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Ribbeting Results: Emergent Infectious Diseases in Wetland Systems

Ariel Horner
University of Central Florida

EMERGENT PATHOGEN DYNAMICS VARY SPATIALLY AND TEMPORALLY IN SUBTROPICAL WETLAND COMMUNITIES

by

ARIEL A. HORNER
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ABSTRACT

North American amphibians have recently been impacted by two major emerging pathogens, the fungus *Batrachochytrium dendrobatidis* (*Bd*) and iridoviruses in the genus *Ranavirus* (*Rv*). Environmental, seasonal and host factors may play important roles in disease dynamics, but few studies incorporate these components into their analyses. Here, we investigated the role of environmental, seasonal, genetic and location effects on driving *Bd* and *Rv* infection prevalence and severity in a biodiversity hot spot, the southeastern United States. We used quantitative PCR to characterize *Bd* and *Rv* dynamics in natural populations of three amphibian species: *Notophthalmus perstriatus, Hyla squirella* and *Pseudacris ornata* and more broadly in multi-species amphibian communities across Florida. We combined pathogen data, genetic and host metrics, and seasonal and environmental variables into statistical models to evaluate how these factors impact infectious disease dynamics. Occurrence, prevalence and intensity of *Bd* and *Rv* varied across species, populations, and sites. *Pseudacris ornata* was found to have high levels of Bd across sites. In Florida, both pathogens were found ubiquitously across sites and seasons and at high levels within three different host families. We conclude that *Bd* and *Rv* are more abundant in the southeastern United States than previously thought and that host, seasonal and environmental factors are all important for predicting amphibian pathogen dynamics. Incorporating seasonal, host and environmental information into conservation plans for amphibians is necessary for the development of more effective management strategies to mitigate the impact of emerging infectious diseases.

ACKNOWLEDGEMENTS

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INTRODUCTION

Emerging infectious diseases have been on the rise over the past 30 years (Daszak et al. 2000). Dobson and Foufopoulos (2001) define an emerging infectious disease (EID) as any infectious disease whose range of hosts, geographical range or prevalence has recently expanded. While many studies have focused on the impacts EIDs have on humans (Farmer 1996), EIDs have had dire consequences on wildlife. All major vertebrate groups (fish, amphibians, reptiles, birds and mammals) have been impacted within the past three decades by EIDs (Montgomery and Montgomery 1988; Gremillion-Smith and Woolf 1988; McClure 1989; Hedrick et al. 1998; Daszak et al. 2000; Gibbons 2000; Altzier et al. 2004; Gozlan et al. 2005; Frick et al. 2010). Infectious pathogens have been major players in the extirpation of sensitive and endangered species, such as the Panamanian Golden Frog (*Atelopus zeteki*) and the black footed ferret (*Mustela nigripes*) (Thorne and Williams 1988; Lips et al. 2010) as well as population declines for many other species (Montgomery and Montgomery 1988; Gremillion-Smith and Woolf 1988; McClure 1989; Roelke-Parker et al. 1996; Cully et al. 1997; Berger et al. 1998; Hedrick et al. 1998; Oldroyd 1999; Daszek 2000; Gibbons 2000; Jensen et al. 2002; Hochachka et al. 2003 Altzier et al. 2004; Gozlan et al. 2005; Frick et al. 2010). Disease has been one of the main factors driving declines in amphibians in recent times (Gibbons 2000; Mendelson et al. 2006). With over 3% of the world's amphibians estimated to have gone extinct and many populations crashing, investigation into the mechanisms behind these declines is important (Alroy 2015). *Batrachochytrium dendrobatitis* (*Bd*), a deadly fungus, has received most of the attention, but a class of iridoviruses collectively known as Ranavirus have also been impacting global amphibian populations.

Bd is a unique fungus belonging to the phylum Chytridiomycota. Chytrid fungi are highly aquatic and unlike other fungi classes, possess flagellated, swimming zoospore (James et al. 2006). Most chytrid fungi infect plants, invertebrates and other fungi or are not pathogenic. *Bd* is different in that it can infect vertebrates, specifically amphibians (Pessier et al. 1999; Gleason et al. 2008). *Bd* is water vectored and implants into the skin of its amphibian host, causing the keratin to thicken within the epidermis (Pessier et al. 2009). As the skin thickens, excessive sloughing occurs. It is hypothesized that the host dies from lack of water uptake and a disruption in electrolyte balance (Voyles at al. 2007). *Bd* infections have been documented on every continent but Antarctica (**Figure 1**) and have deemed responsible for the extinction and decline of several anuran species (Rothermel et al. 2008; Fisher et al. 2009; Alroy 2015)

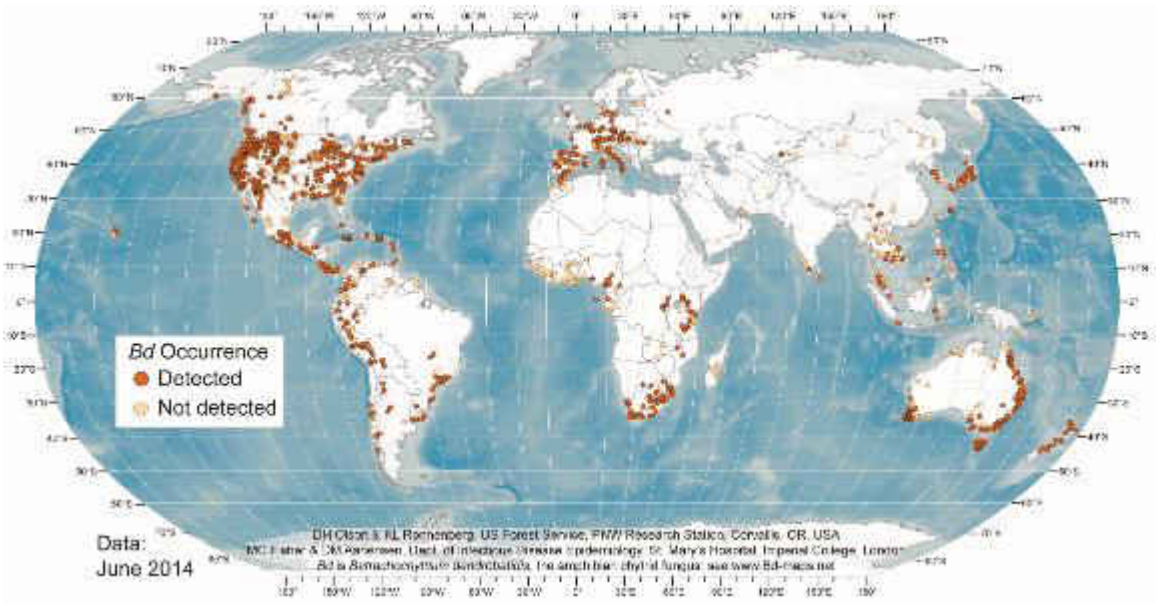

Figure 1. Negative and positive cases of *Bd* worldwide. (Olsen 2014; accessed from http://www.fs.fed.us/pnw/lwm/aem/people/olson.html on Sept 25, 2015)

Ranaviruses are double stranded DNA viruses belonging to the family Iridoviridae (Chinchar 2002). Iridoviruses are icosahedral in shape and enter the cell by fusion with the cell membrane or

through endocytosis (Braunwald et al. 1985). The virus replicates in the cytoplasm of a variety of cells (Chinchar 2002). While other iridoviruses infect invertebrates, iridoviruses in the genus *Ranvirus* only infect ectothermic vertebrates. There are several viruses within the genus *Ranavirus,* but Frog Virus 3 has received the most attention (Chinchar 2002). For the scope of this proposal, whenever Ranavirus (*Rv)* is mentioned, Frog Virus 3 is being referred to. *Rv* was first isolated in the 1960's by Granoff et al. (1966) while studying cancer in *Lithobates pipiens*. One of the major host differences between *Rv* and *Bd* is that *Rv* does not just target amphibians; fish, turtles and other reptiles have been impacted by the virus. *Rv* has been documented in 25 countries and on every continent but Antarctica (**Figure 2**). *Rv* is considered an emerging pathogen, much like *Bd*. Its host range is continually growing and new cases are being discovered worldwide (Daszak et al. 1999). The disease is easily vectored through water or soil, animal on animal interaction or necrophagy (Harp and Petranka 2006; Gray et al. 2009). In amphibians, death ensues due to hemorrhaging and edema of critical organs (Gray et al. 2009; Gray and Chinchar 2015). In turtles, pathology is less specific but damage to the respiratory system is often obse*Rv*ed (Johnson et al. 2008).

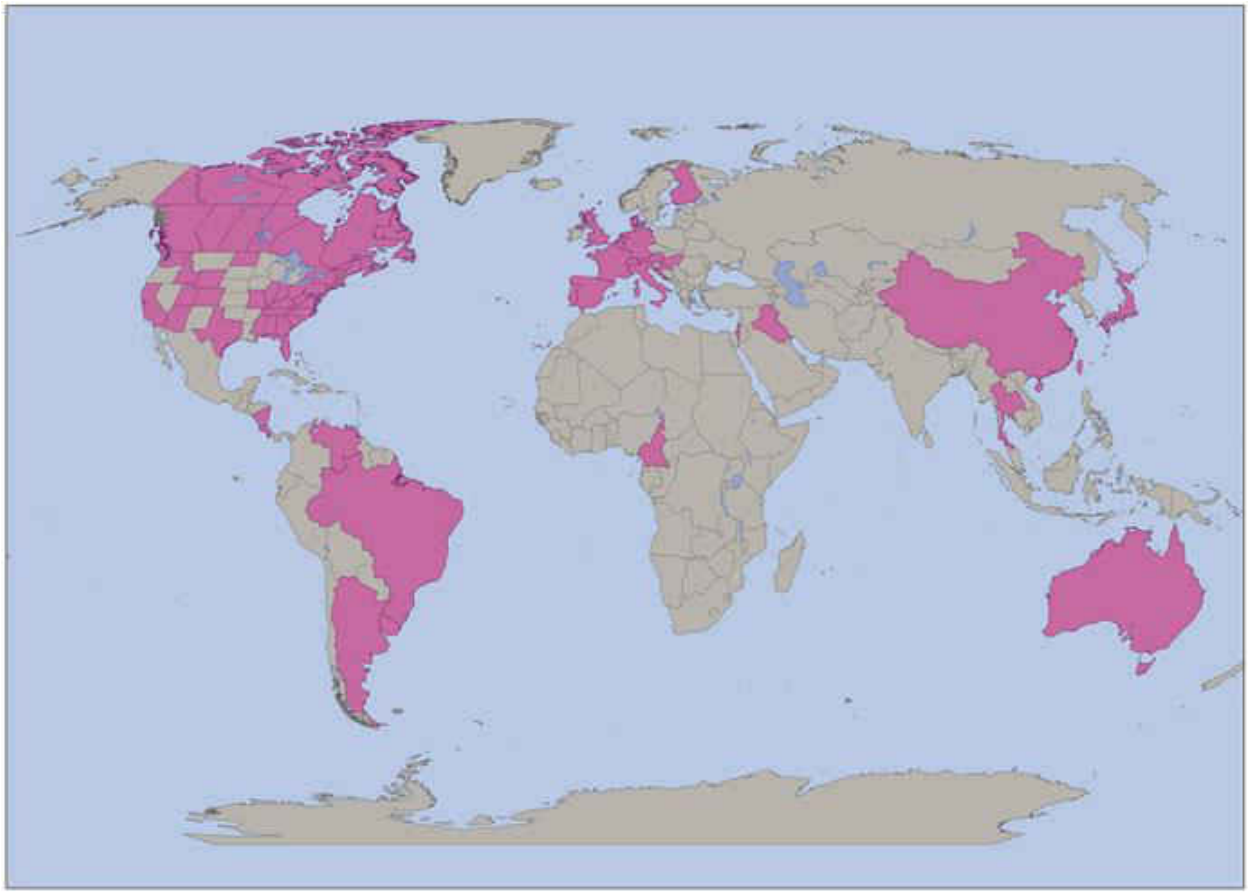

Figure 2. Documented cases of *Rv* worldwide. Pink areas represent countries with positive cases. (Duffus et al. 2015, taken from *Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates)*

While die-offs are happening around the globe, many mortality events have been observed in the United States from *Bd* and *Rv* (Green et al. 2002). *Bd* has mainly impacted ranids and bufonids in western states, including listed species (Daszak et al. 1999; Fellers et al. 2001; Green et al. 2002; Pearl et al. 2007; Muths et al. 2008). *Rv* has caused infection and die-offs in at least 20 different reptile and amphibian species throughout the country, including species under state and federal protection (Green et al. 2002; Johnson et al 2008; Hoverman et al. 2011). In the southeast, gopher frogs (*Lithobates capito*), an IUCN listed species, have been found to be mortally impacted by *Rv* infections (Hoverman et al. 2011; Landsberg et al. 2013; Means et al. 2013). In chelonians, massive die-offs have

been reported for another IUCN listed species, the Eastern box turtle (*Terrapene carolina*), and infections have been documented in the federally listed Gopher tortoise (*Gopherus polyphemus)* *(*Johnson et al. 2008; Allender et al. 2011). Because other anthropogenic factors are already causing declines in North American turtles and tortoises, it is likely that populations, including those of listed species, are susceptible to further decline if confronted with *Rv* (Gibbons et al. 2001).

Because southeastern U.S. states are exceptionally rich in herptile diversity (Hansen et al. 2010), EIDs have the potential to severely impact regional ecosystems and overall biodiversity. At present, EIDs have been documented in multiple amphibian and reptile groups in the southeast (Green et al. 2002; Johnson et al. 2007; Landsberg et al, unpublished data), yet disease monitoring has been limited. Published studies of disease monitoring for southeastern amphibians are limited (Rothermel et al. 2008; Landsberg et al., unpublished data). Additionally, sites are frequently only visited during certain months of the year or at a single time point, and are often not revisited (Rothermel et al. 2008; Landsberg et al., unpublished data). Temporal, spatial, and host-specific variation in pathogen infection have therefore not been assessed.

This study seeks to answer the following questions:

Question 1: *What is the past distribution and prevalence of infection for two prominent ectothermic pathogens (Batraochytrium dendrobatitis [Bd] and Frog Virus 3 [Rv]) in three representative amphibian species throughout their range in the southeast?*

Question 2: *Across three sites in Florida, what is the occurrence, prevalence, intensity, persistence and co-infection of Bd and Rv in mixed anuran communities across space and time?*

CHAPTER 1

Introduction

Infectious disease is a well-known driver of animal declines worldwide (e.g. Daszek 1999; Hochachka et al. 2003). Ectothermic vertebrates, particularly reptiles and amphibians, have been exceptionally impacted by emerging infectious diseases (EIDs) (Daszek et al. 1999). Observations of massive, worldwide, pathogen-associated amphibian die-offs date back to the 1970s and 80s (Barinaga 1990). In North America, amphibians have been impacted by two major emerging pathogens, the fungus *Batrachochytrium dendrobatidis* (*Bd*) and a class of iridoviruses collectively known as *Ranavirus* (*Rv*) (Green et al. 2002; Briggs et al. 2010; Miller, Gray and Storfer 2011; Savage et al. 2011). *Bd* was identified as a disease agent in 1998 (Berger et al. 1998) and *Rv* was classified as an emerging pathogen after 1993 (Fisher et al. 2009; Gray et al. 2009). In North America, *Bd* has mainly impacted anurans in the families Ranidae and Bufonidae, causing mass mortality events for species in these families. Most infections have occurred in the western United States and have impacted already threatened and endangered species (e.g. Daszak et al. 1999; Green et al. 2002; Schrader 2002; Pearl et al. 2007; Muths et al. 2008). *Rv* has caused die-offs in at least 20 different reptile and amphibian species throughout the United States, including species under state and federal protection (Green et al. 2002; Johnson et al. 2008; Hoverman et al. 2011).

In the southeastern United States (hereafter, Southeast), emerging pathogens impacting ectothermic vertebrates have been poorly characterized but have the potential to significantly impact the area. The Southeast is exceptionally rich in amphibian and reptile diversity, hosting more than half of species occurring in the United States (Hansen et al. 2010). EIDs therefore have potential to severely impact this region's ecosystems and overall biodiversity. Presently, EIDs have been documented in

multiple amphibian and reptile groups occurring in the Southeast (Green et al. 2002; Johnson et al. 2007; Landsberg et al. 2013), yet disease monitoring has been limited. One species, the Gopher frog (*Lithobates capito*), is susceptible to *Rv* infections in the lab (Hoverman et al. 2011) and infection and die-offs have been documented for wild populations (Landsberg et al. 2013; Means et al. 2013). However, impacts of pathogens on other species in this area are more enigmatic (Johnson et al. 2008; Chatfield et al. 2012; Rothermel et al. 2012; Means et al. 2013). Because disease monitoring has been limited, museum specimens and other archived biological samples are critical for retrospective pathogen detection and can aid in uncovering when pathogens were first introduced and where they were found in the past (Ouellet et al. 2005).

Climatic variables are significant drivers of pathogen prevalence in wildlife populations, and amphibians and their pathogens are no exception (e.g. Smith and Woolf 1988; Altizer et al. 2004; Savage et al. 2011). Temperature and precipitation are the two major environmental factors that appear to be drivers of *Bd* dynamics. Studies have shown a negative linear relationship between temperature and *Bd* occurrence, prevalence and intensity both in the lab (Woodhams et al. 2003; Ribas et al. 2009) and in natural populations (e.g. Berger et al. 2004; Woodhams et al. 2008; Savage et al. 2011; Sapsford et al. 2013). Additionally, variation in precipitation and humidity have been implicated in the occurrence and prevalence of *Bd,* with increased precipitation and humidity driving patterns (e.g. Johnson et al. 2003; Woodhams and Alford 2005). Most work to date on *Rv* has focused on documenting infections. To our knowledge, there is a paucity of work investigating how environmental factors severe as drivers of *Rv.* Only two studies look at this pattern; one in which salamanders were experimentally infected under different temperature regimes and a negative linear relationship between *Rv* infection intensity and temperature was found (Rojas et al. 2005) and one testing ranid frogs under differing temperature regimes (Echaubard et al. 2014).

There is increasing empirical evidence for a genetic basis to disease resistance in wild vertebrate populations (Wiegertjes 1996; Meagher 1999), although more studies are needed to test this hypothesis in amphibian taxa (Richmond et al. 2009). To date, reduced genetic diversity has been found to increase susceptibility to one pathogen (*Rv)* in a single amphibian species based on neutral microsatellite loci (Pearman and Garner 2005). Additionally, a handful of studies have explored the underlying genetic basis for disease resistance to *Bd*. Tobler and Schmidt (2010) looked at among-population susceptibility to *Bd* in a European frog and inferred that differences in susceptibility among populations had a genetic basis due to differential population responses in a common garden experiment. Similarly, Savage et al. (2015) found that neutral genetic diversity was negatively correlated with *Bd* infection prevalence in a North American frog. Immunogenetic analyses have also found significant associations between specific major histocompatibility complex (MHC) class II alleles and *Bd* tolerance in the lab and in natural systems for multiple species of anurans (Savage and Zamudio 2011; Bataille et al. 2015; Savage and Zamudio 2016). These studies suggest that host genetic diversity underlies differential amphibian population responses to EIDs, but are based on a limited number of taxa.

Despite the importance of environmental and genetic factors in explaining amphibian disease dynamics when investigated separately, few studies incorporate both into a single analysis. Ribas et al. (2009) demonstrated in the lab that both temperature and expression of skin peptides determined how anuran hosts responded to *Bd* infection. Savage et al. (2015) found that environmental factors were responsible for predicting *Bd* infection intensity, while both genetic and environmental factors influenced *Bd* prevalence. This was the first analysis to combine both genetic and environmental factors in a predictive model for EIDs in a natural amphibian system. More studies are needed to confirm the importance of both genetic and environmental factors for explaining amphibian EIDs.

Here, we assess whether EIDs are impacting amphibian populations in the southeastern United States by characterizing *Bd* and *Rv* dynamics using archived samples from three amphibian species: a salamander*, Notophthalmus perstriatus* and two tree frogs, *Hyla squirella* and *Pseudacris ornata*. Each species exhibits unique life history traits and all three occur throughout the Southeast, offering varying perspectives into pathogen dynamics among diverse taxa. *Notopthalmus perstriatus* has a complex life cycle, spending two out of its three life stages in the water (May et al. 2011). Previous studies have observed that *N. perstriatus* and other *Notophthalmus* are susceptible to both *Bd* (Rothermel et al. 2012) and *Rv* (Means et al. 2011). *Hyla squirella* is a common, highly arboreal species that breeds in large aggregates during summer months (Elliot et al. 2009), and little is known about the presence or impact of *Bd* and *Rv* in this species. In contrast, *P. ornata* is an increasingly uncommon species (B. Means pers. comm.), breeds in winter at low densities (Elliot et al. 2009), and is highly susceptible to *Rv* in experimental infection trials (Brenes 2013). Field studies of *P. ornata* tadpoles in northern Florida have also confirmed *Rv* infections in the wild (Means et al. 2013). Previous population genetic analyses found that *N. perstriatus* form distinct East-West groups that do not share haplotypes (May et al. 2011), *H. squirella* genetic structure is heavily determined by habitat structure (Hether and Hoffman 2012), and *P. ornata* genetic structure varies among populations and the species may have been widespread across the Southeast in the past (Degner et al. 2010). By combining these genetic data with environmental and disease variables for each sampled population, we simultaneously assessed the importance of host genetics and environmental variables on predicting disease impact and spread in amphibian populations of the Southeast.

Materials and Methods

Sample collection

All appropriate permits were acquired for the desired field and lab work. Vertebrate animal use was approved by University of Central Florida's IACUC, #06-01W, 09-13W, 09-21W. Samples were collected over various months throughout the Southeast Atlantic Coastal Plain from 1997 to 2010 (Table 4 in Appendix A). *Notophthalmus perstriatus* samples were collected in Florida and Georgia from 1997 to 2000, with additional samples collected in 2008-2010. Samples were collected during various months over the entire collection period (May et al. 2011). *Hyla squirella* samples were collected in Florida and Georgia in 2010 during summer months (Heather et al 2012). *Pseudacris ornata* samples were collected in Florida, Georgia, Alabama, South Carolina and North Carolina between 2006 and 2009 during winter months (Degner et al. 2010). Toe clips were taken from anurans and tail clips were taken from salamanders. Each animal was then released where it was found. For *N. perstriatus*, tissue was either stored in saturated salt buffer (NaCl; 25 mM EDTA, pH 7.5; 20% DMSO), or in DrieRite Desiccant (May et al. 2011). For *H. squirella*, tissue was stored in in anhydrous calcium sulfate (Heather et al 2012). *P. ornata* tissue samples were also stored in anhydrous calcium sulfate (Degner et al 2010). While multiple storage methods were used, long-term storage occurred in a -20C freezer, we ran a random assemblage of samples (10 samples from each species group) and tested elutions via a Microdrop assay in a BioTek plate reader to check for a comparison of the DNA extractions. No significant difference was found between species in amount of DNA present in elutions ($P=.06$).

Pathogen Detection

DNA was extracted from whole tissue samples using DNeasy Blood and Tissue kits (Qiagen) or through the phenol–chloroform method (Degner et al. 2010; May et al. 2011; Hether and Hoffman

2012) and DNA elutions were stored at -20 C or cooler. Taqman quantitative (q)PCR was performed on extracted DNA using the Bio-Rad CFX96 Real-Time System and analyzed with Bio-Rad CFX Manager 3.1 software. Reaction volumes were 25 µL for all standards, samples and controls, consisting of: 8 µL of Bio-Rad Super Mix, 2 µL of 10 µM Forward primer (0.8 µM/ µL), 2 µL of 10 µM Reverse primer (0.8 µM/ µL), 3 µL of Molecular Grade water, 5 µL of 1 µM probe (*Bd* or *Rv*; 0.2 µM/ µL) and 5 µL of standard DNA template or sample DNA template. Cycling conditions were as follows: 95°C for 5 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 min. *Bd* reactions used primers and probes developed by Boyle et al. (2004) and *Rv* reactions used primers and probes developed by Allender et al. (2013). *Bd* and *Rv* reactions were run separately on individual 96-well plates. For absolute pathogen quantification, standard curves were generated from serial dilutions of synthetic pathogen DNA (gBlock Gene Fragments) run in duplicate (Gunawardana et al. 2014; Sandkam et al. 2015). Two negative controls (molecular grade water) were included with each run, as well as a positive control. Samples were first run in pools, which consisted of 5 µL of DNA template from each individual within a population combined, to test for the presence of positives within a population. A result was considered positive if the DNA from 5 µL of the pooled sample amplified before cycle 39 for at least two runs. If a population tested positive, each individual was then tested. All positive samples were run twice and the average of the two values was used in downstream analysis. In rare cases when two runs were inconsistent (one positive and one negative, or more than an order of magnitude difference in infection intensity), a third run was performed and the two most consistent results were retained.

Pathogen Data Analyses

We used two metrics to catalog *Bd* and *Rv* infection across the species ranges for these three amphibians. Prevalence was calculated by dividing the number of infected individuals by the total population sample size, and 95% Clopper–Pearson binomial confidence intervals were calculated using

the package *binom* in R (see Appendix A for R code). To compare prevalence across population and species, we analyzed two-way contingency tables using Fisher's Exact Tests with simulated *P*-values based on 2000 replicates. The second metric, infection intensity, was calculated as the mean number of Genome Equivalents (GE) among duplicate runs. Mean infection intensity per population was measured as the mean infection intensity among infected individuals only. To compare average infection intensity across populations and species, a two-way ANOVA was performed in R v. 3.1.3 (R Core Team 2016). Prevalence and intensity maps were created in ArcGIS v. 10.2.2 to visualize the spatial distribution of infections.

Genetic and Environmental Disease Modeling

For species that tested positive for *Bd* or *Rv* in multiple populations, we used general linear models (GLMs) weighted by population size to predict pathogen prevalence (with quasibinomial error; Warton and Hui 2011) and the natural log of intensity based on genetic and environmental variables, as well as location. For genetic predictor variables, we used average expected heterozygosity (H_E) and allelic richness (AR) as these were found to be important predictors of infection by Savage et al. (2015). Genetic diversity estimates were calculated by investigating diversity at 7 nuclear microsatellites for *P. ornata* (Heather et al 2012), as this species was the only one used in modeling due to its infection prevalence recovered (Table 5 in Appendix A). For environmental predictor variables, we used average precipitation, average temperature, and maximum and minimum temperature per population site for each month of the year. Location factors included latitude and longitude for each population site. Environmental data (current data interpolated from 1960-2000 data) was acquired through Worldclim/Bioclim layers in ArcGIS (Table 6-7 in Appendix A) (Hijmans et al. 2005). Environmental and location data were assessed with Principle Component Analysis (PCA). Additive and interactive models were created and assessed via variance inflation factor (vif) using the *car* package in R, and only

models with values ≤4 were included (Burnham and Anderson 2002). GLMs meeting this criterion were ranked using Akaike information criterion (AICc), and the most informative model was chosen using the lowest AICc value (Burnham and Anderson 2002).

Results

We tested 401 *N. perstriatus* tissue samples from 11 populations, none of which were infected with *Bd* or *Rv*. Among 580 *H. squirella* tissue samples from 20 populations, one individual was infected with *Bd* (Table 6 in Appendix A; Fig 1a) and one individual from a different population tested positive for *Rv* (Table 6 in Appendix A; Fig 1b). Neither *Bd* nor *Rv* prevalence were significantly different among *H. squirella* populations (Fisher Exact test $P = 0.64$). Finally, among 327 *P. ornata* tissue samples from 15 populations, 103 individuals from 10 populations were *Bd* positive (Table 4 in Appendix A; Fig 1c), whereas none tested positive for *Rv* (Table 6 in Appendix A; Fig 1d). *Bd* prevalence among infected *P. ornata* populations ranged from 0.24 to 1.0 (Table 6 in Appendix A). *Bd* prevalence varied significantly among *P. ornata* populations (Fisher Exact test, $P = 0.0005$). Additionally, pooled *Bd* prevalence was significantly different in *P. ornata* compared to *H. squirella* (Fisher Exact test, $P < 0.00001$). Average *Bd* infection intensity among infected populations ranged from 226 to almost eighteen million genome equivalents (GE; Fig 2). Of the 11 infected populations, nine harbored average infections of over 10,000 GE (Table 6 in Appendix A; Fig 2). All but one infected populations had 40% of individuals harboring loads above 10,000 GE and two populations had 100% of individuals with infections above 10,000 GE. *Bd* infection intensity was significantly different among infected *P. ornata* populations (two-way ANOVA, $P < 0.00001$).

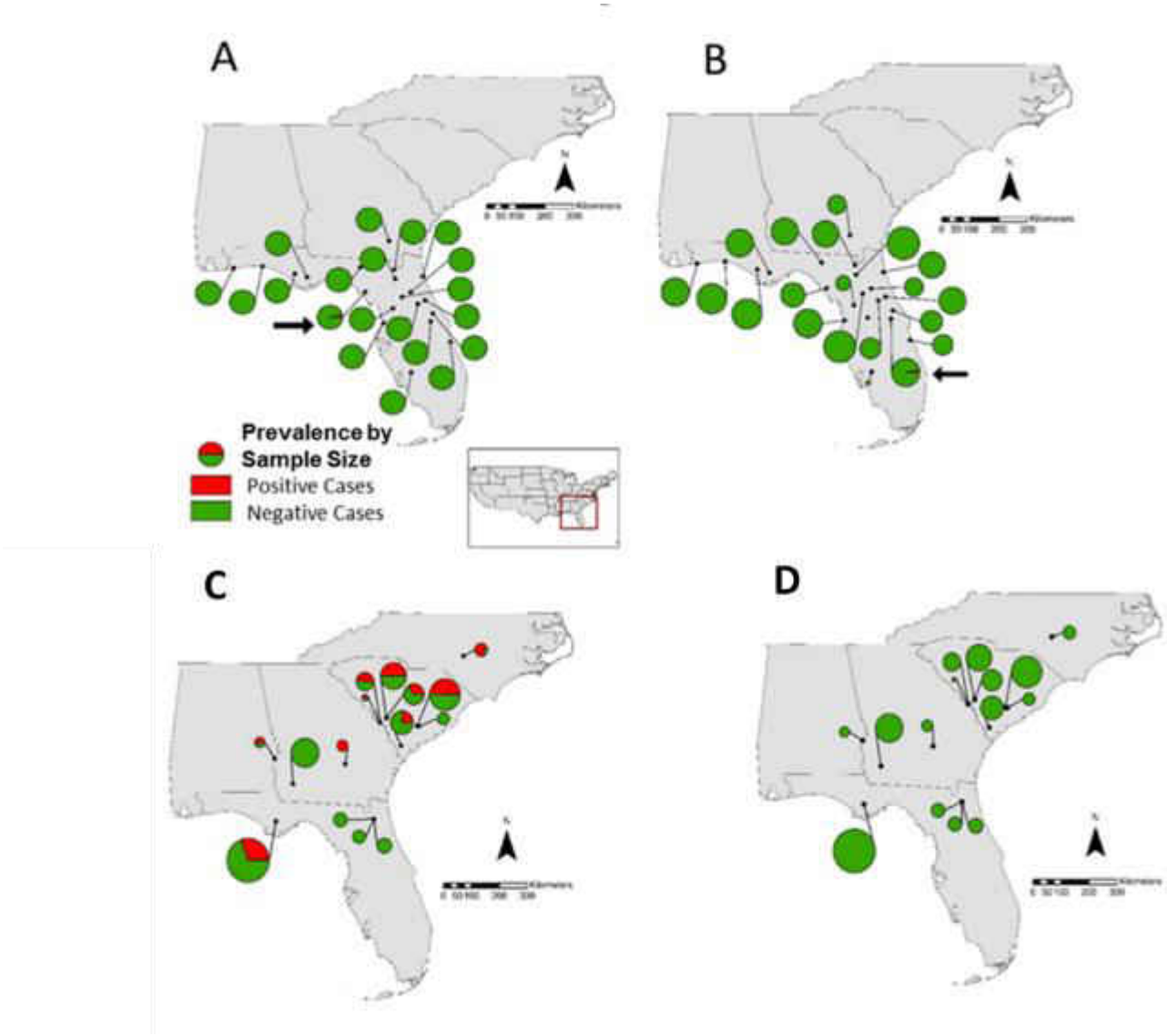

Figure 3. Map of sample localities and (A) *Bd* prevalence in *Hyla squirella,* (B) *Rv* prevalence in *H. squirella,* (C) *Bd* prevalence in *Pseudacris ornata* and (D) *Rv* prevalence in *P. ornata.* Circle size is relative to population size. Arrows point to infected *H. squirella* populations. Green represents proportion of negative cases of the indicated pathogen while red represents proportion of positive cases.

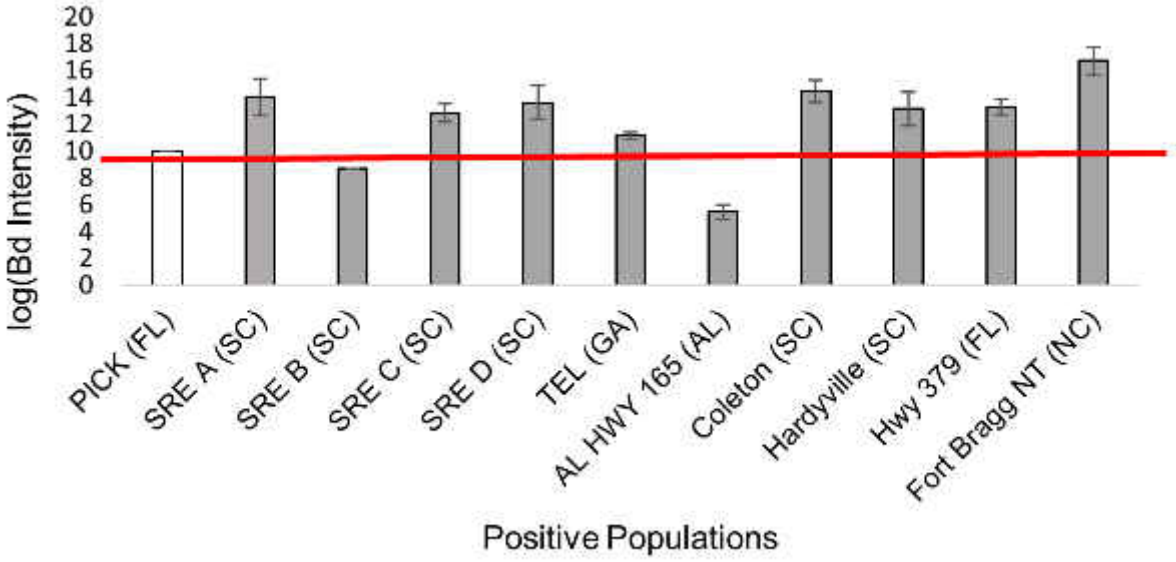

Figure 4. Log-transformed *Bd* average intensities for sampled populations of *H. squirella* and *P. ornata* with standard error of the mean (SEM). White bars indicate average intensity among infected individuals from *H. squirella* populations and black bars denote average intensity among infected individuals from *P. ornata* populations. The red line marks an infection intensity of 10,000 GE.

Because *P. ornata* was the only species harboring pathogens in more than one population, and *Bd* was the only pathogen detected, we limited GLM analyses to *Bd* dynamics within *P. ornata*. Principle component anlaysis of environmental variables across sampled *P. ornata* populations revealed that the first two components explained 89% of variation in our data, with PC1 explaining 76% of the variation and PC2 explaining 13% (Fig 14 in Appendix A). We also ran a principle component analysis on only the temperature variables and found broad concordance with our overall PCA (Figure 15 in Appendix A). For our inclusive PCA, PC1 is associated with decreasing temperature and some increasing precipitation variables and positively associated with latitude, and PC2 is positively associated with winter precipitation and summer temperatures. Since PC1 in our temperature only PCA was in strong concordance with our general PCA, we consider PC1 to be primarily influenced by

temperature and will refer to it thusly from now on. We assessed only additive models with AICs for prevalence and intensity as interactive models showed high variance inflation. We chose the most informative models based on the lowest AIC score in the set (Table 1). PC1, PC2 and average heterozygosity significantly influenced *Bd* prevalence (Fig 16; Table 7 in Appendix A). For our general linear models, which were weighted by population size, we had to combine some populations due to small sample size, leaving us with 11 populations within our models. Our analyses showed that there was a significant relationship between decreasing temperature and an increase in *Bd* prevalence (P = 0.007; Fig 16A). Moreover, there was a significant negative relationship between winter precipitation and summer temperatures and *Bd* prevalence (P = 0.05; Fig 3B). Surprisingly, our model identified a significant relationship between increased average heterozygosity and increased *Bd* prevalence (P = 0.028; Fig 16C, Table 9 in Appendix A). Environmental factors (PC1) significantly influenced *Bd* intensity (Fig 15 and Table 8 in Appendix A). Linear regression displayed a significant relationship between a decrease in temperature and an increase of *Bd* intensity (P = 0.011; Fig 15 and Table 10 in Appendix A). The most informative models for prevalence and intensity were also run using temperature only PC1 and the results were largely similar (Figures 17-18 in Appendix A).

Table 1. Five most informative general linear models and the null models for *Bd* prevalence and *Bd* intensity.

***Bd* Prevalence models**	**AICc**	**dAICc**	**Df**	**Weight**
PC1+PC2+AvgHE	62.4	0	4	0.750
PC1+PC2+AvgHE+AR	64.6	2.2	5	0.249
PC1+PC2+AR	75.6	13.2	3	0.001
PC1+PC2	80.4	18.0	2	<0.001
PC1+AvgHE	82.6	20.3	3	<0.001
NULL	112.6	50.3	1	<0.001
***Bd* Intensity models**	**AICc**	**dAICc**	**Df**	**Weight**
PC1	87.0	0	3	0.5192
PC1+AR	89.6	2.6	4	0.1425
PC1+AvgHE	90.7	3.7	4	0.0823
PC1+PC2	91.3	4.3	4	0.0601
AvgHE	91.8	4.8	3	0.0478
NULL	91.5	4.5	2	0.0559

AvgHE = average heterozygosity, AR = allelic richness, dAICc = delta Akaike information criterion. The difference in AICc between the current model and the most informative model in the set. df = degrees of freedom.

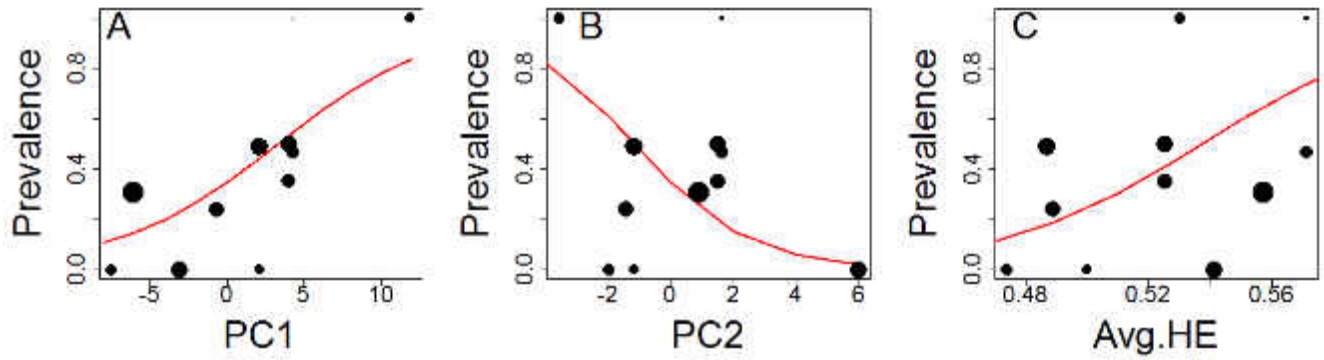

Figure 5. Logistic relationships between *Bd* prevalence and PC1 (A), PC2 (B), and average heterozygosity (C) in populations of *P. ornata.* Increasing values of PC1 correspond most strongly to decreasing values of temperature. Increasing values of PC2 correspond most strongly to increasing winter precipitation. In all panels, each dot corresponds to the observed prevalence in a population and each line corresponds to the best fit logistic model of the relationship between the two variables shown. Points are weighted by population size and are reflected in the size of the point.

Discussion

Our study used both environmental and genetic variables to create a predictive model for chytridiomycosis disease dynamics in the southeastern United States. We identified both concordant and discordant patterns of pathogen prevalence and infection intensity in *N. perstriatus, H. squirella,* and *P. ornata* compared to previous studies (Chatfield et al. 2012; Rothermel et al. 2012; Peterson et al. 2016). First, our data showed limited *Rv* occurrence and high variation in *Bd* infection prevalence within and among our three focal species. Second, we found surprisingly high *Bd* infection intensity in *P. ornata* and *H. squirella*, a strikingly different result compared to the extremely low intensities previously detected (Peterson et al. 2016). Finally, our overall model found that both genetic and environmental variables predict *Bd* prevalence but only environmental variables predict infection intensity. These results are important for understanding the enigmatic story of *Bd* infection in this region, and provide a framework for future management of declining amphibians in the Southeast.

Overall, this study offers insight into pathogen infection history and amphibian disease dynamics in the southeastern United States, a hotspot of amphibian diversity.

Our three focal amphibian species showed largely unique patterns of *Bd* and *Rv* infection prevalence compared to previous studies on the same or similar species. Interestingly, *Rv* was previously found within ponds where *N. perstriatus* populations occur (Means et al. 2013), and a closely related *Notophtahlmus* species in the Southeast, *N. viridescens*, was found to have high *Bd* infection prevalence within sampled populations (Rothermel et al. 2012). In contrast, we found no evidence of *Bd* or *Rv* in *N. perstriatus*, suggesting the presence of genetic, temporal or seasonal differences for our sampled populations. *Hyla squirella* infection dynamics were also surprising as this species breeds in large aggregates in habitats frequented by known *Bd* and *Rv* vector species, particularly *Lithobates catesbianus* (Garner et al. 2006; Elliot et al. 2009). We predicted high pathogen prevalence in *H. squirella*, but instead only found two infected individuals (one *Bd* infected and one *Rv* infected) among all sampled populations. Interestingly, these are the first documented *Bd* and *Rv* infections in *H. squirella* despite previous sampling efforts (Rothermel et al. 2012). These results indicate low overall infection prevalence in *H. squirella*, but the high *Bd* infection intensity we detected in one individual suggests the potential for negative impacts on populations where infection does occur. The only focal species with high pathogen prevalence was *P. ornata,* which exhibited strikingly different infection patterns for *Rv* and *Bd* compared to *H. squirella*. While *Rv* infection was undetectable in *P. ornata*, high *Bd* infection prevalence and intensity occurred in the majority of sampled populations. These results mirror previous studies testing *P. ornata* and other members of *Pseudacris* for pathogens in the Southeast (Rothermel et al. 2012; Peterson et al. 2016).

Life history variation among amphibian species often contributes to variation in pathogen infection prevalence (Kriger and Hero 2007). The distinct life histories among our three study species

may be an underlying factor contributing to the significant differences in *Bd* prevalence and intensity that we observed. In particular, high susceptibility to *Bd* infection in *P. ornata* may be due to the trait of breeding exclusively during rainy periods in the winter months (Elliot et al. 2009), unlike the other two species that are summer breeders. Our data, along with data from several other studies, correlate *Bd* infection with cooler temperatures and higher precipitation in winter months (Woodhams and Alford 2005; Kriger and Hero 2007; Woodhams et al. 2008; Savage et al. 2011; Sapsford et al. 2013). Thus, high contact rates among *P. ornata* individuals during winter breeding aggregations when *Bd* experiences preferred temperatures may be driving the observed high infection prevalence and intensity. Our results, combined with previous monitoring efforts, serve as a valuable baseline should infection outbreaks occur for other southeastern amphibian species and may help elucidate reasons for any enigmatic declines.

Our *Bd* infection intensity data seem to contradict previous studies suggesting that values greater than 10,000 GE lead to mortality, regardless of the amphibian species (Vredenburg et al. 2010; Kinney et al. 2011). We found high *Bd* intensities that surpassed this threshold for the single infected *H. squirella* individual and within most infected *P. ornata* individuals (Fig 2). Although high *Bd* infection prevalence in *Pseudacris* populations is well documented in the literature Ouellet et al. 2005; Rothermel et al. 2012; Peterson et al. 2016), only one other study quantified *Bd* intensity for *P. ornata*; they found low values (<102 GE) for all individuals sampled throughout the United States (Peterson et al. 2016). We uncovered a pattern that is much more extreme; average *Bd* intensities were millions of GE for all but three infected populations (Fig 2) despite the absence of any obser*v*ed disease signs or mortality events (T. Hether pers. comm.). Our data therefore demonstrate that the 10,000 GE proposed mortality threshold is not a standard applicable to every species. Indeed, our findings reinforce recent *Bd* studies in Brazilian amphibian communities (Preuss et al. 2016) and New York

State amphibian communities (Lenker et al. 2014) which showed varying infection intensities across a range of species and seasons without observing any mortality or disease signs in the individuals sampled.

To uncover the driving forces behind the observed patterns of *Bd* infection among our sampled *P. ornata* populations, we incorporated climatic variables and genetic diversity factors into a comprehensive model. *Bd* intensity increased at lower air temperatures, consistent with similar analyses in other species and regions (e.g. Kriger and Hero 2007a; Kriger and Hero 2007b; Sapsford, Alford and Schwarzkopf 2013; Savage et al. 2015). Further, the same variables that influenced *Bd* prevalence for *P. ornata* in our study (temperature, precipitation and average heterozygosity) also explained *Bd* prevalence in *L. yavapaiensis* in Arizona (Savage et al. 2015). However, in contrast to the negative correlation between average heterozygosity and *Bd* prevalence found for *L. yavapaiensis*, we found average heterozygosity was positively correlated with *Bd* prevalence for *P. ornata* (Fig 3C). This pattern is in direct contrast with expectations, as numerous studies across wildlife disease systems have found higher genetic diversity within populations leads to decreased infection prevalence and increased disease resistance (e.g. Lande 1988; Spielman et al. 2004; Pearson and Garner 2005). Our models show that for *P. ornata*, the opposite is true: increased genetic diversity correlates positively with *Bd* prevalence. Two possible explanations exist for this pattern. First, because average heterozygosity increases with larger effective population size, there could be better facilitation of pathogen spread due to density-dependent disease outbreaks (Hochachka and Dhondt 2000; Langwing et al. 2015). This explanation is unlikely for *P. ornata*, however, as this species is generally uncommon and has historically small population sizes (Elliot et al. 2009). Another possible explanation is that *Bd* swept through *P. ornata* populations before our sampling occurred, and selection favoring *Bd* tolerant individuals was strong enough to push tolerant genotypes towards fixation, resulting in decreased

heterozygosity. *Bd* has been present in the United States long enough to make this "genetic purging" (Crnokrak and Barrett 2002) scenario plausible; Ouelett et al. (2005) and Talley et al. (2015) found evidence of *Bd* infections existing in North America as far back as the late 1800s. Our results could thus represent indirect evidence of genetic tolerance to *Bd* evolving in natural amphibian populations (Savage and Zamudio 2016), although further genetic sampling, molecular tests of selection and experimental evidence of *Bd* tolerance are necessary to resolve this hypothesis.

Our data strongly suggest that both genetic and environmental factors should be incorporated, when possible, into models when trying to predict dynamics of infectious pathogens in natural populations. Management plans often only consider genetic or environmental factors when planning for long-term species persistence, but it is becoming increasingly clear that both are important for predicting pathogen impacts. While our study only focuses on amphibians, this modeling framework is applicable and important for other wildlife disease systems. Our results also suggest that *Bd* may be more of a concern for the Southeast than previously thought, at least for some species. There are no documented instances of disease-driven morbidity or mortality in *P. ornata*, yet a majority of sampled individuals were heavily infected with *Bd* and there is evidence of population declines in recent decades (B. Means pers. comm.). Cryptic chytridiomycosis may therefore be an unobserved but causal factor behind population declines and patterns of genetic diversity. Alternately, the *Bd* strain(s) present in the Southeast may currently exist as commensals or sub-lethal pathogens in *P. ornata.* Even under the latter scenario, monitoring *Bd* and other pathogen dynamics is important for future *P. ornata* conservation efforts. If novel biotic or abiotic stressors appear, the additional toll of harboring massive *Bd* intensities may be a tipping point towards extirpation. This may be especially true for populations with low genetic diversity, even if that loss of diversity is due to selection for pathogen tolerance. Means and Means (unpublished data) highlight that habitat destruction and degradation are

threatening *P. ornata* population persistence, and more recently Means et al. 2013 found wild *P. ornata* tadpoles to be heavily infected with *Rv.* Our results suggest *Bd* is a threat for adult frogs, particularly if the same populations are impacted by *Rv* prior to metamorphosis.

Overall, our study highlights how species are differentially impacted by EIDs in the Southeast and how models can be used to infer which environmental and genetic factors are drivers of infection. Infectious disease is often implicated in amphibian population declines only after morbidity and mortality is observed, making the trigger for a disease outbreak difficult to determine retrospectively. It has therefore become increasingly important to characterize and monitor species that have yet to display signs of disease in order to generate a baseline of pathogen dynamics should any future disease outbreak occur. Museum collections and specimens collected for non-disease studies are invaluable for assessing conditions faced by amphibians in the past (Ouelett et al. 2005), and here we utilized these resources to document the presence of two infectious pathogens in two frog species without any prior evidence of disease. Whether ubiquitous *Bd* infections in *P. ornata* reflect post-epidemic adaptation, non-pathogenic *Bd* strains, or virulent, ongoing chytridiomycosis that has gone undetected will require additional analyses. Regardless, our modeling results highlight the combined importance of host genetic variation and climate for determining *Bd* prevalence. Other climatic factors, such as seasonality, may also play a big part in disease dynamics and should be considered in future studies. Our results begin the journey to uncovering amphibian pathogen dynamics and can be used to develop more robust predictive models to assess where pathogens will likely spread and to inform species managers, as well as target suitable future re-introduction sites for amphibians that have been hit the hardest by disease-related declines.

CHAPTER 2

Introduction

A major driver that is negatively impacting biodiversity and creating crisis in human populations is the emergence of infectious diseases (Jones et al. 2008). Many zoonotic pathogens have surfaced since the 1940s, including virulent pathogens like the Ebola virus, and humans and wildlife alike have suffered general health and population declines (Daszak *et al.* 2000, 2001; Jones *et al.* 2008). The presence and severity of these emerging infectious diseases (EIDs) depend on a variety of factors, but three major influences have been found to determine disease spread: host influence, pathogen influence and environmental influence (Stevens 1960). The presence of available hosts and their ecology as well as the ecology of the pathogen in question can influence how diseases persist and proliferate in a system (Retallick and Miera 2007, Schock et al. 2010, Savage et al. 2015, Peterson et al. 2015, Horner et al. 2017). Additionally, climatic factors within the region where diseases emerge, such as seasonal changes in temperature and precipitation, are important in influencing how pathogens affect their intended and unintended hosts (Evengård & Sauerborn 2009, Horner et al. 2017).

It is important to look at as many factors as possible when trying to uncover the causes of disease emergence, because numerous host, pathogen and environmental characteristics can help predict the possibility of pathogens invading new habitats or causing disease outbreaks (Hatcher et al. 2012). Many epidemiological and disease ecology studies tend to focus on a single species (pathogen or host) or a single host-pathogen interaction, despite the often more complex underpinnings to explaining disease dynamics within communities (Wilcox and Gubler 2005, Roche et al. 2012). While it is often difficult to study entire communities, such studies are needed to understand how pathogens impact different hosts and how environmental factors influence those interactions. While studying the

whole ecosystem may not be feasible, the study of indicator species can offer insight into overall ecosystem health (Dufrene and Legendre 1997). Amphibians are considered good indicator species based on their sensitive nature to changes in their environment and quick responses to stress (Carignan and Villard 2001). Amphibian communities have experienced severe declines over the last several decades, indicating stress present within their habitats (Gibbons 2000; Mendelson et al. 2006). While a multitude of factors have been pointed to as the main cause of declines, pathogen infection and disease have been found to be major players (Mendelson et al. 2006). While these declines are unfortunate, these systems offer a great opportunity to better understand how pathogens interact with their hosts.

Here, we looked at anuran communities within central Florida wetlands to gauge the impacts of emerging infectious diseases on native amphibian populations. We investigated not only how one pathogen interacts with its hosts and environment, but how multiple pathogens influence these indicator species within freshwater communities. We studied two emerging infectious pathogens, *Batracochytrium dendrobatidis* (*Bd*) and *Ranavirus* (*Rv*), in amphibian communities. Both pathogens are well-documented to cause mass mortality events in amphibian communities across the globe, and have been shown to be influenced by ecological factors such as spatial and temporal variation (Berger et al. 1998, Woodhams et al. 2008, Sapsford et al. 2013, Savage et al. 2015). For example, *Bd* infection is significantly influenced by temperature and seasonal patterns (Piotrowski et al. 2004, Sapsford et al. 2013, Savage et al. 2015, Horner et al. 2017). *Rv* has not been studied as extensively in this context, but preliminary experiments suggest temperature and host identity influence host infections (Hoverman et al. 2011, Echaubard et al. 2014). While these studies consider how different ecological factors influence infection prevalence and intensity in amphibians, their focus is often narrowed to one or a few hosts and/or one pathogen, leading to a limited scope. We investigated the influence of

multiple pathogens in a suite of hosts, including 11 anuran species across three families, to see how host identity influenced pathogen dynamics within communities. Additionally, we sampled anuran communities at three distinct locations across three years to see how location and seasonal effects influenced pathogen dynamics. This multi-pathogen, multi-host approach over an extended period gives greater insight into how populations respond to a suite of factors, rather than just one.

A major focus of this study was to assess how prevalence and intensity of *Bd* and *Rv* changed over seasons and across sites, as well as how host factors impacted these responses. Other pathogens, like malaria and Lyme, are well known to vary with environmental and host population fluctuations (Fish 1995, Hay et al. 2002). We expected to find higher prevalence and intensity for both *Bd* and *Rv* during the cooler months because of the two pathogens low thermal optima (Piotrowski et al. 2004, Cunningham et al. 2007). We also expected higher infection rates at our higher latitude site for the same reason, as cooler climatic patterns occur at more northern latitudes. Lastly, based on previous studies, we expected host identity to matter for infection prevalence and intensity (Woodhams et al. 2008, Hoverman et al. 2011). Additionally, we wanted to investigate whether these two pathogens infected hosts at the same time (co-infection), as there is the potential for the interactions between pathogens to influence the immune system of the host or for pathogens to affect each other (Thaker et al. 2012, Willsey et al. 2018). However, based on the few anuran co-infection studies conducted to date, we did not anticipate any significant patterns of co-infection (Hoverman et al. 2011, Souza et al. 2012, Whitfield et al. 2013, Warne et al. 2016). Finally, we compared destructive and non-destructive sampling methods and their influence on *Rv* pathogen prevalence and intensity estimates to test whether destructive sampling methods are necessary for accurate estimates of the virus, as is suggested in the literature (Chinchar 2002, Pearman and Garner 2005, Pessier and Mendelson 2010). Based on pathogen biology, we expected liver samples to yield higher and more reliable pathogen

detection compared to skin because *Rv* replicates in the liver (Gray et al. 2009; Gray and Chinchar 2015). By taking a detailed molecular and statistical approach to investigating wetland disease dynamics across multiple seasons, species and amphibian communities, we hope to help shed light on the future of both our study wetlands and their communities, as well as others like them.

Methods and Materials

Sample Collection

Research was conducted under FLDEP permit #09211512 and FLFWC permit # LSSC-15-00054. Vertebrate animal use was approved by University of Central Florida's IACUC #15-29W. Samples were collected from anuran communities from three wetland sites across Florida, USA. Sites were arranged latitudinally across the peninsular region of the state. Our northern site was a sandhill wetland within Mike Roess Gold Head Branch State Park (North), our central site was a cypress wetland within the University of Central Florida's Arboretum (Central), and our southern site was a scrub wetland within Archbold Biological Station's reserve (South). We collected 1,164 toe/tail and liver tissue samples (combined) from 927 individuals between 2015 to 2017, with North sampled 16 times, Central sampled 14 times, and South sampled 5 times, across all four seasons. We sampled from three different anuran families: Ranidae (true frogs), Bufonidae (toads), and Hylidae (tree frogs) (Table 11 in Appendix B). Frogs were captured by hand while walking the entire circumference of each water body during each visit. Each animal was handled using an individual pair of gloves and temporarily placed in an individual Ziploc bag, both which were discarded after capture and processing. This process was used to reduce contamination between samples. Toe clips were taken from adult frogs using sterilized tissue forceps and dissecting scissors, while tail clips were taken from metamorphs if tails were present. All tissue samples were stored in 100% EtOH after field collection. Each animal was processed

with a different pair of gloves and sanitized tools in order to prevent cross-contamination. Animals were released back to the general location where they were found, though every 5th animal per species caught was taken as a whole-body specimen. These animals were humanely euthanized using MS222, and liver samples were taken and stored in 100% EtOH. All samples were stored at -20C until genetic analysis.

Pathogen Detection

DNA was extracted from tissue samples (either toe clip, tail clip, or liver) using DNeasy Blood and Tissue kits (Qiagen) and DNA elutions were stored at -20°C. Taqman quantitative (q)PCR was performed on extracted DNA using the Bio-Rad CFX96 Real-Time System and analyzed through Bio-Rad CFX Manager 3.1 software. Each reaction consisted of 25 µL. Each reaction for all standards, samples and controls, consisted of: 8 µL of Bio-Rad Super Mix, 2 µL of 10 µM Forward primer, 2 µL of 10 µM Reverse primer, 3 µL of Molecular Grade water, 5 µL of 1 µM probe and 5 µL of standard DNA template or sample DNA template. Cycling conditions were: 95°C for 5 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 min. *Bd* reactions used primers and probes developed by Boyle et al. (2004) and *Rv* reactions used primers and probes developed by Allender et al. (2013). *Bd* and *Rv* reactions were run separately on individual 96-well plates. For absolute pathogen quantification, standard curves were generated from serial dilutions of synthetic pathogen DNA (gBlock Gene Fragments) run in duplicate (Gunawardana et al. 2014, Sandkam et al. 2015). Two negative controls (molecular grade water) were included with each run, as well as a known positive control. A result was considered positive for *Bd* if the DNA from 5 µL of the sample amplified before cycle 38 for at least two runs. All positive samples were run twice and the average of the two values was used in the statistical analysis. In rare cases when two runs were inconsistent (one positive and one negative, or more than an order of magnitude difference in infection intensity), a third run was performed, and the two most

consistent results were retained. For *Rv*, samples were also considered positive if the DNA from 5 µL of the sample amplified before cycle 38 for at least two runs. We also compared the tissue and liver samples from whole body specimens and did not consider samples positive unless the liver amplified before cycle 38 for at least two runs, as this is the major organ for viral replication (Chinchar 2002). As we did not have liver tissue for every individual sampled, we could only use this metric on whole body specimens where we had extracted both types of tissue.

Pathogen Analysis and Modeling

We investigated the effect of host family, season and location on *Bd* and *Rv* infection metrics. We also explored two other variables through AICs (species, month; Appendix B Figures 19-26), but family, season, and location were the only factors that possessed sufficient sample size in all groups for a fully quantative comparison and so only family, season, and location were used in analyses. Family was represented by the three sampled anuran families: Ranidae, Bufonidae, and Hylidae. Montly sampling was clustered into three-month seasons: January-March was considered winter, April-June was considered spring, July-September was considered summer, and October-December was considered fall. Location corresponded to the three sample sites: North, Central, and South. Two pathogen infection metrics, prevalence and intensity, were used to measure pathogen infection. Prevalence was calculated by dividing the number of infected individuals by the total population sample size, and 95% Clopper–Pearson binomial confidence intervals were calculated using the package binom in R (see Appendix B for R code). We also used pairwise Fisher's Exact Tests with Bonferroni's correction in order to determine significant prevalence differences between groups. Infection intensity was calculated as the mean number of Genome Equivalents (GE) among duplicate runs. Mean infection intensity was measured as the mean infection intensity among infected individuals only.

We compared preavalence and intensity of *Rv* between tissue and liver samples from individuals who we had both types of samples for by using a paired t test for intensity and a two-sample test for equality of proportions for prevalence. Based on these outcomes (see Results), toe/tail tissue and liver tissue sample values were pooled for the statistical analyses below based on post hoc results.

We ran general linear models with binomial errors for prevalence to assess what factors were influencing the probability of an individual becoming infected with either pathogen for completeness. We chose the most informative model through AICs and looked for which factors were statistically significant (Burnham and Anderson 2002). For intensity, we ran linear models and examined the factors from the most informative model, which was chosen from AIC analysis. We also ran an ANOVA and Tukey's Post Hoc test on the most informative model to re-examine the significance of the factors in the model. We additionally examined co-infection of individuals showing infection with both *Bd* and *Rv*. We ran chi-squared tests to investigate how the prevalence of one pathogen affected prevalence of the other. We also ran linear models on intensity based on infection status with each pathogen, then reassessed our results by running an ANOVA. Additionally, we ran a linear model of *Rv* intensity based on *Bd* intensity, as well as the reverse model of *Bd* intensity based on *Rv* intensity, to test whether pathogen intensities influenced each other. All analyses and figures were generated in R (R v. 3.1.3) (R Core Team 2016).

Results

We collected 927 toe or tail clips and 237 liver samples from three anuran communities from July 2015 to February 2017. We assessed two sampling methods for detecting *Rv* in anuran hosts by comparing *Rv* detection between toe/tail clips and liver samples collected from 237 individuals. *Rv* detection was not significantly different among individuals based on toe versus liver sample assays

(two-sample test for equality of proportion, p= 0.119). Mean *Rv* detection across liver samples was 0.288 (95% BCI = 0.226-0.354) and mean *Rv* detection across toe samples was 0.283 (95% BCI = 0.253-0.315; Figure 6A; Appendix Table 12). We also compared *Rv* intensity values between toe/tail tissue and liver tissue sample, and again found no significant differences between the two categories (paired t-test, p=0.578; Figure 6B, Appendix Table 13; toe/tail log mean intensity 10.06, liver log mean intensity 9.76) There was a small percentage of samples where *Rv* was detected in the liver but not the toe/tail (11%) or in the toe/tail but not the liver (5%). The differences in estimated prevalence between the tissue types across all samples was not significant, however (two-sample equality of proportion test; p=0.947).

Because there were no significant differences observed between the two tissue types for either prevalence or intensity for *Rv*, both sample types for individual anurans were pooled for average calculations. In contrast, *Bd* calculations were interpreted from toe clips only as this pathogen can only infect epidermis. For Ranidae, *Bd* average infection prevalence across populations was 0.14 and *Rv* was 0.34. For Bufonidae across populations, average *Bd* prevalence was 0.23 and average *Rv* prevalence was 0.15. For Hylidae, the average prevalence for *Bd* across populations was 0.93 and for *Rv* was 0.28 (Table 11 in Appendix B). We assessed how prevalence for both pathogens were influenced by family, season and location.

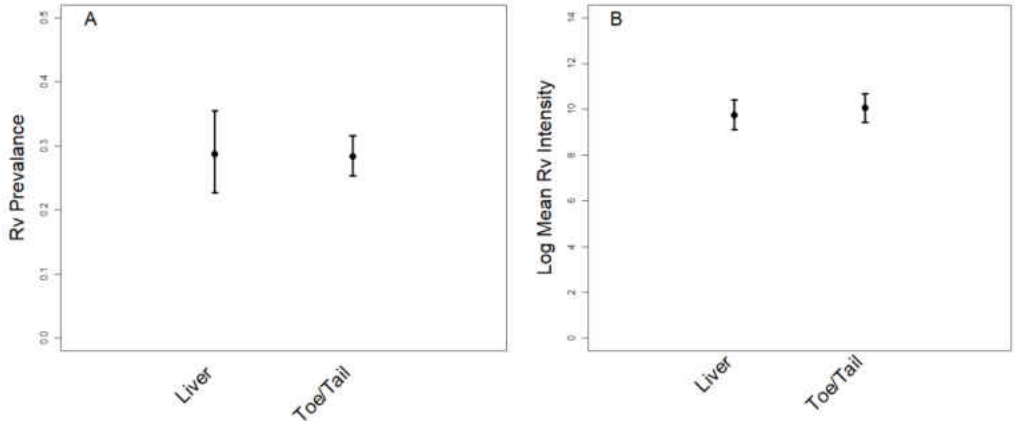

Figure 6. (A)Mean *Rv* prevalence ± binomial confidence intervals across liver and toe/tail tissue samples; (B) Log-transformed mean *Rv* intensity ± standard error liver and toe/tail tissue samples.

Neither *Bd* or *Rv* prevalence showed any statistical differences between the three families sampled and it was not retained in the most informative model of individual probability of infection for *Bd* or *Rv* (Tables 12-15 in Appendix B). When *Bd* and *Rv* were compared to each other, however, hylids and ranids had a significantly higher *Rv* infection prevalence than *Bd* infection prevalence (two-sample proportion test, $p<0.0001$; Figure 7). There was no statistically significant difference between seasons for *Bd* prevalence (Table 20 in Appendix B). For *Rv*, spring prevalence was significantly lower than fall and winter prevalence (Fisher's Exact Test, $p<0.001$, Table 11 in Appendix2). Additionally, *Rv* prevalence was significantly higher than *Bd* prevalence during the summer, fall and winter months (Figure 8). *Bd* prevalence was significantly higher in the North site compared to the South site (Fisher's Exact Test, $p=0.010$). *Rv* prevalance was also significantly higher in the North compared to Central and South, and Central was also significantly higher than South (Fisher's exact test, South-Central $p=0.038$, South-North $p<0.001$, Central-North $p= 0.028$). Additionally, *Rv* prevalence was significantly higher than *Bd* prevalence at the North and Central sites (two-sample proportion test, $p<0.0001$; Figure 9).

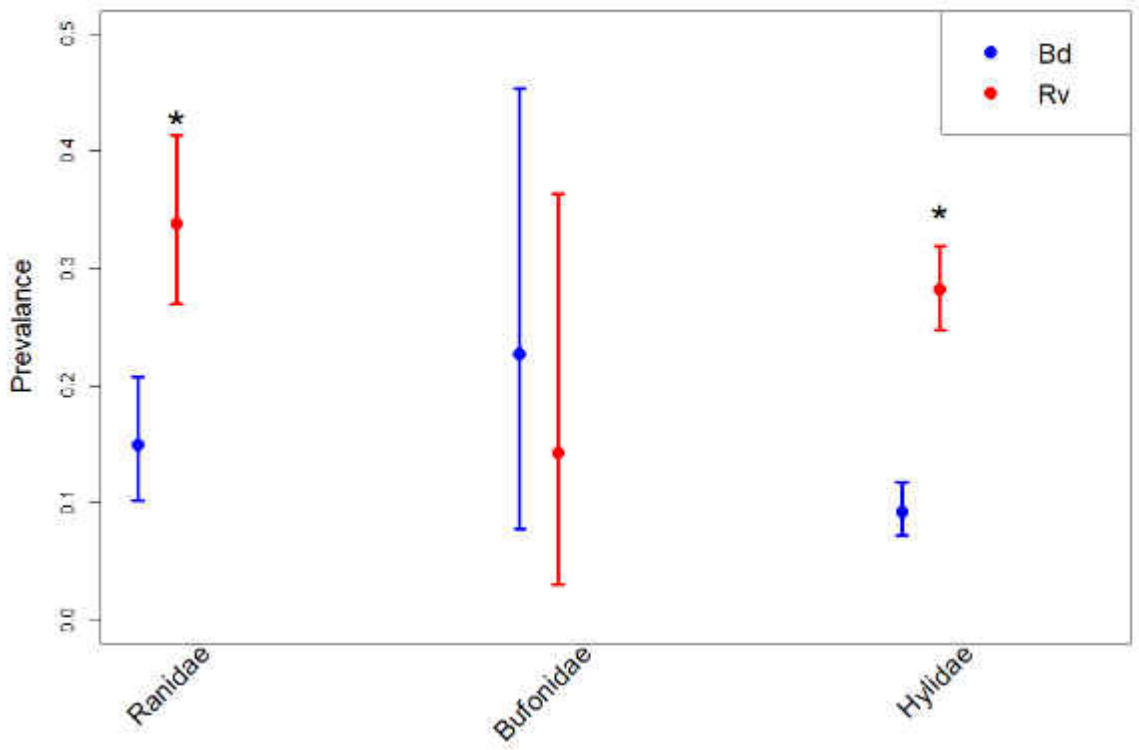

Figure 7. Mean ± binomial confidence interal for *Bd* and *Rv* prevalence in the families Ranidae (N=177), Bufonidae (N=21) and Hylidae (N=630). Asterisks indicate significant differences for *Bd* versus *Rv* prevalence within a family based on two-sample proportion tests.

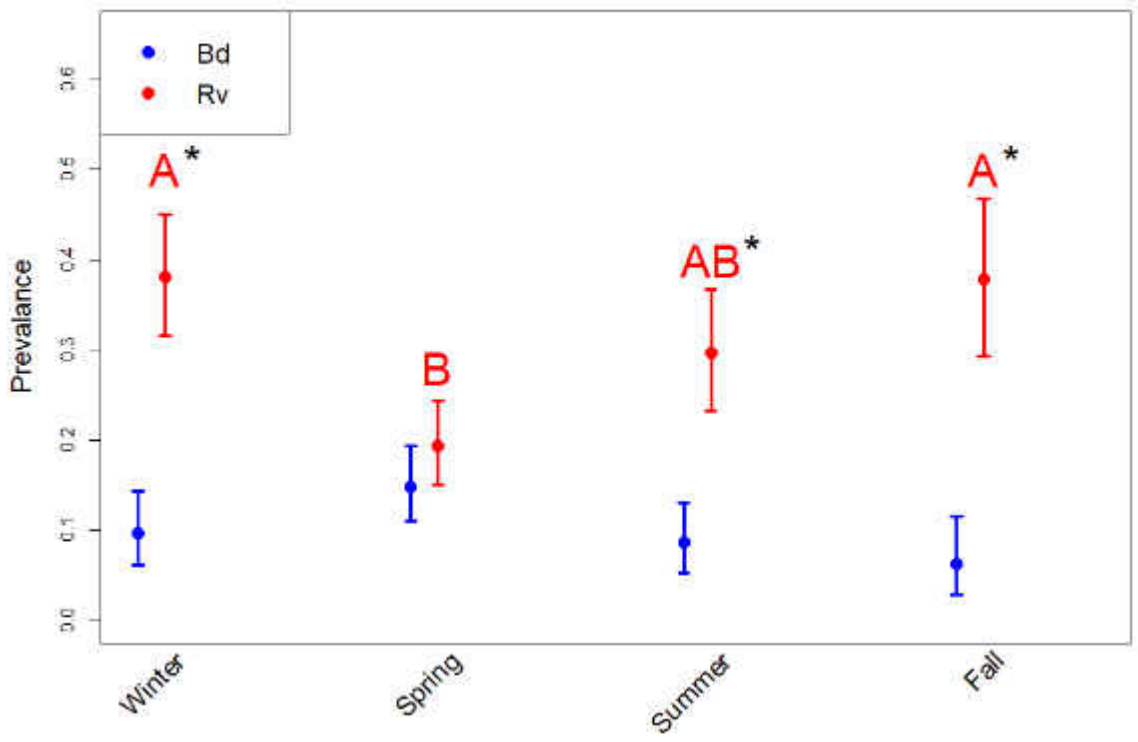

Figure 8. *Bd* and *Rv* prevalence (± 95% binomial confidence intervals) across seasons: Winter (N=210), Spring (N=294), Summer (N=189), and Fall (N=127). Asterisks indicate significant differences for *Bd* versus *Rv* prevalence within a season based on 2-sample proportion tests. Red letters correspond to significant differences in *Rv* prevalence between seasons based on pairwise exact tests.

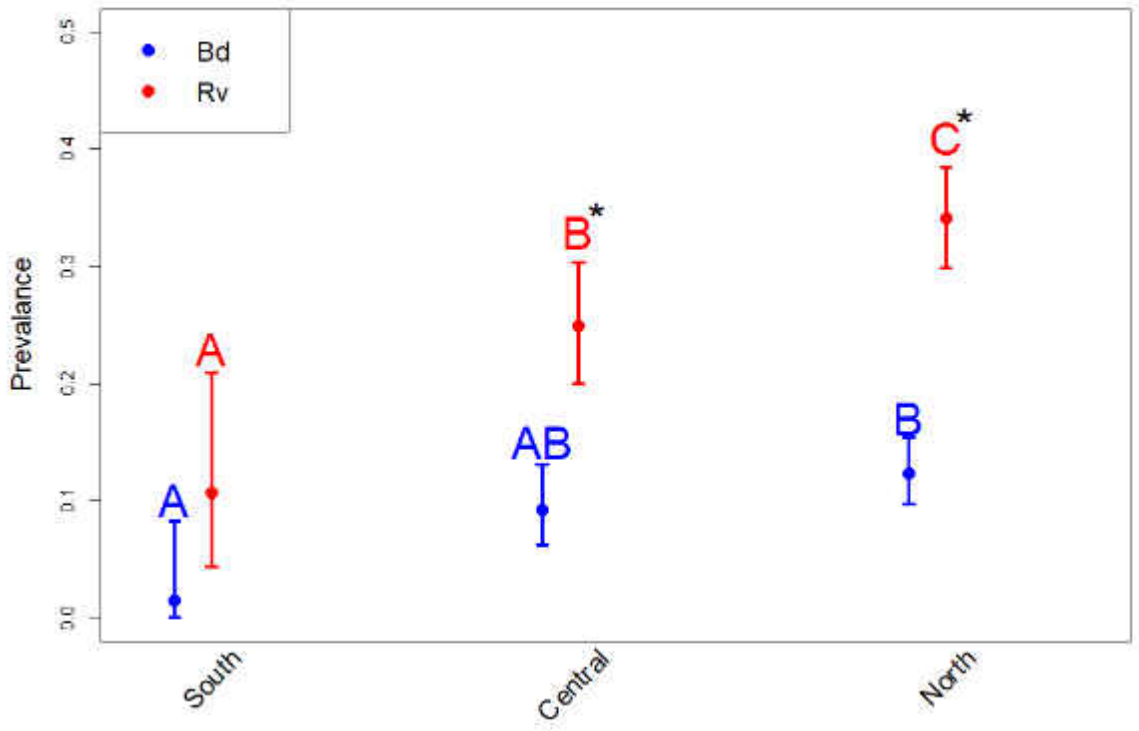

Figure 9. *Bd* and *Rv* prevalence (± binomial confidence intervals) in South (N=65), Central (N=285) and North (N=478) locations. Asterisks indicate significant differences for *Bd* versus *Rv* prevalence within a location based on 2-sample proportion tests, and letters indicate significant differences between groups in pairwise exact tests. Red letters correspond to significant differences in *Rv* prevalence between locations and blue letters correspond to signficnant differences in *Bd* prevalence between locations based on pairwise exact tests.

The most informative model for *Bd* intensity showed significant differences between families and seasons (Table 2, Table 16 in Appendix B), while the most informative model for *Rv* retained differences due to season and location (Table 3). Within family, Hylidae showed significantly higher *Bd* intensities compared to Ranidae (Tukey HSD, p= 0.0006) while no significant differences between family were noted for *Rv* intensity (Figure 10, Tables 18 & 19 in Appendix B). At the species level, *Acris*

gryllus and Hyla squirella had notably high intensities of *Bd* infection within Hylidae (Figure 25 in Appendix B). Across seasons, we found significant differences in intensity for both *Bd* and *Rv* (Table 2, Table 3). In winter, *Bd* intensity was significantly higher than in spring, summer or fall (Tukey HSD p=<0.0001, Figure 11, Table 18 in Appendix B). In *Rv*, spring, summer and winter intensity were all significantly lower than fall intensity (Tukey HSD p=0.008, p=0.004, p=0.04, Figure 11, Table 19 in Appendix B). Location was not retained in the most informative model explaining *Bd* intensity and no significant differences between sites were found, while for *Rv* there was a significant difference between intensity between North and Central sites (p=.045, Figure 12, Tables 18 & 19 in Appendix B)

Table 2. ANOVA results for the most informative model explaining *Bd* intensity.

Predictors	Df	Sum Sq	Mean Sq	F value	P value
Family	2	87.59	43.796	7.9522	**0.0006604**
Season	3	497.33	165.777	30.1009	**1.439e-13**
Residuals	90	495.66	5.507		

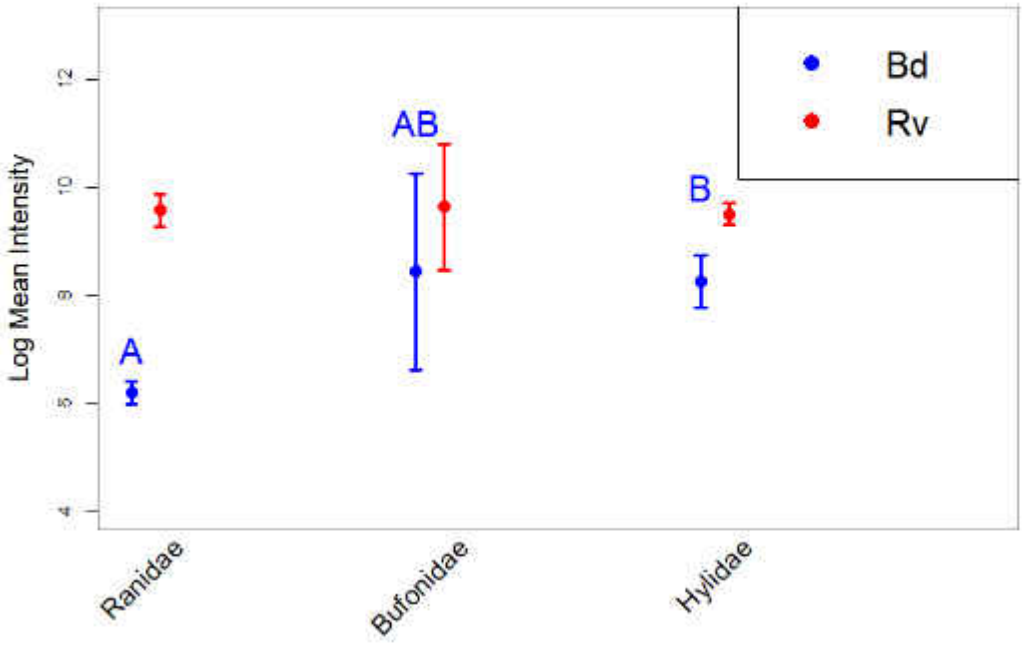

Figure 10. Log transformed mean intensity ± standard errors for *Bd* and *Rv* across families. Blue letters indicate significant differences between families based on Tukey post hoc tests for *Bd*.

Table 3. ANOVA results for the most informative model explaining *Rv* intensity

Predictors	Df	Sum Sq	Mean Sq	F value	P value
Season	3	78.08	26.0258	4.2261	**0.006205**
Location	2	59.57	29.7846	4.8365	**0.008742**
Residuals	235	1447.21	6.1583		

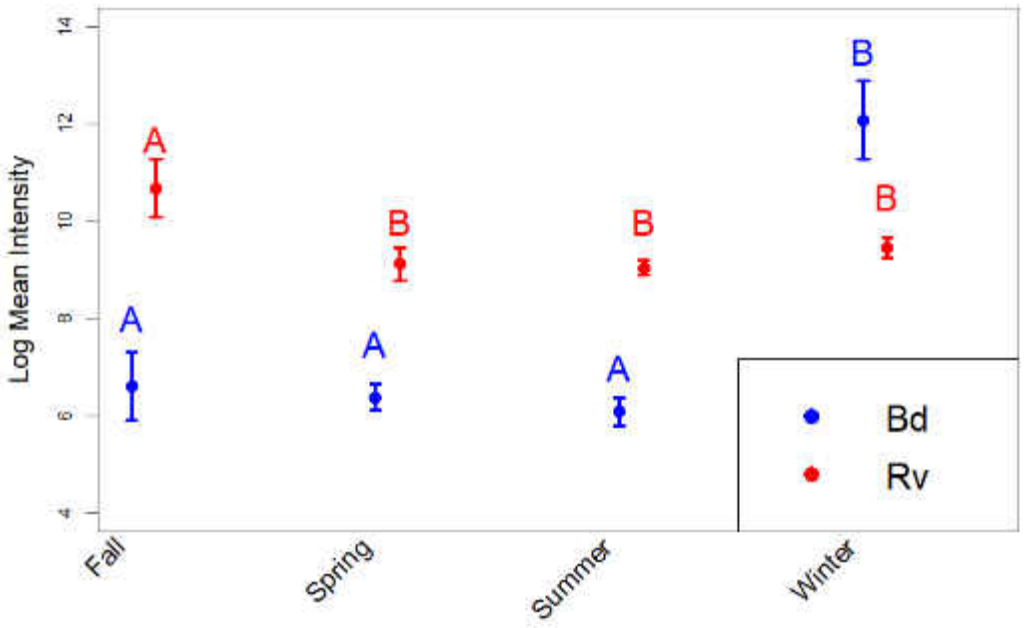

Figure 11. Log transformed means standard errors for *Rv* and *Bd* intensity across seasons. Blue lettersindicate significant differences between seasons in Tukey post hoc tests for *Bd* intensity. Red letters indicate significant differences between seasons in Tukey post hoc tests for *Rv* intensity.

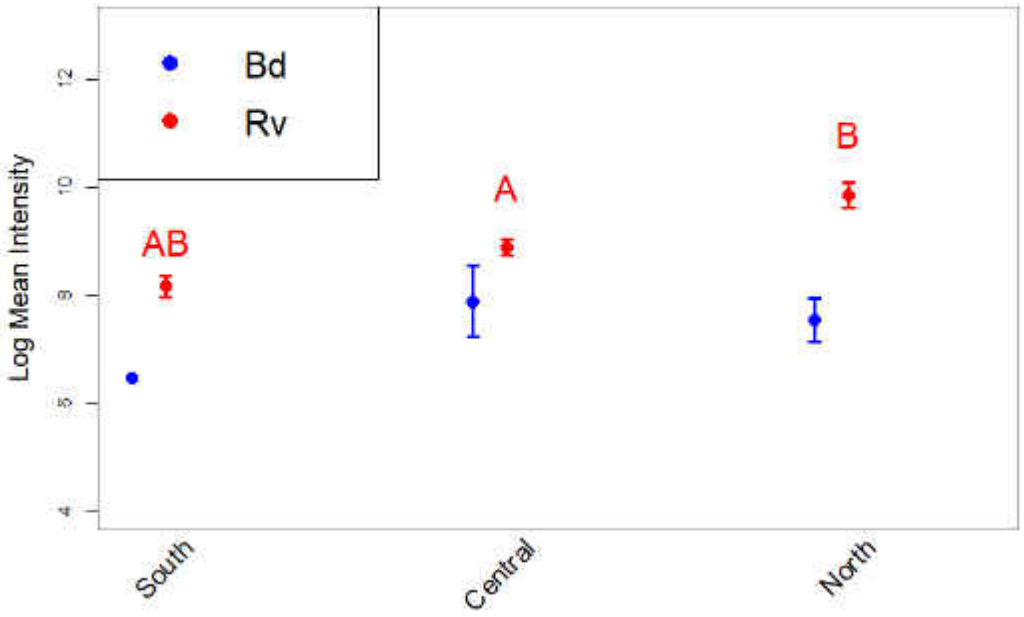

Figure 12. Log transformed mean intensity ± standard errors for *Rv* and *Bd* across Locations. Red lettersindicate significant differences between sites based on Tukey post hoc tests for *Rv* intensity.

Seventeen individuals were co-infected with *Rv* and *Bd*. The presence of one pathogen did not predict infection with the other pathogen (Chi-square test; p= 0.384). Similarly, infection with either *Bd* or *Rv* did not significantly change the intensity of the other infection (ANOVA; *Rv* intensity p=0.737; *Bd* intensity p=0.989, Figure 13). We also assessed how intensity of one pathogen affected the intensity of the other and found a non-significant relationship (p= 0.425, R^2=0.2324).

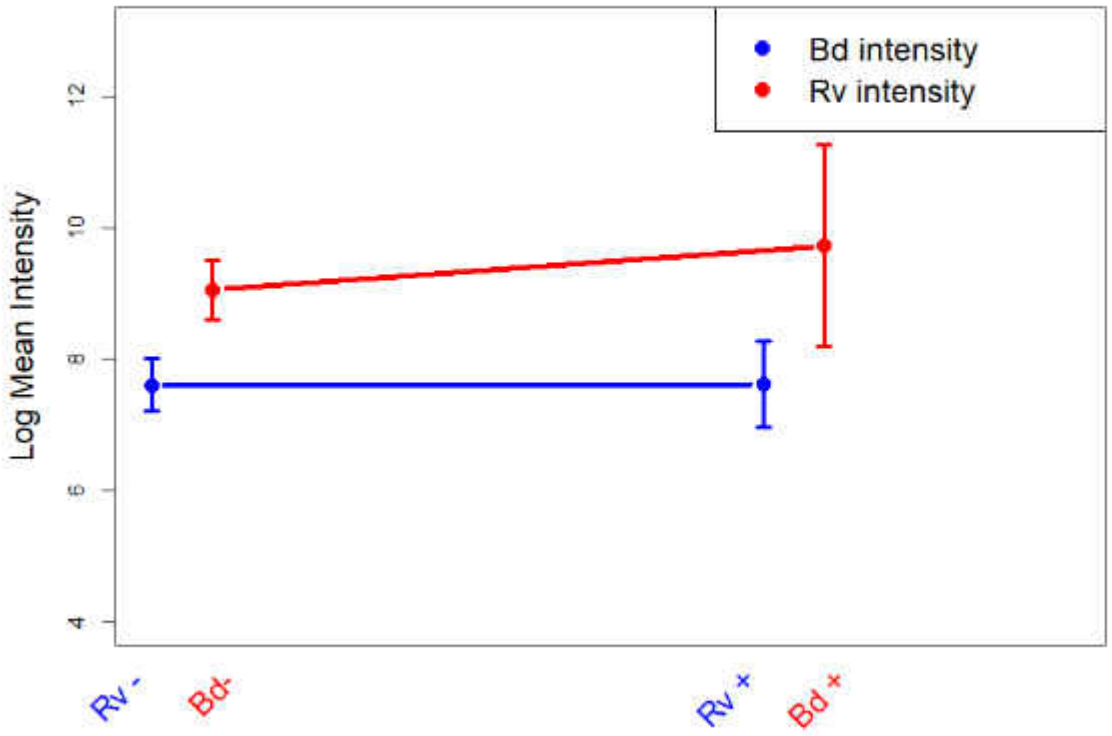

Figure 13. Log mean and standard errors for *Bd* intensity given *Rv* infection status (Blue) and *Rv* intensity given *Bd* infection status (Red).

Discussion

Our study is among the few to monitor multiple pathogens, across seasons and years for a wide range of host species (Buhnerkempe et al. 2015). We found the presence of both *Bd* and *Rv* at all three sites and persistence of both pathogens throughout the year in two of the three sampling areas. Season, family and location had influence on both prevalence and intensity of *Bd* and *Rv* in different

combinations. We found similar results compared to the previous studies done on co-infection where no pattern was found when *Bd* and *Rv* were considered together. We also found supporting results involving detection of *Rv* in different sample types, and a novel result of intensity being equal across tissue types sampled. While our study shows high levels of infection for *Bd* and *Rv* in a suite of hosts, it is important to note that only 12 out of over 900 samples had visible hemorrhaging on their skin, a classic sign of both *Bd* and *Rv* infection. Broader conservation strategies may need to be employed to help amphibians that are already harboring heavy loads of pathogen to survive. Comprehensive monitoring and management should be employed to help preserve declining amphibian populations.

Season was a significant driver for *Bd* intensity and for *Rv* prevalence and intensity. Our results concerning *Bd* mirror the results found for multiple other studies (Berger et al. 1998, Woodhams et al. 2008, Sapsford et al. 2013, Savage et al. 2015, Horner et al. 2017), where winter intensity levels are much higher than any other season. This is most likely because *Bd*'s thermal optimum corresponds with temperatures seen during winter months and reinforces the result found for *Bd* in our first chapter (Horner et al. 2017). For *Rv*, our results are more novel. There are only two studies to date that thoroughly looked at *Rv* and seasonality. Todd-Thompson (2010) found that infection prevalence peaked in the summer. The entire year was not sampled, however, and *Rv* could only be detected at a single sampling site. Hall et all (2018) sampled water through eDNA methods for larval wood frogs and found that *Rv* outbreaks were temperature-dependent and were correlated with rising temperatures. For this study, only larval stages of one species were assessed and ponds were only sampled part of the year (March through July). Our more robust sampling yielded a different result, with *Rv* infections peaking in the fall. Our results may again reflect the temperature optimum that *Rv* displays, which is between 20-30°C (Rojas et al. 2005, Cunningham et al. 2007, Allender et al. 2013, Brand et al. 2016). These are temperatures often experienced not only during Florida autumns but during fall throughout

the southeastern United States. With these results, we again stress the importance of sampling during the entire year and over multiple years to better understand prevalence and intensity patterns of pathogens.

Anuran host family significantly influenced *Bd* intensity alone and had no impact on any of the measured *Rv* dynamics. Specifically, hylids were more likely to have higher *Bd* infection intensity compared to ranids. Overall trends for *Bd* infection across host lineages remain unclear, as most studies focus on one or a few species (often that are closely related) (Green et al. 2002, Pearl et al. 2007, Woodhams et al. 2008, Richmond et al. 2009, Sapsford et al. 2013, Bataille et al. 2015). However, one approach has been to assess family-level *Bd* susceptibility dynamics using database mapping, which has demonstrated that several families (including Hylidae) are over-infected with *Bd* compared to others (Olson et al. 2013). Many studies, however, focus only on ranid frogs as they seem to be one of the most susceptible groups and many populations are experiencing declines in the wild (Daszak et al. 1999; Green et al. 2002, Schrader 2002; Pearl et al. 2007; Muths et al. 2008). Our study is consistent with Olson et al. (2013) in detecting elevated rates of *Bd* infection in hylids compared to other anuran taxa. In contrast with *Bd*, host family did not influence *Rv* dynamics in any way, which contrasts other studies that find ranids are most likely to be infected by *Rv* (Hoverman *et al.* 2011). It is important to note, however, that this was a lab study and other factors were not accounted for. These factors, such as site effects and other natural phenomena like microbial interactions and chemistry of the environment, may outweigh the effect of family on susceptibility to *Rv* infection when compared to lab studies.

We found that sampling location had significant impacts on *Bd* prevalence as well as *Rv* prevalence and intensity. Specifically, we found that *Bd* prevalence was affected most by site, with lower temperatures at these locations leading to higher rates of *Bd* infection, similar to previous

findings for the southeastern USA (Horner et al. 2017). The North site had the highest infection rates out of the three sampling sites for both *Bd* and *Rv,* with rates of prevalence increasing northward as well. This is most likely due to northern latitudes experiencing cooler temperatures more often than the lower latitudes (Kriger and Hero 2007a; Kriger and Hero 2007b; Sapsford, Alford and Schwarzkopf 2013; Savage et al. 2015, Horner et al. 2017). As mentioned previously, the temperature optimums of both pathogens correspond to temperatures experienced at more northern latitudes (Piotrowski et al. 2004, Cunningham et al. 2007). It appears from our findings, as well as those finding from other southeastern USA studies, that *Bd* and *Rv* conform to their preferred thermal thresholds in wetland systems of Florida (Horner et al. 2017). Northern areas with concentrated wetlands should be closely monitored for die-offs and pathogen infection of anurans in order to stay ahead of any catastrophic amphibian declines.

Out of the more than 900 individuals sampled for this project, only 23 individuals were co-infected with *Bd* and *Rv*, despite prevalence of both pathogens being relatively high. Our results corroborate the results of the few other co-infection studies published which also found limited to no significant patterns for explaining co-infection for anuran hosts (Hoverman et al. 2011, Souza et al. 2012, Whitfield et al. 2013, Warne et al. 2016). While co-infection seems to be a common occurrence in other host systems, i.e. plants and insects, some underlying mechanism may be preventing the co-occurrence of these two pathogens inside anurans (Hawley and Altizer 2011). More physiological studies are needed to see how both *Bd* and *Rv* intereact with each other both *in vitro* and inside amphibian hosts.

We found that *Rv* can accurately be detected in multiple sample types, corroborating previous sampling studies of anuran toe clips and their likelihood of producing accurate *Rv* detection (St-Amour and Lesbarrarres 2006). *Rv* replicates in the liver and cause hemorrphaging in the skin of amphibian

hosts (Cunningham et al. 2007), but previous sampling protocols state that *Rv* can not be accurately detected by taking tissue other than liver for sampling (Pessier and Mendelson 2010). Our results suggest that toe or tail clips (both non-lethal skin samples) and liver samples are equivalent for detection, and that non-destructive methods can be used to sample for *Rv* infections, corroborating the research done by St-Amour and Lesbarrarres (2006). We were able to exhibit, for the first time, that intensity of *Rv* infections can also be accurately measured by taking toe and tail clips, as there was no significant difference in intensity when comparing liver tissue to toe/tail tissue samples. Non-destructive sampling methods are important when sampling endangered species and our results suggest this can be done for both *Bd* and *Rv.* As amphibian populations continue to dwindle, it is important to keep populations robust, and non-destructive sampling methods allow populations to only be minimally impacted through sampling (Gonser and Collura 1996).

Our results show that *Bd* and *Rv* are not only present throughout Florida over all four seasons and in multiple hosts, but that these infections are occurring at elevated levels. Despite our results, most individual anurans captured appeared in good health, with some individuals even being recaptured during later sampling trips. We did not witness any noticeable population declines due to mortality over the three years we were conducting this study. It is important to note that our three major sampling areas were in relatively undisturbed areas surrounded by well-managed, contiguous habitat. Even though these individuals had to be under obvious stress from the levels of pathogen they were harboring, they persisted. Taking this observation, we suggest that “curing the disease” may not be the best way to approach this conservation issue and managing large swathes of suitable habitat may be a good approach to combating amphibian disease. We also further stress the need to do comprehensive research on disease systems to better understand them and predict their effects. Our study is a crucial step in understanding the greater community disease ecology of anuran communities

and will aid in informing future management and predictive strategies for combating the amphibian biodiversity crisis.

OVERALL CONCLUSION

Our results from both chapters, coupled with the available literature, suggest that *Bd* and *Rv* respond much like other infectious pathogens and are influenced by their environment. Specifically, seasonality appears to be a major driver of *Bd* dynamics. While these were expected results for *Bd* based on the literature and its ecology, our results for *Rv* were more surprising. Not much is available for seasonal effects on *Rv* besides anecdotal evidence and incomplete observational studies. Our results suggest that both *Bd* and *Rv*, at least in FL, follows their biological "preferences" by increasing their abundance during seasons that mimic their thermal optima (Piotrowski et al. 2004, Cunningham et al. 2007). We offer the first look in to true seasonal dynamics for *Rv* and offer supporting evidence for seasonality and *Bd* dynamics. More studies need to be done, however, to see if a general pattern holds true for seasonality and *Rv* dynamics.

We find strong evidence for high levels of both *Bd* and *Rv* in Florida, and *Bd* in the greater Southeastern United States. In both chapters, high levels of these pathogens were found for both prevalence and intensity. For *Bd*, intensity levels found in Florida and the Southeast exceeded the "10,000 zoospore rule" put forth by Vredenburg et al. (2010). Specifically, for Florida, populations remained intact over multiple years, even though this threshold was reached. We suggest that this rule is not applicable to all species and have evidence that host identity plays a major role in reaction to pathogens. Multiple factors should be considered when studying pathogen dynamics and population sustainability, especially for amphibians.

Most studies for amphibian pathogens are incomplete. Sites are often only visited once or only during mating season, and the rest of the time is unaccounted for. Additionally, few hosts are often compared, and environmental data is often not taken. We encourage a more comprehensive look at disease dynamics by looking at host, environment and pathogen together. The combination of epidemiology and disease ecology on whole communities employs a more wholesome look at systems and how they operate, as well as promoting the idea of "One Health", or overall ecosystem health (Zinsstag et al. 2011). Our research offers a good framework for future studies looking to elaborate on our results.

To sum up, it is clear from our results that amphibians are harboring heavy loads of not one, but two, emerging infectious pathogens. Though it has been understudied in the Southeast, we must take disease dynamics into account when conserving amphibians. Our suggestions for conservationists and managers are to employ more preventative monitoring of amphibian disease before outbreaks take place. For instance, monitoring should be put in place before declines occur, to better understand what causes such outbreaks. Additionally, a more comprehensive view of conservation is necessary to protect amphibians. Whole ecosystems should be protected and managed so disease and recovery through natural selection can take place without additional stressors on the host. This "One Health" approach may be the reason why the populations of anurans at our North site are thriving despite being heavily infected. By conserving these creatures in a more holistic way, we have a chance on helping them survive for future generations.

APPENDIX A:

SUPPLEMENTAL GENETIC DATA, ANALYSES AND R CODE FROM CHAPTER 1

Table 4 Raw data for Pseudacris ornata genetic loci.

Pop	Pop Code	PCRU09		PCRU14		PCRU24		POR105		PTRI29		POR165		PCRU10	
JEN	JEN001	147	147	181	181	263	263	0	0	162	162	0	0	0	0
	JEN002	147	147	181	183	263	265	0	0	162	162	254	268	405	451
	JEN003	141	147	0	0	251	266	270	270	162	162	268	0	449	449
	JEN004	141	145	181	181	263	271	0	0	162	162	226	0	449	449
	JEN005	141	147	181	181	263	263	292	0	162	162	254	268	449	449
	JEN006	141	143	181	181	263	266	272	272	162	162	222	300	449	449
	JEN007	141	143	181	181	263	0	272	272	162	162	234	256	449	479
	JEN008	143	145	181	183	263	263	254	272	162	162	0	0	449	449
	JEN009	143	147	181	181	263	263	272	0	162	162	234	258	383	451
	JEN010	141	141	181	181	263	272	270	270	162	162	0	0	449	449
	JEN011	141	141	181	181	263	263	272	272	162	162	232	258	449	489
	JEN012	143	147	183	183	0	0	272	274	162	162	0	0	449	449
	JEN013	143	147	181	181	263	263	272	272	162	162	248	0	407	449
	JEN014	141	143	181	181	263	263	272	272	162	162	0	0	449	449
	JEN015	141	141	181	181	263	263	230	0	162	162	224	0	449	449
	JEN016	143	147	181	181	263	263	274	274	162	162	234	234	449	449
	JEN017	143	147	181	181	261	265	272	272	0	0	254	268	0	0
	JEN018	147	147	181	181	263	263	278	278	162	162	224	0	449	449
	JEN019	141	141	181	181	263	263	286	0	162	162	224	232	383	449

	JEN020	141	141	181	181	263	263	288	0	162	162	0	0	383	449
	JEN021	143	143	181	181	263	263	270	270	162	162	248	254	449	449
	JEN022	143	147	181	181	263	263	272	0	162	162	0	0	449	449
	JEN023	141	143	181	181	263	263	292	0	162	162	254	254	449	449
	JEN024	143	147	181	183	263	265	274	288	162	162	224	0	383	449
	JEN025	143	147	183	183	263	0	262	270	162	162	232	232	383	449
	JEN026	147	147	181	181	263	263	288	0	162	162	248	254	449	449
	JEN027	143	147	181	181	0	0	270	274	162	162	232	248	489	0
	JEN028	147	147	181	181	0	0	0	0	162	162	232	232	449	449
	JEN029	143	147	181	181	263	263	288	0	162	162	226	232	383	383
SRE A-D	SRE001	143	143	183	183	257	263	226	254	162	162	274	0	449	449
	SRE002	141	153	183	183	263	263	230	306	162	162	232	258	449	449
	SRE003	143	145	183	183	263	263	270	270	162	162	254	0	0	0
	SRE056	141	141	183	183	0	0	0	0	162	162	0	0	0	0
	SRE057	141	143	179	181	263	263	162	306	154	162	240	240	449	449
	SRE058	141	143	181	183	263	263	162	306	154	162	220	220	0	0
	SRE059	143	143	181	183	263	263	158	226	162	162	220	258	449	449
	SRE060	143	143	181	183	257	263	274	278	154	162	220	294	443	443
	SRE061	143	153	183	183	263	265	162	0	162	162	220	232	449	449
	SRE062	143	145	181	183	263	265	254	262	162	162	250	0	449	449
	SRE063	143	143	181	183	257	263	246	266	154	154	224	258	443	449
	SRE064	141	143	181	183	0	0	226	230	162	162	258	258	449	449
	SRE065	143	143	179	181	257	263	174	258	154	162	246	0	443	443
	SRE066	143	145	183	183	263	263	230	266	162	162	248	248	0	0

	SRE067	143	143	183	183	263	263	226	250	162	162	206	248	0	0
	SRE068	143	145	183	183	263	263	162	230	162	162	246	246	0	0
	SRE004	143	143	183	183	263	263	162	262	162	162	240	274	441	443
	SRE005	143	145	183	183	263	263	258	282	162	162	0	0	449	449
	SRE006	143	145	179	183	263	263	0	0	162	162	244	268	449	449
	SRE007	143	143	173	183	263	263	226	278	162	162	236	244	443	449
	SRE008	143	143	183	183	263	263	222	282	162	162	254	0	449	449
	SRE009	141	143	181	183	263	263	222	226	154	162	244	278	383	449
	SRE010	141	143	183	183	263	263	246	258	154	162	244	244	443	449
	SRE011	143	153	0	0	263	263	262	274	162	162	252	280	443	449
	SRE012	141	143	183	183	263	263	230	230	154	162	248	248	449	449
	SRE013	143	143	181	183	263	0	234	0	162	162	206	244	449	449
	SRE014	143	145	183	183	0	0	254	278	162	162	0	0	443	449
	SRE015	143	145	183	183	263	263	162	258	154	162	248	248	449	449
	SRE016	143	145	183	183	263	263	174	204	162	162	244	248	443	449
	SRE017	141	143	181	183	263	263	182	282	162	162	0	0	425	451
	SRE018	143	143	179	183	263	263	230	254	162	162	206	248	443	449
	SRE019	145	145	181	183	263	263	222	258	162	162	218	256	443	449
	SRE020	141	143	183	183	263	263	230	274	162	162	228	252	0	0
	SRE021	143	143	181	181	263	263	250	262	154	162	238	238	443	443
	SRE022	143	153	183	183	263	0	258	286	162	162	250	256	449	449
	SRE023	143	143	183	183	263	263	230	262	154	162	240	250	443	451
	SRE024	141	143	183	183	263	263	254	278	158	162	222	246	443	443
	SRE025	141	143	183	183	263	269	226	250	162	162	244	244	443	449

	SRE026	143	145	179	183	263	263	266	266	162	162	250	256	449	449
	SRE027	143	151	181	183	263	263	226	258	162	162	288	0	0	0
	SRE028	143	143	181	183	263	263	162	278	162	162	226	248	449	449
	SRE029	143	143	181	183	263	263	222	282	154	162	228	232	443	449
	SRE030	143	143	181	181	263	269	246	266	162	162	0	0	383	443
	SRE031	143	155	183	183	263	263	258	262	154	154	230	0	449	449
	SRE032	145	153	183	183	263	263	226	258	162	162	244	250	383	443
	SRE033	143	143	181	183	263	263	254	278	162	162	244	244	443	443
	SRE034	141	143	181	183	263	263	254	282	162	162	220	244	449	449
	SRE035	143	145	181	181	263	263	262	282	162	162	234	252	443	451
	SRE036	143	143	181	183	263	263	222	254	162	162	206	252	451	451
	SRE037	143	143	179	183	263	263	250	278	162	162	232	244	449	449
	SRE038	143	145	183	183	263	263	162	218	162	162	262	0	0	0
	SRE039	143	143	179	181	263	263	222	258	162	162	244	262	449	449
	SRE040	143	151	179	179	0	0	222	290	154	154	238	0	443	449
	SRE041	143	143	183	183	263	263	162	254	162	162	212	218	449	449
	SRE042	143	145	183	183	263	263	262	266	162	162	206	250	383	449
	SRE043	143	145	183	183	263	263	230	262	162	162	224	232	449	449
	SRE044	141	143	179	183	263	263	226	262	162	162	248	262	443	449
	SRE045	143	143	183	183	0	0	162	274	154	162	220	250	449	449
	SRE046	143	143	183	183	263	263	0	0	0	0	220	248	449	449
	SRE047	143	143	183	183	263	263	258	266	162	162	244	300	449	449
	SRE048	143	143	183	183	263	263	226	278	162	162	220	248	449	449
	SRE049	145	153	183	183	263	263	278	282	162	162	212	244	443	451

	SRE050	141	153	183	183	263	263	222	230	162	162	206	206	449	449
	SRE051	143	143	183	183	263	263	254	278	162	162	206	260	443	449
	SRE052	143	143	0	0	263	272	262	266	162	162	264	284	443	449
	SRE053	143	143	181	183	263	263	258	274	162	162	268	0	451	451
	SRE054	143	143	183	183	263	263	162	274	154	162	250	288	449	449
	SRE055	0	0	183	183	263	263	254	256	162	162	274	284	449	449
COLETON SC	Coleton_SC_PO_012_2006	143	145	183	183	263	263	0	0	162	162	236	276	449	499
	Coleton_SC_PO_014_2006	143	143	183	183	263	263	0	0	162	162	236	268	449	449
	Coleton_SC_PO_032_2006	143	145	183	183	263	263	222	234	162	162	240	290	449	449
	Coleton_SC_PO_033_2006	143	145	183	183	263	263	0	0	162	162	240	262	443	443
	Coleton_SC_PO_034_2006	143	153	183	183	263	263	0	0	0	0	240	0	0	0
	Coleton_SC_PO_035_2006	143	143	181	183	263	263	0	0	162	162	244	264	449	449
	Coleton_SC_PO_036_2006	143	145	183	183	263	278	0	0	162	162	236	252	449	449
	Coleton_SC_PO_037_2006	143	145	183	183	263	263	0	0	162	162	0	0	392	449
	Coleton_SC_PO_038_2006	145	145	179	179	263	266	0	0	162	162	256	276	0	0
	Coleton_SC_PO_039_2006	153	153	183	183	263	263	222	258	162	162	240	252	0	0
	Coleton_SC_PO_040_2006	143	145	181	183	263	263	0	0	162	162	236	0	449	449
	Coleton_SC_PO_041_2006	153	153	183	183	263	263	0	0	162	162	264	264	449	449
	Coleton_SC_PO_042_2006	143	143	183	183	263	263	0	0	162	162	236	240	0	0
	Coleton_SC_PO_043_2006	143	145	183	183	263	245	0	0	162	162	264	264	449	449
	Coleton_SC_PO_044_2006	143	153	181	183	0	0	0	0	162	162	236	264	449	457
	Coleton_SC_PO_045_2006	143	153	183	183	263	263	0	0	162	162	244	252	449	449
	Coleton_SC_PO_046_2006	143	153	179	183	263	263	0	0	162	162	244	276	0	0
	Coleton_SC_PO_047_2006	143	145	183	183	263	260	234	246	162	162	236	244	449	449

	Coleton_SC_PO_048_2006	145	145	183	183	263	266	0	0	162	162	264	264	449	449
	Coleton_SC_PO_049_2006	143	145	183	183	263	263	258	262	162	162	276	276	443	443
	Coleton_SC_PO_050_2006	145	153	183	183	263	266	258	262	162	162	256	256	449	449
	Coleton_SC_PO_051_2006	143	143	183	183	263	266	222	262	162	162	236	236	449	449
	Coleton_SC_PO_052_2006	145	145	183	183	263	266	234	280	162	162	240	256	449	449
	Coleton_SC_PO_053_2006	143	145	181	183	263	263	0	0	0	0	0	0	443	443
	Coleton_SC_PO_054_2006	143	143	181	183	244	263	262	264	162	162	236	236	443	449
	Coleton_SC_PO_056_2006	0	0	183	183	263	263	162	246	0	0	244	252	449	449
	Coleton_SC_PO_066_2007	145	145	179	183	263	263	246	246	162	162	220	220	449	449
	Coleton_SC_PO_067_2007	143	145	179	183	263	266	226	242	162	162	0	0	449	449
	Coleton_SC_PO_068_2007	145	153	179	179	263	263	238	242	162	162	284	290	449	449
	Coleton_SC_PO_069_2007	143	143	179	183	263	263	234	238	162	162	244	282	449	449
	Coleton_SC_PO_070_2007	145	145	179	183	263	266	0	0	162	162	220	222	449	449
	Coleton_SC_PO_071_2007	143	145	179	183	263	263	238	258	162	162	264	290	449	449
	Coleton_SC_PO_072_2007	143	143	179	183	263	263	0	0	162	162	224	244	449	449
	Coleton_SC_PO_073_2007	141	143	183	183	263	266	250	286	162	162	220	224	449	449
	Coleton_SC_PO_074_2007	145	153	179	183	263	263	238	242	162	162	186	246	449	449
	Coleton_SC_PO_075_2007	143	145	183	183	263	263	250	250	162	162	232	250	449	469
	Coleton_SC_PO_076_2007	143	143	183	183	263	263	226	226	162	162	226	226	449	449
	Coleton_SC_PO_077_2007	143	143	179	183	263	263	238	258	162	162	246	246	449	449
	Coleton_SC_PO_078_2007	143	145	181	183	263	263	0	0	162	162	220	242	443	449
	Coleton_SC_PO_079_2007	143	145	181	183	263	263	250	262	162	162	236	244	449	449
	Coleton_SC_PO_080_2007	145	145	183	183	263	263	0	0	162	162	244	248	449	449
	Coleton_SC_PO_081_2007	143	143	179	183	263	263	238	258	162	162	246	320	449	451

	Coleton_SC_PO_082_2007	141	143	181	183	263	263	246	262	162	162	224	244	449	449
	Coleton_SC_PO_083_2007	0	0	0	0	263	263	0	0	162	162	232	246	449	449
	Coleton_SC_PO_084_2007	143	143	179	179	263	263	238	242	162	162	246	284	449	449
	Coleton_SC_b_PO_086_SC_2007	143	153	181	183	263	263	234	284	162	162	244	244	443	449
	Coleton_SC_b_PO_089_SC_2007	141	147	183	263	263	263	0	0	162	162	236	264	449	449
	Coleton_SC_b_PO_090_SC_2007	145	153	181	183	263	263	226	262	162	162	264	320	443	449
	Coleton_SC_b_PO_091_SC_2007	143	145	181	183	263	263	246	262	162	162	264	270	443	449
	Coleton_SC_b_PO_092_SC_2007	0	0	0	0	263	263	262	262	162	162	264	244	443	449
	Coleton_SC_b_PO_093_SC_2007	141	145	181	183	263	263	222	272	162	162	244	244	443	449
	Coleton_SC_b_PO_094_SC_2007	143	145	183	183	263	263	246	250	162	162	268	288	0	0
FORT BRAGG	Fort_Brag_NT_001_2007	141	151	179	179	263	272	242	262	162	162	222	254	384	449
	Fort_Brag_NT_002_2007	141	151	179	179	263	272	254	258	162	162	222	234	437	449
	Fort_Brag_NT_003_2007	141	141	179	179	234	272	272	292	162	162	0	0	449	384
	Fort_Brag_NT_004_2007	151	151	179	179	263	272	258	292	162	162	0	0	384	449
	Fort_Brag_NT_005_2007	141	151	179	183	263	263	254	292	162	162	234	236	437	449
	Fort_Brag_NT_006_2007	141	151	179	183	263	263	258	292	154	162	222	234	449	449
	Fort_Brag_NT_007_2007	141	151	179	179	263	263	258	292	162	166	234	258	449	449
	Fort_Brag_NT_008_2007	151	107	179	179	263	272	242	292	162	162	0	0	0	0
HARDYVILLE SC	Hardy_SC_PO_058_2006	143	143	183	183	263	263	262	276	154	154	216	260	384	425
	Hardy_SC_PO_059_2006	0	0	179	181	263	263	264	282	162	162	234	252	449	449
	Hardy_SC_PO_060_2006	0	0	181	183	263	263	154	250	162	162	224	286	449	449
	Hardy_SC_PO_061_2006	0	0	181	183	263	263	162	262	162	162	256	260	384	449

	Hardy_SC_PO_062_2006	145	145	181	183	263	263	154	154	162	162	224	250	449	449
	Hardy_SC_PO_063_2006	143	143	181	183	263	263	162	246	162	162	246	258	449	449
	Hardy_SC_PO_064_2006	143	143	181	183	263	263	254	264	162	162	256	256	449	449
	Hardy_SC_PO_065_2006	143	143	183	183	263	263	258	262	162	162	240	244	449	449
	Hardy_SC_PO_130_2007	143	153	181	183	263	263	262	282	162	162	216	252	425	449
	Hardy_SC_PO_131_2007	143	145	181	183	263	263	254	282	162	162	260	260	449	449
	Hardy_SC_PO_132_2007	143	143	183	183	263	263	162	162	162	162	256	206	449	449
	Hardy_SC_PO_133_2007	141	143	183	183	263	263	250	276	158	162	248	258	449	449
	Hardy_SC_PO_134_2007	145	145	181	183	263	263	154	272	162	162	216	252	449	449
	Hardy_SC_PO_135_2007	143	143	181	183	263	263	254	276	162	162	216	258	449	449
	Hardy_SC_PO_136_2007	143	145	181	183	263	263	226	262	162	162	242	272	384	449
	Hardy_SC_PO_137_2007	143	143	181	183	263	263	242	276	162	162	246	246	0	0
	Hardy_SC_PO_138_2007	143	143	181	183	263	263	222	226	162	162	206	248	449	449
	Hardy_SC_PO_139_2007	141	143	181	183	263	266	0	0	162	162	250	256	449	449
	Hardy_SC_PO_140_2007	143	143	181	183	263	263	246	254	162	162	242	242	449	449
	Hardy_SC_PO_141_2007	145	153	181	183	263	263	276	282	154	162	258	258	449	449
	Hardy_SC_PO_142_2007	143	153	181	183	263	263	162	222	162	162	244	258	449	449
	Hardy_SC_PO_143_2007	143	143	181	183	263	263	250	276	158	162	278	280	443	443
	Hardy_SC_PO_144_2007	143	145	181	183	263	263	262	282	162	162	216	256	443	449
	Hardy_SC_PO_145_2007	143	145	181	183	263	263	0	0	162	162	246	252	449	449
	Hardy_SC_PO_146_2007	143	143	181	183	263	263	276	276	162	162	256	260	0	0
FL HWY 379	HWY_379_EMC_2209_2006	141	141	179	181	263	263	0	0	162	162	266	266	0	0
	HWY_379_EMC_2210_2006	143	147	181	181	263	269	272	272	162	162	248	248	449	449
	HWY_379_EMC_2211_2006	141	143	181	181	263	269	264	264	162	162	0	0	0	0

	HWY_379_EMC_2212_2006	141	141	181	181	263	263	268	268	162	162	248	248	0	0
	HWY_379_EMC_2213_2006	147	147	181	181	263	266	260	260	162	162	262	262	0	0
	HWY_379_EMC_2214_2006	145	145	181	181	263	263	262	268	162	162	222	224	449	449
	HWY_379_EMC_2215_2006	141	157	181	183	263	269	272	272	162	162	228	252	449	449
	HWY_379_EMC_2216_2006	141	141	181	181	263	263	258	258	162	162	246	258	449	449
	HWY_379_EMC_2217_2006	147	157	181	181	263	266	264	264	158	162	254	270	449	449
	HWY_379_EMC_2218_2006	147	147	181	181	263	263	264	268	162	162	248	250	449	449
	HWY_379_EMC_2219_2006	147	151	181	181	263	263	0	0	162	162	236	246	413	413
	HWY_379_EMC_2220_2006	147	147	181	181	263	263	264	264	162	162	248	248	449	449
	HWY_379_EMC_2221_2006	141	147	181	181	263	263	264	264	162	154	254	258	460	460
	HWY_379_EMC_2222_2006	141	141	179	181	263	263	250	272	162	162	0	0	449	449
	HWY_379_EMC_2223_2006	143	157	175	181	263	269	196	260	162	162	250	250	449	449
	HWY_379_EMC_2369_2006	141	147	181	181	260	278	268	268	162	162	234	236	0	0
	HWY_379_EMC_2370_2006	141	145	181	181	266	266	250	250	162	162	234	236	449	449
	HWY_379_EMC_2371_2006	141	143	179	181	263	269	260	264	162	162	228	250	0	0
	HWY_379_EMC_2372_2006	145	143	181	181	0	0	0	0	162	154	244	250	435	435
	HWY_379_EMC_2373_2006	147	147	175	181	266	266	0	0	162	162	220	228	0	0
	HWY_379_EMC_2374_2006	141	141	181	181	266	266	272	272	162	162	232	250	384	449
	HWY_379_EMC_2375_2006	143	143	181	181	263	269	264	264	162	162	258	0	435	449
	HWY_379_EMC_2376_2006	141	141	181	181	266	266	252	276	162	162	224	260	449	449
	HWY_379_EMC_2377_2006	141	141	181	181	263	266	268	272	162	162	244	250	321	321
	HWY_379_EMC_2378_2006	147	147	181	183	266	266	0	0	162	162	250	258	0	0
	HWY_379_EMC_2379_2006	141	143	181	181	263	275	0	0	162	162	230	242	384	449
	HWY_379_EMC_2380_2006	143	143	181	181	263	263	0	0	162	162	226	226	449	449

	HWY_379_EMC_2381_2006	143	145	181	181	263	266	268	268	162	162	260	260	449	449
	HWY_379_EMC_2382_2006	141	147	181	181	263	266	280	280	162	162	224	254	0	0
	HWY_379_EMC_2383_2006	141	143	181	183	263	266	264	264	162	162	238	254	449	449
	HWY_379_PO_001_2006	141	141	181	183	0	0	260	260	162	162	224	258	0	0
	HWY_379_PO_002_2006	141	141	181	183	263	266	0	0	162	162	216	222	449	449
	HWY_379_PO_003_2006	141	143	181	181	266	269	0	0	162	162	230	258	384	489
	HWY_379_PO_004_2006	141	143	181	181	263	266	260	260	162	162	0	0	449	449
	HWY_379_PO_005_2006	141	143	181	181	263	263	0	0	162	162	254	270	0	0
	HWY_379_PO_006_2006	143	145	181	181	263	266	264	272	162	162	240	244	449	449
	HWY_379_PO_007_2006	143	143	181	181	263	266	0	0	162	162	226	226	449	449
	HWY_379_PO_008_2006	143	141	179	181	263	269	0	0	162	162	242	256	388	449
	HWY_379_PO_009_2006	141	147	181	183	242	263	0	0	162	162	0	0	0	0
	HWY_379_PO_010_2006	141	147	181	181	263	266	0	0	162	162	226	240	449	449
	HWY_379_PO_011_2006	143	147	181	181	263	266	0	0	162	162	238	238	0	0
	HWY_379_PO_086_2007	141	141	181	181	263	263	0	0	162	162	244	248	419	489
	HWY_379_PO_087_2007	141	141	181	181	263	266	246	276	162	162	248	248	449	449
	HWY_379_PO_088_2007	141	141	181	183	263	266	0	0	162	162	248	254	0	0
	HWY_379_PO_089_2007	141	147	181	181	263	266	226	252	162	162	224	224	384	449
	HWY_379_PO_090_2007	0	0	0	0	263	263	264	264	0	0	0	0	0	0
	HWY_379_PO_091_2007	141	141	181	181	263	263	268	282	162	162	226	236	0	0
	HWY_379_PO_092_2007	143	145	181	181	263	266	264	264	162	162	226	226	449	449
	HWY_379_PO_093_2007	143	151	181	181	0	0	0	0	162	162	266	268	0	0
	HWY_379_PO_094_2007	145	147	175	179	263	266	0	0	162	162	216	232	384	384
	HWY_379_PO_095_2007	147	147	181	181	263	269	276	306	162	162	216	270	0	0

	HWY_379_PO_096_2007	0	0	0	0	263	263	0	0	162	162	236	236	449	449
	HWY_379_PO_097_2007	141	145	181	181	263	263	260	268	162	162	250	250	449	449
	HWY_379_PO_098_2007	143	147	181	181	263	266	260	260	162	162	246	246	0	0
	HWY_379_PO_099_2007	141	145	181	181	263	266	264	264	162	162	226	246	449	449
	HWY_379_PO_100_2007	141	147	181	181	263	263	264	268	162	162	258	258	392	449
	HWY_379_PO_101_2007	143	143	181	181	263	263	272	280	162	162	242	272	0	0
	HWY_379_PO_102_2007	143	143	181	183	266	275	250	260	162	162	224	224	449	489
	HWY_379_PO_103_2007	147	147	181	181	263	263	260	260	162	162	230	230	0	0
	HWY_379_PO_104_2007	141	141	181	181	263	266	260	276	162	162	224	230	0	0
	HWY_379_PO_105_2007	143	143	181	183	263	263	262	264	162	162	248	248	0	0
	HWY_379_PO_106_2007	141	141	181	181	263	266	252	272	162	162	226	230	0	0
	HWY_379_PO_107_2007	145	147	181	181	266	266	268	268	162	162	244	266	0	0
	HWY_379_PO_108_2007	141	147	181	181	263	266	0	0	162	162	256	258	0	0
	HWY_379_PO_109_2007	141	141	181	181	263	263	262	276	162	162	224	244	384	435
	HWY_379_PO_110_2007	147	147	181	181	263	263	260	264	162	162	244	244	0	0
	HWY_379_PO_111_2007	141	141	181	181	263	263	268	272	162	162	228	250	384	449
	HWY_379_PO_112_2007	143	143	181	181	263	269	258	258	162	162	244	254	0	0
	HWY_379_PO_113_2007	145	147	181	181	263	263	268	268	162	162	226	242	0	0
	HWY_379_PO_114_2007	141	143	181	183	263	263	276	276	162	162	230	236	0	0
	HWY_379_PO_115_2007	143	143	181	181	263	269	262	268	0	0	224	246	0	0
	HWY_379_PO_116_2007	143	143	175	181	266	272	262	262	162	162	226	246	0	0
	HWY_379_PO_117_2007	143	143	181	181	263	263	264	264	162	162	222	248	0	0
	HWY_379_PO_118_2007	143	143	181	183	266	226	264	264	162	162	230	0	0	0
	HWY_379_PO_119_2007	141	147	181	181	263	263	272	272	162	162	236	242	0	0

	HWY_379_PO_120_2007	143	147	181	181	263	263	260	268	162	162	0	0	0	0
	HWY_379_PO_121_2007	143	143	181	181	272	272	260	260	162	162	238	254	0	0
	HWY_379_PO_122_2007	141	147	181	181	263	266	272	272	0	0	244	252	0	0
	HWY_379_PO_123_2007	143	145	181	181	263	266	260	260	162	162	222	228	0	0
	HWY_379_PO_124_2007	141	145	181	181	263	266	0	0	162	162	274	0	0	0
	HWY_379_PO_125_2007	143	145	179	181	263	263	264	242	162	162	238	246	0	0
	HWY_379_PO_126_2007	143	147	181	183	263	266	264	264	162	162	254	258	0	0
	HWY_379_PO_127_2007	145	145	179	181	263	263	264	264	162	162	0	0	0	0
	HWY_379_PO_128_2007	143	147	181	181	263	266	252	264	162	162	240	240	0	0
	HWY_379_PO_129_2007	0	0	0	0	263	266	252	252	162	162	0	0	0	0
POND 51	POND_51_AMH_001_2006	141	151	181	181	263	266	258	258	154	162	250	268	449	449
	POND_51_AMH_002_2006	143	147	181	181	0	0	0	0	154	162	248	250	384	449
	POND_51_AMH_003_2006	143	147	181	181	272	272	0	0	162	162	250	252	384	449
	POND_51_AMH_004_2006	141	143	181	181	263	272	272	272	154	162	248	254	384	449
	POND_51_AMH_005_2006	0	0	0	0	263	263	272	272	162	162	252	264	449	449
	POND_51_AMH_006_2006	143	143	181	181	263	263	258	258	162	162	250	260	328	449
	POND_51_AMH_007_2006	143	143	181	181	263	266	0	0	162	162	250	268	449	449
	POND_51_AMH_008_2006	143	143	181	181	263	263	0	0	162	162	210	268	449	449
	POND_51_AMH_009_2006	0	0	0	0	266	266	272	272	162	162	256	256	449	449
	POND_51_AMH_010_2006	0	0	181	183	263	263	272	272	154	162	248	250	384	449
	POND_51_AMH_011_2006	143	143	181	181	0	0	272	272	162	162	248	254	449	449
	POND_51_AMH_012_2006	141	143	181	181	263	272	0	0	162	162	252	264	384	449
	POND_51_AMH_013_2006	143	147	181	181	263	263	238	238	154	162	252	262	384	449
	POND_51_AMH_014_2006	141	143	181	181	263	263	272	272	154	162	252	248	449	449

	POND_51_AMH_015_2006	141	141	181	183	0	0	0	0	162	162	210	248	449	449
	POND_51_AMH_016_2006	141	141	181	181	266	266	212	212	162	162	256	256	384	449
	POND_51_AMH_017_2007	143	143	183	183	263	263	272	272	162	162	224	248	449	449
	POND_51_AMH_018_2007	143	143	181	183	263	263	0	0	162	162	268	264	386	449
	POND_51_AMH_019_2007	143	143	183	183	263	263	0	0	162	162	232	264	449	449
	POND_51_AMH_020_2007	143	143	181	183	222	263	264	272	162	162	268	250	449	449
	POND_51_AMH_021_2007	143	143	181	181	263	266	272	272	162	162	228	248	443	449
	POND_51_AMH_022_2007	141	143	181	181	263	263	258	258	162	162	250	252	449	449
	POND_51_AMH_023_2007	143	143	181	181	263	269	234	258	162	162	224	224	449	449
	POND_51_AMH_024_2007	141	143	181	181	263	263	0	0	162	162	210	258	449	449
	POND_51_AMH_025_2007	141	141	181	183	263	263	180	238	162	162	254	254	449	449
	POND_51_AMH_026_2007	143	143	181	183	263	263	276	276	162	162	220	276	449	449
	POND_51_AMH_027_2007	0	0	183	183	263	266	290	290	162	162	254	264	449	449
	POND_51_AMH_028_2007	143	143	181	181	263	269	0	0	154	162	210	210	449	449
	POND_51_AMH_029_2007	141	147	181	181	263	263	264	264	162	162	220	250	449	449
	POND_51_AMH_030_2007	141	143	181	181	263	263	272	272	162	162	224	248	384	384
	POND_51_AMH_031_2007	143	147	181	181	263	263	258	276	158	162	226	256	449	449
	POND_51_AMH_032_2007	143	147	181	181	263	263	234	286	162	162	250	254	449	449
	POND_51_AMH_034_2007	141	143	181	181	263	266	294	294	162	162	262	266	449	449
	POND_51_AMH_035_2007	143	143	181	183	263	272	276	276	162	154	210	254	443	449
	POND_51_AMH_036_2007	143	153	183	183	263	266	284	294	154	162	210	272	449	449
	POND_51_AMH_037_2007	143	153	183	183	263	263	256	256	162	162	220	224	328	449
	POND_51_AMH_038_2007	145	153	183	183	263	266	258	258	162	162	230	278	449	449

Table 5. Information on species population locations, number of individuals in populations and year collected.

Species	*Population*	*Latitude*	*Longitude*	*N*	*Year Collected*
Notophthalmus perstriatus	ONF 1	29.383	-81.7956	27	2008
	ONF 2	29.416111	-81.761111	31	1997-2000
	ONF 3	29.060833	-81.803389	44	2009
	ONF 4	29.055833	-81.560389	23	2009
	Berry	29.6877	-82.006831	13	2008
	FD	29.680753	-81.265833	34	2002, 2009-2010
	GSF	29.53475	-82.597861	23	2010
	RSR	28.775278	-81.455833	18	1998, 2010
	FLSHNA	32.578194	-84.269353	10	2009
	JSF	30.001717	-81.015419	14	1999, 2009
	OR1	29.69139	-82.00306	98	1998, 2008
	OR2	29.7222	-81.01651	29	1997-1998, 2008
	OR3	29.01158	-81.01599	17	1997, 1999
	FSMI	n/a	n/a	29	1997
	CB	29.015947	-81.015647	17	1997, 2009
	JOH	29.005383	-81.012447	6	1996
	LA	29.007275	-81.012928	8	1997
	JP	n/a	n/a	1	2009
	TE	29.007278	-81.012714	10	2000
	ANF	30.339556	-84.320467	8	2009
	JJ	31.00435	-84.008817	10	1998
Hyla Squirella	AST	29.1605	-81.5535	20	2010
	CHAR	26.9317	-81.7607	1	2010

	CUT	29.5505	-83.1829	27	2010
	DISS	29.2771	-81.3343	33	2010
	EAPP	30.0282	-84.9879	35	2010
	GRAS	29.0147	-82.3232	10	2010
	GULF	28.539	-82.6171	37	2010
	HIKE	30.3461	-83.3394	33	2010
	LAZY	28.6266	-81.8882	0	2010
	OCK	29.5376	-81.778	16	2010
	OST	28.8461	-81.0936	22	2010
	PALM	27.9213	-80.5515	19	2010
	PEN	30.3196	-87.2634	36	2010
	PICK	n/a	n/a	36	2010
	PINE	30.0503	-81.3978	31	2010
	SAND	30.2744	-82.2845	32	2010
	SPAR	29.3811	-82.042	46	2010
	SR2	30.3849	-86.3761	41	2010
	STAR	29.9711	-82.2559	48	2010
	WAPP	30.1358	-85.3702	41	2010
	WAY	31.2089	-82.4494	16	2010
Pseudacris ornata	SRE A	33.157467	-81.67625	15	2006-2009
	SRE B	33.160517	-81.690650	1	2006-2009
	SRE C	33.289383	-81.48165	32	2006-2009
	SRE D	33.31805	-81.47685	20	2006-2009
	JEN C	30.160083	-81.889267	9	2006-2009
	JEN I	30.15195	-81.8787	10	2006-2009
	JEN J	30.156933	-81.884617	8	2006-2009

SR A	n/a	n/a	2	2006-2009
TEL	31.861444	-82.812075	6	2006-2009
AL HWY 165	32.0371	-85.0839	5	2006-2009
Coleton SC	33.0546	-80.4859	45	2006-2009
Coleton SC b	33.0422	-80.4277	7	2006-2009
Gulf	n/a	n/a	1	2006-2009
Hardyville SC	32.4371	-81.0042	25	2006-2009
Hwy 379	30.0861	-85.0404	85	2006-2009
Pond 51	31.2498	-84.4947	37	2006-2009
Fort Bragg	35.135846	-79.041152	8	2006-2009

Table 6. Principle Component Analysis vector labels and their meanings.

Vector	**Meaning**
TMIN_1	Average minimum temperature for January during observed period
TMIN_2	Average minimum temperature for February during observed period
TMIN_3	Average minimum temperature for March during observed period
TMIN_4	Average minimum temperature for April during observed period
TMIN_5	Average minimum temperature for May during observed period
TMIN_6	Average minimum temperature for June during observed period
TMIN_7	Average minimum temperature for July during observed period
TMIN_8	Average minimum temperature for August during observed period
TMIN_9	Average minimum temperature for September during observed period
TMIN_10	Average minimum temperature for October during observed period
TMIN_11	Average minimum temperature for November during observed period

TMIN_12	Average minimum temperature for December during observed period
TMEAN_1	Average mean temperature for January during observed period
TMEAN_2	Average mean temperature for February during the observed period
TMEAN_3	Average mean temperature for March during the observed period
TMEAN_4	Average mean temperature for April during the observed period
TMEAN_5	Average mean temperature for May during the observed period
TMEAN_6	Average mean temperature for June during the observed period
TMEAN_7	Average mean temperature for July during the observed period
TMEAN_8	Average mean temperature for August during the observed period
TMEAN_9	Average mean temperature for September during the observed period
TMEAN_10	Average mean temperature for October during the observed period
TMEAN_11	Average mean temperature for November during the observed period
TMEAN_12	Average mean temperature for December during the observed period
TMAX_1	Average maximum temperature for January during observed period
TMAX_2	Average maximum temperature for February during observed period
TMAX_3	Average maximum temperature for March during observed period
TMAX_4	Average maximum temperature for April during observed period
TMAX_5	Average maximum temperature for May during observed period
TMAX_6	Average maximum temperature for June during observed period
TMAX_7	Average maximum temperature for July during observed period
TMAX_8	Average maximum temperature for August during observed period
TMAX_9	Average maximum temperature for September during observed period
TMAX_10	Average maximum temperature for October during observed period

TMAX_11	Average maximum temperature for November during observed period
TMAX_12	Average maximum temperature for December during observed period
PREC_1	Average monthly precipitation (mm) for January
PREC_2	Average monthly precipitation (mm) for February
PREC_3	Average monthly precipitation (mm) for March
PREC_4	Average monthly precipitation (mm) for April
PREC_5	Average monthly precipitation (mm) for May
PREC_6	Average monthly precipitation (mm) for June
PREC_7	Average monthly precipitation (mm) for July
PREC_8	Average monthly precipitation (mm) for August
PREC_9	Average monthly precipitation (mm) for September
PREC_10	Average monthly precipitation (mm) for October
PREC_11	Average monthly precipitation (mm) for November
PREC_12	Average monthly precipitation (mm) for December

Table 7. Loading for Principle Component Analysis.

	PC1	PC2
Lat	0.163785842	-0.016999817
Long	0.104347641	-0.263551283
TMIN_1	-0.163735402	-0.043952404
TMIN_2	-0.164485633	-0.039133771
TMIN_3	-0.164698073	-0.024635854
TMIN_4	-0.161974315	-0.04822118
TMIN_5	-0.160221933	-0.050010871

TMIN_6	-0.158395868	-0.054388783
TMIN_7	-0.150559838	-0.09638372
TMIN_8	-0.155368571	-0.086844796
TMIN_9	-0.160898225	-0.08107045
TMIN_10	-0.159334638	-0.102250653
TMIN_11	-0.160938444	-0.088219748
TMIN_12	-0.163797349	-0.049787767
TMEAN_1	-0.164360433	-0.026954085
TMEAN_2	-0.165066237	-0.004347554
TMEAN_3	-0.165015858	0.012589076
TMEAN_4	-0.164680601	0.013925503
TMEAN_5	-0.164274467	0.033118684
TMEAN_6	-0.162065411	0.066910786
TMEAN_7	-0.154031649	0.069476098
TMEAN_8	-0.161019951	0.079270092
TMEAN_9	-0.164812087	0.015408656
TMEAN_10	-0.164678041	-0.030021361
TMEAN_11	-0.164355055	-0.038977525
TMEAN_12	-0.16506398	-0.024312074
TMAX_1	-0.163771461	-0.007038931
TMAX_2	-0.162669409	0.026711441
TMAX_3	-0.159139958	0.059111454
TMAX_4	-0.155516798	0.099994698

TMAX_5	-0.150543691	0.142271259
TMAX_6	-0.125985487	0.233934864
TMAX_7	-0.065283333	0.25636981
TMAX_8	-0.111308031	0.271850715
TMAX_9	-0.149046711	0.164999629
TMAX_10	-0.160021239	0.09140655
TMAX_11	-0.163236266	0.031835862
TMAX_12	-0.16445395	0.002337728
PREC_1	0.029067471	0.358297919
PREC_2	-0.008119454	0.329157561
PREC_3	0.005720686	0.325355
PREC_4	0.033393742	0.312331031
PREC_5	-0.023993824	0.060780024
PREC_6	-0.145777744	-0.144591708
PREC_7	-0.121036447	-0.131243236
PREC_8	-0.1285262	-0.219442967
PREC_9	-0.135055198	-0.188606253
PREC_10	0.025325623	-0.148702897

Table 8. *Bd* and *Rv* infection prevalence with 95% confidence inter*v*als and average *Bd* intensity with standard error for sampled populations of *H. squirella* and *P. ornata*.

Population	*No. Bd infected/No. sampled*	*Bd prevalence (95% CI)*	*Bd intensity*	*Log(Bd intensity (± SE)*	*No. Rv infected/No. sampled*	*Rv prevalence (95% CI)*	*Rv intensity (± SE)*
Hyla squirella							
PICK	1/36	0.03 (0.0007 – 0.15)	20687.9	9.94 (NA)	0/36	0 (0- 0.0973)	0
AST	0/20	0 (0 – 0.17)	0		0/20	0 (0 – 0.17)	0
CHAR	0/1	0 (0 – 0.98)	0		0/1	0 (0 – 0.98)	0
CUT	0/27	0 (0 – 0.13)	0		1/27	0.30 (0.0009 – 0.19)	66.3 (NA)
DISS	0/33	0 (0 – 0.11)	0		0/33	0 (0 – 0.11)	0
EAPP	0/35	0 (0 – 0.10)	0		0/35	0 (0 – 0.10)	0
GRAS	0/10	0 (0 – 0.31)	0		0/10	0 (0 – 0.31)	0
GULF	0/37	0 (0 – 0.09)	0		0/37	0 (0 – 0.09)	0
HIKE	0/33	0 (0 – 0.11)	0		0/33	0 (0 – 0.11)	0

OCK	0/16	0 (0 – 0.21)	0		0/16	0 (0 – 0.21)	0
OST	0/22	0 (0 – 0.15)	0		0/22	0 (0 – 0.15)	0
PALM	0/19	0 (0 – 0.18)	0		0/19	0 (0 – 0.18)	0
PEN	0/36	0 (0 – 0.10)	0		0/36	0 (0 – 0.10)	0
PINE	0/31	0 (0 – 0.11)	0		0/31	0 (0 – 0.11)	0
SAND	0/32	0 (0 – 0.11)	0		0/32	0 (0 – 0.11)	0
SPAR	0/46	0 (0 – 0.08)	0		0/46	0 (0 – 0.08)	0
SR2	0/41	0 (0 – 0.09)	0		0/41	0 (0 – 0.09)	0
STAR	0/48	0 (0 – 0.07)	0		0/48	0 (0 – 0.07)	0
WAPP	0/41	0 (0 – 0.09)	0		0/41	0 (0 – 0.09)	0
WAY	0/16	0 (0 – 0.21)	0		0/16	0 (0 – 0.21)	0
Pseudacris ornata							
SRE A	7/15	0.47 (0.21 – 0.73)	1237326.8	14.03 (1.36)	0/15	0 (0-0.218)	0
SRE B	1/1	1 (0.03 – 1)	5731.7	8.65(NA)	0/1	0 (0-0.975)	0

SRE C	16/32	0.5 (0.32 – 0.68)	360400.5	12.79(0.67)	0/32	0 (0-0.11)	0
SRE D	7/20	0.35 (0.15 – 0.59)	786777.0	13.58(1.26)	0/20	0 (0-.17)	0
JEN C	0/9	0 (0 – 0.34)	0		0/9	0 (0 – 0.34)	0
JEN I	0/10	0 (0 – 0.31)	0		0/10	0 (0 – 0.31)	0
JEN J	0/8	0 (0 – 0.37)	0		0/8	0 (0 – 0.37)	0
TEL	6/6	1 (0.54 – 1)	68099.1	11.13(0.26)	0/6	0 (0-0.46)	0
AL HWY 165	3/5	0.6 (0.15 – 0.95)	226.1	5.42(0.53)	0/5	0 (0-0.52)	0
Coleton SC	22/45	0.49 (0.34 – 0.64)	1818694.7	14.41(0.82)	0/45	0 (0-0.078)	0
Coleton SC b	0/7	0 (0 – 0.41)	0		0/7	0 (0 – 0.41)	0
Gulf	0/1	0 (0 – 0.98)	0		0/1	0 (0 – 0.98)	0
Hardyville SC	6/25	0.24 (0.09 – 0.45)	500392.9	13.12(1.28)	0/25	0 (0-0.137)	0

Hwy 379	26/85	0.31 (0.21 – 0.42)	561268.8	13.24(0.60)	0/85	0 (0-0.0424)	0
Pond 51	0/37	0 (0 – 0.09)	0		0/37	0 (0 – 0.09)	0
Savannah R., Ellentor	0/2	0 (0 – 0.84)	0		0/2	0 (0 – 0.84)	0
Savannah R., Mona	0/5	0 (0 – 0.52)	0		0/5	0 (0 – 0.52)	0
Fort Brag NT	7/8	.88 (0.47 – .99)	17982320.2	16.70(0.70)	0/8	0 (0-0.369)	0

Table 9. Summary estimates of the best fit model for *Bd* prevalence.

	Estimate	*Std. Error*	*z value*	*p value*
(Intercept)	-16.60610	6.10975	-4.129	**0.023**
PC1	0.18931	0.05508	5.221	**0.007**
PC2	-0.54575	0.24129	-3.436	0.050
Avg.HE	30.91722	11.80930	2.618	**0.027**

Null deviance: 87.356	Residual Deviance: 26.463
Null df: 12	Residual df: 9
Dispersion parameter: 2.307298	

Table 10. Summary estimates of the best fit model for *Bd* intensity.

inmod6R	7	1129.943		
inmodR	9	1133.82		
inmod3R	5	1134.23		

Coefficients	Estimate	Std. Error	t value	p value
(Intercept)	8.1948	1.4861	5.514	**0.000182**
PC1	0.7778	0.2559	3.039	**0.011271**

Total dF: 12 Adj r squared: 0.4069

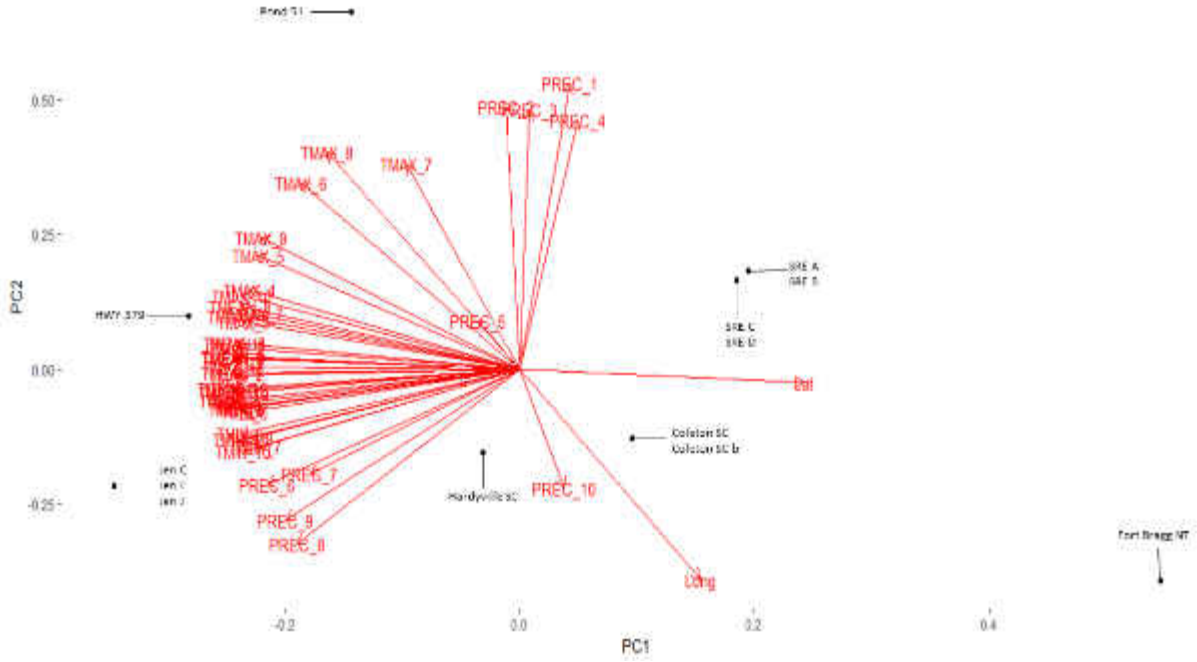

Figure 14. Principle Component Analysis (PCA) of environmental factors (maximum and minimum temperatures, average precipitation, and mean temperature per population sites; 36 temperature variables and 12 precipitation variables from online WorldClim database) and location (latitude and longitude).

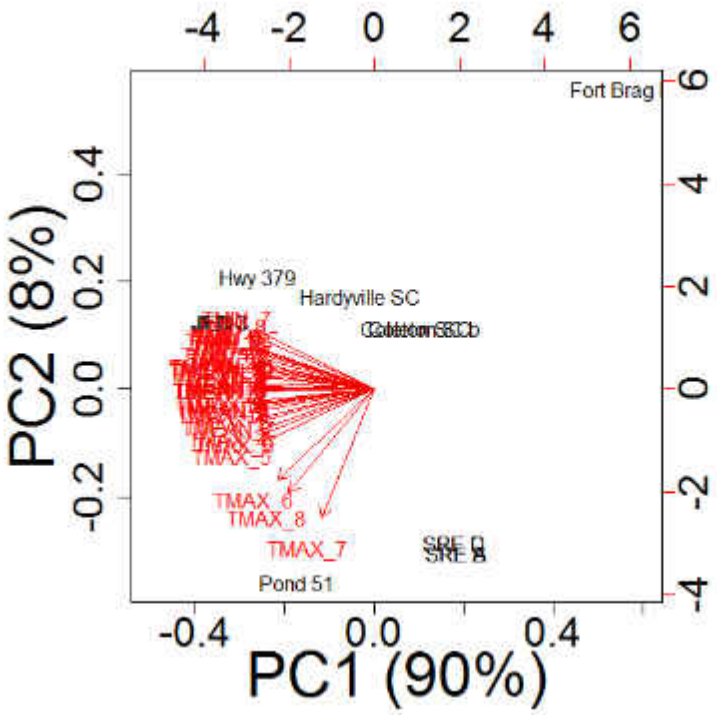

Figure 15. Principle Component Analysis (PCA) of temperature factors (maximum and minimum temperatures, and mean temperature per population sites; 36 temperature variables from online WorldClim database).

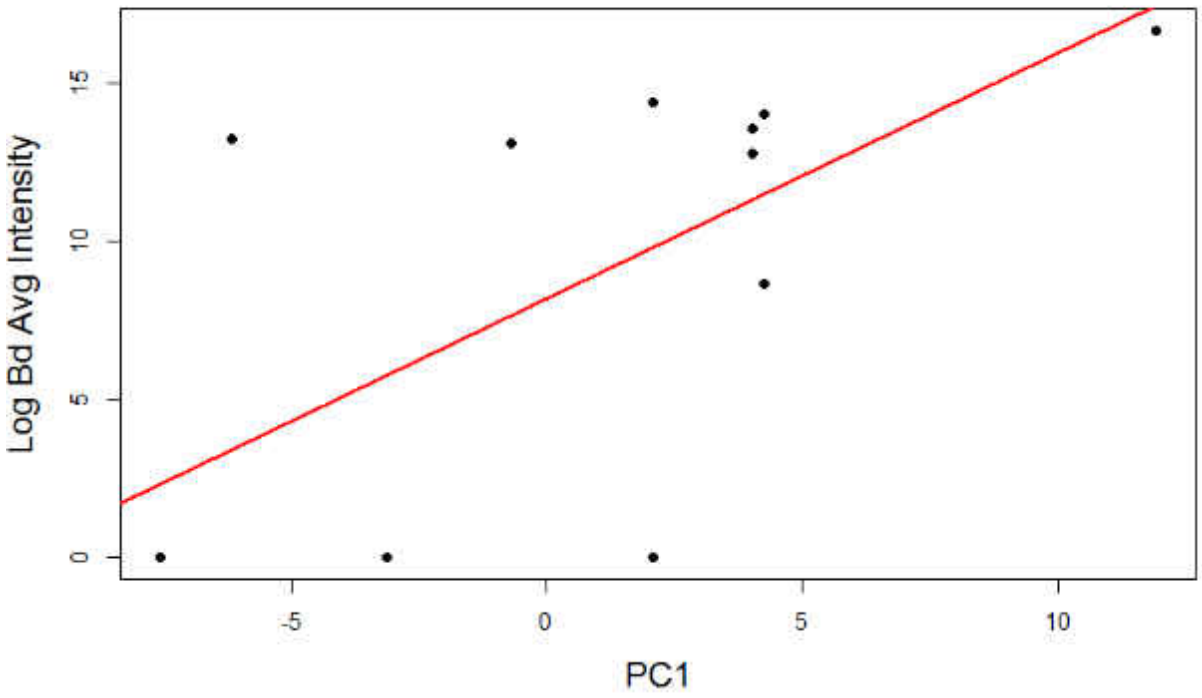

Figure 16. Linear relationship between natural log-transformed average *Bd* infection intensity and PC1.

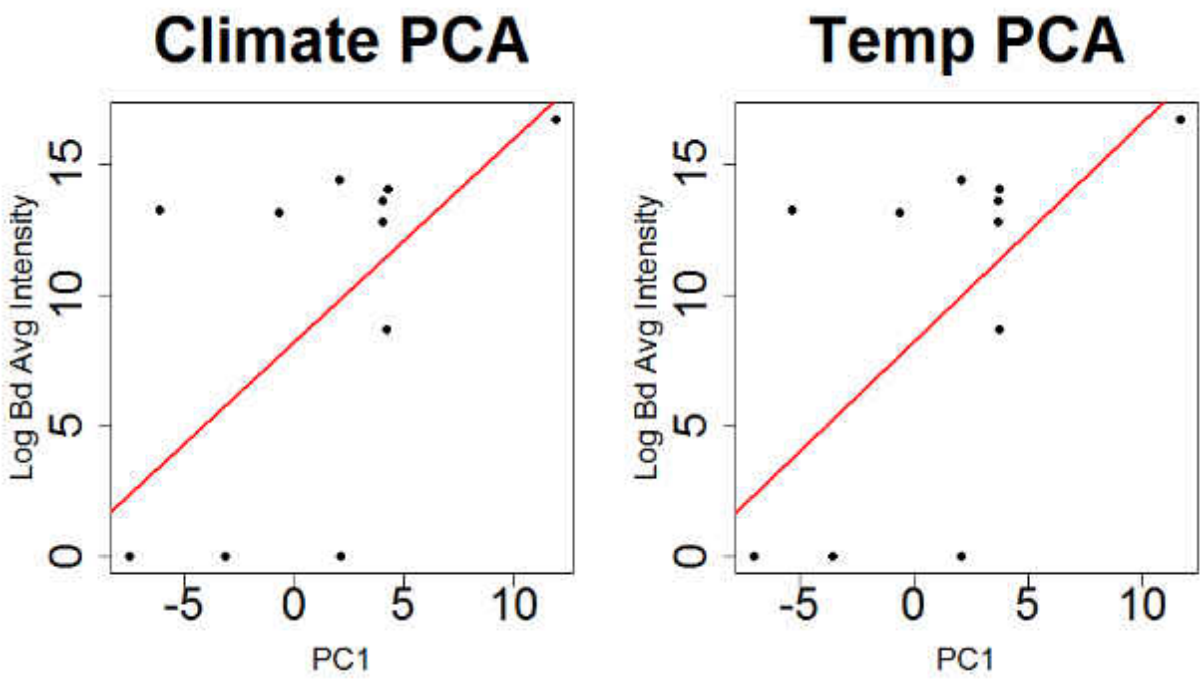

Figure 17. Linear relationship between natural log-transformed average *Bd* infection intensity and PC1, using our all-inclusive PC1 and temperature only PC1.

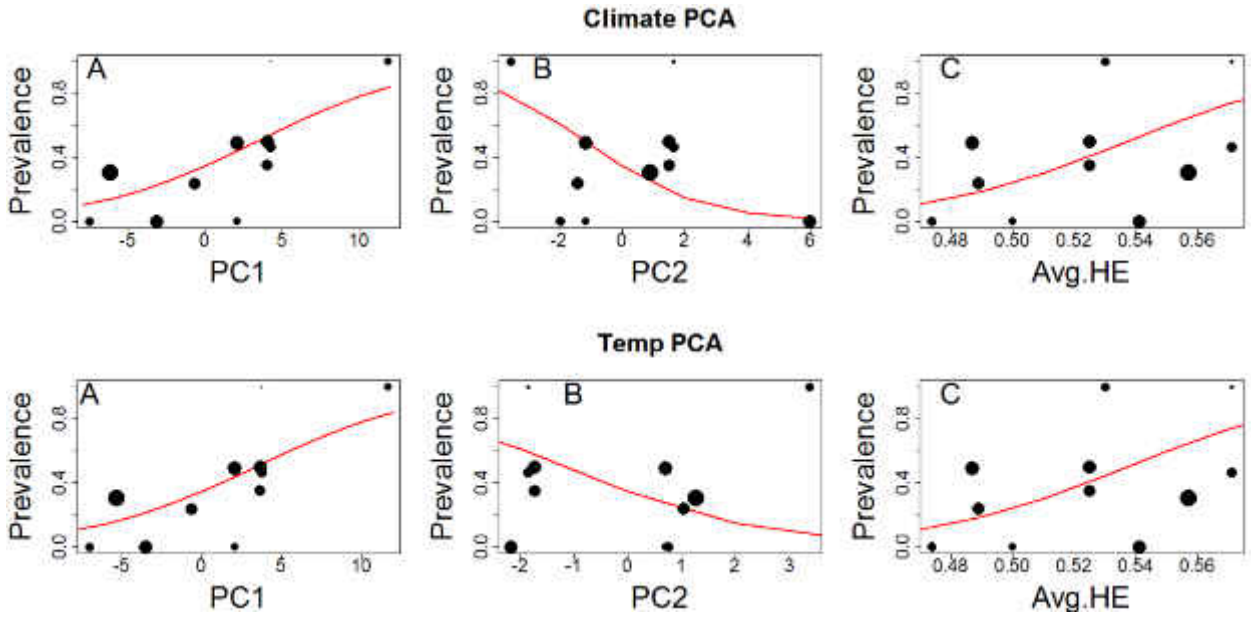

Figure 18. Logistic relationships between *Bd* prevalence and PC1 (A), PC2 (B), and average heterozygosity (C) in populations of *P. ornata.* Increasing values of PC1 correspond most strongly to decreasing values of temperature. The top plots use the inclusive PCA components while the bottom uses the temperature only PCA components. Increasing values of PC2 correspond most strongly to increasing winter precipitation and summer temperatures. In all panels, each dot corresponds to the observed prevalence in a population and each line corresponds to the best fit logistic model of the relationship between the two variables shown. Points are weighted by population size and are reflected in the size of the point.

R Code

#####Standard error########

#function to caluclulate standard error

tapply(intensity$Average.*Bd*.Load,intensity$Pop.ID, se)
se <- function(x) sqrt(var(x)/length(x))
tapply(intensity$Average.*Bd*.Load,intensity$Pop.ID, se)

######BinomCIs###########
#make sure you have installed the package binom first!
library(binom)

binom.confint(x=a, n=b)
# so for example if you have 20 out of 50 individuals testing positive
# then x=20 and n=50
#the results generate eight different CI calculations
#clapper-Person is listed as "exact" in the output

R Code For PCA/GLMS:
####P ornata *Bd* analysis###
data<- read.delim("*Bd* Master List- all species updated_final3_wo_na.txt", na.strings="n/a", header=T)##change what data you are reading##
datapca<-data[11:58] ##limiting data##
row.names(datapca)<-data$Sub.Population
library(bbmle)
library(car)
library(vegan)
library(ggplot2)
library(ggfortify)
##running PCA##
prcomp(datapca, scale=T)
enviropca<-prcomp(datapca, scale=T)
summary(enviropca)

##Teasing apart the PCA##

autoplot(enviropca, loadings=T, loadings.label=T)+ theme(panel.background = element_blank())

biplot(enviropca, cex.axis=2, cex.lab=3, xlab="PC1 (76%)", ylab="PC2 (13%)", xlim=c(-.5, .6))
From this graph we can see that PC1 is inversely related to all temp variables
and a few prep varibles. PC2 is primarily associated directely with winterish precip and
inversely with Prec_10

```
load<-enviropca$rotation ##no Standard dev., just rotation data (loadings)##
axes<-predict(enviropca, newdata=data) ##put data in format for regression##
dat<-cbind(data,axes) ##adds column bind; just adds PC variables to my file##
environintens<-lm(log(Bd.Average.intensity+1)~PC1+PC2+Avg.HE+AR, data=dat) ##linear
regression, have to add 1 to intensity b/c zeros##
summary(environintens)

##if you forget the names of headers##
names(dat)

##Linear(for now) regression##
prev<-lm(Bd.Prevalence~PC1, data=dat)
prevgen<-lm(Bd.Prevalence~Avg.HE+AR, data=dat)
summary(prev)

#####Looking at potential iteraction terms
modelINT1a<-lm(log(Bd.Average.intensity+1)~PC1*PC2, data=dat)
modelINT2a<-lm(log(Bd.Average.intensity+1)~PC1*Avg.HE, data=dat)
modelINT3a<-lm(log(Bd.Average.intensity+1)~PC1*AR, data=dat)
modelINT4a<-lm(log(Bd.Average.intensity+1)~PC2*AR, data=dat)
modelINT5a<-lm(log(Bd.Average.intensity+1)~PC2*Avg.HE, data=dat)
modelINT6a<-lm(log(Bd.Average.intensity+1)~AR*Avg.HE, data=dat)

#Vifs for interactive models
vif(modelINT1a)
vif(modelINT2a)
vif(modelINT3a)
vif(modelINT4a)
vif(modelINT5a)
vif(modelINT6a)


##Models for Intensity to run through AICs##

model1a<-lm(log(Bd.Average.intensity+1)~PC1+PC2, data=dat)
model2a<-lm(log(Bd.Average.intensity+1)~Avg.HE+AR, data=dat)
model3a<-lm(log(Bd.Average.intensity+1)~PC1, data=dat)
model4a<-lm(log(Bd.Average.intensity+1)~PC2, data=dat)
model5a<-lm(log(Bd.Average.intensity+1)~Avg.HE, data=dat)
model6a<-lm(log(Bd.Average.intensity+1)~AR, data=dat)
model7a<-lm(log(Bd.Average.intensity+1)~PC1+PC2+Avg.HE, data=dat)
model8a<-lm(log(Bd.Average.intensity+1)~PC1+PC2+AR, data=dat)
model9a<-lm(log(Bd.Average.intensity+1)~PC1+Avg.HE, data=dat)
model10a<-lm(log(Bd.Average.intensity+1)~PC1+AR, data=dat)
```

```
model11a<-lm(log(Bd.Average.intensity+1)~PC2+AR, data=dat)
model12a<-lm(log(Bd.Average.intensity+1)~PC2+Avg.HE, data=dat)
model13a<-lm(log(Bd.Average.intensity+1)~PC1+Avg.HE+AR, data=dat)
model14a<-lm(log(Bd.Average.intensity+1)~PC2+Avg.HE+AR, data=dat)
model15a<-lm(log(Bd.Average.intensity+1)~PC1+PC2+Avg.HE+AR, data=dat)
model16a<-lm(log(Bd.Average.intensity+1)~1, data=dat)


AICctab(model1a, model2a, model3a, model4a, model5a, model6a, model7a, model8a,
model9a, model10a, model11a, model12a, model13a, model14a, model15a, model16a,
weights=TRUE, base=TRUE)
summary(model3a)

##Plotting Intesnity models##
plot(dat$PC1,log(dat$Bd.Average.intensity+1), xlab="PC1", ylab="Log Bd Avg Intensity",
pch=16, cex.lab=1.5)
abline(model3a, col="red", lwd=2)


#Looking at potenial interactions in prevalence
modelint1b<-glm(Bd.Prevalence~PC1*PC2, data=dat, family=binomial, weight=Number)
modelint2b<-glm(Bd.Prevalence~PC1*Avg.HE, data=dat, family=binomial, weight=Number)
modelint3b<-glm(Bd.Prevalence~PC1*AR, data=dat, family=binomial, weight=Number)
modelint4b<-glm(Bd.Prevalence~PC2*Avg.HE, data=dat, family=binomial, weight=Number)
modelint5b<-glm(Bd.Prevalence~PC2*AR, data=dat, family=binomial, weight=Number)
modelint6b<-glm(Bd.Prevalence~Avg.HE*AR, data=dat, family=binomial, weight=Number)

#looking at potenial interactions in prevalence (vifs)
vif(modelint1b)
vif(modelint2b)
vif(modelint3b)
vif(modelint4b)
vif(modelint5b)
vif(modelint6b)


##Models for Prevalence##

model1b<-glm(Bd.Prevalence~PC1+PC2, data=dat, family=binomial, weight=Number)
model2b<-glm(Bd.Prevalence~Avg.HE+AR, data=dat, family=binomial, weight=Number)
model3b<-glm(Bd.Prevalence~PC1, data=dat, family=binomial, weight=Number)
model4b<-glm(Bd.Prevalence~PC2, data=dat, family=binomial, weight=Number)
model5b<-glm(Bd.Prevalence~Avg.HE, data=dat, family=binomial, weight=Number)
model6b<-glm(Bd.Prevalence~AR, data=dat, family=binomial, weight=Number)
```

```
model7b<-glm(Bd.Prevalence~PC1+PC2+Avg.HE, data=dat, family=binomial,
weight=Number)
model8b<-glm(Bd.Prevalence~PC1+PC2+AR, data=dat, family=binomial, weight=Number)
model9b<-glm(Bd.Prevalence~PC1+Avg.HE, data=dat, family=binomial, weight=Number)
model10b<-glm(Bd.Prevalence~PC1+AR, data=dat, family=binomial, weight=Number)
model11b<-glm(Bd.Prevalence~PC2+AR, data=dat, family=binomial, weight=Number)
model12b<-glm(Bd.Prevalence~PC2+Avg.HE, data=dat, family=binomial, weight=Number)
model13b<-glm(Bd.Prevalence~PC1+Avg.HE+AR, data=dat, family=binomial,
weight=Number)
model14b<-glm(Bd.Prevalence~PC2+Avg.HE+AR, data=dat, family=binomial,
weight=Number)
model15b<-glm(Bd.Prevalence~PC1+PC2+Avg.HE+AR, data=dat, family=binomial,
weight=Number)
model16b<-glm(Bd.Prevalence~1, data=dat, family=binomial, weight=Number)

AICctab(model1b, model2b, model3b, model4b, model5b, model6b, model7b, model8b,
model9b, model10b, model11b, model12b, model13b, model14b, model15b,model16b,
weights=TRUE, base=TRUE)
summary(model7b)

##Plotting Prevalence models##

par( cex.lab=3.5, cex.axis=2.2, mai=c(1,1,1,.3), mfrow=c(1,3))


plot(dat$PC1, data$Bd.Prevalence, ylab="", xlab="", pch=16)
title(ylab="Prevalence", line=4.5, xlab="PC1", at=4.5)
vsize<-seq(-8,12,2)
predPC1<-predict(model7b, type="response", list(PC1=vsize, PC2=rep(mean(dat$PC2),
length(vsize)), Avg.HE=rep(mean(dat$Avg.HE), length(vsize))))
lines(vsize,predPC1, col="red", lwd=2)
mtext(at=-7,line=-2.5,"A", cex=2.4)


plot(dat$PC2, data$Bd.Prevalence,ylab="" , xlab="", pch=16)
title(ylab="Prevalence", line=4.5, xlab="PC2", at=4.5)
vsize<-seq(-4,7,2)
predPC2<-predict(model7b, type="response", list(PC2=vsize, PC1=rep(mean(dat$PC1),
length(vsize)), Avg.HE=rep(mean(dat$Avg.HE), length(vsize))))
lines(vsize,predPC2, col="red", lwd=2)
mtext(at=-2.5,line=-2.5,"B", cex=2.4)

plot(dat$Avg.HE, data$Bd.Prevalence, ylab="", xlab="", pch=16)
title(ylab="Prevalence", line=4.5, xlab="Avg.HE")
```

```
vsize<-seq(.45,.6,.02)
predAvg.HE<-predict(model7b, type="response", list(Avg.HE=vsize,
PC2=rep(mean(dat$PC2), length(vsize)), PC1=rep(mean(dat$PC1), length(vsize))))
lines(vsize,predAvg.HE, col="red", lwd=2)
mtext(at=.48,line=-2.5,"C", cex=2.4)



#####
a<-as.matrix(data[1:20,6:7])
rownames(a)<-data$Sub.Population[1:20]
fisher.test(a, simulate.p.value=T)
fisher.multcomp(a)

b<-as.matrix(data[20:40,6:7])
rownames(b)<-data$Sub.Population[20:40]
fisher.test(b, simulate.p.value=T)


c<-matrix(c(100,1, 257, 579), ncol=2)
fisher.test(c, simulate.p.value=T)
```

APPENDIX B:

ADDITIONAL ANALYSES AND R CODE FROM CHAPETER 2

Table 11. Table showing *Bd* and Ranavirus intensity and prevalence across three species groups. For prevalence, numbers in bold indicate mean prevalence while numbers in parentheses indicate 95% binomial confidence inter*v*als. For intensity, numbers in bold indicate log mean prevalence, numbers in parentheses indicate standard errors.

Family	Species	*Bd* Prevalence	*Bd* Intensity	log *Bd* Intensity	*Rv* Prevalence	*Rv* Intensity	log *Rv* intensity
Ranids	Lithobates capito, Lithobates catesbianus, Lithobates gryllio, Lithobates sphenacephalus	**0.149** (0.102-0.207)	491.76	**6.198** (±0.220)	**0.338** (0.269-0.414)	14472 42	**9.58** (±0.303)
Toads	Anaxyrus terrestris	**0.227** (0.078-0.453)	4628.55	**8.440** (±1.826)	**0.143** (0.030-0.363)	15361.46	**9.63** (±1.17)
Tree Frogs	Acris gryllus, Hyla cinerea, Hyla femoralis, Hyla gratiosa, Hyla squirella, Osteopilus septentrionalis	**0.092** (0.072-0.117)	3881.58	**8.264** (±0.484)	**0.283** (0.248-0.320)	13408.27	**9.50** (±0.199)

Table 12. AIC table for prevalence of *Bd*. Model in bold indicates most informative model

Models	df	AIC	dAIC
Prevalence~Season+Location	**6**	**595.9191**	**0**
Prevalence~Season+Family+Location	8	596.7175	0.7984
Prevalence~Location+Family	5	598.9931	3.074
Prevalence~Location	3	599.939	4.0199
Prevalence~Season+Family	6	601.1742	5.2551
Prevalence~Season	4	602.6909	6.7718
Prevalence~Family	3	604.1093	8.1902
NULL	1	607.82	11.9009

Table 13. Model coefficients for the most informative model for *Bd* individual probability of infection.

Coefficients:				
	Estimate	Std. Error	z value	P-value
(Intercept)	-4.3182	1.03081	-4.189	2.8e-05 ***
Season2	0.49873	0.27737	1.798	0.0722 .
Season3	-0.07368	0.3313	-0.222	0.824
Season4	-0.37434	0.41948	-0.892	0.3722
LocationAP	2.01005	1.02839	1.955	0.0506 .
LocationGSPPL	2.25283	1.01714	2.215	0.0268 *
(Dispersion parameter for binomial family taken to be 1)				
Null deviance: 603.72 on 867degreesof freedom				
Residual deviance: 583.92 on 862 degrees of freedom				

Table 14. AIC table for prevalence of *RV*. Model in bold indicates most informative model

Models	df	AIC	dAIC
Prevalence~Season+Location	**6**	**955.2192**	**0**
Prevalence~Season+Location+Family	8	956.391	1.1718
Prevalence~Season+Family	6	972.8916	17.6724
Prevalence~Season	4	974.4021	19.1829
Prevalence~Location	3	983.8613	28.6421
Prevalence~Location+Family	5	983.8874	28.6682
Prevalnece~Family	3	1000.035	44.816
NULL	1	1000.735	45.5153

Table 15. Model coefficients for the most informative model for *Rv* individual probability of infection

Coefficients:				
	Estimate	Std. Error	z value	P-Value
(Intercept)	-1.5925	0.4327	-3.681	**0.000233 *** **
SeasonSpring	-1.1304	0.247	-4.576	**4.73e-06 *** **
SeasonSummer	-0.5397	0.2522	-2.14	**0.032359 ***
SeasonWinter	-0.2098	0.244	-0.86	0.389983
LocationAP	0.9881	0.4275	2.311	**0.020824 ***
LocationGSPPL	1.5265	0.4174	3.658	**0.000255 *** **
(Dispersion parameter for binomial family taken to be 1)				
Null deviance: 993.20 on 819 degrees of freedom				
Residual deviance: 943.22 on 814 degrees of freedom				

Table 16. AIC table for intensity of *Bd*. Model in bold indicates most informative model

Models	df	AIC	dAIC
Intensity~Family+Season	**7**	**444.0246**	**0**
Intensity~Season	5	444.5167	0.4921
Intensity~Location+Family+Season	9	447.2078	3.1832
Intensity~Location+Season	7	447.6798	3.6552
Intensity~Family	4	504.7281	60.7035
Intensity~Family+Location	6	508.1078	64.0832
NULL	2	508.8433	64.8187
Intensity~Location	4	512.5419	68.5173

Table 17. AIC table for intensity of *RV*. Model in bold indicates most informative model

Rv	df	AIC	dAIC
Intensity~Location+Season	**7**	**1129.943**	**0**
Intensity~Location+Family+Season	9	1133.82	3.877
Intensity~Season	5	1134.23	4.287
Intensity~Location	4	1136.607	6.664
Intensity~Family+Season	7	1138.022	8.079
Intensity~Family+Location	6	1140.509	10.566
NULL	2	1141.84	11.897
Intensity~Family	4	1145.793	15.85

Table 18. Tukey post hoc comparisons of Family, Season, and Location for Bd intensity

Family				
	Difference	Lwr CI	Upr CI	p adj
Toad-Ranid	2.241225	-0.48692	4.969369	0.128693
Hylid-Ranid	2.065505	0.798035	3.332975	**0.000575**
Hylid-Toad	-0.17572	-2.79492	2.443478	0.985998
Season				
	difference	Lwr CI	Upr CI	p adj
Spring-winter	-5.03833	-6.64258	-3.43408	**<2e-16**
Summer-Winter	-5.10791	-7.04616	-3.16965	**<2e-16**
Fall-Winter	-5.12406	-7.57287	-2.67524	**2.4E-06**
Summer-Spring	-0.06958	-1.75733	1.618174	0.999546
Fall-Spring	-0.08573	-2.34147	2.170011	0.999644
Fall-Summer	-0.01615	-2.52046	2.488163	0.999998
Location				
	Difference	Lwr CI	Upr	p adj
AP-ABS	0.027632	-5.70603	5.761291	0.999927
GSPPL-ABS	-0.37119	-6.04701	5.304633	0.98669
GSPPL-AP	-0.39882	-1.66664	0.868996	0.734458

Table 19. Tukey post hoc comparisons of Family, Season, and Location for Rv intensity

Family				
	difference	Lwr CI	Upr CI	p adj
Toad-Ranid	0.058834	-3.41812	3.535788	0.999122
Tree Frog-Ranid	-0.07716	-0.9545	0.800184	0.976559
Tree Frog-Toad	-0.13599	-3.55762	3.285644	0.995166
Season				
	difference	Lwr CI	Upr CI	p adj
Spring-Fall	-1.56593	-2.82898	-0.30288	**0.008241**
Summer-Fall	-1.64778	-2.91598	-0.37959	**0.004975**
Winter-Fall	-1.20252	-2.37965	-0.0254	**0.043188**
Summer-Spring	-0.08186	-1.29494	1.13123	0.998104
Winter-Spring	0.363403	-0.75413	1.48094	0.834605
Winter-Summer	0.44526	-0.67809	1.568608	0.734578
Spring-Fall	-1.56593	-2.82898	-0.30288	**0.008241**
Location				
	Difference	Lwr CI	Upr CI	p adj
AP-ABS	0.625259	-1.70302	2.953533	0.801852
GSPPL-ABS	1.473873	-0.79467	3.742414	0.277608
GSPPL-AP	0.848614	0.012915	1.684313	**0.045644**

Table 20. Fisher Exact Test multiple comparisons of Season, Family, and Location for Bd prevalence. All P-values were Bonferroni corrected for multiple comparisons.

Season	Fall	Spring	Summer
Spring	0.07349	-	-
Summer	1	0.3471	-
Winter	1	0.6795	1

Family	Ranid	Toad
Toad	1	-
Hylid	0.1951	0.2725

Location	ABS	AP
AP	0.12008	-
GSPPL	**0.01041**	0.4223

Table 21. Fisher Exact Test multiple comparisons of Season, Family, and Location for Rv prevalence. All P-values were Bonferroni corrected for multiple comparisons.

Season	Fall	Spring	Summer
Spring	**0.0074**	-	-
Summer	0.8620	0.0675	-
Winter	1	**0.00002**	0.5428

Family	Ranid	Toad
Toad	0.5034	-
Hylid	0.9703	1

Location	ABS	AP
AP	**0.0388**	-
GSPPL	**0.0001667**	**0.02796**

Table 22. A comparison of the mean estimated values for Rv prevalence based on toe tissue and liver tissue.

	Positive	Negative	Mean	Lwr BCI	Upr BCI
Toes only	237	598	0.2838	0.253	0.316
Liver only	59	146	0.2878	0.226	0.355

Table 23. A paired t-test of mean Rv intensity based on toe tissue in comparison to liver tissue

Mean Toe	Mean Liver	Mean diff	Lower	Upper	T value	df	P
10.06042	9.758743	0.3016795	-0.78964	1.392998	0.5612	35	0.5782

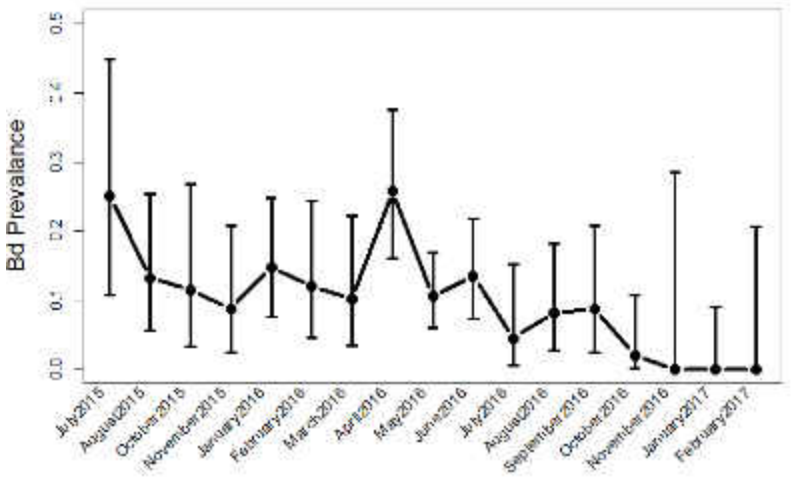

Figure 19. Bd prevalence in each individual month. Error bars correspond to binomial confidence intervals.

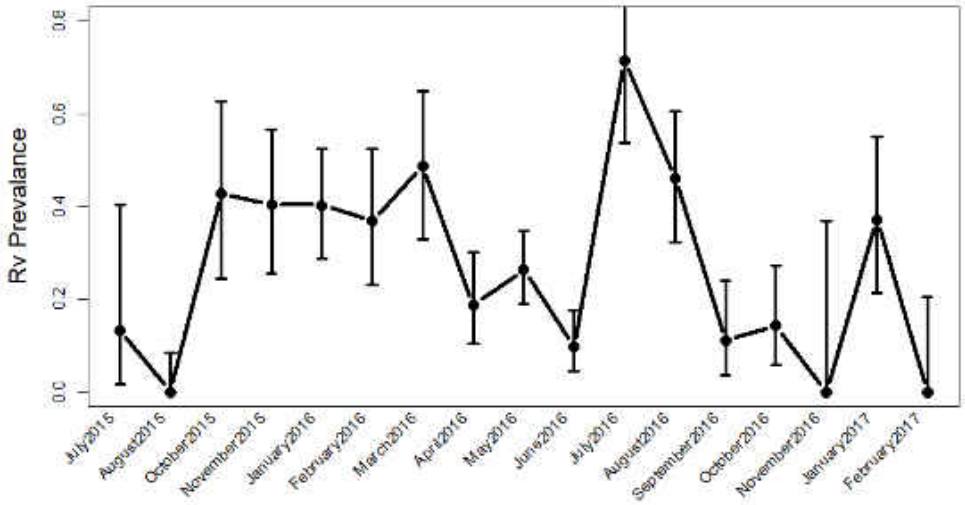

Figure 20. Rv prevalence in each individual month. Error bars correspond to binomial confidence intervals

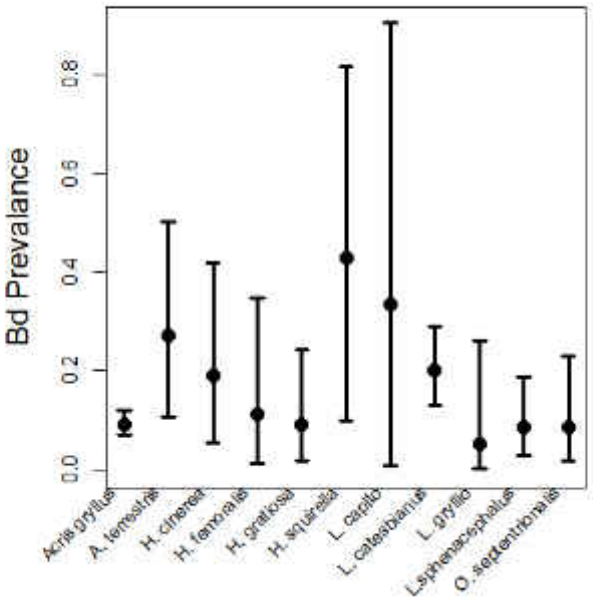

Figure 21. Bd prevalence for each species. Error bars correspond to binomial confidence intervals.

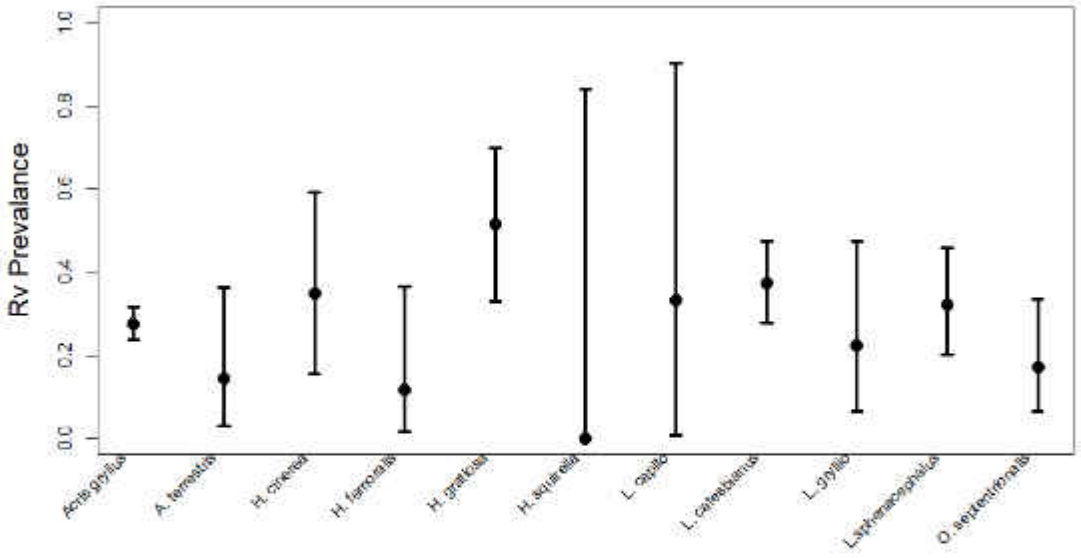

Figure 22. Rv prevalence by species. Error bars correspond to binomial confidence intervals.

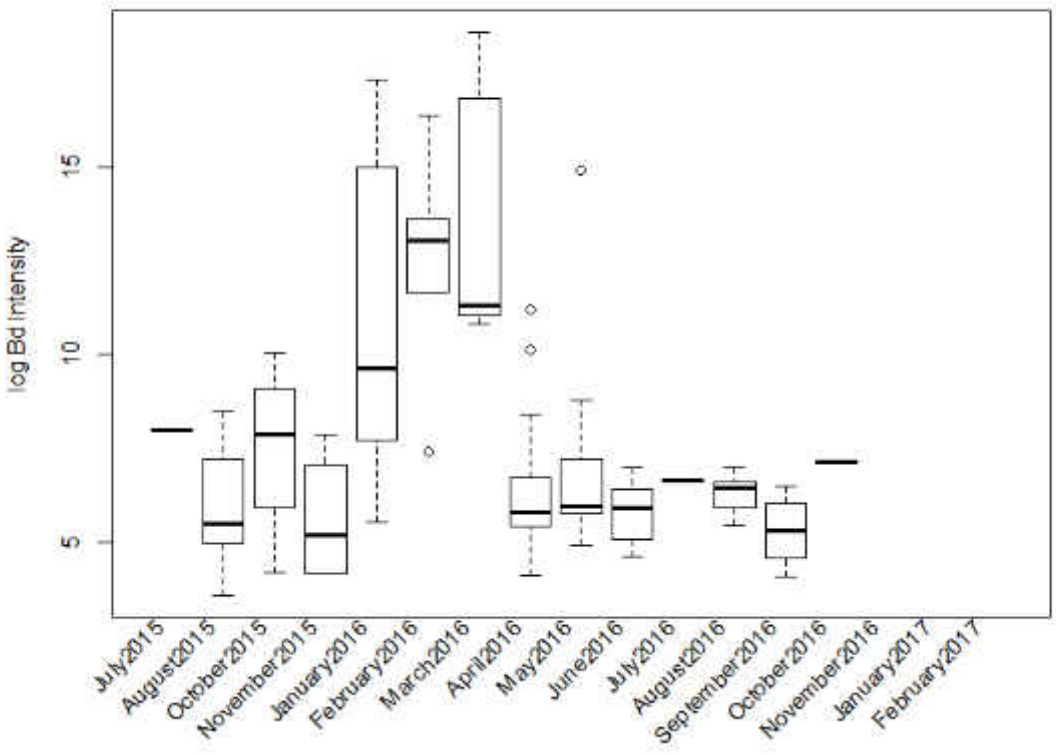

Figure 23. Boxplot of Bd intensity by month

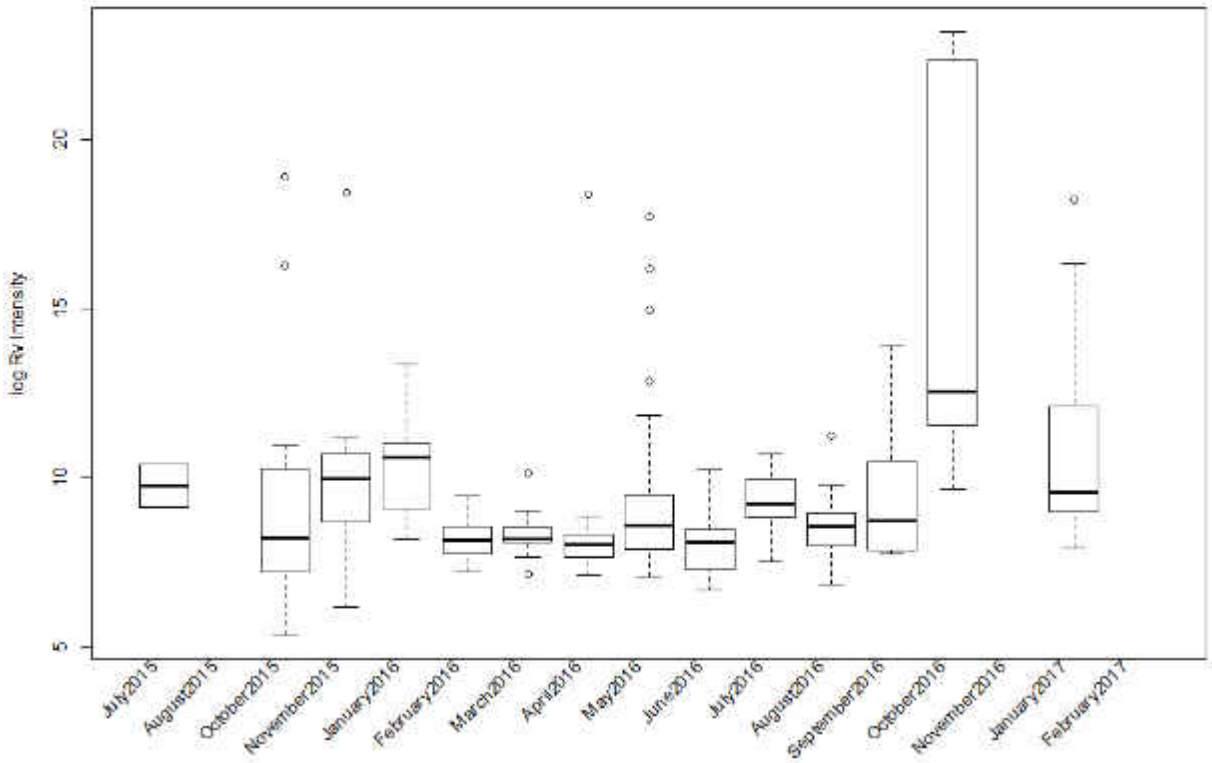

Figure 24. Boxplot of Rv intensity by month

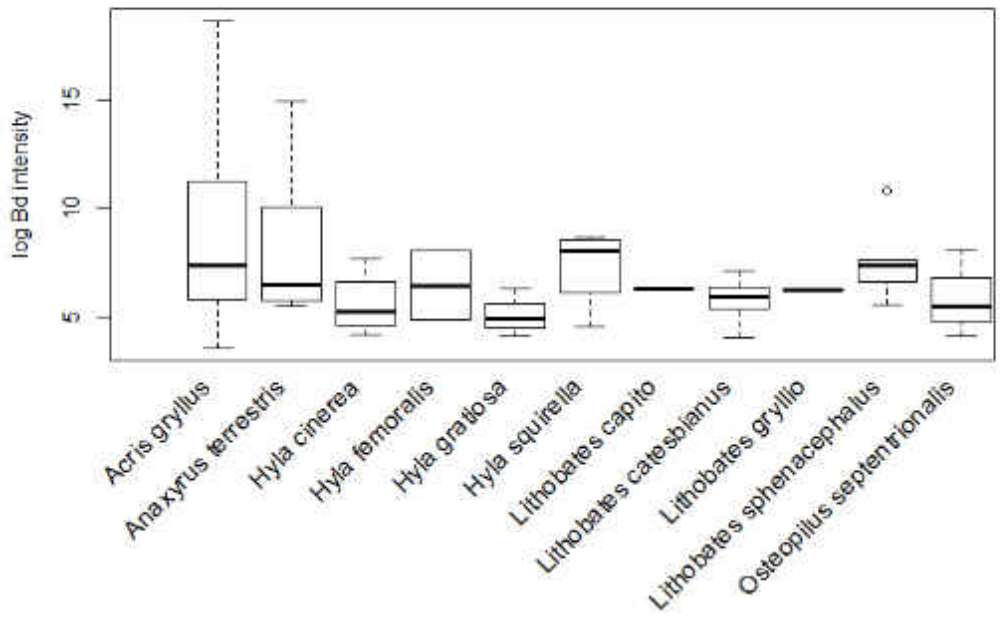

Figure 25. Boxplot of Bd intensity by species

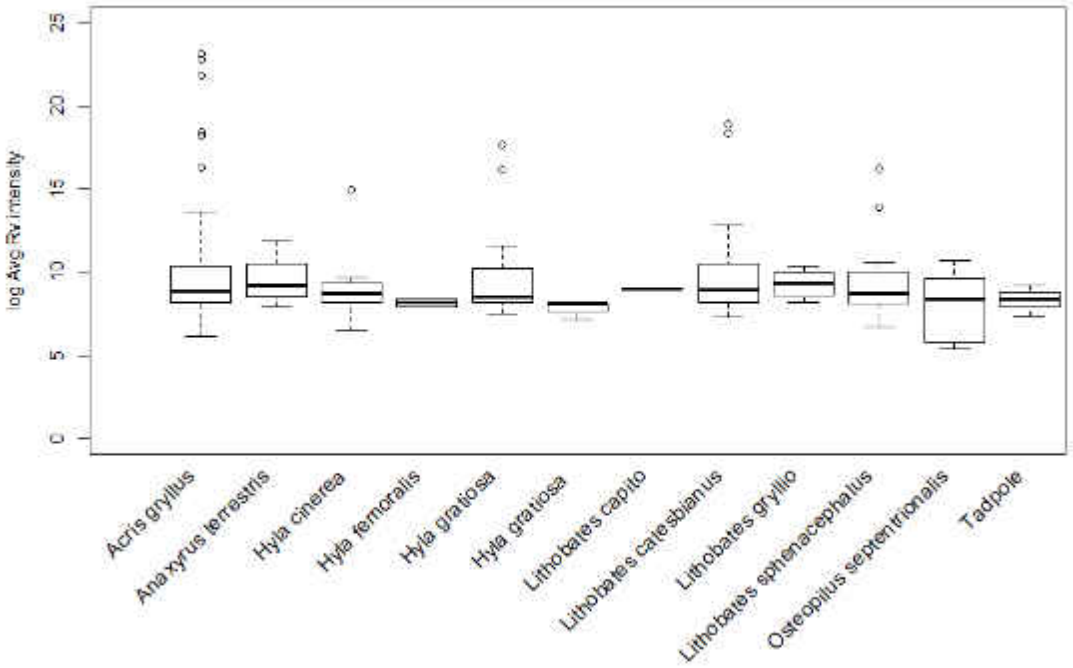

Figure 26. Boxplot of Rv intensity by species.

R Code:

```
##################################################
#####################BD###########################
##################################################

#####INTENSITY###############

#Don't forget to load in your data first!
setwd("C:/Users/Matt/Dropbox/OPS/Ariel")
intensity<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
#ANOVA for Species and Population
intensity<-subset(intensity, Avg.Intensity.Bd>0)

intensity$logload<-log(intensity$Avg.Intensity.Bd)
intensity<-subset(intensity, Location!="FB" & Family!= "Other")
intensity$Family<-factor(intensity$Family)
intensity$Location<-factor(intensity$Location)
inmod<-lm(logload~Location+Family+factor(Season), data=intensity)
inmod1<-lm(logload~Location, data=intensity)
inmod2<-lm(logload~Family, data=intensity)
inmod3<-lm(logload~factor(Season), data=intensity)
inmod4<-lm(logload~Family+Location, data=intensity)
inmod5<-lm(logload~Family+factor(Season), data=intensity)
inmod6<-lm(logload~Location+factor(Season), data=intensity)
inmod7<-lm(logload~1, data=intensity)

AIC(inmod,inmod1,inmod2,inmod3,inmod4,inmod5,inmod6,inmod7)

#inmod5 lowest AIC and has all significant factors

summary(inmod5)
anova(inmod5)
TukeyHSD(aov(logload~Family+factor(Season)+Location, data=intensity))

se <- function(x) sqrt(var(x)/length(x))
#standard errors for the location
tapply(intensity$logload,intensity$Location, mean)
tapply(intensity$logload,intensity$Location, se)

meansL<-as.numeric(tapply(intensity$logload,intensity$Location, mean))
sdevL<-as.numeric(tapply(intensity$logload,intensity$Location, se))
index<-seq(1,length(meansL))
```

```
plot(index, meansL, ylim=c(0,15), xlim=c(1,length(meansL)), pch=c(16,16,16), xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Log Mean Bd Intensity", cex.lab=1.5)
arrows(index, meansL-sdevL, index, meansL+sdevL, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("ABS", "AP", "GSPPL")
text(x = seq(1, 3, by=1), y=rep(-1,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)

#Standard errors for year/month


tapply(intensity$logload,intensity$Year, mean)
tapply(intensity$logload,intensity$Year, se)
t.test(intensity$logload~intensity$Year)
boxplot(intensity$logload~intensity$Year, ylab="Log Mean Bd Intensity", xlab="Year")
boxplot(intensity$logload~intensity$Month,ylab="Log Mean Bd Intensity", xlab="Month Number")




#standard errors for the species
tapply(intensity$logload,intensity$Family, mean)
tapply(intensity$logload,intensity$Family, se)

meansF<-as.numeric(tapply(intensity$logload,intensity$Family, mean))
sdevF<-as.numeric(tapply(intensity$logload,intensity$Family, se))
index<-seq(1,length(meansF))
plot(index, meansF, ylim=c(0,15), xlim=c(1,length(meansF)), pch=c(16,16,16,16), xaxt="n", xlab="",
lwd=4, cex=1.5, ylab="Log Mean Bd Intensity", cex.lab=1.5)
arrows(index, meansF-sdevF, index, meansF+sdevF, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Ranidae", "Bufonidae", "Hylidae")
text(x = seq(1, length(meansF), by=1), y=rep(-1,length(meansF)), par("usr")[1], labels = lablist.x, srt =
45, pos = 2, xpd = TRUE, cex=1.5)




#standard errors for the season
meansS<-as.numeric(tapply(intensity$logload,intensity$Season, mean))
sdevS<-as.numeric(tapply(intensity$logload,intensity$Season, se))
index<-seq(1,length(meansS))
plot(index, meansS, ylim=c(0,15), xlim=c(1,4), pch=c(16,16,16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Log Mean Bd Intensity", cex.lab=1.5)
arrows(index, meansS-sdevS, index, meansS+sdevS, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Fall", "Spring", "Summer", "Winter")
```

```
text(x = seq(1, 4, by=1), y=rep(-1,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)

#Species intensity
par(mai=c(2,1,1,1))
intensityspec<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityspec<-subset(intensityspec, Avg.Intensity.Bd>0)
intensityspec$logload<-log(intensityspec$Avg.Intensity.Bd)
boxplot(intensityspec$logload~intensityspec$Species, xaxt="n", xlim=c(0,11), ylab="log Bd intensity")
text(x = seq(1, 11, by=1), y=rep(2,11), par("usr")[1], labels = levels(intensityspec$Species)[1:11], srt =
45, pos = 2, xpd = TRUE, cex=1.2)
par(mai=c(1.5,1.5,1.5,1.5))


v2b<-with(intensity,paste0(Month, Year))
v3b<-factor(v2b, levels=c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017"))

boxplot(intensity$logload~v3b, ylab="log Bd Intensity", xlab="", xaxt="n")
lablist.x<-c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017")
text(x = seq(1, length(lablist.x), by=1), y=rep(2.8, length(lablist.x)), par("usr")[1], labels = lablist.x, srt =
45, pos = 2, xpd = TRUE, cex=1)


############################
######Prevalance###########
############################

#make sure you have installed the package binom first!
library(binom)

prev<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prev$prev<-prev$Avg.Intensity.Bd
prev$prev[prev$prev>0]<-1
prev$Location<-factor(prev$Location)
prev$Season<-factor(prev$Season)
prev<-subset(prev, Location!="FB" & Family!= "Other")
prev$Family<-factor(prev$Family)
prev$Location<-factor(prev$Location)


##AIC##
```

```
prevmod<-glm(prev~Season+Family+Location,family=binomial,  data=prev)
prevmod1<-glm(prev~Season,family=binomial,  data=prev)
prevmod2<-glm(prev~Family,family=binomial,  data=prev)
prevmod3<-glm(prev~Location,family=binomial,  data=prev)
prevmod4<-glm(prev~Season+Family,family=binomial,  data=prev)
prevmod5<-glm(prev~Season+Location,family=binomial,  data=prev)
prevmod6<-glm(prev~Location+Family,family=binomial,  data=prev)
prevmod7<-glm(prev~1,family=binomial,  data=prev)

AIC(prevmod, prevmod1, prevmod2, prevmod3, prevmod4, prevmod5, prevmod6, prevmod7)

summary(prevmod5)


###BCI for Season
WinterCI<-binom.confint(x=22, n=22+205, methods="exact")
SpringCI<-binom.confint(x=46, n=46+264, methods="exact")
SummerCI<-binom.confint(x=19, n=19+202, methods="exact")
FallCI<-binom.confint(x=9, n=9+134, methods="exact")

meansseason<-c(WinterCI$mean, SpringCI$mean, SummerCI$mean, FallCI$mean)
upperseason<-c(WinterCI$upper, SpringCI$upper, SummerCI$upper, FallCI$upper)
lowerseason<-c(WinterCI$lower, SpringCI$lower, SummerCI$lower, FallCI$lower)

index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,4), pch=c(16,16,16,16), xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Winter", "Spring", "Summer", "Fall")
text(x = seq(1, 4, by=1), y=rep(-.05,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)

###BCI for Location
ABSCI<-binom.confint(x=1, n=65, methods="exact")
APCI<-binom.confint(x=28, n=28+274, methods="exact")
GSPCI<-binom.confint(x=67, n=475+67, methods="exact")

meansseason<-c(ABSCI$mean, APCI$mean, GSPCI$mean)
upperseason<-c(ABSCI$upper, APCI$upper, GSPCI$upper)
lowerseason<-c(ABSCI$lower, APCI$lower, GSPCI$lower)

index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,3), pch=c(16,16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
```

```
lablist.x<-c("ABS", "AP",  "GSPPL")
text(x = seq(1, 3, by=1), y=rep(-.05,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)

#BCI for Family
RanidaeCI<-binom.confint(x=29, n=165+29, methods="exact")
BufonidaeCI<-binom.confint(x=5, n=17+5, methods="exact")
HylidaeCI<-binom.confint(x=62, n=62+605, methods="exact")

meansseason<-c(RanidaeCI$mean, BufonidaeCI$mean, HylidaeCI$mean)
upperseason<-c(RanidaeCI$upper, BufonidaeCI$upper, HylidaeCI$upper)
lowerseason<-c(RanidaeCI$lower, BufonidaeCI$lower, HylidaeCI$lower)

index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,3), pch=c(16,16,16,16), xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Ranidae", "Bufonidae", "Hylidae")
text(x = seq(1, 3, by=1), y=rep(-.05,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)


#BCI for Year
table(prev$prev, prev$Year)
CI2015<-binom.confint(x=22, n=140+22, methods="exact")
CI2016<-binom.confint(x=80, n=625+80, methods="exact")
CI2017<-binom.confint(x=0, n=55, methods="exact")

meansseason<-c(CI2015$mean, CI2016$mean, CI2017$mean)
upperseason<-c(CI2015$upper, CI2016$upper, CI2017$upper)
lowerseason<-c(CI2015$lower, CI2016$lower, CI2017$lower)


index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,3), pch=c(16,16,16,16), xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("2015", "2016", "2017")
text(x = seq(1, 3, by=1), y=rep(-.05,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)



#BCI FOR MONTH
```

```
July2015CI<-binom.confint(x=7, n=28, methods="exact")
August2015CI<-binom.confint(x=7, n=46+7, methods="exact")
October2015CI<-binom.confint(x=4, n=35, methods="exact")
November2015CI<-binom.confint(x=4, n=46, methods="exact")
January2016CI<-binom.confint(x=11, n=64+11, methods="exact")
February2016CI<-binom.confint(x=6, n=50, methods="exact")
March2016CI<-binom.confint(x=5, n=49, methods="exact")
April2016CI<-binom.confint(x=18, n=52+18, methods="exact")
May2016CI<-binom.confint(x=15, n=127+15, methods="exact")
June2016CI<-binom.confint(x=13, n=84+13, methods="exact")
July2016CI<-binom.confint(x=2, n=45, methods="exact")
August2016CI<-binom.confint(x=5, n=61, methods="exact")
September2016CI<-binom.confint(x=4, n=46, methods="exact")
October2016CI<-binom.confint(x=1, n=50, methods="exact")
November2016CI<-binom.confint(x=0, n=11, methods="exact")
January2017CI<-binom.confint(x=0, n=39, methods="exact")
February2017CI<-binom.confint(x=0, n=16, methods="exact")

meansseason<-c(July2015CI$mean, August2015CI$mean, October2015CI$mean,
November2015CI$mean, January2016CI$mean, February2016CI$mean,
March2016CI$mean,April2016CI$mean, May2016CI$mean,  June2016CI$mean, July2016CI$mean,
August2016CI$mean, September2016CI$mean, October2016CI$mean,
November2016CI$mean,January2017CI$mean,February2017CI$mean)
upperseason<-c(July2015CI$upper, August2015CI$upper, October2015CI$upper,
November2015CI$upper, January2016CI$upper, February2016CI$upper,
March2016CI$upper,April2016CI$upper, May2016CI$upper,  June2016CI$upper, July2016CI$upper,
August2016CI$upper, September2016CI$upper, October2016CI$upper,
November2016CI$upper,January2017CI$upper,February2017CI$upper)
lowerseason<-c(July2015CI$lower, August2015CI$lower, October2015CI$lower,
November2015CI$lower, January2016CI$lower, February2016CI$lower,
March2016CI$lower,April2016CI$lower, May2016CI$lower,  June2016CI$lower, July2016CI$lower,
August2016CI$lower, September2016CI$lower, October2016CI$lower,
November2016CI$lower,January2017CI$lower,February2017CI$lower)


index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,length(upperseason)), pch=c(rep(16,
length(upperseason))), xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Bd Prevalance", cex.lab=1.5, type="b")
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017")
text(x = seq(1, length(upperseason), by=1), y=rep(-.03, length(upperseason)), par("usr")[1], labels =
lablist.x, srt = 45, pos = 2, xpd = TRUE, cex=1)
```

```
#BCI By Month

v<-with(prev,paste0(Month, Year))
table(prev$prev, v)

index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,3), pch=c(16,16,16,16), xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("2015", "2016", "2017")
text(x = seq(1, 3, by=1), y=rep(-.05,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)

table(prev$prev,u)


####BCI BY SPECIES

prev<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prev$prev<-prev$Avg.Intensity.Bd
prev$prev[prev$prev>0]<-1
prev$Location<-factor(prev$Location)
prev$Season<-factor(prev$Season)



table(prev$prev, prev$Species)


AgryllusCI<-binom.confint(x=51, n=504+51, methods="exact")
AterrestrisCI<-binom.confint(x=6, n=16+6, methods="exact")
HcinereaCI<-binom.confint(x=4, n=21, methods="exact")
HfemoralisCI<-binom.confint(x=2, n=18, methods="exact")
HgratiosaCI<-binom.confint(x=3, n=33, methods="exact")
HsquirellaCI<-binom.confint(x=3, n=7, methods="exact")
LcapitoCI<-binom.confint(x=1, n=3, methods="exact")
LcatesbianusCI<-binom.confint(x=22, n=22+87, methods="exact")
LgryllioCI<-binom.confint(x=1, n=19, methods="exact")
LsphenacephalusCI<-binom.confint(x=5, n=59, methods="exact")
OseptenrionalisCI<-binom.confint(x=3, n=35, methods="exact")


meansseason<-c(AgryllusCI$mean, AterrestrisCI$mean, HcinereaCI$mean, HfemoralisCI$mean,
HgratiosaCI$mean, HsquirellaCI$mean, LcapitoCI$mean,LcatesbianusCI$mean, LgryllioCI$mean,
LsphenacephalusCI$mean, OseptenrionalisCI$mean)
```

```
upperseason<-c(AgryllusCI$upper, AterrestrisCI$upper, HcinereaCI$upper, HfemoralisCI$upper,
HgratiosaCI$upper, HsquirellaCI$upper, LcapitoCI$upper,LcatesbianusCI$upper, LgryllioCI$upper,
LsphenacephalusCI$upper, OseptenrionalisCI$upper)
lowerseason<-c(AgryllusCI$lower, AterrestrisCI$lower, HcinereaCI$lower, HfemoralisCI$lower,
HgratiosaCI$lower, HsquirellaCI$lower, LcapitoCI$lower,LcatesbianusCI$lower, LgryllioCI$lower,
LsphenacephalusCI$lower, OseptenrionalisCI$lower)


index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.9), xlim=c(1,length(upperseason)), pch=c(rep(16,
length(upperseason))), xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Acris gryllus", "A. terrestris", "H. cinerea", "H. femoralis", "H. gratiosa", "H. squirella","L.
capito","L. catesbianus", "L. gryllio",  "L.sphenacephalus", "O. septentrionalis")
text(x = seq(1, length(upperseason), by=1), y=rep(-.04, length(upperseason)), par("usr")[1], labels =
lablist.x, srt = 45, pos = 2, xpd = TRUE, cex=.9)



##############################################
###################RANAVIRUS#################
##############################################


################Intensity POOLED####################

setwd("C:/Users/Matt/Dropbox/OPS/Ariel")
intensityR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityR<-subset(intensityR, Average.Intensity.Rv>0)
intensityR<-subset(intensityR, Location!="FB" & Family!= "Other")
intensityR$Family<-factor(intensityR$Family)
intensityR$Location<-factor(intensityR$Location)
intensityR$logload<-log(intensityR$Average.Intensity.Rv)
inmodR<-lm(logload~Location+Family+factor(Season), data=intensityR)
inmod1R<-lm(logload~Location, data=intensityR)
inmod2R<-lm(logload~Family, data=intensityR)
inmod3R<-lm(logload~factor(Season), data=intensityR)
inmod4R<-lm(logload~Family+Location, data=intensityR)
inmod5R<-lm(logload~Family+factor(Season), data=intensityR)
inmod6R<-lm(logload~Location+factor(Season), data=intensityR)
inmod7R<-lm(logload~1, data=intensityR)

AIC(inmodR,inmod1R,inmod2R,inmod3R,inmod4R,inmod5R,inmod6R,inmod7R)

####Retain model 6, was prev model 4
```

```
anova(inmod6R)
summary(inmod6R)
TukeyHSD(aov(logload~Family+factor(Season)+Location, data=intensityR))


se <- function(x) sqrt(var(x)/length(x))
#standard errors for the location
tapply(intensityR$logload,intensityR$Location, mean)
tapply(intensityR$logload,intensityR$Location, se)

means<-as.numeric(tapply(intensityR$logload,intensityR$Location, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Location, se))
index<-c(1,2,3)
indexa<-c(1.1,2.1,3.1)
plot(indexa, means, ylim=c(4,13), xlim=c(1,3.5), pch=c(16,16,16), col="red", xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexa, means-sdev, indexa, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansL,  pch=c(16,16,16), col="blue", lwd=4, cex=1.5)
arrows(index, meansL-sdevL, index, meansL+sdevL, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("South", "Central",  "North")
text(x = seq(1.2, 3.2, by=1), y=rep(3.4,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topleft", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)
text(x=1.1, y=9,"AB", col="red", cex=2)
text(x=2.1, y=10,"A", col="red", cex=2)
text(x=3.1, y=11,"B", col="red", cex=2)

#standard errors for the family
tapply(intensityR$logload,intensityR$Family, mean)
tapply(intensityR$logload,intensityR$Family, se)


means<-as.numeric(tapply(intensityR$logload,intensityR$Family, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Family, se))
index<-seq(1,length(means))
indexa<-c(1.1,2.1,3.1)
plot(indexa, means, ylim=c(4,13), xlim=c(1,4), pch=c(16,16,16), xaxt="n", xlab="",col="red", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexa, means-sdev, indexa, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansF,  pch=c(16,16,16),col="blue", lwd=4, cex=1.5, cex.lab=1.5)
arrows(index, meansF-sdevF, index, meansF+sdevF, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("Ranidae", "Bufonidae", "Hylidae")
text(x = seq(1.2, 3.2, by=1), y=rep(3.4,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)
```

```
text(x=1.0, y=7,"A", col="blue", cex=2)
text(x=2.0, y=11.2,"AB", col="blue", cex=2)
text(x=3.0, y=10,"B", col="blue", cex=2)



#standard errors for the season
means<-as.numeric(tapply(intensityR$logload,intensityR$Season, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Season, se))
index<-seq(1,length(means))
indexb<-c(1.1,2.1,3.1,4.1)
plot(indexb, means, ylim=c(4,14), xlim=c(1,4.5), pch=c(16,16,16,16),col="red", xaxt="n", xlab="",
lwd=4, cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexb, means-sdev, indexb, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansS, pch=c(16,16,16,16),col="blue",  lwd=4, cex=1.5,  cex.lab=1.5)
arrows(index, meansS-sdevS, index, meansS+sdevS, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("Fall", "Spring", "Summer", "Winter")
text(x = seq(1, 4.2, by=1), y=rep(3.4,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("bottomright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)
text(x=1.0, y=8,"A", col="blue", cex=2)
text(x=2.0, y=7.5,"A", col="blue", cex=2)
text(x=3.0, y=7,"A", col="blue", cex=2)
text(x=4.0, y=13.5,"B", col="blue", cex=2)
text(x=1.1, y=11.7,"A", col="red", cex=2)
text(x=2.1, y=10,"B", col="red", cex=2)
text(x=3.1, y=10,"B", col="red", cex=2)
text(x=4.1, y=10.5,"B", col="red", cex=2)



v2<-with(intensityR,paste0(Month, Year))
v3<-factor(v2, levels=c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017"))

boxplot(intensityR$logload~v3, ylab="log Rv Intensity", xlab="", xaxt="n")
lablist.x<-c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017")
text(x = seq(1, length(lablist.x), by=1), y=rep(4.5, length(lablist.x)), par("usr")[1], labels = lablist.x, srt =
45, pos = 2, xpd = TRUE, cex=1)
#Species intensity
par(mai=c(2.7,1.5,1,1))
```

```
intensityspecR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityspecR<-subset(intensityspecR, Average.Intensity.Rv>0)
intensityspecR<-subset(intensityspecR, Species!="Green hylid" &Species!="Hyla sp.")
intensityspecR$Species<-factor(intensityspecR$Species)

intensityspecR$logload<-log(intensityspecR$Average.Intensity.Rv)
boxplot(intensityspecR$logload~intensityspecR$Species, xaxt="n",ylim=c(0,25), xlim=c(0,12), ylab="log
Avg Rv intensity")
text(x = seq(1, 12, by=1), y=rep(-2,12), par("usr")[1], labels = levels(intensityspecR$Species)[1:12], srt =
45, pos = 2, xpd = TRUE, cex=1.2)

par(mai=c(1,1,1,1))



################Intensity Toes only####################

setwd("C:/Users/Matt/Dropbox/OPS/Ariel")
intensityR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityR$Toe.intensity<-intensityR$Intensity.Used..toe.tail.
intensityR<-subset(intensityR, Toe.intensity>0)
intensityR<-subset(intensityR, Location!="FB" & Family!= "Other")
intensityR$Family<-factor(intensityR$Family)
intensityR$Location<-factor(intensityR$Location)
intensityR$logload<-log(intensityR$Toe.intensity)
inmodR<-lm(logload~Location+Family+factor(Season), data=intensityR)
inmod1R<-lm(logload~Location, data=intensityR)
inmod2R<-lm(logload~Family, data=intensityR)
inmod3R<-lm(logload~factor(Season), data=intensityR)
inmod4R<-lm(logload~Family+Location, data=intensityR)
inmod5R<-lm(logload~Family+factor(Season), data=intensityR)
inmod6R<-lm(logload~Location+factor(Season), data=intensityR)
inmod7R<-lm(logload~1, data=intensityR)

AIC(inmodR,inmod1R,inmod2R,inmod3R,inmod4R,inmod5R,inmod6R,inmod7R)

####Retain model 6 was previously model 4
anova(inmod6R)
summary(inmod4R)
TukeyHSD(aov(logload~Family+factor(Season)+Location, data=intensityR))


se <- function(x) sqrt(var(x)/length(x))
#standard errors for the location
```

```
tapply(intensityR$logload,intensityR$Location, mean)
tapply(intensityR$logload,intensityR$Location, se)

means<-as.numeric(tapply(intensityR$logload,intensityR$Location, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Location, se))
index<-c(1,2,3)
indexa<-c(1.1,2.1,3.1)
plot(indexa, means, ylim=c(4,13), xlim=c(1,3.5), pch=c(16,16,16), col="red", xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexa, means-sdev, indexa, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansL,  pch=c(16,16,16), col="blue", lwd=4, cex=1.5)
arrows(index, meansL-sdevL, index, meansL+sdevL, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("South", "Central",  "North")
text(x = seq(1.2, 3.2, by=1), y=rep(3.4,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topleft", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)


#standard errors for the family
tapply(intensityR$logload,intensityR$Family, mean)
tapply(intensityR$logload,intensityR$Family, se)


means<-as.numeric(tapply(intensityR$logload,intensityR$Family, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Family, se))
index<-seq(1,length(means))
indexa<-c(1.1,2.1,3.1)
plot(indexa, means, ylim=c(4,13), xlim=c(1,4), pch=c(16,16,16), xaxt="n", xlab="",col="red", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexa, means-sdev, indexa, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansF,  pch=c(16,16,16),col="blue", lwd=4, cex=1.5, cex.lab=1.5)
arrows(index, meansF-sdevF, index, meansF+sdevF, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("Ranidae", "Bufonidae", "Hylidae")
text(x = seq(1.2, 3.2, by=1), y=rep(3.4,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)



#standard errors for the season
means<-as.numeric(tapply(intensityR$logload,intensityR$Season, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Season, se))
index<-seq(1,length(means))
indexb<-c(1.1,2.1,3.1,4.1)
```

```
plot(indexb, means, ylim=c(4,13), xlim=c(1,5), pch=c(16,16,16,16),col="red", xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexb, means-sdev, indexb, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansS, pch=c(16,16,16,16),col="blue",  lwd=4, cex=1.5,  cex.lab=1.5)
arrows(index, meansS-sdevS, index, meansS+sdevS, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("Fall", "Spring", "Summer", "Winter")
text(x = seq(1, 4.2, by=1), y=rep(3.4,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE, cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)



v2<-with(intensityR,paste0(Month, Year))
v3<-factor(v2, levels=c("July2015", "August2015", "October2015", "November2015", "January2016", "February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016", "September2016", "October2016", "November2016","January2017","February2017"))

boxplot(intensityR$logload~v3, ylab="log Rv Intensity", xlab="", xaxt="n")
lablist.x<-c("July2015", "August2015", "October2015", "November2015", "January2016", "February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016", "September2016", "October2016", "November2016","January2017","February2017")
text(x = seq(1, length(lablist.x), by=1), y=rep(4.5, length(lablist.x)), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE, cex=1)
#Species intensity
par(mai=c(2.7,1.5,1,1))
intensityspecR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityspecR$Toe.intensity<-intensityspecR$Intensity.Used..toe.tail.
intensityspecR<-subset(intensityspecR, Toe.intensity>0)
intensityspecR<-subset(intensityspecR, Species!="Green hylid" &Species!="Hyla sp.")
intensityspecR$Species<-factor(intensityspecR$Species)

intensityspecR$logload<-log(intensityspecR$Toe.intensity)
boxplot(intensityspecR$logload~intensityspecR$Species, xaxt="n",ylim=c(0,25), xlim=c(0,12), ylab="log Avg Rv intensity")
text(x = seq(1, 12, by=1), y=rep(-2,12), par("usr")[1], labels = levels(intensityspecR$Species)[1:12], srt = 45, pos = 2, xpd = TRUE, cex=1.2)

par(mai=c(1,1,1,1))



################Intensity Liver Only####################
```

```
setwd("C:/Users/Matt/Dropbox/OPS/Ariel")
intensityR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityR$Liver.Intensity<-intensityR$Intensity.used..Liver.
intensityR<-subset(intensityR, Liver.Intensity>0)
intensityR<-subset(intensityR, Location!="FB" & Family!= "Other")
intensityR$Family<-factor(intensityR$Family)
intensityR$Location<-factor(intensityR$Location)
intensityR$logload<-log(intensityR$Liver.Intensity)
inmodR<-lm(logload~Location+Family+factor(Season), data=intensityR)
inmod1R<-lm(logload~Location, data=intensityR)
inmod2R<-lm(logload~Family, data=intensityR)
inmod3R<-lm(logload~factor(Season), data=intensityR)
inmod4R<-lm(logload~Family+Location, data=intensityR)
inmod5R<-lm(logload~Family+factor(Season), data=intensityR)
inmod6R<-lm(logload~Location+factor(Season), data=intensityR)
inmod7R<-lm(logload~1, data=intensityR)

AIC(inmodR,inmod1R,inmod2R,inmod3R,inmod4R,inmod5R,inmod6R,inmod7R)

####Retain model 3, was previously model 4
anova(inmod3R)
summary(inmod3R)
TukeyHSD(aov(logload~Family+factor(Season)+Location, data=intensityR))


se <- function(x) sqrt(var(x)/length(x))
#standard errors for the location
tapply(intensityR$logload,intensityR$Location, mean)
tapply(intensityR$logload,intensityR$Location, se)

means<-as.numeric(tapply(intensityR$logload,intensityR$Location, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Location, se))
index<-c(1,2,3)
indexa<-c(1.1,2.1,3.1)
plot(indexa, means, ylim=c(4,13), xlim=c(1,3.5), pch=c(16,16,16), col="red", xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexa, means-sdev, indexa, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansL,  pch=c(16,16,16), col="blue", lwd=4, cex=1.5)
arrows(index, meansL-sdevL, index, meansL+sdevL, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("South", "Central",  "North")
text(x = seq(1.2, 3.2, by=1), y=rep(3.4,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topleft", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)
```

```
#standard errors for the family
tapply(intensityR$logload,intensityR$Family, mean)
tapply(intensityR$logload,intensityR$Family, se)


means<-as.numeric(tapply(intensityR$logload,intensityR$Family, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Family, se))
index<-seq(1,length(means))
indexa<-c(1.1,2.1,3.1)
plot(indexa, means, ylim=c(4,13), xlim=c(1,4), pch=c(16,16,16), xaxt="n", xlab="",col="red", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexa, means-sdev, indexa, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansF,  pch=c(16,16,16),col="blue", lwd=4, cex=1.5, cex.lab=1.5)
arrows(index, meansF-sdevF, index, meansF+sdevF, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("Ranidae", "Bufonidae", "Hylidae")
text(x = seq(1.2, 3.2, by=1), y=rep(3.4,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)



#standard errors for the season
means<-as.numeric(tapply(intensityR$logload,intensityR$Season, mean))
sdev<-as.numeric(tapply(intensityR$logload,intensityR$Season, se))
index<-seq(1,length(means))
indexb<-c(1.1,2.1,3.1,4.1)
plot(indexb, means, ylim=c(4,13), xlim=c(1,5), pch=c(16,16,16,16),col="red", xaxt="n", xlab="", lwd=4,
cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5)
arrows(indexb, means-sdev, indexb, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
points(index, meansS, pch=c(16,16,16,16),col="blue",  lwd=4, cex=1.5,  cex.lab=1.5)
arrows(index, meansS-sdevS, index, meansS+sdevS, length=0.05, angle=90, code=3, lwd=3, col="blue")
lablist.x<-c("Fall", "Spring", "Summer", "Winter")
text(x = seq(1, 4.2, by=1), y=rep(3.4,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=2)



v2<-with(intensityR,paste0(Month, Year))
v3<-factor(v2, levels=c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017"))
```

```
boxplot(intensityR$logload~v3, ylab="log Rv Intensity", xlab="", xaxt="n")
lablist.x<-c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017")
text(x = seq(1, length(lablist.x), by=1), y=rep(4.5, length(lablist.x)), par("usr")[1], labels = lablist.x, srt =
45, pos = 2, xpd = TRUE, cex=1)
#Species intensity
par(mai=c(2.7,1.5,1,1))
intensityspecR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
intensityspecR$Liver.Intensity<-intensityspecR$Intensity.used..Liver.

intensityspecR<-subset(intensityspecR, Liver.Intensity>0)
intensityspecR<-subset(intensityspecR, Species!="Green hylid" &Species!="Hyla sp.")
intensityspecR$Species<-factor(intensityspecR$Species)

intensityspecR$logload<-log(intensityspecR$Liver.Intensity)
boxplot(intensityspecR$logload~intensityspecR$Species, xaxt="n",ylim=c(0,25), xlim=c(0,12), ylab="log
Avg Rv intensity")
text(x = seq(1, 12, by=1), y=rep(-2,12), par("usr")[1], labels = levels(intensityspecR$Species)[1:12], srt =
45, pos = 2, xpd = TRUE, cex=1.2)

par(mai=c(1,1,1,1))



#########PREVALANCE###############

library(binom)

prevR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prevR$prevR<-prevR$Average.Intensity.Rv
prevR$prevR[prevR$prevR>0]<-1
prevR$Location<-factor(prevR$Location)
prevR$Season<-factor(prevR$Season)
prevR<-subset(prevR, Location!="FB" & Family!= "Other")
prevR$Family<-factor(prevR$Family)
prevR$Location<-factor(prevR$Location)

##AIC##
prevRmod<-glm(prevR~Season+Family+Location,family=binomial,  data=prevR)
prevRmod1<-glm(prevR~Season,family=binomial,  data=prevR)
prevRmod2<-glm(prevR~Family,family=binomial,  data=prevR)
prevRmod3<-glm(prevR~Location,family=binomial,  data=prevR)
prevRmod4<-glm(prevR~Season+Family,family=binomial,  data=prevR)
```

```
prevRmod5<-glm(prevR~Season+Location,family=binomial,  data=prevR)
prevRmod6<-glm(prevR~Location+Family,family=binomial,  data=prevR)
prevRmod7<-glm(prevR~1,family=binomial,  data=prevR)

AIC(prevRmod, prevRmod1, prevRmod2, prevRmod3, prevRmod4, prevRmod5, prevRmod6,
prevRmod7)
#retain prevRmod 5, was previously prevRmod
summary(prevRmod5)

#BCI FOR SEASON
table(prevR$prev, prevR$Season)

WinterCIR<-binom.confint(x=80, n=80+130, methods="exact")
SpringCIR<-binom.confint(x=57, n=57+237, methods="exact")
SummerCIR<-binom.confint(x=56, n=56+133, methods="exact")
FallCIR<-binom.confint(x=48, n=48+79, methods="exact")

meansseasonR<-c(WinterCI$mean, WinterCIR$mean,SpringCI$mean, SpringCIR$mean,
SummerCI$mean, SummerCIR$mean, FallCI$mean, FallCIR$mean)
upperseasonR<-c(WinterCI$upper,WinterCIR$upper, SpringCI$upper,SpringCIR$upper,
SummerCI$upper,SummerCIR$upper, FallCI$upper, FallCIR$upper)
lowerseasonR<-c(WinterCI$lower,WinterCIR$lower,SpringCI$lower, SpringCIR$lower,
SummerCI$lower,SummerCIR$lower, FallCI$lower, FallCIR$lower)

index<-c(1,1.1,2,2.1,3,3.1,4,4.1)
plot(index, meansseasonR, ylim=c(0,.65), xlim=c(1,4.5), pch=c(16,16,16,16, 16,16,16,16),
col=c("blue","red","blue","red","blue","red","blue","red"), xaxt="n", xlab="", lwd=4,
cex=1.5,cex.lab=1.5, ylab="Prevalance")
arrows(index, lowerseasonR, index, upperseasonR, length=0.05, angle=90, code=3,
lwd=3,col=c("blue","red","blue","red","blue","red","blue","red"))
lablist.x<-c("Winter", "Spring", "Summer", "Fall")
text(x = seq(1.2, 4.2, by=1), y=rep(-.04,4), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topleft", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=1.5)
text(x=1.1, y=.5, "A", col="red", cex=2.5)
text(x=1.2, y=.52, "*", col="black", cex=2.5)
text(x=2.1, y=.28, "B", col="red", cex=2.5)
text(x=3.1, y=.4, "AB", col="red", cex=2.5)
text(x=3.25, y=.42, "*", col="black", cex=2.5)
text(x=4.1, y=.5, "A", col="red", cex=2.5)
text(x=4.2, y=.52, "*", col="black", cex=2.5)

#BCI FOR LOCATION
table(prevR$prev, prevR$Location)
```

```
ABSCIR<-binom.confint(x=7, n=58+7, methods="exact")
APCIR<-binom.confint(x=71, n=214+71, methods="exact")
GSPCIR<-binom.confint(x=163, n=315+163, methods="exact")

meansseason<-c(ABSCI$mean,ABSCIR$mean,APCI$mean,APCIR$mean,GSPCI$mean,GSPCIR$mean)
upperseason<-c(ABSCI$upper,ABSCIR$upper,APCI$upper,APCIR$upper,GSPCI$upper,GSPCIR$upper)
lowerseason<-c(ABSCI$lower,ABSCIR$lower,APCI$lower,APCIR$lower,GSPCI$lower,GSPCIR$lower)

index<-c(1,1.1,2,2.1,3,3.1)
plot(index, meansseason, ylim=c(0,.5), xlim=c(.9,3.5), pch=c(16,16,16,16,16,16),
col=c("blue","red","blue","red","blue","red"), xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Prevalance",
cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3,
col=c("blue","red","blue","red","blue","red"))
lablist.x<-c("South", "Central", "North")
text(x = seq(1.2, 3.2, by=1), y=rep(-.03,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topleft", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=1.5)

text(x=1.1, y=.23, "A", col="red", cex=2.5)
text(x=2.15, y=.35, "*", col="black", cex=2.5)
text(x=2.1, y=.33, "B", col="red", cex=2.5)
text(x=3.1, y=.41, "C", col="red", cex=2.5)
text(x=3.15, y=.43, "*", col="black", cex=2.5)

text(x=1.0, y=.101, "A", col="blue", cex=2.5)
text(x=2.0, y=.151, "AB", col="blue", cex=2.5)
text(x=3.0, y=.171, "B", col="blue", cex=2.5)

#BCI FOR FAMILY
table(prevR$prev, prevR$Family)
RanidaeCIR<-binom.confint(x=60, n=117+60, methods="exact")
BufonidaeCIR<-binom.confint(x=3, n=18+3, methods="exact")
HylidaeCIR<-binom.confint(x=178, n=178+452, methods="exact")

meansseason<-c(RanidaeCI$mean, RanidaeCIR$mean,BufonidaeCI$mean,BufonidaeCIR$mean,
HylidaeCI$mean, HylidaeCIR$mean)
upperseason<-c(RanidaeCI$upper,RanidaeCIR$upper, BufonidaeCI$upper, BufonidaeCIR$upper,
HylidaeCI$upper, HylidaeCIR$upper)
lowerseason<-c(RanidaeCI$lower, RanidaeCIR$lower, BufonidaeCI$lower,BufonidaeCIR$lower,
HylidaeCI$lower, HylidaeCIR$lower)

index<-c(1,1.1,2,2.1,3,3.1)
```

```
plot(index, meansseason, ylim=c(0,.5), xlim=c(1,3.5), pch=c(16,16,16,16,16,16),
col=c("blue","red","blue","red","blue","red"), xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Prevalance",
cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3,
col=c("blue","red","blue","red","blue","red"))
lablist.x<-c("Ranidae","Bufonidae","Hylidae")
text(x = seq(1.2, 3.2, by=1), y=rep(-.025,3), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd", "Rv"), cex=1.5)
text(x=1.1, y=.43, "*", cex=2.5)
text(x=3.1, y=.35, "*", cex=2.5)

#text(x=1.1, y=.3, "N=194", cex=1.5)
#text(x=3.1, y=.3, "N=667", cex=1.5)




v<-with(prevR,paste0(Month, Year))
table(prevR$prev, v)




July2015CI<-binom.confint(x=2, n=15, methods="exact")
August2015CI<-binom.confint(x=0, n=42, methods="exact")
October2015CI<-binom.confint(x=12, n=12+16, methods="exact")
November2015CI<-binom.confint(x=17, n=17+25, methods="exact")
January2016CI<-binom.confint(x=29, n=29+43, methods="exact")
February2016CI<-binom.confint(x=17, n=17+29, methods="exact")
March2016CI<-binom.confint(x=20, n=41, methods="exact")
April2016CI<-binom.confint(x=13, n=56+13, methods="exact")
May2016CI<-binom.confint(x=35, n=98+35, methods="exact")
June2016CI<-binom.confint(x=9, n=92, methods="exact")
July2016CI<-binom.confint(x=25, n=35, methods="exact")
August2016CI<-binom.confint(x=24, n=24+28, methods="exact")
September2016CI<-binom.confint(x=5, n=45, methods="exact")
October2016CI<-binom.confint(x=7, n=49, methods="exact")
November2016CI<-binom.confint(x=0, n=8, methods="exact")
January2017CI<-binom.confint(x=13, n=22+13, methods="exact")
February2017CI<-binom.confint(x=0, n=16, methods="exact")
```

```
meansseason<-c(July2015CI$mean, August2015CI$mean, October2015CI$mean,
November2015CI$mean, January2016CI$mean, February2016CI$mean,
March2016CI$mean,April2016CI$mean, May2016CI$mean,  June2016CI$mean, July2016CI$mean,
August2016CI$mean, September2016CI$mean, October2016CI$mean,
November2016CI$mean,January2017CI$mean,February2017CI$mean)
upperseason<-c(July2015CI$upper, August2015CI$upper, October2015CI$upper,
November2015CI$upper, January2016CI$upper, February2016CI$upper,
March2016CI$upper,April2016CI$upper, May2016CI$upper,  June2016CI$upper, July2016CI$upper,
August2016CI$upper, September2016CI$upper, October2016CI$upper,
November2016CI$upper,January2017CI$upper,February2017CI$upper)
lowerseason<-c(July2015CI$lower, August2015CI$lower, October2015CI$lower,
November2015CI$lower, January2016CI$lower, February2016CI$lower,
March2016CI$lower,April2016CI$lower, May2016CI$lower,  June2016CI$lower, July2016CI$lower,
August2016CI$lower, September2016CI$lower, October2016CI$lower,
November2016CI$lower,January2017CI$lower,February2017CI$lower)


index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.8), xlim=c(1,length(upperseason)), pch=c(rep(16,
length(upperseason))), xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Rv Prevalance", cex.lab=1.5, type="b")
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("July2015", "August2015", "October2015", "November2015", "January2016",
"February2016","March2016","April2016", "May2016",  "June2016", "July2016", "August2016",
"September2016", "October2016", "November2016","January2017","February2017")
text(x = seq(1, length(upperseason), by=1), y=rep(-.05, length(upperseason)), par("usr")[1], labels =
lablist.x, srt = 45, pos = 2, xpd = TRUE, cex=1)




#BCI for Species



prevR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prevR$prevR<-prevR$Average.Intensity.Rv
prevR$prevR[prevR$prevR>0]<-1
prevR$Location<-factor(prevR$Location)
prevR$Season<-factor(prevR$Season)



table(prevR$prevR, prevR$Species)
```

```
AgryllusCI<-binom.confint(x=145, n=145+380, methods="exact")
AterrestrisCI<-binom.confint(x=3, n=21, methods="exact")
HcinereaCI<-binom.confint(x=7, n=13+7, methods="exact")
HfemoralisCI<-binom.confint(x=2, n=17, methods="exact")
HgratiosaCI<-binom.confint(x=16, n=16+15, methods="exact")
HsquirellaCI<-binom.confint(x=0, n=2, methods="exact")
LcapitoCI<-binom.confint(x=1, n=3, methods="exact")
LcatesbianusCI<-binom.confint(x=38, n=38+64, methods="exact")
LgryllioCI<-binom.confint(x=4, n=18, methods="exact")
LsphenacephalusCI<-binom.confint(x=18, n=18+38, methods="exact")
OseptenrionalisCI<-binom.confint(x=6, n=35, methods="exact")


meansseason<-c(AgryllusCI$mean, AterrestrisCI$mean, HcinereaCI$mean, HfemoralisCI$mean,
HgratiosaCI$mean, HsquirellaCI$mean, LcapitoCI$mean,LcatesbianusCI$mean, LgryllioCI$mean,
LsphenacephalusCI$mean, OseptenrionalisCI$mean)
upperseason<-c(AgryllusCI$upper, AterrestrisCI$upper, HcinereaCI$upper, HfemoralisCI$upper,
HgratiosaCI$upper, HsquirellaCI$upper, LcapitoCI$upper,LcatesbianusCI$upper, LgryllioCI$upper,
LsphenacephalusCI$upper, OseptenrionalisCI$upper)
lowerseason<-c(AgryllusCI$lower, AterrestrisCI$lower, HcinereaCI$lower, HfemoralisCI$lower,
HgratiosaCI$lower, HsquirellaCI$lower, LcapitoCI$lower,LcatesbianusCI$lower, LgryllioCI$lower,
LsphenacephalusCI$lower, OseptenrionalisCI$lower)


index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,1), xlim=c(1,length(upperseason)), pch=c(rep(16,
length(upperseason))), xaxt="n", xlab="", lwd=4, cex=1.5, ylab="Rv Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Acris gryllus", "A. terrestris", "H. cinerea", "H. femoralis", "H. gratiosa", "H. squirella","L.
capito","L. catesbianus", "L. gryllio",  "L.sphenacephalus", "O. septentrionalis")
text(x = seq(1, length(upperseason), by=1), y=rep(-.04, length(upperseason)), par("usr")[1], labels =
lablist.x, srt = 45, pos = 2, xpd = TRUE, cex=.9)



##########################################################
###################CONINFECTION#########################
##########################################################

####prevalance of rv based on bd status####

summary(glm(prevR$prevR~factor(prev$prev), family=binomial))
chisq.test(table(prevR$prevR, prev$prev))
```

```
BdPosCI<-binom.confint(x=23, n=71+23, methods="exact")
BdNegCI<-binom.confint(x=235, n=225+541, methods="exact")

meansseason<-c(BdNegCI$mean,  BdPosCI$mean)
upperseason<-c(BdNegCI$upper, BdPosCI$upper)
lowerseason<-c(BdNegCI$lower, BdPosCI$lower)

index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(0,3), pch=c(16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Rv Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Bd -", "Bd +")
text(x = seq(1, 2, by=1), y=rep(-.05,2), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)



########prevalance of Bd based on rv status########

summary(glm(prev$prev~factor(prevR$prevR), family=binomial))
chisq.test(table(prev$prev, prevR$prevR))
RvPosCI<-binom.confint(x=23, n=225+23, methods="exact")
RvNegCI<-binom.confint(x=71, n=541+71, methods="exact")

meansseason<-c(RvNegCI$mean,  RvPosCI$mean)
upperseason<-c(RvNegCI$upper, RvPosCI$upper)
lowerseason<-c(RvNegCI$lower, RvPosCI$lower)

index<-seq(1,length(upperseason))
plot(index, meansseason, ylim=c(0,.5), xlim=c(0,3), pch=c(16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Bd Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Rv -", "Rv +")
text(x = seq(1, 2, by=1), y=rep(-.05,2), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)

####intensity of ranavirus based on Bd status####

intensityR$prev<-intensityR$Avg.Intensity.Bd
intensityR$prev[intensityR$prev>0]<-1
intenscomodRGB<-lm(logload~factor(prev), data=intensityR)
summary(intenscomodRGB)
anova(intenscomodRGB)
means<-as.numeric(tapply(intensityR$logload,intensityR$prev, mean))
```

```
sdev<-as.numeric(tapply(intensityR$logload,intensityR$prev, se))
index<-seq(1,length(means))
plot(index, means, ylim=c(0,15), xlim=c(0,3), pch=c(16,16,16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Log Mean Rv Intensity", cex.lab=1.5)
arrows(index, means-sdev, index, means+sdev, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Bd -", "Bd +")
text(x = seq(1, 2, by=1), y=rep(-1,2), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)


####intensity of Bd based on ranavirus status####

intensity$prev<-intensity$Average.Intensity.Rv
intensity$prev[intensity$prev>0]<-1
intenscomodBGR<-lm(logload~factor(prev), data=intensity)
summary(intenscomodBGR)
anova(intenscomodBGR)

meansB<-as.numeric(tapply(intensity$logload,intensity$prev, mean))
sdevB<-as.numeric(tapply(intensity$logload,intensity$prev, se))
index<-seq(1,length(meansB))
indexc<-c(1.1,2.1)
plot(index, meansB, ylim=c(4,13), xlim=c(1,2.5), pch=c(16,16,16,16), col="blue", xaxt="n", xlab="",
lwd=4, cex=1.5, ylab="Log Mean Intensity", cex.lab=1.5, type="b")
arrows(index, meansB-sdevB, index, meansB+sdevB, length=0.05, angle=90, code=3, lwd=3,
col="blue")
points(indexc, means, ylim=c(0,15), xlim=c(0,3), pch=c(16,16,16,16),col="red", xaxt="n", xlab="",
lwd=4, cex=1.5, ylab="Log Mean Bd Intensity", cex.lab=1.5, type="b")
arrows(indexc, means-sdev, indexc, means+sdev, length=0.05, angle=90, code=3, lwd=3, col="red")
lablist.x<-c("Rv -", "Bd-", "Rv +", "Bd +")
text(x = c(1,1.15,2,2.15),col=c("blue", "red", "blue", "red"), y=rep(3.2,2), par("usr")[1], labels = lablist.x,
srt = 45, pos = 2, xpd = TRUE, cex=1.5)
legend("topright", col=c("blue", "red"), pch=c(16,16), legend=c("Bd intensity", "Rv intensity"), cex=1.5)

#intensity-intensity model
intensityRc<-subset(intensityR, Average.Intensity.Rv>0 & Avg.Intensity.Bd>0)
intintmod<-lm(log(Average.Intensity.Rv)~log(Avg.Intensity.Bd), data=intensityRc)
summary(intintmod)



###Fisher Mulitple Comparisons
setwd("C:/Users/Matt/Dropbox/OPS/Ariel")

library(RVAideMemoire)
```

```
prevR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prevR$prevR<-prevR$Average.Intensity.Rv
prevR$prevR[prevR$prevR>0]<-1
prevR$Location<-factor(prevR$Location)
prevR$Season<-factor(prevR$Season)
prevR<-subset(prevR, Location!="FB"& Family!= "Other")
prevR$Location<-factor(prevR$Location)

prev<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prev$prev<-prev$Avg.Intensity.Bd
prev$prev[prev$prev>0]<-1
prev$Location<-factor(prev$Location)
prev$Season<-factor(prev$Season)
prev<-subset(prev, Location!="FB" & Family!= "Other")
prev$Location<-factor(prev$Location)

fisher.multcomp(table(prev$Season, prev$prev), p.method="bonferroni")
fisher.multcomp(table(prev$Family, prev$prev), p.method="bonferroni")
fisher.multcomp(table(prev$Location, prev$prev), p.method="bonferroni")
fisher.multcomp(table(prev$Year, prev$prev), p.method="bonferroni")


fisher.multcomp(table(prevR$Season, prevR$prevR), p.method="bonferroni")
fisher.multcomp(table(prevR$Family, prevR$prevR), p.method="bonferroni")
fisher.multcomp(table(prevR$Location, prevR$prevR), p.method="bonferroni")
fisher.multcomp(table(prevR$Year, prevR$prevR), p.method="bonferroni")




####LIVER and TOE Comps
prevR<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)##
prevR$prevR<-prevR$Intensity.used..Liver.
prevR$prevR[prevR$prevR>0]<-1
prevR$Location<-factor(prevR$Location)
prevR$Season<-factor(prevR$Season)
prevR<-subset(prevR, Location!="FB" & Family!= "Other")
prevR$Family<-factor(prevR$Family)
prevR$Location<-factor(prevR$Location)


prevR$prevRT<-prevR$Intensity.Used..toe.tail.
prevR$prevRT[prevR$prevRT>0]<-1
prevR$Location<-factor(prevR$Location)
```

```
prevR$Season<-factor(prevR$Season)
prevR<-subset(prevR, Location!="FB" & Family!= "Other")
prevR$Family<-factor(prevR$Family)
prevR$Location<-factor(prevR$Location)



Liver<-binom.confint(x=59, n=146+59, methods="exact")
Toes<-binom.confint(x=237, n=598+237, methods="exact")


meansseason<-c(Liver$mean,  Toes$mean)
upperseason<-c(Liver$upper, Toes$upper)
lowerseason<-c(Liver$lower, Toes$lower)
par(mfrow=c(1,2))

index<-seq(1,length(upperseason))
par(mai=c(1.2,1.2,1.2,.5))
plot(index, meansseason, ylim=c(0,.5), xlim=c(0,3), pch=c(16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Rv Prevalance", cex.lab=1.5)
arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
lablist.x<-c("Liver", "Toes")
text(x = seq(1, 2, by=1), y=rep(-.05,2), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
text(x=.1, y=.5, "A", cex=1.75)


dati<-read.csv("Thesis_Dataset_Master_File48_averageacross_AH.csv", header=T)
dati$logtailp1<-log(dati$Intensity.Used..toe.tail.+1)
dati$loglivp1<-log(dati$Intensity.used..Liver.+1)
dati2<-subset(dati, logtailp1>0 &  dati$loglivp1>0)




t.test(dati2$logtailp1, dati2$loglivp1, paired=T)


 toesImean<-mean(dati2$logtailp1[!is.na(dati2$logtailp1)])
 liverImean<-mean(dati2$loglivp1[!is.na(dati2$loglivp1)])
 se <- function(x) sqrt(var(x)/length(x))
 toesIse<-se(dati2$logtailp1[!is.na(dati2$logtailp1)])
 liverIse<-se(dati2$loglivp1[!is.na(dati2$loglivp1)])
```

```
  meansseason<-c(liverImean,  toesImean)
  upperseason<-c(liverImean+liverIse, toesImean+toesIse)
  lowerseason<-c(liverImean-liverIse, toesImean-toesIse)

  index<-seq(1,length(upperseason))
  par(mai=c(1.2,.9,1.2,1.2))
  plot(index, meansseason, ylim=c(0,14), xlim=c(0,3), pch=c(16,16), xaxt="n", xlab="", lwd=4, cex=1.5,
ylab="Rv Intensity", cex.lab=1.5)
  arrows(index, lowerseason, index, upperseason, length=0.05, angle=90, code=3, lwd=3)
  lablist.x<-c("Liver", "Toes")
  text(x = seq(1, 2, by=1), y=rep(-.9,2), par("usr")[1], labels = lablist.x, srt = 45, pos = 2, xpd = TRUE,
cex=1.5)
  text(x=.1, y=14, "B", cex=1.75)
```

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