Batrachochytrium dendrobatidis prevalence across different habitats in northwest Ecuador in the direct developing frog *Pristimantis achatinus*

MASTERS THESIS
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ABSTRACT: Amphibians are among the most threatened taxa with approximately 43% of its species being threatened with extinction (Li et al. 2021; Kriger et al. 2007). Two main drivers of amphibian population declines are habitat loss from anthropogenic forces and disease, however; the interaction between these two drivers remains under investigated in many crucial regions deemed as biodiversity hotspots that have also been exploited (Becker et al. 2011). Loss of biodiversity also has consequences on host susceptibility to disease and disease transmission (Searle et al. 2011). Chytridiomycosis is the amphibian diseased caused by *Batrachohytrium dendrobatidis (Bd)* and has led to many amphibians' population declines, predominantly in South America (Li et al. 2021). We investigated how different habitats, and community composition effected prevalence of *Bd* in the terrestrial frog *Pristimantis achatinus (P. achatinus)* in the Mache Chindul Reserve of northwest Ecuador. Prevalence of *Bd* was investigated following a standardized swabbing protocol (Hyatt et al. 2007) via 29 different sites, each site was composed of a paired pasture and forest transect separated by a hard forest edge. We observed no significant effect of habitat type or species richness on *Bd* prevalence. We also observed no effect of habitat type on species richness. Given their high abundance and equivalent rates of infection in all habitat types sampled, we suggest *P. achatinus* may serve as an important vector of *Bd* in our project area.

INTRODUCTION

Over the previous decade approximately 43% of amphibian species have been categorized as threatened by the IUCN (Li et al. 2021; IUCN 2022). Scientists have listed some of the main drivers of amphibian diversity loss as deforestation, climate change, and the emergence of infectious diseases such as chytridiomycosis (Becker et al. 2011; Kilburn et al. 2010). Chytridiomycosis is one of the most prevalent amphibian diseases worldwide - this disease infects the keratinized skin of amphibians on every continent except for Antarctica and it has been responsible for catastrophic population declines in the neotropics (Kilburn et al. 2010; Rebollar et al. 2014).

The Neotropical region encompasses several biodiversity hotspots, each which provide crucial habitats to 2,916 native amphibian species (Li et al. 2021). Despite the importance of the variable ecosystems throughout the Neotropics its landscapes are some of the most heavily exploited by anthropogenic activity (Stuart et al. 2008; Alvarado-Rybak et al. 2021). The Chocó-Darien Global Ecoregion (CGE) of northwest South America is a priority for conservation due to its high endemism and ongoing threats of deforestation (Fagua et al. 2019). In addition to the loss of habitat from deforestation, infectious diseases have been spreading throughout the region which has consequently resulted in many anuran populations die offs in the neotropics (León et al. 2019).

Chytridiomycosis is caused by the pathogenic fungus *Batrachochytrium dendrobatidis (Bd)* and infection occurs when waterborne zoospores of *Batrachochytrium* infect the keratinized skin of amphibians and cause abnormal cell loss, hyperkeratosis, and skin sloughing (Sonn et al. 2019; Kilburn et al. 2010). *Bd* is considered a generalist pathogen because it can be found in a range of different environments of varying qualities (Li et al. 2021; Hill et al. 2021). *Bd* infection load and individual disease outcomes are heavily influenced by environmental factors such as temperature, precipitation, and host species richness (Lambertini et al. 2020). Individual susceptibility also varies greatly across hosts depending on life stage, species, and breeding behavior (Li et al. 2021). Host species life history also plays a large role in disease dynamics for an individual and the evolution behind different host responses is associated with tradeoffs between pathogen exposure and disease defenses (Mesquita et al. 2017; Rumschlag et al. 2020).

Deforestation and chytridiomycosis are two commonly studied drivers of amphibian population declines, however; few studies to date have investigated these drivers simultaneously, despite the overlapping consequences (Rawien et al. 2022; Menéndez-Guerrero et al. 2013). Habitat disturbances from deforestation disrupts the connectedness of aquatic and terrestrial environments which can promote inbreeding, cause changes to microclimate conditions, and alter host-community structure and disease transmission pathways making amphibians more susceptible to infections (Sonn et al. 2019; Antonelli et al. 2018; Becker et al. 2020). Host disease susceptibility is influenced when microhabitat conditions shift out of a species thermal optimum which puts physiological stress on an individual and lowers their defenses against infection (Menéndez-Guerrero et al. 2013). The strong influence abiotic determinants have on disease transmission and host susceptibility make understanding how infection rates vary across individuals in relation to habitat a priority in research for conservation (Lambertini et al. 2020; Rebollar et al. 2014). Changes in biodiversity and species richness resulting from deforestation can also play a role in disease transmissions (Searle et al. 2011). Dilution effects transpire when biodiversity and disease risk are inversely correlated, and amplification effects occur when there is a positive correlation between disease risk and biodiversity (Searle et al. 2011). The overlapping consequences of deforestation, biodiversity, and diseases highlight the importance of including community structure into disease dynamics studies (Searle et al. 2011; Urgiles et al. 2021).

Pristimantis achatinus (P. achatinus) is a direct developing, generalist species that inhabits a range of environments throughout South America (Székely et al. 2016; Acosta-Galvis et al. 2020). *Pristimantis* is the most speciose genus of vertebrate taxa and approximately 30% of its species are endemic to Ecuador (Székely et al. 2016). Individuals can be found in primary forest, secondary forest, and pasture landscapes (Székely et al. 2016; Acosta-Galvis et al. 2020). *Pristimantis achatinus* does not require bodies of water that act as *Bd* reservoirs for survival because individuals lay their eggs in moist patches of leaf litter on the forest floor (Ribeiro et al. 2020). This species is incredibly abundant throughout northwest Ecuador and has been successful in both pasture and forest environments (Mendoza et al. 2015). The wide distribution of *P. achatinus* across various habitats with previously recorded *Bd* presence makes this species an ideal study system to further investigate how environmental factors influence patterns of infection in an understudied population (Burrowes et al. 2017; Moura-Campos et al. 2021).

Our goal in this study was to gain a better understanding of how *Bd* is affecting *P. achatininus* populations in habitats of varying quality. To do so, we sampled *Bd* from individuals encountered in different habitat types along forest edges in northwest Ecuador. We also surveyed each point for anuran species richness and abundance. We hypothesized that there would be more infected individuals in forest habitats as opposed to pastures, and that sites with higher species richness will also have more infected individuals. The data from this study will benefit conservation management strategies for this biodiversity hotspot and provide a deeper understanding of how *Bd* is influencing *P. achatinus* populations. This will help researchers make predictions about the future outlook of this species.

METHODS

Study site

Fieldwork was conducted from June to July in 2021 and from June to August in 2022 in and around the 65,000-ha reserve Mache-Chindul Ecological Reserve (REMACH) in Esmeraldas Province, Ecuador in the Chocó Biogeographic zone. This region undergoes its wet season from January to June and receives about 2000-3000 mm of yearly rainfall (Clark et al. 2006). All sampling took place within the privately owned research station Fundación para la Conservación de los Andes Tropicales (FCAT). The landscape surrounding FCAT contains fragments of intact Humid Premontane and Humid Tropical Forest zones, regenerating pastures, active cattle pastures, cacao, and other crops. Despite the altered landscapes, this critical region remains a biodiversity hotspot and exhibits extremely high levels of amphibian endemism (Clark et al. 2006; Longo et al. 2015).

For the collection period of 2021 we sampled 33 different sites consisting of forest and pasture habitats. Every site was sampled once for diurnal collection and once for nocturnal collection over an 8-week period. We sampled every location for 15-minute periods and swabbed any individual caught within a 10m circumference of the marked point. All individuals were caught by hand and swabbed for *Bd* following the standardized protocol discussed in the ***Bd* Detection** section of this paper.

For the 2022 field season we established 29 paired transects, each of which ran perpendicular to “hard edges” between forest and pasture habitat. Paired transects originated at the forest-pasture transition and extended 30m x 10m in opposite directions, i.e., one transect into forest and a second into pasture habitats. To ensure all points were independent of each other, paired transects were established at least 125 meters apart. This distance was formulated from previous literature which states motile zoospores of *Bd* rarely travel farther than 2cm in an aquatic environment before encysting and 100% of zoospores face mortality within 3 hours of drying, which makes long-term persistence in ephemeral ponds unlikely, and supports that 125 meters on terrestrial land is sufficient spacing between sites (Kriger et al. 2007; Bishir et al. 2018). The mean elevation of all transects was 456 +/- 62m (range: 305 - 535). The was no effect of elevation on richness or probability of *Bd* infection ($p > 0.3$ for both tests), so we excluded this variable from additional analysis.

Richness surveys and Sample Collection

Visual-encounter surveys (VES) were conducted at each transect, once diurnally and once nocturnally to assess community structure (Hutchens et al. 2009). We randomized the sites visited for sampling, the same site was never sampled more than once a day and neighboring sites were never visited consecutively, the goal in doing this was to avoid recording the same individual twice. The VES were completed by JL and KP and consisted of slowly walking the entire area of each transect, lifting substrate, listening for audio cues, checking trees and other vegetation, and checking under any possible covers such as rocks that individuals may be utilizing.

Bd Detection

Swabbing for *Batrachochytrium dendrobatidis (Bd)* occurred at night. *Pristimantis achatinus* individuals were captured and swabbed to detect for the *Bd* pathogen using the standard *Bd* swabbing protocols in Hyatt (2008) and Kriger (2007). Briefly, using sterile specimen swabs, individuals were swabbed 5 times each in each of the following locations (1) dorsal surface (2) sides, from groin to armpit (3) ventral surface (4) undersides of thighs (5) undersides of each foot. After an individual was swabbed, it was placed in a clean 20 x 25 cm plastic bag until the collection period ended to avoid re-capture. Swabs were kept in 96 percent ethanol at 0 degrees Celsius until analysis.

Laboratory analysis

All swabs were transported to Universidad San Francisco de Quito where collaborator JG ran PCR analyses to test for presence of infection. DNA was extracted from swabs following a standard PCR Thermal Cycler protocol (Chan et al. 2016). Molecular detection of *Bd* was done by following a modified Boyle *et al.*, 2004 protocol for the qPCR assay.

Statistical Analysis

We recorded richness and abundance for all species, for accurate identification of species individuals that could not be identified in the field, lateral, dorsal, and ventral photographs were taken, and individuals were later classified with the help of three resources: the Museum of

Zoology of the Pontificia Universidad Católica del Ecuador, BioWeb Ecuador, previous literature with photo vouchers, and assistance from local experienced experts. We checked the data for normality using the Shapiro-Wilk normality test, then ran a two-tailed t-test to evaluate any difference in species richness between forest and pasture habitats, we then ran a chi-squared test to look for any difference in *Bd* prevalence between the years 2021 and 2022 for *P. achatinus*. To calculate rate of infection between habitats we divided the total number of forest sites with more infected individuals by the total number of sites in the study and multiplied that sum by 100 to determine the percent rate of infection ((12 / 29) * 100). We ran a binomial generalized linear mixed model (GLMM) looking at presence or absence of *Bd* as a response variable and species richness and habitat type as predictors. We also included an interaction effect between habitat type and species richness and included a random effect of transect type (pasture vs. forest) in the model to control for the paired design of the transects.

Statistical analyses were run in R v 4.1.1 using packages GLMM and GGPLOT2.

RESULTS

Species Richness

We recorded 768 individuals from 17 species, 10 genera, and 5 families across our 29 paired sampling sites (table1). *Pristimantis achatinus* made of 75.6% of the recorded individuals. Two of the recorded species are listed as vulnerable by the IUCN, *Pristimantis rosadoi* and *Leptodactylus peritoaktites* (IUCN 2022). The mean species richness forest sites was 2.55 +/ 1.23 and for pasture sites mean species richness was 2 +/ 1.09 (range: 1 – 6 species). There was no difference in species richness between pasture and forest sites (paired t-test: t (57) = 1.744, P

= 0.086). There was also no statistical difference in individual abundance between forest and pasture sites (paired t-test: t (58) = 0.215, P = 0.830). Mean abundance for forest sites was 13.1 +/ 105.5, pasture sites had a mean abundance of 12.5 +/ 102.6).

Bd Detection

Bd sampling took place from June to July in 2021 and 2022 for *P. achatinus*. Infection rate was higher in 2022 than in 2021 (59% vs. 38%; $X^2 = 10.127$, $P = 0.002$).

Bd was widespread across all sampling sites and the paired t-test resulted in no statistical difference in the rate of infection between pasture vs. forest transects (t (57) = 55.48, P = 0.500); the mean infection rate for both pasture and forest sites was 61.2%.

There were no statistically significant impacts of both species' richness (P = 0.630) and habitat type (P = 0.185) on *Bd* prevalence (Fig. 1). There was no significant interaction effect between species richness and habitat on *Bd* prevalence (P = 0.145) and the random effect of transect had little influence on *Bd* prevalence between transects.

DISCUSSION

The goal of this study was to investigate how different habitats and community composition effect the prevalence of *Bd* in populations of *P. achatinus* within the Chocó Biogeographic zone. There has been little research to date examining *Bd* prevalence around the Mache Chindul reserve in northwest Ecuador and the available literature on *Bd* in terrestrial frog species is extremely limited. The lack of knowledge pertaining to *Bd* distribution in this region made this study a priority for conservation. We aimed to close some of the existing gaps in research by taking a further look at the overlapping consequences of biodiversity loss and altered habitats on disease prevalence. Our decision to study an abundant, generalist species of frog that is a known carrier of *Bd* helped us take a closer look at how *Bd* could be spreading around the reserve. Choosing a broadly distributed, highly successful species also accounted for any possible bias in the study associated with a specialized species and their environmental limitations.

We found no major difference in species richness between forest and pasture habitats. However, the maximum number of species recorded was 6 and this level of biodiversity was only observed once in a pasture habitat. Species abundances also did not differ between habitat types. The highest abundance recorded occurred at a transect with an available annual water source, however; the high abundance observed was due to sampling a breeding location for the toad species *Rhinella alata* shortly after a population tadpoles metamorphosed into toadlets.

Between the collection periods of 2021 and 2022 we found a significant difference in infection rates across *P. achatinus* populations. *Bd* was abundant in pasture dwelling individuals in both 2021 and 2022 which alludes to the idea that *P. achatinus* individuals may be acting as transmission vectors in environments where *Bd* cannot survive independent of a host. This raises concern for specialized species that are more vulnerable to lethal infection, especially those that have habitat overlap with *P. achatinus* populations.

Habitat type and community composition had no effect on *Bd* prevalence. This result may correlate to the high abundance of *P. achatinus* and this species ability to persist in a range of different environments as well. There have not been any documented *P. achatinus* population declines for this specific area of the Chocó Biogeographic zone which suggests that individuals persisting with infection may be spreading infection between environments, causing infection rates to be similar. However, we remain unsure if populations and infected individuals move between habitats or if they tend to travel between habitats of similar environmental conditions. Although infection rates did not differ significantly between habitats, we observed the beginning of a trend where higher species richness was associated with a lower probability of *Bd* in an individual (Fig. 1). This observed trend supports the Dilution Effect Hypothesis which states greater biodiversity is correlated with lowered risk of disease and a decreased rate of pathogen transmission (Searle et al. 2011).

The results of this study highlight both the broad distribution of *Bd* around the Mache Chindul Reserve, and the high infection rates across different populations of *P. achatinus*. Our study also suggests that *Bd* prevalence is increasing over time around the reserve. Our finding that habitat type does not affect *Bd* prevalence differs from previous literature stating *Bd* should have increased success and abundances in non-degraded environments (Agostini et al. 2015; Smith et al. 2009). Future directions should include revisiting the Mache Chindul Reserve and resampling *P. achatinus* populations to determine the yearly rate at which *Bd* is increasing in this species. Future studies should also focus on different life stages and sex of *P. achatinus* individuals, in addition to infection intensity to better assess how *Bd* effects individuals at different stages. A deeper understanding of how *Bd* effects individuals relative to life stage will be useful for making predictions about the stability of future populations.

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LITERATURE CITED

Acosta-Galvis, A. R., Saldarriaga-Gómez, A. M., Ramírez, B., & Vargas-Ramírez, M. (2020). A new Terrarana frog of genus *Pristimantis* from an unexplored cloud forest from the eastern Andes, Colombia. *ZooKeys, 961*, 129–156. https://doi.org/10.3897/zookeys.961.51971

Agostini, M. G., & Burrowes, P. A. (2015). Infection patterns of the CHYTRID fungus, Batrachochytrium dendrobatidis, on Anuran assemblages in agro-ecosystems from Buenos Aires Province, Argentina. *Phyllomedusa: Journal of Herpetology*, *14*(2), 113. https://doi.org/10.11606/issn.2316-9079.v14i2p113-126

Alvarado-Rybak, M., Lepe-Lopez, M., Peñafiel-Ricaurte, A. *et al.* Bioclimatic and anthropogenic variables shape the occurrence of *Batrachochytrium dendrobatidis* over a large latitudinal gradient. *Sci Rep* **11**, 17383 (2021). https://doi.org/10.1038/s41598-021-96535-w

Antonelli A, Ariza M, Albert J, Andermann T, Azevedo J, Bacon C, Faurby S, Guedes T, Hoorn C, Lohmann LG, Matos-Maraví P, Ritter CD, Sanmartín I, Silvestro D, Tejedor M, ter Steege H, Tuomisto H, Werneck FP, Zizka A, Edwards SV. 2018. Conceptual and empirical advances in Neotropical biodiversity research. *PeerJ* 6:e5644 https://doi.org/10.7717/peerj.5644

Becker, G., & Zamudio, K. (2011). Tropical amphibian populations experience higher disease risk in natural habitats. *PNAS*, *108*(24), 9893–9898. https://doi.org/https://doi.org/10.1073/pnas.1014497108

Bishir, S. C., Hossack, B. R., Fishback, L. A., & Davenport, J. M. (2018). Post-breeding movement and habitat use by wood frogs along an Arctic–subarctic ecotone. *Arctic, Antarctic, and Alpine Research*, *50*(1). https://doi.org/10.1080/15230430.2018.1487657

Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T., & Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, *60*, 141–148. https://doi.org/10.3354/dao060141

Burns, T.J., Scheele, B.C., Brannelly, L.A., Clemann, N., Gilbert, D. and Driscoll, D.A. (2021), Indirect terrestrial transmission of amphibian chytrid fungus from reservoir to susceptible host species leads to fatal chytridiomycosis. Anim. Conserv., 24: 602-612. https://doi.org/10.1111/acv.12665

Burrowes, P. A., Martes, M. C., Torres-Ríos, M., & Longo, A. V. (2017). Arboreality predicts *batrachochytrium dendrobatidis* infection level in tropical direct-developing frogs. *Journal of Natural History*, *51*(11-12), 643–656. https://doi.org/10.1080/00222933.2017.1297504

Chan K, Wong P-Y, Yu P, Hardick J, Wong K-Y, Wilson SA, et al. (2016) A Rapid and Low-Cost PCR Thermal Cycler for Infectious Disease Diagnostics. PLoS ONE 11(2): e0149150. https://doi.org/10.1371/journal.pone.0149150

Clark, J. L., et al. (2006). "Floristic checklist of the Mache-Chindul mountains of Northwestern Ecuador." Contributions from the United States National Herbarium: 1-180.

Fagua, J. C., & Ramsey, R. D. (2019). Geospatial modeling of land cover change in the Chocó-darien global ecoregion of South America; one of most biodiverse and rainy areas in the world. *PLOS ONE*, *14*(2). https://doi.org/10.1371/journal.pone.0211324

Hyatt, Alex & Boyle, D & Olsen, Veronica & Boyle, David & Berger, Lee & Obendorf, David & Dalton, A & Kriger, K. & De Los Heros, Martin & Hines, Harry & Phillott, R & Campbell, Ria & Marantelli, Gerry & Gleason, Frank & Coiling, A. (2007). Diagnostic assay and sampling protocols for the detection of Batrachochytrium dendrobatidis. Diseases of aquatic organisms. 73. 175-92. 10.3354/dao073175.

Hutchens, S.J. and DePerno, C.S. (2009), Efficacy of Sampling Techniques for Determining Species Richness Estimates of Reptiles and Amphibians. Wildlife Biology, 15: 113-122. https://doi.org/10.2981/08-024

IUCN. 2022. The IUCN Red List of Threatened Species. Version 2022-1. https://www.iucnredlist.org. Accessed on [2022].

Jongsma, G. F., Hedley, R. W., Durães, R., & Karubian, J. (2014). Amphibian diversity and species composition in relation to habitat type and alteration in the Mache–Chindul Reserve, Northwest Ecuador. *Herpetologica*, *70*(1), 34. https://doi.org/10.1655/herpetologica-d-12-00068

Kilburn, V.L., Ibáñez, R., Sanjur, O. *et al.* Ubiquity of the Pathogenic Chytrid Fungus, *Batrachochytrium dendrobatidis*, in Anuran Communities in Panamá. *EcoHealth* **7**, 537–548 (2010). https://doi.org/10.1007/s10393-010-0634-1

Kleemann J, Zamora C, Villacis-Chiluisa AB, Cuenca P, Koo H, Noh JK, Fürst C, Thiel M. Deforestation in Continental Ecuador with a Focus on Protected Areas. *Land*. 2022; 11(2):268. https://doi.org/10.3390/land11020268

Kriger, K.M. and Hero, J.-M. (2007), The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. Diversity and Distributions, 13: 781-788. https://doi.org/10.1111/j.1472-4642.2007.00394.x

Lambertini, C., Becker, C. G., M. Belasen, A., Valencia-Aguilar, A., Nunes-de-Almeida, C. H., Betancourt-Román, C. M., Rodriguez, D., da Silva Leite, D., Oliveira, I. S., Gasparini, J. L., Ruggeri, J., Mott, T., Jenkinson, T. S., James, T. Y., Zamudio, K. R., & Toledo, L. F. (2021). Biotic and abiotic determinants of Batrachochytrium dendrobatidis infections in amphibians of the Brazilian Atlantic Forest. *Fungal Ecology*, *49*, 100995. https://doi.org/10.1016/j.funeco.2020.100995

Longo, A. V., Savage, A. E., Hewson, I., & Zamudio, K. R. (2015). Seasonal and ontogenetic variation of skin microbial communities and relationships to natural disease dynamics in declining amphibians. *Royal Society Open Science*, *2*(7), 140377. https://doi.org/10.1098/rsos.140377

Mendoza, Á. M., Ospina, O. E., Cárdenas-Henao, H., & García-R, J. C. (2015). A likelihood inference of historical biogeography in the world's most diverse terrestrial vertebrate genus: Diversification of direct-developing frogs (Craugastoridae: Pristimantis) across the Neotropics. *Molecular Phylogenetics and Evolution*, *85*, 50–58. https://doi.org/10.1016/j.ympev.2015.02.001

Menéndez-Guerrero, P.A. and Graham, C.H. (2013), Evaluating multiple causes of amphibian declines of Ecuador using geographical quantitative analyses. Ecography, 36: 756-769. https://doi.org/10.1111/j.1600-0587.2012.07877.x

Moura-Campos, D., Greenspan, S. E., DiRenzo, G. V., Neely, W. J., Toledo, L. F., & Becker, C. G. (2021). Fungal disease cluster in tropical terrestrial frogs predicted by low rainfall. *Biological Conservation*, *261*, 109246. https://doi.org/10.1016/j.biocon.2021.109246

Pontifical Catholic University of Ecuador. 2021. QCAZ Museum of Zoology amphibian collection database. Version 2021.0. Available at <https://bioweb.bio/portal/> Access: January 31, 2021.

Rebollar EA, Hughey MC, Harris RN, Domangue RJ, Medina D, Ibáñez R, et al. (2014) The Lethal Fungus *Batrachochytrium dendrobatidis* Is Present in Lowland Tropical Forests of Far Eastern Panamá. PLoS ONE 9(4): e95484. https://doi.org/10.1371/journal.pone.0095484

Ribeiro, J. W., Siqueira, T., DiRenzo, G. V., Lambertini, C., Lyra, M. L., Toledo, L. F., Haddad, C. F., & Becker, C. G. (2020). Assessing amphibian disease risk across tropical streams while accounting for Imperfect Pathogen Detection. *Oecologia*, *193*(1), 237–248. https://doi.org/10.1007/s00442-020-04646-4

Rumschlag, S. L., & Boone, M. D. (2020). Amphibian infection risk changes with host life stage and across a landscape gradient. *Journal of Herpetology*, *54*(3). https://doi.org/10.1670/19-107

Searle, C. L., Biga, L. M., Spatafora, J. W., & Blaustein, A. R. (2011). A dilution effect in the emerging amphibian pathogen *batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences*, *108*(39), 16322–16326. https://doi.org/10.1073/pnas.1108490108

Smith, K. G., Lips, K. R., & Chase, J. M. (2009). Selecting for extinction: nonrandom disease-associated extinction homogenizes amphibian biotas. *Ecology Letters*, *12*, 1069–1078. https://doi.org/10.1111/j.1461-0248.2009.01363.x

Sonn, J. M., Utz, R. M., and Richards-Zawacki, C. L.. 2019. Effects of latitudinal, seasonal, and daily temperature variations on chytrid fungal infections in a North American frog. *Ecosphere* 10(11):e02892. 10.1002/ecs2.2892

Urgiles, V.L., Ramírez, E.R., Villalta, C.I. *et al.* Three Pathogens Impact Terrestrial Frogs from a High-Elevation Tropical Hotspot. *EcoHealth* **18**, 451–464 (2021). https://doi.org/10.1007/s10393-021-01570-8

Species	Forest Transects ≥ 1 Individual	Total Forest Individuals	Pasture Transects ≥ 1 Individual	Total Pasture Individuals	Total Transects	Total Individuals	IUCN Rating
Pristimantis achatinus	**30**	**259**	**30**	**322**	**60**	**581**	**LC**
Pristimantis rosadoi	**2**	**2**	**0**	**0**	**2**	**2**	**VU**
Pristimantis illotus	**1**	**1**	**1**	**2**	**2**	**3**	**NT**
Pristimantis latidiscus	**2**	**2**	**0**	**0**	**2**	**2**	**LC**
Pristimantis walkeri	**5**	**5**	**0**	**0**	**5**	**5**	**LC**
Craugastor longirostris	**2**	**2**	**0**	**0**	**2**	**2**	**LC**
Pristimantis subsigillatus	**0**	**0**	**1**	**1**	**1**	**1**	**LC**
Pristimantis esmeraldas	**3**	**6**	**2**	**2**	**5**	**8**	**LC**
Rhinella alata	**7**	**53**	**6**	**26**	**13**	**79**	**DD**
Epipedobates boulengeri	**8**	**28**	**0**	**0**	**8**	**28**	**LC**
Hyloxalus awa	**7**	**22**	**1**	**1**	**8**	**23**	**LC**
Oophaga sylvatica	**4**	**7**	**4**	**6**	**8**	**13**	**NT**
Boana rosenbergi	**0**	**0**	**2**	**2**	**2**	**2**	**LC**
Engystomops pustulatus	**0**	**0**	**1**	**1**	**1**	**1**	**LC**
Leptodactylus ventrimaculatus	**1**	**2**	**6**	**8**	**7**	**10**	**LC**
Leucostethus bilsa	**2**	**2**	**1**	**1**	**3**	**3**	**LC**
Leptodactylus peritoaktites	**0**	**0**	**2**	**2**	**2**	**2**	**VU**

Table 1: Anuran species richness and abundance recorded from VES at each site and their IUCN red list ratings. (LC- least concern, NT- near threatened, VU- vulnerable, DD- data deficient)

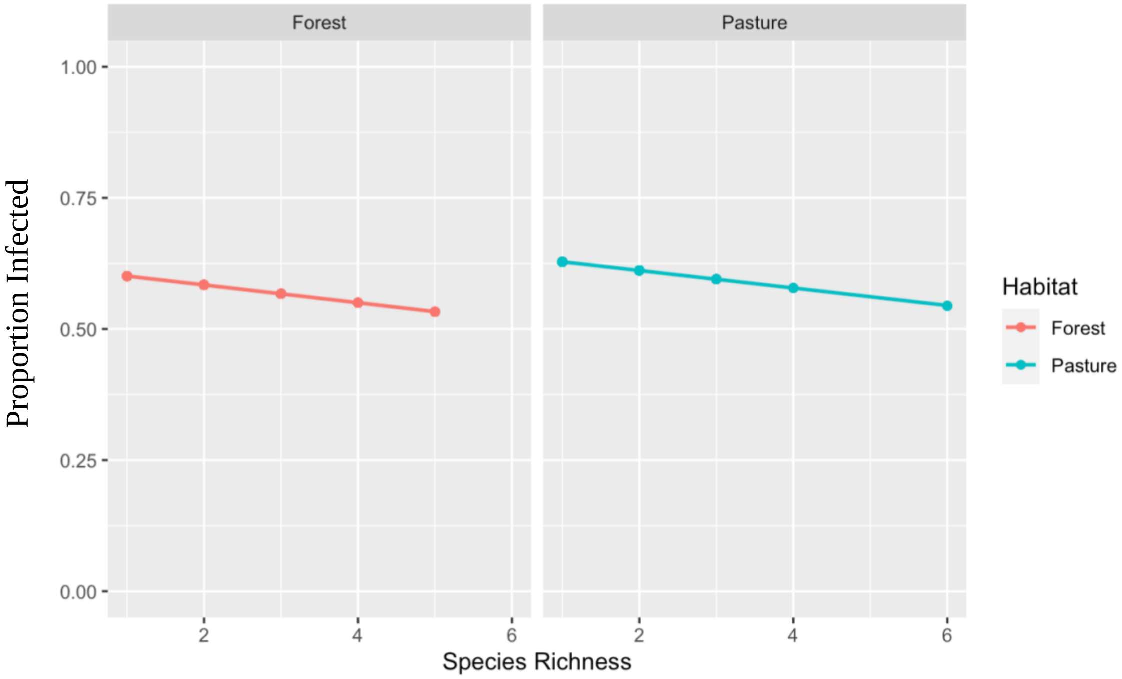

Figure 1: Proportion of infected individuals relative to habitat and species richness.