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Original Contribution

Pathogen Dynamics in an Invasive Frog Compared to Native Species

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Abstract: Emerging infectious diseases threaten the survival of wildlife populations and species around the world. In particular, amphibians are experiencing population declines and species extinctions primarily in response to two pathogens, the fungus *Batrachochytrium dendrobatidis* (*Bd*) and the iridovirus *Ranavirus* (*Rv*). Here, we use field surveys and quantitative (q)PCR to compare infection intensity and prevalence of *Bd* and *Rv* across species and seasons on Jekyll Island, a barrier island off the coast of Georgia, USA. We collected oral and skin swabs for 1 year from four anuran species and three families, including two native hylids (*Hyla cinerea* and *Hyla squirella*), a native ranid (*Rana sphenocephala*), and the invasive rain frog *Eleutherodactylus planirostris*. *Bd* infection dynamics did not vary significantly over sampling months, but *Rv* prevalence and intensity were significantly higher in fall 2014 compared to spring 2015. Additionally, *Rv* prevalence and intensity were significantly higher in *E. planirostris* than in the other three species. Our study highlights the potential role of invasive amphibians as drivers of disease dynamics and demonstrates the importance of pathogen surveillance across multiple time periods and species to accurately capture the infectious disease landscape.

Keywords: Amplification host, *Eleutherodactylus planirostris*, Emerging infectious diseases, Invasive species, Seasonality

Introduction and Purpose

Emerging infectious diseases (EIDs) present significant threats to the survival of diverse host species across the world (Daszak et al. 2000; Jones et al. 2008). Amphibians have declined and experienced extinction events at a rapid rate since at least the 1970s (Kiesecker et al. 2001; Stuart et al. 2004; Keesing et al. 2010), with infectious diseases being one of the greatest contributors to their decline worldwide (Daszak et al. 2003; Pounds et al. 2006). Pathogen-associated anuran declines are primarily attributed to two major pathogens, the fungus *Batrachochytrium dendrobatidis* (*Bd*) and the iridovirus *Ranavirus* (*Rv*) (Berger et al. 1998; Stuart et al. 2004). *Bd* is a waterborne fungal pathogen that infects amphibian epidermis and causes the disease chytridiomycosis (Berger et al. 1998). Chytridiomycosis has already caused the decline or

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extinction of over 500 amphibian species worldwide (Skerratt et al. 2007; Olson et al. 2013). *Rv* is a large double-stranded DNA virus that infects amphibians, fishes, and turtles (Johnson et al. 2008; Gray et al. 2009; Brenes et al. 2014). Anurans with high *Rv* infection intensity often exhibit liver necrosis, swelling, erythema, and hemorrhages along the hind legs, ventral region, and in some internal organs (Chinchar 2002; Miller et al. 2011; Lesbarrères et al. 2012). Amphibian hosts that are susceptible to *Rv* usually die due to extensive organ necrosis because *Rv* infects multiple cell and tissue types (Gray et al. 2009; Miller et al. 2011).

The emergence, spread, and persistence of infectious diseases can be attributed to numerous host and environmental interactions (Casadevall and Pirofski 2001). Seasonal variation relating to photoperiod, precipitation, temperature, or humidity can affect host susceptibility or the pathogen's ability to infect its host (Zapata et al. 1992; Dowell 2001; Bowden et al. 2007). Numerous studies document higher *Bd* prevalence in cooler months (Berger et al. 2005; Kriger and Hero 2007; Savage et al. 2011; Ruggeri et al. 2015), and these findings are consistent with in vitro laboratory trials (Piotrowski et al. 2004) demonstrating that *Bd* reaches highest pathogenicity at cooler temperatures (17–25°C). In contrast, most *Rv* seasonality studies find that amphibian epidemics occur in spring or early summer, coinciding with temperature increases and metamorphosis (Green et al. 2002). Higher temperatures may damage hosts by increasing viral replication rates. For example, common frog tadpoles showed elevated *Rv* mortality and pathogen intensity at 20°C compared to 15°C (Bayley et al. 2013). Other studies find contrasting results, where higher temperatures increase host immunity and decrease viral titers (Rojas et al. 2005).

Many studies investigating *Bd* and *Rv* simultaneously in wild amphibians are conducted at locations where disease outbreaks have previously been observed, prompting pathogen testing because of known die-off events (Crawford et al. 2010; Cheng et al. 2011; Rosa et al. 2017). However, studying disease dynamics in amphibian populations not currently experiencing die-offs is also important for understanding why pathogens can persist in certain areas without leading to disease outbreaks, whereas in other populations infection leads to mass mortality events. In particular, knowing which species serve as reservoir hosts and why they are able to survive without exhibiting any clinical signs or population declines will lead to a better understanding of global amphibian declines. Furthermore, understanding baseline patterns of pathogen prevalence and severity prior to any observed die-offs will facilitate conservation and management efforts in amphibian populations if future disease outbreaks ever take place.

Previous research for both *Bd* and *Rv* highlights species-specific differences in pathogen prevalence and disease susceptibility (Hoverman et al. 2010; Searle et al. 2011; Gervasi et al. 2013; Savage et al. 2018). For example, some species such as the American bullfrog (*Rana catesbeiana*) carry *Bd* without displaying any clinical signs, while other species have experienced population declines with 100% mortality (Daszak et al., 2003; Bielby et al. 2008). Similarly, in three species of larval anurans, *Rv* susceptibility and mortality rates were significantly different under controlled experimental conditions (Hoverman et al. 2010). In this study, we surveyed four species, including two native tree frogs (*Hyla cinerea* and *Hyla squirella*), one native ranid frog (*Rana sphenocephala*), and one invasive species of rain frog (*Eleutherodactylus planirostris*). The three native species are abundant throughout the Southeastern USA, while the invasive species has extended through Florida and has a few isolated colonies in Alabama, Georgia, and Hawaii in its invasive USA range (Heinicke et al. 2011). All four species have unique natural histories and represent three families, allowing us to broadly assess pathogen dynamics across a barrier island ecosystem. *Hyla cinerea* is highly arboreal and breeds aquatically in permanent ponds between March and September (Nichols 2008). Field studies of *H. cinerea* have never reported the presence of *Bd* (Rothermel et al. 2008), and a laboratory study infecting adult *H. cinerea* with *Bd* found no clinical signs of chytridiomycosis (Brannelly et al. 2012). In contrast, *H. cinerea* mortality due to *Rv* infection has been documented in a wild population (Miller et al. 2011). *Hyla squirella* is also highly arboreal and breeds aquatically (Virden 2003); no documentation of *Bd* or *Rv* infection has been reported in this species (Horner et al. 2017). *Rana sphenocephala* breeds in permanent ponds, mostly between November and March (Meade 2008). Field studies report *Bd* infection in *R. sphenocephala*, but at low prevalence (Daszak et al. 2005; Rothermel et al. 2008). *Rv* infections and mortality events have also been documented within *R. sphenocephala* (Miller et al. 2011; Hoverman et al. 2012; Landsberg et al. 2013). *Eleutherodactylus planirostris* is a direct-developing species from Cuba that breeds terrestrially under moist leaf cover (Olson et al. 2012). To date, a single study found *Bd* infection in invasive *E. planirostris* in Florida (Rizkalla 2010). *Rv* has not been detected in *E. planirostris*.

Here, we sampled three native species and one invasive species to characterize *Bd* and *Rv* dynamics on Jekyll Island, Georgia, USA, which is a barrier island (an island that forms a barrier between the mainland and the ocean). Because of continual exposure to oceanic mechanisms such as tide, wind, current, and storm, multiple environmental zones can be found on barrier islands that depend on their distance from the mainland and the size of the island. Jekyll Island is a 23 km^2 mixed-energy barrier island characterized by being wide at one end and narrow at the other. To characterize amphibian pathogen dynamics across Jekyll Island, we compared infections across species, sampling locations, sampling month, and sample type. Given the widespread global distribution of these two pathogens, even where disease outbreaks are absent, we predicted that both pathogens would be present on the island. We also predicted higher *Bd* infection prevalence and intensity in cooler months and higher *Rv* infection prevalence and intensity in warmer months, consistent with previous seasonal studies of these pathogens (Green et al. 2002; Savage et al. 2011). Finally, we predicted low overall pathogen prevalence and intensity due to the lack of documented amphibian die-offs in the Southeastern USA. Due to the wide range of variation in susceptibility among amphibian species, populations, and individuals (Savage and Zamudio 2011; Bataille et al. 2015), we did not make specific predictions about species variation in pathogen dynamics.

Materials and Methods

Sample Collection

We collected anuran swab samples from 26 localities across Jekyll Island, GA, USA, in 2014 and 2015. Sampling occurred from August to November 2014 and April to July 2015 in order to represent fall and spring periods of amphibian activity. Within the seasonal sampling periods, sites were visited monthly. Selected locations represented typical habitats on a semi-developed barrier island. Locations were selected to include a mix of undeveloped locations and those with varying levels of human visitation. While we included localities to represent different ecological habitats and levels of human activity and to capture the variance of natural and impacted systems, we were unable to standardize effort relative to sampling technique or produce even sampling sizes among sites, months, and sampling techniques that would have facilitated our analysis of the effects of habitat and anthropogenic activity.

At each site, we used multiple sampling techniques, including laying out rectangular plywood boards (“cover boards,” 10 sites), placing PVC pipes (see below, 12 sites), conducting nighttime call surveys (11 sites), and visual encounter surveys with hand captures (all sites). Employing a diversity of sampling methods increased the likelihood that we would detect the target species that included both terrestrial and arboreal representatives. A combination of arboreal PVC pipes and shoreline pipes was distributed across the 12 localities where pipes were used to locate and sample anurans. We constructed arboreal PVC pipes adapted from Boughton et al. (2000) that were 3.81 and 5.10 cm (1.5″ and 2″, respectively) in diameter, and the retreats were 60 cm with caps. Shoreline pipes were placed along the edge of water bodies (Boughton et al. 2000). Water filled the bottom of the pipe, while the other three quarters remained above water. The pipes were uncapped on both sides to allow water to enter the bottom and for tree frogs to passively enter and exit from the top. In addition to the pipe array, frogs were collected at night during and following rain events at 11 sites where individuals were hand-captured.

Upon capture, we collected separate oral and skin swabs using fine-tipped swabs (Medical Wire Co. #MW113). Body swabs were collected via an epidermal swab targeting the abdominal midline, between the hind legs, and the webbing of the back feet totaling in 30 swab strokes per frog (Vredenburg and Briggs 2009). A new pair of gloves were used for each frog handled to reduce cross-contamination and disease spread (Vredenburg and Briggs 2009). Swabs were then stored in the Whirl-paks in a −20°C freezer until DNA extraction and pathogen quantification. All frogs were released at the site of capture immediately after sampling.

Pathogen Quantification

DNA was extracted from swab samples using Qiagen DNeasy Blood and Tissue kits (Qiagen Corporation, Maryland, USA) using the manufacturer’s protocol. TaqMan quantitative PCR (qPCR) was then performed on extracted DNA using the Bio-Rad CFX96 Real-Time System (Bio-Rad, Hercules, California, USA). Standard curves were generated from serial dilutions of synthetic pathogen DNA (gBlock gene fragments) run in duplicate for absolute pathogen quantification, and we included two negative

controls (molecular grade water) and two positive controls (known infected frogs) on all runs. *Bd* and *Rv* reactions were run independently on 96-well plates using primers and probes developed by Boyle et al. (2004) for *Bd* reactions and primers and probes developed by Allender et al. (2013) for *Rv* reactions. All standards, samples, and controls were tested in 25 μL volumes composed of 8 μL of Bio-Rad Supermix, 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 TaqMan probe (*Bd* or *Rv*; 0.2 μM/μL), and 5 μL of standard DNA template or sample DNA template. All reactions were run under the following cycling conditions: 95°C for 5 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. We first ran pools consisting of 5 μL DNA per sample for all samples from a given locality to first determine pathogen occurrence. All samples from each positive pool were then tested individually for both pathogens. All samples were considered positive for *Bd* or *Rv* if they amplified before cycle 38 across at least two independent runs. If the first two runs were inconsistent, samples were run a third time and the average of the two most consistent runs was retained as the pathogen infection intensity value.

Statistical Analyses

All data analysis was conducted in R using the packages binom, bbmle, and ggplot2 (R Core Team 2014). Infection prevalence (the proportion of positive individuals out of the total number sampled) and infection intensity [the average number of genomic equivalents (GE) measured on an individual] for both *Bd* and *Rv* were analyzed using ANOVA with Tukey HSD post hoc tests to determine significance of each individual factor. Potential explanatory variables included in the ANOVA were species, location, month, average monthly precipitation, average monthly temperature, average minimum monthly temperature, and average maximum monthly temperature, and these factors were chosen based on those explanatory variables that have previously been shown to influence pathogen presence and abundance in amphibian systems. Additionally, we used a two-sided *t* test with unequal variances to compare oral and body swabs. All climate variables (precipitation, average temperature, minimum temperature, and maximum temperature) were obtained from the WorldClim/Bioclim ArcGis dataset (current data interpolated from 1960 to 2000 data; Supplemental Information 1). To compare how these factors may be acting in combination to influence prevalence of each pathogen in the system, we ran each variable individually and we then step-wise added each variable into generalized linear models where each model had a one factor or a combination of two, three, four, five, six, or seven (our global model) different factors (GLMs; family = binomial) that accounted for all possible combinations between explanatory variables as well as a null model. For infection intensity, we used the same approach with logarithmically scaled Gaussian linear models so that all possible combinations between explanatory variables were present. We compared the AICc values of the GLMs using AICctab in R using the package bbmle (R Core Team 2014). We considered the best explanatory models to be the model with the lowest AICc score, as well as any other models that differed from the top model by < 2 AICc values, following Johnson et al. (2012).

Results

Amphibian swab samples were collected in all targeted months from all sampling localities. All individuals we swabbed for this study appeared healthy and were asymptomatic at the time of sampling. We obtained oral and body swabs from 215 individuals across the 26 sampling localities (Fig. 1). A total of 17 individuals were positive for *Bd* (8%) and 11 individuals were positive for *Rv* (5%) in at least one sample type (Table 1; Fig. 1). Prevalence varied among species, months, and pathogens, with nearly identical infection dynamics in the two tree frog species (Tables 1, 2). As expected, animal activity and our sample sizes varied among months due to the influence of abiotic conditions, with notable decreases in anuran detection in hotter and drier months (August–October 2014; Table 2; Supplemental Information 1). Nine of 59 *H. cinerea* were positive for *Bd* (8%; average infection intensity = 244 GE) and one was positive for *Rv* (1%; average infection intensity = 66 GE). Two of 22 *H. squirella* were positive for *Bd* (9%; average infection intensity = 1111 GE) and one was positive for *Rv* (4%; average infection intensity = 2 GE). Infection dynamics in *R. sphenocephala* were also similar, with six of 97 individuals positive for *Bd* (6%; average infection intensity = 9273 GE) and none positive for *Rv*. In contrast, *E. planirostris* showed higher *Rv* prevalence than the other three species. Among 37 total sampled individuals, one was *Bd* positive (3%; average infection intensity = 124 GE) whereas 9 were *Rv* positive (24%; average infection intensity = 344 GE).

Table 1. *Bd* and *Rv* Infections Detected by qPCR and Grouped by Species and Sample Type.

Species	*Bd*			*Rv*		
	Body swabs	Oral swabs	All individuals	Body swabs	Oral swabs	All individuals
H. cinerea	3/59 (0.05)	6/59 (0.10)	8/59 (0.14)	1/59 (0.02)	0/59 (0.00)	1/59 (0.02)
H. squirella	1/22 (0.04)	2/22 (0.09)	2/22 (0.09)	0/22 (0.00)	1/22 (0.04)	1/22 (0.04)
R. sphenocephala	0/97 (0.00)	6/95 (0.06)	6/97 (0.06)	0/97 (0.00)	0/95 (0.00)	0/97 (0.00)
E. planirostris	1/37 (0.03)	0/35 (0.00)	1/37 (0.03)	4/37 (0.11)	6/35 (0.17)	9/37 (0.24)
Total	4/215 (0.04)	12/213 (0.06)	17/215 (0.08)	5/215 (0.02)	9/213 (0.04)	11/215 (0.05)

Total values for species and sample types are also included. Infection prevalence is shown in parentheses.

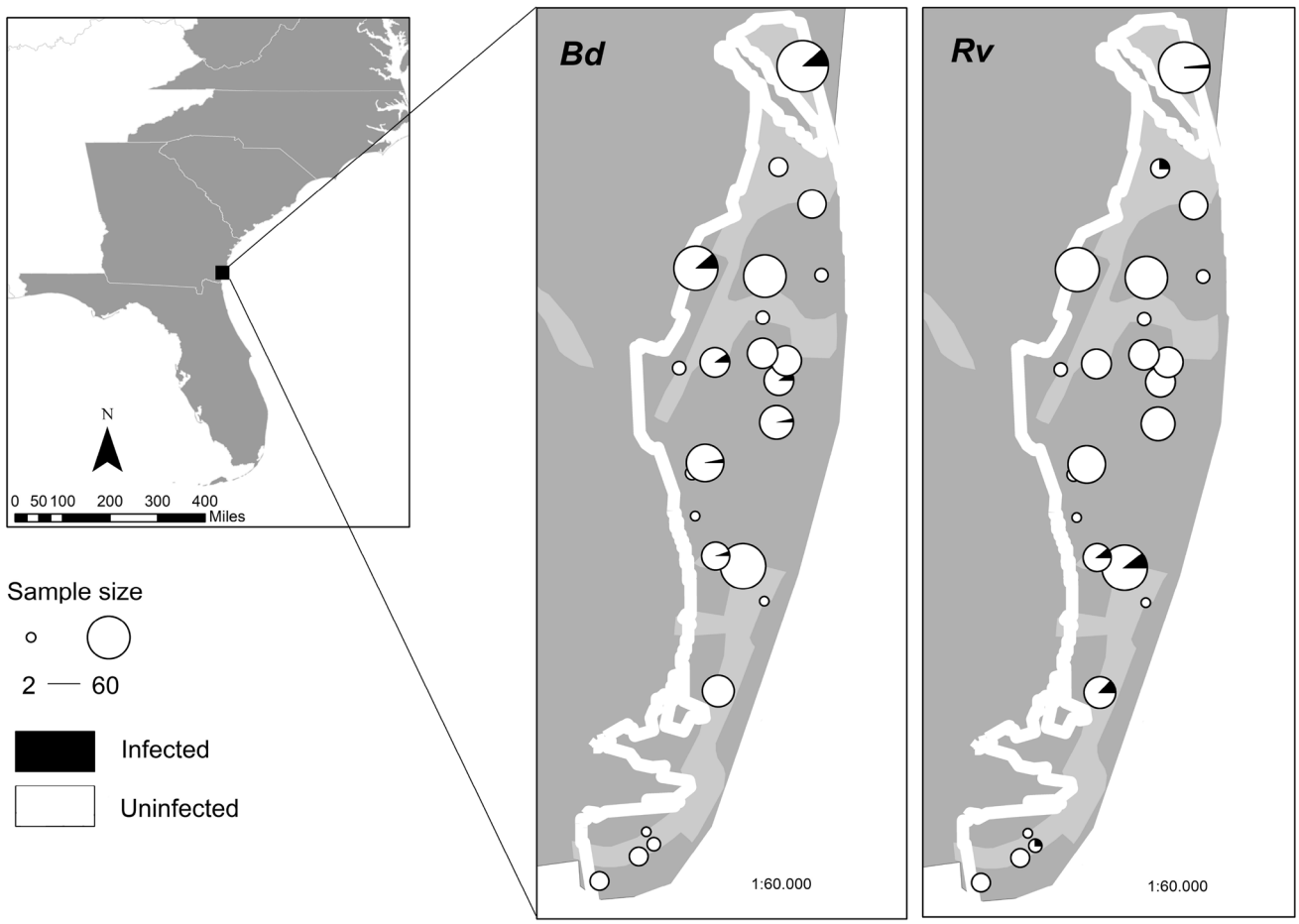

Figure 1. *Bd* and *Rv* prevalence across sampling sites on Jekyll Island, GA, USA, from August of 2014 to July of 2015. Black color shows the proportion of infected individuals, and white color shows the proportion of uninfected individuals for *Bd* (left panel) and *Rv* (right panel). Circle size is proportionate to sample size, and each pie chart combines all species and sampling dates for that location.

Bd prevalence differed significantly for multiple factors: average precipitation ($F = 9.258$, $P = 0.0026$, positive relationship), mean temperature ($F = 8.744$, $P = 0.0035$, positive relationship), maximum temperature ($F = 8.463$, $P = 0.0040$, positive relationship), and minimum temperature ($F = 8.937$, $P = 0.0031$, positive relationship). *Bd* prevalence did not differ significantly among swab types, although oral swabs tended toward higher pathogen detection rates (t value = 1.96, $P = 0.067$). *Rv* prevalence was driven by a different combination of factors, including

Table 2. *Bd* and *Rv* Infections Detected by qPCR and Grouped by Sampling Month, Sample Type, and Total Number of Samples and Individuals.

Month	*Bd*			*Rv*		
	Body swabs	Oral swabs	All individuals	Body swabs	Oral swabs	All individuals
August 2014	1/9 (0.11)	0/9 (0.00)	1/9 (0.11)	2/9 (0.22)	0/9 (0.00)	2/9 (0.22)
September 2014	0/1 (0.00)	0/1 (0.00)	0/1 (0.00)	1/1 (1.00)	0/1 (0.00)	1/1 (1.00)
October 2014	0/7 (0.00)	0/7 (0.00)	0/7 (0.00)	2/7 (0.28)	3/7 (0.43)	3/7 (0.43)
November 2014	0/18 (0.00)	0/18 (0.00)	0/18 (0.00)	0/18 (0.00)	2/18 (0.11)	2/18 (0.11)
April 2015	0/28 (0.00)	0/28 (0.00)	0/28 (0.00)	0/28 (0.00)	1/28 (0.03)	1/28 (0.03)
May 2015	0/42 (0.00)	1/42 (0.02)	1/42 (0.02)	0/42 (0.00)	0/42 (0.00)	0/42 (0.00)
June 2015	3/39 (0.08)	6/38 (0.16)	7/39 (0.18)	1/39 (0.02)	0/38 (0.00)	1/39 (0.02)
July 2015	2/71 (0.03)	6/70 (0.08)	8/71 (0.11)	0/71 (0.00)	1/70 (0.01)	1/71 (0.01)
Total	6/215 (0.03)	13/213 (0.06)	17/213 (0.08)	6/215 (0.03)	7/213 (0.03)	11/215 (0.05)

Infection prevalence is shown in parentheses.

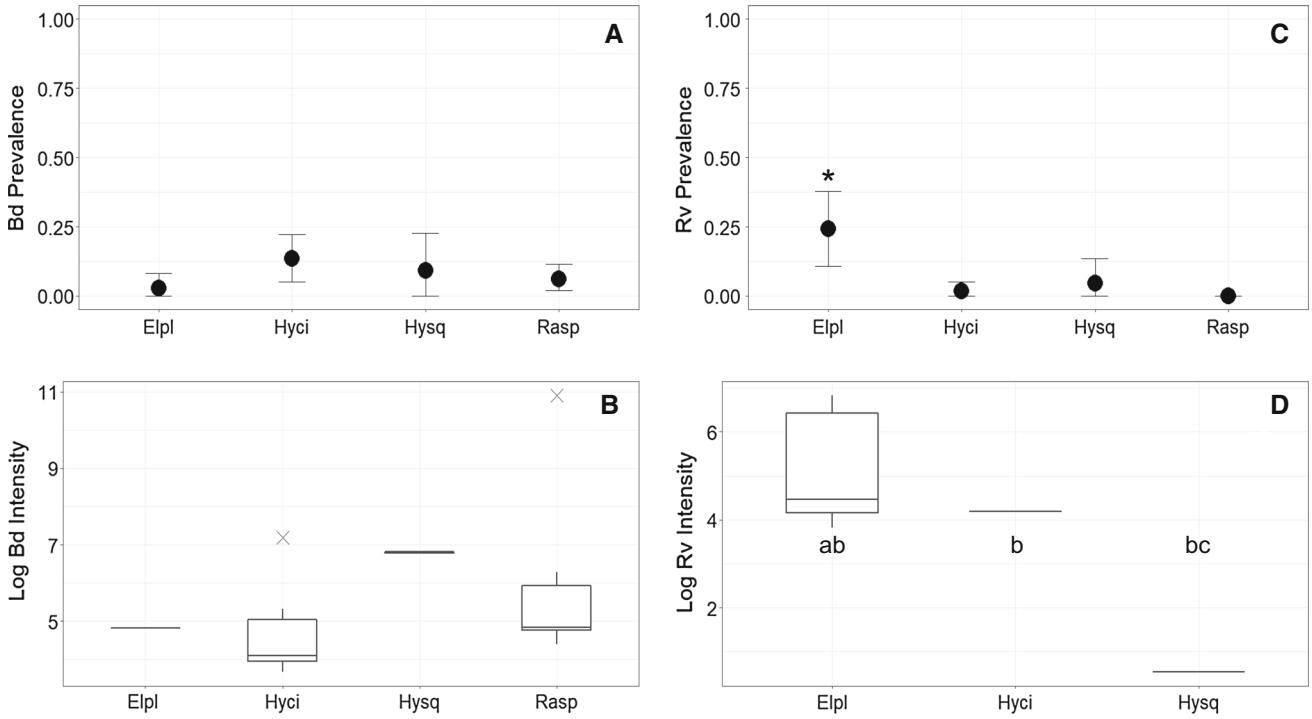

Figure 2. *Bd* and *Rv* prevalence (± 95% confidence interval) and log-transformed mean infection intensity (± standard error) among species. **a** *Bd* prevalence, **b** *Bd* intensity, **c** *Rv* prevalence, and **d** *Rv* intensity. Asterisks or different letters indicate significantly different values based on ANOVA with Tukey HSD post hoc tests. Black X symbols indicate the presence of an outlier in the data. Different letters indicate statistically significant differences.

month ($F = 9.144$, $P < 0.0001$) and species ($F = 13.58$, $P < 0.0001$). Specifically, *E. planirostris* had significantly higher *Rv* prevalence than all other sampled species (Tukey HSD = 13.58, all $P < 0.002$; Fig. 2). Across sampling months, September and October 2014 showed significantly higher *Rv* prevalence than any other sampling month (Tukey HSD = 12.41, all $P < 0.008$; Fig. 3).

Bd infection intensity did not differ significantly based on species, month, swab type, yearly precipitation, or temperature. In contrast, *Rv* intensity differed significantly

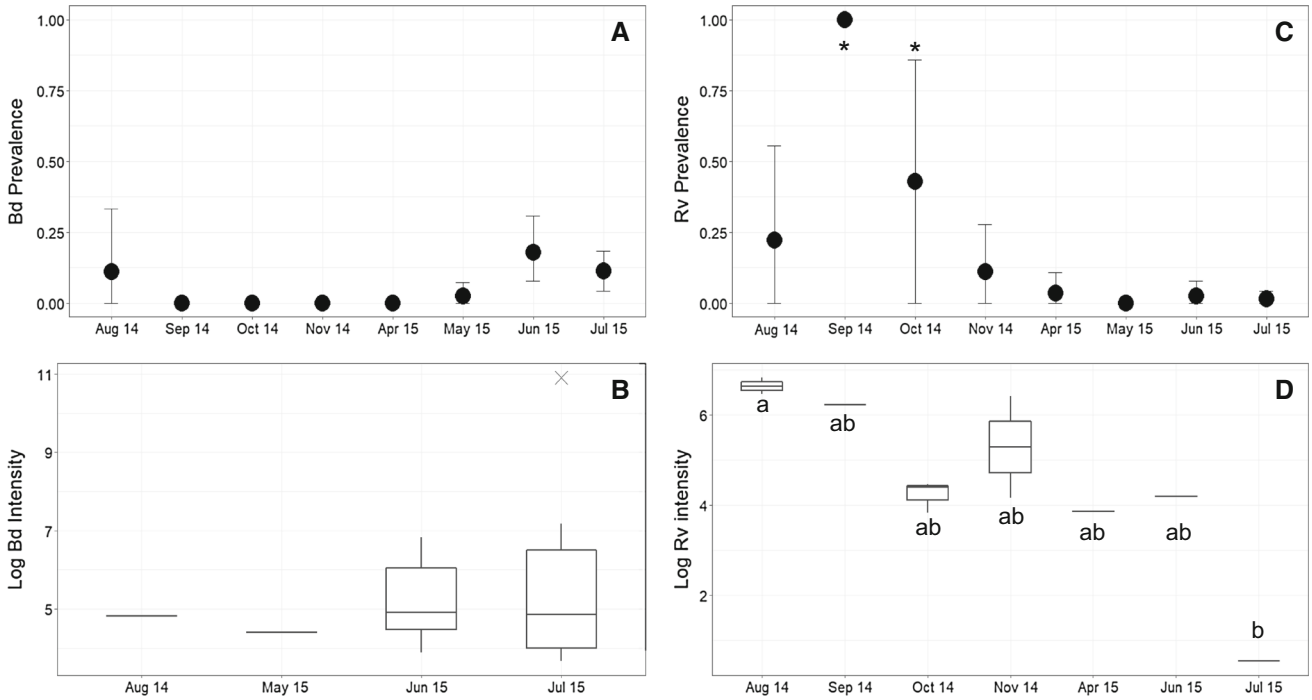

Figure 3. *Bd* and *Rv* prevalence (± 95% confidence interval) and log-transformed mean infection intensity (± standard error) across sampled months. **a** *Bd* prevalence, **b** *Bd* intensity, **c** *Rv* prevalence, and **d** *Rv* intensity. Asterisks indicate significantly different values based on ANOVA with Tukey HSD post hoc tests. Black X symbols indicate the presence of an outlier in the data. Different letters indicate statistically significant differences.

across species ($F = 6.18$, $P = 0.02$), with significantly higher infection intensity in *E. planirostris* compared to *H. squirella* (Tukey HSD = 6.286, $P = 0.014$; Fig. 2). *Rv* intensity also differed significantly across sampling months ($F = 6.844$, $P = 0.042$), but only the comparison between August 2014 and July 2015 was significant (Tukey HSD = 6.844, $P = 0.02$; Fig. 3). Coinfection was rare, with only one *E. planirostris* individual showing simultaneous *Bd* and *Rv* infection among all anurans sampled.

Using model selection, we found *Bd* prevalence was best explained by models that included species, minimum temperature, precipitation, maximum temperature, and average temperature (Table 3). For *Bd* infection intensity, the best explanatory model was the null model, indicating that none of the included variables can explain the observed infection intensity data (Table 3). Multiple models were best able to explain *Rv* prevalence, including models that had the following variables: (1) species, (2) species and minimum temperature, (3) species and mean temperature, (4) species and maximum temperature, and (5) minimum temperature, mean temperature, and species (Table 4). In contrast, a single model best explained *Rv* infection intensity, and included four variables: minimum temperature, species, location, and precipitation (Table 4).

Discussion

Understanding disease ecology within a community of hosts and pathogens is crucial to understanding when, where, and whether an outbreak is most likely to occur. Despite all individuals in this study appearing healthy at the time of collection with no signs of infection, numerous individuals were infected with at least one of the two pathogens we tested for that are known to cause amphibian declines. Infection prevalence was low or absent in most sampled localities, implying *Bd* and *Rv* are not severely affecting amphibian populations on this island. However, we identified factors that significantly influenced infection prevalence and intensity across months and species, revealing patterns that may be useful in predicting and mitigating future disease outbreaks. For example, as climatic patterns shift, or invasive populations spread, we now have baseline infection dynamics that we can track changes from to assess whether an outbreak is on the horizon.

Table 3. Top Ten *Bd* Prevalence and Intensity Model Values with Their Respective dAICc Values and Weights.

Bd prevalence			*Bd* intensity		
Model	dAICc	Weight	Model	*dAICc*	Weight
Min. temperature	**0.0**	**0.0220**	**Null**	**0.0**	**0.2129**
Mean temperature + min. temperature + precipitation	**0.0**	**0.0217**	Max. temperature	2.5	0.0623
Max. temperature + min. temperature + precipitation	**0.0**	**0.0217**	Mean temperature	2.5	0.0616
Max. temperature + mean temperature + precipitation	**0.0**	**0.0216**	Min. temperature	2.5	0.0606
Max. temperature + mean temperature + min. temperature	**0.1**	**0.0208**	Precipitation	2.6	0.0567
Mean temperature	**0.2**	**0.0199**	Min. temperature + precipitation	5.8	0.0119
Max. temperature	**0.7**	**0.0155**	Mean temperature + precipitation	5.8	0.0118
Mean temperature + max. temperature	**1.0**	**0.0136**	Max. temperature + precipitation	5.8	0.0114
Precipitation + min. temperature	**1.2**	**0.0118**	Mean. temperature + min. temperature	5.9	0.0112
Min. temperature + species	**1.4**	**0.0112**	Mean temperature + max. temperature	5.9	0.0110

The models that best predict *Bd* prevalence are within 2 AICc values from the top model and are indicated in bold text.

Table 4. Top Ten *Rv* Prevalence and Intensity Models with Their Respective dAICc Values and Weights.

Rv prevalence			*Rv* intensity		
Model	dAICc	Weight	Model	dAICc	Weight
Species	**0.0**	**0.0253**	**Min. temperature + species + location + precipitation**	**0.0**	**0.7112**
Species + min. temperature	**0.2**	**0.0233**	Month + location + species + mean temperature	3.9	0.1003
Species + mean temperature	**0.2**	**0.0225**	Month + location + species + max. temperature	7.1	0.0201
Species + max temperature	**0.4**	**0.0211**	Precipitation + location + species + min. temperature	8.4	0.0104
Mean temperature + species + max temperature	**0.4**	**0.0209**	Precipitation + species + location + month	9.5	0.0104
Precipitation + species	**0.6**	**0.0184**	Precipitation + species + location + mean temperature	10.4	0.0040
Max. temperature + species + min. temperature	**0.8**	**0.0171**	Mean temperature + location + month	10.5	0.0037
Mean temperature + species	**1.3**	**0.0131**	Precipitation + mean temperature + location + month	10.5	0.0037
Min. temperature + species + precipitation	**1.5**	**0.0117**	Max. temperature + mean temperature + location + month	10.5	0.0037
Min. temperature + species + mean temperature + max. temperature	**1.6**	**0.0115**	Precipitation + species + location + mean temperature	10.8	0.0032

The models that best predict *Rv* prevalence and intensity are within 2 AIC values from the top model and are indicated in bold text.

Bd prevalence differed significantly based on environmental conditions for the month during which the surveys were conducted and was higher in warmer and wetter months. This pattern contradicts the majority of *Bd* studies which find that prevalence typically increases in cooler months (e.g., Savage et al. 2011); however, the association between higher moisture and increased *Bd* prevalence is consistent with both laboratory and field studies of *Bd* (Lips et al. 2004; Piotrowski et al. 2004). In contrast, *Bd* intensity did not differ significantly regarding any of the factors we measured. This difference is likely due to the low number of positives detected from this study (only 17 total positives), limiting our power to identify any significant trends. Previous studies have found climatic variables to drive *Bd* infection intensity, even when host factors are most important for determining *Bd* infection prevalence (Savage et al. 2015; Horner et al. 2017). Thus, we predict more extensive sampling would reveal that temperature and humidity are important drivers of *Bd* infection intensity on Jekyll island, but at present, our limited sampling does not support this pattern.

Rv prevalence and intensity both differed significantly across sampled species. Namely, the invasive species *E. planirostris* had higher *Rv* infection prevalence and intensity compared to the three native species. Invasive species such as *E. planirostris* can have strong effects on shaping the disease communities in the areas they colonize (Pitt et al. 2005; Crowl et al. 2008; Strauss et al. 2012). For example, *Rana catesbeiana* and the African clawed frog (*Xenopus laevis*) can increase the overall presence of *Bd* or *Rv* in a location and may act as amplification hosts (Hanselmann et al. 2004; Robert et al. 2007; Schloegel et al. 2009; Moutou and Pastoret 2010). *Eleutherodactylus planirostris* in particular has been documented with high pathogen prevalence and intensity in other locations in which they have colonized (Beard et al. 2009). More research is needed to understand the role *E. planirostris* plays in shaping the pathogen landscape throughout its invasive range and potentially triggering disease outbreaks within native hosts. More broadly, we need to increase focus on the role of invasive amphibians in contributing to the rise of EIDs by acting as disease amplifiers or reservoir hosts.

Rv prevalence also differed significantly based on sampling month, with significantly elevated prevalence in September and October of 2014 which on Jekyll Island had average mean temperatures of 26°C and 21°C, respectfully (Supplemental Information 1). This pattern contrasts our prediction based on the overall pattern that *Rv* tends to occur in warmer months of the year, which in Jekyll Island would primarily be the months June–August, which had the highest average temperatures (27–28°C) and maximum temperatures (31°C–32°C) during the study period (Green et al., 2002; Supplemental Information 1). However, experimental infection studies show that the relationship between temperature and *Rv* susceptibility is complex and variable among studies (Brand et al. 2016; Brunner et al. 2017); therefore, further research across climatic regimes and host species is merited on this topic. Furthermore, precipitation was highest in September 2014 when *Rv* prevalence was highest, suggesting that rainfall is more important than temperature for promoting the spread of this waterborne pathogen. If *Rv* is truly linked to cooler temperatures and *Bd* to warmer temperatures in this system, this association helps to explain why we may have only detected one example of coinfection of *Bd* and *Rv*. These two pathogens may have disparate times when infection typically occurs and may overlap only occasionally when one pathogen is decreasing replication and the other is increasing (Gray et al. 2007; Schloegel et al. 2009). However, coinfection may still be important in this system because of a third amphibian EID, Perkinsea, a protist pathogen that infects larval amphibians. Perkinsea often coinfects individuals that also have *Rv* (Landsberg et al. 2013; Isidoro-Ayza et al. 2017). Perkinsea may also have caused more die-offs than *Bd* in North America (Isidoro-Ayza et al. 2017), suggesting that future pathogen surveillance efforts on Jekyll Island and throughout North America should include a molecular test for Perkinsea (Karwacki et al. 2018) to better capture the overall pathogen landscape.

Our study of Jekyll Island provides insight into disease dynamics of a barrier island that serves as a popular tourist destination. Island ecosystems are particularly interesting locations to study EIDs because they are isolated, small, and often house imperiled ecosystems. The demographic and genetic isolation of islands increases the risk of population inbreeding, leading to a decrease in genetic heterozygosity and an increase in infection and morbidity rates (Frankham 2008; Gray et al. 2009). The small surface area of islands also increases contact between infected individuals, potentially increasing the prevalence of these two pathogens on the island (Gray et al. 2009). However, we have minimal empirical data for understanding amphibian pathogens on islands, and mostly understand amphibian pathogens across broader spatial scales within continents. For example, *Bd* is most prevalent in mid-to-high eleva-

tions in tropical areas and can spread in waves (Lips et al. 2008). *Rv* is also found at high elevations, but with no clear pattern of spatial dispersal in anurans (Gray et al. 2009; Brunner et al. 2015). On Jekyll Island, *Bd* is more prevalent in the north, while *Rv* is more prevalent in the south. This is consistent with *E. planirostris* being the putative vector of *Rv*, as the species was potentially first introduced via the main entry road onto the island which is located at the southern end. We do not have historical samples enabling us to explicitly test this scenario, and the small overall size of the island and autocorrelation among sampling sites makes spatial analyses tenuous. Additionally, Jekyll Island may show spatial variation in prevalence due to its multiple environmental zones. Barrier islands are constantly exposed to oceanic mechanisms causing them to endure constant change, which particularly impacts drumstick-shaped islands causing them to experience a gradient in habitat north to south due to their unique shape (Hoyt 1967). Despite these considerations, the introduction of *E. planirostris* on Jekyll Island highlights that non-native species have the potential of spreading EIDs and stresses the importance of disease control in delicate ecosystems.

Conclusion

Despite the low *Bd* and *Rv* prevalence and lack of disease signs among all sampled anurans, both pathogens were present on Jekyll Island, demonstrating the importance of monitoring habitats that may appear to be disease free. We found significant month-to-month variation, suggesting seasonal pathogen drivers that do not match the typically observed patterns of elevated *Bd* in cool months and elevated *Rv* in warm months; instead, *Rv* was significantly more prevalent in cool months and *Bd* showed higher prevalence in warm months. Further, we found significantly elevated *Rv* infections in the one invasive species sampled, suggesting invasive species may contribute to the spread of amphibian pathogens. Due to sample size limitations within each species and sampling location, we were unable to analyze the effect of habitat type or level of disturbance (habitat degradation or human activity) on pathogen prevalence or intensity. This initial study provides a baseline for future sample designs and temporal monitoring efforts that will support inquiry into the influence of these factors. It is essential to initiate and continue long-term monitoring of amphibian populations to understand pathogen threats and the role that invasive species like *E. planirostris* have in altering disease dynamics in native communities.

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