INCORPORATING ENVIRONMENTAL FACTORS INTO DISCUSSIONS OF DIVERSITY-DISEASE RELATIONSHIPS

by

AUSTIN LEE COLEMAN

(Under the Direction of Stacey Lance)

ABSTRACT

Understanding how biodiversity influences pathogens has global implications for predicting disease outbreaks and preventing additional biodiversity loss. The dilution effect hypothesis suggests that more diverse communities constrain pathogen infection due to an increased abundance of incompetent hosts. I investigated the relationships between spatial and temporal changes in amphibian biodiversity and prevalence of ranavirus, an important pathogen implicated in amphibian declines. I gathered amphibian community data for 20 ephemeral wetlands using standardized trapping methods. For a subset of individuals (2,210), I quantified presence and load of ranavirus using quantitative PCR. Results indicate wetland canopy cover and air and water temperature values are the most informative predictors of ranavirus occurrence. Daily ambient air temperature, anuran size, emergent vegetation and canopy, and prevalence were the best predictors of viral load. These results suggest rising temperatures could be very problematic for amphibians and the importance of community interactions may be masked by environmental factors.

INDEX WORDS: Dilution effect, amphibians, ranavirus, community, diversity, environmental factors

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AUSTIN LEE COLEMAN

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AUSTIN COLEMAN

Major Professor:	Stacey Lance
Committee:	Andrew Park
	Krista Capps

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

For my family.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

AMPHIBIAN DECLINES

In the midst of what most scientists agree is the Earth's sixth mass extinction, biodiversity loss has become a concern for conservation (Wake & Vredenburg, 2008). Specifically, amphibian population declines far exceed those of other vertebrate groups, with 30% of all species listed as threatened by the International Union for Conservation of Nature (IUCN, NatureServe, & Conservation International, 2004; Wake & Vredenburg, 2008). The primary factors responsible or amphibian declines include habitat destruction (Lehtineni, Galatowitsch, & Tester, 1999), environmental contaminants (Egea-Serrano, Relyea, Tejedo, & Torralva, 2012), pet trade (Schlaepfer, Hoover, & Dodd Jr, 2005) and disease (Lips et al., 2006). Emerging infectious diseases such as Ranavirus (RV) and Chytridiomycosis (Bd) are the most common diseases associated with population declines and localized extinctions in amphibians (Berger et al., 1998; Lesbarreres et al., 2012; Stuart et al., 2004). Currently, RV has global distribution and representation across six continents (Duffus et al., 2015) and is the focus of my thesis research.

RANAVIRUS IN AMPHIBIANS

Allan Granoff (1923–2012) isolated the first ranaviruses in the 1960's (Granoff, Came, & Breeze, 1966). Ranaviruses, in the family Iridoviridae, are large double-stranded DNA viruses that affect ectothermic vertebrates (Gray & Chinchar, 2015; Gray,

Miller, & Hoverman, 2009; T. Williams, Barbosa-Solomieu, & Chinchar, 2005). There are many identified strains, however, frog virus 3 (FV3) is the strain most commonly associated with amphibian die-offs (Chinchar et al., 2005). Characteristics of RV infection include systemic hemorrhaging and organ necrosis which often lead to kidney and liver failure (Gray et al., 2009). Infection can reduce survival (Jesse L. Brunner, Richards, & Collins, 2005a) and cause sub lethal effects such as changes in growth and development (Echaubard, Little, Pauli, & Lesbarréres, 2010) and can potentially increase effects of other stressors such as contaminants (Blaustein et al., 2011; Blaustein, Romansic, Kiesecker, & Hatch, 2003). Transmission routes for RV in amphibians are diverse and include contact with contaminated soils, water, or other infected organisms (Harp et al., 2006; Brunner et al., 2005; Brunner et al., 2009) and consumption of live or dead infected organisms (Harp et al., 2006; Jancovich et al., 1997). Vertical transmission has not been confirmed, however, it has not yet been ruled out and findings show varied results (J. L. Brunner, Schock, & Collins, 2007; Jesse L. Brunner & Collins, 2009).

Viability of RV and susceptibility to disease can be affected by many biotic and abiotic factors such as amphibian innate immune response (Grayfer, Andino, Chen, Chinchar, & Robert, 2012; Morales et al., 2010), amphibian life stages (Jesse L. Brunner et al., 2005a; Love et al., 2016), seasonality (Hoverman, Mihaljevic, Richgels, Kerby, & Johnson, 2012), temperature (Rojas, Richards, Jancovich, & Davidson, 2005; Speare & Smith, 1992), and hydroperiod dynamics (Warne, Crespi, & Brunner, 2011; Winzeler, 2018). Ranavirus can infect amphibians at most life stages with the exception of eggs, which may have lower susceptibility due to the protein capsule coating the eggs or the membrane around the embryo (Haislip, Gray, Hoverman, & Miller, 2011). Although die-

offs have been reported in adult frog and newt populations in Europe (Teacher, Cunningham, & Garner, 2010), infection effects are typical worse in the larval and metamorphic life stages due to already compromised immune function (D. Miller, Gray, & Storfer, 2011).

Many studies have investigated the influence of anthropogenic impacts (Love et al., 2016; North, Hodgson, Price, & Griffiths, 2015) and environmental conditions (Gahl & Calhoun, 2008; Jun et al., 2009; La Fauce, Ariel, Munns, Rush, & Owens, 2012; Warne et al., 2011) on RV prevalence. However, a majority of the work done has involved mortality event responses (Geng et al., 2011; Green, Converse, & Schrader, 2002; Miaud et al., 2016), between species transmission (Brenes, Gray, Waltzek, Wilkes, & Miller, 2014), and viral phylogeny (Duffus & Andrews, 2013; Eaton et al., 2007; Hoverman, Gray, Haislip, & Miller, 2011). Although RV has a wide host breadth and global distribution (Duffus et al., 2015), few studies have looked at the influence of community composition or community diversity on RV prevalence and intensity.

THESIS OBJECTIVES

Recent work on emerging infectious diseases has established a link between increased biodiversity and reduced risk of disease to humans and wildlife through a dilution effect (CITE). The primary dilution effect hypothesis states that more diverse host communities may contain a higher proportion of incompetent hosts and therefore, decrease the overall risk and intensity of infection through transmission interference and/or susceptible host regulation (Johnson, Ostfeld, & Keesing, 2015; F. Keesing, Holt, & Ostfeld, 2006; Ostfeld & Keesing, 2000; Zargar, Chishti, Ahmad, & Rather, 2015). Despite the fact that global declines of amphibians (Stuart et al., 2004) have been tied to

infectious disease (CITE) there has been almost no work examining relationships between diversity and disease in amphibians. Ranavirus has been responsible for die-offs in captive and wild amphibians over the past two decades (Kolby et al., 2014; Lesbarreres et al., 2012). Thus, it is critical to determine how amphibian community ecology impacts RV dynamics and whether additional losses in biodiversity are predicted to increase the impacts of RV. My objectives were to investigate 1) patterns of RV prevalence and intensity across the landscape, 2) how amphibian community assembly influences RV infection patterns, and 3) whether abiotic environmental factors are important for predicting disease prevalence and intensity. To address these questions, I examined amphibian communities and RV prevalence and intensity in 20 ephemeral wetlands on the United States Department of Energy Savannah River Site in South Carolina.

CHAPTER 2

INCORPORATING ENVIRONMENTAL FACTORS INTO DISCUSSIONS OF DIVERSITY-DISEASE RELATIONSHIPS

Coleman, A. L., Scott, D. E., Park, A. W., Capps, K. A., Beasley, R. J., and S. L. Lance

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ABSTRACT

Understanding how biodiversity influences pathogens has global implications for predicting disease outbreaks and preventing additional biodiversity loss. The dilution effect hypothesis suggests that more diverse communities constrain pathogen infection due to an increased abundance of incompetent hosts. Ranavirus is an emerging infectious disease that infects ectothermic vertebrates. I aim to explore the relationship between amphibian community structure and the prevalence, distribution, and intensity of RV infection. Previous research supports dilution effect for amphibian diseases, but was conducted in areas with low host diversity. The United States Department of Energy's Savannah River Site (SRS) in South Carolina contains 100's of wetlands and over 30 species of pond-breeding amphibians. Previous surveys indicate widespread occurrence of RV on the SRS. My objectives included characterizing the amphibian communities of 20 wetlands and investigating the relationship between spatial and temporal changes in amphibian biodiversity and Ranavirus prevalence. In 2016, I gathered amphibian community data for 20 ephemeral wetlands using standardized capture methods. I captured more than 30,000 individual amphibians representing 21 species. For a subset of individuals captured (2,210), I quantified presence and load of Ranavirus using quantitative PCR. Using Boosted Regression Tree analysis I found canopy cover and air and water temperature values are the most informative predictors of ranavirus occurrence. Daily ambient air temperature, anuran size, emergent vegetation and canopy, and prevalence were the best predictors of viral load. These results suggest rising temperatures could be very problematic for amphibians and the importance of community interactions may be masked by environmental factors.

INTRODUCTION

Understanding how biodiversity influences pathogen occurrence and intensity has global implications for predicting disease outbreaks and preventing additional loss of biodiversity. Host-pathogen dynamics can be strongly influenced by community composition, assembly, and diversity (F. Keesing et al., 2006; Felicia Keesing et al., 2010). Diversity-disease relationships can involve a decrease (dilution effect) or increase (amplification effect) in disease risk with increasing biodiversity (F. Keesing et al., 2006). Although dilution has been observed (Johnson, Preston, Hoverman, & Richgels, 2013a; Lagrue & Poulin, 2015; Swaddle & Calos, 2008) and conditions for amplification have been suggested (E. Miller & Huppert, 2013b; Scheele, Hunter, Brannelly, Skerratt, & Driscoll, 2017), the dilution effect has received more attention as the potential to link biodiversity conservation to improved human health has obvious appeal (Felicia Keesing et al., 2010). The primary dilution effect hypothesis states that more diverse host communities may contain a higher proportion of incompetent hosts and therefore, decrease the overall risk and intensity of infection through transmission interference and/or susceptible host regulation (Johnson, Ostfeld, et al., 2015; F. Keesing et al., 2006; Ostfeld & Keesing, 2000; Zargar et al., 2015). Recently, a large-scale meta-analysis has provided broad evidence in support of dilution effect and maintains that this phenomenon is naturally occurring and common in nature (Civitello et al., 2015).

Investigations into the dilution effect have provided insight into potential mechanisms regulating disease dynamics, however, applying concepts of community ecology to disease ecology is still in its infancy (Civitello et al., 2015; Johnson, De Roode, & Fenton, 2015; Venesky, Liu, Sauer, & Rohr, 2014). There is still an ongoing

debate regarding the generality of the dilution effect (Z. Y.X. Huang, Van Langevelde, Estrada-Peña, Suzán, & De Boer, 2016; Ostfeld, 2013; Randolph & Dobson, 2012b; Wood et al., 2014; Zargar et al., 2015) and there remains a need to examine underlying mechanisms and patterns across scales (Z. Y.X. Huang et al., 2016; Zheng Y.X. Huang, Yu, Van Langevelde, & De Boer, 2017; Zheng Y X Huang et al., 2013; Ostfeld, 2013; Randolph & Dobson, 2012a). In particular, the dilution effect may be context dependent such that diversity itself may have less of an effect than specific community composition yet many studies do not distinguish between functional diversity, species richness, relative abundance, or combined indices like Shannon or Simpson (Randolph & Dobson, 2012b). Another concern is that while some studies examine overall prevalence of a pathogen in a community (Dobson, 2004), others focus on risk of infection to a specific species of interest (Becker, Rodriguez, Longo, Talaba, & Zamudio, 2012). Studies so far have not investigated these from the point of view of the community as well as a focal host. It is possible that different variables impact prevalence across communities and focal species, thus it is important to examine both in a single system. Additionally, few studies of diversity-disease relationships have addressed the role of physiochemical properties known to influence community metrics (Z. Y.X. Huang et al., 2016).

While community-level interactions within and among species likely determine the realized community composition, environmental conditions may govern the potential pool of species or mediate local species loss depending on physiological tolerances (Connelly et al., 2011; Guderyahn, Smithers, & Mims, 2016; R. Semlitsch, Scott, Pechmann, & Gibbons, 1996; Welborn et al., 1996; Werner, Skelly, Relyea, & Yurewicz, 2007a). This often occurs via environmental filtering, a process where physiological

tolerances mediate species existence in a particular region or habitat (Menge & Sutherland, 1976). Changes in environmental conditions can have direct influence on host and pathogen physiology (Almodóvar, Nicola, Ayllón, & Elvira, 2012; Brown, Goekjian, Poulson, Valeika, & Stallknecht, 2009; Poulin & Mouillot, 2003). Parasite richness and distribution is tightly linked to the host, therefore, any change influencing the physiology of the host will directly impact the pathogen (Adlard, Miller, & Smit, 2015). Abiotic factors including climate (temperature, humidity, etc.), pH, and salinity have all been shown to influence pathogen distributions (Becker et al., 2012; Brown et al., 2009; Liu, Rohr, & Li, 2013). Temperature fluctuations, in particular, have a direct effect on ectothermic host physiological mechanisms such as growth (Álvarez & Nicieza, 2002), behavior (Brattstrom, 1979), immune responses to pathogens (T. R. Raffel, Rohr, Kiesecker, & Hudson, 2006)-resulting in differences in susceptibility (Brand et al., 2016), infection intensity and mortality (Allender, Mitchell, Torres, Sekowska, & Driskell, 2013; Brand et al., 2016; Jun et al., 2009; Rojas et al., 2005). Understanding how community diversity affects pathogen transmission is integral to predicting host-pathogen dynamics and disease emergence. However, many biotic and abiotic factors known to influence community assembly are being largely ignored in studies of diversity-disease relationships and this presents a large gap limiting our ability to achieve a holistic view in predicting disease emergence and outcomes.

Here, I use a natural system characterized by a multi-host pathogen, Ranavirus (RV), and complex assemblages of amphibians residing in ephemeral wetlands of the southeastern United States to quantify the relative contributions of biotic and abiotic factors on disease risk. This system is ideal for examining relationships between

diversity and disease. Ranavirus has the potential to infect at least 14 families and more than 70 species (Gray et al., 2009; D. Miller et al., 2011), but susceptibility and competency varies across species (Hoverman et al., 2011; Hoverman, Gray, & Miller, 2010; Reeve, Crespi, Whipps, & Brunner, 2013). Ephemeral wetlands in the southeastern United States contain species rich assemblages of greater than 30 potential species of pond-breeding amphibians that vary spatially and temporally on the landscape (Gibbons et al., 2006; R. Semlitsch et al., 1996; Sharitz, 2003; Snodgrass, Bryan, & Burger, 2000). This system allowed me to investigate relationships at the community-level as well as from the point of view of a focal host species. Amphibians are experiencing global declines (Stuart et al., 2004) and RV has been responsible for die-offs in captive and wild amphibians over the past two decades (Kolby et al., 2014; Lesbarreres et al., 2012). Thus, it is critical to determine how amphibian community ecology impacts RV dynamics and whether additional losses in biodiversity are predicted to increase the impacts of RV. My objectives were to investigate 1) patterns of RV prevalence and intensity across the landscape, 2) how amphibian community assembly influences RV infection patterns, and 3) whether abiotic environmental factors are important for predicting disease prevalence and intensity. To address these questions, I examined amphibian communities and RV prevalence and intensity in 20 wetlands.

METHODS

Study Site

The location of this study was the United States Department of Energy's Savannah River Site (SRS). The SRS was acquired by the federal government in 1950 and closed to the public a year later. The site encompasses 777 km^2 and includes portions

of Aiken, Barnwell and Allendale counties in South Carolina. Over 80% of the site remains largely unimpacted from site operations and contains over 30 species of pond breeding amphibians and over 400 ephemeral wetlands (Gibbons et al., 2006; R. Semlitsch et al., 1996; Sharitz, 2003). Pond-breeding amphibians have been the subject of numerous studies on the SRS (Croshaw & Scott, 2006; Daszak et al., 2005; Gibbons et al., 2006; Love et al., 2016; Peterman, Anderson, Drake, Ousterhout, & Semlitsch, 2014; Scott, 1994; R. Semlitsch et al., 1996). Importantly, RV is known to occur on the SRS and to vary in prevalence across wetlands (Love et al., 2016).

Data Collection

Amphibian community assessment and sample collection

I surveyed amphibian communities in 20 ephemeral wetlands on the SRS (Figure 1) from January to July of 2016. I established four groups of five wetlands that were closer in proximity to ensure all wetlands in a group could be sampled in a single day. Each group of wetlands was sampled for one week per month for 6 months. Each month, I sampled the groups in order from A – D, such that wetlands were sampled every four weeks. I kept the sampling order consistent each month, rather than randomize the order, to avoid waiting longer than 30 days to re-visit a wetland, as this limited the possibility that I would miss a species with short development periods. Each week of sampling, I deployed minnow traps at all five wetlands on day 1, checked them for occupancy on days two to five, and then closed them on day five. The number of minnow traps in a wetland was scaled to reflect relative wetland perimeter (small-10 traps, medium-20 traps, and large-30 traps; Table 1). In the northeast quadrant of each wetland I placed minnow traps every 10 m at a depth of ~15-20 cm leaving a pocket of air in the top

(Figure 2). I conducted a dipnet survey around the entire perimeter of each wetland once per month in water approximately ~ 0.5 m deep (Figure 2). Briefly, every 20 m, I conducted 3 sweeps—in front of me, and to my left and right. I recorded species identification and the number of individuals per species for each trap and dipnet sweep. Additionally, I collected 1 individual per species per trap/dipnet sweep), brought them back to the lab at the University of Georgia Savannah River Ecology Laboratory where I euthanized individuals in a 3% MS-222 solution per IACUC (AUP #: A2015 12-009-R2) to test them for RV. In some cases, identifying individuals to the species level was not possible. Therefore, when unsure of species identity, I used a subset of individuals that all were considered to be of the same unknown species, to identify species using DNA barcoding (see Laboratory Analysis-Species Identification section below).
Developmental stage has been shown to influence the infection occurrence in larval amphibians (Love et al., 2016). Therefore, for each individual, I measured snout-vent length (SVL) and noted developmental stage. I divided the stages into larval with no limbs, larval with hind limbs, larval with front limbs, metamorphosed individuals, and adults. Community metrics have been shown to influence pathogen dynamics so I also calculated several metrics to describe the amphibian communities. I calculated the observed species richness, cumulative species richness, Shannon's diversity, Peilou's evenness, the abundance of species captured, and the relative abundance of species captured for each wetland and sampling period combination.

Environmental data collection

Each month, I collected environmental data, including percent canopy cover, wetland drying score, descriptors of dominant emergent vegetation and canopy, and

water temperature in each wetland. Canopy cover was estimated once per month using a spherical densitometer (Lemmon, 1956), and by averaging four measurements at 0.5 m depth in four locations around the perimeter (NE, SE, NW, and SW). When accessing the center of the wetland did not require a boat, I also took four measurements in the center of the wetland. In keeping with previous studies of wetlands on the SRS (Snodgrass, Komoroski, Bryan, & Burger, 2000), I used a binary drying score (0: water absent; 1: water present). As many species of amphibian are impacted by temporal variation in hydroperiod (Koprivnikar, Paull, & Johnson, 2014; Snodgrass, Komoroski, et al., 2000; Werner, Skelly, Relyea, & Yurewicz, 2007b), I combined our drying scores with those collected in previous studies (Snodgrass, Komoroski, et al., 2000) to calculate a drying score. I obtained daily mean ambient air temperatures from the SRS-Atmospheric Technologies Group (https://www.srs.gov/weather/index.html) and I collected daily minimum, maximum, and mean water temperature values every two hours by attaching one iButton temperature logger (iButtonLink, LLC. Whitewater, WI,USA) encased in Plasti-Dip (Plasti Dip International, Blaine, Minnesota, USA) to the side of a minnow trap ~10 cm below the surface in each wetland.

Laboratory Analyses

DNA extraction

For each individual, I removed a portion of liver, oral disc, and tail with sterilized dissection scissors and combined the tissues to approximately 10-25 mg. I extracted DNA using buffers from Qiagen DNEasy Blood and Tissue kit (Qiagen, Valencia, California, USA) and a modified tissue extraction protocol using Econospin® mini spin columns (Epoch Life Science, Inc., Missouri City, Texas, USA) and eluting samples in

50 µl AE buffer. Eluted DNA was analyzed for DNA concentrations on a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware, USA) and stored at -20°C. Prior to use in PCR, I normalized all samples to a DNA concentration of 50 ng/µl.

Species identification

When species identification was completed using DNA barcoding, I used a standard barcoding protocol targeting a partial fragment of the mitochondrial cytochrome oxidase subunit, following methods outlined in (Che et al., 2012). Amplifications were performed using GeneAmp 9700 thermal cyclers (Applied Biosystems). To prepare amplicons for sequencing I combined 10 µl of PCR product with 2 units of Exonuclease I (New England BioLabs) and 10 units of shrimp alkaline phosphatase I (New England BioLabs) and incubated them for 20 min at 37°C and then 15 min at 80°C. I sequenced in both directions using a modified protocol for BigDye®Terminator Cycling Sequencing Ready Reaction Mix v3.1 (Applied Biosystems). Reactions were run in a total volume of 10 µL and contained 1.0 µl Big Dye, 0.75X Big Dye sequencing buffer, 10.0 µM Primer, and 2.0 µl of cleaned amplicon. After cleaning the sequencing reactions with Sephadex® G-50 (Sigma-Aldrich, Inc., Darmstadt, Germany) I ran them on a 3130xl Genetic Analyzer (Applied Biosystems and Hitachi, Ltd., Foster City, CA, USA). The resulting sequences were edited in Geneious V8.1.9 (Kearse et al., 2012) and submitted to NCBI's GenBank database. I set a percent match threshold at 98% to be conservative with my identification (O'Bryhim, Parsons, & Lance, 2017).

Quantitative PCR for detection and quantification of ranavirus

To detect and quantify RV loads, I used a quantitative PCR approach targeting a conserved portion of the major capsid protein for FV3-like ranavirus. I followed the protocol described in Allender et al. (2013) with two exceptions: my primers and hydrolysis probe came from IDT© 2018 (Integrated DNA Technologies, Inc., Skokie, Illinois, USA) and I used Bio-Rad Sso^{TM} Universal Probes Supermix (Matthew C. Allender, Bunick, & Mitchell, 2013). I ran all samples and negative controls in triplicate on a 96 well plate using an BioRad iCycler IQ real-time PCR detection system (BioRad, Hercules, California, USA), and standards from previously cultured FV-3 virus that was isolated from a marbled salamander from the SRS (Winzeler, 2018). To produce standard curves I ran a serial dilution of our stock culture with a starting concentration of 1.75×10^5 PFU. Each standard was replicated at least three times with the two lowest standards (1.75×10^1 , $1.75 \times 10^{0)}$ replicated four and five times respectively. To be considered positive, two of the sample replicates had to have a threshold cycle (Ct) value lower than the Ct of the lowest standard (Love et al., 2016; Winzeler, 2018).

Statistical Analyses

I calculated RV prevalence as the number of individuals from all species testing positive divided by the total numbers of individuals collected from all wetlands. I calculated prevalence by species, by wetland, by sampling period, and by each species/ wetland/ sampling period combination. For all prevalence calculations I incorporated 95% Clopper-Pearson binomial confidence intervals using the *binom* package in R. For each individual that tested positive, I calculated mean viral load as the mean starting quantity values from triplicate qPCR runs.

I conducted all statistical analysis using RStudio V 3.4.3 and ran Boosted Regression Trees (BRTs) for data analysis (Elith, Leathwick, & Hastie, 2008). I chose BRTs because these models deal very well with missing data, different types of predictor variables (binary, numeric, categorical, etc.), nonlinear relationships, and interaction effects between predictors. As opposed to standard regression methods that usually produce a single predictive model, BRTs improve predictive performance by using multiple simplified models for prediction (boosting). BRTs require the user to optimize values for two hyperparameters- tree complexity and learning rate. The learning rate controls the contribution of every tree to the full model and tree complexity determines if interactions are fitted and how they are fitted. More parameter selection details are provided in Elith, Leathwick, and Hastie (2008). Model predictions are evaluated using area under the curve calculations (AUC) for the data, cross-validation using AUC (cv-AUC), and AUC (test-AUC) for the withheld test data. When developing BRTs, a cross-validation method is used for model development and model evaluation. Cross-validation tests the model on portions of the provided data (training data-80%) as well as the withheld data (test data-20%) with a 10-fold cross validation value. BRTs report several deviance estimates: mean total deviance, mean residual deviance, and cross-validation deviance (reported in Results section). BRTs do not produce *P* values. Rather, they use recursive binary splits to calculate the percent relative influence for each predictor. The model estimates percent relative influence based on how often a predicting variable is chosen by the model and how much the model improves when that variable is included in the model (Buston & Elith, 2011; Elith et al., 2008). After

calculating the percent relative influence, the model scales the values such that all influence values sum to 100%.

Prevalence of RV

I developed two separate models with specific hyperparameters (Table 2, Table 3) to predict infection occurrence at the species- and landscape-scale. I trained models for 126 and 107 predicting variables, respectively (Table 4, Table 5) across two response variables to predict RV dynamics- overall RV presence/absence and a RV presence/absence response for a focal species (*Lithobates sphenocephalus).* I selected *L. sphenocephalus* because it is common among wetlands, its presence was consistent between wetlands through time, and the RV prevalence in this species was similar to the overall prevalence of RV infection across all species (13%, 15%, respectively). I used RV presence/absence (2,210 observations) and the focal species response (985 observations) to predict infection occurrence across spatial and temporal scales, community metrics, and environmental predictors. The overall occurrence model split data into testing (442 observations/61 positive) and training data (1,786 observations / 281 positive). The focal species model split data into testing (197 observations / 21 positive) and training data (788 observations / 106 positive). I trained two distinct models for the response variables which were run using all available predictors and using the function *gbm.step* from R dismo package (Hijmans & Elith, 2015). I created dummy variables for wetland identity, species identity, and sampling period, as I was interested if these factors influenced the ability of the model to predict infection. Therefore, I characterized each factor by a binary predictor (0/1) to indicate association of an individual with specific levels of that predictor. I selected either the most influential 6

variables chosen by the model or the variables displaying >5% relative influence for further discussion.

Viral load analysis

I used a reduced dataset consisting of only individuals that tested positive for infection (334 observations), to explore the variables mediating viral load when an individual becomes infected. I attempted to train a BRT model to investigate what predictors are important for predicting variation in viral load intensity; however, the model was unable to train or divide the data into logical binary splits, be evaluated for model deviance estimates, or evaluate the predictive ability of the training and test datasets using the reduced number of data points. . Therefore, I modeled viral load intensity using generalized linear models (GLM) containing biologically relevant predictors (Warton & Hui, 2011).

For species-level infection load GLMs, only 13 species had representation of greater than four replicates to be included; therefore I used the data associated with these for further modeling. I used the natural log of the mean viral load to meet normality assumptions. Prevalence values had a non-linear effect on viral load as continuous variables and were transformed into categorical variables (Pasta, 2009), and binned into four distinct groups (Prevalence 1 = 0-10%, Prevalence 2 = 11-20%, Prevalence 3 = 21-30%, Prevalence 4 = 31-40%, and Prevalence 5= 41-76%). The vegetation variable integrated canopy and aquatic vegetation data; it was separated into four categories: closed hardwood canopy, mixed softwood and hardwood 50-80% closed, mixed softwood and hardwood 50-80% open, and primarily open herbaceous. Though sampling period was significant in all of the models, Spearman's rank correlations indicated that

sampling period was correlated with more fine scale variables, such as temperature (rho=0.91). Therefore, sampling period was removed from all of the models. Mean daily ambient air temperature, rather than water temperature was used in the models because I only had four months of data for water temperature. Strong correlations between air and water temperature (rho=0.92) supported this decision. I assessed additive GLMs with AICc values for viral load because our interactive models displayed high variance inflation (Burnham & Anderson, 2002). The most informative models were chosen based on the lowest AICc scores and dAICc < 2. I employed Type III ANOVAs with a Wald test to identify the most informative models. Tukey's post hoc multiple comparison tests were run for each categorical variable showing a significant relationship to determine which levels were significantly different from one another. Following model selection, ANOVA, and multiple comparisons, I employed a chi-squared ANOVA to ensure the most informative model was significantly different than the null model.

RESULTS

Summary

Overall, we captured 31,038 individual amphibians from seven families and 21 species (Table X). A subset of 2,210 (17 species-excluding ambystomatid salamanders and two-toed amphiuma; Table 6) individuals was tested for the presence of RV and estimation of viral load. Prevalence of RV varied among species (Table 7), wetlands (Table 8) and sampling period (Table 9). We detected RV in 12 of 17 species, and in 342 out of 2210 individuals for an overall prevalence rate of 15.5 ± 14.0 –17.1%. The overall species prevalence ranged from 0 - 35% after excluding *Hyla avivoca*, a species for which we only captured a single, infected individual. Four species made up 87% of all

RV-positive samples: *Anaxyrus terrestris* (35%), *Pseudacris nigrita* (32%), *P. crucifer* (24%), and *Scaphiopus holbrookii* (21%). Ranavirus prevalence among wetlands ranged from 0 – 76%. Overall prevalence varied seasonally, reaching its peak in April (24%) and May (23%) (Table 9).

Predictors of RV occurrence

I ran a BRT model using all 2,210 observations (342 positive for infection). Minimal variation between the cv-AUC (0.877 ± 0.01) and test-AUC (0.878) indicate model overfitting was not apparent and that the model successfully predicted training data and test data (Table 10, Table 11). The area under the curve (AUC, Figure 3) for the full model (full model training data AUC= 0.927) suggested canopy cover, snout-vent length (a proxy for amphibian size), and temperature provided sufficient information for predicting the occurrence of RV.

Overall, environmental factors, rather than individual- or community-level metrics, were most important to predicting RV occurrence. Canopy cover was the strongest predictor, followed by the three temperature values I kept in the model (Figure 4). The relationship between canopy cover and RV occurrence was bimodal, suggesting individuals in wetlands characterized by very low canopy (< 20%) and high canopy cover (> 90%) had increased odds of testing positive for RV (Figure 5). In general, the odds of an individual testing positive were greater at higher temperatures, but the relationships of temperature and RV presence varied across the different measures. When minimum water temperatures were above 15°C, the odds of RV presence appeared to increase and remain high with increasing temperature (Figure 5). The odds of RV occurrence were very high once mean ambient air temperature reached 25°C.

Canopy cover and snout-vent length were the only biotic factors that impacted the odds of individuals testing positive. One individual-level predictor, SVL, was also a relatively strong predictor (>5% relative importance)—larger individuals had lower odds of having RV. No measures of amphibian communities explained more than 5% of the variation in RV presence. However, several of these measures did explain between 1 and 5% (Figure 4).

Predictors of RV in focal species

I ran the focal species BRT model using only *L. sphenocephalus* (985 observations; 127 positive for RV). Minimal variation between the cv-AUC (0.81±0.022) and test-AUC (0.863) suggest that overfitting was not an issue and the model successfully predicted training data and data withheld from the model (Table 12, Table 13). Similar to the analysis of community-level data, environmental factors had the highest relative influence in predicting RV occurrence in *L. sphenocephalus* (Figure 7). Model results also indicate the odds of *L. sphenocephalus* individuals testing positive for RV were influenced by temperature, canopy cover, SVL, and being from a particular wetland (wetland #17; full model training data AUC=0.894, Figure 6). Minimum daily water temperature had the highest relative influence followed closely by mean ambient air temperature and then canopy cover (Figure 7). Snout-vent length and being from a specific wetland (Wetland 17) also had relative influence values greater than 5% (Figure 7). All temperature values with > 5% relative influence displayed a similar trend (Figure 8); when temperatures exceeded 15°C, the odds of RV occurrence increased for this species. The relationship of canopy cover and infection occurrence was slightly different in the focal species model than in the full model (Figure 8), as *L. sphenocephalus* in

wetlands with > 90% or < 10% canopy coverage showed equally high odds for RV presence. Similar to patterns documented in the full community, canopy cover and snout-vent length were the only biotic variables with >5% influence on the presence of RV. In contrast to the full model where size had a negative relationship to the presence of RV, medium sized *L. sphenocephalus* had increased odds of infection. There were also numerous biotic variables, including community-level metrics, which influenced the presence of infection (1-5%; Figure 7).

Predictors of viral load

The most informative model (Table 14) explaining variation in and predicting viral load included RV prevalence, mean daily ambient air temperature, SVL, and wetland vegetation; yet, it left a large proportion of the variation unexplained (McFadden Pseudo $R^2 = 0.09$, Nagel Kerke Pseudo $R^2 = 0.27$). Type III ANOVA with a Wald test revealed significant results for three of the four predictors in the most informative model (RV prevalence ($p < 0.001$), mean daily ambient air temperature ($p < 0.001$), and vegetation ($p = 0.03$). Tukey's multiple comparisons test for RV prevalence revealed significant differences in viral loads between prevalence classes, such that the highest mean viral loads were observed in wetlands with a prevalence between 31 and 40% (Prevalence 4; Figure 9) and differed most from Prevalence 1 (0-10%). Individual viral loads also had a positive relationship with increasing ambient air temperature (Figure 10). Although the final model includes SVL, when examined independently it was not a statistically significant predictor ($p = 0.08$; Figure 11). Viral loads were significantly higher in individuals from wetlands with both closed hardwood canopy and open herbaceous sites than mixed softwood and hardwood with 50 – 80% closed ($p = 0.0001$)

and mixed softwood and hardwood with 50 – 80% herbaceous vegetation types ($p = 0.002$; Figure 12). Based on the most informative model, prevalence, ambient air temperature, SVL, and vegetation all had significant effects on variation in mean viral loads (Figure 13). A Chi-squared ANOVA indicates that the most informative model was significantly different than our null model ($p < 0.0001$).

DISCUSSION

Overall patterns of ranavirus prevalence

Overall, the prevalence of RV I documented in wetlands on the SRS was 15.5%; much lower than found in previous studies there (37.4% ; Love et al., 2016) and in the southeastern U.S. (60%; Hoverman, Gray, Miller, & Haislip, 2012). Although my overall prevalence was much lower than in the Love et al. study (2016), both studies found a high prevalence in *A. terrestris* indicating that it may be a very important carrier (Love et al., 2016). Surprisingly, *L. catesbeianus* had no detectable infection. *Lithobates catesbeianus* has been strongly associated with infection prevalence and is considered to be a reservoir host maintaining transmission throughout the year (Gray et al., 2009; Hoverman, Gray, et al., 2012). In a previous study (Love et al. 2016) the prevalence of RV in *L. catesbeianus* was ~29% which emphasizes the dynamic nature of RV. Variation in prevalence at the wetland level was high and ranged from 0-76% across all species and time points. Variation in prevalence at the wetland level ranged from 0-100% in a recent study on the SRS (Love et al., 2016). Prevalence varied by season where February and March remained low, subsequently peaked during April and May, and then decreased in June and July.

Biotic and abiotic predictors of disease dynamics

Based on my study, canopy cover and temperature are the most important predictors of RV occurrence in amphibians on the SRS. Amphibians in wetlands with open and closed canopies had much higher odds of testing positive for RV and having higher viral loads. Interestingly, Becker et al (2012) and Raffel et al 2010 also found that amphibian pathogen prevalence, in this case the chytrid fungus, was higher in closed canopy wetlands. However, they attributed this to temperature changes across canopy cover gradients (Becker et al., 2012; Thomas R. Raffel, Michel, Sites, & Rohr, 2010). Because I used a BRT approach with temperature variables in the model, my results indicate that canopy cover is important independent of temperature. Clearly, temperature was an important predictor of RV, but canopy cover had a separate influence on RV occurrence. Closed hardwood canopy wetlands had higher average prevalence (23%) than open herbaceous wetlands (10%), however, trends for closed hardwood wetlands may have been driven by one wetland with high prevalence (Wetland 3, prevalence = 76%). Canopy cover influences the amount and type of leaf litter, vegetation, and nutrients available in wetlands (Plenzler & Michaels, 2015). Compounds and traits in leaf litter can impact wetland communities across trophic levels by affecting algal abundance, zooplankton density, and can subsequently altering patterns of amphibian growth (Stoler & Relyea, 2011, 2016). Increased canopy cover is associated with decreasing dissolved oxygen, water temperature, vegetation abundance and diversity, and algal genera (Plenzler & Michaels, 2015) -all of which can impact the body condition and health of amphibians. Amphibian distribution, reduced growth and development has been strongly linked to associations with leaf littered wetlands (Werner et al., 2007b; B.

K. Williams, Rittenhouse, & Semlitsch, 2008). In addition to affecting the individual health of larval amphibians, the leaf litter and vegetation in wetlands can alter the diversity and abundance of predators and prey (Plenzler & Michaels, 2015) and thus impact the risk of predation to larval amphibians. On the SRS, open canopies in ephemeral wetlands are more often associated with a diversity of amphibian predators including Ambystomatid salamanders, dragonfly larvae, Belastomatids, and crayfish. Presence of predation risk has shown to alter activity levels and microhabitat use in larval amphibians (Relyea, 2001; Skelly & Werner, 1990) often resulting in amphibian aggregation which can induce stress and alter pathogen transmission dynamics (R. D. Semlitsch & Gavasso, 1992). It is also possible that predators could have been targeting infected or competent host species. Given the ability of RV to cause die-offs in ectothermic vertebrates (Gray et al., 2009; Miaud et al., 2016), it is very important to further examine how canopy cover may be impacting pathogen dynamics.

As previously mentioned, in addition to canopy cover, higher ambient air and water temperature are important predictors of RV occurrence. Specifically, there was an elevated risk of infection at temperatures breaching 15°C. Temperature can have direct impacts on both host and pathogen physiology (Rojas et al., 2005; Speare & Smith, 1992). In amphibians, temperature impacts growth, development (Harkey & Semlitsch, 2017), and immune response (T. R. Raffel et al., 2006). Increased temperatures could spark quicker growth and development necessary for amphibians to metamorphose and escape desiccation in ephemeral wetland resulting in a tradeoff where less energy is put toward immune response. Simultaneously, RV's replication occurs between 12 and 32°C

(G. V Chinchar, 2002; Speare & Smith, 1992). Thus, prolonged periods of higher temperatures could prove problematic for amphibian taxa susceptible to RV.

Viral load

We found ambient air temperature, vegetation type, and prevalence to be important predictors of increased viral loads. While increasing air temperature was associated with an increase in occurrence of RV it was actually weakly associated with a decreasing viral load (Brand et al., 2016; G. V Chinchar, 2002). The reason for this is not entirely clear, but it is possible that our water temperatures exceeded the ideal range for RV replication. Viral loads were positively related to prevalence of RV and were highest when prevalence was between 31% and 40%. This relationship could stem from a dose response of viral particles (Jesse L. Brunner, Richards, & Collins, 2005b). Because RV is directly transmitted through the environment and contact with infected hosts (J. L. Brunner et al., 2007; Gray et al., 2009; Robert, George, De Jesús Andino, & Chen, 2011), as prevalence increases, the effective wetland-level dose should be relatively higher and thus, increase odds of contact with infected individuals and viral particles in the water column. However, it is important to note this prevalence class was represented by only one wetland and could have been driven by dynamics inside that wetland.

Diversity-disease implications

Previous work has shown strong evidence for a dilution effect across systems and that communities can play a role in influencing pathogen dynamics (Civitello et al., 2015; Haas, Hooten, Rizzo, & Meentemeyer, 2011; Johnson, Lund, Hartson, & Yoshino, 2009; E. Miller & Huppert, 2013a; Ostfeld & Keesing, 2012). I did not find any evidence of a dilution effect and no single community-level metric was an important predictor of RV

occurrence. However, in both models, many community parameters were associated with lower relative influence. Other studies have found community-level metrics and environmental factors to be important individually, however, very few have investigated them at the same time (Becker et al., 2012; Brand et al., 2016; Johnson, Preston, Hoverman, & Richgels, 2013b; Savage, Becker, & Zamudio, 2015). These findings support previous studies regarding environmental influences on pathogen dynamics (Altizer et al., 2006; Becker et al., 2012; Cohen et al., 2016; Rojas et al., 2005) and call into question the relative importance of community interactions. A concern regarding the dilution effect is if or how it operates across scales (Z. Y.X. Huang et al., 2016). Some studies have suggested that a dilution effect is common at localized scales, however, as scale increases, environmental factors become more important for disease predictions (Cohen et al., 2016). Diversity-disease relationships have gained support and may be great for investigations in to conservation at localized scales. A dilution effect may exist, but it may not be as important to disease prediction relative to environmental factors at larger scales especially as anthropogenic impacts and effects of climate change become more pervasive threats.

CHAPTER 3

CONCLUSIONS

RANAVIRUS IN AMPHIBIANS

Overall the prevalence of RV I documented in amphibians inhabiting wetlands on the SRS was 15.5%; much lower than found in a previous study (37.4%; Love et al., 2016). However, like in the Love et al. (2016) study I found a high prevalence in *A. terrestris* (35%) indicating that it may be a very important carrier. *Lithobates catesbeianus* had no detectable infection in my study. *Lithobates catesbeianus* has been strongly associated with infection prevalence and is considered to be a reservoir host maintaining transmission throughout the year (Gray et al., 2009; Hoverman, Gray, et al., 2012). In a previous study (Love et al. 2016) the prevalence of RV in *L. catesbeianus* was 29% which emphasizes the need to examine the same wetlands across temporal scales to better understand RV dynamics. Variation in prevalence at the wetland level was high and ranged from 0 - 76% across all species and time points. Variation in prevalence at the wetland level ranged from 0 -100% in a recent study on the SRS (Love et al., 2016). Ranavirus occurrence also displayed variation seasonally. Prevalence varied by season and was low in February and March, peaked during April and May, then decreased in June and July. These results emphasize the dynamic nature of RV infection variation across species, sites, and seasons. Long term investigations directed toward the spatial and temporal dynamics of RV infection will help determine amphibian disease risk.

My results indicate that canopy cover and temperature play a role in RV occurrence. Along with canopy and temperature, prevalence has an effect on the intensity of infection. Canopy cover (Becker et al., 2012; Thomas R. Raffel et al., 2010) and temperature (Brand et al., 2016; Forrest & Schlaepfer, 2011; Rojas et al., 2005) have been shown to influence infection in amphibian diseases. It is possible that canopy cover's influence on deposition of leaf litter has an effect on amphibian growth and development (Stoler & Relyea, 2011, 2016). Along with impacting larval amphibian health, leaf litter and lack of leaf litter can alter the diversity and abundance of amphibian predators (Plenzler & Michaels, 2015). The presence of predators has shown to impact amphibian activity and stress (Relyea, 2001; Skelly & Werner, 1990). Amphibians are experiencing global declines due to many threats. Included on the list of threats are emerging infectious diseases such as RV. Habitat destruction and deforestation could prove even more problematic by altering canopy cover in ephemeral wetland systems. My results indicate higher temperatures increased odds of having RV. As the temperatures rise due to climate change, ephemeral wetlands will experience longer periods of increased temperatures (Mulholland & Best, 1997) and altered hydrology (Brooks, 2000, 2004, 2009) that could exacerbate the effects of RV in amphibians. Future studies should be directed at understanding the mechanism behind canopy cover's interaction with RV as well as studies determining how increased temperatures may direct host distributions and subsequently, pathogen distributions.

DIVERSITY-DISEASE RELATIONSHIPS

Previous work has shown strong evidence for a dilution effect across systems and that communities can play a role in influencing pathogen dynamics (Civitello et al., 2015;

Haas et al., 2011; Johnson et al., 2009; E. Miller & Huppert, 2013a; Ostfeld & Keesing, 2012). Other studies have found community-level metrics and environmental factors to be important individually, however, very few have investigated them at the same time (Becker et al., 2012; Brand et al., 2016; Johnson et al., 2013b; Savage et al., 2015). I did not find any evidence of a dilution effect and no community-level metrics were important predictors of RV occurrence. However, many community predictors did display lower relative influence. This is not to say that community interactions are not influencing pathogen transmission or that dilution does not play a role. These findings simply call into question the relative importance of the role of community interactions in pathogen transmission. It is possible that a dilution effect exists in this system, but it may not be as important to disease prediction relative to environmental factors at larger scales. Especially as anthropogenic impacts and effects of climate change become more pervasive threats, it is important to begin incorporating environmental conditions into discussions of diversity- disease relationships.

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Table 1: The 20 wetlands included in the study and their respective area, perimeter, and the number of minnow traps deployed per wetland. Area and perimeter values were taken from GIS layer data.

Wetland	Wetland ID	Area(ha)	Perimeter(m)	Trap Totals
Sampling group A				**90**
Bay12	7	0.91	533.123	10
Dry Bay	20	7.27	1038.471	30
Ellenton Bay	12	12.14	1117.283	30
Ginger's Bay	9	1.99	316.412	10
Squirrel Bay	14	1.33	372.635	10
Sampling group B				**80**
Bay128	2	1.91	347.799	10
Castor Bay	18	5.1	1043.131	20
Flamingo Bay	19	11.15	1290.885	30
Linda's Pond	5	0.9	254.865	10
Rainbow Bay	4	1.21	315.424	10
Sampling group C				**90**
Bay 92	3	5.54	748.733	20
Bay 93	13	4.25	796.458	20
Bay 100	16	6.26	916.859	20
Bay 120	8	1.19	419.059	10
Thunder Bay	17	8.64	902.758	20
Sampling group D				**90**
Bay 52	11	3.71	708.715	20
Bay 5144	6	1.46	411.932	10
Bay 5179	1	1.53	550.667	10
Mona Bay	10	11.65	1247.99	30
Sarracenia Bay	15	4.48	793.649	20

Table 2. List of the hyper parameters and values used in the overall RV prevalence BRT model.

RV Prevalence BRT Model Hyperparameters	Hyperparameter Values
Tree complexity	3
Learning rate	0.01
Bag fraction	0.7
Total models	1500

Table 3. Hyper parameters and values used in the focal species RV BRT Model.

RV focal species BRT model hyperparameters	Hyperparameter values
Tree complexity	3
Learning rate	0.003
Bag fraction	0.75
Total models	1700

Table 4. List of predicting variables applied to the RV prevalence BRT. Sample level indicates the resolution of the predictor. Percent missing data was calculated by the number of empty values for each observation.

Model Predictor	Description	Sample Level	%Missing
RV.Status	Binary response	Individual	0
SQMean(viral load)	Numeric Response	Individual	0
Prevalence	Percent	Site	0
Vegetation	Vegetation descriptions	Site	0
SoilType	Dominant soil type for each wetland	Site	0
Stage	Developmental stage for the individual	Individual	0
AvgTemp	Mean daily ambient air temp	Daily Site	18.7
MinTemp	Minimum daily water temp	Daily Site	18.7
MaxTemp	Maximum daily water temp	Daily Site	18.7
MeanTemp	Mean daily water temp	Daily Site	18.7
DryingScore	Overall percent of 0's and 1's	Site	0
CumRich	Cumulative species richness	Monthly Site	0
CanopyCover	Percent canopy cover	Site	0
Area	Wetland area	Site	0
Perimeter	Wetland perimeter	Site	0

H	Shannon's Diversity index	Monthly Site	0
S	Observed species richness	Monthly Site	0
J	Peilou's evenness	Monthly Site	0
SVL	Snout-Vent Length	Individual	0
WetAltID.1	Wetland Dummy	Individual	0
WetAltID.2	Wetland Dummy	Individual	0
WetAltID.3	Wetland Dummy	Individual	0
WetAltID.4	Wetland Dummy	Individual	0
WetAltID.5	Wetland Dummy	Individual	0
WetAltID.6	Wetland Dummy	Individual	0
WetAltID.7	Wetland Dummy	Individual	0
WetAltID.8	Wetland Dummy	Individual	0
WetAltID.9	Wetland Dummy	Individual	0
WetAltID.10	Wetland Dummy	Individual	0
WetAltID.11	Wetland Dummy	Individual	0
WetAltID.12	Wetland Dummy	Individual	0
WetAltID.13	Wetland Dummy	Individual	0
WetAltID.14	Wetland Dummy	Individual	0
WetAltID.15	Wetland Dummy	Individual	0
WetAltID.16	Wetland Dummy	Individual	0
WetAltID.17	Wetland Dummy	Individual	0
WetAltID.18	Wetland Dummy	Individual	0
WetAltID.19	Wetland Dummy	Individual	0

WetAltID.20	Wetland Dummy	Individual	0
Month.Apr	Month Dummy	Individual	0
Month.Feb	Month Dummy	Individual	0
Month.Jul	Month Dummy	Individual	0
Month.Jun	Month Dummy	Individual	0
Month.Mar	Month Dummy	Individual	0
Month.May	Month Dummy	Individual	0
Species.4	Species Dummy	Individual	0
Species.9	Species Dummy	Individual	0
Species.20	Species Dummy	Individual	0
Species.21	Species Dummy	Individual	0
Species.24	Species Dummy	Individual	0
Species.26	Species Dummy	Individual	0
Species.27	Species Dummy	Individual	0
Species.28	Species Dummy	Individual	0
Species.29	Species Dummy	Individual	0
Species.31	Species Dummy	Individual	0
Species.32	Species Dummy	Individual	0
Species.34	Species Dummy	Individual	0
Species.35	Species Dummy	Individual	0
Species.38	Species Dummy	Individual	0
Species.39	Species Dummy	Individual	0
Species.41	Species Dummy	Individual	0

Species.42	Species Dummy	Individual	0
PA2	Presence/Absence of species 2	Individual	0
PA3	Presence/Absence of species 3	Individual	0
PA4	Presence/Absence of species 4	Individual	0
PA5	Presence/Absence of species 5	Individual	0
PA6	Presence/Absence of species 6	Individual	0
PA8	Presence/Absence of species 8	Individual	0
PA9	Presence/Absence of species 9	Individual	0
PA20	Presence/Absence of species 20	Individual	0
PA21	Presence/Absence of species 21	Individual	0
PA24	Presence/Absence of species 24	Individual	0
PA26	Presence/Absence of species 26	Individual	0
PA27	Presence/Absence of species 27	Individual	0
PA28	Presence/Absence of species 28	Individual	0
PA29	Presence/Absence of species 29	Individual	0
PA31	Presence/Absence of species 31	Individual	0
PA34	Presence/Absence of species 34	Individual	0
PA35	Presence/Absence of species 35	Individual	0
PA38	Presence/Absence of species 38	Individual	0
PA39	Presence/Absence of species 39	Individual	0
PA41	Presence/Absence of species 41	Individual	0
PA42	Presence/Absence of species 42	Individual	0
AB2	Absolute abundance of species 2	Monthly Site	0

AB3	Absolute abundance of species 3	Monthly Site	0
AB4	Absolute abundance of species 4	Monthly Site	0
AB5	Absolute abundance of species 5	Monthly Site	0
AB6	Absolute abundance of species 6	Monthly Site	0
AB8	Absolute abundance of species 8	Monthly Site	0
AB9	Absolute abundance of species 9	Monthly Site	0
AB20	Absolute abundance of species 20	Monthly Site	0
AB21	Absolute abundance of species 21	Monthly Site	0
AB24	Absolute abundance of species 24	Monthly Site	0
AB26	Absolute abundance of species 26	Monthly Site	0
AB27	Absolute abundance of species 27	Monthly Site	0
AB28	Absolute abundance of species 28	Monthly Site	0
AB29	Absolute abundance of species 29	Monthly Site	0
AB31	Absolute abundance of species 31	Monthly Site	0
AB34	Absolute abundance of species 34	Monthly Site	0
AB35	Absolute abundance of species 35	Monthly Site	0
AB38	Absolute abundance of species 38	Monthly Site	0
AB39	Absolute abundance of species 39	Monthly Site	0
AB41	Absolute abundance of species 41	Monthly Site	0
AB42	Absolute abundance of species 42	Monthly Site	0
RA2	Relative abundance of species 2	Monthly Site	0
RA3	Relative abundance of species 3	Monthly Site	0
RA4	Relative abundance of species 4	Monthly Site	0

RA5	Relative abundance of species 5	Monthly Site	0
RA6	Relative abundance of species 6	Monthly Site	0
RA8	Relative abundance of species 8	Monthly Site	0
RA9	Relative abundance of species 9	Monthly Site	0
RA20	Relative abundance of species 20	Monthly Site	0
RA21	Relative abundance of species 21	Monthly Site	0
RA24	Relative abundance of species 24	Monthly Site	0
RA26	Relative abundance of species 26	Monthly Site	0
RA27	Relative abundance of species 27	Monthly Site	0
RA28	Relative abundance of species 28	Monthly Site	0
RA29	Relative abundance of species 29	Monthly Site	0
RA31	Relative abundance of species 31	Monthly Site	0
RA34	Relative abundance of species 34	Monthly Site	0
RA35	Relative abundance of species 35	Monthly Site	0
RA38	Relative abundance of species 38	Monthly Site	0
RA39	Relative abundance of species 39	Monthly Site	0
RA41	Relative abundance of species 41	Monthly Site	0
RA42	Relative abundance of species 42	Monthly Site	0

Table 5. List of predicting variables applied to the focal species (*Lithobates sphenocephalus*) RV prevalence BRT. Sample Level indicates the resolution of the predictor. Percent missing data was calculated by the number of empty values for each observation.

Model Predictor	Description	Sample Level	%Missing
RV.Status	Binary response	Individual	0
Prevalence	Percent	Site	0
Vegetation	Vegetation descriptions	Site	0
SoilType	Dominant soil type for each wetland	Site	0
Stage	Developmental stage for the individual	Individual	0
AvgTemp	Mean daily ambient air temp	Daily Site	18.7
MinTemp	Minimum daily water temp	Daily Site	18.7
MaxTemp	Maximum daily water temp	Daily Site	18.7
MeanTemp	Mean daily water temp	Daily Site	18.7
DryingScore	Overall percent of 0's and 1's	Site	0
CumRich	Cumulative species richness	Monthly Site	0
CanopyCover	Percent canopy cover	Site	0
Area	Wetland area	Site	0
Perimeter	Wetland perimeter	Site	0
H	Shannon's Diversity index	Monthly Site	0
S	Observed species richness	Monthly Site	0

J	Peilou's evenness	Monthly Site	0
SVL	Snout-Vent Length	Individual	0
WetAltID.1	Wetland Dummy	Individual	0
WetAltID.2	Wetland Dummy	Individual	0
WetAltID.3	Wetland Dummy	Individual	0
WetAltID.4	Wetland Dummy	Individual	0
WetAltID.5	Wetland Dummy	Individual	0
WetAltID.6	Wetland Dummy	Individual	0
WetAltID.7	Wetland Dummy	Individual	0
WetAltID.8	Wetland Dummy	Individual	0
WetAltID.9	Wetland Dummy	Individual	0
WetAltID.10	Wetland Dummy	Individual	0
WetAltID.11	Wetland Dummy	Individual	0
WetAltID.12	Wetland Dummy	Individual	0
WetAltID.13	Wetland Dummy	Individual	0
WetAltID.14	Wetland Dummy	Individual	0
WetAltID.15	Wetland Dummy	Individual	0
WetAltID.16	Wetland Dummy	Individual	0
WetAltID.17	Wetland Dummy	Individual	0
WetAltID.18	Wetland Dummy	Individual	0
WetAltID.19	Wetland Dummy	Individual	0
WetAltID.20	Wetland Dummy	Individual	0
Month.Apr	Month Dummy	Individual	0

Month.Feb	Month Dummy	Individual	0
Month.Jul	Month Dummy	Individual	0
Month.Jun	Month Dummy	Individual	0
Month.Mar	Month Dummy	Individual	0
Month.May	Month Dummy	Individual	0
PA2	Presence/Absence of species 2	Individual	0
PA3	Presence/Absence of species 3	Individual	0
PA4	Presence/Absence of species 4	Individual	0
PA5	Presence/Absence of species 5	Individual	0
PA6	Presence/Absence of species 6	Individual	0
PA8	Presence/Absence of species 8	Individual	0
PA9	Presence/Absence of species 9	Individual	0
PA20	Presence/Absence of species 20	Individual	0
PA21	Presence/Absence of species 21	Individual	0
PA24	Presence/Absence of species 24	Individual	0
PA26	Presence/Absence of species 26	Individual	0
PA27	Presence/Absence of species 27	Individual	0
PA28	Presence/Absence of species 28	Individual	0
PA29	Presence/Absence of species 29	Individual	0
PA31	Presence/Absence of species 31	Individual	0
PA34	Presence/Absence of species 34	Individual	0
PA35	Presence/Absence of species 35	Individual	0
PA38	Presence/Absence of species 38	Individual	0

PA39	Presence/Absence of species 39	Individual	0
PA41	Presence/Absence of species 41	Individual	0
PA42	Presence/Absence of species 42	Individual	0
AB2	Absolute abundance of species 2	Monthly Site	0
AB3	Absolute abundance of species 3	Monthly Site	0
AB4	Absolute abundance of species 4	Monthly Site	0
AB5	Absolute abundance of species 5	Monthly Site	0
AB6	Absolute abundance of species 6	Monthly Site	0
AB8	Absolute abundance of species 8	Monthly Site	0
AB9	Absolute abundance of species 9	Monthly Site	0
AB20	Absolute abundance of species 20	Monthly Site	0
AB21	Absolute abundance of species 21	Monthly Site	0
AB24	Absolute abundance of species 24	Monthly Site	0
AB26	Absolute abundance of species 26	Monthly Site	0
AB27	Absolute abundance of species 27	Monthly Site	0
AB28	Absolute abundance of species 28	Monthly Site	0
AB29	Absolute abundance of species 29	Monthly Site	0
AB31	Absolute abundance of species 31	Monthly Site	0
AB34	Absolute abundance of species 34	Monthly Site	0
AB35	Absolute abundance of species 35	Monthly Site	0
AB38	Absolute abundance of species 38	Monthly Site	0
AB39	Absolute abundance of species 39	Monthly Site	0
AB41	Absolute abundance of species 41	Monthly Site	0

AB42	Absolute abundance of species 42	Monthly Site	0
RA2	Relative abundance of species 2	Monthly Site	0
RA3	Relative abundance of species 3	Monthly Site	0
RA4	Relative abundance of species 4	Monthly Site	0
RA5	Relative abundance of species 5	Monthly Site	0
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RA27	Relative abundance of species 27	Monthly Site	0
RA28	Relative abundance of species 28	Monthly Site	0
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RA31	Relative abundance of species 31	Monthly Site	0
RA34	Relative abundance of species 34	Monthly Site	0
RA35	Relative abundance of species 35	Monthly Site	0
RA38	Relative abundance of species 38	Monthly Site	0
RA39	Relative abundance of species 39	Monthly Site	0
RA41	Relative abundance of species 41	Monthly Site	0
RA42	Relative abundance of species 42	Monthly Site	0

Table 6: The ephemeral wetland species included in the disease analysis and their respective species code designations.

Species	Species Code
Bufonidae	
Anaxyrus terrestris	21
Hylidae	
Acris gryllus	24
Hyla avivoca	32
H. chrysoscelis	31
H. cinerea	27
H. femoralis	29
H. gratiosa	28
Pseudacris crucifer	26
P. nigrita	34
P. ornata	35
Microhylidae	
Gastrophryne carolinensis	38
Ranidae	
Lithobates catesbeianus	39
L. clamitans	41
L. sphenocephalus	42
Salimandridae	
Notophthalmus viridescens	9
Scaphiopodidae	
Scaphiopus holbrookii	20
Sirenidae	
Siren intermedia	4

Table 7. Summary of samples included in the analyses with the number of each species analyzed (N), the number that tested positive for ranavirus (N_{RV}), and the lower and upper Clopper-Pearson binomial confidence limits for percent prevalence presented (CI%).

Species	**N**	**N_{RV}**	**Prevalence (%)**	**CI%**	**Mean Viral Load**
Bufonidae	**154**	**48**	**31**	**24.0-39.1**	**44407.30**
Anaxyrus terrestris	116	40	35	25.9-43.9	44407.30
Hylidae	**744**	**151**	**20**	**17.5-23.4**	**59,434.43**
Acris gryllus	69	0	0	0-5.2	0
Hyla avivoca	1	1	100	2.5-100	51,7872.69
H. chrysoscelis	3	0	0	0-70.6	0
H. cinerea	3	0	0	0-70.8	0
H. femoralis	15	0	0	0-21.8	0
H. gratiosa	104	12	12	6.1-19.3	8.05
Pseudacris crucifer	328	79	24	19.6-29.1	16,982.34
P. nigrita	166	53	32	24.9-39.6	26.22
P. ornata	124	6	5	1.8-10.2	23.53
Microhylidae	**14**	**2**	**14**	**1.8-42.8**	**27.25**
Gastrophryne carolinensis	14	2	14	1.8-42.8	27.25
Ranidae	**1075**	**133**	**12**	**10.5-14.5**	**40,095,937.91**
Lithobates catesbeianus	25	0	0	0-13.7	0
L. clamitans	65	6	9	3.5-19.0	20.25
L. sphenocephalus	985	127	13	10.9-15.1	120,287,793.5
Salimandridae	**153**	**8**	**5**	**2.3-10.0**	136.63
Notophthalmus viridescens	153	8	5	2.3-10.0	136.63
Scaphiopodidae	**69**	**0**	**0**	**0-5.2**	**3.5**
Scaphiopus holbrookii	38	8	21	9.6-37.3	3.5
Sirenidae*/Siren intermedia*	**1**	**0**	**0**	**0-97.5**	**0**
Grand Total	**2210**	**342**	**15.5**	**14.0-17.1**	----------

Table 8. Summary of samples included in the analyses with the number of samples analyzed from each wetland (N), the number that tested positive for ranavirus (N_{RV}), and the lower and upper Clopper-Pearson binomial confidence limits for percent prevalence presented (CI).

Wetland	N	N_{RV}	Prevalence (%)	CI (%)	Mean Viral Load
Sampling group A	**411**	**28**	**7**	**0.05-0.10**	**14.22**
Bay12	107	3	3	0.6-8.0	48.09
Dry Bay	42	2	5	0.6-16.2	37.93
Ellenton Bay	159	21	13	8.4-19.5	4.923
Ginger's Bay	19	1	5	0.1-26.0	23.96
Squirrel Bay	84	1	1	0-6.5	13.43
Sampling group B	**282**	**28**	**10**	**0.07-0.14**	**807,295.40**
Bay128	77	16	21	12.4-31.5	1279639.97
Castor Bay	93	3	3	0.7-9.1	54.67
Flamingo Bay	76	8	11	4.7-19.7	95.82
Linda's Pond	24	1	4	0-21.1	14.07
Rainbow Bay	12	0	0	0-26.4	0
Sampling group C	**637**	**214**	**34**	**0.30-0.37**	**85787.41**
Bay 92	46	35	76	61.2-87.4	51.78
Bay 93	64	17	27	16.3-39.1	4.17
Bay 100	80	19	24	14.0-17.1	4.37
Bay 120	208	51	25	19.0-30.9	55.25
Thunder Bay	239	92	39	32.3-45.0	86,805.65
Sampling group D	**880**	**72**	**8**	**0.06-0.10**	**15.53**
Bay 52	208	14	7	3.7-11.0	6.44
Bay 5144	107	16	15	8.8-23.1	15.58
Bay 5179	176	35	20	14.3-26.6	7.04
Mona Bay	224	6	3	0.9-5.7	4.19
Sarracenia Bay	165	1	0.6	0-3.3	1.60
Grand Total	**2210**	**342**	**15.5**	**14.0-17.1**	**----------**

Table 9. Summary of samples included in the analyses with the number of samples analyzed each month (N), the number that tested positive for ranavirus (N_{RV}), and the lower and upper Clopper-Pearson binomial confidence limits for percent prevalence presented (CI).

Month	**N**	**N_{RV}**	**Prevalence (%)**	**CI (%)**	**Mean Viral Load**
February	291	13	5	2.4-7.5	45.29
March	352	19	5	3.3-8.3	11.28
April	699	164	24	20.4-26.8	87,063.99
May	519	118	23	19.2-26.6	370,116.24
June	194	28	14	9.8-20.2	15.73
July	155	0	0	0-2.4	0
Grand Total	**2210**	**342**	**15.5**	**14.0-17.1**	**----------**

Table 10. Summary of the predictive performance and model evaluation statistics of the RV prevalence BRT model (training data n= 1768). The model performance was evaluated using mean total deviance, mean residual deviance, cross validation deviance, training data correlation, cross validation correlation, area under the curve (AUC) for training data, and cross validation AUC. Parentheses contain the standard error of the mean.

Model Evaluation Statistics	Model Evaluation Values
Number of infection positive	281
Number of infection negative	1487
Mean total deviance	0.876
Mean residual deviance	0.486
Estimated CV deviance	0.576 ± 0.019
Training data correlation	0.68
CV correlation	0.596 ± 0.022
Training AUC score	0.927
CV AUC score	0.877 ± 0.01
Predictive deviance	0.531

Table 11. Summary of the predictive performance and model evaluation statistics of the RV prevalence BRT model test data (test data n=442). The testing data for this model were withheld during the model run.

Test Model Evaluation Statistics	Test Model Evaluation Values
Number of infection positive	61
Number of infection negative	381
Test-AUC	0.878
Test correlation	0.599

Table 12. Summary of the predictive performance and model evaluation statistics of the focal species RV BRT model (training data n= 894). The model performance was evaluated using mean total deviance, mean residual deviance, cross validation deviance, training data correlation, cross validation correlation, AUC for training data, and cross validation AUC. Parentheses contain the standard error of the mean.

Model Evaluation Statistics	Model Evaluation Values
Number of infection positive	106
Number of infection negative	788
Mean total deviance	0.79
Mean residual deviance	0.501
Estimated CV deviance	0.605 ± 0.024
Training data correlation	0.635
CV correlation	0.521 ± 0.036
Training AUC score	0.894
CV AUC score	0.81 ± 0.022
Predictive deviance	0.467

Table 13. Summary of the predictive performance and model evaluation statistics of the focal species RV BRT model test data (test data n=197).

Test Model Evaluation	Test Model Evaluation Values
Number of infection positive	21
Number of infection negative	176
Test-AUC	0.863
Test correlation	0.576

Table 14. Most informative general linear model statements for mean viral load of RV. Prevalence= prevalence binned into 5 classes, AvgTemp=mean daily ambient air temperature, SVL= snout-vent length, Vegetation= canopy and vegetation types binned into 4 classes, DryingScore= indication of hydroperiod calculated as the percent time wetland held water.

RV Mean Viral Load Model Statements	AICc	dAICc	Df	Weight
Prevalence +AvgTemp+SVL+Vegetation	1040.9	0.0	11	0.1833
Prevalence+ AvgTemp+SVL+DryingScore+Vegetation	1041.2	0.3	12	0.1560
Prevalence+AvgTemp+DryingScore+Vegetation	1041.7	0.8	11	0.1242
Prevalence+AvgTemp+Vegetation	1042.0	1.1	10	0.1080
NULL MODEL	1084.8	43.9	2	<0.001
FULL MODEL	1051.6	10.7	25	<0.001

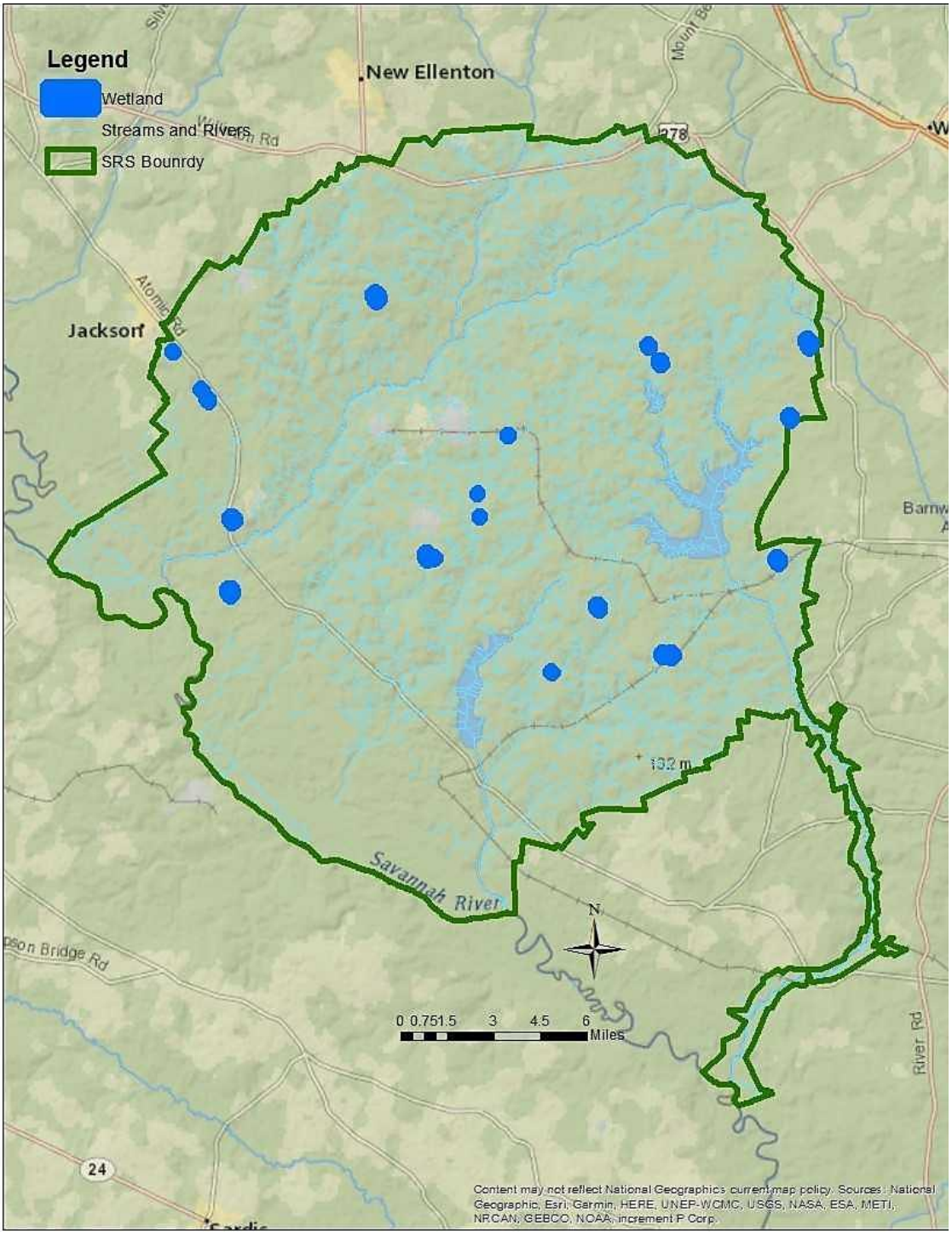

Figure 1. Map of the SRS depicting locations of study wetlands.

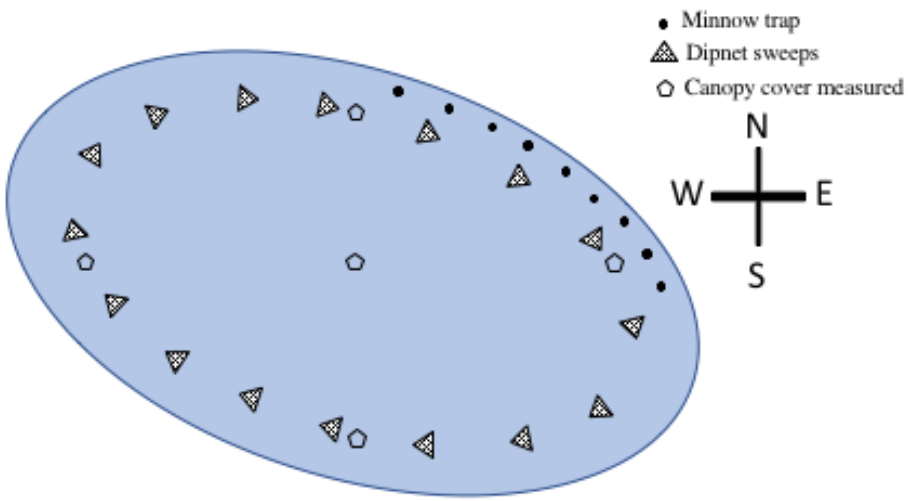

Figure 2. Diagram depicting standardized amphibian sampling methods in wetlands. Minnow traps were deployed every 10 m in the northeast corner in shallow water (~20 cm), dipnet sweeps were performed ~0.5 m deep around the entire wetland with three sweeps conducted every 20m. Canopy cover was measured in the center of the wetland and at 0.5 m deep at each cardinal direction.

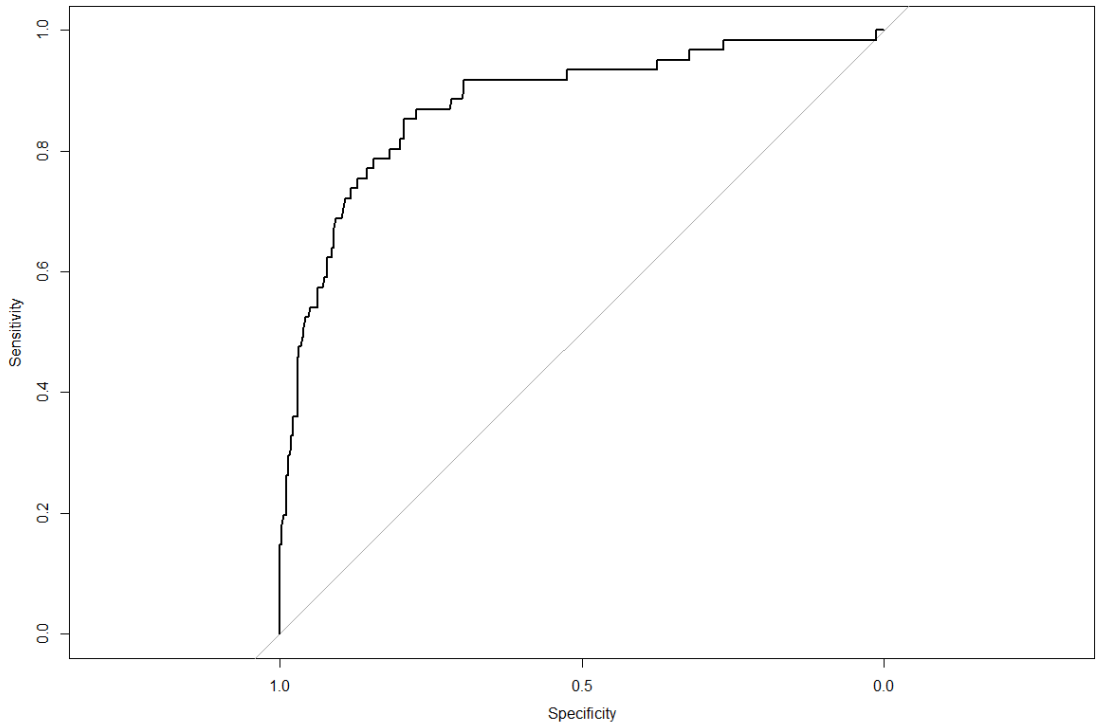

Figure 3. Receiver operating characteristics curve (ROC) for the overall RV prevalence BRT. The curve uses testing data to visualize how well the model correctly identifies test observations. ROC plots the sensitivity (true positive) over the specificity (true negative). ROC score for the RV prevalence BRT was 0.87.

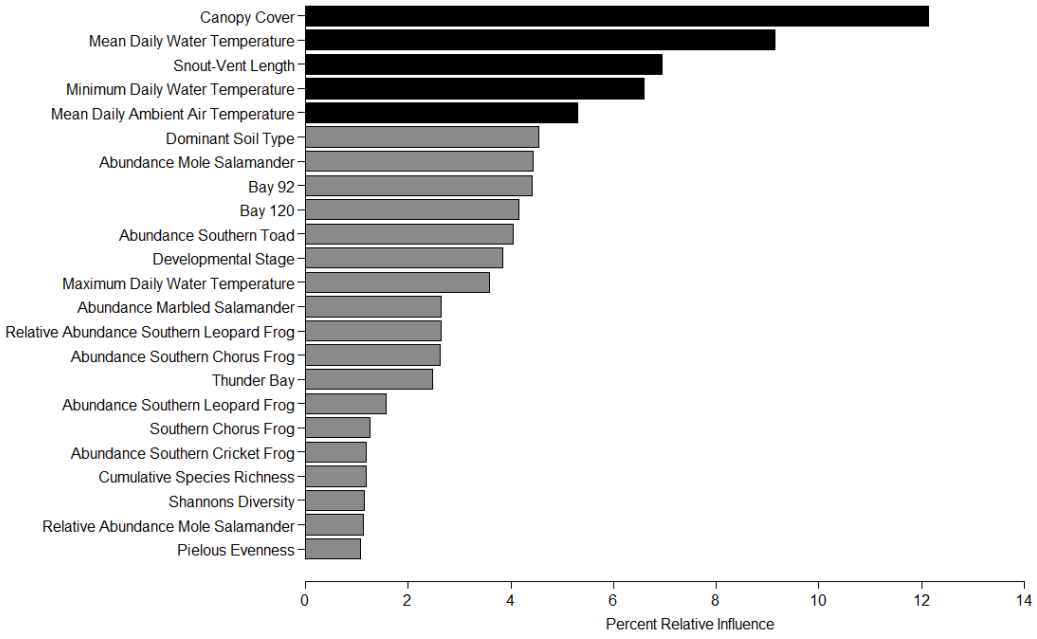

Figure 4. Relative influence of predictor variables in explaining the overall prevalence of RV across species and wetlands. Predictors shaded black represent variables with greater than 5% relative influence. Predictors shaded in grey have a percent relative influence of less than 5%, but greater than 1%.

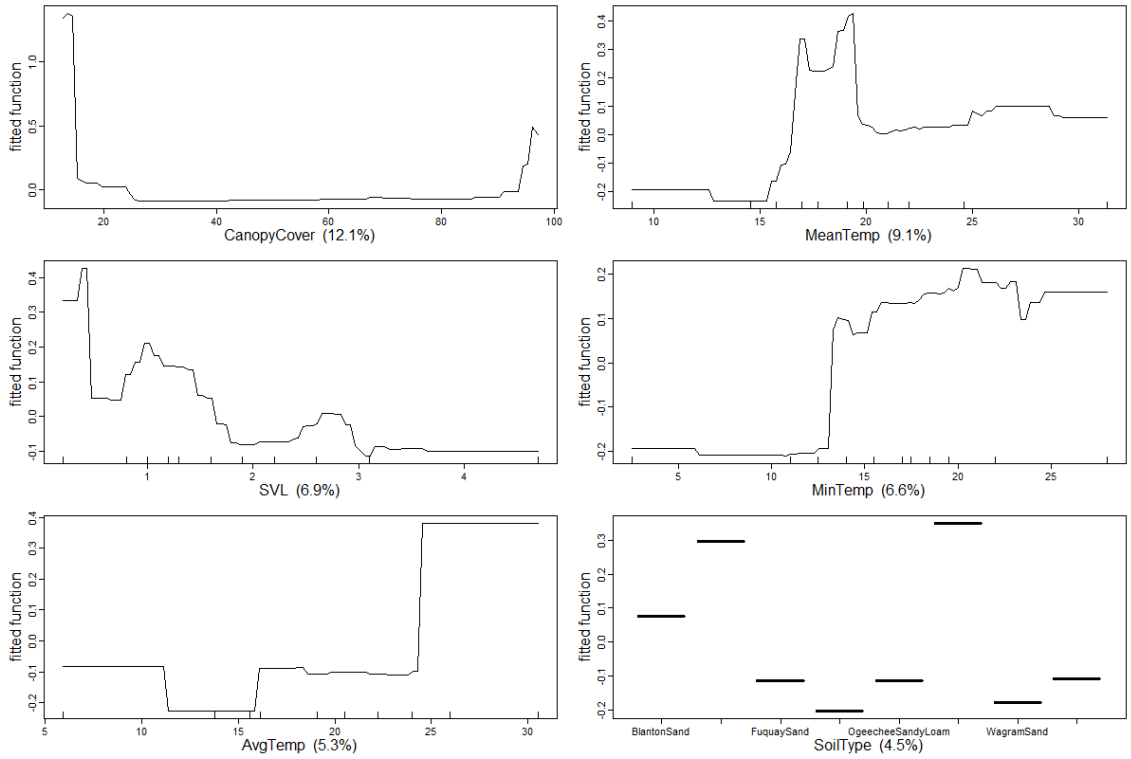

Figure 5. Relationships between predictors of interest and the log odds ratio of individual amphibians testing positive for RV when holding all other predictors at their mean values. Each graph is a partial dependency plot with high values on y-axis indicating increased probability of testing positive for RV.

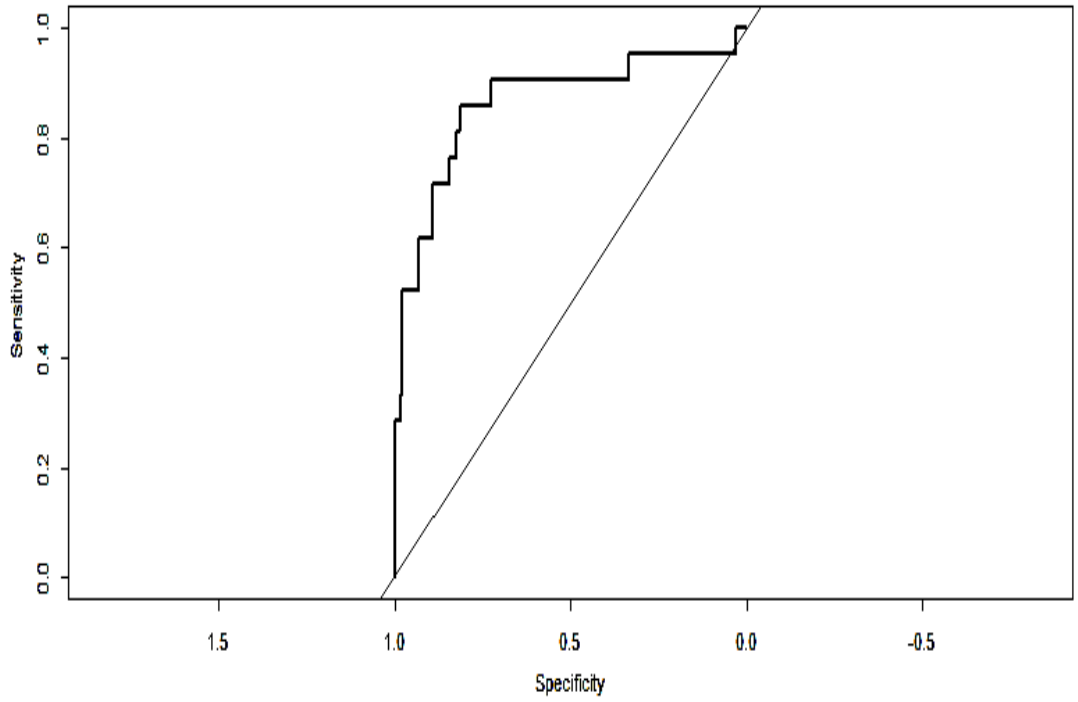

Figure 6. Receiver operating characteristics curve (ROC) for the focal species RV BRT. The curve uses testing data to visualize how well the model correctly identifies test observations. ROC plots the sensitivity (true positive) over the specificity (true negative). ROC score for the RV prevalence BRT was 0.86.

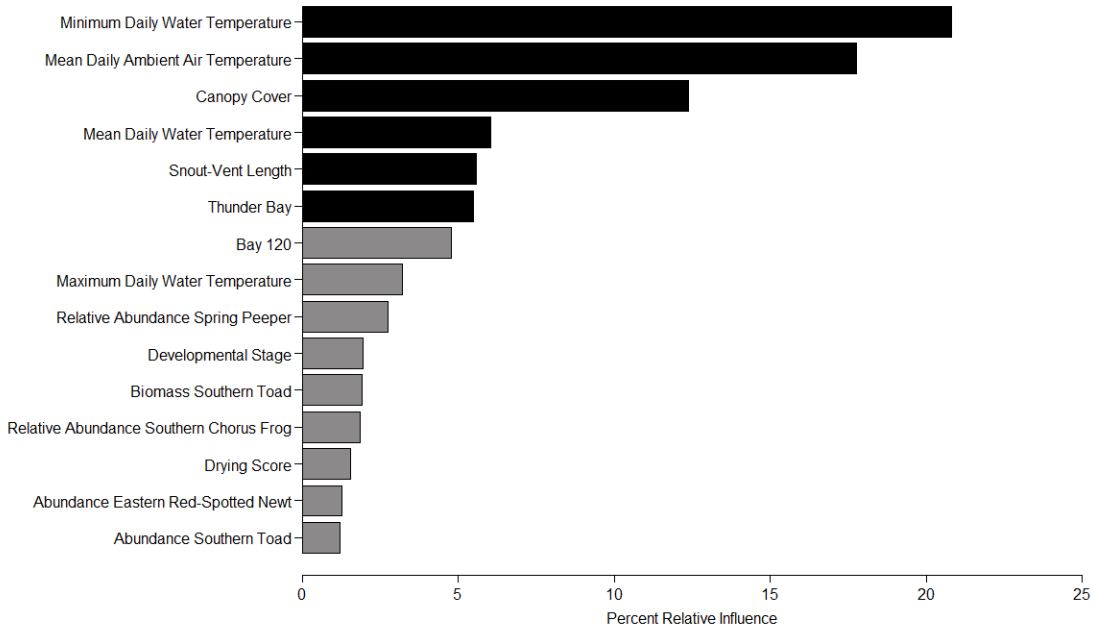

Figure 7. Relative influence of predictor variables in explaining the prevalence of RV in the focal species, *L. sphenocephalus*. Predictors shaded black represent variables with greater than 5% relative influence. Predictors shaded in grey have a percent relative influence of less than 5%, but greater than 1%.

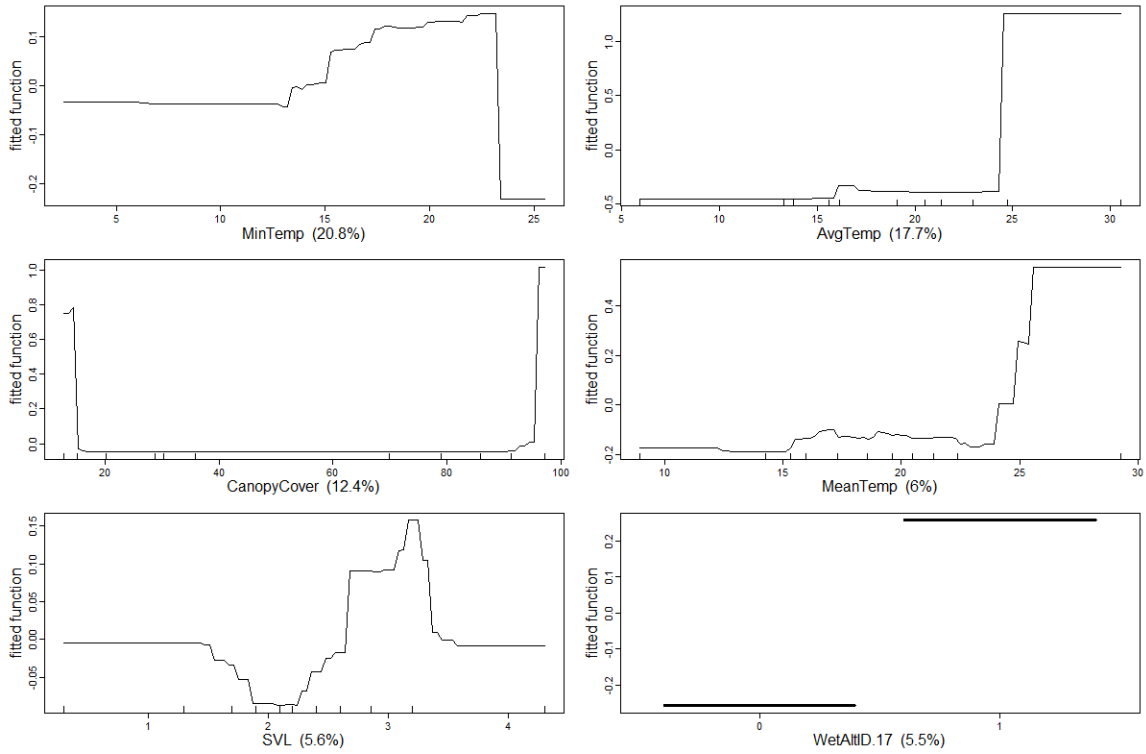

Figure 8. Relationships between predictors of interest and the log odds ratio of *L. sphenocephalus* individuals testing positive for RV when holding all other predictors at their mean values. Each graph is a partial dependency plot with high values on y-axis indicating increased probability of testing positive for RV.

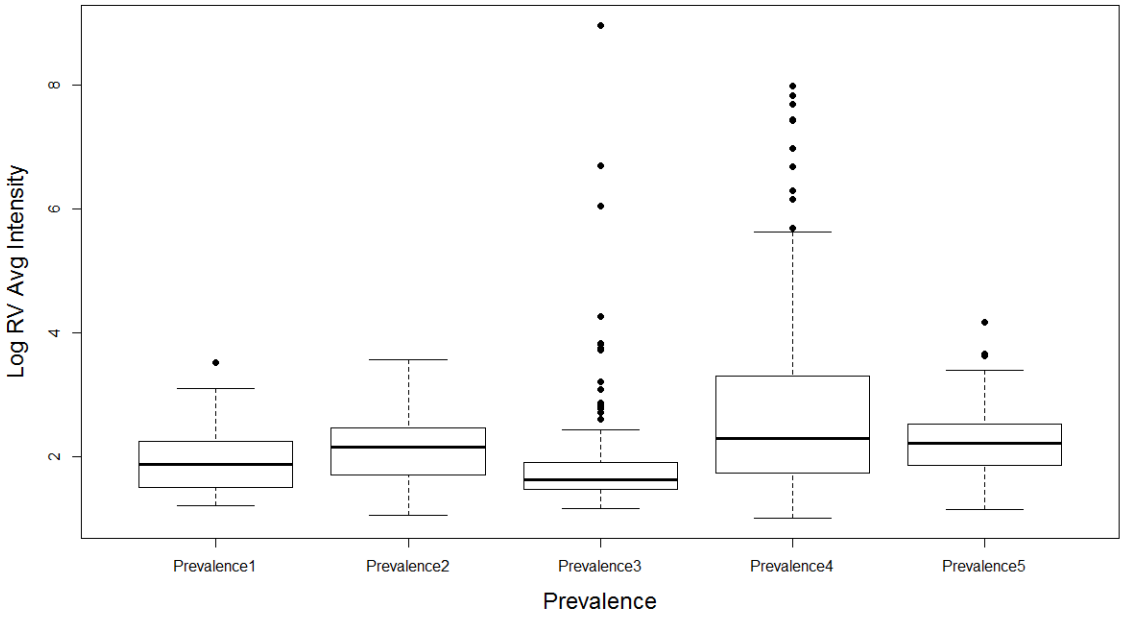

Figure 9. The relationship between viral load and prevalence classes from GLMs. Prevalence was turned into a class variable (1= 0-10%, 2=11-20%, 3= 21-30%, 4=31-40%, and 5=41-76%).

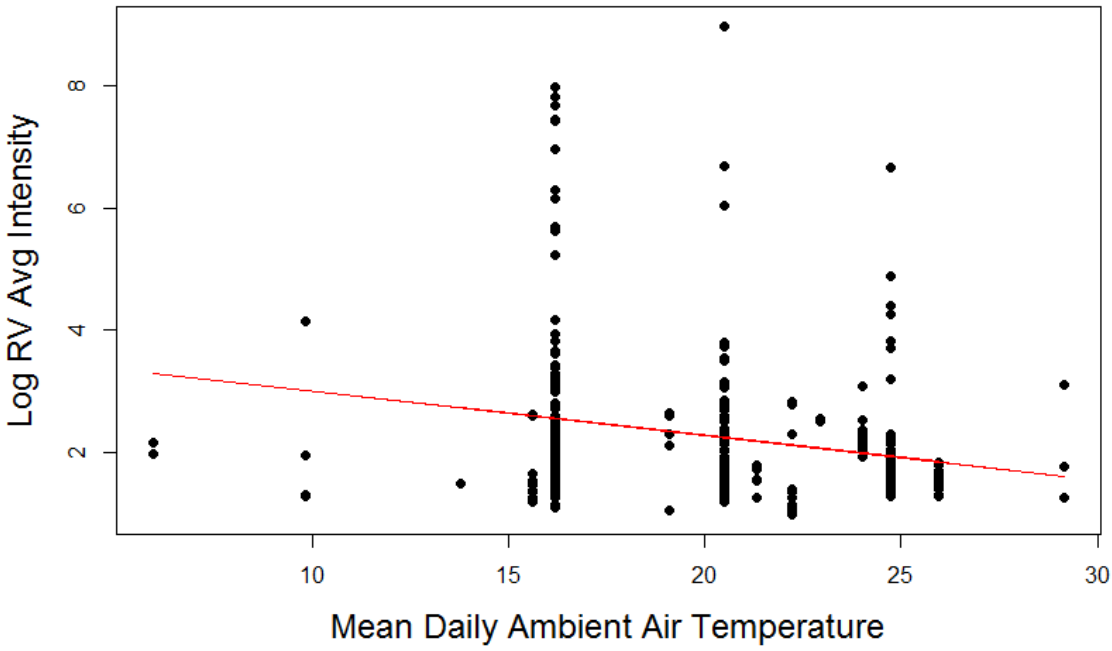

Figure 10. The regression relationship between Log RV average intensity and mean daily ambient air temperature. The relationship indicates a weak trend where increasing temperatures result in decreased viral loads.

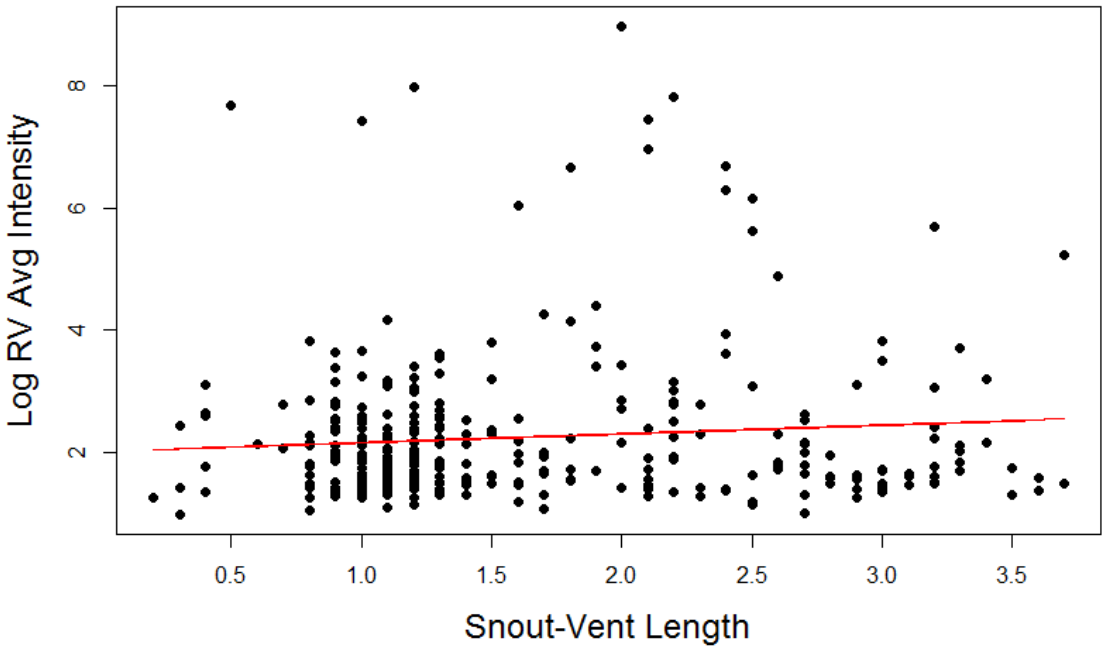

Figure 11. The regression relationship between Log RV average intensity and snout-vent length. The relationship indicates a weak trend where increasing snout-vent length results in increasing viral loads.

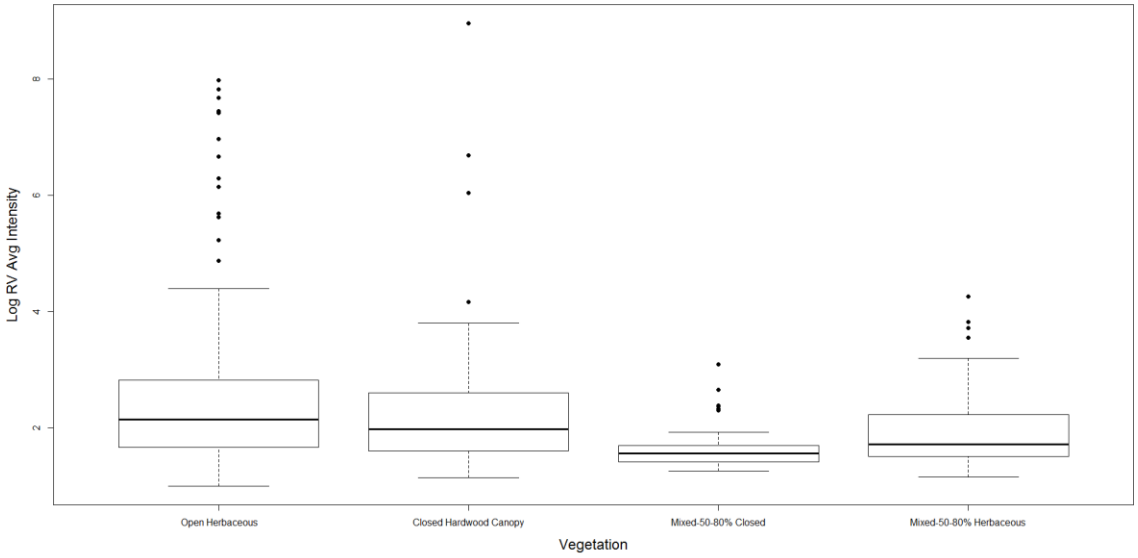

Figure 12. Log RV average intensities for individuals associated with wetlands associated with four vegetation types. Open herbaceous wetlands and closed hardwood canopy wetlands had individuals with significantly higher average viral loads.

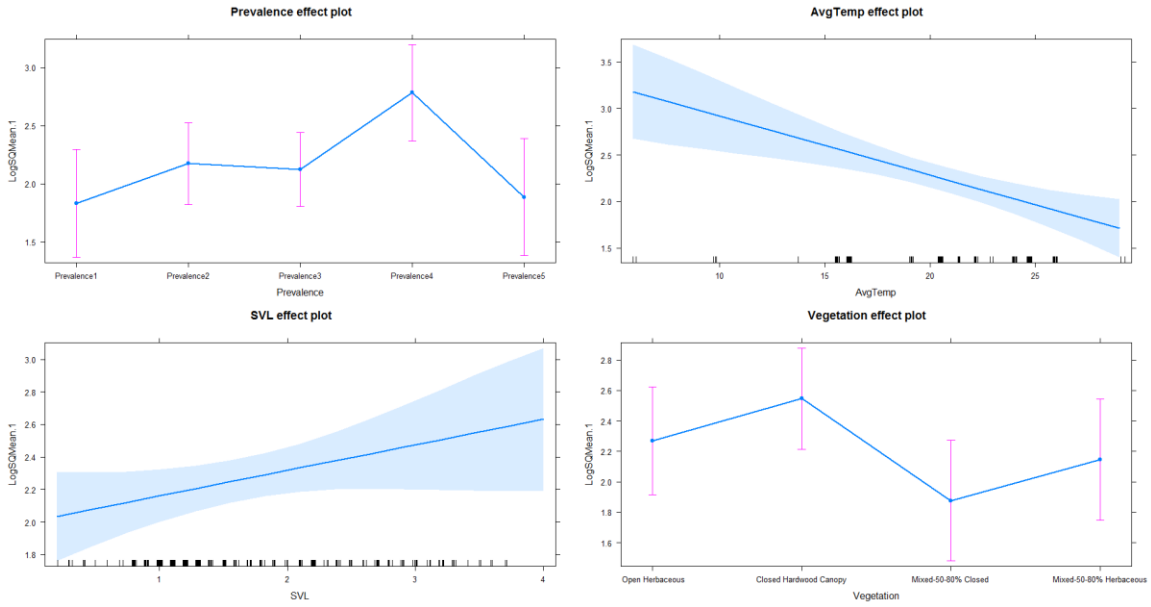

Figure 13. Effects plots (*effects package-R)* for all four variables from the most informative GLM explaining trends in average viral loads.