



**Response of an *Eleutherodactylus coqui* Population to Hurricane Maria:  
Demography and Disease Dynamics**

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## **Dedication**

To my beloved grandfather, Roberto Jiménez, who supported me since day one.



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## Introduction

There is an overwhelming amount of evidence indicating declines and local extinctions of amphibian populations as a direct effect of global climate shifts (Stuart et al. 2005, Mendelson et al. 2006). Recently, a review suggested that amphibians are the least studied vertebrate taxon concerning how they respond to extreme climatic events and showed they are negatively affected by phenomena such as cyclones and droughts (Maxwell et al. 2019). Amphibians are especially vulnerable to local abiotic regimes, and changes in these may limit their survival because of the physiological constraints intrinsic to their evolutionary history (Vitt and Cadwell 2014). Also, it has been suggested that the effect of global warming may be higher for tropical ectotherms because they tend to be thermoconformers, adapted to narrower temperature ranges than temperate species (Navas 1996a, 1996b, Huey et al. 2009). However, amphibian declines have also been associated with other anthropogenic causes, such as habitat loss/change (Bolaños et al. 2008, Hof et al. 2011, Bishop et al. 2012), invasive species (Bishop et al. 2012, Nunes et al. 2019), over-exploitation (Carpenter et al. 2009), pesticides and environmental toxins (Bishop et al. 2012), and emerging and re-emerging infectious disease (Bishop et al. 2012, Kolby and Daszak 2016, Scheele et al. 2019). In particular, amphibians are declining drastically due to a pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (Bd, Longcore et al. 1999) that causes a skin disease known as chytridiomycosis (Berger et al. 1998). Consequently, amphibian populations are disappearing at an alarming rate, which has raised the attention of conservation biologists around the world (Mendelson et al. 2006, Skerratt et al. 2007, Wake and Vredenburg 2008, Scheele et al. 2019).

Amphibians have are valuable to humankind as a food source, global pet trade, medicine, and ecosystem services (Bishop et al. 2012, O’Hanlon et al. 2018). They are also considered bioindicators because they can determine the conditions of the ecosystem they inhabit (Dunson et al. 1992, Welsh and Ollivier 1998). Studies show that amphibians are especially sensitive to environmental change because their unprotected permeable skin makes them prone to desiccation and toxin absorption (Dunson et al. 1992, Welsh and Ollivier 1998, Vitt and Cadwell 2014, Wake and Koo 2018). Precisely because of their fragile skin, it makes amphibians hard to mark and follow through time. Various techniques have been used to mark adults, such as toe-clipping (Stewart and Woolbright 1996, Burrowes et al. 2004), radiotelemetry (McGarrity and Johnson 2010), and pit tags (Pyke 2005), but these techniques have not been used successfully for earlier ontogenetic stages. Marking young metamorphs or juveniles of direct-developing frogs is especially challenging due to their small body size. Following early age classes through time is necessary for understanding aspects of population biology from demography that can inform life tables, to disease dynamics. Addressing synergistic effects in amphibian populations, like extreme climatic disturbances and disease, is complex and requires following individuals through time. Under a climate change scenario, it is important to understand how amphibians will respond to the synergies of environmental and anthropogenic origin.

In September of 2017, Hurricane Maria hit Puerto Rico as a strong category 4 storm with maximum sustained winds of 155 mph. High energy winds spread at 280 km/hr over the island along with heavy rainfall resulting in catastrophic flooding in many areas (NOAA 2017) and considerable forest damage across the island (Hall et al. 2020,

Burrowes et al. 2021). In this thesis, we sampled the effect of Hurricane Maria on a highland population of the Puerto Rican endemic frog *Eleutherodactylus coqui*. In particular, we addressed potential changes to the population structure and to the disease dynamics at El Yunque National Forest. Long-term population trends and several aspects of host-pathogen interactions for *E. coqui*–*Bd*, have been studied at this locality (Burrowes et al. 2004, Longo et al. 2009, Longo and Burrowes 2010) Although other studies have addressed the consequence of hurricanes to *coqui* populations (Woolbright 1991, Klawinski et al. 2014), to our knowledge, no study has questioned the effect of environmental disasters, like hurricanes, in the dynamics of endemic pathogens such as *Bd*. Predicting the outcome for forest-dwelling amphibians is challenging because the environmental interactions between amphibians and this pathogen are complex. In the Brazilian Atlantic forest, Becker and Zamudio (2011) found that amphibians were at lower risk of chytridiomycosis in open forest edge or in cleared areas because of the increased temperature caused by the lack of canopy was unfavorable for *Bd* growth. Thus, we might expect that the storm blowout could result in lower prevalence and intensities of *Bd* infection among *coqui* frogs. Other studies have shown that increased physiological stress leads to added vulnerability of amphibians to pathogens, including *Bd* (Rollins-Smith et al. 2011, Woodhams et al. 2020). Hence, after the habitat devastation and associated environmental stressors brought about by Hurricane Maria, we could also expect greater susceptibility to *Bd* in *E. coqui* hosts.

The first two chapters of this thesis contribute to baseline data for population structure and prevalence and infection intensity in highland *E. coqui*, respectively. Assessing how hurricanes influence the population structure of tropical animals may

reveal underlying mechanisms of persistence of under intensifying global change phenomena. This is especially important for species of amphibians persisting in enzootic conditions, because like *E. coqui*, they may seem relatively stable, but they are still susceptible to Bd, and environmental disturbances may exacerbate their vulnerability (Burrowes et al. 2004, Pounds et al. 2006). The last chapter evaluates the effectiveness of a marking technique for juveniles of *E. coqui*. Identifying a marking system that can effectively follow young metamorphs as they transition to adulthood critical for studying aspects of population dynamics and demography. By re-sampling marked young individuals in a cohort, we can learn about Bd infection levels at a specific age, how infection changes through time, and how many juveniles in a cohort survive to the next generations (sub-adults and adulthood). With this information, we can infer the contribution of younger ontogenetic stages of direct-developing frogs towards the enzootic persistence of Bd infection, something that has not been done before. Overall, this thesis provides information that serves as baselines to predict, compare, and evaluate future changes to biodiversity in tropical areas.

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**Response of an *Eleutherodactylus coqui* Population to Hurricane Maria:  
Demography and Disease Dynamics**

**Chapter 1: Effect of Hurricane Maria on the population structure and demographics of *Eleutherodactylus coqui***

## Abstract

The Caribbean region is expected to face an increase in frequency and intensity of climatic disturbances, such as hurricanes. Amphibians and reptiles are especially vulnerable to local abiotic regimes and their response to changes are limited by physiological constraints intrinsic to their evolutionary history. In September of 2017, El Yunque National Forest in Puerto Rico experienced extensive forest damage by the effect of hurricane Maria. In this study, we assessed the effect of hurricane Maria on the abundance, age-specific survivorship, detection probability, and expected gains of a well-studied high-elevation population of *Eleutherodactylus coqui*. We hypothesize that the population structure would change due to the canopy loss and consequent habitat disturbances caused by the storm blowout. To measure abundance estimates, we used a multi-state N-mixture model to analyze the changes in age-specific demographics on *E. coqui* population. We found that hurricane Maria had a negative impact on local expected gains and abundance estimates of *E. coqui* that caused a disruption in the typical demographic pattern as age classes transition through the warm-wet and cool-dry seasons in Puerto Rico. However, no drastic declines were observed. These results point to plasticity in the response of *E. coqui* to severe environmental events. Recording changes in high-elevation *E. coqui* population structure may offer insight to how other endangered sympatric species would respond to such phenomena. As stronger and more frequent tropical storms are predicted soon, this study provides baseline data that can help predict, compare, and evaluate future changes to biodiversity in critical areas.

Keywords: Severe environmental effects, Hurricane Maria, *Eleutherodactylus coqui*, population structure

## Introduction

Tropical weather disturbances, such as hurricanes, are expected to increase in frequency and intensity (Goldenberg et al. 2001, Kossin et al. 2020). Consequently, to these storms, a dramatic alteration of habitat composition may occur, resulting in changes in the dynamics of populations. Several studies on various taxa have recorded hurricane-induced changes in tropical regions showing shifts in population abundance, species richness, and diversity (e.g. Woolbright 1991, Fogarty et al. 2009, Schriever et al. 2009, Edmunds 2019, Marroquín-Páramo et al. 2021). Although animal populations vary in their response to hurricanes, some herpetofauna assemblages may exhibit resilience until a specific hurricane intensity threshold is overcome (Marroquín-Páramo et al. 2021). The cumulative effects of two hurricanes (within 4 years) in a dry tropical forest in Mexico was associated to a significant decrease in abundance of anurans, lizards, and snakes, but the effect on species richness varied by group depending on the forest formation (Marroquín-Páramo et al. 2021). In addition, this study suggested that hurricanes can affect long-term colonization and contribute to the extinction of amphibian and reptile populations (Marroquín-Páramo et al. 2021).

As ectotherms, amphibians and reptiles are especially vulnerable to local abiotic regimes and their response to changes are limited by physiological constraints intrinsic to their evolutionary history (Vitt and Cadwell 2014). It has been suggested that the effect of global warming may be greater for tropical ectotherms because they tend to be thermoconformers, adapted to narrower temperatures ranges than temperate species (Navas 1996a, 1996b, Huey et al. 2009). In the Caribbean, the effect of recent climate

change is evidenced the rise of air temperatures (Stephenson et al. 2014, Menéndez-Guerrero et al. 2020), and changes in rain patterns (Burrowes et al. 2004, Neelin et al. 2006). A study in the eastern highlands of Puerto Rico, documented an increase in temperature at El Yunque of approximately 1°C in the last 30 years of the past century, as well as an increase in the number of dry periods (Burrowes et al. 2004). This increase in temperature has been associated to physiological responses of *Eleutherodactylus coqui* frogs such as changes in their vulnerability to disease (Longo and Burrowes 2010), and in the characteristics of male advertisement calls in an elevational gradient (Narins and Meenderink 2014). Thus, tropical, terrestrial direct developing frogs in the genus *Eleutherodactylus* may be especially susceptible to the temperature changes that the loss of canopy due to hurricane blowouts may cause.

In September of 2017, El Yunque National Forest experienced extensive forest damage due to the sequential effect of indirect category 5 hurricane Irma and direct category 4 hurricane Maria (Burrowes et al. in press, Hall et al. 2020). Hurricane Maria swept over Puerto Rico with high energy winds of up to 280 km/h and heavy rains, resulting in catastrophic flooding in many areas (NOAA 2019). This hurricane produced the single largest maximum rainfall event in Puerto Rico since 1956, and the highest precipitation out of 129 storms that have impacted the island since that year (Keellings and Hernández Ayala 2019). Studies on the response of lowland *Eleutherodactylus coqui* to other, less intense hurricanes in Puerto Rico provide the basis to formulate hypotheses on the effect of hurricane Maria at higher elevations where the forest damage may have been more severe. In the Luquillo Experimental Forest (LEF) a study on the effect of hurricane Hugo (category 3 in 1989) revealed an increase in adults,

albeit at a smaller body size, and a decrease in juveniles (Woolbright 1991, 1996). The increment of adults was attributed to a greater availability of retreat sites among the fallen debris, and to a decrease in invertebrate predators; the reduction of juveniles was explained by a decrease in reproductive activity due to changes in microclimate (Woolbright 1991). Also, at the Luquillo Experimental Forest, another study evaluated the effect of two consequences of hurricanes: canopy disturbance and increased deposition of debris on a population of *E. coqui* (Klawinski et al. 2014). In contrast to the results of Woolbright (1991), this study found that while canopy disturbances were detrimental to *E. coqui* abundance in experimental plots, an increase in debris in the forest floor had no effect (Klawinski et al. 2014).

The effects of climatic disturbances are complex and tend to affect many ecosystem services simultaneously (Lugo 2008). Thus, to study the consequence of a particular event on the dynamics of a population for which previous data is available provides an opportunity to associate observed responses to specific environmental changes. These findings have the potential to advance our understanding of evolutionary processes and allow us to make informed recommendations to conservation practitioners on species that need to be prioritized after severe climatic events. In this study, we assessed the effect of Hurricane Maria on the abundance, age-specific survivorship, detection probability, and expected gains of a well-studied highland population of *Eleutherodactylus coqui* in el Yunque National Forest. Based on previous findings, we hypothesize that the population structure would change due to the canopy loss and consequent habitat disturbances caused by the storm blowout. Particularly, we expected to find a decrease in the abundance of juveniles and sub-adults, and an



increase of adult *E. coqui* individuals (Woolbright 1991). Since juvenile frogs have a larger surface to volume ratio than the adult frogs, they are more susceptible to desiccation, and this may be exacerbated by increased ambient temperatures brought about by the loss of canopy (Klawinski et al. 2014, Burrowes et al. 2021). Because seasonal changes in temperature and rainfall in Puerto Rico have the potential to affect movement patterns (Woolbright 1985), abundance (Stewart 1995, Burrowes et al. 2004), reproduction (Joglar 1998) and vulnerability to disease (Longo et al. 2010) of *Eleutherodactylus coqui*, we will evaluate the impact of hurricane Maria through time taking into consideration the seasonal change pre and post-hurricane Maria.

## Methods

### *Focal Species*

The common coquí frog, *Eleutherodactylus coqui*, is a widespread native species throughout Puerto Rico including its full elevational gradient from 0 to 1189 m.a.s.l. (Joglar 1998). Body size correlates with elevation, where low elevation populations are smaller compared to high elevation populations (Narins and Hurley 1982, Joglar 1998). At our study site, the average size for females and males is 46.4 and 36.4 mm respectively (Joglar 1998). The marked sexual dimorphism by size in this species, with female body size 27.5–29% larger than males, is possibly a result of reproductive constraints related to the production of large direct-developing eggs by females and the cost of calling by males (Woolbright and Stewart 1987, Woolbright 1989, Joglar 1998). *E. coqui* male reproductive behavior has a fitness trade-off between calling and parental

care, both events of high energy consumption (Woolbright and Stewart 1987, Joglar 1998).

Juveniles attain reproductive maturity within a year after hatching (Woolbright 1996), and adults reproduce year round, although an increase in reproduction has been observed during the warm-wet (WW) season (May–December) in comparison with the cool-dry (CD) season (January–April) of Puerto Rico (Woolbright 1989, Joglar 1998). Age classes of *E. coqui* can be distinguished by body size and these vary with elevation (Joglar 1998). At our study site at El Yunque we consider juveniles individuals with snout-vent length (SVL) up to 17.99 mm, subadults those with SVL 18–27.99 mm, and adults those larger than 28 mm. Population densities of *E. coqui* can vary widely, and in a study at El Verde forest at 351 m, they recorded 1–24 adults and 1–230 frogs of all sizes in 100 m<sup>2</sup> (Stewart and Pough 1983). At times, juveniles and subadults can be more abundant than adults and are more likely to be predated by large invertebrates (Formanowicz et al. 1981, Stewart and Pough 1983). Conspecifics, other amphibians, reptiles, birds, and mammals are also common predators of *E. coqui* (Joglar 1998), and in turn adult coqui frogs are considered the most important nocturnal predators in Puerto Rico consuming about 114,000 prey items per hectare (Stewart and Woolbright 1996). Overall, these traits highlight the ecological importance of *E. coqui* at various trophic levels of the tropical forest food web (Stewart and Woolbright 1996).

### *Study Site and Field Methods*

Repeated surveys were conducted in the Palo Colorado Forest at El Yunque National Forest in eastern Puerto Rico (19.11°N, 66.60°W) at an elevation of 661 m.

The Palo Colorado forest formation is classified as a subtropical wet forest association with an annual rainfall of approximately 2000 – 4000 mm (Ewell and Whitmore 1973). The pass of hurricanes Irma and Maria over Puerto Rico in September 2017 caused considerable damage to the forest (Hall et al. 2020), and potentially disturbed the ecology of many of its inhabitants including the *Eleutherodactylus* (Woolbright 1991, Klawinski et al. 2014, Burrowes et al. 2021)

Two 50 x 3 m transects (300 m<sup>2</sup>) within the Palo Colorado forest were surveyed bimonthly from September 2016 to July 2017 (Pre-Hurricane) and from May 2018 to September 2019 (Post-Hurricane). Both before and after the hurricane our sampling included the transitions between three seasons WW, CD, and the next WW season. Field work began around dusk (18:30–17:00 hrs.) and consisted of two persons conducting a visual encounter survey in the transects for 2.0–2.5 hours in each of two 50 m linear transects. Whenever possible, individuals were caught on-sight using nitrile powder-free gloves and placed temporarily in plastic bags. The microhabitat and the specific location in the transect where the frogs were found were recorded on small pieces of write-in-the rain paper that were placed inside the bag. Frogs were transported to the laboratory at the University of Puerto Rico where further data were recorded per individual: body size (SVL), measured with calipers while the frogs were still in the bag, age classes, determined by body size as described above, and sex of adults.

All field work was conducted under permits from the Department of Natural and Environmental Resources of Puerto Rico DRNA 2017-IC-036–037; DRNA 2018-IC-067–68, and approved IACUC protocol (3012-05-23-2018).

## *Data Analysis*

A multi-state N-mixture model was used to estimate abundance and changes in age-specific demographics on *E. coqui* population, measuring the effect of hurricane Maria. We applied this model, because it allows for estimation of abundances based on repeated count data only, without the need for marking individuals. Before the hurricane, we tried to mark all age classes in the population using a unique color-code combination of Visual Implant Elastomers (VIE's) to identify individuals upon recapture. Although this technique has been used successfully in geckos (Kondo et al. 2006) and in amphibian tadpoles (Bainbridge et al. 2015, Fouilloux et al. 2020), we found that it was not appropriate for *E. coqui* because the colored marks moved across the body from left to right, and between articulations, making individual codes indistinguishable. This problem with VIE's has also been reported for other amphibians (Moosman and Moosman Jr 2006, Bainbridge et al. 2015).

An extension of the Dail-Madsen model (Zipkin et al. 2014) was used because it assumes that class-specific (Juveniles and Adults) count data is available at a given location and time, and that locations are independent of each other accounting for observational error. For this model we made the following assumptions: (1) the population is closed at locations  $j$  within each season but not across seasons, (2) frog detections within a location  $j$  are independent, (3) all frogs within locations  $j$  during each season have the same detection probability, (4) all locations  $j$  are spatially independent, (5) open population dynamics with births, deaths, immigrations, and emigrations that abide to Markov's property (i.e., the abundance of frogs at time  $t$  depends only on the

abundance of frogs at time  $t-1$ ), and that (6) the number of individuals that survive at locations  $j$  and the number of individuals that are gained (via birth and immigration) are independent.

Specifically, we modeled  $N_{i,j,t}$ , the true abundance of each age class ( $i$ ) in location ( $j$ ) at time ( $t$ ). For this, we considered age-specific abundances of the life history of *E. coqui* defined as  $i=1$  for juveniles and subadults (non-reproductive class) from now on referred to as juveniles, and  $i=2$  for adults (reproductive class). For a given age class ( $i$ ), locality ( $j$ ), replicate sampling event ( $k$ ) and time ( $t$ ) we modeled  $n_{i,j,k,t} \sim \text{Bin}(N_{i,j,t}, p_i)$  where  $p_i$  is the age-specific detection probability, accounting for imperfect detection (see Table 1.1 for model nomenclature). We also include detection as a random effect to accommodate for the extra variation in the detection probabilities by sampling a normal distribution.

For the first month of sampling ( $t=1$ ) we modeled using a discrete distribution for count data taken at our study site. As in Zipkin et al. (2014), we assumed an abundance estimated as  $N_{i,j,1} \sim \text{Pois}(\lambda_i)$  for the  $j$  sampled locations. The following months ( $t \geq 2$ ) were modeled differently by allowing transitions between classes, and by taking into consideration the number of individuals that survive ( $S$ ) and are gained ( $G$ ) in the population.  $T_{i,j,t}$  is the category that accounts for the stochastic transition class representing recruitment of juveniles (Table 1.1). Thus, the age-specific and total population abundances at a given location  $j$  are determined by the number of juveniles that are gained and survived, minus those that transition to adulthood:  $N_{1,j,t} = G_{1,j,t} + S_{1,j,t} - T_{1,j,t}$  and by the number of adults that survived plus those acquired from recruitment:  $N_{2,j,t} = S_{2,j,t} + T_{1,j,t}$ .

To estimate model parameters, we used a Bayesian approach as in Zipkin et al. (2014) with a modification on the use of survival probability priors. Because we had reliable informative priors on survival probability for both age classes of *E. coqui* (Stewart and Woolbright 1996, Langhammer 2013) we used these to estimate survival probabilities before the hurricane. However, since the effect of this climatic disturbance on the population was not known at the time of this study, we used vague priors for both age classes after the hurricane. We applied Markov chain Monte Carlo (MCMC) to our Bayesian analysis in order to sample from posterior distributions for each of the parameters using JAGS software through RStudio Version 1.3.1073 with the 'jagsUI' package<sup>1</sup> (Plummer 2003, Rstudio 2020). We ran three parallel Markov chains for 500,000 iterations with 50,000 burn-in iterations and 25,000 adaptation iterations. Chains were thinned by 10 and we assessed convergence using diagnostic plots and the Gelman-Rubin statistic ( $\hat{R}$ , successful convergence based on all  $\hat{R}$  values  $< 1.1$ ).

To test for differences between parameter estimates before and after hurricane Maria, we computed the proportion of MCMC iterations where one parameter is greater than the other, following Ruiz-Gutiérrez et al. (2010). Extreme values (close to 0 and 1) suggest that the parameter estimates are significantly different and values near 0.50 suggest that they are similar (Table S1). This value can be directly interpreted as the probability that the first parameter is greater than the other parameter, for example, a probability of 0.80 is interpreted as 80% probability that parameter  $\mu_{\text{pre-Maria}} > \mu_{\text{post-Maria}}$ . The model fit metrics were calculated using Bayesian Posterior Predictive Checks (BPPC), which uses a Bayesian probability value close to 0.50 to indicate good fit.

## Results

Demographic parameters for a population of *Eleutherodactylus coqui* studied before and after hurricane Maria were modeled taking into consideration the seasonal transitions. Estimates of detection probabilities, total abundances, expected gains of juveniles and adults, the transition probability from juveniles to adults, and age-specific survivorship were adjusted for detection probabilities. We obtained a good fit for our model with BPPC probabilities of 0.507437 and 0.5449704 respectively for juvenile and adult parameter estimates. We observed a hurricane effect on the detection probability of both age classes. Before hurricane Maria, the detection probability for adults decreased with time as the seasons transitioned from WW to CD (Table 1.1). In contrast, the detection probability of juveniles increased in the CD season, probably due to a greater number of juveniles as result of reproduction in the previous WW season (Table 1.1). After the hurricane, detection probabilities of both age classes changed. We found that adult detection probability during the WW was higher than CD season, while juvenile detection probability was higher during the CD season as expected, but lower than before the hurricane (Table 1.1).

The estimated changes in age-specific total abundances of individuals through time, marked by seasons and the impact of hurricane Maria are illustrated in Figure 1.1 and values are reported in Table 1.2. Abundance was estimated by the N-mixture model considering both juvenile and adults gains, detection probability and survivorships during our Pre- and Post- hurricane Maria sampling periods. Pre-María abundance estimates concur with trends observed in previous studies on *E. coqui* at the same

locality (Woolbright 1985, Joglar 1998, Longo and Burrowes 2010). We found a greater number of adults during the WW season (2016), followed by an increase of juvenile abundances in the CD season (2017) when adult abundances remained stable (Fig. 1.1). Abundance estimates for the next WW season (2017) increase for the adults and decrease for juveniles, most likely due to recruitment of surviving juveniles to adulthood (Fig. 1.1, Before hurricane). This pattern of change in total abundances throughout the seasons is disrupted after hurricane Maria (Fig. 1.1, Post-Maria). The usual peak in juvenile abundance in the CD season was not observed in 2019, and in contrast to pre-hurricane times, the number of juveniles declined (Fig. 1.1). Juvenile abundance during the CD season pre-hurricane had an 83% probability of being higher than after hurricane Maria (Table S1). It is important to note that Figure 1.1 compares the results of two sampling periods pre- and post-hurricane Maria that were interrupted by eight months when we were unable to get to our study site to sample. Thus, Figure 1.1 should not be interpreted as a continuum, but as comparison of the population structure in two similar time frames, before and after a severe climatic event.

Expected gains were estimated in the population by allowing the number of individuals gained per location ( $j$ ) to vary by age class, season, and hurricane. Expected gains of juveniles occur via birth and immigration, while those for the adults occur via recruitment of juveniles. Before the hurricane, expected gains of juvenile were higher in the transition from the WW 2016 to the CD 2017 season than in the shift from the CD to WW 2017 seasons (Table 1.2, pre-hurricane). In contrast, adult expected gains per location were higher as the population transitioned from the CD–WW seasons of 2017 (Table 1.2, pre-hurricane). After the hurricane, expected gains through the seasonal



changes decreased for both age classes in comparison with pre-hurricane estimates (Table 1.2). There was an 85% probability of higher juvenile gains per location during the WW–CD transition before, than after the hurricane, and 75% probability of higher adult gains during the CD–WW seasonal transition without a hurricane effect (Table S1). This agrees with the increase in estimated abundances (Figure 1.2) and detection probabilities for both age classes (Table 1.2).

The hurricane effect on the survivorship of *E. coqui* differed among age classes. The mean adult survival probability before hurricane Maria was higher than after (Table 1.2) with a 78% higher probability of survivorship in pre-hurricane times (Table S1). Although, juvenile survivorship decreased after Maria, the effect was not drastic (Table 1.2). Finally, the transition probability from juvenile to adulthood was greater before the hurricane than after, suggesting that the consequences of hurricane Maria affected recruitment into adulthood (Table 1.2).

## Discussion

This study documents the changes in demographic and abundance parameters observed in a highland population of *Eleutherodactylus coqui* in Puerto Rico that survived hurricane Maria in 2017. Overall, we found that the population structure changed after Hurricane Maria (Fig 1.1), with an increase in the adult abundance and a decrease in the juvenile abundance. Our model revealed a hurricane effect as a marked disruption in the way abundances of adults and juveniles changed through time as the seasons transition from WW to CD in Puerto Rico (Fig. 1.1). The change in the pattern

of age-specific abundances was concurrent with the values estimated by our model for detection probabilities, expected gains through seasonal shifts, survival probability of adults, and transition probability of juveniles to adulthood. Without a severe hurricane effect on the forest, juveniles are expected to increase during the CD season due to births that take place mostly during the previous WW season when reproduction is highest (Woolbright 1985, Joglar 1998, Longo and Burrowes 2010). Hence, adults are expected to increase in the following WW season as surviving juveniles' transition to adulthood. This pattern is evidenced by our data collected before hurricane Maria (Fig. 1.1 -before Hurricane) and supported by our model's pre-hurricane parameter estimates (Table 1.2). However, after the hurricane, we did not observe the typical peak in juveniles in the CD season followed by the increase in adults in the following WW season (Fig. 1.1 -after Hurricane), and the expected gains of both age classes in the corresponding seasonal shifts decreased after Maria (Table 1.2). In addition, adult survivorship estimates decreased, and the transition probability of juveniles to adulthood was lower, suggesting that hurricane-related alterations to the forest were detrimental to recruitment.

It is noteworthy that the initial abundances, those estimated by our model for the first season of sampling before and after the hurricane, indicate an increase in the abundance of both adults and juveniles after Maria (Figure 1.1, Table 1.2, Post-hurricane). Thus, rather than a drastic decline in *E. coqui* numbers due to the hurricane, this study shows a change in the pattern of population structure as seasons progress that could have negative repercussions to future population growth. The significant decrease in expected gains per locality (Table S1) and the lower transition probability

(recruitment) observed after hurricane Maria are evidence of a demographic effect that may result in a future population decline. A previous study that assessed the effect of a less intense hurricane on a lowland population of this species revealed that adults increased while juveniles decreased potentially due to less reproductive activity (Woolbright 1991). Our results are not fully comparable to this study because the time frame was different, immediately after hurricane Hugo, versus eight months after hurricane Maria, and the duration of our sampling included two seasonal changes before and after the hurricane. Nonetheless, we also document a decrease in the number of juveniles with low detection probabilities and lower total abundance in the CD season of 2019 after hurricane Maria. With respect to the adult population, our results are complex and need to be considered in context to the disruption in the typical demographic effect across seasons that was observed after the hurricane (Fig.1.1). When we were able to return to the forest to commence post-hurricane sampling efforts, we found higher adult initial abundances, as observed by Woolbright (1991), who attributed this to an increase in retreat sites due to the higher complexity of forest debris, and/or a decrease in invertebrate predators (Woolbright 1991). It is also possible, that a sampling bias may produce more adults and less juveniles after a strong storm blowout. Adults, may be easier to observe while active on trunks and branches stripped from vegetation, and juveniles who are mostly active in the forest floor (Burrowes et al. 2017), may be harder to find amongst the complexity of the fallen debris in the forest floor (Klawinski et al. 2014). However, our model revealed that the survival probability of adults decreased after hurricane Maria, most likely due to a decline in the probability of juveniles transitioning to adulthood.

Although our data show that Hurricane Maria had an impact on the typical demographic changes that are expected as seasons transition from the times that favor reproduction (WW) to harsher times for the amphibians (CD), no drastic declines were observed. A possible explanation for this is that *E. coqui* is a widely distributed species, known to be an ecological generalist (Joglar 1998), and thus, expected to have the genetic diversity to adapt to environmental changes. This is remarkable from an evolutionary perspective because the hurricane Maria caused a severe canopy loss at our study site that resulted in a significant increase in forest ambient temperatures, as well as those in typical *E. coqui* retreat sites (Burrowes et al. in press). This effect is expected to be more detrimental in mountain areas because high-elevation *E. coqui* are more sensitive to temperature increases than lowland conspecifics (Delgado and Burrowes in press). Furthermore, juveniles, with higher surface-to-volume ratios, are expected to be more vulnerable to the increase in forest temperatures as those brought by hurricane blowouts. Nonetheless, our results point to plasticity in the response of *E. coqui* to severe environmental disturbances (O'Neill et al. 2018), as the population persisted after a strong (category 5-4) storm like hurricane Maria (Fig. 1.1). Other amphibian species that occur at our study site, such as *E. hedricki* and *E. portoricensis*, are classified as endangered by the International Union for the Conservation of Nature (IUCN 2021) and may be much more severely affected by the habitat and climatic disturbances caused by hurricanes. With stronger and more frequent storms predicted to occur in the Caribbean (Kossin et al. 2020), studies like the one presented herein serve as baselines to predict, compare and evaluate future changes to biodiversity in tropical areas.

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## Tables



**Table 1.1:** Parameter definitions in the N-mixture model applied to study abundance and demographic changes of a population of *Eleutherodactylus coqui*.

Parameter	Definition
Juvenile survival: $S_{1,j,t} \sim \text{Bin}(N_{1,j,t-1}, \omega_1)$	The number of juveniles at location $j$ that survive from time $t-1$ to $t$ and remain at $j$ . This is modeled with respect to the apparent survival probability of juveniles, which is dependent on the population's local abundance at time $t-1$ .
Adult survival: $S_{2,j,t} \sim \text{Bin}(N_{2,j,t-1}, \omega_2)$	The number of adults at location $j$ that survive from time $t-1$ to $t$ and remain at $j$ . This is modeled with respect to the apparent survival probability of adults, which is dependent on the population's local abundance at time $t-1$ .
Transition of juveniles to adulthood: $T_{1,j,t} \sim \text{Bin}(S_{1,j,t}, \phi)$	The number of juveniles at location $j$ that transition to adults and remain at $j$ . This is modeled with respect to the probability that a surviving juvenile transition to adult.
Recruitment to adulthood: $G_{1,j,t} \sim \text{Pois}(\gamma N_{2,j,t-1})$	The number of juveniles gained to adulthood by recruitment at location $j$ and remain at $j$ . This is modeled with respect to the product of the arrival rate and the abundance of adults at location $j$ at time $t-1$ .
Juvenile total abundance: $N_{1,j,t} = G_{1,j,t} + S_{1,j,t} - T_{1,j,t}$	The total abundance of juveniles at a given location $j$ determined by the number of juveniles that were gained to the population and survived, minus those that transition to adulthood.
Adult total abundance: $N_{2,j,t} = S_{2,j,t} + T_{1,j,t}$	The total abundance of adults at a given location $j$ determined by the number of adults that survived plus those acquired from recruitment.

**1.2 Table:** Mean estimates and 95% confidence intervals of age-specific parameters generated by the N-mixture model for abundances and demographics of *Eleutherodactylus coqui* before and after hurricane Maria. WW and CD are the warm-wet and cool-dry seasons respectively, the n-dash (–) indicates seasonal transitions. For code details of the N-mixture model applied see Table S2.

<b>Model Parameters</b>	<b>Age-classes</b>	<b>Pre-Hurricane</b>			<b>Post-Hurricane</b>		
<b>Detection probability</b>	Adult	<b>WW</b> 0.186 (0.121–0.264 95% CI)	<b>CD</b> 0.122 (0.057–0.218 95% CI)		<b>WW</b> 0.182 (0.115–0.258 95% CI)	<b>CD</b> 0.187 (0.101–0.325 95% CI)	
	Juvenile	0.129 (0.070–0.209 95% CI)	0.154 (0.079–0.256 95% CI)		0.106 (0.059–0.172 95% CI)	0.135 (0.071–0.234 95% CI)	
<b>Initial abundances</b>	Adult	1.160 (0.701–1.653 95% CI)			1.802 (1.406–2.287 95% CI)		
	Juvenile	1.256 (0.744–1.878 95% CI)			1.453 (0.903–2.067 95% CI)		
<b>Total abundances</b>	Adult	<b>WW2016</b> 73 (50–113 95% CI)	<b>CD2017</b> 86 (50–164 95% CI)	<b>WW2017</b> 137 (99–202 95% CI)	<b>WW2018</b> 139 (97–215 95% CI)	<b>CD2019</b> 110 (69–185 95% CI)	<b>WW2019</b> 114 (79–178 95% CI)
	Juvenile	82 (52–143 95% CI)	137 (87–242 95% CI)	94 (58–161 95% CI)	100 (59–173 95% CI)	97 (63–160 95% CI)	62 (38–105 95% CI)
<b>Expected gains per location</b>	Adult	<b>WW–CD</b> 0.047 (-1.761–1.580 95% CI)	<b>CD–WW</b> 0.756 (-1.139–1.800 95% CI)		<b>WW–CD</b> -0.447 (-2.188–1.086 95% CI)	<b>CD–WW</b> 0.074 (-1.690–1.337 95% CI)	
	Juvenile	1.390 (0.185–2.285 95% CI)	0.264 (-1.497–1.476 95% CI)		0.402 (-1.521–1.731 95% CI)	-0.588 (-2.232–0.752 95% CI)	
<b>Survival probability</b>	Adult	0.820 (0.557–0.892 95% CI)			0.688 (0.204–0.912 95% CI)		
	Juvenile	0.767 (0.302 - 0.903 95% CI)			0.794 (0.377 - 0.992 95% CI)		
<b>Transition probability</b>	Juvenile to Adult	0.481 (0.029 - 0.920 95% CI)			0.417 (0.027 - 0.827 95% CI)		

**Table S1:** Summary of results from the test for differences between parameter estimates before and after hurricane Maria, computed from the proportion of MCMC iterations where one parameter is greater than the other (Ruiz-Gutiérrez et al. 2010). Extreme values (close to 0 and 1) suggest that the parameter estimates are significantly different and values near 0.50 suggest that they are similar. This value can be directly interpreted as the probability that the first parameter is greater than the other parameter, for example, a probability of 0.80 is interpreted as 80% probability that parameter  $\mu_{\text{pre-Maria}} > \mu_{\text{post-Maria}}$ . WW1 refers to the first warm-wet season and WW2 refers to the second warm-wet season within our sampling periods.

<b>Model Parameter (Pre-hurricane vs. Post-hurricane)</b>	<b>Mean</b>
<b>Expected gains</b>	
<i>Juvenile WW-CD</i>	0.8538
<i>Juvenile CD-WW</i>	0.7878
<i>Adult WW-CD</i>	0.6617
<i>Adult CD-WW</i>	0.7546
<b>Total abundances per time-step</b>	
<i>Juvenile WW1</i>	0.2911
<i>Juvenile CD</i>	0.8294
<i>Juvenile WW2</i>	0.8636
<i>Adult WW1</i>	0.0135
<i>Adult CD</i>	0.2377
<i>Adult WW2</i>	0.7537

**Table S2:** Code for N-mixture model on abundance estimates before and after Hurricane Maria. This code was written for this study with the help of G. V. DiRenzo based on Zipkin et al. (2014).

```
# 1. Load libraries -----
# Load library for analysis
library(jagsUI)
library(ggplot2)
library(reshape2)
library(tidyverse)

# 2. Set informative priors -----
# Set priors using parameter estimates from Penny Langhammer's dissertation
# Most of those parameters were calculated as rates we need to convert rates to
probabilities
# Adult lower bound has a 1.25 daily mortality rate
# Convert daily mortality rate to daily mortality probability
low.mort.prob = 1-exp(-1.25*1)
# Mortality probability to survival probability
low.surv.prob = 1 - low.mort.prob
# Convert from daily to monthly Adult survival probability
# Lower estimate
adult.low = low.surv.prob^30
# Adult upper bound has a 3.75 mortality rate
# Daily mortality rate to daily mortality probability
up.mort.prob = 1-exp(-3.75*1)
# Mortality probability to survival probability
up.surv.prob = 1 - up.mort.prob
# Adult upper monthly survival probability
adult.up =(1 - (1- exp(-3.75e-3)))^30
# Juv lower bound has a 1.1 mortality rate
# daily mortality rate to daily mortality probability
juv.low.mort.prob = 1-exp(-1.1*1)
# Mortality probability to survival probability
juv.low.surv.prob = 1 - juv.low.mort.prob
# Juvenile lower monthly survival probability
juv.low = juv.low.surv.prob^30
# Juvenile upper bound has a 3.3 mortality rate
# Juvenile upper monthly survival probability
juv.up =(1 - (1- exp(-3.3e-3)))^30

# 3. Write the model in JAGS language -----
sink("./Coqui_model_v5.txt")
cat("
model{
```

```

##### Priors #####

# Define the priors for each state
for(s in 1:n.states){ # Por cada clase de edad
  for(h in 1:n.hurc){ # Por cada nivel de huracan
    for(k in 1:2){ # 1 = WW to CD; 2 = CD to WW
      # Detection probability - on logit scale
      alpha_p[s, k, h] ~ dnorm(0, 0.77)
      # Expected gain - intercept
      gamma_int[s, k, h] ~ dnorm(0, 1)
    }
  }
  # Initial abundance
  a.lambda[s, h] ~ dnorm(0, 1)
}
}
for(h in 1:n.hurc){ # Por cada nivel de huracan
  # Transition probability
  omega[h] ~ dunif(0, 1)
}
# Random effect for detection probability
for(s in 1:n.states){ # Por cada clase de edad
  for(i in 1:n.site){
    for(j in 1:n.surv){
      for(k in 1:n.season){
        for(h in 1:n.hurc){
          rand[s, i, j, k, h] ~ dnorm(0, tau[s, h])
        }
      }
    }
  }
}
for(s in 1:n.states){ # Por cada clase de edad
  for(h in 1:n.hurc){
    tau[s, h] <- 1/(sd[s, h] * sd[s, h])
    sd[s, h] ~ dgamma(0.1, 0.1)
    #dunif(0, 5)
    #dt(0, (1/(2^2)), 1)T(0,)
  }
}
# Survival probabilities with informative priors
phi[1,1] ~ dunif(juv.low,juv.up) # Survival before Hurricane Juvenile
phi[1,2] ~ dunif(0, 1) # Survival after Hurricane Juvenile
phi[2,1] ~ dunif(adult.low,adult.up) # Survival before Hurricane Adult
phi[2,2] ~ dunif(0, 1) # Survival after Hurricane Adult
#----- Ecological model
#---- First season- estimating the initial abundance per age class

```

```

for(i in 1:n.site){
  for(h in 1:n.hurc){
    N1[i, 1, h] ~ dpois(lambda1[i,h])
    log(lambda1[i,h]) <- a.lambda[1, h]
    N2[i, 1, h] ~ dpois(lambda2[i,h])
    log(lambda2[i,h]) <- a.lambda[2, h]
  }
}
#----- All other seasons
# We assume open population dynamics
# Animals are born, die, immigrate, emigrate, and transition from preadult to adult
for(i in 1:n.site){
  for(k in 2:n.season){ # Por cada epoca
    for(h in 1:n.hurc){ # Por cada nivel de huracan
      #----- Juvenile
      # Total survivors
      S1[i,k-1,h] ~ dbin(phi[1, h]^time[k-1,h], N1[i, k-1, h])
      # Survive & become adult
      T12[i,k-1,h] ~ dbin(omega[h]^time[k-1,h], S1[i,k-1,h])
      # Expected number of individuals gained = birth + immigration
      G1[i,k-1,h] ~ dpois(Ga1[i, k-1, h])
      log(Ga1[i, k-1, h]) <- gamma_int[1, k-1, h]
      #----- Adult
      # Total survivors
      S2[i,k-1,h] ~ dbin(phi[2, h]^time[k-1,h], N2[i,k-1,h]) # Total survivors
      # Expected number of individuals gained = immigration
      G2[i,k-1,h] ~ dpois(Ga2[i, k-1, h])
      log(Ga2[i, k-1, h]) <- gamma_int[2, k-1, h]
      #----- Totals
      N1[i, k, h] <- S1[i, k-1, h] + G1[i, k-1, h] - T12[i, k-1, h]
      N2[i, k, h] <- S2[i, k-1, h] + G2[i, k-1, h] + T12[i, k-1, h]
    }
  }
}
#----- Observation model
for(i in 1:n.site){
  for(j in 1:n.surv){
    for(k in 1:n.season){
      for(h in 1:n.hurc){
        # Observed data
        # We are feeding the model y1 and y2 below in the win.data object
        y1[i, j, k, h] ~ dbin(p[1, i, j, k, h], N1[i, k, h])
        logit(p[1, i, j, k, h]) <- alpha_p[1, season.index[k], h] + rand[1, i, j, k, h]
        y2[i, j, k, h] ~ dbin(p[2, i, j, k, h], N2[i, k, h])
        logit(p[2, i, j, k, h]) <- alpha_p[2, season.index[k], h] + rand[2, i, j, k, h]
        # Simulated data
      }
    }
  }
}

```

```

# We are generating values for y1.new and y2.new using the parameters estimated
above from the observed data
y1.new[i, j, k, h] ~ dbin(p[1, i, j, k, h], N1[i, k, h])
y2.new[i, j, k, h] ~ dbin(p[2, i, j, k, h], N2[i, k, h])
}
}
}
}
# Cantidad derivada
# Sumar la abundancia de ranas en todos los sitios por la clase de edad
for(k in 1:n.season){
for(h in 1:n.hurc){
N1_tot[k, h] <- sum(N1[ , k, h])
N2_tot[k, h] <- sum(N2[ , k, h])
}
}
##### Bayesian posterior predictive check#####
for(i in 1:n.site){
for(j in 1:n.surv){
for(k in 1:n.season){
for(h in 1:n.hurc){
# Generated the expected number of observed individuals
# The expected number of observed individuals = the detection probability * the true
number of observed individuals
eval1[i, j, k, h] <- p[1, i, j, k, h] * N1[i, k, h]
eval2[i, j, k, h] <- p[2, i, j, k, h] * N2[i, k, h]
# Calculate the difference between the observed data & the expected number of
observed individuals
# Residual values of the observed data
E1[i, j, k, h] <- pow((y1[i, j, k, h] - eval1[i, j, k, h]),2) / (eval1[i, j, k, h] + 0.5)
E2[i, j, k, h] <- pow((y2[i, j, k, h] - eval2[i, j, k, h]),2) / (eval2[i, j, k, h] + 0.5)
# Calculate the difference between the simulated data & the expected number of
observed individuals
# Residual values of the simulated data
E.new1[i, j, k, h] <- pow((y1.new[i, j, k, h] - eval1[i, j, k, h]),2) / (eval1[i, j, k, h] + 0.5)
E.new2[i, j, k, h] <- pow((y2.new[i, j, k, h] - eval2[i, j, k, h]),2) / (eval2[i, j, k, h] + 0.5)
} # h
} #k
} #j
} #i

# Next, we sum up the residuals of the observed & simulated data
# Pre adult
zzzfit1 <- sum(E1[,,,])
zzzfit1.new <- sum(E.new1[,,,])
# Adult

```

```

zzzfit2      <- sum(E2[,,,])
zzzfit2.new <- sum(E.new2[,,,])
# And last, we compare the sum of the residuals between the observed and simulated
data
# We want fit.diff1 and fit.diff2 to be close to 0.5
fit.diff1 <- step(zzzfit1 - zzzfit1.new)
fit.diff2 <- step(zzzfit2 - zzzfit2.new)
}
", fill = TRUE)
sink()

```

```

# 4. Bundle the data and initial values -----

```

```

# standardize time
std.meses <- (meses - mean(meses))/sd(meses)
# Bundle data
win.data <- list(y1 = matriz_prea,
  y2 = matriz_ad,
  n.site = nrow(matriz_prea),
  n.surv = ncol(matriz_prea),
  n.season = dim(matriz_prea)[3],
  n.hurc = dim(matriz_prea)[4],
  n.states = 2,
  time = meses,
  months = std.meses,
  season.index = c(1, 2, 1),
  # Using the values from above
  juv.low = juv.low,
  juv.up = juv.up,
  adult.low = adult.low,
  adult.up = adult.up
)

```

```

# Supply initial values for the JAGS function

```

```

# 5. Set MCMC settings -----

```

```

# MCMC settings
ni <- 500000 # Number of iterations
nb <- 50000 # Burning periods
nt <- 10 # Thinning rate
nc <- 3 # Number of chains
na <- 25000 # Adaptation period

```

```

# 6a. Analyze the data -----

```

```

# Analyze the data
## This takes ~ 142.161 minutes to run
# out <- jags(data = win.data,
#   inits = inits,
#   parameters.to.save = params,
#   model.file = "./Coqui_model_v5.txt",

```



```

#   n.chains = nc,
#   n.thin = nt,
#   n.iter = ni,
#   n.burnin = nb,
#   parallel = TRUE)
## Save the output
# 6b. Load in the previous model run -----
# Load the previous model run
# 7. Check the output -----
# Check the model fit metrics
# This is the Bayesian p-value we calculated (also known as a Bayesian posterior
predictive check)
out$mean$fit.diff1
out$mean$fit.diff2
out$mean$zzzfit1
out$mean$zzzfit1.new
# Check the output
# 8. Look at the model estimates -----
# Vector with parameter names in the same order as model estimates
# Make names a factor
names <- factor(names, levels = names)
# Label what scale to use
scale <- c(rep("Log scale", times = 4),
           rep("Prob scale", times = 4),
           rep("Log scale", times = 8),
           rep("Prob scale", times = 10))
# Model mean
mod.mean <- c(
  out$mean$a.lambda,
  out$mean$phi,
  out$mean$gamma_int,
  out$mean$omega,
  plogis(out$mean$alpha_p)
)
# Model lower 95% CI
mod.q2.5 <- c(
  out$q2.5$a.lambda,
  out$q2.5$phi,
  out$q2.5$gamma_int,
  out$q2.5$omega,
  plogis(out$q2.5$alpha_p)
)
# Model upper 95% CI
mod.q97.5 <- c(
  out$q97.5$a.lambda,
  out$q97.5$phi,

```

```

out$q97.5$gamma_int,
out$q97.5$omega,
plogis(out$q97.5$alpha_p)
)
# Combine the names, truth, and model output
dat <- data.frame(names = names,
  scale = scale,
  mod.mean = mod.mean,
  mod.q2.5 = mod.q2.5,
  mod.q97.5 = mod.q97.5)
# Set colors for truth and estimates
cols <- c("Estimated" = "black")
# 9. Compare parameter estimates before & after the hurricane
# To quantify differences between parameter estimates, we computed the proportion of
MCMC iterations where one parameter is greater than the other parameter following
Ruiz-Gutierrez et al. (2010). This value can be directly interpreted as the probability that
the first parameter is greater than the other parameter Extreme values (close to 0 and
1) suggest that the parameters are significantly different and values near 0.50 suggest
that estimates are similar.

# Transition probability pre- vs post- hurricane
# omega[h]
mean(out$sims.list$omega[, 1] > out$sims.list$omega[,2])
sd(out$sims.list$omega[, 1] > out$sims.list$omega[,2])
# Compare the expected number of individuals gained per site
## gamma_int[s, k, h]
# Juvenile
# pre-hurricane vs. post-hurricane
# Transition WW - CD
mean(out$sims.list$gamma_int[,1,1,1] > out$sims.list$gamma_int[,1,1,2])
sd(out$sims.list$gamma_int[,1,1,1] > out$sims.list$gamma_int[,1,1,2])
# Juvenile
# pre-hurricane vs. post-hurricane
# Transition CD - WW
mean(out$sims.list$gamma_int[,1,2,1] > out$sims.list$gamma_int[,1,2,2])
sd(out$sims.list$gamma_int[,1,2,1] > out$sims.list$gamma_int[,1,2,2])
# Adult
# pre-hurricane vs. post-hurricane
# Transition WW - CD
mean(out$sims.list$gamma_int[,2,1,1] > out$sims.list$gamma_int[,2,1,2])
sd(out$sims.list$gamma_int[,2,1,1] > out$sims.list$gamma_int[,2,1,2])
# Adult
# pre-hurricane vs. post-hurricane
# Transition CD - WW
mean(out$sims.list$gamma_int[,2,2,1] > out$sims.list$gamma_int[,2,2,2])
sd(out$sims.list$gamma_int[,2,2,1] > out$sims.list$gamma_int[,2,2,2])

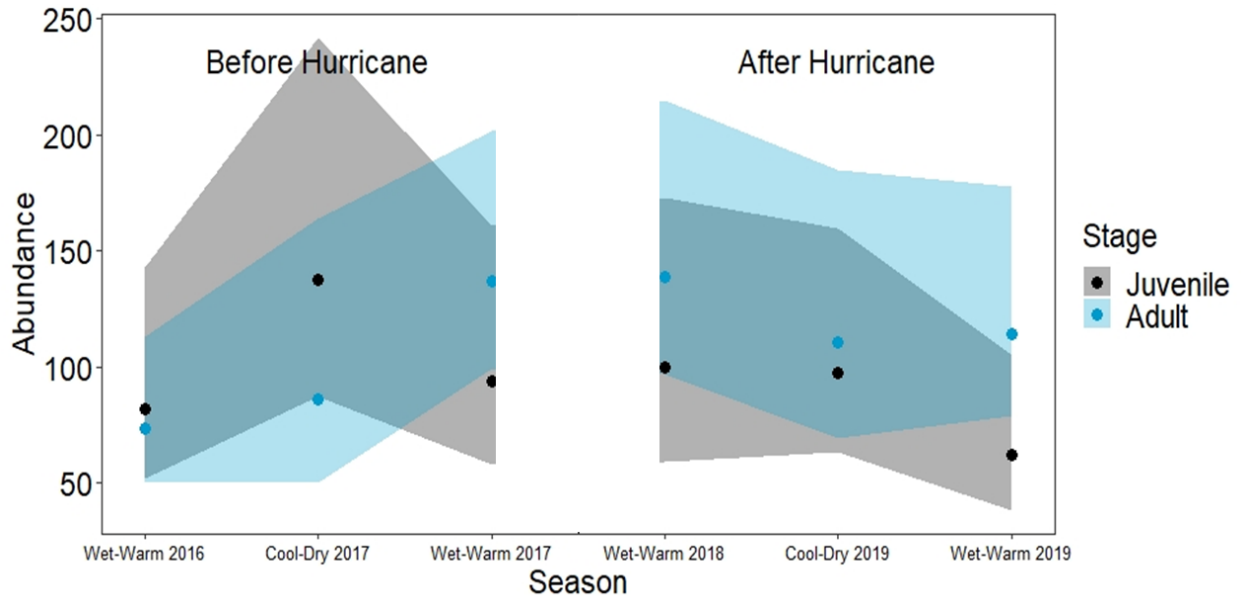
```

```

# Compare the abundance of adults and juveniles at each timestep
# Juvenile
# WW1 comparison
# Pre-hurricane vs post-hurricane
mean(out$sims.list$N1_tot[ ,1, 1] > out$sims.list$N1_tot[ ,1, 2])
sd(out$sims.list$N1_tot[ ,1, 1] > out$sims.list$N1_tot[ ,1, 2])
# Juvenile
# CD comparison
# Pre-hurricane vs post-hurricane
mean(out$sims.list$N1_tot[ ,2, 1] > out$sims.list$N1_tot[ ,2, 2])
sd(out$sims.list$N1_tot[ ,2, 1] > out$sims.list$N1_tot[ ,2, 2])
# Juvenile
# WW2 comparison
# Pre-hurricane vs post-hurricane
mean(out$sims.list$N1_tot[ ,3, 1] > out$sims.list$N1_tot[ ,3, 2])
sd(out$sims.list$N1_tot[ ,3, 1] > out$sims.list$N1_tot[ ,3, 2])
# Adults
# WW1 comparison
# Pre-hurricane vs post-hurricane
mean(out$sims.list$N2_tot[ ,1, 1] > out$sims.list$N2_tot[ ,1, 2])
sd(out$sims.list$N2_tot[ ,1, 1] > out$sims.list$N2_tot[ ,1, 2])
# Adults
# CD comparison
# Pre-hurricane vs post-hurricane
mean(out$sims.list$N2_tot[ ,2, 1] > out$sims.list$N2_tot[ ,2, 2])
sd(out$sims.list$N2_tot[ ,2, 1] > out$sims.list$N2_tot[ ,2, 2])
# Adults
# WW2 comparison
# Pre-hurricane vs post-hurricane
mean(out$sims.list$N2_tot[ ,3, 1] > out$sims.list$N2_tot[ ,3, 2])
sd((out$sims.list$N2_tot[ ,3, 1] > out$sims.list$N2_tot[ ,3, 2]))
# End script

```

**Figure**



**Fig. 1.1:** Season-specific abundances estimated by N-mixture model considering both juveniles and adults gains, detection probability and survivorships during our Pre- and Post- hurricane Maria sampling periods. The separation indicates an interruption of 8 months of sampling. The dots indicate means, and the shadowed areas cover the 95 % confidence intervals.

**Chapter 2: Effect of Hurricane Maria on Bd prevalence and infection intensity in a highland population of *Eleutherodactylus coqui***

## Abstract

Amphibians are declining drastically due, in part, to a pathogenic fungal parasite named *Batrachochytrium dendrobatidis* (*Bd*). In addition, extreme climatic events, like hurricanes, are increasing in frequency and intensity and this points to the need to understand the way they influence host-pathogen dynamics. Studies in the Americas have advanced our understanding on how environmental factors affect virulence and dispersion of *Bd* in wild populations. However, in some amphibian communities where *Bd* is enzootic, the dynamics of the disease may be driven by pathogen overdispersion. Under these circumstances, it is important to understand how *Bd* infection intensity and prevalence influences host survival and reproduction because this may explain the underlying mechanisms of persistence or extinction. In this study, we evaluate the effect of severe natural disturbances on the dynamics of *Bd* infections across seasons in a population of highland *Eleutherodactylus coqui* in Puerto Rico. We found significant differences in *Bd* infection intensities in the population of *E. coqui* after the hurricane, suggesting a hurricane effect on the vulnerability of hosts. In addition, a zero-inflated regression model distinguished the effects of the hurricane, seasons and their interaction on the population-level prevalence of *Bd*. Our work highlights the value of long-term field studies because they provide time-sensitive data necessary to evaluate population responses to global change.

Keywords: Host-pathogen dynamics, chytridiomycosis, tropical amphibians

## Introduction

Amphibians represent an entire vertebrate class that currently exemplifies what has been referred to as the sixth-mass extinction (Mendelson et al. 2006, Ceballos et al. 2017). In addition to common anthropogenic causes such as habitat degradation, overharvesting, invasive species, and changes in land use, amphibians are declining drastically due to a pathogenic fungal parasite—*Batrachochytrium dendrobatidis* (*Bd*) (Skerratt et al. 2007, Scheele et al. 2019). This pathogen causes a skin disease in amphibians known as chytridiomycosis (Berger et al. 1998). At present, no method has been described to effectively eradicate chytridiomycosis in the wild. Although *Bd* is a generalist pathogen that parasitizes all amphibians, not all hosts are equally affected by chytridiomycosis (Daszak et al. 1999, Kolby and Daszak 2016). Studies have found that the combination of a host's evolutionary history, ecological habitats, local environment (especially temperature and moisture), and *Bd*'s physiological requirements can determine the impact of chytridiomycosis on amphibian populations (Daszak et al. 1999, Fisher et al. 2009, James et al. 2015). A few studies have been able to document changes in amphibian assemblages before, during (epizootic), and after (enzootic) the appearance of *Bd*. Among these, the best documented cases are El Copé, Panama (Lips et al. 2006); Sierra Nevada, CA, USA (Briggs et al. 2010, Vredenburg et al. 2010); tropical Australia (Retallick et al. 2004), and Panama (Voyles et al. 2018). These studies have provided important information on the dynamics of *Bd*, and motivated further research about the response of naïve species to this deadly pathogen, the taxonomic and ecological signatures of host susceptibility, and the mechanisms that may drive



certain species to extinction, others to decline drastically, and yet others to persist while still vulnerable to *Bd*. At present, various strains of *Bd* have been identified with different degrees of pathogenicity (Farrer et al. 2011, James et al. 2015), and a hyper-pathogenic strain known as the global pandemic lineage of *Bd* (*Bd*-GPL) has spread from Southeast Asia to all over the world causing drastic declines, even in pristine areas (O'Hanlon et al. 2018, Scheele et al. 2019). The prevalence of *Bd* in amphibian communities is associated with cooler ambient temperature because it grows best between 17-25 °C (Piotrowski et al. 2004). Thus, in the Neotropics, amphibians are at the highest risk of *Bd* infection in montane humid forests where humidity is high, and the temperature is optimum for *Bd* growth (Ron 2005, Liu et al. 2013).

Studies in the Americas have advanced our understanding on how environmental factors affect virulence and dispersion of *Bd* in wild populations (Lips et al. 2006, Briggs et al. 2010, James et al. 2015). We know that temperature, precipitation, and altitude influence *Bd*'s growth, and consequently host-pathogen interactions at given sites (Murray et al. 2009, James et al. 2015). In addition, seasonal variability may facilitate persistence of populations in spite of *Bd* due to host-pathogen trade-offs (Murray et al. 2009, Longo et al. 2010). For example, in tropical Australia the wet season is cooler, benefiting growth and dispersion of *Bd* zoospores and resulting in the highest *Bd* infection burdens for amphibians (Kriger and Hero 2007). In contrast, in Puerto Rico the dry season is the coolest, favoring *Bd* growth, and affecting amphibians that tend to clump in the few humid pockets of the forest (Longo et al. 2009). In a climate change scenario, the Caribbean region is expected to experience more frequent and stronger periods of drought and, as a consequence, amphibian populations may be severely

affected (IPPC 2018). Much work is still needed to understand how certain amphibians can persist with *Bd*, especially in direct-developing amphibians that seem to have survived the epizootic but remain susceptible to chytridiomycosis (Longo and Burrowes 2010, Longo et al. 2013a). A study on a Panama community of amphibians that was highly deteriorated by a *Bd* epizootic in 2003, showed that *Bd* virulence has not attenuated over time, but suggests that some persisting species may have evolved resistance (Voyles et al. 2018).

In some amphibian communities where *Bd* is enzootic, the dynamics of the disease may be driven by pathogen overdispersion (Grogan et al. 2016, Catenazzi et al. 2017). Overdispersion of *Bd* results in a population characterized by having many individuals with low infection burdens, and few with high infection loads. However, despite low *Bd* infection burdens, populations under enzootic conditions could still be at risk of declines if faced with a major disturbance (Longo and Burrowes 2010, Grogan et al. 2016). Under these circumstances, it is important to understand how *Bd* infection intensity and prevalence influences host survival and reproduction because this may explain the underlying mechanisms of persistence or extinction.

In this study, we evaluate the effect of severe natural disturbances on the dynamics of *Bd* infections across seasons in a population of highland *Eleutherodactylus coqui* in Puerto Rico. We studied the changes in *Bd* prevalence and infection intensities before and after the pass of two strong hurricanes within two weeks in 2017, but especially, after the devastating effect of hurricane Maria. Previous studies showed that strong hurricanes can affect coqui populations by changing local temperature regimes and habitat structure (Woolbright 1991, Klawinski et al. 2014). After hurricane Hugo

(category 3 in 1998), a population of *Eleutherodactylus coqui* at the Luquillo Experimental Forest (LEF) of Puerto Rico, revealed an increase in adults and a decrease in juveniles (Woolbright 1991, 1996). The proliferation of adults was attributed to a greater availability of retreat sites among the fallen debris and a decrease in invertebrate predators, while the reduction of juveniles was explained by a decrease in reproductive activity due to changes in microclimate (Woolbright 1991). Another study experimentally evaluated the effect of two consequences of hurricanes, canopy disturbance and increased deposition of debris in the forest floor, on a population of coquis in the LEF in Puerto Rico (Klawinski et al. 2014). In contrast to the results of Woolbright (1991), this study found that while canopy disturbances were detrimental to *E. coqui* abundance in experimental plots, an increase in forest debris had no effect (Klawinski et al. 2014).

None of these studies questioned the effect of these environmental disasters in the dynamics of endemic pathogens, like *Bd*. Given the susceptibility of *E. coqui* to *Bd* infections during drought conditions (Longo and Burrowes 2010, Longo et al. 2013a), and the higher vulnerability of juveniles than adults to chytridiomycosis (Longo and Burrowes 2010, Burrowes et al. 2017), there were several potential outcomes for *Bd* infections after the hurricane. One possibility was that amphibians could fair better against *Bd* due to increased temperature as consequence of the lack of canopy cover in the forest (Becker and Zamudio 2011). In contrast, we could hypothesize that physiological stress in response to habitat devastation, changes in predator-prey abundance, and temperature increase could contribute to increased vulnerability of hosts to pathogens (Rollins-Smith et al. 2011). Our results show that although the

hurricane did influence the prevalence and infection intensities of *Bd*, this effect interacted with seasons, and eventually subsided as the forest recuperated and/or the frogs were able to adapt to the changes.

## **Methods**

### *Study Site and Field Methods*

Repeated surveys were conducted in Palo Colorado Forest at El Yunque National Forest (EYNF) in eastern Puerto Rico (19.11°N, 66.60°W) at an elevation of 661 m. The Palo Colorado formation is characterized as a subtropical wet forest association with an annual rainfall of approximately 2000 – 4000 mm by Ewell and Whitmore (1973). However, with the pass of hurricanes Irma and Maria over Puerto Rico on September 2017, caused considerable forest damage has been recorded across the island (Hall et al. 2020) and was observable at our study site at EYNF (Burrowes et al. 2021).

Two 3 X 50 m transects (300 m<sup>2</sup>) within the Palo Colorado forest were surveyed between September 2016 – July 2017 (Pre-Hurricane) and May 2018 – September 2019 (Post-Hurricane). Field work began around dusk (18:30–17:00 hrs) and consisted of two people conducting a visual encounter survey in the transects during 1.5–2.0 hours while attempting to catch all frogs. Individuals were caught on-sight with nitrile powder-free gloves and placed in plastic bags with their corresponding microhabitat and location in the transect recorded on small notes of write-in-the rain paper, which were deposited in their respective bags. Frogs were transported to the laboratory of the

University of Puerto Rico where we continued to collect further data per individual: body size by measuring the snout-to-vent length (SVL), and sex of adults (those individuals with SVL  $\geq$  28 mm). Finally, all frogs were sampled for presence of *Bd* by swabbing their venter, feet and toes using Medical wire cotton swabs as described by (Kriger et al. 2006). Every time a different frog was handled, we used a new pair of powder-free nitrile gloves to prevent cross-contamination (Hyatt et al. 2007).

#### *Bd sampling, DNA extraction and qPCR techniques*

We extracted DNA from tissue swabs using 50  $\mu$ L PrepMan Ultra extraction reagent (Life Technologies, Inc.). Then, we followed standard methods for *Bd* diagnosis and quantification via quantitative Polymerase Chain Reaction (qPCR) (Boyle et al. 2004). We ran assays with *Bd* standards made from a 146 bp synthetic fragment (gBlock, IDTDNA; ITS Hap01; Longo et al. 2013b) and created a serial dilution ranging from  $10^6$  to 10 copies. To facilitate comparisons with studies done in other parts of the world where other *Bd* strains occur, we report the number of ITS1 copies per sample, rather than the genomic equivalents of *Bd* zoospores (Longo et al. 2013b)

All field work was conducted under permits from the Department of Natural Resources, DRNA 2017-IC-036, 2017-IC-037, and 2018-IC-001, 2018-IC-002 to collect animals and tissues samples in Puerto Rico, and corresponding approved IACUC protocol (3012-05-23-2018).

## Data Analysis

We analyzed differences in *Bd* infection for three seasons warm-wet (WW) to cool-dry (CD) and the next warm-wet (WW) as they transitioned before (2016–2017) and after (2018–2019) hurricane Maria. It was important to examine the seasonal effect, because it has been shown that susceptibility of *E. coqui* to *Bd* varies seasonally (Longo et al. 2009, Longo and Burrowes 2010), and we wanted to know if the hurricane affected this pattern. Thus, *Bd* prevalence and infection intensities were grouped by age class (juveniles versus adults), season (WW versus CD), and hurricane (Pre- versus Post-Maria). To compare population level *Bd* prevalence and levels of *Bd*-infection intensities we divided *Bd* load (ITS1 copy numbers) by categories in the following manner- Low: >0 to 665; Moderate: >665 to 6500; High >6500. We used a Wilcoxon rank sum test with continuity correction to evaluate differences in *Bd* infection intensity before and after the hurricane and used chi-square statistic to test for a potential association between hurricane condition and the distribution of *Bd* infection intensities in *E. coqui* hosts. Finally, we used a zero-inflated regression (negative binomial with log-link) model to evaluate the relative contribution of the hurricane (Pre- versus Post-Maria), seasons (WW versus CD) and age (juveniles versus adults) on *Bd* infection load (Log (number of ITS1 copies +1)). We used this model because *Bd* is enzootic in Puerto Rico, and infection intensities tend to be over-dispersed in the population with a large proportion of individuals not infected at a given time (Longo et al. 2013, Burrowes et al. 2017). We report the best model based on results from a likelihood ratio test. All the statistics and graphs were performed using R statistical computing software (R Core Team 2021).

## Results

Infection intensities of the pathogenic chytrid fungus in the population of *E. coqui* differed significantly after the hurricane, suggesting a hurricane effect on the vulnerability of hosts to *Bd* ( $W = 11919$ ,  $P < 2.2e^{-16}$ ). In addition, the distribution of the levels of infection also changed significantly ( $X^2 = 18.862$ ,  $df = 3$ ,  $P = 0.00021$ ) indicating that among infected individuals there was a higher percentage of frogs with high *Bd* load after hurricane Maria (Fig. 2.1).

A look at the level of *Bd* infection by age class is consistent with a hurricane effect showing that a significantly higher proportion of the adults ( $X^2 = 18.3533$ ,  $df = 3$ ,  $P=0.000372$ ) and the juveniles ( $X^2 = 21.3257$ ,  $df = 3$ ,  $P=0.00009$ ) in the population carried higher infection loads after hurricane Maria Fig. 2.2. Regardless of age class, the percentage of animals infected versus not infected (prevalence) was not significantly different between pre- and post- hurricane (Fig. 2.2).

Infection intensities through the seasonal transitions also changed after the hurricane (Fig. 2.3). The expected pattern in non-hurricane times is that infection intensities increase in the CD season, when harsh conditions favor clumping of infected individuals in retreat sites within humid pockets of the forest (Longo et al. 2010). This difference was not significant during the WW (2016) – CD (2017) seasonal transition, most likely because *Bd* is already endemic in this population (Longo and Burrowes, 2010, Burrowes et al. 2017). Results from the first sampling opportunity after the hurricane (eight months after Maria) in the WW season of 2018, show an increase in *Bd* loads for both age classes (juveniles:  $W = 25$ ,  $P = 2.981e^{-11}$ , adults  $W = 0$ ,  $P = 4.782e^{-11}$ ).

10, Fig. 2.3). The levels of infection remain high throughout the CD season of 2019, and do not return to pre-hurricane times until the warmer and wetter conditions return (WW-2019), approximately two years after this climatic and environmental disturbance.

Results from the zero inflated negative binomial regression model distinguished only the effect of hurricane Maria as significant predictor of *Bd* loads in *E. coqui* during our study period (Table 2.1). The negative coefficient estimated for hurricane (Pre-Maria, -0.68538) implies that *Bd* infection levels were lower for all individuals before the hurricane (Table 2.1). Contrary to our expectations, neither season, age class, nor their interactions, predicted the *Bd* burdens carried by *E. coqui* as they overcame a severe hurricane effect (Table 2.1). In addition, the model recognized that hurricane (Pre-Maria), season (WW), and the interaction between hurricane and season significantly contributed more zeros to the distribution suggesting an effect of these factors and their interaction on the population-level prevalence of *Bd*, with lower values before the hurricane and during WW seasons. Prevalence is scored as 0 for non-infected individuals and 1 for those with positive *Bd* loads. Thus, factors that significantly predict zero-inflated *Bd* infection loads are associated to the prevalence of this pathogen, rather than with the distribution of infection loads among vulnerable hosts (Grogan et al. 2016).

## Discussion

Extreme climatic events, like hurricanes, are increasing in frequency and intensity (IPPC 2018) and this points to the need to understand the way they influence organisms. The dynamics of *Batrachochytrium dendrobatidis* prevalence and the variation of its infection



intensities in a population of highland *Eleutherodactylus coqui* at El Yunque, have been studied extensively in the past (Burrowes et al. 2004, 2008, 2017, Longo et al. 2009, 2015, Longo and Burrowes 2010), but never after a severe climatic disturbance. Having empirical data on *E. coqui* population structure and corresponding *Bd* infection status for three seasons before hurricane Maria was key to study the effect of hurricane Maria at our study site. Our model revealed a significant hurricane effect evidenced by greater prevalence and higher *Bd* loads (>6500 ITS1-copies) for the entire population after the hurricane (Fig. 2.1., Table 2.1). Although, age class was not a significant predictor of *Bd* infection during the time of this study (Table 2.1), both juveniles and adults had significantly higher *Bd* loads after the storm consistent with a hurricane effect on the vulnerability of hosts to *Bd*, regardless of age (Fig. 2.1).

Studies have shown that during the cooler and drier season (CD), *E. coqui* generally carry higher *Bd* burdens, and that this effect is exacerbated at times of extended droughts (Longo et al. 2009). Results from our sampling before hurricane Maria (2016–2017) are encouraging, in that they show a *Bd* prevalence of 38 % versus 55 % reported for previous years (Longo and Burrowes 2010), and that they show only a slight increase in *Bd* infections in the CD season of 2017. These data suggest that before hurricane Maria, *E. coqui* were responding to *Bd* in a manner consistent with extended enzootic conditions of this pathogen, and/or that they had evolved potential defense mechanisms against *Bd* (Longo and Zamudio 2017, Rollins-Smith 2017, Voyles et al. 2018). However, after hurricane Maria, we see a significant increase in higher *Bd* loads in the entire population that is also detected in juveniles and in adults (Fig.2.1 and 2.2), suggesting a disruption of the host-pathogen dynamics that prevailed in this

system before a severe climatic event occurred. *Bd* levels increased significantly in the WW (2018) for both age classes and *Bd* burdens remained high during the following CD (2019) season among susceptible hosts (Fig. 2.3). The effect of extreme atmospheric events on wildlife have shown changes in behavior (Abernathy et al. 2019), habitat use (Luja and Rodríguez-Estrella 2010, Klawinski et al. 2014, Boucek et al. 2017, Burrowes et al. 2021), survivorship (Van de Pol et al. 2010), population structure (Woolbright 1996, Matich and Heithaus 2012), and skin symbionts (Woodhams et al. 2020) among others, that point to various selective forces as drivers of these changes.

For *E. coqui* persisting with *Bd* at our study site at El Yunque, the effect of hurricane Maria brought about severe canopy devastation resulting in decreased perching and reproductive habitat for frogs, as well as a significant increase in understory temperatures (Burrowes et al. 2021). Field work in the first months after the hurricane revealed that male frogs were still calling, but adults were difficult to observe because they were not active in their typical understory nocturnal sites, and juveniles, common in pre-hurricane surveys, were rare. This was probably a consequence of a decrease in arboreal vegetation such as bromeliads and palm-frond axils that fell with hurricane winds, as well as the loss of moss-covered tree trunks and branches that before the hurricane were concealed by leaves overhanging from the canopy, providing ideal hiding places for active frogs. These changes in habitat have the potential to translate in physiological stress for the frogs that can result in immune depression and hence, higher vulnerability to pathogens like *Bd* (Rollins-Smith 1998, Rohr et al. 2008, Rollins-Smith et al. 2011). If the hurricane blowout constrained *E. coqui* frogs mostly to the forest floor where *Bd* is most likely to occur (Burrowes et al. 2017), both adults and

juveniles would be at higher risk of chytridiomycosis. We considered two potential outcomes of hurricane Maria on the *Bd* dynamics among *E. coqui*. (1) a positive effect where frogs could fair better because higher forest temperatures would halt *Bd* growth (Piotrowski et al. 2004), or (2) increased vulnerability of hosts confronting high physiologically stress due to environmental changes. Our results point to the second hypothesis, highlighting a significant hurricane effect on the *Bd*-loads of hosts. Other than temperature and habitat loss discussed above, changes in the food web may have caused lack of prey or excess of predators (Woolbright 1991), resulting in additional stress.

Although we did not observe a drastic decline in *E. coqui* abundance after the hurricane Maria, we did observe a significant decrease in expected gains per locality and lower recruitment from juveniles to adulthood (Ch. 1, Table 1.1). These changes are evidence of a demographic effect that may be associated to the higher *Bd* infection loads observed after the storm (Fig. 2.2). We present the first study that addresses the change in the dynamics of chytridiomycosis in amphibians affected by a severe climatic event. Our findings suggests that a category 5–4 hurricane, like Maria, was an important driver of increased susceptibility to a deadly pathogen like *Bd* even in a generalist species like *E. coqui*. Our work highlights the value of long-term field studies because they provide time-sensitive data necessary to evaluate population responses to global change. At this point we do not have explicit recommendations that may help mitigate the effect of hurricanes on host-pathogen dynamics. However, we expect that our findings will inform prioritization in management strategies in the face of future climatic events.

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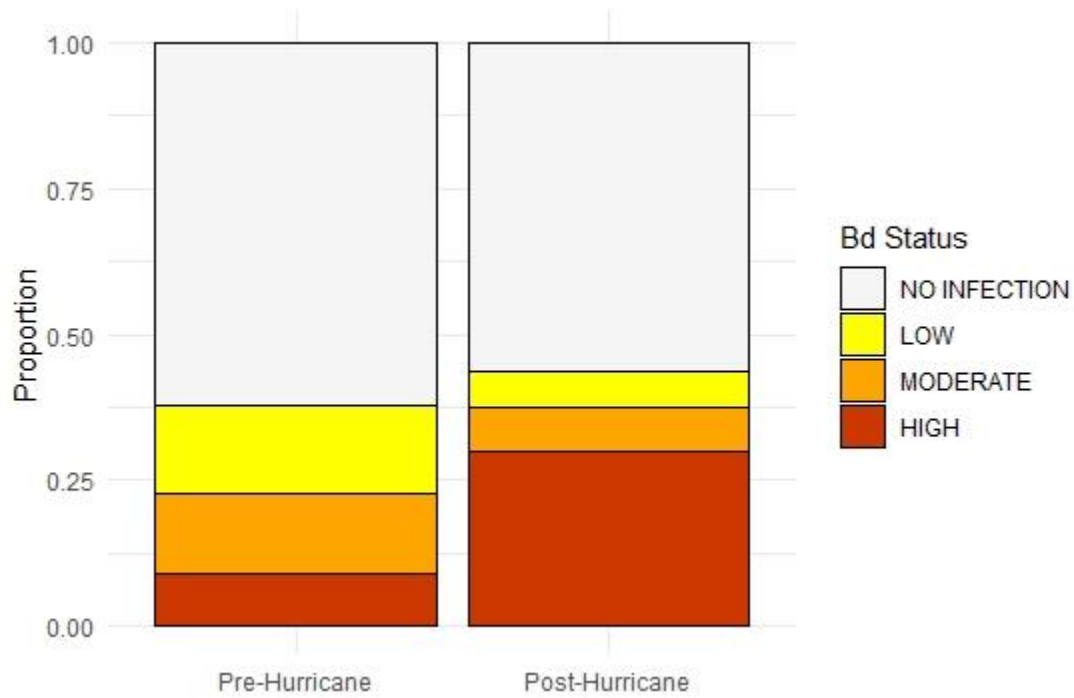
## Table

**Table 2.1:** Summary of Zero inflated negative binomial regression model\* to evaluate the effect of several factors on the *Bd* load carried by *E. coqui* hosts.

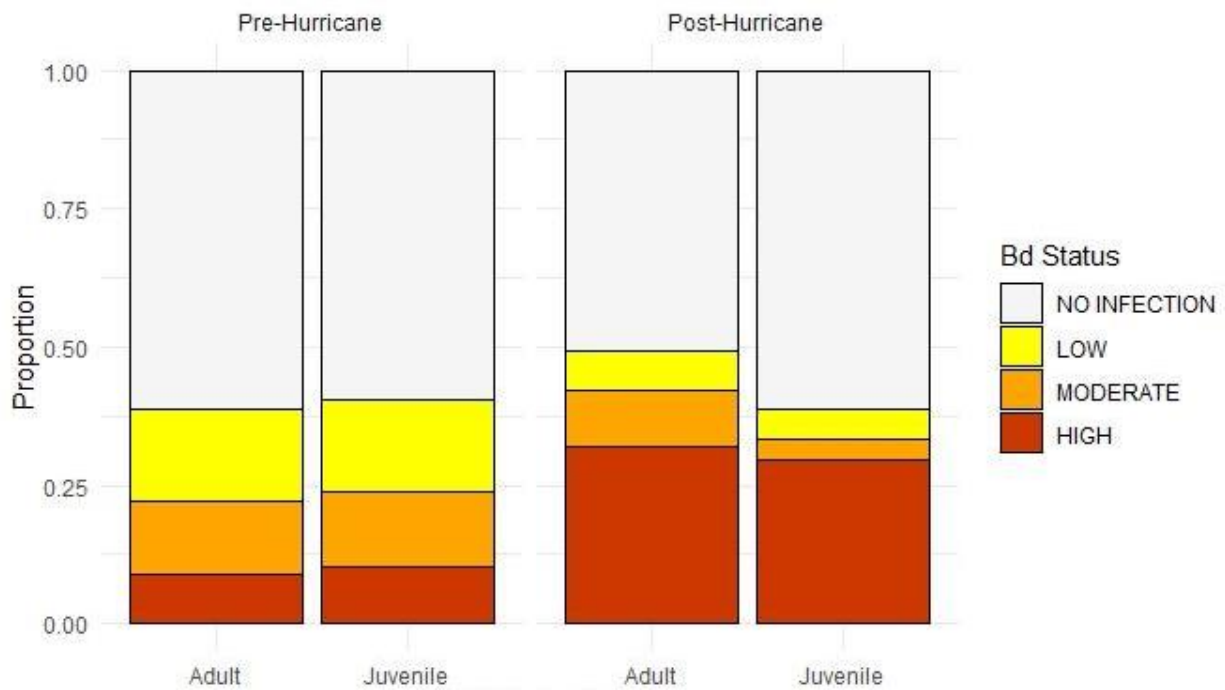
	Estimated std.	Error	z value	P value
<i>Count model coefficients (negative binomial with log-link)</i>				
<b>Intercept</b>	2.69553	0.09714	27.750	<2e-16 ***
<b>Hurricane (Pre-Maria)</b>	-0.68538	0.12204	-5.616	1.95e-08 ***
<b>Season (WW)</b>	-0.14959	0.10563	-1.416	0.157
<b>Age (Juv)</b>	0.03165	0.14670	0.216	0.829
<b>Hurricane x Season (Pre-Maria*WW)</b>	-0.02157	0.14390	-0.150	0.881
<b>Hurricane x Age (Pre-Maria *Juv)</b>	-0.10584	0.17641	-0.600	0.549
<b>Season x Age (WW*Juv)</b>	0.04217	0.16695	0.253	0.801
<b>Hurricane x Season x Age (Pre-Maria *WW*Juv)</b>	0.17883	0.21630	0.827	0.408
<b>Log(theta)</b>	1.96742	0.11536	17.054	<2e-16 ***
<i>Zero inflated model coefficients</i>				
<b>Intercept</b>	0.6926	0.2612	2.652	0.0080 **
<b>Hurricane (Pre-Maria)</b>	-0.7870	0.3311	-2.377	0.0175 *
<b>Season (WW)</b>	-0.7260	0.2902	-2.501	0.0124 *
<b>Age (Juv)</b>	0.2579	0.3870	0.667	0.5051
<b>Hurricane x Season (Pre-Maria *WW)</b>	1.7185	0.3802	4.520	6.18e-06 ***
<b>Hurricane x Age (Pre-Maria*Juv)</b>	0.2191	0.4641	0.472	0.6369
<b>Season x Age (WW*Juv)</b>	0.1356	0.4502	0.301	0.7633
<b>Hurricane x Season x Age (Pre-Maria *WW*Juv)</b>	-0.8091	0.5612	-1.442	0.1494

\**Bd* infection load = hurricane + season +  $\alpha$ . For the model, theta= 7.1522, number of iterations in Broyden-Fletcher-Goldfarb-Shanno (BFGS) optimization = 18 and log-likelihood = -2102 on 17 degrees of freedom. Asterisks and period denote the significance level: '\*\*\*' <0.001, '\*\*' <0.01, '\*' <0.05.

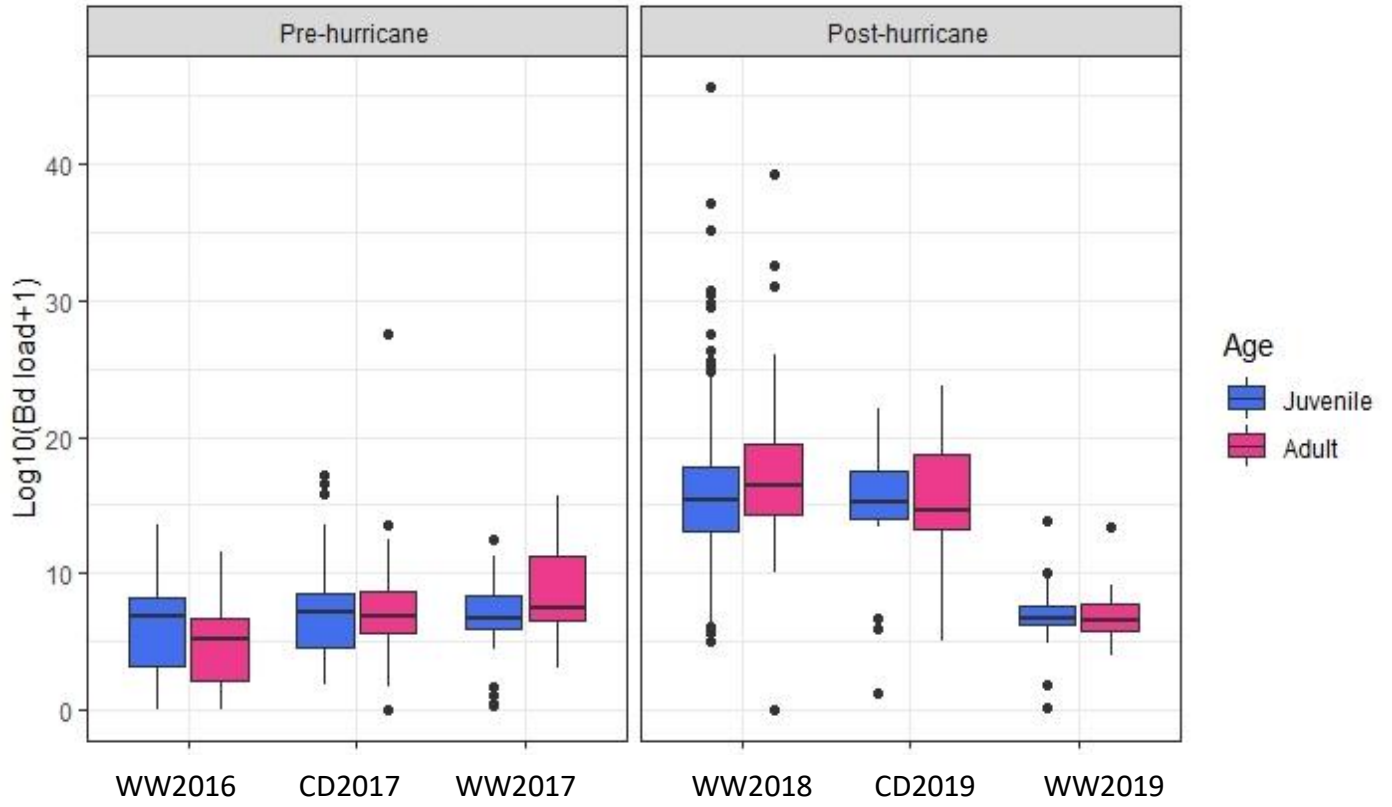
## Figures



**Figure 2.1:** *Bd* infection status in a population of *E. coqui* before and after Hurricane Maria. Infection intensity categories are defined by *Bd* load (ITS1 copy numbers) where Low: >0 to 665; Moderate: >665 to 6500; and High >6500.



**Figure 2.2:** *Bd* infection status by age class in a population of *E. coqui* before and after Hurricane Maria. Infection intensity categories are defined by *Bd* load (ITS1 copy numbers) where Low: >0 to 665; Moderate: >665 to 6500; and High >6500.



**Figure 2.3:** Seasonal variation in *Bd* load of infected frogs, by age class before and after Hurricane Maria.

**Chapter 3: An effective way to mark cohorts of juvenile terrestrial direct-developing frogs**

## Abstract

Marking juveniles of terrestrial direct-developing frogs is challenging because of their small size (< 18 mm) and fragility. This difficulty has limited studies on demography or population dynamics where empirical data on the survivorship of juveniles or their recruitment to adulthood are missing. In a controlled laboratory experiment we tested the survivorship of wild caught juvenile *Eleutherodactylus coqui* Thomas, 1966 to marking with a single colour Visual Internal Elastomer (VIE) in the thigh with and without additional ventral skin-swabbing for disease or microbiome monitoring. Results revealed 100 % survival in all groups, and all juveniles remained unharmed, moved freely, and fed actively during three days after treatments suggesting that this type of manipulation does not cause direct mortality. After 17 months of the experiment, we have recaptured 11 % of the marked juveniles as adults indicating that they can survive to recruitment age. We propose the use of a single VIE colour as a method to mark and follow date-specific cohorts of juvenile direct-developing frogs or young metamorphs until they reach older and larger age classes. This marking method can be used safely together with skin swabbing and provide valuable information for studies on population biology and age-specific response to environmental or disease stressors.

Keywords: Amphibian, Direct-developers, Juveniles, cohorts, Marking, VIE



## Introduction

Amphibians are difficult to mark permanently because their skin is thin and permeable making them vulnerable to intoxication (by paints or polishes that may work well for reptiles), or to skin rupture when using external tags (Donnelly et al., 1994). A variety of techniques have proven effective for long-term marking and identification of amphibian adults such as toe-clipping (e.g. Woolbright, 1996; McCarthy and Parris, 2004; Phillot et al., 2007; Longo and Burrowes, 2010; Burrowes et al., 2011; Grafe et al., 2011), pattern mapping or photography (e.g. Arntzen et al., 2004; Carafa and Biondi, 2004; Del Lama et al., 2011; Šukalo et al., 2013), passive integrated transponder (PIT) tagging (e.g. Camper and Dixon, 1988; Pope et al., 2001; Arntzen et al., 2004; Schulte et al., 2007), genotyping with microsatellites (Ringler et al., 2015), and visual internal elastomers (VIE), a UV-fluorescent polymer injected subcutaneously (e.g. Fogarty and Vilella, 2002; Moosman and Moosman, 2006; Campbell et al., 2009). The latter method has also proven successful when marking anuran larvae (Donnelly et al., 1994), and yielded high survivorship of *Bufo bufo* Linnaeus, 1758 tadpoles, especially when marked in in the upper tail (Iannella et al., 2017). However, marking young metamorphs or juveniles of direct-developing frogs is especially challenging due to their small body size. The biology and ecology of *Eleutherodactylus coqui* Thomas, 1966, a terrestrial direct-developing frog native to Puerto Rico, is well known (reviewed by Joglar, 1998), and although several studies have addressed population densities of this species (e.g. Stewart and Woolbright, 1996; Woolbright, 1996; Fogarty and Vilella, 2002; Burrowes et al., 2004; Woolbright et al. 2006), there are no studies on

demography, nor models on its population dynamics based on empirical data on juvenile survivorship and recruitment. Perhaps one of the reasons for this is the difficulty encountered when trying to mark the small juveniles. Young *E. coqui* froglets range in body size from 4–18 mm, depending on the geographic location in the island because in the lowland specimens are smaller than in the highlands (Joglar, 1998). While adults and subadults of *E. coqui* have responded well to mark and recapture studies using a variety of toe-clipping codes (Stewart and Woolbright, 1996; Woolbright, 1996; Burrowes et al., 2004; Burrowes et al., 2011), we found it extremely difficult to toe-clip juveniles because the manipulation necessary often resulted in damage and eventual death of individuals. As far as using a unique code consisting of a combination of two or more VIE colours to mark *E. coqui* frogs, our experience was similar to that of Fogarty and Vilella (2002). We were successful at recapturing adult and subadults, but we failed to recapture juveniles. Thus, to our knowledge, an effective method to mark juveniles of *E. coqui* is currently unavailable.

Herein we present the results of an experiment to test the viability of a single colour VIE injection as a method to mark juveniles of terrestrial direct developing frogs. This method does not aim to identify individuals, but rather to recognize and follow young cohorts until they reach an older, reproductive age class. Thus, even if the elastomer moves between body parts, as has been reported for adults marked with multiple colour codes (Moosman and Moosman, 2006; Bainbridge et al., 2015), a single colour VIE would still allow for the identification of a cohort marked on a particular dated period. We hypothesized that survivorship would be compromised by the degree of

manipulation, and that larger (older) juveniles would fare better than the smaller (younger) individuals.

An effective technique to safely mark immature froglets, that allows for their identification as they survive to reproductive age classes, is necessary to inform studies on the population dynamics of amphibians (Skelly and Richardson, 2010; Phillot et al., 2013). In current times, the global amphibian declines due to disease (Skerratt et al., 2007; Scheele et al., 2019) and/or other factors associated to global change phenomena (Stewart, 1995; Cushman, 2006; Becker et al., 2010; Burrowes, 2009) are evident. Thus, baseline population biology studies including recruitment information are critical to understand the mechanisms by which diverse factors (anthropogenic, biotic or abiotic) may contribute to the decline of populations and potentially lead to the loss of biodiversity.

## **Materials and Methods**

We collected juveniles (also referred to as froglets) of *E. coqui* at El Yunque National Forest in Puerto Rico (18.3016°N, 65.7854°W) at an elevation of 661 m, where a population has been censused and studied for the incidence of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* Longcore et al., 1999 for over 20 years (Burrowes et al., 2004; Longo and Burrowes, 2010; Burrowes et al., 2017). Froglets, of snout-vent length (SVL) ranging from 9–18 mm, were captured in the wild and brought to the laboratory (Fig. 1). Because collecting many juveniles of *E. coqui* at one time can be challenging, especially after the forest devastation caused by hurricane Maria

(Burrowes et al., in press), we worked with smaller sample sizes but conducted three independent trials of this experiment in June 2018, November 2018, and August 2019. In each trial a total of 18 juveniles were divided in three treatment groups, each including six juveniles within the full range of body sizes: two of SVL 9–12 mm, two of SVL 12.1–16 mm, and two of SVL 15.1–18 mm. Treatments consisted of (1) VIE only: juveniles were marked with a single VIE in yellow colour injected subcutaneously on the left thigh using a small, gauge 29, syringe (Fig. 1); (2) VIE + Swab: frogs were marked as above and also gently swabbed four times in the ventral area using cotton swabs (Medical Wire); and (3) Control: frogs were not marked or swabbed. We followed company's (Northwest Marine Technology Inc.) instructions for preparation of visual internal elastomers for injection, a procedure that requires mixing the coloured polymer with a fixative and used a fresh pair of gloves with each individual throughout the experiment. Swabbing was included in the experimental design as an additional manipulation, because we are especially interested in monitoring the prevalence and infection intensity of *B. dendrobatidis* in hosts, which is done by obtaining a sample of epidermal tissue on cotton swabs (Hyatt et al., 2007). It is important to highlight that swabbing of *E. coqui* juveniles should be done gently and not exceed five strokes to prevent rupture of the skin (Burrowes unpub.). Skin swabs can also be used to sample for other pathogens, as well as the general microbiome (e.g. Longo and Zamudio, 2017; García-Recinos et al., 2019). Thus, determining if simultaneous VIE marking and swabbing was safe for froglets would be useful when considering these methods for future studies.

We kept the juveniles overnight in the plastic bag in which they were collected in the field, and treatments were applied the next morning in the laboratory. Afterwards, each juvenile was housed separately in a (15 x 10 cm) plastic container previously cleaned and rinsed with a 10 % chlorine bleach solution. We perforated six holes on the lid of the containers to allow for air circulation and lined the bottoms with a clean white paper towel sprayed by aged tap water to maintain humidity. The side of each container was labelled with the treatment type and the froglet's SVL and placed in our animal care room at room temperature (25 °C) and 12-hour light regime for three days. Every morning for these three consecutive days, each froglet was checked for survivorship, stimulated with a thin glass rod to examine its ability to respond to stimulus and test for lethargy or limping, sprayed with aged tap water, and fed with approximately ten fruit flies. Feces and unconsumed flies were removed daily from the containers. On the fourth day of the treatment, all froglets were returned to the field where they were originally collected. In subsequent monthly amphibian monitoring efforts at the same site, we looked for marked juveniles, subadults or adults with a single yellow VIE marking, when found, we noted the position of the VIE in the body.

## **Results**

We obtained 100 % survivorship of a total of 54 juveniles in all treatments during the three independent trials of the experiment. We did not encounter problems marking or swabbing the juveniles (Fig 2). Regardless of the treatment received, all individuals responded to stimulus by moving away and without any indication of lethargy or limping

that would suggest a selection disadvantage in the wild. While larger individuals consumed all their prey (with only two exceptions when 2 flies were left uneaten), the mid-sized and smallest juveniles left on average 5 and 3 flies respectively every time they were fed. We found no relation between treatment and food consumption that would suggest that marking and/or swabbing affected the appetite of juvenile frogs. Contrary to our hypothesis, we did not find an effect associated to body size or degree of manipulation. Because juvenile *E. coqui* are fragile, and typically difficult to maintain in the laboratory (Colon-Piñeiro et al., 2017), we assumed that if they survived for three days after treatment, unharmed and with no signs of lethargy or limping, the treatments could not be considered a direct cause of mortality. Seventeen months after the last experiment, between August 2019 and January of 2021, we had recaptured 6 of the 54 VIE-marked juveniles (now as adults) at our study site in Puerto Rico. In three of the six adults recaptured, the yellow marking had moved from the thigh to the flank area (on the of the same side in two of the individuals and to the opposite side in one), indicating that in *E. coqui* VIE markings move around. Thus, the use of multiple VIE colour codes in specific body parts to identify individuals can lead to confusion as has been reported for other frogs (Moosman and Moosman, 2006; Bainbridge et al., 2015).

## **Discussion**

We showed that marking juveniles of terrestrial direct development frogs with a single VIE colour did not kill or cause any damage on juveniles of *E. coqui*. The fact that they remained healthy and consumed fruit flies readily for three consecutive days

suggests that the VIE marking method, as well as the manipulation required for marking in this manner and simultaneously swabbing their ventrum, does not cause mortality to these small froglets. However, juveniles of *E. coqui* are prey to many invertebrates (Stewart and Woolbright, 1996; Joglar, 1998) and studies have shown that this age class is the most vulnerable to chytridiomycosis (Longo and Burrowes, 2010; Burrowes et al., 2017). Thus, if field studies were to yield low recapture rates of juveniles marked with a single VIE colour and/or swabbed, it may be the result from death by natural reasons rather than the marking technique. Nonetheless, 17 months after the experiments in the lab, we have obtained 11 % recapture rate of single-VIE marked juveniles suggesting that this kind of cohort marking can inform recruitment into adulthood. A study on *Allobates femoralis* in French Guyana, revealed a much lower survival rate (3.72 %) from tadpoles to adults that had been identified via microsatellite genotyping (Ringler et al., 2015). Thus, although our recapture data is still preliminary, it suggests that juveniles marked with a single VIE survive to adulthood.

Marking juveniles of terrestrial direct-development frogs with a single VIE colour allows for the identification of young cohorts in a population. For example, in a particular study, one can choose to mark all juveniles within a dated period with a yellow VIE, while subadults are marked with blue and use different colours for the next year. By distinguishing the year's, or the season's juveniles in a population, we can then use data from capture-recapture methods to estimate survivorship from one age class to the next and make predictions about the reproductive growth of the population based on empirical data.

Since both subadults (SVL 18–28 mm) and adults (> 28 mm) of *E. coqui* can be marked and effectively identified when recaptured using toe-clipping, or even more accurately with mini (1.4 x 8 mm) passive integrated transponders (Burrowes and Aleman, unpub), we would need to rely on VIE marking only for cohorts of juveniles. With individuals of all age classes effectively and safely marked, we can also learn about growth rates at different stages, identify age classes that are more susceptible to stressors (e.g. disease, hurricanes, droughts, forest fragmentation, etc.), and ask a variety of ecological and evolutionary questions informed by data on population dynamics. From a conservation standpoint, these studies could advise management decisions that may result in the protection of species before they fall into critical numbers that merit listing them as vulnerable, threatened, or worse by the International Union for the Conservation of Nature (IUCN). In conclusion, based on the positive results of our experiment, we recommend the use of a single colour injection of VIE as an effective way to mark (and recapture) date-specific cohorts of juveniles of direct-developing frogs. This method can be used in conjunction with swabbing the ventral area of individuals and may be applied to young metamorphs of other species of anurans.



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**Figure**



**Figure 3.1:** Juvenile of *Eleutherodactylus coqui* Thomas, 1966 before receiving one of the treatments in the laboratory (A), and view of a juvenile of *E. coqui* under UV radiation showing the fluorescence of its single yellow VIE marking in the thigh (B).