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The influence of landscape and environmental factors on ranavirus epidemiology in a California amphibian assemblage

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Abstract

1. A fundamental goal of disease ecology is to determine the landscape and environmental processes that drive disease dynamics at different biological levels to guide management and conservation. Although ranaviruses (family *Iridoviridae*) are emerging amphibian pathogens, few studies have conducted comprehensive field surveys to assess potential drivers of ranavirus disease dynamics.
2. We examined the factors underlying patterns in site-level ranavirus presence and individual-level ranavirus infection in 76 ponds and 1,088 individuals representing five amphibian species within the East Bay region of California.
3. Based on a competing-model approach followed by variance partitioning, landscape and biotic variables explained the most variation in site-level presence. However, biotic and individual-level variables explained the most variation in individual-level infection.
4. Distance to nearest ranavirus-infected pond (the landscape factor) was more important than biotic factors at the site level; however, biotic factors were most influential at the individual level. At the site level, the probability of ranavirus presence correlated negatively with distance to nearest ranavirus-positive pond, suggesting that the movement of water or mobile taxa (e.g., adult amphibians, birds, reptiles) may facilitate the movement of ranavirus between ponds and across the landscape.
5. Taxonomic richness associated positively with ranavirus presence at the site level, but vertebrate richness associated negatively with infection prevalence in the host population. This might reflect the contrasting influences of diversity on pathogen colonisation versus transmission among hosts.
6. Amphibian host species differed in their likelihood of ranavirus infection: American bullfrogs (*Rana catesbeiana*) had the weakest association with infection while rough-skinned newts (*Taricha granulosa*) had the strongest. After accounting for host species effects, hosts with greater snout–vent length had a lower probability of infection.
7. Our study demonstrates the array of landscape, environmental, and individual-level factors associated with ranavirus epidemiology. Moreover, our study helps illustrate that the importance of these factors varies with biological level.

KEYWORDS
dilution effect, emerging infectious diseases, iridovirus, multimodel inference, reservoir species

1 | INTRODUCTION

Infectious diseases are increasingly recognised as important components of communities and ecosystems, yet their emergence in humans, wildlife and plants across the globe has sparked concern because of their potentially devastating effects on populations (Daszak, Cunningham, & Hyatt, 2000; Dobson & Foufopoulos, 2001; Jones et al., 2008). While decades of research have demonstrated the important roles of landscape and environmental (e.g., abiotic conditions and species interactions) processes in driving disease dynamics (reviewed in Poulin, 1998, 2007), a perpetual challenge in disease ecology is that the individual factors studied and their relative importance can be highly system-specific. For example, climate change is cited as a major influence on vector-borne diseases (Githeko, Lindsay, Confalonieri, & Patz, 2000; Rogers & Randolph, 2006), flooding can influence the prevalence of cholera (reviewed in Ahern, Kovats, Wilkinson, Few, & Matthies, 2005), and loss of biodiversity can influence the prevalence of Lyme disease (Keesing, Holt, & Ostfeld, 2006; Keesing et al., 2010; Ostfeld & Keesing, 2000). Thus, for many emerging diseases, there is a need to conduct comprehensive field surveillance studies that combine assessments of key epidemiological parameters (e.g., presence, infection, pathogen load) with landscape and environmental data to determine the potential drivers of disease patterns across the landscape. Determining which factor—or groups of factors—is most influential can help to develop predictions, increase our knowledge base for host–pathogen interactions and inform management and conservation.

Recent studies have highlighted the importance of investigating the influence of factors at multiple biological levels of organisation because of contrasting results between levels (e.g., site- [higher level] versus individual level [lower level]; Borcard, Legendre, Avois-Jacquet, & Tuomisto, 2004; Cohen et al., 2016; Dunn, Davies, Harris, & Gavin, 2010; Johnson, De Roode, & Fenton, 2015; Schotthoefer et al., 2011). It has been hypothesised that abiotic factors influence distributional patterns at higher levels, whereas biotic factors (e.g., species interactions) influence distributional patterns at lower levels (Cohen et al., 2016; Levin, 1992; Mcgill, 2010; Rahbek, 2004; Wiens, 1989). Accordingly, abiotic (e.g., temperature, precipitation, altitude) and biotic (e.g., host richness) factors were highly important in predicting the distribution of three pathogens (the pathogenic fungus *Batrachochytrium dendrobatidis* [Bd], West Nile virus and the bacterium that causes Lyme disease [*Borrelia burgdorferi*]) at higher levels, but biotic factors were more important at lower levels (Cohen et al., 2016)). Landscape factors, such as connectivity among habitat patches, can also influence disease dynamics and the dispersal of pathogens. For example, the movement of the pathogenic fungus Bd through amphibian assemblages across the landscape suggests that dispersal plays a key role at regional levels (Laurance, Mcdonald, & Speare, 1996; Lips, Diffendorfer, Mendelson, & Sears, 2008; Vredenburg, Knapp, Tunstall, & Briggs, 2010). Therefore, evaluating which factors are most influential to the distribution of diseases, and at what levels of organisation, is important to gain a clear understanding of what controls the spread of diseases among hosts *and* across the landscape.

Ranaviruses (family *Iridoviridae*) are viral pathogens of amphibians, fishes, and reptiles that have been implicated in mortality events across the globe (Duffus et al., 2015). Over the last two decades, reports of mortality events in amphibian populations have gradually increased in the literature (Duffus et al., 2015). Consequently, experimental studies and field surveys have been initiated to explore the potential drivers of ranavirus disease dynamics. Recent reviews have highlighted environmental factors that could influence ranaviral disease dynamics (Brunner, Storfer, Gray, & Hoverman, 2015). For example, abiotic factors such as land use (e.g., cattle grazing and urbanisation), water quality and contaminants from runoff (e.g., nutrients, pesticides, heavy metals) are associated with increased prevalence of ranavirus in experimental studies and in the field (Forson & Storfer, 2006a,b; Kerby, Hart, & Storfer, 2011; Kerby & Storfer, 2009; North, Hodgson, Price, & Griffiths, 2015). In the United Kingdom (U.K.), deeper ponds were associated with an increased incidence of die-off events (North et al., 2015). However, few studies have broadly explored the role of pond characteristics on ranavirus occurrence or prevalence (Hoverman, Gray, Miller, & Haislip, 2012), particularly within an entire amphibian assemblage. In addition to abiotic factors, biotic factors (e.g., competition, predation, reservoir species) likely play a role in ranavirus distribution and dynamics. For instance, American bullfrogs (*Rana catesbeiana*; phylogenetic taxonomy reviewed in Yuan et al., 2016) and fishes are implicated as potential reservoirs for the pathogen (Brunner et al., 2015). It has also been hypothesised that predators can increase disease risk by inducing physiological stress that compromises immune function (Reeve, Crespi, Whipps, & Brunner, 2013). While there are many hypothesised abiotic and biotic drivers of ranavirus emergence, there have been few attempts to assess the relative importance of these factors using large-scale field patterns for this pathogen.

The influences of landscape processes on ranavirus dynamics have received relatively little attention (Gahl & Calhoun, 2008; Hoverman, Gray, et al., 2012; North et al., 2015; Price, Garner, Cunningham, Langton, & Nichols, 2016). Given that amphibians are often characterised by metapopulation dynamics (Gulve, 1994), the movement of infected hosts between breeding sites in close proximity to each other could influence spatial patterns in ranavirus occurrence on the landscape. Spatial models explained more variation than non-spatial models for ranavirus mortality events in the U.K. (North et al., 2015; Price et al., 2016). However, no spatial relationships were observed for mortality events in Acadia National Park, Maine, U.S.A (Gahl & Calhoun, 2008). An additional challenge is that most studies on the distribution of ranaviruses come from mortality events detected by scientists or members of the public. This sparse and non-random selection of samples provides only scarce insight into the baseline epidemiology of ranaviruses in amphibian populations or across the landscape, and environmental processes underlying these patterns.

In the current study, our primary objective was to quantify the influence of a suite of landscape, abiotic and biotic variables on ranavirus disease dynamics in amphibian assemblages. To this end, we conducted comprehensive field surveys of 76 ponds to collect data on infection presence and prevalence within each amphibian population and obtain corresponding information on the biological and environmental characteristics associated with epidemiological observations. We sought to broadly evaluate the influence of an array of factors on ranavirus epidemiology, and how these factors influenced pathogen dynamics between two biological levels, by collecting data from multiple amphibian host species and at both the individual and population (pond) levels. To determine the relative influence of landscape, abiotic, and biotic factors on ranavirus, we used model selection and multimodel averaging followed by variance partitioning, thereby allowing us to assess the joint effects of hypothesised covariates and how they varied between the site level and individual level.

2 | METHODS

2.1 | Study area and species

We examined patterns of ranavirus presence and infection in amphibian assemblages in the East Bay region of California (Figure 1; Hoverman, Mihaljevic, Richgels, Kerby, & Johnson, 2012; Johnson, Preston, Hoverman, & Richgels, 2013; Richgels, Hoverman, & Johnson, 2013). We sampled 93 ponds in managed parks and protected areas within three counties (i.e., Alameda, Contra Costa, and Santa Clara); ranavirus infection status of ponds was unknown prior to sampling. We selected ponds that were smaller (< 2 ha) and likely to contain amphibian assemblages (Hoverman, Mihaljevic, et al., 2012). Ponds were discrete and well-bounded entities and did not have above-ground water flow among them in the summer months. The timing of visitation to ponds was determined by researcher availability and other logistical constraints and was therefore not spatiotemporally randomised. The amphibian assemblage in this region is composed of seven species: northern Pacific tree frogs (*Hyliola regilla*), western toads (*Anaxyrus boreas*), American bullfrogs (*R. catesbeiana*), California newts (*Taricha torosa*), rough-skinned newts (*T. granulosa*), California red-legged frogs (*Rana draytonii*) and California tiger salamanders (*Ambystoma californiense*). Given the threatened status of California red-legged frogs and California tiger salamanders, we recorded them during surveys but excluded them from ranavirus sampling.

2.2 | Field sampling and measurements during site visits

We conducted field surveys from May to August 2013 using the field sampling protocols of Hoverman, Mihaljevic, et al. (2012). In brief, we used a combination of visual encounter surveys, dipnet sweeps and habitat-stratified seine hauls to sample the ponds (Johnson, Preston, Hoverman, & Richgels, 2013; Richgels et al., 2013). We

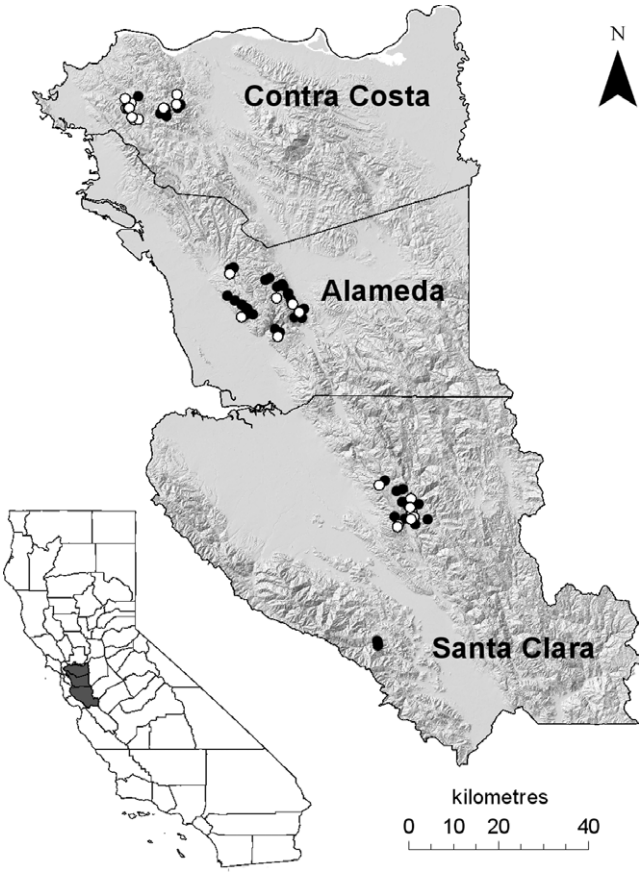

FIGURE 1 Study area and ponds included in site-level analyses (n = 76) in three counties (Alameda, Contra Costa and Santa Clara) of the East Bay region of California in 2013. Black points represent sites with ranavirus presence (those included in site- and individual-level analyses), and white points represent sites without ranavirus presence (those only included in site-level analyses)

disinfected all gear (e.g., nets and waders) with 15% bleach (10 min contact time) between sites. We identified amphibians to species, fishes to genus or species, and macroinvertebrates to order, family or genus in the field (Table S1). At each pond, we randomly selected about 10 individuals per species for ranavirus screening (mean = 20 total amphibians per site, range = 1–84). We sampled metamorphic anurans (Gosner stage 25–32; Gosner, 1960) and late-stage larval newts (2–4 T; Calhoun, Bucciarelli, Kats, Zimmer, & Johnson, 2017) to maintain similarity in life stages among species because we were unable to collect metamorphic newts. Therefore, we controlled for differences among life stages in our sampling and did not hypothesise these differences would influence our observed patterns.

We necropsied each amphibian and sampled kidney and liver tissues for ranavirus; we flame-sterilised equipment between individuals. For each individual, we pooled the liver and kidney tissues and extracted DNA using DNeasy Blood and Tissue Kits (Qiagen). To quantify infection status for each individual, we used quantitative polymerase chain reaction (Wuerthner, Hua, & Hoverman, 2017). Our qPCR mixture included a 1.0 μl mixture of each primer at 10 pmol/μl (rtMCP-F [5′-ACA CCA CCG CCC AAA AGT AC-3′] and rtMCP-R [5′-CCG TTC ATG ATG CGG ATA ATG-3′]), and a

fluorescent probe (rtMCP-probe [5′-CCT CAT CGT TCT GGC CAT CAA CCA-3′]), and 6.25 μl of TaqMan® Universal PCR Master Mix (Applied Biosystems). We added 2.5 μl of DNA-grade water and 2.5 μl of template DNA to achieve a final volume of 12.25 μl. We used a Bio-Rad real-time qPCR system (Bio-Rad) to perform qPCR. We included a standard curve and a negative (virus-free) water sample in each qPCR. We used a synthetic double-stranded DNA standard, which is conserved among *Ranavirus* species, by synthesising a 250-bp fragment of the major capsid protein (MCP) gene (gBlocks Gene Fragments; Integrated DNA Technologies). For the standard curve, we prepared a log-based dilution series (4.014×10^5–4.014×10^2 viral copies μl^{-1}). We ran standard curve samples and unknowns in duplicate. We considered duplicated unknowns that peaked before 40 cycles (the point at which standards stop amplifying and results become unreliable) to be ranavirus positive and reran any unknowns with mixed (positive and negative) results. There were no mixed results after the rerun.

We measured an array of landscape, abiotic and biotic predictor variables that we considered to be potential factors affecting ranavirus epidemiology, given the available literature (Table 1). Our landscape variable was distance to nearest ranavirus-infected pond (other than the pond the individual was found within). To calculate this distance, we recorded latitude and longitude of each site and measured Euclidean distance to nearest ranavirus-infected pond, which was determined after sampling, using the function "dist" in the R package "stats" (R Core Team, 2017). From the generated distance matrix, we deleted columns representing distances of each pond to ponds classified as ranavirus-negative, and sorted to isolate distance to nearest ranavirus-infected pond for each pond and individual within each pond. This method is limited in that not all ponds in the landscape were sampled; thus, other ranavirus-positive sites could occur, but not have been visited. However, our sampling scheme sought to sample all neighbouring ponds within a contiguous area (e.g., a park or protected area), such that these estimates are likely to capture general patterns related to colonisation potential.

We assessed pond permanence, per cent forest or wetland surrounding ponds, pond area and water quality factors at each site. For pond permanence, we classified ponds as "temporary" if they were observed going dry during direct field visits (2011–2013) or using historical images in Google Earth (Johnson, Hoverman, Mckenzie, Blaustein, & Richgels, 2013); ponds that held water throughout the course of the study were classified as "permanent." We measured conductivity (S/m), total dissolved solids (mg/L), salinity (mg/L) and pH with a YSI meter (Model 556; Yellow Spring Instrument, Yellow Springs, Ohio, USA). We quantified total nitrogen (mg/L), dissolved organic carbon (mg/L) and total ammonia (mg/L) using standard methods (https://instaar.colorado.edu/research/labs-groups/arikaree-environmental-lab/free-play/; Johnson, Hoverman, et al., 2013). We used principal component analysis (PCA) to reduce dimensionality of the seven abiotic water quality variables. Water quality variables, except pH, were log-transformed to reduce positive skewness and scaled and centred, before conducting the PCA. We retained only the first two components from PCA for further analyses, which had eigenvalues greater than one (Guttman–Kaiser criterion) and proportion of variance greater than the "broken-stick" percentage (Table S2; Yeomans & Golder, 1982; Legendre & Legendre, 2012). Principal component 1 had high loadings for total dissolved solids (loading = −0.58), salinity (−0.57) and conductivity (−0.54). Principal component 2 was associated with total nitrogen (loading = 0.64), dissolved organic carbon (0.58), ammonium (0.46) and pH (0.14). We calculated the percentage of area within a 1 km radius of each pond classified as forested (sum of all forest types) and wetland (open water) using ArcGIS and the National Landcover Database (Homer et al., 2015; Johnson, Preston, Hoverman, & Richgels, 2013) because of our interest in the influence of intact forest and wetlands surrounding focal ponds. We calculated pond surface area (m^2; hereafter, area) by walking the perimeter of the pond with a handheld GPS using the track function. Area was base-10 log-transformed to meet assumptions of normality for analyses.

We represented the biotic community with per cent vegetation cover on pond shorelines (hereafter, per cent shoreline vegetation),

TABLE 1 Predictor variables included to investigate patterns in landscape (L), abiotic (A), biotic (B) and individual-level (I) influences on site-level ranavirus presence and individual-level ranavirus infection in amphibian assemblages in the East Bay region of California in 2013. Individual-level influences were only included in the individual-level ranavirus infection analyses. Water quality principal components 1 and 2 are the product of reducing the dimensionality of seven water quality parameters. Numbers of *A. boreas*, *H. regilla*, *R. catesbeiana*, *T. granulosa* and *T. torosa* are the numbers of western toads, Pacific tree frogs, American bullfrogs, rough-skinned newts and California newts, respectively, examined for ranavirus at each site

	Variable	Type
1	Distance to nearest ranavirus-infected pond (km)	L
2	Per cent forest surrounding	A
3	Per cent wetland surrounding	A
4	Water quality: principal component 1	A
5	Water quality: principal component 2	A
6	Pond area (m^2)	A
7	Pond permanence (permanent or temporary)	A
8	Amphibian density (measured as catch per unit effort)	B
9	Cattle presence	B
10	Number of *A. boreas*	B
11	Number of *H. regilla*	B
12	Number of *R. catesbeiana*	B
13	Number of *T. granulosa*	B
14	Number of *T. torosa*	B
15	Fish presence	B
16	Per cent shoreline vegetation	B
17	*Rana catesbeiana* presence	B
18	Taxonomic richness	B
19	Vertebrate richness	B
20	Snout–vent length (mm)	I
21	Species identity	I

taxonomic richness, vertebrate richness, amphibian density measured as catch per unit effort, number of amphibians (all species combined) examined for ranavirus, and the presence or absence of fishes, cattle and non-native *R. catesbeiana*. We visually estimated per cent shoreline vegetation at each site. We determined vertebrate richness by counting the number of amphibian and fish taxa. Taxonomic richness included all amphibians, fishes, and macroinvertebrates (detailed methods in Johnson et al., 2016). We calculated amphibian density by counting the number of individuals of each amphibian species during dip net sweeps and dividing by the total number of sweeps completed. We also included the number of each species examined for infection (*H. regilla*, *A. boreas*, *R. catesbeiana*, *T. torosa* or *T. granulosa*) in site-level analyses to determine if the number of each species examined at each site (a proxy for species composition) influenced the presence of ranavirus. We also included snout–vent length (mm) and species identity (*H. regilla*, *A. boreas*, *R. catesbeiana*, *T. torosa* or *T. granulosa*) in individual-level analyses.

2.3 | Data analysis

Our response variable for site-level analyses was ranavirus presence defined as one or more amphibians of any species infected with ranavirus within a pond. We excluded ponds with incomplete environmental data. We also modelled individual-level infection status (infected or not infected) to allow us to incorporate both individual-level (e.g., body size) and site-level covariates (landscape, abiotic and biotic). Our response variable for individual-level analyses was ranavirus infection defined as an individual having detectable ranavirus infection. We limited our individual-level infection analyses only to ponds where ranavirus was detected, which included infected and uninfected individuals. Therefore, we excluded sites where ranavirus was not detected.

First, we individually assessed the influence of 21 and 17 predictor variables on ranavirus presence and infection, respectively, in amphibian assemblages with univariate generalised linear models fitted with a binomial distribution (yes or no for ranavirus presence or infection) and logit link (Tables S3 and S4). This approach allowed us to identify associations between individual predictor variables and ranavirus presence and infection, separately, prior to comparing competing models and conducting multimodel inference. To keep global models for ranavirus presence and infection tractable, we only included predictor variables with p-values $< .10$ from univariate analyses into global models.

We used mixed effects models using the R function "glmer" in the R package "lme4" (R v3.4.3; Bates, Mächler, Bolker, & Walker, 2015; R Core Team, 2017; Zuur, Leno, Wlaker, Saveliev, & Smith, 2009) fitted with a binomial distribution and logit link to analyse ranavirus presence and infection global models. We centred and scaled all continuous predictor variables to facilitate comparison of coefficients among predictor variables and improve numerical stability. For snout–vent length of amphibians, we centred and scaled within each species to account for differences in snout–vent length among species. We did not include interaction terms in global models because we did not hypothesise strong interactions between or among predictor variables, and to keep models tractable. We included amphibian density (measured as catch per unit effort) in ranavirus infection and presence global models, and total number of amphibians (all species combined) examined for ranavirus at each site in the ranavirus presence global model, as fixed effects to account for differences in the number of amphibians sampled and examined among sites, which influences detection likelihood. We base-10 log-transformed the total number of amphibians examined per site prior to analyses to meet assumptions of normality. We also included sampling date in both global models to account for differences in time of year that ponds were sampled. For analyses of individual-level infection, in which site was a random intercept term, we nested observations from different amphibian individuals and species within the same site.

We used the "dredge" function in the R package "MuMIn" to separately create a set of all possible submodels from ranavirus presence and infection global models, determine the best-supported models and calculate model averages for parameters from the best-supported models (multimodel inference; Burnham & Anderson, 2004; Barton, 2018). We compared submodels separately for ranavirus presence and infection analyses with an information-theoretic approach using Akaike's information criterion (AIC; Burnham & Anderson, 2004; Mazerolle, 2016). We used AIC corrected for small sample sizes (AIC_C) for both analyses because the number of observations divided by number of parameters was low for most ranavirus presence models ($n/K < 40$; Anderson & Burnham, 2002; Burnham & Anderson, 2004). Moreover, it is generally recommended to use AIC_C because it converges to AIC with large samples sizes like those included in ranavirus infection analyses (Anderson & Burnham, 2002; Burnham & Anderson, 2004). We report model-averaged parameter estimates (β), standard errors (*SE*), adjusted *SE* and relative importance of each predictor variable averaged from top models (ΔAIC_C, < 4 AIC_C units) derived from each global model (ranavirus presence or infection). Additionally, we estimated the variance in site-level ranavirus presence and individual-level ranavirus infection accounted for by landscape, abiotic, biotic or individual variables in global models with the "varpart" function in the R package "vegan" (Borcard, Legendre, & Drapeau, 1992; Schotthoefer et al., 2011).

We investigated normality of response and predictor variables using kernel density plots and Q-Q plots, checked assumptions of all top models and checked normality of model residuals against fitted values for top models. We tested for collinearity between predictor variables included in global models using Pearson's correlation coefficients and tested for multicollinearity among predictor variables in both global models with variance inflation factors with the R package "car" (Fox & Weisberg, 2011). We also calculated dispersion parameters to examine overdispersion in global models for ranavirus presence and prevalence. We investigated spatial autocorrelation of site-level ranavirus presence and residuals of ranavirus presence and infection global models using Moran's I test in the R package "spdep" (Bivand, 2014; Borcard et al., 1992; Schotthoefer et al., 2011). Raw databases are available as supplementary files (Database S1 and S2)

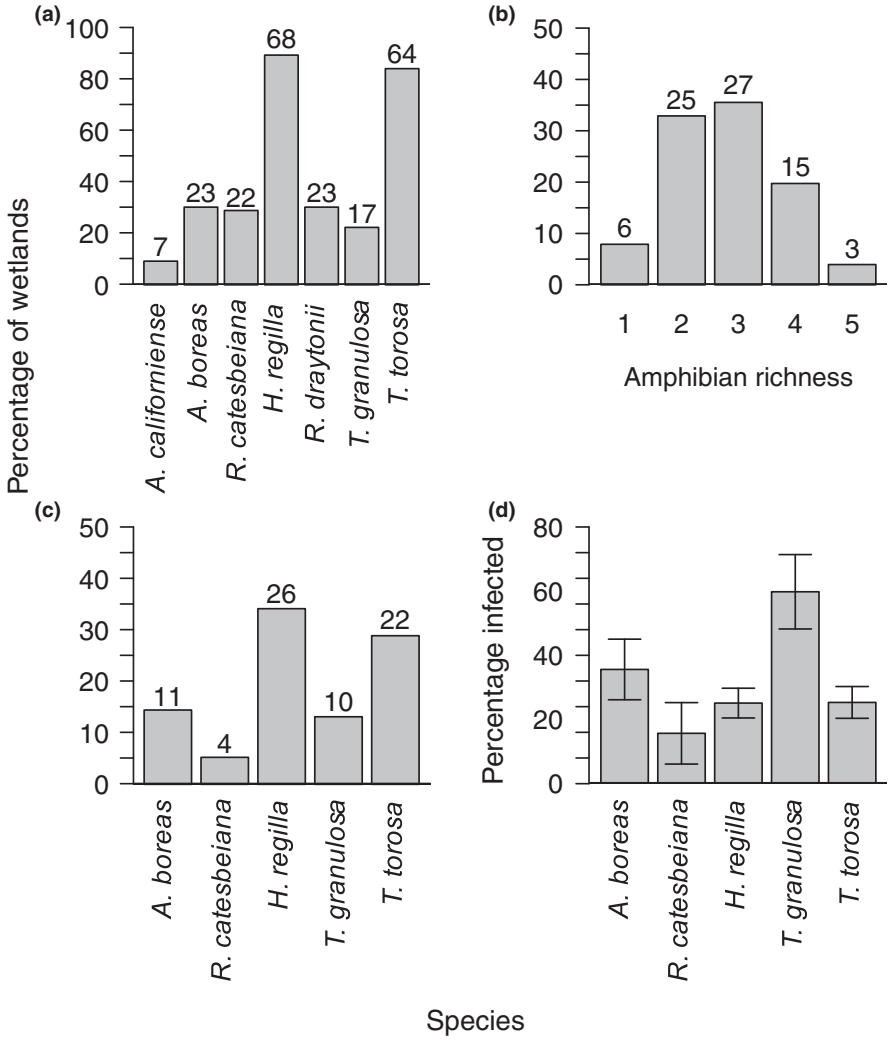

FIGURE 2 Per cent of ponds with each species (a), species richness at ponds (b), per cent of ponds with ranavirus-infected hosts for each species (c) and mean per cent of hosts infected with ranavirus per pond (with 95% confidence intervals) of those collected of each species (d) in amphibian assemblages in the East Bay region of California in 2013. Numbers above bars indicate number of ponds with each species or species richness (n = 76). For plots (a), (c) and (d): *Ambystoma californiense*, California tiger salamander; *Anaxyrus boreas*, western toad; *Rana catesbeiana*, American bullfrog; *Hyliola regilla*, northern Pacific tree frog; *Rana draytonii*, California red-legged frog; *Taricha granulosa*, rough-skinned newt; *Taricha torosa*, California newt

and at the Purdue University Research Repository (PURR, http://purr.purdue.edu).

3 | RESULTS

3.1 | Sampling overview

In total, our site-level analyses included 76 ponds and 1,376 amphibians sampled for ranavirus representing five species. We removed 17 of the 93 originally surveyed sites from site-level analyses because they had incomplete site- or individual-level covariate data, or both. We sampled only one site in May (1%, n = 1), most sites in June (26%, n = 19) and July (56%, n = 41), and some sites in August (16%, n = 12); sampling date was not correlated with ranavirus presence or infection (p > 0.704). The most common amphibian species among ponds were *H. regilla* and *T. torosa*, and most sites (68%, n = 52) had two or three amphibian species (Figure 2). Thirty-three per cent of tested amphibians were positive for ranavirus (n = 456 of 1,376). At least one infected individual occurred at 67% of ponds (n = 51 of 76) and an average of 50% of individuals (95% CI = 41–59%) were infected with ranavirus at each pond. For individual-level analyses, we removed 25 sites (including 288 individuals) where ranavirus was not present; thus, we reduced our individual-level sample size to 1,088 individuals. The percentage of infected individuals at ponds where ranavirus was detected varied among species; *T. granulosa* had the highest average percentage of individuals infected (mean = 60%, 95% CI = 48%–71%) followed by *A. boreas* (36%, 26%–45%), *T. torosa* (25%, 20%–30%), *H. regilla* (25%, 20%–30%) and *R. catesbeiana* (16%, 6%–25%). We observed non-native *R. catesbeiana* at 29% (n = 22) of ponds, and fishes (i.e., *Gambusia affinis*, *Lepomis macrochirus*, *Carassius auratus*, *Ictalurus* spp. or *Micropterus* spp.) at 26% of ponds (n = 20).

3.2 | Model selection and multimodel inference

Univariate analyses determined that landscape (distance to nearest ranavirus-infected pond), abiotic (per cent wetland within 1 km of pond) and biotic (amphibian density, taxonomic richness, number of *H. regilla* examined for infection, number of *A. boreas* examined for infection and total number of amphibians examined for ranavirus) variables were associated with, and included in the global model for, site-level ranavirus presence. For individual-level ranavirus infection, univariate analyses demonstrated that abiotic (pond permanence and per cent forest), biotic (*R. catesbeiana* presence, and vertebrate and

TABLE 2 Model-averaged coefficients for centred and scaled predictor variables from a subset of models (delta AICc < 4 points, 8 of 64 models) of site-level ranavirus presence in amphibian assemblages in the East Bay region of California in 2013. Coefficients are arranged by ascending *p*-value, then alphabetically. "Distance" is distance to nearest ranavirus-infected pond (km), "Total dissected" is the total number of amphibians (all species combined) examined for ranavirus as each site, and "Amphibian density" was measured as catch per unit effort. Numbers of *A. boreas* and *H. regilla* are the number of western toads and Pacific tree frogs, respectively, examined for ranavirus at each site. "Num. mod." is the number of models that include that predictor variable, "Importance" is proportion of models within the model subset that contain that variable, "*SE*" is standard error, and "Adj. *SE*" is adjusted standard error. Coefficients with $p \leq .05$ are shaded in grey

Variable	Num. mod	Importance	Estimate	*SE*	Adj. *SE*	*z*	*p*
Distance	8	1.00	−0.26	0.05	0.05	5.39	.001
Taxonomic richness	8	1.00	0.12	0.04	0.05	2.62	.008
Amphibian density	4	0.59	0.09	0.05	0.06	1.69	.090
Sampling date	8	1.00	−0.03	0.05	0.05	0.72	.471
Number of *A. boreas*	2	0.18	0.03	0.05	0.05	0.60	.547
Number of *H. regilla*	2	0.17	0.03	0.06	0.06	0.51	.608
Total dissected	8	1.00	0.02	0.06	0.06	0.24	.807
Per cent wetland	2	0.15	0.00	0.06	0.06	0.07	.945

taxonomic richness) and individual-level (snout–vent length and species identity) variables were associated with and included in the global model. From the global models, the "dredge" function produced 64 models comprised of eight landscape, abiotic, and biotic variables for ranavirus presence and 256 models comprised of eight landscape, abiotic, biotic and individual-level variables for ranavirus infection (Tables S3 and S4). For ranavirus presence, eight models were within four AIC_C of the best-supported model (Table S5). For individual-level ranavirus infection analysis, 37 models were within four AIC_C of the best-supported model (Table S6).

Landscape and biotic variables had the strongest associations with site-level ranavirus presence in our best-supported models (Table 2). Distance to nearest ranavirus-infected pond and taxonomic richness were included in all best-supported models, while amphibian density and pond area were only included in half of the best-supported models. Ponds that were farther from a ranavirus-infected pond had a lower likelihood of ranavirus presence ($\beta = -0.26 \pm 0.05$ [model-averaged coefficient ± adjusted *SE*]; Figure 3). Ponds with greater taxonomic richness had a higher likelihood of ranavirus presence ($\beta = 0.12 \pm 0.04$). Variance partitioning analyses demonstrated that the landscape variable, distance to nearest ranavirus-infected pond, explained the most variance (adjusted R^2 from variance partitioning = .18) and the biotic variables (taxonomic richness, amphibian density, number of *H. regilla* examined for infection, number of *A. boreas* examined for infection and total number of amphibians examined for infection), explained a smaller portion of variance ($R^2 = .09$) in site-level ranavirus presence (Table 3).

The best-supported models for individual-level ranavirus infection included abiotic, biotic and individual-level predictor variables (Table 4). Snout–vent length, species identity and vertebrate richness had the strongest associations with ranavirus infection. Species differed in their likelihood of ranavirus infection. *Rana catesbeiana*, which was the reference level in the species identity variable, had the lowest likelihood of ranavirus infection ($\beta = -2.09 \pm 0.75$; Figure 4). *Taricha torosa* ($\beta = 1.82 \pm 0.61$), *H. regilla* ($\beta = 2.24 \pm 0.61$), *A. boreas* ($\beta = 2.75 \pm 0.62$) and *T. granulosa* ($\beta = 2.99 \pm 0.69$) had higher likelihood of ranavirus infection relative to *R. catesbeiana*. Additionally, hosts with greater snout–vent length were less likely to be infected ($\beta = -0.40 \pm 0.10$). Finally, hosts in ponds with greater vertebrate richness, while controlling for host density, were marginally less likely to be infected ($\beta = -0.58 \pm 0.31$). Variance partitioning demonstrated that individual-level variables explained the most variation in ranavirus infection (species identity and snout–vent length; adjusted $R^2 = .04$; Table 3) followed by biotic variables

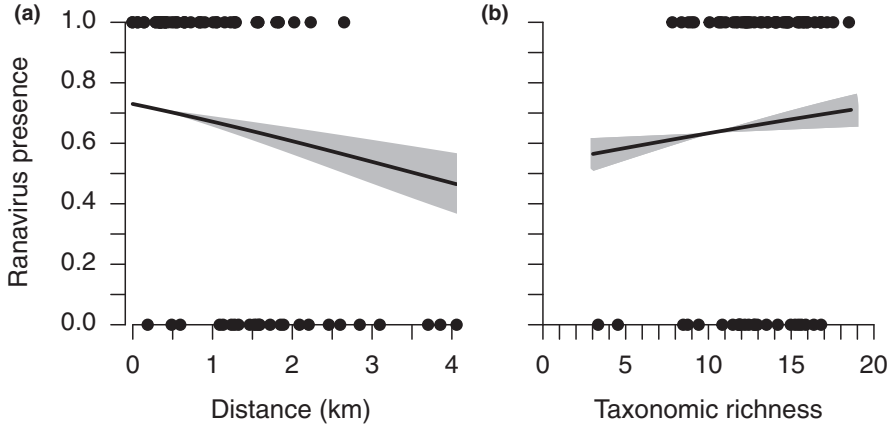

FIGURE 3 Model-averaged (eight models) predicted probability of site-level ranavirus presence (with 95% confidence bands; $n = 76$) in amphibian assemblages in the East Bay region of California in 2013 with increasing (a) distance to nearest ranavirus-infected pond (distance, km), and (b) taxonomic richness in ponds in 2013. Points for taxonomic richness are jittered to reduce overlap

TABLE 3 Results of variance partitioning analyses quantifying the amount of unique variation (adjusted R^2) attributed to landscape, abiotic, biotic and individual-level (individual) variables, and the shared variation between and among the variable subsets, for site-level ranavirus presence and individual-level ranavirus infection. Individual-level variables were only included in individual-level analyses, and probability values can only be calculated for landscape, abiotic, biotic and individual-level components. An asterisk (*) and bold font indicate $p < .01$, and two asterisks (**) and bold font indicate $p < .001$ for that comparison

	Ranavirus	
	Presence	**Infection**
Spatial (S)	**0.190****	
Abiotic (A)	−0.007	0.002
Biotic (B)	**0.086***	**0.029****
Individual (I)		**0.043****
SA	0.105	
SB	−0.007	
AB	−0.002	0.034
AI		0.003
BI		0.008
ABI		0.029
SAB	0.015	
Residuals	0.621	0.853

(*R. catesbeiana* presence, taxonomic richness and vertebrate richness; adjusted $R^2 = .03$).

After accounting for model covariates, no spatial autocorrelation was observed for ranavirus presence in site-level observations based on Moran's I ($p = .865$). Additionally, residuals for ranavirus presence and infection models with the most support were not spatially autocorrelated based on Moran's I ($p > .792$). Collinearity between predictor variables was low; however, and as expected, collinearity was highest between distance to nearest ranavirus-infected pond and the per cent wetland surrounding ponds in both analyses ($\rho = 0.64$ and 0.61). Variance inflation factors (VIFs) for all predictor variables in ranavirus presence and infection global models indicated low multicollinearity among variables (VIFs < 2.27). Overdispersion was not observed in site-level ranavirus presence and individual-level infection global models (dispersion parameters < 1).

4 | DISCUSSION

For any infectious disease, it is critical to identify the landscape and environmental factors that influence the distribution of the pathogen. This information can advance our understanding of disease emergence and strategies for management and conservation. Here, we examined the factors underlying patterns in site-level ranavirus presence and individual-level ranavirus infection in amphibian assemblages with comprehensive field surveillance data. Ranavirus was widespread throughout our study site, and our analyses demonstrated that site- and individual-level patterns in ranavirus epidemiology were more strongly associated with landscape and biotic factors (aspects of species richness) than abiotic factors.

At the landscape level, ponds in closer proximity to ranavirus-positive ponds were more likely to support ranavirus and have higher infection prevalence. To date, the influence of landscape processes on ranavirus dynamics is poorly understood. Disease risk might be greatest for ponds near other infected ponds, which has been found in other amphibian disease systems. For example, the movement of the pathogenic fungus Bd through amphibian assemblages across the landscape suggests that dispersal probably plays an important role (Laurance et al., 1996; Lips et al., 2008; Vredenburg et al., 2010). Previous research has found equivocal results related to the spatial clustering of ranavirus-associated mortality events

TABLE 4 Model-averaged coefficients for centred and scaled predictor variables from a subset of models (delta AICc < 4 points, 37 of 256 models) of individual-level ranavirus infection in amphibian assemblages in the East Bay region of California in 2013. Coefficients are arranged by ascending *p*-value, then alphabetically. "Num. mod." is the number of models that include that predictor variable, "Importance" is proportion of models within the model subset that contain that variable, "*SE*" is standard error, and "Adj. *SE*" is adjusted standard error. Coefficients with $p \leq .05$ are shaded in grey

Variable	Num. mod.	Importance	Estimate	*SE*	Adj. *SE*	*z*	*p*
Snout–vent length	37	1.00	−0.40	0.10	0.10	4.11	<.001
Spp. identity—*A. boreas*	37	1.00	2.75	0.62	0.62	4.44	<.001
Spp. identity—*H. regilla*	37	1.00	2.24	0.61	0.61	3.69	<.001
Spp. identity—*R. catesbeiana*	37	1.00	−2.09	0.74	0.74	2.80	<.001
Spp. identity—*T. granulosa*	37	1.00	2.99	0.69	0.69	4.34	<.001
Spp. identity—*T. torosa*	37	1.00	1.82	0.61	0.61	2.98	.003
Vertebrate richness	24	0.71	−0.58	0.31	0.31	1.86	.061
Taxonomic richness	21	0.52	−0.40	0.28	0.28	1.44	.156
Per cent forest	19	0.51	−0.41	0.29	0.29	1.41	.156
Pond permanence	18	0.39	−0.66	0.61	0.62	1.08	.281
R. catesbeiana presence	12	0.23	−0.29	0.72	0.72	0.41	.675
Sampling date	11	0.20	0.05	0.16	0.16	0.36	.720

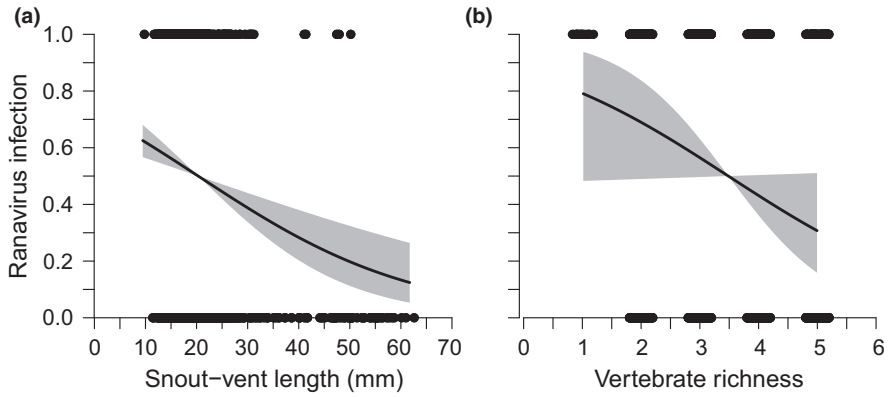

FIGURE 4 Model-averaged (37 models) predicted probability of individual-level ranavirus infection (with 95% confidence bands; n = 1,088) in amphibian assemblages in the East Bay region of California in 2013 with increasing (a) snout–vent length and (b) vertebrate richness. Points for vertebrate richness are jittered to reduce overlap

(Gahl & Calhoun, 2008; North et al., 2015). Movement of infected amphibians among ponds could distribute ranavirus from infected ponds to other nearby ponds. Amphibians can metamorphose from ponds with ranavirus infections and the returning adults can harbour infections (Brunner, Schock, Davidson, & Collins, 2004). For instance, a reconstructed ranavirus emergence event in the U.K. demonstrated a localised spread from nearby ponds with distances spread similar to known amphibian and frog dispersal distances (Price et al., 2016). While this suggests that infected hosts can move ranaviruses across the landscape, the movement patterns of infected hosts have not been explored. Given that the dispersal ability of most amphibians is relatively limited (Blaustein, Wake, & Sousa, 1994; Wells, 2010), the probability of infected hosts reaching distant ponds is relatively low. In our study, there was a ~20% reduction in ranavirus presence at about 2 km.

Ponds near ranavirus-positive ponds might have more frequent introductions of the virus into the system, thereby increasing exposure and infection probabilities. Movement of other taxa (e.g., reptiles, birds, humans), either via sublethally infected hosts or uninfected taxa transporting ranaviruses on their surfaces, could also distribute ranaviruses across the landscape (reviewed in Brunner et al., 2015). However, the transfer of ranaviruses on the surface of uninfected taxa might be rare given that ranavirus can be rapidly degraded in the environment by naturally occurring plankton and microbes (Johnson & Brunner, 2014) and when wetland drying occurs (Brunner, Schock, & Collins, 2007). Ranaviruses could also be distributed across the landscape when rain events and flooding occur, which can connect nearby wetlands through the movement of water. Future research examining the movement of ranavirus-infected hosts and other sources of ranavirus dispersal among wetlands will provide critical information on how ranaviruses move across the landscape and influence disease risk.

The influence of biodiversity on disease risk has been a major focus of recent disease ecology research (Johnson, Ostfeld, & Keesing, 2015; Keesing et al., 2006). Although rarely considered in ranavirus studies, we found that factors related to species richness were associated with ranavirus patterns. In our study, taxonomic richness correlated positively with the probability of ranavirus presence at the site level, whereas vertebrate richness correlated negatively with individual-level ranavirus infection prevalence. Greater taxonomic richness could increase the likelihood that ranavirus is introduced into a wetland (e.g., via mobile taxa) or the probability of successfully establishing in a species, as also found in other studies of parasites (e.g., Johnson, Preston, Hoverman, & Lafonte, 2013; Johnson et al., 2016; Rottstock, Joshi, Kummer, & Fischer, 2014). Additionally, more diverse wetlands might support more potential reservoirs for ranavirus infection; however, there was no evidence that fishes or *R. catesbeiana* were associated with patterns in ranavirus infection. The negative association between vertebrate richness and infection is suggestive of a dilution effect, which has been observed in other amphibian disease systems (trematodes and Bd; Johnson, Preston, Hoverman, & Lafonte, 2013; Rohr et al., 2015; Searle, Biga, Spatafora, & Blaustein, 2011; Venesky, Liu, Sauer, & Rohr, 2014), yet our field data lack estimates of transmission within the communities to confirm this mechanism. Moreover, whether diversity inhibits transmission and subsequent disease risk often depends strongly on the type of transmission involved (e.g., density-dependent or density-independent) as well as whether communities assemble additively or substitutively (i.e., does total host abundance increase with diversity or remain constant?; Dobson, 2004; Johnson, Ostfeld, et al., 2015; Mihaljevic, Joseph, Orlofske, & Paull, 2014). Further research would be required to investigate these points specifically for ranaviruses, as well as to obtain more high-resolution estimates of infection over time. These are essential data for quantifying field-based transmission patterns, but are limited for wild populations (Brunner et al., 2015). Some prior investigations of ranavirus in amphibians suggest that transmission could be density-dependent or density-independent (Brunner et al., 2007, 2015; Greer, Briggs, & Collins, 2008). Because this is the first study to document associations between species richness and ranavirus dynamics, the mechanisms underlying these patterns require further investigation.

Although environmental stressors have frequently been hypothesised as drivers of ranavirus epidemiology (Brunner et al., 2015; Gray, Miller, Schmutzer, & Baldwin, 2007; Greer & Collins, 2008), we found no significant interactions between ranavirus occurrence and the factors representing environmental stressors that we measured in this study. For instance, factors associated with cattle (e.g.,

cattle presence, reduced shoreline vegetation, increased ammonia) did not influence ranavirus presence or infection in our analyses. Additionally, there was no association with the amount of forest surrounding the ponds. Lastly, there was no evidence that non-native *R. catesbeiana* or fishes contributed to ranavirus patterns, despite the postulated importance of these groups as reservoirs of ranavirus and other amphibian pathogens in other regions (Brunner et al., 2015).

Individual-level factors, such as amphibian species identity, were important in explaining infection prevalence. *Rana catesbeiana* exhibited the lowest likelihood of infection among the five species sampled in these ponds. *Rana catesbeiana* had only 16% overall infection prevalence, even after accounting for site-level differences. This outcome is complemented by findings from laboratory experiments where *R. catesbeiana* were relatively resistant to ranavirus infection compared to other amphibian species (Hoverman, Gray, Haislip, & Miller, 2011). For the remaining species in the assemblage, there is a need to conduct experimental studies examining their susceptibility to ranaviruses. The total number of amphibians sampled and examined for ranavirus, as well as the species composition of sampled amphibian communities, might also influence ranavirus presence and infection. These variables were not strongly influential in our final models, but might influence the likelihood of ranavirus presence and infection at the site and individual level. Future studies should investigate how variation in these biotic variables influences ranaviral disease dynamics.

We observed that larger host body size (greater snout–vent length) was negatively associated with the probability of ranavirus infection, even after accounting for species-level differences in body size. This observation coincides with an observation that body size was negatively associated with Bd infection (Gervasi et al., 2017) and frequent observations that juveniles might be more prone to infection than adults (i.e., with larger body sizes) in amphibians and fishes (Ariel & Owens, 1997; Cullen & Owens, 2002; Cullen, Owens, & Whittington, 1995; Jensen, Holopainen, Tapiovaara, & Ariel, 2011). Larger body size may be an indicator of a more developed immune system, which could prevent infections from establishing (Gervasi et al., 2017; Miller, Gray, & Storfer, 2011). Future field- and laboratory-based studies investigating relationships among size, development and ranavirus infection will undoubtedly benefit our understanding of ranavirus infection in amphibians.

5 | CONCLUSIONS

Despite more than a decade of research on ranavirus–amphibian interactions, our understanding of the factors underlying ranavirus epidemiology in natural systems remains limited. While numerous factors have been proposed as drivers of infection, it still remains unclear why the outcome of a ranavirus outbreak can vary from no obvious mortality to a massive die-off event (Brunner et al., 2015). Moreover, the predominant focus on ranavirus-associated mortality events has failed to capture baseline epidemiological patterns across the landscape. Using a data set from 76 ponds, five amphibian species and 1,376 individuals, our results illustrate that multiple factors explained ranavirus epidemiology in our system. In particular, landscape factors explained more variance at higher biological levels (site level) while biotic and individual-level factors explained more variance at lower biological levels (individual level). Our findings are similar to those suggested for other disease systems and highlight the importance of investigating factors influencing disease epidemiology at multiple biological levels (Cohen et al., 2016; Johnson, De Roode, et al., 2015; Schotthoefer et al., 2011). Several variables such as cattle presence and water chemistry parameters, which are often cited to influence ranavirus epidemiology (Forson & Storfer, 2006a,b; Kerby & Storfer, 2009; Kerby et al., 2011), were not influential in our study. Additionally, the variables we included in our analyses explained scant variability in ranavirus presence and infection. Therefore, further experimental and field-based investigations of proposed and novel factors will undoubtedly help broaden our understanding of the dynamics of this emerging infectious pathogen and benefit management and conservation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

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