicting ecological niches of species (Elith *et al.* 2006; Ortega-Huerta & Peterson 2008) and has been proven effective even on data sets with small sample sizes (Hernandez *et al.* 2006; Pearson *et al.* 2007; Wisz *et al.* 2008; Benito *et al.* 2009; Kumar & Stohlgren 2009). Maxent is a machine learning algorithm that requires presence records only and uses random background points to sample environmental conditions in the study area. The model is probabilistic and calculates the probability of presence: 0 being the lowest and 1 the highest. By automatically including interactions among predictor variables, the model is nonparametric and can account for nonlinearities. Maxent ranked as the best-performing model algorithm in a recent comparison of 16 different modelling methods encompassing data on birds, terrestrial plants, bats and reptiles (Elith *et al.* 2006; Kumar *et al.* 2009). We used Maxent (version 3.3.3.e; Phillips, Anderson & Schapire 2006; http://www.cs.princeton.edu/~schapire/maxent/) as our SEM model of choice for studying the patterns of mortality associated with WNS. Duplicate WNS presence records (one of 81) were removed using Maxent's 'remove duplicate' function. We trained the model for a smaller extent (Training Extent) that covered WNS-positive and WNS-negative locations (Fig. S1, Supporting information). This Training Extent was estimated by drawing a minimum convex polygon around the WNS-positive and WNS-negative locations (Fig. 1). The model trained for the Training Extent was projected to the whole study area (Fig. 1). This was done to limit the model training region to the area that was actually sampled for WNS presence and for drawing random background points or pseudo-absences (Phillips & Dudik 2008; Phillips *et al.* 2009; VanDerWal *et al.* 2009). We ran the model with 100 replicate runs and used 70% of the data for training and 30% of the data for testing in each run. Predictions from 100 models were averaged to produce final maps of probability of presence of WNS mortality. Geographic pattern in uncertainty of the predictions was mapped by calculating the coefficient of variation of projected probability of occurrence among 100 replicate runs (Fig. S2, Supporting information).

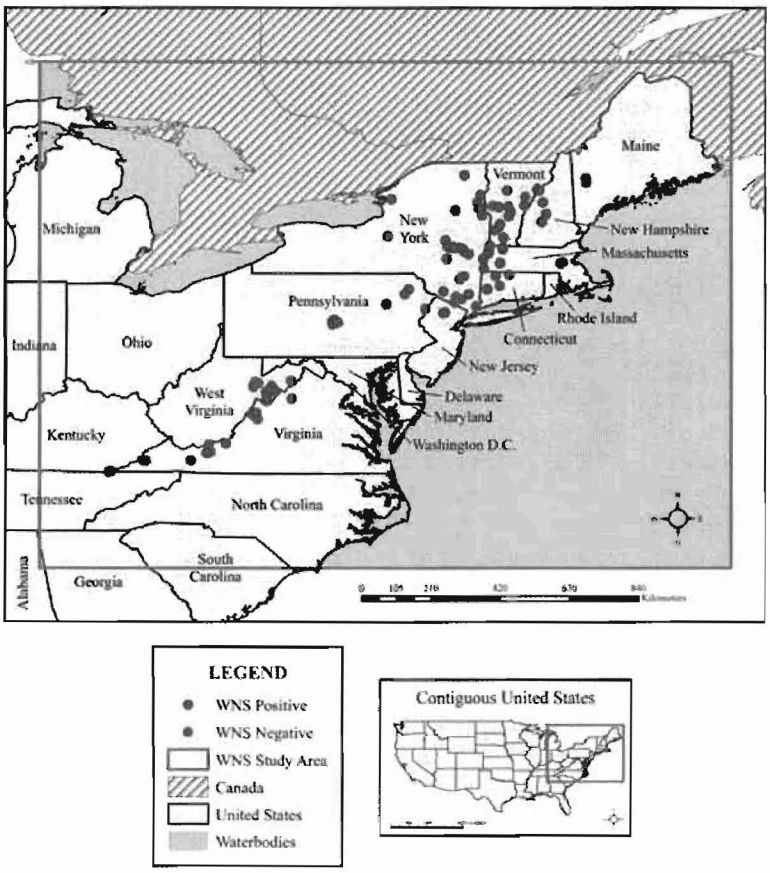

Fig. 1. Study site map and location of positive (presence) and negative (absence) points for sites with bat white-nose syndrome (as of May 2010). White-nose syndrome (WNS)-positive points represent locations where bats had been diagnosed with disease by histopathology, or mortality and clinical signs of disease were observed. WNS-negative points are locations where no WNS diagnosis or clinical signs and mortality had been recorded.

The accuracy of the model was assessed using the area under the receiver operating characteristic (ROC) curve (AUC), which is a threshold-independent measure of model discrimination ability and varies from 0 to 1 (Fielding & Bell 1997). An AUC value of 0·5 or less represents a model with predictions no better than random, and values close to 1 represent high discrimination. To assess the relative importance of environmental variables, we used two procedures included in Maxent: per cent variable contributions and jackknife estimation (see details in Phillips, Anderson & Schapire 2006). We also examined the response curves showing the relationships between different environmental predictor variables and the probability of WNS presence. We examined the predictors for cross-correlations by calculating Pearson correlation coefficients (r). We removed 27 highly cross-correlated variables ($r \geq 0{\cdot}9$) and included only one predictor in the model from a set of highly cross-correlated variables (Table S2, Supporting information). The decision to include a variable from a set of highly cross-correlated variables was made based on its potential biological relevance to the presence of WNS. The initial model was run with the remaining 34 variables, but the final 'pruned' model included only 15 predictors; 19 variables were dropped on the basis of their lower predictive power [i.e. lower percentage contribution ($< 1{\cdot}0$) and lower regularized training gain] in the initial full Maxent model (Table 2 and Table S1, Supporting information).

In November 2011, we retested our model predictions based on county-level data showing the subsequent occurrence of WNS reported by the US Fish and Wildlife Service (http://www.fws.gov/whitenosesyndrome/maps/WNSMap_10-03-11_300dpi.jpg) on 3 October 2011. No occurrence data from these newly affected counties were used in the model calibration. We overlaid 24 counties where WNS was reported after our initial analysis and calculated the maximum probability of WNS presence predicted by the Maxent model for any 1-km cell within a county; the mean of predicted probability for all 1-km cells within a county was also calculated.

Results

Maxent yielded a highly predictive model with an average test AUC value of 0·85 (±0·03; Fig. 2) and contained 15 environmental variables. This model predicted highly suitable environmental conditions for WNS in parts of New York, Vermont, south-western North Carolina and northern Georgia (Fig. 2). The jackknife procedure showed that land use/land cover type, frequency of precipitation (proportion of days that had any precipitation) and annual temperature range at the site of WNS-positive hibernacula had the most predictive power (i.e. higher training gains and test AUC values; Fig. 3a,b). Land use/land cover type, mean temperature of wettest quarter and

Table 1. Number of bat hibernacula classified as positive or negative for bat white-nose syndrome (WNS), by state, which were used as presence locations to model the habitat associations of WNS mortality

State	WNS positive	WNS negative
Connecticut	3	2
Maine	N/A	2
Massachusetts	7	1
New Hampshire	5	0
New Jersey	3	N/A
New York	34	3
Pennsylvania	8	N/A
Vermont	12	2
Virginia	5	8
West Virginia	4	13
Total	81	31

elevation (standard deviation) also highly influenced the final model, with 17%, 15% and 13% contributions, respectively (Table 2). WNS probability of occurrence was higher for barren lands (areas with < 15% vegetation cover). Other influential predictors, in decreasing order of importance, were as follows: frequency of precipitation (12·2%), annual temperature range (8·5%) and precipitation of wettest month (8·1%; Table 2).

Individual response curves describing the relationships between probability of WNS presence and the top predictor variables are shown in Fig. 4. The response curve for elevation (standard deviation) showed an increasing probability of WNS presence with increasing elevational heterogeneity (Fig. 4a). The highest probability of WNS presence was observed when the standard deviation in elevation (within 1-km^2 cell) was around 210 m, and the lowest probability was when there was lower variation in elevation within a 1-km^2 cell (Fig. 4a). The response curve for frequency of precipitation (i.e. proportion of days in a year that had any precipitation) showed peak probability of WNS presence at about 0·30 (precipitation in 30% of days per year) (Fig. 4b). The probability of WNS presence was highest when precipitation during the wettest month was < 100 cm and declined rapidly at higher amounts (Fig. 4c). Probability of WNS presence was highest when the mean temperature of the wettest quarter was between −2 and 17 °C (Fig. 4d) and when mean annual temperature ranged between 38 and 40 °C (Fig. 4e). The test of model predictions based on county-level WNS occurrence data in November 2011 showed excellent performance of the model with 71% of the confirmed counties (17 of 24) having at least one cell that had probability more than 0·50 (Fig. S3 and associated Table S3, Supporting information).

Discussion

CAVEATS

Species–environmental matching models present a first approximation of habitat suitability of an organism or, in the case of WNS, mortality associated with a disease-causing organism. The model appears to be more transferable in areas close to the Training Extent and less transferable in distant areas. Uncertainty in model predictions was higher in southern North Carolina and north-eastern South Carolina (Fig. S2, Supporting information), which could be due to (i) the variation observed in the predictions among replicates based on the

Table 2. Estimates of relative contributions of the environmental variables to the final Maxent model; values shown are averages (±SD) over 100 replicate runs. See Table S1 (Supporting information) for details about the environmental variables (e.g. native spatial resolution and sources of data)

Variable	Percentage contribution	Permutation importance
Land use/land cover types	17·1 (3·7)	11·1 (2·4)
Mean temperature of wettest quarter (°C; Bio8)	15·4 (2·5)	10·2 (3·0)
Elevation (standard deviation)	12·6 (4·0)	10·8 (4·6)
Frequency of precipitation (number of wet days/total days in a year)	12·2 (2·7)	14·7 (4·6)
Annual temperature range (°C; Bio7)	8·5 (3·8)	25·5 (6·7)
Precipitation of wettest month (cm; Bio13)	8·1 (4·0)	1·1 (1·5)
Precipitation of driest month (cm; Bio14)	6·9 (2·4)	7·4 (2·5)
Northness (cosine of aspect)*	5·3 (2·3)	2·9 (1·8)
Eastness (sine of aspect)*	3·2 (1·2)	2·8 (1·4)
Compound topographic index	2·9 (1·9)	1·3 (0·9)
Distance to water	2·2 (1·0)	3·5 (1·6)
Enhanced Vegetation Index (EVI) seasonal amplitude (difference between the maximal value and the base level; Tan *et al.* 2008)	1·8 (1·3)	2·5 (1·8)
Normalized Difference Vegetation Index (NDVI) base level (average of the left and right minimum values; Tan *et al.* 2008)	1·4 (1·1)	0·3 (0·4)
Temperature seasonality (Bio4)	1·3 (0·7)	4·0 (2·2)
Mean temperature of driest quarter (°C; Bio9)	1·1 (0·9)	1·9 (1·7)

*We transformed circular variable aspect (0–360°) into a linear north-south gradient (northness) and an east-west gradient (eastness) by performing cosine and sine transformations, respectively. Northness ranges from −1 (south-facing) to 1 (north-facing), and Eastness from −1 (west-facing) to 1 (east-facing) (Gutierrez *et al.* 2005; Kumar, Stohlgren & Chong 2006).

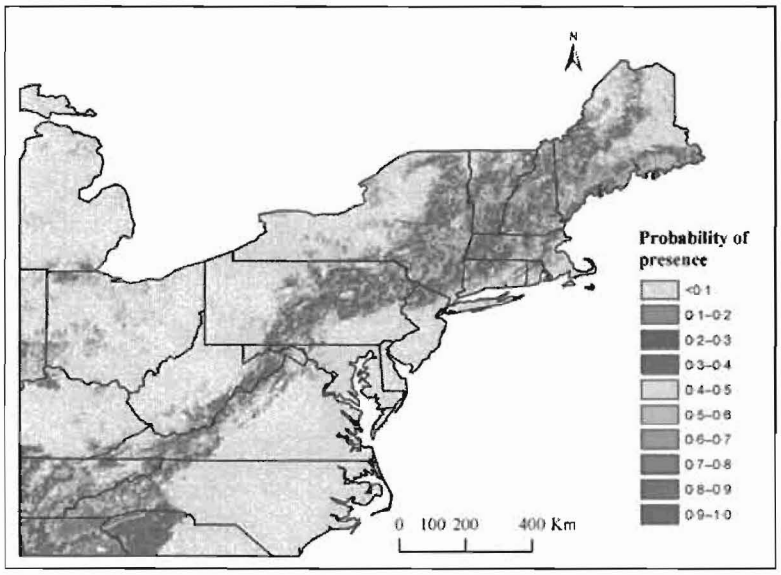

Fig. 2. Predicted probability of presence of bat white-nose syndrome (WNS) associated mortality [average test AUC = 0·85 (±0·03); average training AUC = 0·96 (±0·01)] in north-eastern United States. Predictions are averages over 100 replicate runs. Highly suitable areas for WNS presence are predicted in areas that are higher in elevation and topographically heterogeneous, drier and colder during winter, and more seasonally variable than surrounding habitats.

random splitting of the data; (ii) the effects of model extrapolations far beyond the Training Extent (Stohlgren *et al.* 2011) (Fig. S1, Supporting information). The recent emergence and rapid spread of WNS suggest that the disease has not reached equilibrium in populations of hibernating bats of North America, so an iterative approach to modelling and disease surveillance seems warranted. The locations of bat hibernacula are often unknown, and there are undoubtedly WNS-positive sites in the affected region that have not been reported. Mortality and clinical signs of disease may also sometimes go unnoticed at affected hibernacula. Other types of error and bias effects in the study include the following: (i) GPS instrument error or measurement inaccuracies and some 'fuzzed' locations for privacy purposes (assumed to be minor with a 1-km^2 modelling resolution); (ii) misreporting or failure to report infected and non-infected sites (assumed to be minor based on reputable sources); and (iii) resolution of predictor variables. Despite these issues, the predictive power of the Maxent results suggest that, given the available data, we have a good first approximation of environmental conditions outside bat hibernacula that may be associated with WNS mortality. However, these results do not account for differences in disease among species or pathogen variants, and caution should be taken in extrapolating them to other geographic regions or future conditions. In particular, resource management decisions, such as where to institute universal precautions to limit disease spread or focus conservation actions, should not be made based on our results until they are validated.

In addition to caveats associated with the modelling approach we took, we only investigated one of the several hypotheses for explaining differences in mortality observed as WNS spreads. As discussed above, other explanations for variation in mortality include differences among bats (hosts), differences in the fungus (pathogen) and/or differences in the way that the pathogen spreads among hosts and geographic regions or environmental conditions.

ENVIRONMENTAL FACTORS INTERPRETED

Our results showed that fifteen of the 61 environmental factors considered in the Maxent model were strongly associated with observed patterns in WNS mortality (Table 2), thus lending support to the hypothesis that environmental conditions might restrict the distribution of WNS mortality relative to the overall pattern of infection by *G. destructans*. Land use and land cover type was one of the top predictors of WNS mortality and showed that higher WNS probability of occurrence was associated with 'barren land (rock/sand/clay; area with < 15% of vegetation cover)'; this finding may have more to do with caves and abandoned mines often occurring in this land cover type than being a biologically important predictor of the disease process. Our analysis indicated that WNS mortality is strongly influenced by mean temperature of the wettest quarter year (Table 2), as well as a combination of frequency of precipitation, annual temperature range, elevational heterogeneity and temperature seasonality. In general, model results suggest that the probability of WNS mortality is greatest at sites that are higher in elevation and topographically heterogeneous, drier and colder during winter, and more seasonally variable than surrounding habitats. The fungus *G. destructans* apparently does its greatest damage to bats while they are hibernating and bats in colder regions must hibernate for longer periods (Humphries, Thomas & Speakman 2002). Conditions outside hibernacula may play an important role in surviving exposure

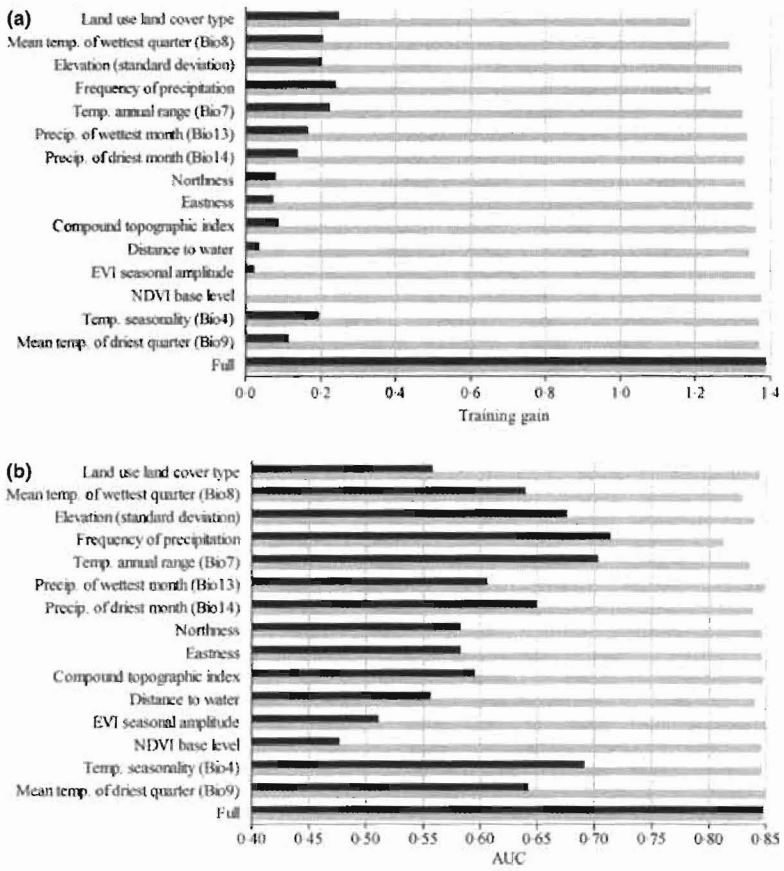

Fig. 3. Environmental variable contributions to (a) training gain and (b) AUC of the final model for white-nose syndrome (WNS). The *x*-axis represents a measure of model predictive ability using 'training gain' and 'AUC'. Dark black bars indicate how well a model performs using only that variable compared to 'full' model, and light grey bars indicate how well a model performs excluding that variable. Values shown are average over 100 replicate runs.

to *G. destructans*, because insects upon which bats rely for food are not active in extreme cold and bats expend more energy staying warm when active and flying at colder temperatures. Furthermore, evidence suggests that bats infected with *G. destructans* during hibernation suffer from dehydration (Cryan *et al.* 2010; Willis *et al.* 2011), so that drier conditions outside hibernacula and a lack of unfrozen water for drinking may enhance the detrimental effects of disease. When outside conditions are less harsh during winter, bats infected by *G. destructans* may have more opportunities to escape from the now-vulnerable state of hibernation to feed and/or drink. On the basis of our modelling results, we hypothesize that there is a threshold of winter length and severity outside bat hibernacula, below which bats might be more likely to survive fungal infection through the hibernation season.

CAUTIOUS CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Maxent modelling may provide a good first approximation of the environmental factors influencing mortality associated with WNS in north-eastern North America. This is the first attempt to do so, and an iterative approach to data collection and modelling is strongly suggested. Unfortunately, the epizootic of WNS is in an early stage. Until the full extent of the epizootic is realized, SEM models may be unstable in space and time. Model results indicate that, in the presence of *G. destructans*, WNS mortality is probable across a large portion of north-eastern North America. Although this result may seem irrelevant considering the widespread mass mortality already being observed in the region, the model results offer hope that future spread and impacts of the disease can be predicted with greater precision. For example, at the time of writing (March 2012), extensive WNS mortality has not yet been noted outside the areas of high probability of occurrence predicted by our model. During the winter of 2010/2011, WNS mortality was observed at new sites as far west as Indiana and western Tennessee and Kentucky (USGS 2011a,b), yet mortality was not reported at sites in Oklahoma and Missouri where *G. destructans* had been detected in *M. velifer* and *M. grisescens*, respectively, during the winter of 2009/2010. Despite increased surveillance, reports of mass mortality were generally not as widespread in south-eastern states during winter of 2010/2011 as they had been during prior winters in north-eastern areas (http://www.fws.gov/whitenosesyndrome/). Winter conditions

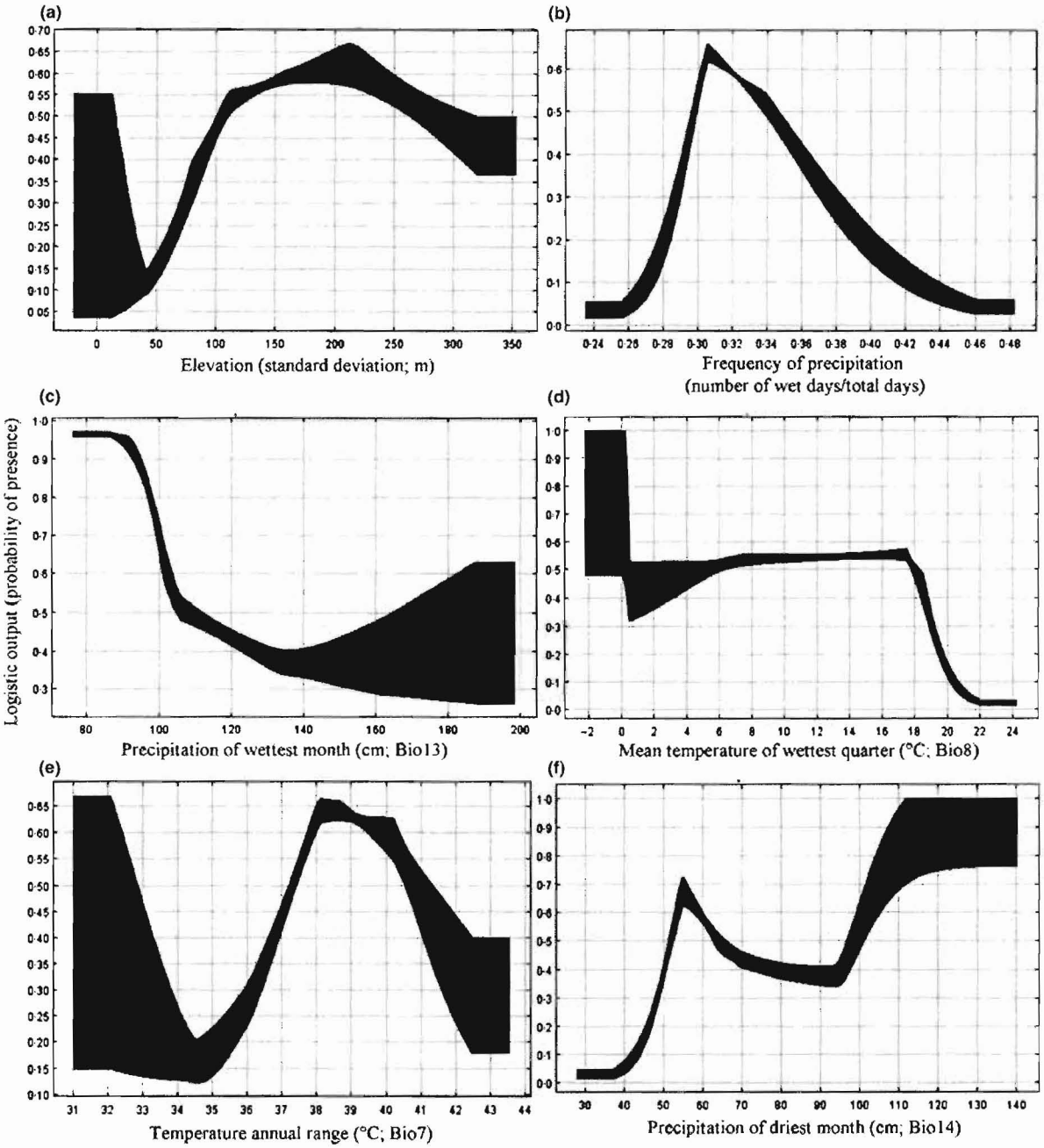

Fig. 4. Relationships between top environmental predictors and the probability of occurrence of white-nose syndrome mortality in bats; (a) elevation (standard deviation, m), (b) frequency of precipitation, (c) precipitation of wettest month (cm), (d) mean temperature of wettest quarter (°C), (e) temperature annual range (°C), and (f) precipitation of driest month (cm). Each of the following curves represents a different Maxent model created using only the corresponding variable. Blue margins are ± 1 SD calculated over 100 replicates.

throughout south-eastern North America during 2010/2011 were strongly influenced by the La Niña Southern Oscillation climate pattern and were generally warmer and drier than average (http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/lanina/us_impacts/ustp_impacts.shtml). Combined with our modelling results, which suggest colder and drier conditions are associated with WNS mortality, the apparent slowing spread of WNS in southern regions during the winter of 2010/2011 may be partially attributable to climatic variation. It is possible that levels of mortality comparable with those observed since the emergence of WNS in north-eastern North America may be seen in more southern regions when winter climate conditions return to more typical patterns. On the other hand, environmental conditions outside

hibernacula in new regions where the fungus occurs might be such that bats are able to stave off disease and mortality. If the latter scenario unfolds, one interpretation might be that *G. destructans* is only capable of killing bats and causing disease if certain environmental conditions co-occur with the fungus. In such a case, a SEM modelling approach, combined with field surveillance and ground-truthing, could be used to test this hypothesis and, if not disproven, determine the environmental conditions associated with mortality. Understanding the environmental conditions associated with WNS mortality will be important for managing the disease, because management and conservation actions (e.g. institution of universal precautions) can then be prioritized to focus on areas where disease mortality is most likely to impact bat populations. Maps produced in this study can be used in the selection of priority monitoring sites for conservation. Furthermore, if there are environmental conditions under which bats infected by *G. destructans* can survive the disease as our model suggests, clearly defining such conditions will help us judge whether risky management actions (e.g. treating infected bats and their environments with pharmaceutical compounds) are likely to do more harm than good. Our approach provides a powerful new tool for understanding and tracking the continent-wide effects of WNS on populations of hibernating bats in North America. In addition, a similar approach might be useful for determining whether the presence of *G. destructans* without associated mortality on several species of bats in Europe is attributable to differing environmental conditions or some other factor, such as natural immunity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. (a) The Training Extent defined using minimum convex polygon around the WNS positive and negative sites, and (b) Maxent model predictions for the Training Extent.

Fig. S2. Geographic patterns in the uncertainty of model projections of presence of bat white-nose syndrome, expressed as the coefficient of variation (CV) of projected probability of occurrence among 100 replicate runs.

Fig. S3. Test of model predictions based on county level data showing the subsequent occurrence of WNS reported by the US Fish and Wildlife Service.

Table S1. Environmental variables considered in Maxent model for predicting mortality associated with bat white-nose syndrome.

Table S2. Cross-correlations among environmental predictor variables.

Table S3. Maximum probability of WNS presence predicted by the Maxent model for any 1 km cell within a newly affected county, as well as mean of predicted probability for all 1 km cells within each county.

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