The influence of landscape and environmental factors on ranavirus epidemiology in amphibian assemblages

Running title: *Ranavirus epidemiology in amphibians*

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ABSTRACT

Aim To quantify the influence of a suite of landscape, abiotic, biotic, and host-level variables on ranavirus disease dynamics in amphibian assemblages at two biological levels (site and host-level).

Location Wetlands within the East Bay region of California, USA.

Methods We used competing models, multimodel inference, and variance partitioning to examine the influence of 16 landscape and environmental factors on patterns in site-level ranavirus presence and host-level ranavirus infection in 76 wetlands and 1,377 amphibian hosts representing five species.

Results The landscape factor explained more variation than any other factors in site-level ranavirus presence, but biotic and host-level factors explained more variation in host-level ranavirus infection. At both the site- and host-level, the probability of ranavirus presence correlated negatively with distance to nearest ranavirus-positive wetland. At the site-level, ranavirus presence was associated positively with taxonomic richness. However, infection prevalence within the amphibian population correlated negatively with vertebrate richness. Finally, amphibian host species differed in their likelihood of ranavirus infection: American Bullfrogs had the weakest association with infection while Western Toads had the strongest. After accounting for host species effects, hosts with greater snout-vent length had a lower probability of infection.

Main conclusions Strong spatial influences at both biological levels suggest that mobile taxa (e.g., adult amphibians, birds, reptiles) may facilitate the movement of ranavirus among hosts and across the landscape. Higher taxonomic richness at sites may provide more opportunities for colonization or the presence of reservoir hosts that may influence ranavirus presence. Higher

host richness correlating with higher ranavirus infection is suggestive of a dilution effect that has been observed for other amphibian disease systems and warrants further investigation. Our study demonstrates that an array of landscape, environmental, and host-level factors were associated with ranavirus epidemiology and illustrates that their importance vary with biological level.

Key words amplification effect, disease dynamics, dilution effect, dilution host, emerging infectious diseases, generalized linear model, multimodel inference, Iridovirus, reservoir species

INTRODUCTION

Infectious diseases are increasingly recognized 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 *et al.*, 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 is cited as a major influence on vector-borne diseases (Githeko *et al.*, 2000; Rogers & Randolph, 2006; Rohr *et al.* 2011; Mordecai et al. 2017), flooding can influence the prevalence of cholera (reviewed in Ahern *et al.*, 2005), and loss of biodiversity can influence the prevalence of Lyme disease (Ostfeld & Keesing, 2000; Keesing *et al.*, 2006; Keesing *et al.*, 2010). 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 (Rohr *et al.* 2015.

Recent studies have highlighted the importance of investigating the influence of factors at multiple biological levels of organization because of contrasting results between levels (e.g., site- versus individual-level; Borcard *et al.*, 2004; Dunn *et al.*, 2010; Schotthoefer *et al.*, 2011; Liu *et al.* 2013; Johnson *et al.*, 2015a; Cohen *et al.*, 2016). It has been hypothesized that abiotic factors influence distributional patterns at larger levels whereas biotic factors (e.g., species interactions) influence distributional patterns at smaller levels (Wiens, 1989; Levin, 1992; Rahbek, 2004; McGill, 2010; Cohen *et al.*, 2016). 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*), but biotic factors were more important at smaller 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 *et al.*, 1996; Lips *et al.*, 2008; Rohr *et al.* 2008; Vredenburg *et al.*, 2010; Liu *et al.* 2013). Therefore, evaluating which factors are most influential to the distribution of diseases, and at what levels of organization, is important to gain a clear understanding of what controls the spread of diseases among hosts *and* across the landscape.

Ranaviruses 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 *et al.*, 2015). For example, abiotic factors such as land use (e.g., cattle grazing and urbanization), 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; Forson & Storfer, 2006b; Kerby & Storfer, 2009; Kerby *et al.*, 2011; North *et al.*, 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 wetland characteristics on ranavirus occurrence or prevalence (Hoverman *et al.*, 2012a), 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*) and fish are implicated as potential reservoirs for the pathogen (Brunner *et al.*, 2015). It has also been hypothesized that predators can increase disease risk by inducing physiological stress that compromises immune function (Reeve *et al.*, 2013). Thus, while there are many hypothesized 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 *et al.*, 2012a; North *et al.*, 2015). Given that amphibians are often characterized 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 either detected by scientists or members of the public. This non-random selection of samples provides only sparse insight into the baseline epidemiology of ranaviruses in amphibian populations or 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 93 wetlands 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. By collecting data from multiple amphibian host species and at both the individual and population (wetland) levels, 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. To determine the relative influence of landscape, abiotic, and biotic factors on ranavirus, we used model selection and multi-model averaging followed by variance partitioning, thereby allowing us to assess the joint effects of hypothesized covariates and how they varied between the site-level and individual host-level.

METHODS

Study area and species

We examined patterns of ranavirus presence and infection in wetland amphibian

assemblages located in the East Bay region of California (Figure 1; Hoverman *et al.*, 2012b; Johnson *et al.*, 2013b; Richgels *et al.*, 2013). We sampled 93 wetlands in managed parks and preserves within three counties (i.e., Alameda, Contra Costa, and Santa Clara; Johnson *et al.*, 2016). We selected wetlands that were smaller (< 2 ha) and likely to contain amphibian assemblages (Hoverman *et al.*, 2012b). Visitation to wetlands was haphazard, but was not spatiotemporally randomized because of logistical constraints. 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.

Field sampling, assessing ranavirus infection, and determining environmental variables

We conducted field surveys from May–August 2013 using the field sampling protocols of Hoverman et al. (2012b). In brief, we used a combination of visual encounter surveys, dipnet sweeps, and habitat-stratified seine hauls to sample the wetlands (Johnson *et al.*, 2013b; Richgels *et al.*, 2013). We disinfected all gear (e.g., nets and waders) with 15% bleach between sites. In the field, we identified amphibians to species, fishes to genus or species, and macroinvertebrates to order, family, or genus (Supplementary Table S1). At each wetland, we randomly selected up to 20 individuals (larvae, metamorphs, or both) per species for ranavirus screening. We necropsied individuals and sampled a portion of kidney and liver tissue for ranavirus. Equipment was flame sterilized between individuals. For each individual, ranaviral DNA was extracted from the combined liver and kidney tissue sample and infection was determined using standard

177 quantitative PCR protocols (Forson & Storfer, 2006b).

178 We used an array of landscape, abiotic, and biotic predictor variables to represent
179 environmental influences on ranavirus dynamics guided by theory and previous investigations
180 (Table 1). Our landscape variable was distance to nearest ranavirus-infected wetland (other than
181 the wetland the individual was found in). To calculate this distance, we recorded latitude and
182 longitude of each site and measured Euclidean distance to nearest ranavirus-infected wetland
183 using the R function 'dist'. From the generated distance matrix, we deleted columns representing
184 distances of each wetland to uninfected wetlands, and sorted to isolate distance to nearest
185 ranavirus-infected wetland for each wetland and individual within each wetland. This method is
186 limited in that not all wetlands in the landscape were sampled; thus, ranavirus-positive sites
187 could occur, but not have been visited. However, our sampling scheme sought to sample all
188 neighboring wetlands within a contiguous area (e.g., a park or preserve), such that these
189 estimates are likely to capture general patterns related to colonization potential.

190 We assessed wetland permanence (permanent or temporary), percent forest or wetland
191 surrounding wetlands, wetland area, and water quality factors at each site. We asses wetland
192 permanence (permanent or temporary) based on water depth, wetland area, and with additional
193 verification from historical images in Google Earth (Johnson *et al.*, 2013c). We measured
194 conductivity (S/m), total dissolved solids (mg/l), salinity (mg/l), and pH with a YSI meter
195 (Model 556; Yellow Spring Instrument, Yellow Springs, Ohio, USA). We quantified total
196 nitrogen (mg/l), dissolved organic carbon (mg/l), and total ammonia (mg/l) using standard
197 methods (http://snobear.colorado.edu/Kiowa/Kiowaref/procedure.html; Johnson *et al.*, 2013c).
198 We used PCA to reduce dimensionality of the seven abiotic water-quality variables that we
199 measured. Water-quality variables, except pH, were log-transformed to reduce positive

skewness, and scaled and centered, 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 (Supplementary Table S2; Yeomans & Golder, 1982; Kindt & Coe, 2005; 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, to a lesser extent, pH (0.14). We calculated the percentage of area within a 1-km radius of each wetland classified as forested (sum of all forest types) and wetland (open water) using ArcGIS and the National Landcover Database (Johnson *et al.*, 2013b; Homer *et al.*, 2015) because of our interest in the influence of intact forest and wetlands surrounding focal wetlands. We calculated wetland 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 percent vegetation cover on wetland shorelines (hereafter, percent shoreline vegetation), taxonomic richness, vertebrate richness, amphibian catch per unit effort (herein, CPUE), and the presence or absence of fishes, cattle, and non-native *R. catesbeiana*. We visually estimated percent 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 CPUE by counting the number of individuals of each amphibian species during dip net sweeps and dividing by number of sweeps completed. We also included snout-vent length (mm), and species identity (*H. regilla*, *A. boreas*, *R. catesbeiana*, *T. torosa*, or

223 *T. granulosa*) into host-level analyses. Snout-vent length was scaled and centered among species

224 *Data analysis*

225 Our response variable for site-level analyses was ranavirus presence defined as one or
226 more amphibians of any species infected with ranavirus within a wetland. We excluded wetlands
227 with incomplete environmental data. Our response variable for host-level analyses was ranavirus
228 infection defined as an individual having detectable ranavirus infection. We limited our ranavirus
229 infection analyses only to wetlands where ranavirus was detected, which included infected and
230 non-infected individuals. Therefore, we removed individuals from sites where ranavirus was not
231 detected.

232 We assessed the influence of predictor variables on ranavirus presence and infection in
233 amphibian assemblages with generalized linear models fitted with a binomial distribution and
234 logit link. We conducted all analyses in program R v3.3.1 (R Development Core Team, 2015).
235 We included base-10 log-transformed total number of individuals examined for ranavirus at each
236 site as a fixed term to account for differences in the number of animals examined, which was
237 expected to influence detection likelihood. For analyses of host-level infection, we used mixed
238 effects models using the R package 'lme4' (Zuur *et al.*, 2009; Bates *et al.*, 2014) in which site
239 was a random intercept term, thereby allowing us to nest observations from different amphibian
240 species within the same site. We modeled host-level infection status (infected or not infected) to
241 allow us to incorporate both host-level (e.g., body size) as well as site-level covariates
242 (landscape, abiotic, and biotic). To keep models tractable, we initially used univariate analysis to
243 identify associations between specific predictors variables and site-level ranavirus presence and
244 host-level ranavirus infection. For univariate variable selection analyses, we used mixed model
245 forms mentioned above (compared to correlations). Predictor variables with P-values < 0.10

246 from these univariate analyses were combined together into a global model. We centered and
247 scaled all continuous predictor variables to facilitate comparison of coefficients among predictor
248 variables and improve numerical stability. For snout-vent length of amphibian hosts, we centered
249 and scaled within each species to account for differences in snout-vent length among species. We
250 did not include interaction terms in global models because we did not hypothesize strong
251 interactions between or among predictor variables, and to keep models tractable. We tested for
252 collinearity between predictor variables included in the global models using Pearson's
253 correlation coefficients, and tested for multicollinearity among predictor variables in both global
254 models with variance inflation factors with the R package 'car'. We also calculated dispersion
255 parameters to examine overdispersion in global models for ranavirus presence and prevalence.
256 Additionally, we estimated the variance in site-level ranavirus presence and host-level ranavirus
257 infection accounted for by landscape, abiotic, biotic, or individual variables in global models
258 with the 'varpart' function in the R package 'vegan' (Borcard *et al.*, 1992; Schotthoefer *et al.*,
259 2011). We used the dredge function in the R package 'MuMIn' to create a set of all possible sub-
260 models from ranavirus presence and infection global models and determine the best-supported
261 model from the subset of predictor variables (Bartón, 2010). We compared models separately for
262 ranavirus presence and infection analyses with an information-theoretic approach using Akaike's
263 Information Criterion (AIC; Burnham & Anderson, 2004; Mazerolle, 2016). We used AIC
264 corrected for small sample sizes (AIC_C) for both analyses because the number of observations
265 divided by number of parameters was low for most ranavirus presence models ($n/K < 40$;
266 Anderson & Burnham, 2002; Burnham & Anderson, 2004). Moreover, it is generally
267 recommended to use AIC_C because it converges to AIC with large samples sizes such as we
268 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). 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 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' (Borcard *et al.*, 1992; Schotthoefer *et al.*, 2011; Bivand, 2013).

RESULTS

Sampling overview

In total, our site-level analyses included 76 wetlands and 1,377 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 host-level covariate data, or both. The most common amphibian species among wetlands were *H. regilla* and *T. torosa*, and most sites (35%) had three amphibian species (Fig. 2). Thirty-two percent of tested amphibians were positive for ranavirus (*n* = 441 of 1,377). At least one infected individual occurred at 67% of wetlands (*n* = 51 of 76) and an average of 61% of individuals were infected at wetlands with ranavirus infection (95% CI = 53–68%). For host-level analyses, we removed 288 individuals from 25 sites where ranavirus was not present; thus, we reduced our host-level sample size to 1,089 individuals. The percentage of infected individuals at wetlands 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* =

292 22) of wetlands and fish presence (i.e., *Gambusia affinis*, *Lepomis macrochirus*, *Carassius*
293 *auratus*, *Ictalurus* spp., or *Micropterus* spp.) was observed at 26% of wetlands (n = 20).
294
295 *Model selection and multimodel inference*
296 Univariate analyses determined that landscape (distance to nearest ranavirus-infected
297 wetland), abiotic (percent wetland), and biotic (CPUE and taxonomic richness) were associated
298 with, and included in the global model for, site-level ranavirus presence. For host-level ranavirus
299 infection, univariate analyses demonstrated that landscape (distance to nearest ranavirus-infected
300 wetland), abiotic (percent wetland), biotic (*R. catesbeiana* presence and vertebrate richness), and
301 host-level (snout-vent length and species identity) were associated with, and included in the
302 global model for, host-level ranavirus infection. From the global models, we produced 16 total
303 models comprised of four landscape and abiotic variables for site-level analysis of ranavirus
304 presence and 64 total models comprised of six landscape, abiotic, biotic, and host-level variables
305 for host-level analysis of ranavirus infection using the dredge function in R (Supplementary
306 material Appendix 1, Tables A3 and A4). For site-level ranavirus presence analysis, four models
307 were within 4 AIC_C of the best-supported model (Supplementary material Appendix 1, Table
308 A5). For host-level ranavirus infection analysis, eight models were within 4 AIC_C of the best-
309 supported model (Supplementary material Appendix 1, Table A6).
310 Landscape and biotic variables had the strongest associations with site-level ranavirus
311 presence in our best-supported models (Table 2). Distance to nearest ranavirus-infected wetland
312 and taxonomic richness were included in all best-supported models, while CPUE and nearby
313 wetland area were only included half of the best supported-models. Wetlands that were farther
314 from the nearest ranavirus-infected wetland had a lower likelihood of ranavirus presence (β = -

315 0.26 ± 0.05 [model-averaged coefficient ± adjusted SE]; Fig. 3). Wetlands with greater
316 taxonomic richness had a higher likelihood of ranavirus presence (β = 0.12 ± 0.04). Variance
317 partitioning analyses demonstrated that the landscape variable, distance to nearest ranavirus-
318 infected wetland, explained the most variance (adjusted R^2 from variance partitioning = 0.18)
319 and the biotic variable, taxonomic richness, explained a smaller portion of variance (R^2 = 0.09)
320 in site-level ranavirus presence (Table 3).

321 The best-supported models for host-level ranavirus infection prevalence included
322 landscape, abiotic, biotic, and host-level predictor variables (Table 4). Distance to nearest
323 ranavirus-infected wetland, snout-vent length, species identity, and vertebrate richness had the
324 strongest associations with ranavirus infection. Hosts in wetlands that were further from the
325 nearest ranavirus-infected wetland had the lowest likelihood of ranavirus infection (distance β = -
326 1.40 ± 0.38; Fig. 4). Hosts in wetlands with greater vertebrate richness, while controlling for host
327 density, were less likely to be infected (β = -0.61 ± 0.31). Additionally, species differed in their
328 likelihood of ranavirus infection. *Rana catesbeiana*, which was the reference level in the species
329 identity variable, had the lowest likelihood of ranavirus infection *(*β = -4.09 ± 0.90; Fig. 5).
330 *Taricha torosa* (β = 2.66 ± 0.84), *P. regilla* (β = 3.02 ± 0.84), *A. boreas* (β = 3.72 ± 0.85), and *T.*
331 *granulosa* (β = 4.03 ± 0.92) had higher likelihood of ranavirus infection relative to *R.*
332 *catesbeiana*. Finally, hosts with greater snout-vent length were less likely to be infected (β = -
333 0.40 ± 0.11). Variance partitioning demonstrated that the biotic variable, taxonomic richness
334 explained the most variation in ranavirus infection at the host-level (adjusted R^2 = 0.06) followed
335 by landscape (adjusted R^2 = 0.04) and host-level variables (species identity and snout-vent
336 length; adjusted R^2 = 0.03; Table 3).

337 No spatial autocorrelation was observed for ranavirus presence (*P* = 0.865) in site-level

observations based on Moran's I. Additionally, residuals for ranavirus presence and infection models with the most support were not spatially autocorrelated based on Moran's I ($P > 0.792$). Collinearity between predictor variables was low; however, and as expected, collinearity was highest between distance to nearest ranavirus-infected wetland and the amount of nearby wetland area 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 host-level infection global models (dispersion parameters < 1).

DISCUSSION

For any infectious disease, it is critical to identify the landscape and environmental factors that influence the distribution of the pathogen to develop a broader understanding of disease emergence and strategies for management and conservation. Here, we examined the landscape and environmental factors underlying patterns in site-level ranavirus presence and host-level ranavirus infection in amphibian assemblages in the East Bay region of California during 2013. We used comprehensive field surveillance data, rather than observations of mortality events commonly used to describe patterns in ranavirus disease dynamics, and model selection with multimodel inference to determine ranavirus epidemiology in the assemblage. Ranavirus was widespread throughout our study site and our analyses demonstrated that site- and host-level patterns in ranavirus epidemiology were more strongly associated with landscape and biotic factors (aspects of species richness), rather than abiotic factors.

At the landscape level, wetlands in closer proximity to ranavirus-positive wetlands 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

361 for wetlands in close proximity to other infected wetlands, which has been found in other
362 amphibian disease systems. For example, the movement of the pathogenic fungus Bd through
363 amphibian assemblages across the landscape suggests that dispersal probably plays an important
364 role (Laurance *et al.*, 1996; Lips *et al.*, 2008; Rohr *et al.* 2008; Vredenburg *et al.*, 2010; Liu *et al.*
365 2013). Previous research has found equivocal results related to the spatial clustering of ranavirus-
366 associated mortality events (Gahl & Calhoun, 2008; North *et al.*, 2015). Our findings suggest
367 that the movement of infected amphibians among wetlands could distribute ranavirus from
368 infected wetlands to other nearby wetlands. Amphibians can metamorphose from wetlands with
369 ranavirus infections and the returning adults can harbor infections (Brunner *et al.*, 2004). For
370 instance, a reconstructed ranavirus emergence event in the U.K. demonstrated a localized spread
371 from nearby ponds with distances spread similar to known amphibian and frog dispersal
372 distances (Price *et al.*, 2016). While this suggests that infected hosts can move ranavirus across
373 the landscape, the movement patterns of infected hosts have not been explored. Given that the
374 dispersal ability of most amphibians is relatively limited (Blaustein *et al.*, 1994; Wells, 2010),
375 the probability of infected hosts reaching distant wetlands is relatively low. In our study, there
376 was a 20 and 60% reduction in ranavirus presence and infection, respectively, at about 2 km.
377 Wetlands in close proximity to ranavirus-positive wetlands might have more frequent
378 introductions of the virus into the system thereby increasing exposure and infection probabilities.
379 Movement of other taxa (e.g., reptiles, birds, humans) either via sublethally infected hosts or
380 immune taxa transporting ranavirus on their surfaces could also distribute ranavirus across the
381 landscape (reviewed in Brunner *et al.*, 2015). However, the transfer of ranavirus on the surface
382 of immune taxa might be rare given that ranaviruses can be rapidly degraded in the environment
383 by naturally occurring plankton and microbes (Johnson & Brunner, 2014) and when wetland

384 drying occurs (Brunner *et al.*, 2007). Ranavirus could also be distributed across the landscape
385 when rain events and flooding occur, which can connect nearby wetlands through the movement
386 of water. Future research examining the movement of ranavirus-infected hosts and other sources
387 of ranavirus dispersal among wetlands will provide critical information on how ranavirus moves
388 across the landscape and influences disease risk.

389 The influence of biodiversity on disease risk has been a major focus of recent disease
390 ecology research (Keesing *et al.*, 2006; Civitello *et al.* 2015; Johnson *et al.*, 2015b). Although
391 rarely considered in ranavirus studies, we found that biotic factors broadly related to species
392 richness were associated with ranavirus patterns. In our study, taxonomic richness correlated
393 positively with the probability of ranavirus presence at the site-level whereas vertebrate richness
394 was correlated negatively with host-level ranavirus infection prevalence. Greater taxonomic
395 richness could increase the likelihood that ranavirus is introduced into a wetland (e.g., via mobile
396 taxa) or the probability of successfully establishing in a species, as also found in other studies of
397 parasites (e.g., Johnson *et al.*, 2013a; Rottstock *et al.*, 2014; Johnson *et al.*, 2016). Additionally,
398 more diverse wetlands might support potential reservoirs for ranavirus infection, although there
399 was no evidence that fish or non-native Bullfrog were associated with patterns in ranavirus
400 infection. The negative association between vertebrate richness and infection is suggestive of a
401 dilution effect, yet our field data lack estimates of transmission within the communities to
402 confirm this mechanism. The dilution effect has been observed in other amphibian disease
403 systems (trematodes and B. dendrobatidis; Searle *et al.*, 2011; Johnson *et al.*, 2013a; Venesky *et*
404 *al.*, 2014a,b; Rohr *et al.*, 2015) and therefore might also occur for ranavirus. Because this is the
405 first study to document associations between species richness and ranavirus dynamics, the
406 mechanisms underlying these patterns are in need of further investigation with controlled

407 experiments.

408 Although environmental stressors have frequently been hypothesized as drivers of
409 ranavirus epidemiology (Gray *et al.*, 2007; Greer & Collins, 2008; Brunner *et al.*, 2015), we
410 found no significant interactions between ranavirus occurrence and the factors representing
411 environmental stressors that we measured in this study. For instance, factors associated with
412 cattle (i.e. cattle presence, reduced shoreline vegetation, increased ammonia) did not influence
413 ranavirus presence or infection in our analyses. Additionally, there was no association with the
414 amount of forest surrounding the wetlands, which functions as an indicator of habitat integrity.
415 Lastly, there was no evidence that non-native *R. catesbeiana* or fishes contributed to ranavirus
416 patterns, despite the postulated importance of these groups as reservoirs of ranavirus and other
417 amphibian pathogens in other regions (Brunner *et al.*, 2015).

418 Host-level factors such as amphibian species identity were also a major factor in
419 explaining infection prevalence. *Rana catesbeiana* exhibited the lowest likelihood of infection
420 among the five species sampled in these wetlands. *Rana catesbeiana* had only 3% overall
421 infection prevalence, even after accounting for site-level differences. Using *R. catesbeiana* as the
422 references species, infection tended to be higher in the remaining species. Our findings are
423 similar to previous laboratory experiments where *R. catesbeiana* were relatively resistant to
424 ranavirus infection compared to other amphibian species (Hoverman *et al.*, 2011). For the
425 remaining species in the assemblage, there is a need to conduct experimental studies examining
426 their susceptibility to ranavirus. Preliminary results from our research group have found high
427 levels of susceptibility to infection and high mortality in *H. regilla,* and moderate infection and
428 mortality in *A. boreas* (N.M. Hambalek, personal communication).

429 We observed that larger host body size (greater snout-vent length) reduced 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 or Bd-induced death (Rohr *et al.* 2013; Gervasi *et al.*, 2017), but positive (Raffel *et al.* 2013; McMahon *et al.* 2014) and non-linear relationship (Raffel *et al.* 2010) have also been observed. It also coincides with frequent observations that juveniles might be more prone to infection than adults (i.e., with larger body sizes) in amphibians and fishes (Cullen *et al.*, 1995; Ariel & Owens, 1997; Cullen & Owens, 2002; Jensen *et al.*, 2011). Larger body size may be an indicator of a more-developed immune system, which could prevent infections from establishing (Miller *et al.*, 2011; Gervasi *et al.*, 2017). Future field-based and experimental studies investigating relationships among size, development, and ranavirus infection will undoubtedly benefit our understanding of ranavirus infection in amphibians.

CONCLUSIONS

Despite decades 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 dataset from 76 wetlands, five amphibian species, and 1,377 hosts, our results illustrate that landscape and biotic factors were most important for explaining ranavirus epidemiology. In particular, landscape factors explained more variance at larger (site-level) biological levels while biotic and host-level factors explained more variance at smaller biological levels (host-level). Our findings are similar to those suggested for other disease distributions and highlight the importance of

453 investigating factors influencing disease epidemiology at multiple biological levels (Schotthoefer
454 *et al.*, 2011; Johnson *et al.*, 2015a; Cohen *et al.*, 2016). Several variables such as cattle presence
455 and water chemistry parameters, that are often cited to influence ranavirus epidemiology (Forson
456 & Storfer, 2006a; Forson & Storfer, 2006b; Kerby & Storfer, 2009; Kerby *et al.*, 2011), were not
457 influential in our study. Additionally, the variables we included in our analyses explained scant
458 variability in site-level ranavirus presence and host-level ranavirus infection. Therefore, further
459 experimental and field-based investigations of proposed and novel factors will undoubtedly help
460 broaden our understanding of the dynamics of this emerging infectious pathogen and benefit
461 management and conservation.

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680

SUPPORTING INFORMATION

Table S1. Sampled amphibian, fish, and macroinvertebrate species in the East Bay Region of California in 2013.

Table S2. Results of principal component analysis for seven abiotic water variables and 'broken-stick' test.

Table S3. Results of univariate variable selection for correlation between site-level ranavirus presence and 14 predictor variables.

Table S4. Results of univariate variable selection for correlation between host-level ranavirus infection and 16 predictor variables

Table S5. Summary statistics for the four top-ranked models for site-level ranavirus presence.

Table S6. Summary statistics for the eight top-ranked models for host-level ranavirus presence.

DATA ACCESSIBILITY STATEMENT

Data used for spatial analyses and mapping available from the National Land Cover Database (https://catalog.data.gov/dataset/national-land-cover-database-nlcd-land-cover-collection) and the State of California Geoportal (http://portal.gis.ca.gov/geoportal). Ranavirus infection and microhabitat data included in this study are available from the Pangea database (data deposited, will insert link when posted and after review).

BIOSKETCH

Brian Tornabene is a PhD Student at the University of Montana in Missoula, Montana, USA. His work focuses on natural history, ecotoxicology, and population and disease ecology—often

703 with herpetofauna. Currently, he is investigating the influence of brine contamination from oil
704 and gas development on amphibian communities.
705
706 **TABLES**
707 Table 1. Predictor variables included to investigate patterns in landscape (L), abiotic (A), biotic
708 (B), and host-level (H) influences on site-level ranavirus presence and host-level ranavirus
709 infection in amphibian assemblages in the East Bay region of California in 2013. Host influences
710 were only included in the host-level ranavirus infection analyses. Principal components 1 and 2
711 are the product of reducing the dimensionality of seven water quality parameters.

	Variable	Type
1	Distance to nearest ranavirus-infected wetland (km)	L
2	Percent forest surrounding	A
3	Percent wetland surrounding	A
4	Principal component 1	A
5	Principal component 2	A
6	Wetland area (m^2)	A
7	Wetland permanence (permanent or temporary)	A
8	Amphibian catch per unit effort	B
9	Cattle presence	B
10	Fish presence	B
11	Percent shoreline vegetation	B
12	*Rana catesbeiana* presence	B
13	Taxonomic richness	B

14	Vertebrate richness	B
15	Snout-vent length (mm)	H
16	Species identity	H

712
713

714 Table 2. Model-averaged coefficients for centered and scaled predictor variables from a subset of
715 models (delta AICc < 4 points, 4 of 16 models) of site-level ranavirus presence in amphibian
716 assemblages in the East Bay region of California in 2013. Coefficients are arranged by ascending
717 *P*-value, then alphabetically. "Distance" is distance to nearest ranavirus-infected wetland (km),
718 "Num. mod." is the number of models that include that predictor variable, "Importance" is
719 percent of models within the model subset that contain that variable, "SE" is standard error, and
720 "Adj. SE" is adjusted standard error. Coefficients with $P \leq 0.05$ are shaded in grey.

Variable	Num. mod.	Importance	Estimate	SE	Adj. SE	*z*	*P*
Distance	4	1.00	-0.26	0.05	0.05	5.24	< 0.001
Taxonomic richness	4	1.00	0.12	0.04	0.04	2.61	< 0.001
Catch per unit effort	2	0.57	0.09	0.05	0.05	1.65	0.099
Total dissected	4	1.00	0.03	0.06	0.06	0.59	0.554
Percent wetland	2	0.23	0.01	0.06	0.06	0.10	0.919

721

722 Table 3. Results of variance partitioning analyses quantifying the amount of unique variation
723 (adjusted R^2) to landscape, abiotic, biotic, and host-level (Host) variables, and the shared
724 variation between and among the variable subsets, for site-level ranavirus presence and host-
725 level ranavirus infection. Host-level variables were only included in host-level analyses and
726 probability values can only be calculated for landscape, abiotic, biotic, and host-level
727 components. An asterisk (*) indicates $P < 0.01$ and two asterisks (**) indicate $P < 0.001$

Variance component	Ranavirus	
	Presence	Infection
Landscape (L)	0.190**	0.042**
Abiotic (A)	-0.007	-0.001
Biotic (B)	0.086*	0.058**
Host (H)		0.031**
LA	0.105	0.027
LB	-0.007	-0.021
LH		0.014
AB	-0.002	0.002
AH		0.000
BH		0.036
LBH		0.009
ABH		0.002
LAH		0.009
LAB	0.015	-0.001
LABH		0.002

Residual	0.621	0.811

728

729 Table 4. Model-averaged coefficients for centered and scaled predictor variables from a subset of
730 models (delta AICc < 4 points, 8 of 64 models) of host-level ranavirus infection in amphibian
731 assemblages in the East Bay region of California in 2013. Coefficients are arranged by ascending
732 *P*-value, then alphabetically. "Distance" is distance to nearest ranavirus-infected, "Num. mod." is
733 the number of models that include that predictor variable, "Importance" is percent of models
734 within the model subset that contain that variable, "SE" is standard error, and "Adj. SE" is
735 adjusted standard error. For species identity (Spp. identity), "ANBO" is *A. boreas*, "HYRE" is *H.*
736 *regilla*, "RACA" is *R. catesbeiana* (the reference level), "TAGR" is *T. granulosa*, and "TATO"
737 is *T. torosa*. Coefficients with $P \leq 0.05$ are shaded in grey.

	Num. mod	Importance	Estimate	SE	Adj. SE	z	P
Distance	8	1.00	-1.40	0.38	0.38	3.68	< 0.001
Snout-vent length	8	1.00	-0.40	0.11	0.11	3.52	< 0.001
Spp. identity - ANBO	8	1.00	3.72	0.85	0.85	4.36	< 0.001
Spp. identity - HYRE	8	1.00	3.02	0.84	0.84	3.60	< 0.001
Spp. identity - RACA	8	1.00	-4.09	0.90	0.91	4.52	< 0.001
Spp. identity - TAGR	8	1.00	4.03	0.92	0.92	4.40	< 0.001
Spp. identity - TATO	8	1.00	2.66	0.84	0.84	3.17	0.001
Vertebrate richness	4	0.71	-0.61	0.30	0.31	1.99	0.047
R. catesbeiana presence	4	0.39	-0.78	0.75	0.75	1.04	0.298
Percent Wetland	4	0.39	-0.56	0.59	0.59	0.96	0.337

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739

740 **FIGURE LEGENDS**

741 Fig. 1. Study area and wetlands included in site-level analyses (*n* = 76) in three counties
742 (Alameda, Contra Costa, and Santa Clara) of the East Bay region of California in 2013. Black
743 points represent sites with ranavirus presence (those included in host-level analyses) and white
744 points represent sites without ranavirus presence. Ranavirus was not detected at the southwest
745 sites (those not included in the bottom inset map) within Santa Clara county.

746

747 Fig. 2. Percent of wetlands with each species (a), species richness at wetlands (b), percent of
748 wetlands with ranavirus infected hosts for each species (c), and mean percent of hosts infected
749 with ranavirus per wetland (with 95% CI) of those collected of each species (d) in amphibian
750 assemblages in the East Bay region of California in 2013. Numbers above bars indicate number
751 of wetlands with each species or species richness (*n* = 76). For plots (a), (c), and (d): AMCA,
752 *Ambystoma californiense* (California Tiger Salamander); ANBO, *Anaxyrus boreas* (Western
753 Toad); RACA, *Rana catesbeiana* (American Bullfrog); HYRE, *Hyliola regilla* (Northern Pacific
754 Tree Frog); RADR, *Rana draytonii* (California Red-legged Frog); TAGR, *Taricha granulosa*
755 (Rough-skinned Newt); TATO, *Taricha torosa* (California Newt).

756

757 Fig. 3. Model-averaged predicted probability of site-level ranavirus presence (*n* = 76) in
758 amphibian assemblages in the East Bay region of California in 2013 with increasing (a) distance
759 to nearest ranavirus-infected wetland (Distance, km), and (b) taxonomic richness in wetlands in
760 2013. Points for taxonomic richness are jittered to reduce overlap.

761

762

763 Fig. 4. Model-averaged predicted probability of host-level ranavirus infection (*n* = 1,089) in
764 amphibian assemblages in the East Bay region of California in 2013 with increasing (a) distance
765 from nearest ranavirus-infected wetland (Distance, km), (b) snout-vent length, and (c) vertebrate
766 richness. Points for vertebrate richness are jittered to reduce overlap.
767
768 Fig. 5. Model-averaged predicted probability of ranavirus infection with standard error (*n* =
769 1,089) for amphibian hosts in the East Bay region of California in 2013. For species: ANBO,
770 *Anaxyrus boreas* (Western Toad); HYRE, *Hyliola regilla* (Northern Pacific Tree frog); RACA,
771 *Rana catesbeiana* (American Bullfrog; the reference level); TAGR, *Taricha granulosa* (Rough-
772 skinned Newt); and TATO, *Taricha torosa* (California Newt).
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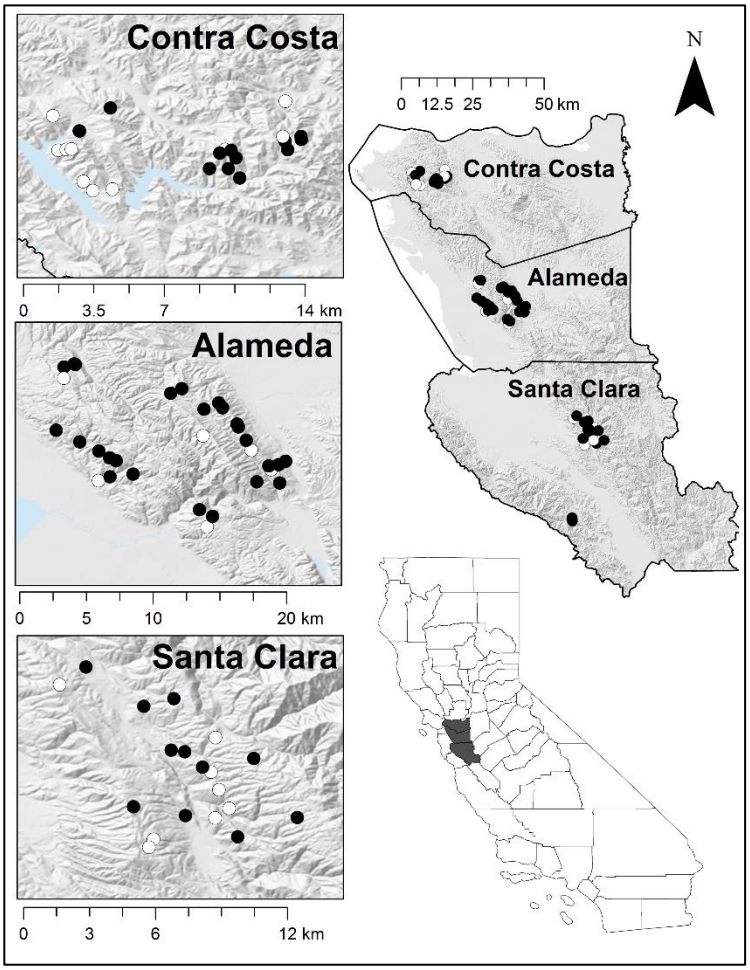
786 **FIGURES**

787



788

Fig. 1

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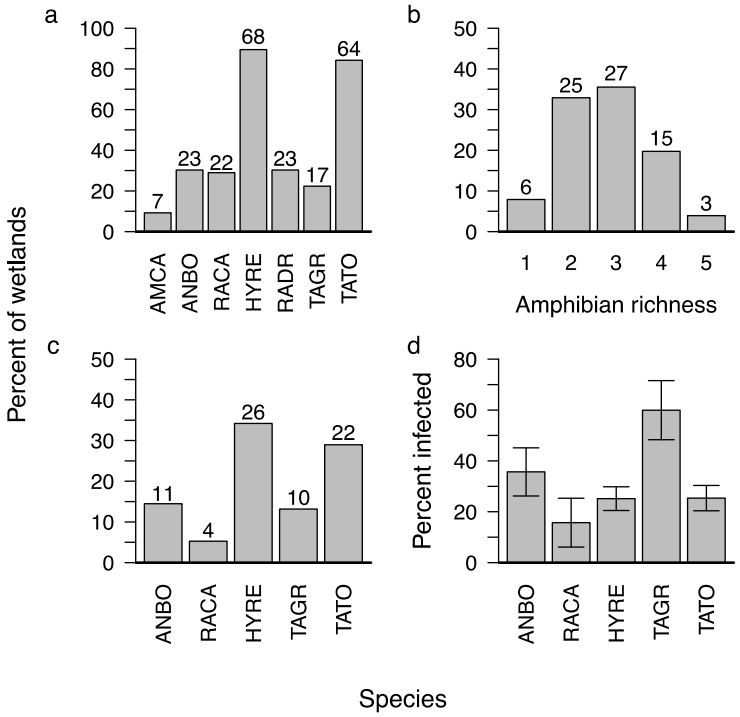

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Fig. 2

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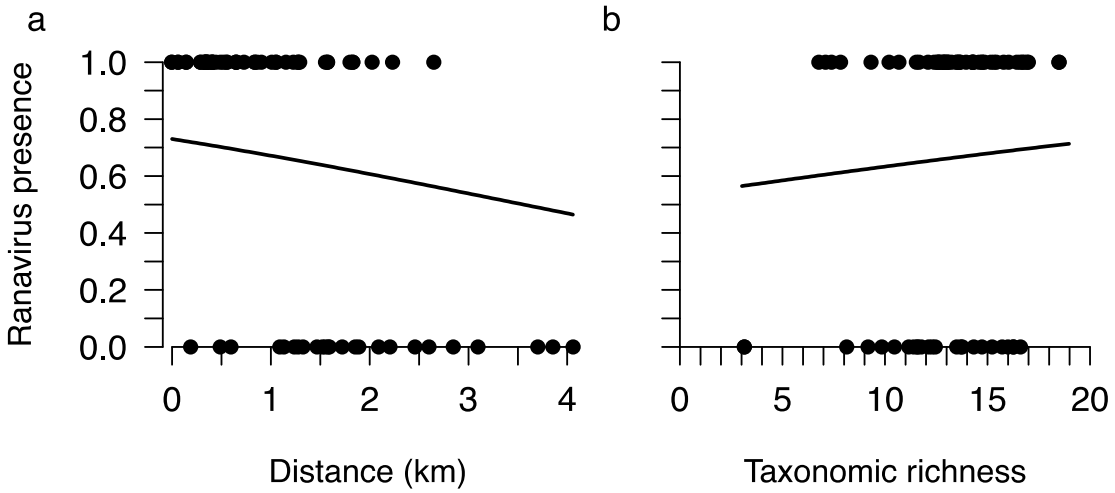

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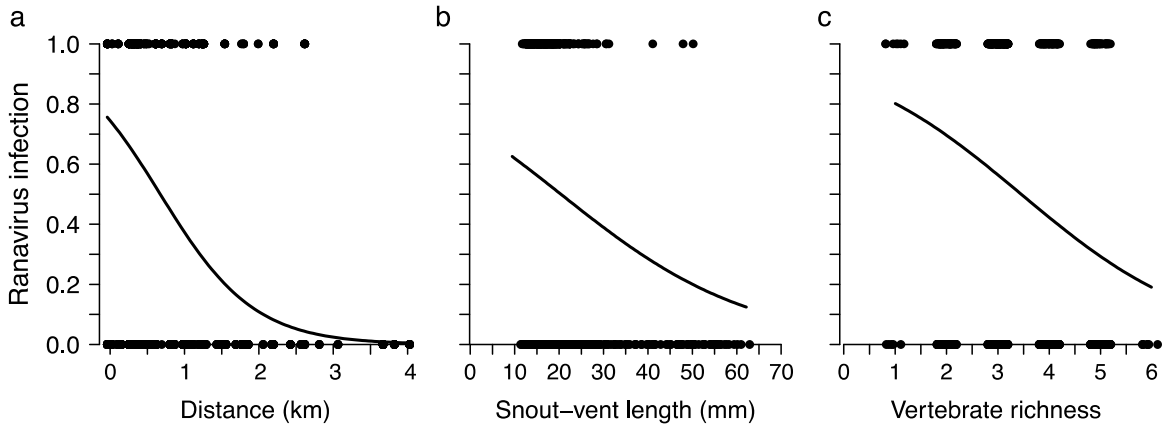

Fig. 4

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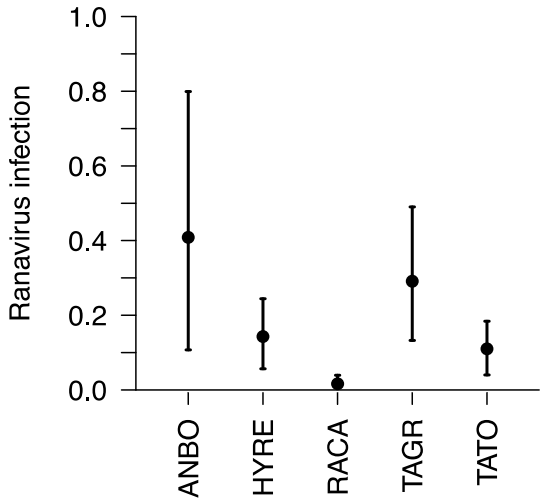

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Fig. 5

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