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RESEARCH PAPER

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The influence of pesticide use on amphibian chytrid fungal infections varies with host life stage across broad spatial scales

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Abstract

Aim: Widespread pesticides might influence pathogen distributions across landscapes via effects on host–pathogen interactions. Empirical research supports two hypotheses regarding effects of pesticides on amphibians and the aquatic fungal pathogen *Batrachochytrium dendrobatidis* (Bd): (a) pesticides can be toxic to Bd, reducing infection risk of aquatic larval amphibians, and (b) exposure to pesticides early in amphibian development can increase disease risk after metamorphosis. The aim of this research is to evaluate whether these patterns from laboratory experiments are consistent across host species and occur in the field at broad spatial scales.

Location: Contiguous U.S.A.

Time period: 1998–2009.

Major taxa studied: Amphibian hosts and Bd.

Methods: Our data included 3,946 individuals evaluated for Bd infection across 49 amphibian species, which resulted in 155 estimates of Bd prevalence in populations. We used multimodel inference to examine associations between Bd infection prevalence in amphibian populations and (a) total pesticide use, (b) pesticide use by type (herbicide, insecticide or fungicide), and (c) the most commonly used pesticide compounds across life stages, controlling for several factors documented to affect the distribution of Bd.

Results: Consistent with laboratory findings, our results indicate that use of multiple herbicide compounds is most closely associated with low infection risk in the aquatic larval stage but high risk in the terrestrial post-metamorphic stage when controlling for insecticide and fungicide use. We caution against assuming that insecticides and fungicides do not influence Bd distributions, because use of herbicides, insecticides and fungicides are all correlated positively.

Main conclusions: The effects that pesticides can have on disease distributions are complex and should be considered strongly at broad scales and across host species, especially in environments in which use and exposure are widespread. Accurate predictions of disease distributions may lead to more effective management strategies to limit disease spread.

KEYWORDS

Batrachochytrium dendrobatidis, disease distributions, herbicides, host–pathogen interactions, pathogen, pesticides, wildlife disease

1 | INTRODUCTION

The emergence of infectious diseases threatens public health, global economies and wildlife populations (Binder, 1999; Daszak, Cunningham, & Hyatt, 2000; Morens, Folkers, & Fauci, 2004). Therefore, understanding factors that determine distributions of infectious diseases is crucial if we are to design effective management strategies to limit disease spread. Anthropogenic activities are predicted to be major determinants of infectious disease distributions (Daszak, Cunningham, & Hyatt, 2001; Jones et al., 2008). Although mounting evidence suggests that changes to climate and land use type can influence distributions of disease (Lafferty, 2009; Martin & Boruta, 2013; Rohr et al., 2011), the influence of chemical contaminants on disease distributions remains relatively undetermined (Lawler et al., 2006). For wildlife populations in freshwater ecosystems, chemical contaminants, including pesticides, are a widespread abiotic factor that might influence the distributions of disease occurrence by affecting host–pathogen interactions. Pesticides can have simultaneous positive and negative effects on parasite transmission; the net effect of these factors determines the influence of pesticides on disease risk in wildlife populations (Rohr, Raffel, Sessions, & Hudson, 2008). For instance, pesticides can increase the incidence of pathogen infections (Christin et al., 2003; Pettis, vanEngelsdorp, Johnson, & Dively, 2012) via the disruption of host immune systems (Blakley, Brousseau, Fournier, & Voccia, 1999; Rohr, Schotthoefer, et al., 2008). Alternatively, exposure to pesticides can also decrease pathogen viability via direct negative effects of pesticides on pathogen survival and reproduction (Lafferty & Kuris, 1999; Morley, Irwin, & Lewis, 2003), pointing to the complex nature of the effects of pesticides on host–pathogen interactions.

For hosts with complex life cycles, host life stage could determine the net effects of pesticides on disease risk if the relative balance between the effects of pesticides on host susceptibility and pathogen viability changes throughout the development of the host. If the net effect of pesticides changes with host life stage, we might expect pesticides to be negatively associated with infections for stages in which the negative effects of pesticides are greater on pathogen viability compared with host immunity. Alternatively, we might expect a positive association between pesticides and infection prevalence for host stages in which the negative impact of pesticides is greater on host immunity compared with pathogen viability.

Understanding the influence of pesticides on disease dynamics in amphibian populations at broad spatial scales is markedly important because amphibians are facing global declines that are caused, in part, by a fungal pathogen, *Batrachochytrium dendrobatidis* (Bd) that causes the disease chytridiomycosis. *Batrachochytrium dendrobatidis* has been linked to population declines, mass mortality and species extinctions in hosts (Lips et al., 2006; Skerratt et al., 2007), and its effects on hosts can be altered by environmental conditions, including pesticide exposure, that may influence pathogen viability or host immune response (Gaietto, Rumschlag, & Boone, 2014; Wise, Rumschlag, & Boone, 2014). Although the presence of Bd in North America dates back to 1888 (Talley, Muletz, Vredenburg, Fleischer, & Lips, 2015), we lack an understanding of the variation in host susceptibility across environmental gradients and the role that pesticides might play in mediating the occurrence of Bd.

Experimental evidence suggests that pesticide exposure during critical developmental windows in early life can have effects on the immune system in adult stages (Rohr & Palmer, 2005, 2013 ; Rohr, Sager, Sesterhenn, & Palmer, 2006) and that there are differential effects of pesticide exposure on amphibian–Bd interactions over aquatic larval and terrestrial post-metamorphic life stages. For instance, in the aquatic larval life stage of amphibians, pesticides can have direct negative effects on Bd, which might result in an overall decrease in the risk of infection for aquatic larvae. In fact, Bd growth on infected tadpoles can be reduced by pesticide exposure (McMahon, Romansic, & Rohr, 2013) and can even result in clearance of Bd from the host (Hanlon, Kerby, & Parris, 2012). These negative effects of pesticides on Bd are likely to be driven by reduced Bd growth and production of Bd zoospores, the aquatic infective stage of the pathogen (Hanlon et al., 2012; McMahon et al., 2013). *Batrachochytrium dendrobatidis* infects keratinized cells in amphibians, which occur only in the mouthparts of tadpoles (Voyles, Rosenblum, & Berger, 2011), suggesting that susceptibility to Bd infection is low in this early-life stage. As tadpoles metamorphose into the terrestrial life stage, the incidence of keratinized cells increases as the epidermis develops, and Bd infection can move from the mouthparts of the tadpole to the entire surface of the body (McMahon & Rohr, 2015), suggesting that susceptibility to infection and disease development increases in the terrestrial host life stage (Rachowicz & Vredenburg, 2004). In the terrestrial post-metamorphic life stage, pesticide exposure during early life is associated with increased Bd-induced mortality, which may be driven by disruption of the immune system. For example, early-life pesticide exposures can lead to increased Bd-induced mortality of terrestrial hosts, which is caused by reduced tolerance to infection; this finding points to a cost of pesticide exposure that could be induced by disruption of the immune system (Rohr et al., 2013). Although these experimental studies support differential effects of pesticide exposure on amphibian–Bd interactions over aquatic larval and terrestrial post-metamorphic life stages, we do not know whether these laboratory patterns are consistent across amphibian species and occur in natural populations at broad spatial scales.

The objective of the present study was to evaluate associations between pesticide use and the prevalence of Bd infection in amphibian populations across host life stages in the U.S.A. We used publically available data, including 155 field observations of Bd infection prevalence and corresponding estimates of pesticide use at the county level. We used multimodel inference approaches to test for associations of (a) total pesticide use, (b) pesticide use by type (herbicide use, insecticide use, fungicide use), and (c) the most commonly used pesticide compounds within type and Bd infection prevalence in amphibian populations across life stages, controlling for the influence of environmental (vegetation, precipitation, temperature) and biotic (host family) factors. Based on the experimental evidence reviewed previously concerning persistent and life stage-dependent effects of pesticides

on Bd infection risk in amphibians, we predicted that in the aquatic larval stage of amphibians, Bd prevalence would be negatively associated with pesticide use, and in the terrestrial post-metamorphic stage, Bd prevalence would be positively associated with pesticide use.

2 | METHODS

2.1 | Response and predictor variables

We obtained a spatially explicit dataset of amphibian populations surveyed for Bd infection from Bd Maps (www.bd-maps.net) in 2013. *Batrachochytrium dendrobatidis* survey sites were included in our analyses if five or more individuals were surveyed at a given site between 1992 and 2012, life-stage information of amphibians was provided, and survey sites were located in the contiguous U.S.A. The resulting dataset comprised 3,946 individuals evaluated for Bd infection, across 49 species, which resulted in 155 observations of Bd infection prevalence at the county level in 78 counties (Figure 1). As pesticide use, our predictor variable of interest, is given at the county level (described below), we coarse grained Bd prevalence to represent unique infection prevalence measures of species, life stages, and survey years at the county level. Bd infection prevalence, which served as the response variable in all of our statistical models, was arcsine–square-root transformed for each analysis.

To provide a conservative estimate of pesticide use, we used U.S.A. county-level low pesticide use (as opposed to high) estimates from 1992 to 2012 obtained from the Estimated Annual Agricultural Pesticide Use dataset provided by the Pesticide National Synthesis Project of the National Water-Quality Assessment Program (U.S. Geological Survey) (https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/). Preliminary analyses showed the effects of high pesticide use estimates were indistinguishable from low-use estimates (data not shown). We classified the pesticide compounds as herbicide, insecticide, or fungicide using the primary use type classifications provided by Pesticide Action Network Pesticide Database (https://www.pesticideinfo.org/). We included plant growth regulators and defoliants as herbicides and insect growth regulators as

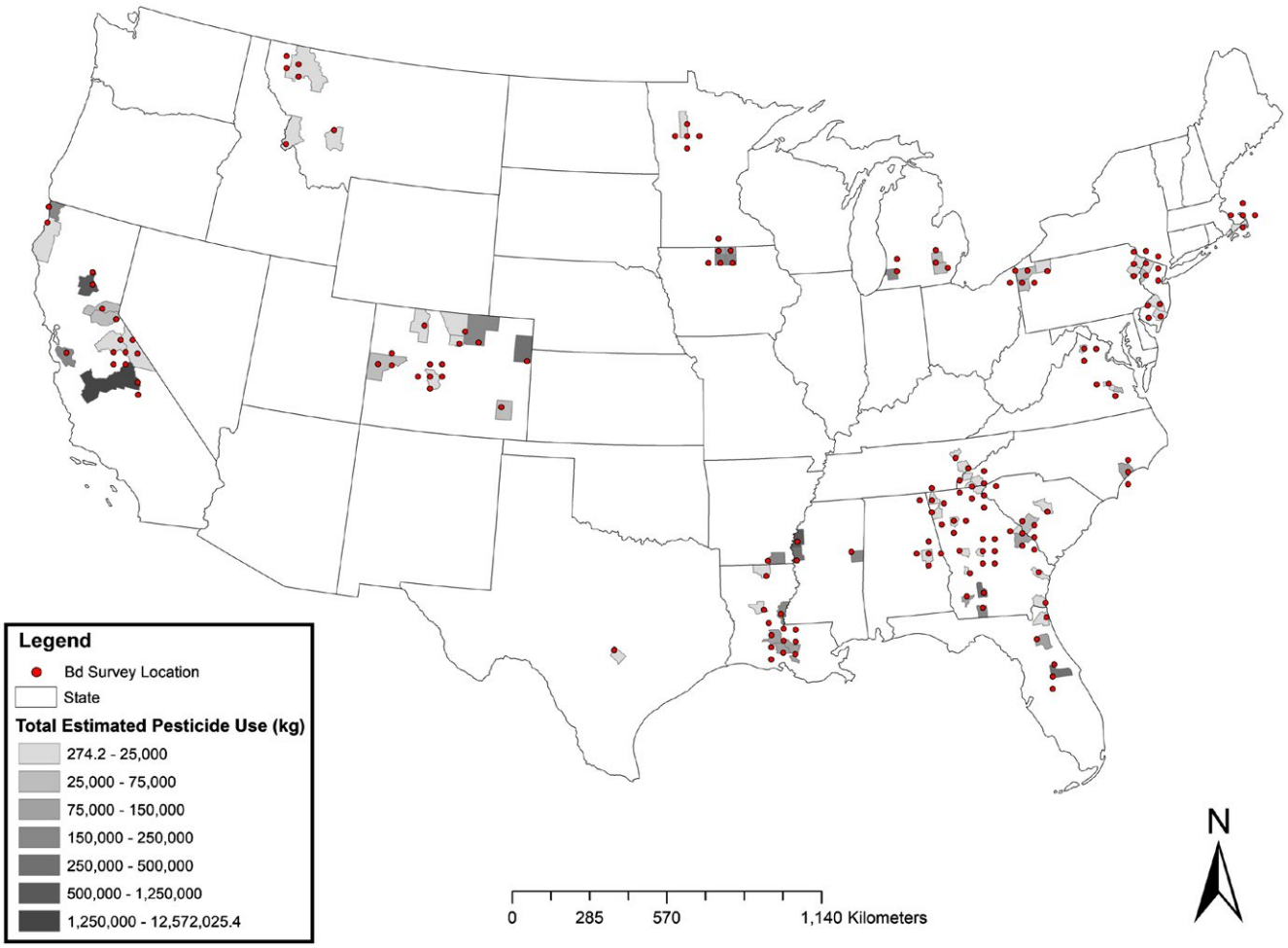

FIGURE 1 Map depicting *Batrachochytrium dendrobatidis* survey locations in amphibian populations and estimated total pesticide use at the county level in the contiguous U.S.A. For ease of visualization for locations in which surveys have been conducted over multiple years, survey locations have been arranged to prevent overlap of points, and total pesticide use within counties has been averaged across years. Map projection is Albers equal area conic [Colour figure can be viewed at wileyonlinelibrary.com]

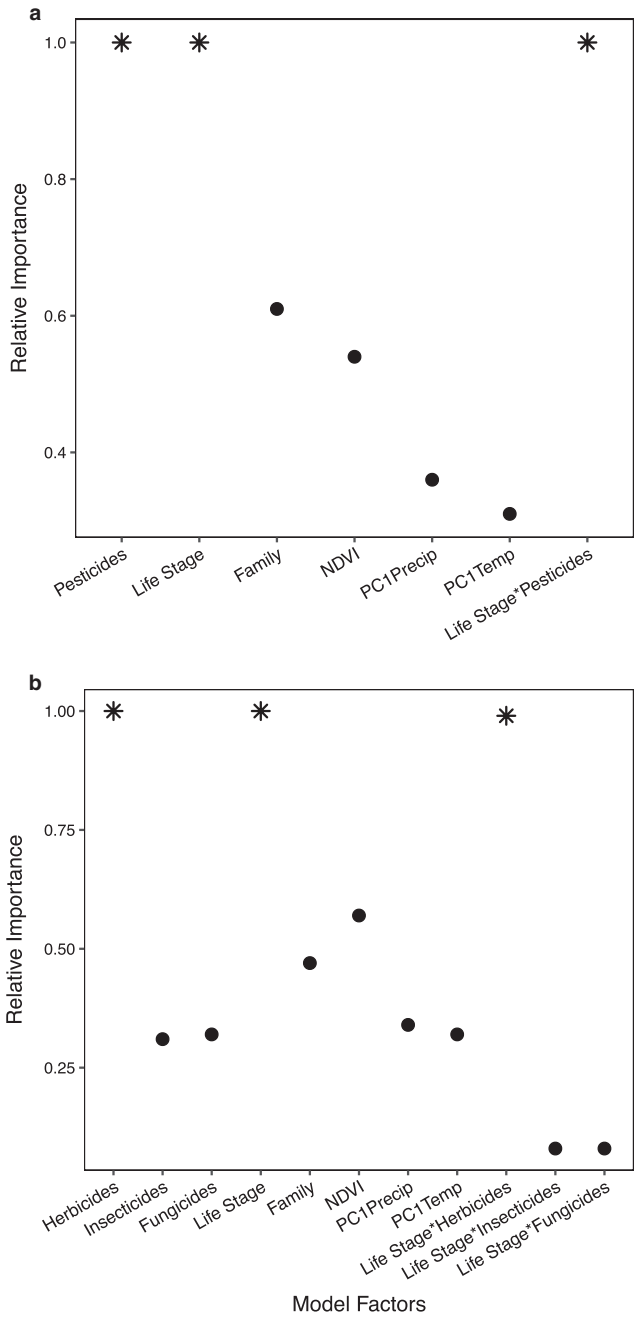

FIGURE 2 Relative importance of factors included in model comparisons evaluating the influence of (a) total pesticide use, and (b) herbicide, insecticide and fungicide use, and model covariates [family, normalized difference vegetation index (NDVI), precipitation (PC1Precip.) and temperature (PC1Temp.)] on *Batrachochytrium dendrobatidis* infection prevalence across amphibian host life stages in the contiguous U.S.A. Asterisks indicate significance of the factor ($p < 0.05$) from model averaging

insecticides. We excluded mineral and biologic fungicides (e.g., bacteria) because we were interested in non-target effects of synthetic fungicides on host responses to Bd that might influence infection prevalence. For a given site in a given county, we summed low-use estimates across pesticide types at the county level. In statistical models, pesticide usages were transformed using the natural logarithm.

To estimate local vegetative habitat, we used annual interpolated normalized difference vegetation index (NDVI) values that describe canopy photosynthetic activity across the entire growing season with respect to a given location for each year in which Bd surveys were conducted. Thus, the defined growing season was based on the phenology of vegetative growth for a given location. The NDVI data were taken from the Advanced Very High Resolution Radiometer Remote Sensing Phenology collection made available by the U.S. Geological Survey (https://earthexplorer.usgs.gov/). Data for the following abiotic factors were downloaded from WorldClim (https://www.worldclim.org/): 30-year means (1960–1990) of annual total precipitation, precipitation of the wettest month, precipitation of the driest month, annual mean temperature, mean diurnal temperature range, maximum temperature of the warmest month and minimum temperature of the coldest month. The NDVI, precipitation and temperature measures were extracted at survey site locations using ArcMap 10.4. We coarse grained NDVI, precipitation, and temperature values to the county level by taking averages across sites when prevalence values of the same species, life stage, and survey years occurred in the same county. We reduced our three precipitation measures and four temperature measures into a single precipitation measure and a single temperature measure using principal components analyses by extracting the first axis for precipitation measures (99.3% of the total variation, hereafter PC1Precip.) and for temperature measures (88.1% of the total variation, hereafter PC1Temp.).

2.2 | Generalized least squares models

All analyses were conducted in R version 3.2.1 (R Core Team, 2015). Generalized least squares (GLS) multiple regression models were fitted using the GLS function ("nlme" package; Pinheiro, Bates, DebRoy, Sarker, & R Core Team, 2016) with full maximum likelihood fit and an exponential spatial correlation structure. Results of the analyses did not change if a Gaussian or spherical spatial error structure was used. In all models, observations were weighted based on the number of individuals surveyed within a county. We constructed three sets of models to evaluate the influence of (a) total pesticide use, (b) pesticide use by type (herbicide use, insecticide use, fungicide use), and (c) the most commonly used herbicide compounds on Bd infection prevalence across host life stages. Models for total pesticide use and pesticide use by type included the following covariates to control for the effects of biotic and environmental factors: host family, NDVI, PC1Precip., and PC1Temp. To simplify models of pesticide compounds, we included host family and NDVI as the only biotic and environmental covariates, because these covariates were relatively important in models of total pesticide use and pesticide use by type (relative importance score > 0.45; Figure 2). To determine whether the results were sensitive to the selection of a Gaussian error distribution and associated arcsine−square-root transformation, all analyses were duplicated using generalized linear multiple regression models (GLMs) that were fitted using the GLM function ("stats" package; R Core Team, 2015) with a binomial error distribution and a logit link function (Supporting Information Appendix S1). The results of these binomial

analyses that did not account for spatial autocorrelation did not substantially differ from those presented in the present manuscript (Supporting Information Tables S1.1 and S1.2, Figures S1.1–S1.3).

To evaluate the influence of total pesticide use across life stages of amphibian hosts on the prevalence of Bd infection, we constructed a model with predictor variables including total pesticide use, host life stage, the interaction of life stage and pesticides, and all covariates. To evaluate which type of pesticide drove the effects of total pesticide use on the prevalence of Bd infection across life stages, we constructed a model with predictor variables including herbicide use, insecticide use, fungicide use, host life stage, the two-way interactions of host life stage with herbicide, insecticide or fungicide use, and all covariates. When controlling for the effects of insecticide and fungicide use, herbicide use was the best predictor of Bd infection prevalence across host life stages. To determine which commonly used herbicide compounds drove the effects of herbicide use, we first gathered the use estimates for the top five most-used herbicide types in our dataset and constructed five models that included the following predictor variables: herbicide compound (glyphosate, atrazine, metolachlor-s, ethephon, or sodium chlorate), herbicide use minus the compound of focus, insecticide use, fungicide use, host life stage, the interaction of host life stage and herbicide compound of focus, host family, and NDVI. For estimates of use in counties in which a compound estimate was not given, we assumed no use of that compound for the given site.

2.3 | Multimodel inference and comparisons of goodness-of-fit

To avoid relying on a single model to draw conclusions about the importance of predictors on prevalence, we used multimodel inference ("MuMin" package; Barton, 2016), which fits models using all combinations of predictors and ranks models by second-order Akaike information criteria corrected for small sample sizes (AICc; dredge function) for models including total pesticide use and pesticide use by type. Candidate models for total pesticide use included the following predictor variables: total pesticide use, host life stage, the interaction of life stage and pesticides, and all covariates (host family, NDVI, PC1Precip. and PC1Temp.). Candidate models for pesticide use by type included the following predictor variables: herbicide use, insecticide use, fungicide use, host life stage, the two-way interactions of host life stage with herbicide, insecticide or fungicide use, and all covariates (host family, NDVI, PC1Precip. and PC1Temp.). The AICc, ΔAICc and Akaike weights for each candidate model were calculated. To compare the influence of model factors across all candidate models, Akaike weights for each factor were summed across models to determine relative importance scores (Burnham & Anderson, 2002). The *p*-values were calculated from full model-averaged parameter estimates with statistical shrinkage (Burnham & Anderson, 2002). This method of parameter estimation treats the absence of a predictor in a model as a coefficient equal to zero. The chances of type I error and upward bias that can be caused using only a single (or few) top model(s) are reduced (Burnham & Anderson, 2002). Nagelkerke pseudo-R^2 values were calculated to assess the variance explained of the top-performing models (with a ΔAICc equal to zero). To determine the relative amount of variance each predictor explained, in top-performing models of total pesticide use and pesticide use by type, we removed the predictor of interest and recalculated the Nagelkerke pseudo-R^2. Comparison of the pseudo-R^2 of the full model with the pseudo-R^2 of the model with the predictor of interest removed gives an estimate of the variance explained by the predictor of interest. To evaluate the effect size of pesticides by total or by type on infection prevalence, we present standardized model-averaged coefficients (Table 1) and provide partial regression plots from the top-performing models including total pesticide use and use by pesticide type.

To determine the relative contribution of each of the top five herbicide compounds to the patterns of total herbicide use on Bd prevalence across host life stages, we used a log-likelihood ratio test to compare the goodness-of-fit of two models: one that included the interaction between the focal herbicide compound and host life stage and one that did not. To evaluate the effect size of herbicide compounds on infection prevalence, we provide partial regression plots for each herbicide compound and the sum of the most used herbicide compounds across host life stages controlling for covariates.

3 | RESULTS

3.1 | The influence of total pesticides

Total pesticide use, life stage, family, and the interaction of pesticide use and life stage were associated significantly with the prevalence of Bd infection in amphibian populations when controlling for covariates (Table 1; Figure 2a). The relative importance scores for pesticide use, life stage, and the interaction of pesticide use and life stage were greater than all covariates, including host family, NDVI, precipitation, and temperature (Figure 2a). In the best-fitting model controlling for covariates, the impact of pesticides depended on life stage (Table 2; Figure 3a; Nagelkerke pseudo-R^2 = 0.36). The variance of the model was best explained by the interaction of life stage and pesticides [Nagelkerke pseudo-R^2 of the full model minus Nagelkerke pseudo-R^2 of the model with focal predictor (interaction of life stage and pesticides) removed = 0.14], followed by life stage (0.09), family (0.08), NDVI (0.03), and pesticides (0.00). For the aquatic larval life stage of the hosts, Bd infection prevalence decreased with increasing pesticide use, but for the terrestrial post-metamorphic life stage of hosts, the prevalence of Bd infection increased with pesticide use (Figure 3a). The effect of pesticides was significantly negative in the aquatic stage (Table 1; pesticides: *z*-value = 2.70, $p = 0.007$) and significantly positive in the terrestrial life stage (*z*-value = 3.86, $p < 0.001$).

3.2 | The influence of pesticide use by type

Although pesticide use by type was positively correlated (herbicide use vs. insecticide use: Pearson's correlation coefficient = 0.77, herbicide use vs. fungicide use = 0.60, insecticide use vs. fungicide use = 0.82), the influence of total pesticide use on Bd infection prevalence across host life stages seemed to be driven by herbicide use in

TABLE 1 Model averaged coefficients, standard error, *z*-statistics and *p*-values with statistical shrinkage from multimodel inference analyses evaluating the effects of total pesticide use and pesticide use by type on *Batrachochytrium dendrobatidis* infection prevalence in amphibian populations in the contiguous U.S.A

Variable	Coefficient	*SE*	*z*	*p*
Total pesticide use				
(Intercept)	0.2972	0.0958	3.48	**<0.001**
Pesticides	−0.1521	0.0559	2.70	**0.007**
Life stage (terrestrial)	0.1014	0.0698	3.66	**<0.001**
Family (Hylidae)	−0.1039	0.1190	0.87	0.385
Family (Plethodontidae)	−0.1580	0.1536	1.03	0.305
Family (Ranidae)	−0.0421	0.0774	0.54	0.589
Family (Salamandridae)	0.0739	0.1459	0.50	0.615
NDVI	−0.0264	0.0332	0.79	0.428
PC1Precip.	−0.0067	0.0257	0.26	0.797
PC1Temp.	0.0125	0.0305	0.41	0.684
Pesticides × Life stage (terrestrial)	0.3019	0.0688	4.36	**<0.001**
Type of pesticide use				
(Intercept)	0.3001	0.0934	3.67	**<0.001**
Herbicides	−0.1368	0.0541	2.51	**0.012**
Insecticides	0.0015	0.0422	0.04	0.972
Fungicides	−0.0075	0.0317	0.24	0.813
Life stage (terrestrial)	0.0782	0.0708	3.80	**<0.001**
Family (Hylidae)	−0.0724	0.1067	0.68	0.499
Family (Plethodontidae)	−0.1247	0.1526	0.82	0.415
Family (Ranidae)	−0.0394	0.0751	0.52	0.602
Family (Salamandridae)	0.0422	0.1262	0.33	0.740
NDVI	−0.0287	0.0343	0.84	0.403
PC1Precip.	−0.0095	0.0276	0.34	0.733
PC1Temp.	0.0071	0.0270	0.26	0.793
Herbicides × Life stage (terrestrial)	0.2961	0.0707	4.16	**<0.001**
Insecticides × Life stage (terrestrial)	0.0015	0.0363	0.04	0.968
Fungicides × Life stage (terrestrial)	0.0021	0.0243	0.08	0.933

Note. Coefficients and standard errors are standardized. *p*-values lessthan 0.05 are in bold. In the baseline model, the host family is Bufonidaeand the life stage aquatic. PC1Precip. and PC1Temp. = first axis from aprincipal components analysis for precipitation and temperature measures,respectively; NDVI = normalized difference vegetation index.

comparison to insecticide and fungicide use (Table 1). Herbicide use, life stage, and the interaction of herbicide use and life stage were significant predictors of prevalence, controlling for insecticide use, fungicide use, the interaction of insecticide and fungicide uses with life stage, and all other covariates (Table 1). The relative importance scores for herbicide use, life stage, and the interaction of herbicide use and life stage were greater than all other factors in the model, including family, NDVI, precipitation, temperature, insecticide use, fungicide use, and the interaction of insecticide or fungicide use with life stage (Figure 2b). Similar to the effect of total pesticide use, herbicide use was associated with decreased prevalence of infection in the aquatic larval life stage and increased prevalence of infection in the terrestrial post-metamorphic life stages (Table 2; Figure 3b; Nagelkerke pseudo-R^2 = 0.37). The variance of the model was best explained by the interaction of life stage and pesticides [Nagelkerke pseudo-R^2 of the full model minus Nagelkerke pseudo-R^2 of the model with focal predictor (interaction of life stage and pesticides) removed = 0.15], followed by life stage (0.10), family (0.07), NDVI (0.04), and herbicide use (0.00). The effect of herbicides was significantly negative in the aquatic stage (Table 1) and significantly positive in the terrestrial life stage (*z*-value = 3.30, $p < 0.001$). We caution against assuming that insecticides and fungicides do not influence Bd distributions because use of herbicides, insecticides, and fungicides are correlated positively.

3.3 | The influence of herbicide compounds

The five most commonly used herbicides in the dataset include glyphosate (33% of total herbicide use based on weight), atrazine (10%), metolachlor-s (5%), ethephon (5%), and sodium chlorate (4%). Including the interaction between focal herbicide compound and life stage improved the goodness-of-fit compared with the same model without this interaction for four of the five herbicides [glyphosate (log-likelihood ratio = 17.55, $p < 0.001$), atrazine (log-likelihood ratio = 9.10, $p = 0.002$), metolachlor-s (log-likelihood ratio = 14.0, $p < 0.001$), ethephon (log-likelihood ratio = 2.65 $p = 0.104$), and sodium chlorate (log-likelihood ratio = 6.10, $p = 0.01$)]. Similar to the effect of total pesticides and herbicides, glyphosate use was negatively associated with Bd prevalence in the aquatic stage and positively associated in the terrestrial stage (Figure 4a). Both atrazine and metolachlor-s use were negatively associated with Bd prevalence in the larval stage but did not appear to be associated with infections in the terrestrial stage (Figure 4b,c). In contrast, ethephon and sodium chlorate use did not have a strong influence on Bd prevalence in the aquatic larval stage, but were positively associated with Bd prevalence in the terrestrial post-metamorphic stage (Figure 4d,e). The influence of the sum of the top five most-used herbicide compounds matches closely with the pattern of overall herbicide use on Bd prevalence (Figure 4f).

4 | DISCUSSION

Pesticides represent a major ecological disturbance to communities in aquatic environments and can shape distributions of organisms across landscapes (Beketov, Kefford, Schafer, & Liess, 2013; Liess & Von Der Ohe, 2005; Schäfer et al., 2007). Our study demonstrates that pesticide use can be associated with patterns of infection prevalence in

TABLE 2 Model comparison results for models with ΔAICc values less than two testing predictors including total pesticides and types of pesticides

Model	AICc	ΔAICc	Akaike weight
Models including total pesticide use			
Family + Life stage + Pesticides + NDVI + Pesticides × Life stage	163.28	0.00	0.19
Family + Life stage + Pesticides + PC1Temp. + Pesticides × Life stage	164.36	1.08	0.11
Life stage + Pesticides + Pesticides × Life stage	164.62	1.34	0.10
Family + Life stage + Pesticides + NDVI + PC1Temp. + Pesticides × Life stage	165.18	1.90	0.07
Family + Life stage + Pesticides + Pesticides × Life stage	165.24	1.96	0.07
Life stage + Pesticides + NDVI + Pesticides × Life stage	165.25	1.97	0.07
Models including types of pesticides			
Family + Life stage + Herbicides + NDVI + Herbicides × Life stage	162.48	0.00	0.08
Life stage + Herbicides + Herbicides × Life stage	163.21	0.73	0.06
Life stage + Herbicides + NDVI + PC1Precip. + Herbicides × Life stage	163.45	0.97	0.05
Life stage + Herbicides + NDVI + Herbicides × Life stage	163.71	1.23	0.04
Family + Life stage + Herbicides + PC1Temp. + Herbicides × Life stage	164.21	1.73	0.03
Family + Life stage + Herbicides + Herbicides × Life stage	164.42	1.94	0.03

Note. Models evaluated the influence of total pesticide use, and pesticide use by type (herbicide, insecticide and fungicide use) on the prevalence of *Batrachochytrium dendrobatidis* infection in the contiguous U.S.A. PC1Precip. and PC1Temp. = first axis from a principal components analysis for precipitation and temperature measures, respectively; NDVI = normalized difference vegetation index.

wildlife at broad spatial scales, which is consistent with the body of experimental research on pesticides and amphibian–Bd interactions. We show a negative relationship between pesticide use and the prevalence of Bd infection in the aquatic larval life stage and a positive relationship between pesticide use and the prevalence of Bd infection in the terrestrial post-metamorphic life stage. When compared across types of pesticide use, our analyses suggest that the combined influence of the most commonly used herbicides are the primary determinants of these differential effects of pesticides on infection prevalence across host life stages.

Hosts and pathogens in freshwater systems are likely to be exposed to pesticides in the aquatic environment, where the presence of contaminants, including pesticides, is common because of aerial deposition and agricultural runoff (Gilliom & Hamilton, 2006). In the amphibian–Bd system, when pesticides are present, the balance between hosts and pathogens in the aquatic environment might be tipped in favour of hosts because of direct negative effects of pesticides on pathogen viability, which explains the possible mechanism for the observed negative association between pesticide use and the prevalence of infection in the aquatic larval stage of hosts in the present study. Several pesticides, including atrazine (the second most used herbicide compound in the U.S.A. and in our dataset), have been shown to have direct negative effects on Bd growth, survival (Hanlon & Parris, 2012; McMahon et al., 2013), and production of zoospores, the aquatic infective stage of Bd (Hanlon & Parris, 2012).

However, as hosts develop, they can suffer delayed negative effects of early-life pesticide exposure well into adulthood, increasing their overall risk of disease development in the terrestrial post-metamorphic life stage. Pesticide exposure can have delayed effects on organisms (Jones, Hammond, & Relyea, 2009; Rohr & Palmer, 2005) and can disrupt host–pathogen interactions, leading to an increase in infectious disease risk (Rohr & McCoy, 2010; Rohr et al., 2006; Wise et al., 2014). We propose that the observed positive effect of pesticide use, mainly driven by herbicide use, on the prevalence of Bd infection is consistent with the body of empirical research showing persistent negative effects of early-life exposures to pesticides on infectious disease risk; for instance, post-metamorphic amphibians can suffer increased mortality to Bd as a result of early-life exposure to atrazine caused by reduced tolerance to infection, suggesting a long-term cost of pesticide exposure (Rohr et al., 2013).

Interestingly, herbicides, as opposed to insecticides or fungicides, were most correlated with the observed patterns between pesticide use and infection prevalence. We propose that the power to detect an effect of herbicides might be greater than that for insecticides or fungicides because herbicides are used in greater amounts in the U.S.A. (Grube, Donaldson, Kiely, & Wu, 2011), increasing the likelihood of exposure in natural systems. In a given year, herbicides are used more than five times as much as insecticides or fungicides as measured by the mass of the active ingredient (Grube et al., 2011). Therefore, herbicide exposure of natural host–pathogen populations might be more likely to occur and result in measureable effects in comparison to exposure to insecticides or fungicides.

Our results show that the combined effects of the most commonly used herbicides together drive the observed patterns of total herbicide use on infection prevalence. The association between individual herbicide compounds and infection prevalence across life stage either closely matched the overall pattern of total herbicide use (e.g., glyphosate) or showed a similar pattern to the influence of total herbicide use in at least one of the host life stages (e.g., atrazine, metolachlor-s, ethephon, sodium chlorate). For instance, atrazine and metolachlor-s

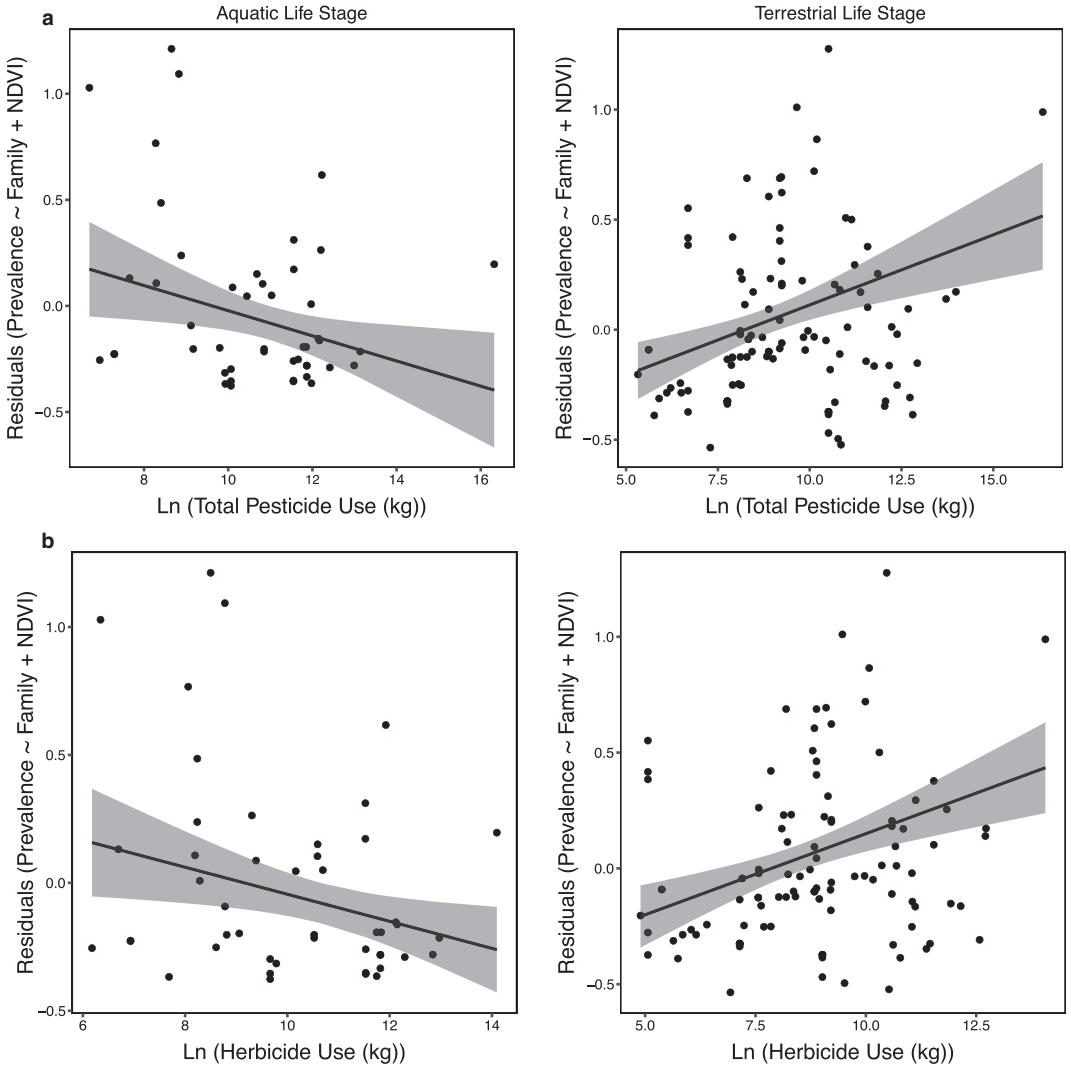

FIGURE 3 Partial regressions showing (a) the influence of total pesticide use, and (b) herbicide use on *Batrachochytrium dendrobatidis* infection prevalence in amphibian populations across aquatic and terrestrial amphibian life stages in the contiguous U.S.A. controlling for effects of host family and normalized difference vegetative index (NDVI). Models shown are the models with a change in Akaike information criterion corrected for small sample sizes (ΔAICc) equal to zero from model comparisons. Prevalence has been arcsine–square-root transformed in the figures. Grey bands represent 95% confidence intervals

have negative effects on infection prevalence in the aquatic larval life stage but no strong effect in the terrestrial post-metamorphic life stage, whereas ethephon and sodium chlorate have positive effects on infection prevalence in the terrestrial post-metamorphic life stage but no strong effect in the aquatic larval life stage. The top five most commonly used herbicides in our dataset composed the majority of the total use of herbicides, so when we examine the influence of the sum of these herbicide compounds on infection prevalence across host life stages (Figure 4f), unsurprisingly, it closely matched the patterns for total herbicide use (Figure 3b).

4.1 | Caveats and limitations

It is important to recognize that (a) the results presented in the present study are correlative, (b) we cannot rule out the effects of insecticides and fungicides, and (c) a substantial amount of variation remains to be explained in the present system. First, although the patterns observed in the present study are consistent with some laboratory experiments, we are limited in our ability to draw cause-and-effect conclusions because the results of the present study are correlative in nature. We call for additional empirical studies to be conducted to test for the effects of pesticides on infection prevalence across host species to determine whether or not differential effects of pesticides according to host life stages are capable of driving patterns of infections.

Second, we caution against assuming that the lack of relationships between Bd prevalence and insecticide and fungicide is evidence that these pesticide types do not influence Bd distributions. Instead, we highlight that herbicide, insecticide, and fungicide use are positively correlated at the county level across the U.S.A. Given that estimates of pesticide use are derived, at least in part, from land use data (Thelin & Stone, 2013), counties in which herbicide use is high are likely to be counties with increased agricultural land use; therefore, these

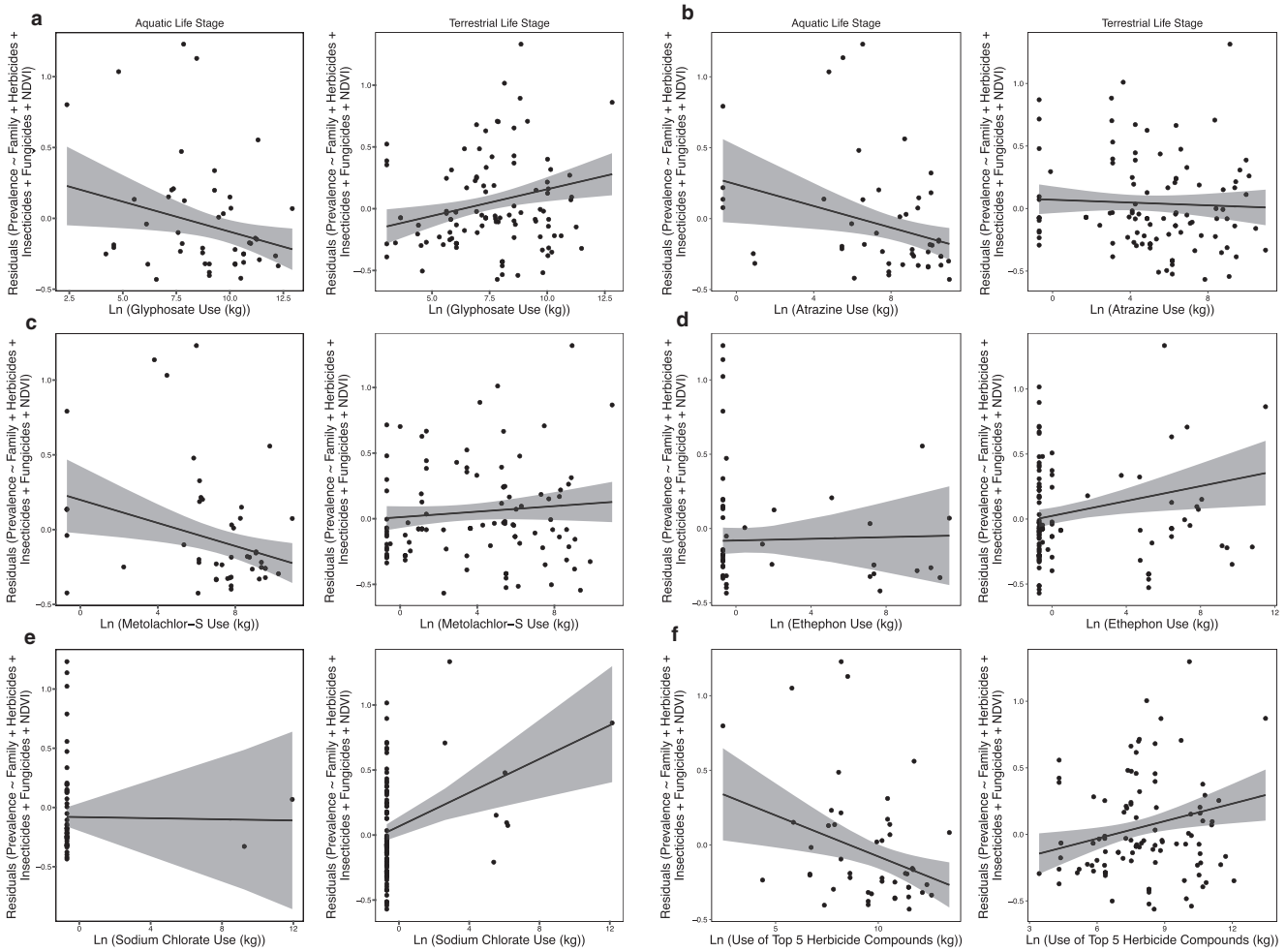

FIGURE 4 Partial regressions showing the influence of (a) glyphosate use, (b) atrazine use, (c) metolachlor-s use, (d) ethephon use, (e) sodium chlorate use, and (f) the sum of these top five herbicide compounds on the prevalence of *Batrachochytrium dendrobatidis* infection in amphibian populations across life stages in the contiguous U.S.A., controlling for the effects of family, normalized difference vegetative index (NDVI), herbicide use (minus the compound or group of focus), insecticide use and fungicide use. Prevalence has been arcsine−square-root transformed in the figures. Grey bands represent 95% confidence intervals

counties also have high insecticide and fungicide use. The influence of the most abundant pesticide type, namely herbicides, gives rise to the model that best predicts infection prevalence. Even though our models control for the use of other pesticide types when testing for a focal pesticide type, because of the positive correlation among the pesticide use types, we are hesitant to disregard the potential influence of insecticides and fungicides on Bd distributions. For instance, similar results of a decreased risk of Bd infection in the larval stage and an increased risk of Bd infection in the terrestrial stage have been associated with fungicides. Using a combination of experiments and field surveys, Rohr, Brown, Battaglin, McMahon, and Relyea (2017) observed that low concentrations of the fungicides azoxystrobin, chlorothalonil, and mancozeb were directly toxic to Bd in culture and exposure to fungicides in the larval host stage increased Bd loads in the terrestrial host stage. Field surveys found that Bd prevalence in the terrestrial life stage was positively associated with measured fungicide concentrations in pond water. These findings suggest that fungicides can also shape patterns of Bd infections at the landscape level.

Finally, a considerable amount of variation in the prevalence of Bd infection remains to be explained in this system. Although we evaluated and controlled for several alternative hypotheses that could influence patterns of infection prevalence, including host family, temperature, precipitation, and vegetation, many other factors could contribute to the distribution of Bd in amphibians. These additional factors include but are not limited to the following: acquired resistance of the host to infection (McMahon et al., 2014), dispersal abilities of the host and pathogen (Lekberg, Koide, Rohr, Aldrich-Wolfe, & Morton, 2007), parasite and host diversity (Johnson, Hartson, Larson, & Sutherland, 2008), and consideration of environmental factors that act at different spatial scales (Cohen et al., 2016). Even pesticides themselves could have simultaneous direct and indirect effects on hosts and pathogens in this system. For instance, pesticides might influence aquatic algae and macroinvertebrate communities, which are important food sources for amphibian hosts that could indirectly influence infection patterns. Evaluation of these additional factors falls outside the scope of the present study.

4.2 | Conclusions

Pesticides can be a major driver of communities in freshwater ecosystems (McMahon et al., 2012), but studies on how pesticides are associated with distributions of pathogens at large spatial scales in wildlife are rare. The value of the present study is that we evaluated the influence of pesticides on infection patterns across thousands of individual hosts from 49 species at the national scale. Consistent with the body of experimental evidence in this system, our research illustrates how pesticides can be associated with distributions of infectious pathogens over broad spatial scales via effects that vary over the life span of a host, which highlights the complex nature of the impact of contaminants on natural systems. Although our study evaluates the potential influence of pesticides on the likelihood of infection in hosts, we have not evaluated the fitness consequences of pesticides on hosts exposed to parasites, which may occur through the physiological mechanisms of resistance or tolerance (Raberg, Graham, & Read, 2009). For instance, pesticide exposure of Bd-infected or Bd-exposed amphibian hosts may result in increased host mortality. With their impacts on pathogen viability and host immunity, the effects of pesticides on infectious disease distributions should be given more attention, particularly at broad scales and across host species. Accurate predictions of disease distributions may lead to the most effective management strategies to limit the spread of diseases to vulnerable populations.

DATA ACCESSIBILITY

The derived dataset used for these analyses will be made publically available via Figshare upon manuscript acceptance.

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