

**THE EFFECT OF HURRICANE MARÍA ON THE *ELEUTHERODACTYLUS*
COMMUNITY AND OCCUPANCY MODELLING OF *E. RICHMONDI*:
A BIOACOUSTICS MONITORING STUDY AT THE CARITE STATE FOREST**

By

Diana Carolina López Hernández

A Thesis submitted in a partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE
In
BIOLOGY**

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS

2019

Approved by:

Alberto R. Puente Rolón, Ph.D.
President, Graduate Committee

Date

Fernando J. Bird-Picó, Ph.D.
Member, Graduate Committee

Date

Raúl E. Macchiavelli, Ph.D.
Member, Graduate Committee

Date

Ana Vélez Díaz, M.S.
Interim Biology Department Director

Date

Alexandra Gregory Crespo, Ph.D.
Graduate Office Representative

Date

© *Diana Carolina López Hernández, 2019*

ABSTRACT

Hurricane María restructured Puerto Rico's forests, including vegetation and animal communities. One of the main links on the trophic chains of these forests are amphibians of the *Eleutherodactylus* genus. A species whose populations have been declining is *Eleutherodactylus richmondi*. Finding new populations and understanding its ecology is necessary for its conservation. Using bioacoustics and occupancy modelling, this study quantifies the effect of Hurricane María on the *Eleutherodactylus* assemblages, and it identifies a new population of *E. richmondi* and its site occupancy. Results showed that there was a change in the acoustic environment after the hurricane due to changes in insect and amphibian calling activity. Most species decreased their calling activity, except *E. antillensis* and *E. brittoni*. Occupancy modelling showed that *E. richmondi* occupies sites with high percent canopy cover and terrain slope. This study showed how acoustic communities react to disturbances and added necessary information on *Eleutherodactylus richmondi*.

RESUMEN

El Huracán María cambió la estructura de los bosques de Puerto Rico, incluyendo su vegetación y comunidades de animales. Uno de los principales eslabones de las cadenas tróficas de estos bosques son los anfibios del género *Eleutherodactylus*. Una de las especies de este género, que ha estado disminuyendo en los números de sus poblaciones es *E. richmondi*. Encontrar nuevas poblaciones de esta especie y entender su ecología es necesario para su conservación. Este estudio cuantifica el efecto del Huracán María en las comunidades de *Eleutherodactylus*, e identifica una nueva población de *E. richmondi* y su ocupación. Los resultados muestran que ocurrió un cambio en el paisaje acústico luego del huracán, debido al cambio de actividad acústica de los insectos y anfibios. La mayoría de las especies de anfibios disminuyeron su actividad acústica, a excepción de *E. antillensis* y *E. brittoni*. El modelo de ocupación mostro que *E. richmondi* ocupa sitios con un porcentaje alto de cobertura del dosel y de inclinación del terreno. Este estudio muestra como las comunidades acústicas reaccionaron al disturbio y añadió información necesaria sobre la ecología de *Eleutherodactylus richmondi*.

ACKNOWLEDGEMENTS

First of all I would like to thank my parents for always supporting me in all of my endeavors and ideas. I would like to express thanks to my graduate committee. Dr. Alberto R. Puente-Rolón for ideas, support, contacts, equipment and fieldwork help. Dr. Fernando J. Bird-Picó for his guidance and ideas, not only about this project but also life and career advice since the beginning of my master's degree. Dr. Raúl E. Macchiavelli for his help with the statistical analysis of this project and for introducing me to the field of biometrics. Thanks to Dr. Neftalí Ríos López also for access to his library of research papers and for accompanying us in the first field work survey. Of course, I would like to thank Fish and Wildlife Services for permission to use the recordings gathered in 2017 as pre-hurricane data, and for providing access to the ARBIMON platform. Thanks to Jan Paul Zegarra for brainstorming ideas which ultimately led to this project. Thanks to the Biology Department for the "Dr. Peter Castro" scholarship and to Iniciativa Herpetologica for providing me with an equipment grant for the dataloggers used in this project. Special thanks to Dra. Alexandra Gregory for her help, life and professional advice, teachings, and overall support. Thanks to Kenneth Xavier Rodriguez for his friendship and hard work and support during fieldwork. To Rhianna Smith for her friendship and big sister mentorship. Thanks to Donato Seguí for always lending an ear and offer advice. And last but not least, I give special thanks to my wife Mariné Lugo Ruiz for her help with fieldwork, ideas, support and patience. Without her help and support I would not have been able to do this project.

TABLE OF CONTENTS

Abstract.....	iii
Resumen.....	iv
Acknowledgements.....	v
List of Tables.....	viii
List of Figures.....	ix
List of Annexes.....	x
Introduction.....	1
Literature Review.....	4
Disturbances.....	4
2017 Atlantic Hurricane Season.....	5
Effects on Forests.....	7
Effects on Animal Communities.....	9
Amphibians: Importance and Vulnerability.....	11
<i>Eleutherodactylus richmondi</i>	14
Bioacoustics.....	15
Occupancy Models.....	17
Chapter 1: The effect ff Hurricane María on the soundscapes, richness and calling activity of the <i>Eleutherodactylus</i> assemblages of The Carite State Forest.....	19
Abstract.....	20
Introduction.....	21
Methods.....	23
Results.....	28

Discussion.....	38
Chapter 2: Identification of habitat features associated with site occupancy of <i>Eleutherodactylus richmondi</i> in the Carite State Forest with the use of bioacoustics and occupancy modelling....	43
Abstract.....	44
Introduction.....	45
Methods.....	48
Results.....	53
Discussion.....	58
General conclusions and recommendations.....	62
Literature Cited.....	64
Appendixes.....	71

LIST OF TABLES

Table 1. Survey structure of the 31 sampled sites for pre-post hurricane comparison..	23
Table 2. Average maximum, minimum frequency (kHz) and duration (s) used to create species-specific identification models in ARBIMON.	32
Table 3. Occurrence and average nightly call frequency of each species of <i>Eleutherodactylus</i> present in the study before and after Hurricane María.	35
Table 4. Maximum, minimum, average and standard deviation of covariate measurements.	50
Table 5. Model selection table for the single-species single-season occupancy model of <i>Eleutherodactylus richmondi</i> .	53
Table 6. Estimates for the most parsimonious occupancy model of <i>E. richmondi</i> .	54

LIST OF FIGURES

Figure 1. Rainfall (inches) from Hurricane María	6
Figure 2. Forest disturbance intensity after Hurricane María.	8
Figure 3. Hierarchical cluster analysis of the soundscapes of sites measured before and after the hurricane..	30
Figure 4. Average number of frequency bins with activity >0 per site and frequency category, divided by year before and after the hurricane.	31
Figure 5. Model based average number of species present per site	34
Figure 6. Average nightly calling activity per site. Sites used were the ones where each of the species was present both before and after the hurricane.....	37
Figure 7. Predicted relationship between detection probability and relative humidity.	54
Figure 8. Predicted relationship between probability of occupancy and the covariates..	55
Figure 9. Average diel calling activity of <i>E. richmondi</i>	56
Figure 10. Model based average 24h calling activity of <i>E. richmondi</i> per site.....	57

LIST OF APPENDIXES

Appendix A. Coordinates of sites used for 2017-2018 comparisons.....	71
Appendix B. Location of the sites used for the pre and post-hurricane comparisons.....	72
Appendix C. Coordinates of sites used for occupancy modelling and day activity description of <i>E. richmondi</i>	73
Appendix D. Location of the sites surveyed for occupancy modelling of <i>E. richmondi</i>	74
Appendix E. Diagram of Automatic Microphones	75

INTRODUCTION

A disturbance is defined as a disruption on the flux of an ecosystem by an external force (Lugo, 2008). Hurricanes are large-scale disturbances that affect the Caribbean (Walker, et al., 1991; Vilella & Fogarty, 2005). In 2017, the Atlantic hurricane season was extremely active with an above average number of major hurricanes (Klotzbach & Bell, 2017). On September 20th of 2017 Category 4 Hurricane María, one of the most notable storms of the season, made landfall on Puerto Rico. The impact of hurricanes have a profound effect on plants, soils, landforms, and animals (Walker, et al., 1991).

Rearrangement of the forest structure can affect animal populations by altering the food supply and microclimate of the forest floor (Waide, 1991). One of the vertebrate groups that numerically dominate most forests in Puerto Rico are the anurans of the genus *Eleutherodactylus* (Klawinski, et al., 2014). These amphibians serve as environmental bio-indicators (Burrowes, et al., 2004) and play an important role in forest ecosystem dynamics (Semlitsch, 2003). Population changes of *Eleutherodactylus* species are influenced by abiotic factors such as seasonal changes in daylength, rainfall, and temperature (Stewart, 1995). Subtle changes in these variables can result in warmer and drier conditions, acting as potential stressors for the *Eleutherodactylus* fauna (Burrowes, et al., 2004). This dependency on local environmental factors makes them more prone to local extinctions after disturbances (Ríos-López & Aide, 2007).

Studies of the influence that hurricanes have on the *Eleutherodactylus* species community of Puerto Rico have shown that species respond differently to the disturbance. After Hurricane Hugo populations of *E. coqui* and *E. hedricki* increased in numbers (Woolbright, 1991). On the other hand, other studies conducted after disturbances detected that populations of *E. coqui*, *E.*

antillensis, *E. brittoni*, and *E. richmondi* decreased in numbers (Vilella & Fogarty, 2005; Klawinski, et al., 2014). One of the species, *Eleutherodactylus richmondi* has showed a population decline from different locations after both of the previously mentioned hurricanes, Hugo (Woolbright, 1991), and Georges (Vilella & Fogarty, 2005). Furthermore, populations from this species have been showing a steady decline and disappearances since 1986 (Zegarra-Vila, 2007). The International Union for Conservation of Nature (IUCN) has classified *E. richmondi* as critically endangered (Angulo, 2008) whereas The Puerto Rico Department of Natural and Environmental Resources (DRNA) classified the species as Vulnerable (DRNA, 2016). Research concerning the current state of this species is imperative considering that few populations are known and most of them have gone extinct (Villanueva-Rivera, 2006). Although there are studies of the effect of hurricanes on *Eleutherodactylus spp.*, most of these are focused on the Luquillo Experimental Forest (LEF) (Woolbright, 1991) and one in the central mountain range (Vilella & Fogarty, 2005). This leaves an information gap on the effect of large scale disturbances on the amphibian community of other highly diverse areas of Puerto Rico. Therefore, it is crucial to determine the current state of *Eleutherodactylus* species populations in other diverse areas, especially after the impact of Hurricane María.

The study of *Eleutherodactylus* species in Puerto Rico requires large scale sampling of areas. This large scale sampling is rarely feasible due to it requiring significant time, resources, and manpower. Passive Acoustic Monitoring (PAM) by the deployment of automatic digital recorders can help in large scale sampling (Aide, et al., 2013). PAM has been used to map out the distribution of threatened bird species (Campos-Cerqueira & Aide, 2016), to evaluate the impact of industrial development on anurans and birds (Deichmann, et al., 2017), to measure the calling activity of anurans (Ospina, et al., 2013), to measure the impact of traffic noise on animal

communities (Herrera-Montes & Aide, 2011), and to measure the effect that human activities have on biodiversity (Herrera-Montes, 2018).

In this study, I use passive acoustic monitoring and bioacoustics to: (1) Quantify the effect that Hurricane María had on the amphibian assemblages in and around Carite State Forest; (2) Identify habitat features associated with site selection of *E. richmondi* on the south portion of the Carite State Forest; and (3) Describe the diurnal calling activity shown by *E. richmondi* on the south portion of the Carite State Forest. This forest is predicted to have high vertebrate species diversity (Gould, et al., 2008) and was affected by the direct impact of this major hurricane. This study will help to understand the dynamics of how amphibians in Puerto Rico deal with disturbances of this magnitude, a proxy that shows the level of adaptation they have to the fluxes of their environment.

LITERATURE REVIEW

Disturbances

All natural communities are dynamic systems (Sousa, 1984), affected by abiotic or biotic physical forces better known as disturbances (Rykiel, 1985). A disturbance can affect all levels of biological organization by causing a disruption in the ecosystem and its communities, altering its physical environment, and changing its resource and substrate availability; turning disturbances into a major shaping force for composition and structure of the environment they affect (White & Jentsch, 2001). The effects that disturbances have on the ecosystem can be assessed according to the severity, frequency, duration, scale, and points of interaction with the ecosystem (Lugo, 2008). These characteristics can be altered by climate variables, meaning that climate change (White & Jentsch, 2001). Changes in temperature and precipitation can influence the occurrence, timing, frequency, duration, and intensity of disturbances (cited by Dale, et al., 2001). The impact that disturbances have on the ecosystem give a high importance to its research because of the insight they provide into key ecological mechanisms (Zimmerman, et al., 1996) and the crucial role they have in maintaining biodiversity (Tanner, et al., 1991).

These disturbances can come from natural or human sources. Examples of natural disturbances are wildfires, landslides, flooding, treefalls, droughts and windstorms (Zimmerman, et al., 1996). Hurricanes are tropical cyclones with sustained winds that have a global role in the heat balance of the Earth by transporting excess tropical heat to higher latitudes and regulating the climatic homeostasis of the planet (Lugo, 2008). The effects of a hurricane on ecosystems depend on the intensity of the hurricane, its diameter, its velocity of forward movement, the susceptibility of the ecosystems to damage, and the degree of protection afforded by topographic feature. This

tropical phenomena is considered to be a common disturbance for Caribbean ecosystems (Tanner, et al., 1991). Over the last 100 years there have been approximately 800 hurricanes and tropical storm passages through the Caribbean (Lugo, 2008). The frequency in which they affect the Caribbean basin and its islands may be altered due to climate change. By evaporating more water and transporting it to higher latitudes, global warming may accelerate the hydrologic cycle, therefore could possibly produce more intense and frequent storms (cited by Dale, et al., 2001).

2017 Atlantic Hurricane Season

The Atlantic Hurricane belt is an area in the Atlantic Ocean prone to be affected by hurricanes during the season that goes from June 1st to November 30th (National Hurricane Center, 2015). Islands in this area are subjected to hurricanes in intervals of every 10 to 60 years (Waide, 1991). This hurricane basin shows the largest year-to-year variability compared to the rest of the tropical cyclone basins around the globe (Klotzbach & Bell, 2017).

Characterized by its above average number of named storms, hurricanes and major hurricanes, the 2017 Atlantic Hurricane season was marked as extremely active, with 51.25 hurricane days and 6 major hurricanes formed (Category 3, 4, 5) (Klotzbach & Bell, 2017). Hurricanes and storms pass close to Puerto Rico every 2-5 years (cited by Beard, et al., 2005) and it gets directly hit by a hurricane every decade (Lugo, 2008). In 2017 Puerto Rico was affected by 2 of the 6 major hurricanes of the season, Hurricane Irma on September 6 and Hurricane María on September 20. Irma's center was positioned 92.6km to the north of the northern shore of Puerto Rico. Even if the eye did not make direct landfall on the Island, its outer rings brought high wind speeds up to 48kt, and rainfall averaging 10-15 inches at high elevations between the 5 and 7th of September (Cangialosi, et al., 2018).

The second hurricane that affected Puerto Rico was María, which formed on September 12 at the west coast of Africa, on the 18th of September it increased its wind speed by 65kt in 24 hours, making it the sixth-fastest intensifying hurricane in the Atlantic basin record. Just before landfall with the southeast coast of Puerto Rico, hurricane María tripled its eye diameter from 16.7km to 51.9, contributing to its weakening into a category 4 but increasing the diameter of the hurricane's highest winds. The hurricane's eye entered the Island at Yabucoa at 1015 UTC on September 20 with 135kt winds (Pasch, et al., 2018), making it the first category 4 hurricane to make landfall since 1932 and the strongest hurricane to make landfall in Puerto Rico since San Felipe II in 1928 (Klotzbach & Bell, 2017). The trajectory of the hurricane on the Island went from southeast to northwest and it produced heavy rainfall up to 96.62cm at one location (Figure 1). The center emerged into the Atlantic at 1800 UTC on September 20 with a category 3 intensity (Pasch, et al., 2018). This decline in intensity happens when a hurricane hits land because it requires warm water to maintain its strength (Lugo, 2008).

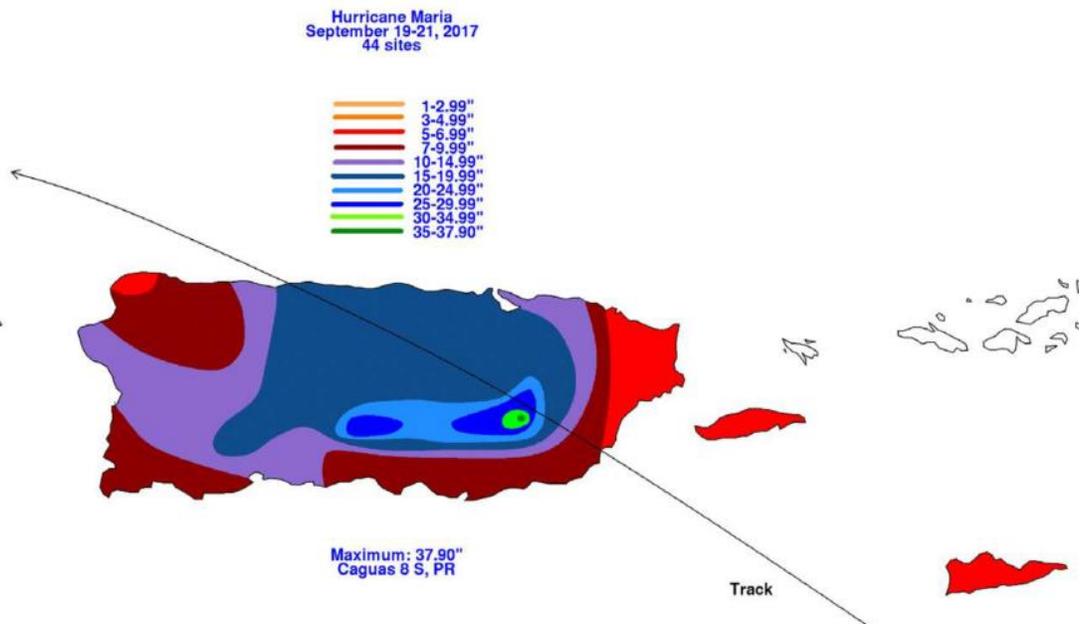


Figure 1. Rainfall (inches) from Hurricane María. Source: Pasch, et al. (2018).

Effects on forests

The path of Hurricane María over this Island left intense forest disturbance (Figure 2). These disturbances can have a profound effect on plants, soils, landforms, and animals (Walker, et al., 1991), causing long lasting effects on the terrestrial biota environment (Waide, 1991). The two main components of a hurricane are rain and wind, and the interaction of these two factors cause visible and invisible effects on forests (Lugo, 2008). The former effect can be seen as the alteration and visible change of the vegetation structure in the forest (Tanner, et al., 1991). The change in vegetation structure starts with defoliation. Defoliation is the loss of leaves and branches off of trees caused by the dissipation of wind energy on the canopy of the forest (Lugo, 2008), and it is one of the most noticeable effects that tropical cyclones have on forested areas. This results in the formation of new forest gaps (Tanner, et al., 1991) and an input of large amounts of fresh, non-senesced biomass to the forest floor (Klawinski, et al., 2014). Both of these factors have an effect on the microclimate of the forest. The increased amount of biological debris and leaf litter can lead to an increase in some soil nutrients (Tanner, et al., 1991) and a higher heterogeneity of the landscape (Lugo, 2008).

Tanner (1991) mentions that the opening of the canopy creates gaps with increased light penetration which subsequently increases soil and air temperature. These factors in combination with the new input of leaf litter can result in moister soils where the tree gaps are. An increase in moisture can consequently alter the microbial activity of the soil affecting nutrient cycling and affecting animal populations. The increase in light availability on the forest floor also acts as an aid for new colonist plant species. After a gap is created new germination will occur, it can come from newly dispersed seeds, buried seeds, or sprouts of the old surviving trees (Connell, 1989). New colonization then adds variability and the future structure of the forest canopy becomes a

mosaic of early successional species and old regenerating species (Walker, et al., 1991). High sustained winds can also cause tree uprooting, with trees that are larger in diameter having a higher probability of being uprooted (Walker, et al., 1996). This uprooting can affect soil morphology and its characteristics, nutrient availability, and regeneration trends, all of which can help a forest quickly recover via succession (Lugo, 2008). Walker, et. al. (1991) mention that even if there was a high level of defoliation and uprooting during Hurricane Hugo in 1989, only 4% of the trees left upright died, and 77% of the trees that were uprooted started sprouting after 54 weeks. In addition to defoliation and uprooting, rainfall during a hurricane can reach intense levels, causing flooding and saturating the soil which in turn makes it mechanically weak, generates erosion and triggers landslides (Tanner, et al., 1991). Landslides structurally change the landscape and they require more time to go back to forest conditions because its colonization by plants depends on the availability of the seeds and propagules, as well as on the organic matter and nutrients present on the soil (Walker, et al., 1996).

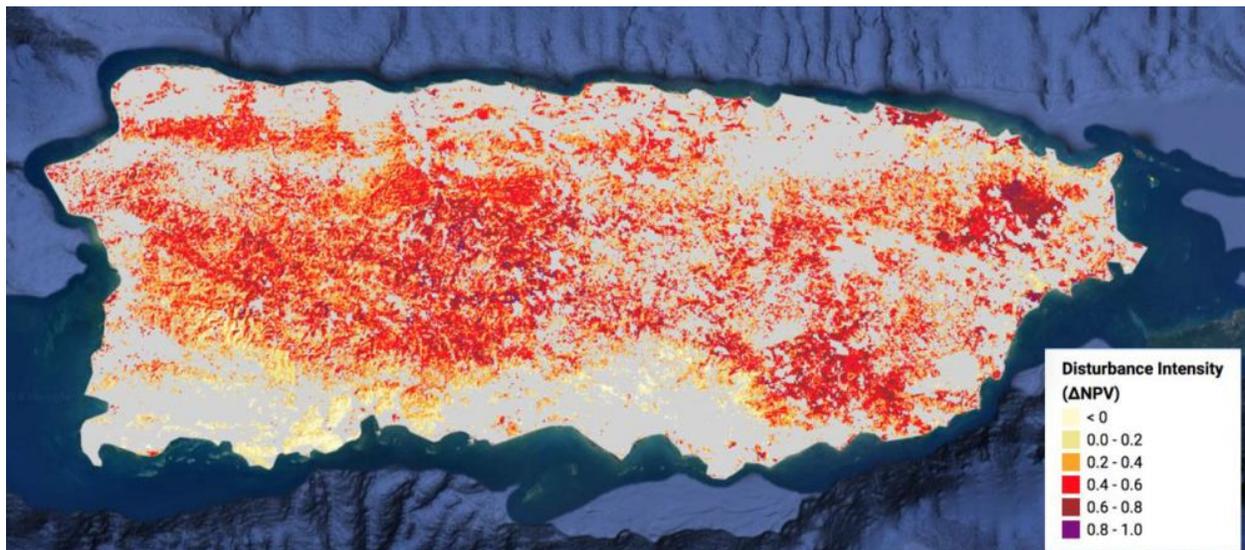


Figure 2. Forest disturbance intensity after Hurricane María. Grey areas represent non-forested areas or areas with cloud cover (Feng, et al., 2018).

Effect on animal communities

The structure of an ecosystem is partly shaped by animal assemblages. Animal population attributes can then be affected by the rearrangement of the forest structure due to disturbances. Waide (1991) mentions that animal populations are affected directly and indirectly by a hurricane. A direct effect is physical damage during the storm that leads to the death of the individual. Indirect effects on the other hand are alterations in microhabitat structure leading to a change in resource availability (Zimmerman, et al., 1996). Microclimate modifications caused by vegetation restructuring, an increase in light and temperature of the understory, variation in habitat heterogeneity and changes in food supply (Waide, 1991) are a few of the indirect effects that cause microclimatic modifications and can lead to a restructuring of the animal community. Lugo (2008) presented examples on population variations of fruit eating bats, snails, stick insects, lizards, frogs, and birds due to the impact of a hurricane.

Because of the canopy loss, the forage space of organisms is compressed, and there is an influx of biomass to the understory, altering then the stratification of animal resources (Wunderle, 1999). Animals move to available and suitable habitats when responding to the changes in microenvironment. An example of this is seen in the shift of habitat use by *Anolis stratulus*, a canopy lizard that started foraging on the forest floor after the opening of the canopy (cited by Lugo, 2008). A change in the movement pattern of the Puerto Rican boa (*Chilabothrus inornatus*) was detected after hurricane Georges by Wunderle (2004), resulting in the radio-tagged boas increasing their movement and becoming more visible to observers after the hurricane, due to the shift from damaged to less damaged sites in response to changes in foliage and prey abundance.

Effects on animals due to diet restrictions are suffered mostly by nectarivores and frugivores (Lugo, 2008). Fruits and flowers are lost due to defoliation and loss of tree branches. Gannon et al. (2006) measured the population of bats before and after hurricane Hugo, the results showed that fruit eating bats decreased in numbers and took 5 years for it to get to its pre-hurricane levels. This variation was due to the reduction in fruit and flower availability for months after the storm. That same fruit availability factor affected populations of Puerto Rican parrots after hurricane Hugo, causing the breeding season to be delayed by a month and a half after the cyclone (Wunderle, 1999). Higher temperatures and the abundant input of leaf litter are factors that could increase the abundance of insects after a hurricane, providing a good food source for insectivores and omnivores (Waide, 1991). This variability in food resources increases the need for feeding plasticity, or the flexibility to be able to eat from different food sources depending on the resource availability. This hypothesis was proven by Waide (1991) on insectivorous birds feeding on different kinds of arthropods after a hurricane, suggesting that there is feeding plasticity in Caribbean birds.

Plasticity can be seen as an opening or enlargement of a particular niche, which in part can limit specialization and diversity (White & Jentsch, 2001). The reduction of species specialization then favors generalists; this can explain why there is an abundance of generalist species on the small Caribbean Islands that are susceptible to repeated hurricanes (Waide, 1991). Because of the differences in ability that certain species have when responding to disturbances, adaptation to them plays an important role on animal survival and consequently on natural selection. Giving large scale disturbances an important role on the evolution of species affected by them. Species have evolved a diverse spectrum of abilities that were shaped by past exposures to disturbances (White & Jentsch, 2001). These evolutionary responses provide a key to understanding the structure of

the communities that are chronically disrupted (Waide, 1991). Taking into account the effect of hurricanes on forests and animal communities, Lugo (2008) summarizes the ecological role of hurricanes in six main effects: increase the heterogeneity of the landscape and variability of its processes, hurricanes change the ecological space available to organisms, they set the organisms in motion, they redirect succession and rejuvenate the landscape, they shape forest structure and influence species composition, and at last, they induce evolutionary change through natural selection.

Amphibians: Importance and vulnerability

Secondary succession is the process of reestablishment of a community after its disturbance, this is the result of interspecific competition, producing an environment in which new dominant species are competitively superior (Horn, 1974). New animal assemblages are then composed primarily of disturbance adapted species (Hernández-Ordóñez, et al., 2015; Suazo-Ortuño, et al., 2018). Located in the hurricane belt, Puerto Rico is susceptible to the repeated impact of hurricanes. Waide (1991) explains that his chronic disturbance places island forests into a state of continual secondary succession, hence explaining the relatively low diversity of species in the subtropical forests of Puerto Rico when compared to continental forests. The vertebrate groups that numerically dominate most forests in this Island are the lizards of the *Anolis* genus, and amphibians of the *Eleutherodactylus* genus (Klawinski, et al., 2014), which makes them the dominant consumers of the forest trophic webs (Waide, 1991). According to Hedges (1993) *Eleutherodactylus* frogs not only dominate Puerto Rican forests, they dominate the West Indian frog fauna. And their enormous success is most likely explained by their life cycle. Frogs within

this genus do not have a biphasic life cycle, they are terrestrial-breeding hatching directly into froglets, and can spend their entire life far away from water (Woolbright, 1997).

Dominating terrestrial ecosystems provides these amphibians with an important ecological role. Amphibians act as an important link in trophic chains, contributing to the energy flow. Being ectotherms the energy they ingest is efficiently converted to biomass that reaches higher trophic levels (cited by Whiles, et al., 2006). Also, amphibians are important as secondary and tertiary consumers, serving also as food for vertebrate and invertebrate predators. Of the *Eleutherodactylus* genus, *E. coqui* is the most abundant species in Puerto Rico, with one of the highest known amphibian population density in the world (20,570 frogs/ha), constituting the largest component on the nocturnal biomass of all vertebrates in the forest (Stewart, 1995). This anuran species consumes an average of 114,000 invertebrates per hectare per night (Beard, et al., 2005; Stewart M. M., 1995). Predatory amphibians can help reduce the spread of illnesses carried by mosquitoes, hence influencing the regulation of disease transmission and pest outbreaks (Hocking & Babbitt, 2014). Furthermore, amphibians act as bioindicators of the health of the forest ecosystem (Burrowes, et al., 2004) because of their highly permeable skin and sensitivity to changes in environmental conditions (Ríos-López & Aide, 2007).

Despite the lack of aquatic habitat dependency, *Eleutherodactylus* species do require moist and cool environments to avoid desiccation (Burrowes, et al., 2004). This dependency on moisture comes from their permeable skin, and it makes them sensitive to environmental changes (Ríos-López & Aide, 2007); pollution, increased ultraviolet radiation (Halliday, 2008). Warmer and drier conditions turn into potential stressors for *Eleutherodactylus* assemblages (Burrowes, et al., 2004). Relative low vagility, narrow habitat tolerances, and high vulnerability to pathogens are other factors that make them susceptible to environmental changes (Cushman, 2006). This sensitivity to

environmental stressors makes them vulnerable to disturbances. Ríos-López & Aide (2007) studied the herpetofaunal assemblage in a reforestation project, they found that the increase in vegetation heterogeneity and decrease in microclimate variability facilitate herpetofaunal succession after a disturbance. After the impact of hurricane Jova on the Pacific Coast of Mexico, Suazo-Ortuño et al. (2018) measured the response of herpetofaunal assemblages to the disturbance. They found marked changes in richness and composition of communities of frogs, lizards and snakes, with the modification of vegetation being the main significant factor. The modification of vegetation after a hurricane is mainly due to the fact that defoliation and debranching create forest gaps. Forest gaps allows the entrance of more light and change microclimate conditions; this does not necessarily translate to a negative effect. The reduction of canopy cover in an Australian rainforest after a tropical cyclone was shown to increase temperature and water loss in the understory, reducing the infection risk of frogs to *B. dendrobatidis* by 11-28% (Roznik, et al., 2015). Barker & Rios-Franceschi, (2014) found that site occupancy was negatively associated with high temperatures and seasonal precipitations on the populations of *E. portoricensis* at the Cayey and Luquillo mountains.

In Puerto Rico, amphibian population responses to hurricanes have been measured both experimentally (Klawinski, et al., 2014) and observationally (Vilella & Fogarty, 2005; Woolbright, 1991). Klawinski, et al. (2014) manually mimicked the conditions resulting from the impact of hurricane Hugo, including opening up the canopy and adding debris and leaf litter on experimental plots at the Luquillo Experimental Forest. The results showed that debris deposition had no significant effect on *E. coqui* density but that the canopy disturbance had a significant negative effect on their population density. Following Hurricane Georges in 1998, Vilella & Fogarty (2005) reported changes in pattern of diversity and abundance of the frog assemblages in the Cordillera

Central of Puerto Rico. Their results showed that only *Eleutherodactylus coqui* had an increase in abundance, and three other species (*E. antillensis*, *E. brittoni*, and *E. richmondi*) showed the greatest decreases. After hurricane Hugo in 1989, Woolbright (1991) found that the population densities of *Eleutherodactylus* frogs were affected by the hurricane at the Luquillo Experimental Forest. *E. coqui* and *E. hedricki* increased while the abundance of *E. richmondi* significantly decreased.

Eleutherodactylus richmondi

Eleutherodactylus richmondi or ground coqui (locally referred as coqui caoba – mahogany) is an endemic species of Puerto Rico. This species inhabits the forest floor, hiding under low boulders, under leaf litter, and around fallen logs (Zegarra-Vila, 2007; Stewart & Woolbright, 1996). It is restricted to the wet forests of Puerto Rico (Heatwole, et al., 1965) at an altitude between 40 to 1158 meters (cited by Zegarra-Vila, 2007) with an approximate maximum population density of 100 individuals per hectare (Stewart & Woolbright, 1996). Villanueva (2006) found that its peak of calling activity was between sunset and midnight, a result that contradicts Drewry & Rand (1983), who found that the activity was increasing during the night with a peak after midnight.

Populations of this amphibian have been showing a steady decline and disappearances, according to publications since 1986 (Zegarra-Vila, 2007). On the IUCN red list *E. richmondi* is classified as critically endangered (Angulo, 2008) and as Vulnerable in the DRNA, the latter meaning that it is facing a high risk of extinction in the wild on an immediate future (DRNA, 2016). The decline of the population of this species has also been observed after natural disturbances. After Hurricane Hugo in 1989, Woolbright (1991) monitored *Eleutherodactylus*

populations using count abundance, his results suggested that the population density of *E. richmondi* decreased by 83%. A similar result was found by Vilella & Fogarty (2005), they found a decrease in the relative abundance of this species at the Cordillera Central after the impact of hurricane Georges. Both of these studies place microclimate changes as the main contribution for this decline. The decline and high probability of extinction of this species has been attributed to its specialization in morphology and ecology (Joglar & Burrowes, 1996). Assessment of this species has been mostly focused at the LEF (Luquillo Experimental Forest) (Drewry & Rand, 1983; Woolbright, 1991; Stewart & Woolbright, 1996), and partly at the Maricao State Forest (Zegarra-Vila, 2007) and Cordillera Central (Vilella & Fogarty, 2005). Sampling areas for this species have also been limited to easy access areas near roads and trails, leaving the possibility that there could be populations in better condition that have not been mapped (Villanueva-Rivera, 2006). The need for a more in depth, not dependent on trails or roads, mapping of the distribution of this species in places that this have not been done before is paramount to create new conservation management strategies. Villanueva (2006) created a suitability map for the species and it shows 24.4km² of possible suitable area in the Carite State Forest. There have not been any studies that try to measure the distribution of *E. richmondi* in the Carite State Forest, an area predicted to have high vertebrate species diversity (Gould, et al., 2008) and that was affected by the direct impact of Hurricane María.

Bioacoustics

The study of *Eleutherodactylus* species in Puerto Rico requires large scale sampling of areas. Due to their independence from bodies of water, these amphibians do not necessarily aggregate around water and can be spread across the landscape (Woolbright, 1997). Studies that

investigate patterns of biodiversity on large scales help to understand the factors that determine species distribution, richness and abundance (Ficetola, et al., 2013). Understanding these variables aids the conservation assessment of species. Large scale studies allow the description of biogeographical and habitat availability pattern of species (Ficetola, et al., 2014). Sampling large scale areas is rarely feasible, requiring significant time, resources, and manpower. These logistical constraints stress the necessity of improving methodology to increase data collection over large areas and through time (Aide, et al., 2013). Passive acoustic monitoring by the deployment of automatic digital recorders can help free us of these logistical restraints. Automatic recorders allow for the simultaneous survey of multiple sites (Herrera-Montes & Aide, 2011), for 24 hours a day (Aide, et al., 2013), in a non-invasive way (Depraetere, et al., 2012), subsequently generating a large amount of high-quality data (Acevedo & Villanueva-Rivera, 2006). The large amount of sound data that is generated would require intense expert work on manually analyzing the hours of recordings; Which at the same time could create a problem of observer bias because experts vary in their abilities to identify species (Aide, et al., 2013). The use of the ARBIMON (Automated Remote Biodiversity Monitoring Network) online platform can help reduce the time for analyzing recordings; this system allows the user to create machine learning algorithms to automate the identification of the species (Aide, et al., 2013). A soundscape is the combination of all biotic and abiotic sounds and it works as an acoustic signature for each site (Campos-Cerqueira & Aide, 2017). It has been demonstrated that when more species are present there is an increase in diversity of signals across the spectrum of frequencies (Farina, et al., 2014), offering the potential to be used as a measuring tool for acoustic community dynamics (Deichmann, et al., 2017).

Occupancy modelling

Occupancy models are useful for wildlife population studies of species distribution, range, and metapopulation dynamics (Bailey & Adams, 2005). Occupancy is a useful state variable that is defined as the proportion of an area that is occupied by a certain species (Mackenzie, et al., 2006). Species in the wild are rarely detected with perfect accuracy regardless of the sampling technique employed, especially when working with cryptic animals. But not detecting a species does not necessarily mean that the species was not present, it just means that it was not detected during that sampling timeframe. Without a correction for this imperfect detection probability, the status of the species will not be accurately reflected by the data (Mackenzie, et al., 2006). Other techniques used to assess wildlife population status are index count and capture-recapture. Index counts assume that the detection probability is constant, an assumption that is unlikely to be true. Capture-recapture, on the other hand, does account for detection probability but it requires physically marking an animal to identify it, an invasive technique that could lead to sub-lethal effects (Crossland, et al., 2005). Occupancy modelling corrects for imperfect detectability by using presence/absence data from repeated observations at each site (Bailey & Adams, 2005), and at the same time does not require marking of animals. Using this technique is less invasive and requires less effort because its base requirement is presence or absence of the animal within a sampling unit, for example, the hearing of a call would be sufficient evidence of presence (Crossland, et al., 2005).

The main model parameters are occupancy, which is the probability of the site being occupied by the target species, and detectability, the probability of detecting the species during each survey (Mackenzie, et al., 2006; Bailey & Adams, 2005). The detectability may vary among

study sites and may be affected by certain weather variables. These variables can be related to the parameter estimates using the logistic equation or logit-link function (Bailey & Adams, 2005; MacKenzie & Bailey, 2004). As with every statistical model, occupancy models have critical assumptions. Mckenzie (2006) summarizes it in 4 main assumptions: (1) Sites are closed to changes in occupancy, meaning that the species is present at the site for the duration of the sampling season; (2) Sites are independent, detecting a species on one site does not affect detecting it on another site; (3) There is no unexplained heterogeneity in occupancy, if the probability of occupancy is different across sites, it can be explained by covariates; and (4) There is no unexplained heterogeneity in detectability, differences in detectability across surveys can be explained by covariates. Violating these assumptions can cause biases in the results. Occupancy modelling has been successfully used in the comparison of distribution of anurans across elevational gradients and land uses in Puerto Rico (Monroe, et al., 2017), in the estimation of detection probability of terrestrial salamanders (Bailey, et al., 2004), in the monitoring of the cryptic frog *Leiopelma hochstetteri* (Crossland, et al., 2005), and in the estimation of population declines of *E. portoricensis* in the central mountain range of Puerto Rico (Barker & Rios-Franceschi, 2014). This model has also been successfully used in combination with call surveys to monitor frog populations (Pellet & Schmidt, 2005), and threatened birds (Campos-Cerqueira & Aide, 2016). In the latter, Campos-Cerqueira and Aide (2016) proved that it is possible to generate unbiased and precise occupancy models by using automatic identification models and manually validating only 4% of the recordings. This expands the window of possibilities for large-scale ecological surveys.

CHAPTER 1:

**THE EFFECT OF HURRICANE MARÍA ON THE SOUNDSCAPES, RICHNESS AND
CALLING ACTIVITY OF THE *ELEUTHERODACTYLUS* ASSEMBLAGES OF THE
CARITE STATE FOREST**

ABSTRACT

Hurricane María had an effect on the vegetation and animals of the Puerto Rican Forests. A group that dominates these forests are the *Eleutherodactylus* frogs. This study quantifies the effect that Hurricane María had on their assemblages by the use of passive acoustic monitoring, soundscape analysis, and calling activity comparisons. Results showed changes in the soundscapes of the sites sampled before and after the hurricane. An in depth analysis showed that the changes were due to an increase in the activity of insects and changes in amphibian calling activity. *E. antillensis* and *E. brittoni* were the only colonizer species increasing in occurrence and calling activity. *E. portoricensis* did not change its activity. *E. richmondi*, *E. wightmanae*, *E. coqui* and *E. cochranæ* decreased their calling activity post-hurricane. Variation in response by each species can be attributed to differences in abilities and habitat selection, consequently giving disturbances a role in natural selection.

INTRODUCTION

Characterized by its above average number of major hurricanes, the 2017 Atlantic Hurricane season was considered an extremely active one (Klotzbach & Bell, 2017). On September 20th of 2017 Hurricane María made landfall on Puerto Rico, making it the first Category 4 hurricane to make landfall since 1932, and the strongest hurricane on the Island since 1928 (Klotzbach & Bell, 2017). These intense natural disturbances have a profound effect on plants, soils, landforms, and animals (Walker, et al., 1991). The restructuring of the forest due to a disturbance can come from massive defoliation, which increases litterfall and alters the microclimate due to the opening of the canopy (Klawinski, et al., 2014). It can also produce changes in rainfall patterns, nutrient cycling, and food availability (Waide, 1991; Vilella & Fogarty, 2005). Thus, new animal assemblages are primarily composed of disturbance adapted species (Hernández-Ordóñez, et al., 2015; Suazo-Ortuño, et al., 2018).

One of the vertebrate groups that numerically dominate most forests in Puerto Rico are the amphibians of the genus *Eleutherodactylus* (Klawinski, et al., 2014). They are the dominant consumers (Waide, 1991), which help to reduce mosquitoes, hence influencing the regulation of disease transmission and pest outbreaks (Hocking & Babbitt, 2014). Whilst additionally serving as food for vertebrate and invertebrate predators (Stewart, 1995). Population changes of *Eleutherodactylus* species are influenced by abiotic factors such as seasonal changes in daylength, rainfall, and temperature (Stewart, 1995). Subtle changes in these variables can result in warmer and drier conditions, acting as potential stressors for the population biology of the *Eleutherodactylus* fauna (Burrowes, et al., 2004). This sensitivity to environmental stressors makes them vulnerable to disturbances.

The influence disturbance has on community attributes of forest frogs in Puerto Rico has been measured, both experimentally (Klawinski, et al., 2014) and observationally (Vilella & Fogarty, 2005; Woolbright, 1991). Klawinski et al. (2014) manually mimicked the conditions of Hurricane Hugo in the Luquillo Experimental Forest (LEF) and showed that the opening of the canopy was the dominant aspect reducing densities of *E. coqui*. Woolbright (1991) measured the population densities of *Eleutherodactylus* frogs in the LEF after the impact of Hurricane Hugo in 1989, and observed an increase in densities of *E. coqui* and *E. hedricki*, while the abundance of *E. richmondi* significantly decreased. Another study from Vilella and Fogarty (2005) reported changes in diversity patterns and abundance of the frog assemblages in the Cordillera Central of Puerto Rico following Hurricane Georges in 1998. They observed that only *E. coqui* had an increase in abundance, and three other species (*E. antillensis*, *E. brittoni*, and *E. richmondi*) decreased. Because of the growing concern due to the climate-model projections pointing to an increase in drier and warmer conditions (Monroe, et al., 2017) and a possible increase in more intense and frequent storms (cited by Dale, et al., 2001), it is to understand how the amphibian assemblages reacted to the impact of major hurricane impacts.

This study is based in and around the Carite State Forest, a forest predicted to have high vertebrate species diversity (Gould, et al., 2008), that has not been studied after disturbances, and that was affected by the direct impact of this major hurricane. The objective of this chapter is to quantify the effect that Hurricane María had on the amphibian assemblages in and around the Carite State Forest by using bioacoustics. My two main hypothesis are: (1) there will be a change in the acoustic environment of the sites measured after the hurricane and (2) the calling activity of the *Eleutherodactylus* species will change following the hurricane.

METHODS

Study area and site selection

The study area was located in and around the Carite State Forest located on the southeast of Puerto Rico within the municipalities of Cayey and Guayama. The annual mean rainfall for this forest is of 1453 mm and its average temperature is 22.9°C. Lugo (2005) classified the area as Mountain Wet Forest on Volcanic Substrate (Ríos-López, et al., 2015). This study builds off a previous collaborative project between US Fish and Wildlife Service (USFWS) and Sieve Analytics (Aide, et al., 2017). The project was focused in the search for the golden coqui and the elfin woods warbler in the Sierra de Cayey (Aide, et al., 2017). The initial sampling of that project was done by USFWS at 90 sites between the months of April, May and late August of 2017. Recordings from 31 of those 90 sites were used as pre-hurricane data. Post-hurricane data was collected at the same 31 sites between the months of April, May and early September of 2018. Studied sites were located between 325 and 825m above sea level. The 2018 sampling was divided using the same structure as the 2017 USFW project, which consisted of five sampling periods within those months: two sampling periods in April and May, and one sampling period in early September. The detailed sampling structure comparing both years can be seen in Table 1. Location and coordinates of the sites can be seen in Appendix B and Appendix A respectively.

Table 1. Survey structure of the 31 sampled sites. Post hurricane surveys were carried out on approximately similar dates to the pre-hurricane survey.

2017		2018	
Month	Days	Month	Days
April	3 - 10	April	7 - 13
	18 - 25		21 - 27
May	9 - 16	May	9 - 15
	23 - 30		23 - 29
August	22 - 29	September	2 - 9

Acoustic surveys, storage and processing

A portable recorder was deployed to gather acoustic data in each of the 31 sites. The recorders consisted of a condenser microphone (Monoprice – Model 600200) connected directly to cellular phone (BLU A4) via a TRRS jack. The phone was plugged to an external battery pack (20,000mAh), and it was enclosed in a weather-proof box. The automatic recordings were programmed to record in mono audio for one minute every 10 minutes from 4:00pm to 7:00am using the ARBIMON Touch App for Android (<https://play.google.com/store/apps/details?id=touch.arbimon.com.arbimontouch&hl=en>). Each recorder was strapped to a tree at a height of 1.5m. The recorders gathered acoustic data for an average of 4.8 ± 0.8 nights. One of the sites was eliminated from the study due to acoustic device damage in the 2018 sampling.

All recordings were stored and analyzed using the ARBIMON II web platform (<https://arbimon.sieve-analytics.com>). Recordings were visually inspected and damaged recordings were eliminated. A total of 27,938 recordings were used for the analysis. The soundscape analysis was performed by aggregating recordings from each site and year. The recordings were aggregated at the time scale of hour of the day with a bin size of 86Hz, with an amplitude filtering threshold of 0.02Hz to filter out background noise. The hours used for this analysis were from 4pm to 6am. Each soundscape created is a matrix of acoustic activity with 3,584 time/frequency bins (14h x 256 frequency bins). The matrix was normalized by dividing the number of peaks in the frequency bins by the total number of recordings collected during each hour interval. This controls for the unequal number of recordings used per site-year soundscape. To locate in which frequencies there had been changes in use, the soundscape matrix was divided into 3 frequency regions. The frequency regions were chosen in accordance to the results of Campos-Cerqueira & Aide (2017), and visual observations of the recordings. The first region was

between 0.02 - 4 kHz and were marked as the frequencies used by amphibians, nocturnal birds, and running water. The second region was between 4 - 7 kHz and were the frequencies of most overlap between amphibians and insects. The third region was of sounds over 7 kHz and were frequencies used only by insects, particularly cicadas.

To pinpoint which species were causing the change in the soundscapes before and after the hurricane, I created a species-specific identification model within the ARBIMON platform for each of the *Eleutherodactylus* species present in the study. This was achieved by reviewing audio spectrograms and listening to a randomly selected subgroup of five recordings per day of each site and year (approx. 3,000 recordings). In each of the recordings of this subset I validated the presence or absence of each of the species and created a training set of their most common call. Each of the species-specific models were adjusted to reduce false positives and were ran through the 27,938 recordings. Generated results were manually post validated before analyzing.

Statistical Analysis

Soundscapes

To compare the soundscapes of each site pre and post hurricane, I employed the hierarchical clustering methodology used by Deichmann, et al. (2017). Clusters were calculated using Euclidean distance and the complete distance linkage method. Hierarchical clustering was performed on 60 soundscapes, one per year (2017 and 2018) for each of the 30 sites surveyed. This grouping allowed for comparison of soundscape similarities of the same site before and after the hurricane. Significant clusters were estimated via approximate unbiased and bootstrap probabilities. All hierarchical cluster analysis was done using the package pvclust (Suzuki & Shimodaira, 2006) in R (version 3.5.0). To pinpoint in which frequency bands there had been

changes in acoustic activity before and after the hurricane, the average number of frequency bins with an activity >0 at each site, year, and frequency category (0.02-4kHz, 4-7kHz, >7 kHz) were compared. This was done by using a generalized linear mixed model with a negative binomial distribution and logarithmic link function. This analysis was done using the GLIMMIX procedure in SAS UniversityEdition (ver.9.4).

Species Richness and Calling Activity

Species richness and species occurrence were calculated. Species richness was defined as the total number of *Eleutherodactylus* species per site and species occurrence was the proportion of sites in which each species was present. Richness was compared before and after the hurricane first paired by site, followed by an analysis of the effects in each individual site. Both analysis were performed using a generalized linear mixed model with a Poisson distribution and a logarithmic link function.

On the sites where there was no change in number of species present before or after the hurricane, calling activity of each species present was calculated. This was done to determine if there was a pattern of increase or decrease in calling activity even if there was not a complete removal or addition of a species. As done by Ospina, et al. (2013), detection frequency was calculated as a proxy for calling activity over a period of time. This frequency was calculated as the proportion of recordings where a species was present divided by the total number of recordings per night. Detection frequency was analyzed separately for all species of *Eleutherodactylus* found in the study. A generalized linear mixed model with a binomial distribution was used to determine if there was a significant change in calling activity of each species before and after the hurricane,

and across sites. All analyses were done using the GLIMMIX procedure in SAS UniversityEdition (ver.9.4) and the maximum likelihood approach was based on Laplace approximation.

RESULTS

Community level

Hierarchical cluster analysis grouped soundscapes from 30 sites measured before and after the hurricane into 58 clusters. Eleven of these 58 clusters consisted of statistically similar soundscapes, with an approximately unbiased p-value over 0.95. Of the 11 significant clusters, 10 were groups of soundscapes from the same year, six of those were clusters from sites measured after the hurricane and four were clusters from sites measured in 2017. Only one of the significant clusters consisted of soundscapes of the same site (Site 35) measured before and after the hurricane. Although the rest of the soundscapes were not significantly similar, the same grouping structure can be seen. The majority of soundscapes measured before the hurricane were grouped together. Overall, of the 30 pre-hurricane soundscapes, 83% were clustered together, the rest were either separated (E2) or clustered with post-hurricane soundscapes (Figure 3).

To evaluate changes in acoustic activity of the community and better explain the cluster analysis, the average number of frequency bins with an activity >0 before and after the hurricane were compared (Figure 4). The average number of frequency bins used was dependent on the frequency category (<4 , 4-7, >7 kHz), site, and year ($F:11.55$ $p<.0001$). In the low frequency category <4 kHz, the amount of frequency bins used before the hurricane ranged from a minimum of 4.4 to 16.6 bins. After the hurricane this range went from 9.0 to 22.0 frequency bins. Out of the 30 sites measured, eight showed a significant increase in the amount of frequency bins used after the hurricane. In this frequency category, the rest of the sites showed no significant change in activity. In the frequencies between 4 and 7kHz, five of the 30 sites underwent a significant change in the amount of frequency bins with activity >0 . Of those five sites, only one showed a significant decrease in the frequency bins used (site E1). Overall in the mid-frequencies category, the range

of bins used in 2017 went from 2.1 to 23.3, and in 2018 it ranged from 3.5 to 23.1 bins. The most dramatic changes in bins used can be seen in the category of high frequencies. Before the hurricane the range of bins used in this category went from 0.06 to 28.5, and after the hurricane it went from a minimum of 0.00 to a maximum of 82.2. Fourteen out of 30 sites showed a significant change in the amount of frequency bins used in the high frequency range of over 7kHz. Ten out of those 14 sites had an increase in the number of bins with activity, and four sites showed a decrease. The four sites that showed dramatic increases in insect activity were sites E2, E4, E6 and G4.

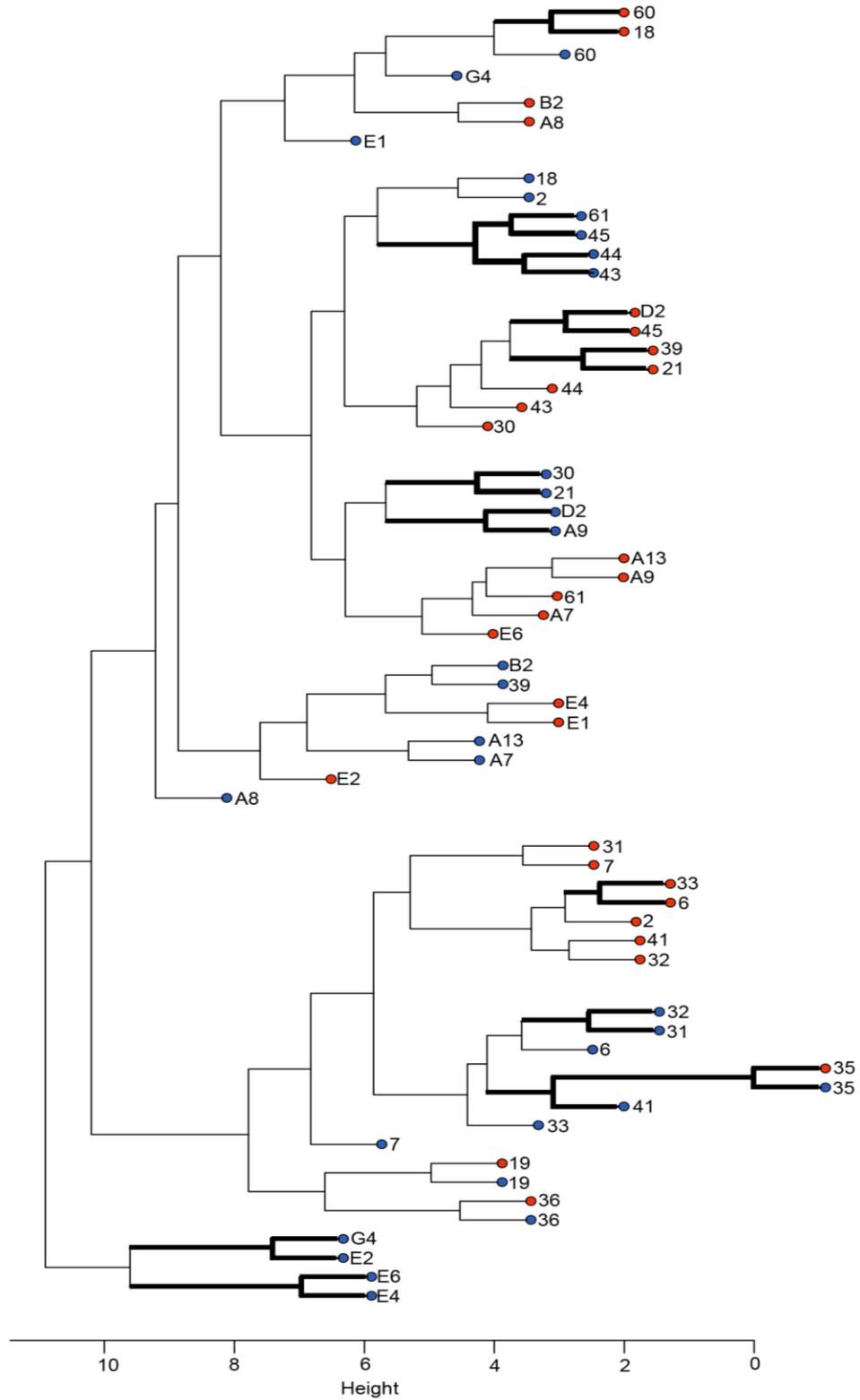


Figure 3. Hierarchical cluster analysis of the soundscapes of sites measured before and after the hurricane. Sites identified with red dots are pre-hurricane soundscapes and blue dots are post-hurricane soundscapes. Clusters with high approximately unbiased p-values (>0.95) are considered significant and are marked with thick lines.

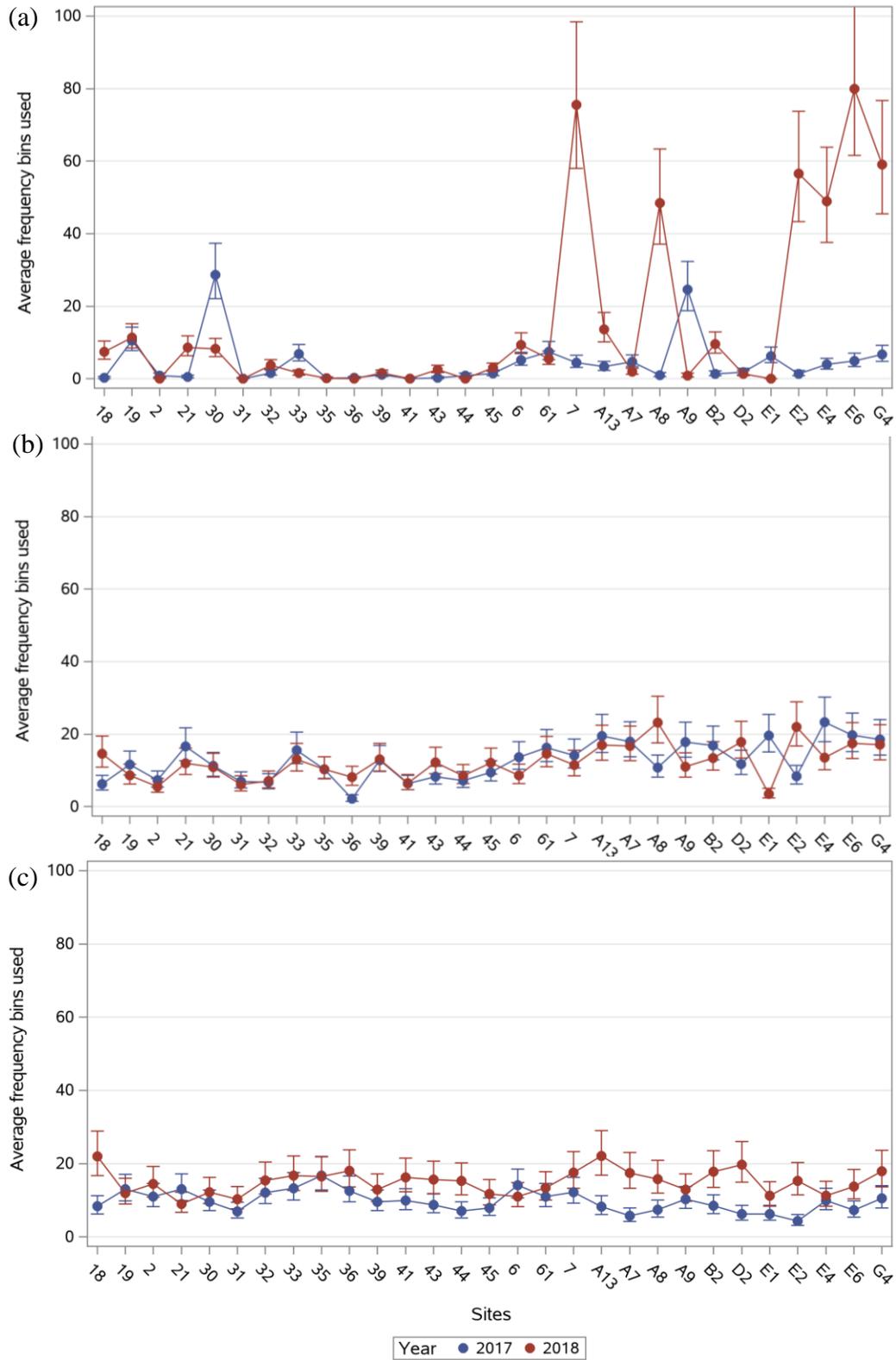


Figure 4. Average number of frequency bins with activity >0 per site and frequency category, divided by year before and after the hurricane. 95% confidence intervals are shown. (a) High frequencies over 7kHz, used by insects. (b) Mid frequencies used by insects and amphibians, between 4 and 7kHz. (c) Low frequencies under 4kHz, used by amphibians and nocturnal birds.

Species level

Species specific identification models

A total of eight species were detected and seven species-specific identification models were created (Table 2). Although *Eleutherodactylus gryllus* was found, the identification model was not created due to its low occurrence throughout the study. This species was detected only in nine of the 27938 recordings. The few false positive results in the generated matrix for the *E. brittoni* model were mostly due to insects using similar frequencies. For *E. antillensis* and *E. portoricensis*, the false positives were mostly present at dawn and dusk because of its similarity to the complex common coquí retreat call. The sound of the rain, and the similar use of frequency by *E. brittoni*, were responsible for the false positives generated for the *E. richmondi* model. False positives generated in both the models of *E. wightmanae* and *E. cochranae* came from insects, and their own calls due to their similar frequency use.

Table 2. Average maximum, minimum frequency (kHz) and duration (s) used to create species-specific identification models. Estimates of precision and accuracy of each model are presented. Accuracy= (true positives + true negatives) / total ; Precision= true positives/ (true positives + false positives).

<i>Eleutherodactylus spp.</i>	Max (kHz)	Min (kHz)	Duration (s)	Accuracy	Precision
<i>E. brittoni</i>	5.80	3.68	0.27	0.89	0.99
<i>E. antillensis</i>	3.20	1.50	0.15	0.90	0.97
<i>E. portoricensis</i>	2.95	1.27	0.33	0.86	0.90
<i>E. richmondi</i>	6.32	3.20	0.17	0.83	0.93
<i>E. wightmanae</i>	4.24	2.80	1.50	0.82	0.97
<i>E. cochranae</i>	4.70	3.37	0.32	0.73	0.86
<i>E. coqui</i>	3.06	1.04	0.67	0.94	0.96
<i>E. gryllus*</i>	8.25	6.49	0.16	---	---

*Identification model was not created due to low occurrence.

Species Richness and occurrence

Sites had from two to four species, with the majority of sites having the presence of two species both before and after the hurricane. When paired by site, the average number of species before and after the hurricane was not significantly different (F: 0.66, p-val: 0.4187). There was a highly significant interaction between each site and the effect of year (F: 12.42; $p < 0.001$) (Figure 5). A total of 30% of the sites had a significant change in the average number of *Eleutherodactylus* species. Of those nine sites, seven had a significant increase in number of species and two sites showed a decrease. Site A13 went from two to three species, with the appearance of *E. antillensis* in the recordings of 2018. Site 18 showed an increase in detection of *E. brittoni*, and Site E2 showed an increase in the detection of *E. antillensis*. Sites 36, 43, 44, and 61 had a significant increase in species richness because of the detection of *E. brittoni* in 2018, a species that was not detected in any recordings of those sites before the hurricane. The sites that had a significant decrease in the number of species were site 21 and site 60. Site 21 showed no presence of *E. wightmanae* after the hurricane, a species that was present before it. The recordings of site 60 during the 2018 survey showed no presence of *E. richmondi*, this species was present at this site before the hurricane.

Only *Eleutherodactylus brittoni* and *Eleutherodactylus coqui* had high occurrence (>0.60) in both the 2017 and 2018 surveys (Table 3). Overall, *E. coqui* had the highest occurrence due to its presence in all the 30 sites both before and after the hurricane. Both *Eleutherodactylus brittoni* and *E. antillensis* increased its occurrence after the hurricane. The species *E. brittoni* showed the second highest occurrence both before and after the hurricane, going from 0.80 to 0.93 in 2018. This increase was due to its appearance in four sites where it was not present before. One species that did not change in occurrence was *E. wightmanae*, because it disappeared in one site but was

detected on another where it was not present before. The only species that decreased in occurrence were *E. richmondi* and *E. gryllus* (lowest occurrence), this was due to the lack of detection of both species on one site in which they were present before the hurricane.

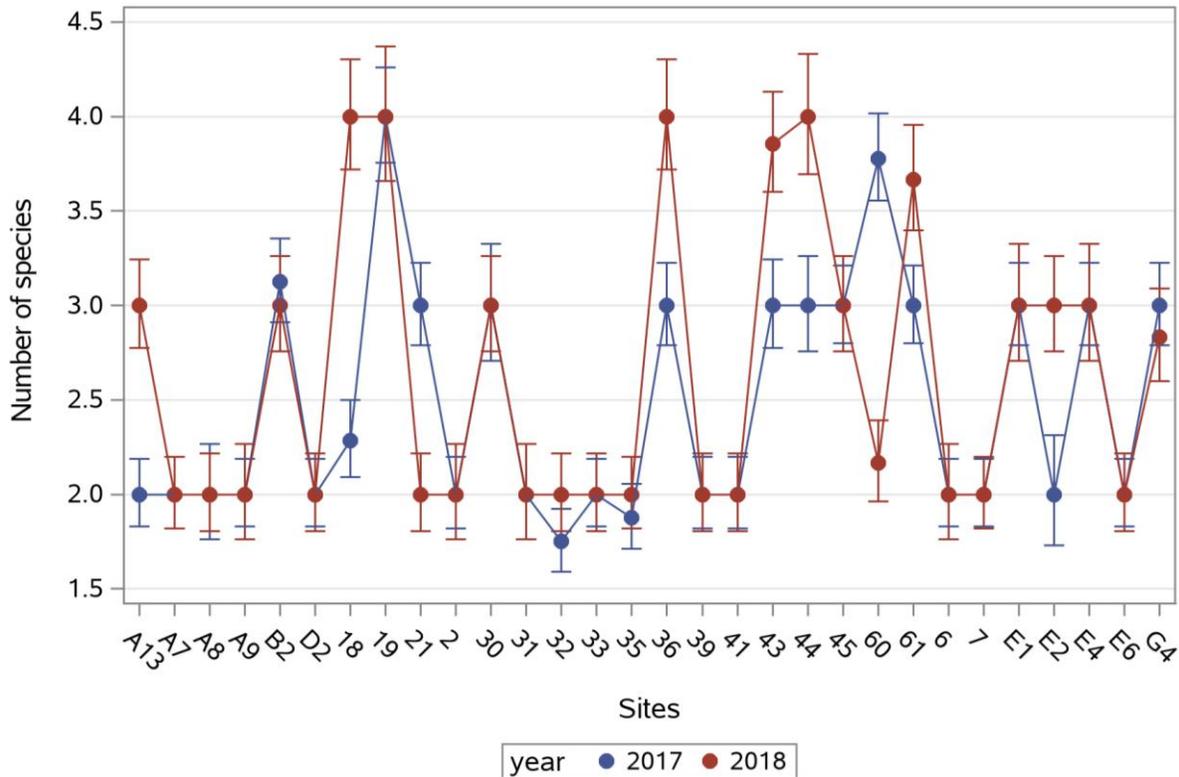


Figure 5. Model based average number of species present per site, before and after the hurricane. 95% confidence intervals are shown.

Calling activity

Table 3 shows the average nightly calling activity of all species of *Eleutherodactylus* found in the study from before and after Hurricane María. Because of its low occurrence and calling activity of *E. gryllus* throughout the study, the comparison before and after the hurricane could not be done. This species had appeared in one site (Site B2) during 2017 and represents 1% of all recordings. Four species: *E. coqui*, *E. wightmanae*, *E. richmondi*, and *E. cochranae* had a significant decrease in the average nightly calling activity after the hurricane. This change in

calling activity was dependent on each of the sites where each of the species were present (Figure 6). *Eleutherodactylus coqui* had a significant change in calling activity after the hurricane in 17 out of the 30 sites where it was present. In 12 of those 17 sites this species decreased its calling activity and increased its activity in the five remaining sites (Figure 6b). *Eleutherodactylus wightmanae* had a significant calling activity decrease, going from 87% to 77% in average calling activity per night. In the eight sites where this species was present in both years, five had a significant change in activity. In only one of those sites this change was due to an increase of its activity (Site 18) (Figure 6b). Before and after the hurricane *E. richmondi* was constantly present in four sites. This species had a significant calling activity decrease in two of those sites. There was no significant calling activity change in the remaining sites (Figure 6e). Finally, *E. cochranae* appeared in two sites, and in both of these sites it had a significant decrease (Figure 6d).

Table 3. Occurrence and average nightly call frequency of each species of *Eleutherodactylus* present in the study before and after Hurricane María. Call frequency (number of recordings where species was present / total of nightly recordings) was used as a proxy for calling activity. The p-value is used to compare the overall average nightly calling activity before and after the hurricane.

<i>Eleutherodactylus</i> <i>spp.</i>	2017			2018			p-value
	Occurrence	Calling activity	Std. error	Occurrence	Calling activity	Std. error	
<i>E. coqui</i>	1.00	0.84	0.005	1.00	0.79	0.007	<.0001
<i>E. brittoni</i>	0.80	0.71	0.011	0.93	0.91	0.006	<.0001
<i>E. wightmanae</i>	0.33	0.87	0.009	0.33	0.77	0.013	<.0001
<i>E. antillensis</i>	0.13	0.50	0.027	0.17	0.76	0.016	<.0001
<i>E. richmondi</i>	0.17	0.93	0.008	0.13	0.86	0.016	<.0001
<i>E. portoricensis</i>	0.10	0.89	0.011	0.10	0.87	0.012	0.2888
<i>E. cochranae</i>	0.07	0.53	0.015	0.07	0.30	0.019	<.0001
<i>E. gryllus</i>	0.03	0.01	---	0.00	0.00	---	---

The only two species that showed an increase in calling activity were *E. brittoni* and *E. antillensis*. For *E. antillensis*, there was an increase in calling activity in two of the four sites where it was constantly present. There was no change in the remaining two sites (Figure 6f). A total of 24 sites were used by *E. brittoni* throughout the study. This species had a nightly calling activity change in 23 out of those 24 sites. In 83% of the sites where activity showed a change it was due to an increase in its nightly calling activity (Figure 6a). The only species that showed no change in nightly calling activity throughout this study in the three sites where it was found was *E. portoricensis* (Figure 6g).

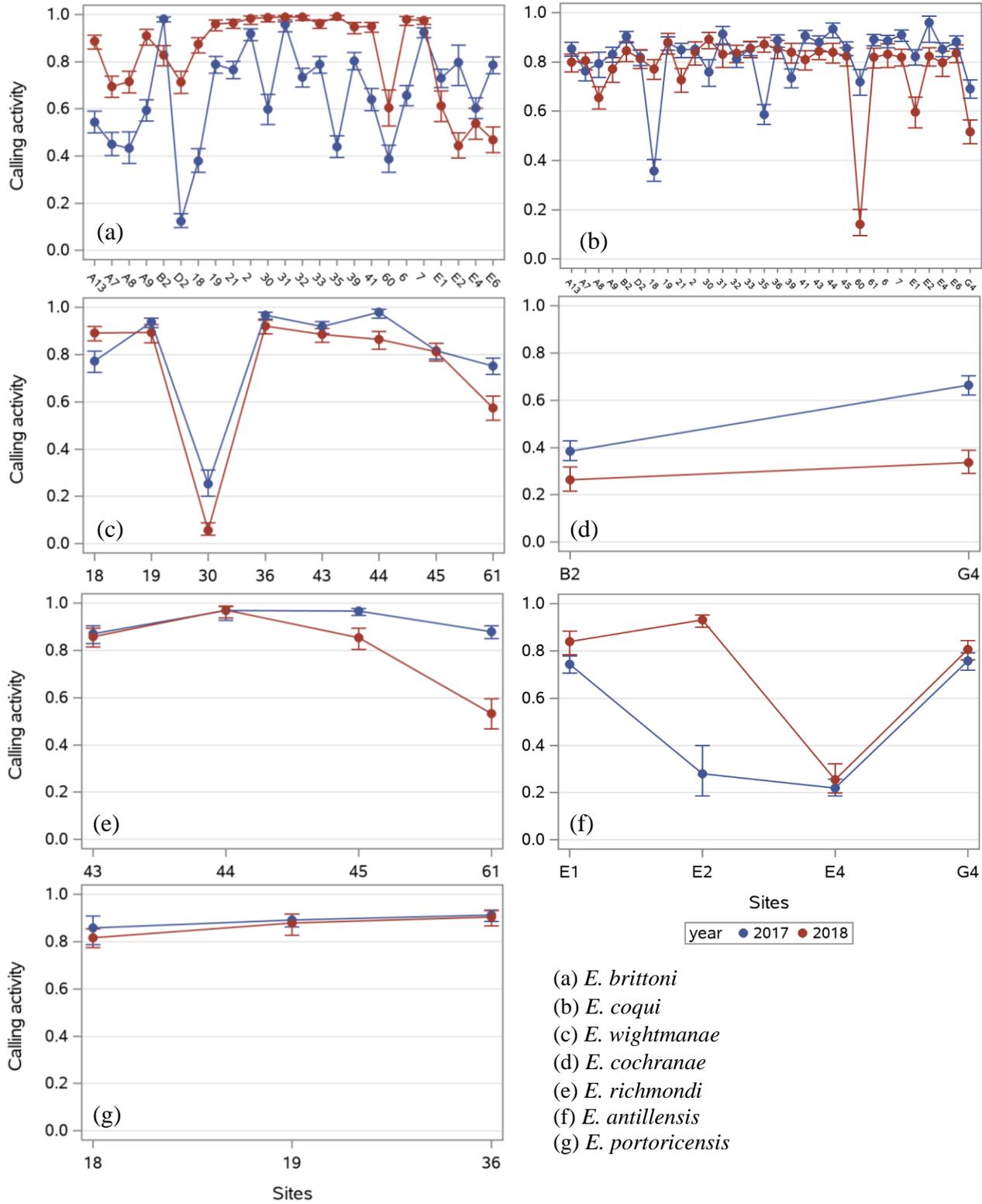


Figure 6. Average nightly calling activity per site. Sites used were the ones where each of the species was present both before and after the hurricane. Calling activity was defined as the proportion of recordings in which a species was present over the total number of recordings per night. Each graph represents the nightly calling activity of a different species. 95% confidence interval is shown.

DISCUSSION

Soundscapes are the aggregation of sounds of a particular site and they provide a unique signature of the habitat (Campos-Cerqueira & Aide, 2017) and can be used to assess biodiversity turnover (Farina & Pieretti, 2012). The lack of 2017-2018 grouping indicates that the acoustic signature of most sites changed following the hurricane, and that sites recorded during the same year share a more similar acoustic structure. The hypothesis that soundscapes would show changes after the hurricane is then accepted. Because it describes the frequencies and amplitude of the sonic signals produced by its members (Farina & James, 2016), the change in the soundscape structure can be partly due to species-specific variations. An increase or decrease in calling activity (Ospina, et al., 2013), variation of vocalizations (Ernstes & Quinn, 2016), and localized colonization or extinctions of vocal species (Herrera-Montes & Aide, 2011), can cause a change in the acoustic environment of a site.

Dividing the soundscape into three main frequency categories allowed for a more in depth analysis of the soundscapes. Vocal species can limit acoustic overlap in both frequency and time (cited by Farina & James, 2016). The average number of frequency bins is used as a proxy for activity in the soundscape, more activity means a higher number of frequency bins and vice versa. Results from the frequency bins comparison showed that the use of frequency bins did change after the hurricane, and this change was site-specific. The high frequency category results indicate a dramatic increase in average number of frequency bins used, where a third of the sites sampled had a significant increase in high frequency activity, and only four showed a significant decrease. This suggests that, on the sites with lower number of frequency bins used after the hurricane, there is a decrease in the activity of nocturnal vocal insects that dominate the use of these frequencies. Comparatively, the sites that had higher use of frequency bins then have a higher insect calling

activity. This last condition can be clearly seen in the sites that were sampled in the last survey of 2018 (September), and were in the lowest elevation (around 300m). Altitude could be a factor if we take into account that insect species have been detected as indicator species in low elevation sites (Campos-Cerqueira & Aide, 2017). Given that it has been found that hurricanes affect insect population numbers, either lowering them or having dramatic outbreaks (cited by Lugo, 2008) then it is also a possibility that, since the sites were the last sampled, they had more time to recover after the disturbance, and therefore, had more insect activity. The increase in frequencies used by insects could then be a factor of altitude or successional time.

Results from the mid frequency and low frequency categories (Figure 4b and 4c) can be more easily explained in combination with the results from *Eleutherodactylus* species richness and calling activity. Throughout the study, seven *Eleutherodactylus* species were consistently found, and all of them use acoustic frequencies of less than 7kHz. The change in frequency bin use after the hurricane in mid and low frequency categories can then be attributed to a change in the calling activity pattern or localized colonization/extinction from any of these species. The rest of the sites that showed an increase in frequency bin use after the hurricane, but were not colonized by new species, were those in which the calling activity (i.e. detection frequency) of *E. brittoni* and *E. antillensis* increased. Although the general species richness throughout the study did not change, the change of richness was site-dependent. The only species that was not found after the hurricane was *E. gryllus*, a cloud forest bromeliad-dweller (Joglar, 1998). An inference cannot be directly made on the cause of this localized extinction because of the already low occurrence of this species before the impact of Hurricane María. The other species that appeared in fewer sites after the hurricane was *E. richmondi*. The colonizer species were found to be *E. brittoni* and *E. antillensis*,

these were the only ones that increased their occurrences. And the rest of the species were found at the same amount of sites before and after the disturbance.

Site-dependency was also seen in results of calling activity. The detected species of *Eleutherodactylus* did not necessarily change its calling activity in the same way in all of the sites. Henceforth, the habitat structure of each site was an important factor in determining how a species reacts to a large-scale disturbance. In addition to being the only colonizer species, *E. brittoni* and *E. antillensis* were also the only species that increased their calling activity after the hurricane. This result is most likely explained by their habitat preference. Both of these species prefer grasses and open areas (Joglar, 1998). Defoliation and debranching after a hurricane results in the formation of new forest gaps (Tanner, et al., 1991) and an input of large amounts of fresh, non-senesced biomass to the forest floor (Klawinski, et al., 2014). Both of these factors, together with an increase in light availability on the forest floor, act as an aid for new colonist plant species. These new colonist grasses open the door for the new colonization of grass-dwelling *Eleutherodactylus*. In contrast to these results, Vilella and Fogarty (2005) found that *E. brittoni* and *E. antillensis* decreased in relative abundance after Hurricane Georges. The reason for these contrasting results could come from the difference in wind speed and amount of rainfall between both hurricanes. Hurricane Georges made landfall as a category 3 with 115 mile per hour winds (USGS, 1999), and María as a category 4 with winds up to 156 miles per hour, just below the threshold of category 5 (Pasch, et al., 2018). Defoliation is a function of maximum daily wind speed (cited by Lugo, 2008). Consequently, this difference in wind speed can lead to a substantial change in amount of defoliation. Rainfall also differed between both hurricanes; Georges dropped an average of 56cm (USGS, 1999), and María 86cm (Pasch, et al., 2018). Having more forest gaps

and rain may have favored understory growth after Hurricane, an ideal habitat for *E. brittoni* and *E. antillensis*.

Forest floor-dwellers *Eleutherodactylus richmondi* and *Eleutherodactylus wightmanae* decreased their calling activity after the hurricane, a result that is similar to the ones of Woolbright (1991) after Hurricane Hugo, and Vilella and Fogarty (2005) after hurricane Georges. This decrease in calling activity could be explained by the change in the forest floor microclimate after the disturbance (Vilella and Fogarty, 2005). After a hurricane, the opening of the canopy adds leaf litter to the soil and increases light penetration and temperatures, consequently resulting in moister soils (Tanner, 1991). Which in turn can lead to the restructuring of the animal community by adding habitat heterogeneity and changes in food supply (Waide, 1991). The way in which a species responds to the disturbance, positively or negatively, will be determined by adaptation to these microhabitat changes and new food resources (Woolbright, 1991; Suazo-Ortuño, et al., 2018).

Eleutherodactylus cochranae and *Eleutherodactylus coqui* both decreased their calling activity after the hurricane. Responses of *E. cochranae* to the effects of large scale disturbances have not been reported before. However, this species of coqui calls from bromeliads up to 2.5m from the ground (cited by Joglar, 1998), and these plants are susceptible to hurricane winds. Therefore, their absence could affect *E. cochranae* calling activity. The result that the calling activity of *E. coqui* decreased after the hurricane goes in concordance with Klawinski et al. (2014) but contrasts with the one from Vilella and Fogarty (2005) at Maricao, and Woolbright (1991) at the LEF. The increase in abundance of *E. coqui* reported by Vilella and Fogarty (2005) after Hurricane Georges was not significant. Woolbright (1991) reported an increase in *E. coqui* abundance one year after Hurricane Hugo. Recordings from this study were taken approximately

six months after Hurricane María, the contrast in results could possibly be due to successional time. Klawinski et al. (2014) reported that after a significant canopy opening, the negative effect on the frog community can be seen up to one year from the disturbance. Experimental results after manually mimicking the effects of Hurricane Hugo identified that the opening of the canopy and concomitant microclimatic changes was the main cause of *E. coqui* declines (Klawinski et al., 2014). There was almost a complete canopy opening after Hurricane María resulting from the defoliation, breaking and uprooting of trees (NASA/Goddard, 2018).

The only species that did not show changes in calling activity 6 months after the Hurricane near the Carite State Forest was *Eleutherodactylus portoricensis*. Barker & Ríos-Franceschi, (2014) found that site occupancy was negatively associated with high temperatures and seasonal precipitations on the populations of *E. portoricensis* at the Cayey and Luquillo mountains. Ríos-López & Aide (2007) studied the herpetofaunal assemblage in a reforestation project, they found that the increase in vegetation heterogeneity and decrease in microclimate variability facilitates herpetofaunal succession after a disturbance. It is possible that the rainfall pattern of Hurricane María, in addition to the increase in habitat heterogeneity, allowed this species to feed and reproduce, consequently keeping the same calling activity. There is sufficient evidence to accept the second hypothesis due to the change in richness and calling activity seen after the hurricane. Soundscape analysis has been used before to measure the effects that human disturbance has on animal communities (Deichmann, et al., 2017; Herrera-Montes & Aide, 2011). And now with this study, it has also proved to be an important tool to understand how a community reacts to natural large scale disturbances.

CHAPTER 2:

**IDENTIFICATION OF HABITAT FEATURES ASSOCIATED WITH SITE
OCCUPANCY OF *ELEUTHERODACTYLUS RICHMONDI* IN THE
CARITE STATE FOREST**

ABSTRACT

Amphibian populations worldwide are facing declines, this phenomenon can also be seen in Puerto Rico. *Eleutherodactylus richmondi* is an endemic species of anuran whose populations have been reported as declining or extinct. Finding new populations of this species and understanding its specialized ecology is necessary for its conservation. This study uses passive acoustic monitoring and occupancy modelling to identify site occupancy of *E. richmondi*, and to describe its diurnal calling activity. Results showed that the percent canopy cover and steep gradient of terrain slope are important habitat features associated with site occupancy. This population showed diurnal calling activity from 10% to 90% at different sites, proving that habitat site-specific variables are the mediators to its variation in diurnal calling activity. This study described a population of *E. richmondi* on the southeast part of the Carite State Forest, with the potential to be pivotal in the study of this species.

INTRODUCTION

Amphibians are the taxonomic group with the highest number of critically endangered species in the world (Campos-Cerqueira & Aide, 2017). They are currently facing worldwide population declines, range contractions, and species extinctions (Semlitsch, 2003). In Puerto Rico, amphibian population declines have been happening since 1970 (Stallard, 2001). These extinctions could have a deterring effect on other vertebrates (Joglar, 1998) because amphibians have an important role in forest ecosystem dynamics (Semlitsch, 2003). They are one of the dominant consumers of the forest food webs (Waide, 1991), and serve as food for vertebrate and invertebrate predators (Stewart, 1995).

Eleutherodactylus richmondi is one of the endemic anurans of Puerto Rico and its populations have been showing a steady decline and disappearances since 1986 (Zegarra-Vila, 2007). Today this species is classified as Critically Endangered by the International Union for Conservation of Nature (IUCN) (Angulo, 2008) and as Vulnerable by the Puerto Rico Department of Natural and Environmental Resources (DRNA) (DRNA, 2016). Assessment of *E. richmondi* has been mostly focused at the LEF (Luquillo Experimental Forest) (Drewry & Rand, 1983; Woolbright, 1991; Stewart & Woolbright, 1996), at the Maricao State Forest (Zegarra-Vila, 2007) and the Cordillera Central (Vilella & Fogarty, 2005). Besides the lack of variability in the choice of sampling areas for these species, these studies have been limited to easy areas near roads and trails, leaving the possibility that there are populations in better condition that have not been discovered (Villanueva-Rivera, 2006).

A decline of the population of *Eleutherodactylus richmondi* has also been documented after natural disturbances. After Hurricane Hugo in 1989, Woolbright (1991) monitored

Eleutherodactylus populations at the Luquillo Experimental Forest, and his results suggested that the population density of *E. richmondi* decreased by 83%. A similar result was found by Vilella and Fogarty (2005), they found a decrease in the relative abundance of this species at the Cordillera Central after the impact of hurricane Georges. The decline and high probability of extinction of this species has been attributed to its specialization in morphology and ecology (Joglar & Burrowes, 1996). *E. richmondi* is a forest floor-dweller that inhabits pockets associated with mud slopes and boulders (Woolbright, 1997), and under leaf litter and around fallen logs (Stewart & Woolbright, 1996; Rivero, 1998). The identification and quantification of forest floor habitat characteristics related to the site occupancy of this species is necessary for the improvement of its distribution models and conservation management.

The methodology of this chapter is based on the use of passive acoustic monitoring and occupancy modelling, two active areas of ecological research that have been effectively used together (Campos-Cerqueira & Aide, 2016). Passive acoustic monitoring allows for a large scale sampling of areas by reducing its logistical problems. It allows for the simultaneous survey of multiple sites (Herrera-Montes & Aide, 2011), for 24 hours a day (Aide, et al., 2013), in a non-invasive way (Depraetere, et al., 2012), subsequently generating a large amount of high-quality data (Acevedo & Villanueva-Rivera, 2006). These data can then be analyzed and post-validated to be used in combination with occupancy modelling. Occupancy modelling is a useful tool for wildlife population studies of species distribution, range, and metapopulation dynamics (Mackenzie, et al., 2006; Bailey & Adams, 2005). This statistical tool corrects for imperfect detectability by using presence/absence data from repeated observations at each site (Bailey & Adams, 2005), and at the same time does not require marking of animals.

Populations of *Eleutherodactylus richmondi* located in this forest have not been quantified. This study will aid in the location of new populations of *E. richmondi*, and in the understanding of its site selection criteria. This study comes as a response to the need for a more in depth mapping for this species. A step that is paramount for the creation of new conservation management strategies for this highly understudied species. The objectives of this chapter are to use bioacoustics to: (1) identify habitat characteristics associated with site occupancy of *E. richmondi* on the south portion of the Carite State Forest; and (2) describe the diurnal calling activity shown by *E. richmondi* on the south portion of the Carite State Forest.

METHODS

Study area and site selection

The study area was located in the southwest portion of the Carite State Forest, located on the southeast of Puerto Rico within the municipalities of Cayey and Guayama. Lugo (2005) classifies this forest as Mountain Wet Forest on Volcanic Substrate (Ríos-López, et al., 2015). A total of 28 sites were visited in three separate sampling occasions during the months of July and August of 2018 (Appendix C and Appendix D). During the survey of this location the total rainfall was 66.3mm (USGS Station 50039995), and the temperature and humidity averaged $23.3 \pm 1.0^{\circ}\text{C}$ and $98.2 \pm 5.0\%$ respectively. The average altitude of the sampled sites was $596 \pm 25\text{m}$ above sea level. All sampled sites were separated by a minimum of 100m. Canopy cover was low due to debranching and defoliation that resulted from the hurricane impact, which at the same time produced an abundance of leaf litter and understory growth.

Acoustic survey

A portable recorder was deployed to gather acoustic data in 31 sites. The recorders consisted of a condenser microphone (Monoprice – Model 600200) connected directly to cellular phone (BLU A4) via a TRRS jack. The phone was plugged to an external battery pack (20,000mAh), and it was enclosed in a weather-proof box. Twenty of the automatic recorders were programmed to record in mono audio for one minute every 10 minutes for 24 hours, and eight were programmed to record one minute every 10 minutes from 4pm to 7am. All recorders were programmed using the ARBIMON Touch App for Android (<https://play.google.com/store/>

apps/details?id=touch.arbimon.com.arbimontouch&hl=en). Each recorder was strapped to a tree at a height of 1.5m. The recorders gathered acoustic data for an average of 4.6 ± 0.8 nights.

The recordings were stored and analyzed using the ARBIMON II web platform (<https://arbimon.sieve-analytics.com>). I visually inspected the recordings and eliminated the damaged ones. A total of 17269 recordings were used for the analysis. Within the ARBIMON platform I created a species-specific identification model of *Eleutherodactylus richmondi*. This was achieved by reviewing audio spectrograms and listening to a randomly selected subgroup of 5 recordings per day of each site (approx. 1500 recordings). In each of the recordings of this subset I validated the presence or absence of *Eleutherodactylus richmondi* and created a training set of its common call. The species-specific model was adjusted to reduce false positives and was ran through the 17269 recordings. This generated a presence/non-presence matrix.

Covariates

Temperature and humidity data was recorded using an automatic data logger (Tempo Disc®) at each site during the survey time. The loggers recorded a data point every 10 minutes which were then averaged per day. Daily precipitation data was provided by the weather station at the Carite Dam (USGS Station 50039995), located approximately 1km away from the sites. At each site, altitude, canopy cover, horizontal cover, slope, and leaf litter depth were measured. These habitat variables were chosen because they have been proven to provide a means to weigh suitable micro-climatic conditions for amphibians (Monroe, et al., 2017). Although altitude was noted, it was not used in the analysis due to the low altitude difference between sites (Max. difference = 108m). Measurements of slope and leaf litter depth were taken at 3 points randomly chosen and were averaged per site. Slope was measured in degrees using a clinometer. Percent

canopy cover was measured using the android app HabitApp (Version 1.1). Measures of horizontal cover were taken to describe the way in which vegetation was arranged. These were taken 5m away from the recorder on all four cardinal directions using a vegetation profile board as described by (Nudds, 1977). The board was 2.5m tall, divided in five 0.5m parts and each part had 60 squares of alternating colors. Non obstructed squares were counted and percent covered was calculated. Percent cover up to one meter was used in the analysis due to *E. richmondi* inhabiting mostly the forest floor and up to 1 m (Stewart & Woolbright, 1996; Zegarra-Vila, 2007). Table 4 shows covariate values.

Table 4. Maximum, minimum, average and standard deviation of covariate measurements used for occupancy modelling.

	Min	Max	Mean	Stdev
Canopy Cover (%)	10.50	76.00	54.30	15.26
Incline (°)	1.40	49.75	22.14	11.36
Leaf Litter (cm)	1.33	12.67	5.67	2.36
Horizontal Cover (%)	38.33	98.75	73.64	14.14
Temperature (°C)	20.64	26.98	23.34	1.03
Relative humidity (%)	83.12	108.45	98.19	5.01
Rain (mm)	0.00	22.86	3.56	5.84

Occupancy Modelling

A hierarchical single-species single-season occupancy model was used to estimate occupancy, probability of the site being occupied by the target species, and detection, probability of detecting the species during each survey (Mackenzie, et al., 2006; Bailey & Adams, 2005). Because occupancy models account for imperfect detections but not false positives, all positive detections from the generated presence/non-presence matrix were post validated. The model parameters may vary among study sites and may be affected by certain weather variables. To potentially explain variation between sites, the site-specific covariates that were included in the

occupancy parameter were canopy cover, horizontal cover, slope, and leaf litter depth. Observation level covariates that were added to explain detection heterogeneity between sample occasions were temperature, humidity and precipitation.

As used by Monroe, et al. (2017) and Campos-Cerqueira & Aide (2016), and recommended by Mackenzie, et al. (2006), a two-step approach was used to determine the most parsimonious model. The first step was to model the detection parameter while keeping the occupancy parameter constant. To achieve this, I compared models where the detection parameter was constant, or where it varied according to observation level covariates: temperature, humidity, rain, and their combinations. The best model was determined by using the Akaike Information Criterion (AIC) (Burnham & Anderson, 2002). Once the best ranked model for detection was chosen, that parameter was kept constant while testing site-level covariates (slope, horizontal cover, litter depth and canopy cover) on the occupancy parameter. The best fitting model was chosen using AIC. To check the adequacy of model fit, the selected model was subsequently assessed for goodness-of-fit using the parametric bootstrap procedure implemented by MacKenzie & Bailey (2004). All occupancy modelling and fitting was done in R (version 3.5.0) using the package Unmarked (Fiske & Chandler, 2011).

Day activity

To describe the diurnal calling activity of *E. richmondi* at the southwest portion of the Carite State Forest, the sites in which this species was present were selected. A total of fifteen of the sites were selected using two criteria: the species was present, and the sites were recorded for one minute every 10 minutes for 24 hours. The species-specific model of *E. richmondi* that was previously generated was run through the recordings of those 15 sites. The generated

presence/non-presence matrix was post validated. Detection frequency was calculated as a proxy for calling activity. This frequency was calculated as the proportion of recordings in which *E. richmondi* was present over the total number of recordings per hour. The 24 hour period was divided in two categories: Hours from 5pm to 5am were considered “Night”, and hours from 6am to 5pm were considered “Day”. To determine if the diurnal calling activity was similar to the nocturnal, and to determine if this pattern was similar in all of the sites, a Generalized Linear Mixed Model with a Binomial distribution and a logit link function was used. This analysis was done in SASUniversityEdition (ver.9.4) using the GLIMMIX procedure and the maximum likelihood using Laplace approximation. Each covariate was analyzed using an analysis of variance to see if they showed differences between sites.

RESULTS

The naïve probability of occupancy and detection of *Eleutherodactylus richmondi* was 0.6790, and 0.935 respectively. Modelling the detection parameter while keeping occupancy constant proved that detection was affected by observation level covariates. The model with the highest support for the detection part was the one that included the effect of humidity. The second part of the model selection approach was to then keep the humidity covariate constant and test for each site-specific covariate on the occupancy parameter (Table 5). Slope proved to be a good predictor of the occupancy variation of *Eleutherodactylus richmondi*, models that included this covariate performed better and appeared in the first six best ranked models. The most parsimonious model was the one that included the effect of slope and percent of canopy cover on the occupancy parameter, and humidity on detection. (Table 5). The fit of this model was proved to be adequate (p-val=0.129) after verifying its goodness-of-fit using a parametric bootstrap procedure.

Table 5. Model selection table for the single-species single-season occupancy model of *Eleutherodactylus richmondi*. Model parameters are occupancy (ψ) and detection (p). Proven to be a good predictor for detection, the observation level covariate kept constant to model detection was relative humidity (Hum). Site-specific covariates are: % Canopy cover (CC), slope, leaf litter depth (Litter), and horizontal cover (HC).

Model	AIC	deltaAIC	AICwt	cumltyWt
$\psi(\text{CC+Slope}), p(\text{Hum})$	79.15	0.00	0.57	0.57
$\psi(\text{CC+Slope+Litter}), p(\text{Hum})$	81.11	1.96	0.22	0.79
$\psi(\text{HC+CC+Slope+Litter}), p(\text{Hum})$	82.94	3.79	0.09	0.88
$\psi(\text{Slope}), p(\text{Hum})$	83.71	4.56	0.06	0.93
$\psi(\text{Slope+Litter}), p(\text{Hum})$	85.30	6.15	0.03	0.96
$\psi(\text{HC+Slope}), p(\text{Hum})$	85.56	6.41	0.02	0.98
$\psi(\text{CC}), p(\text{Hum})$	88.52	9.37	0.01	0.99
$\psi(\text{CC+Litter}), p(\text{Hum})$	88.97	9.81	0.00	0.99
$\psi(\text{Litter}), p(\text{Hum})$	90.06	10.91	0.00	1.00
$\psi(\text{CC+HC}), p(\text{Hum})$	90.50	11.35	0.00	1.00
$\psi(\text{Litter+HC}), p(\text{Hum})$	90.78	11.63	0.00	1.00
$\psi(\text{HC}), p(\text{Hum})$	91.80	12.65	0.00	1.00

Results showed that the negative relationship between humidity and the probability of detection did not prove to be significant ($p=0.1550$) (Figure 7). The decrease in detection when the environment becomes oversaturated could be due to the sound of the rain. There is suggestive evidence that the percent canopy cover and the degree of slope of the terrain are positively associated with the occupancy of *E. richmondi*. (Table 6). The probability of occupancy is predicted to increase when canopy cover increases, while slope remains constant. The same pattern can be seen for the slope covariate. The probability of occupancy increases when slope increases, while % canopy cover remains constant. Plots of the relationship between probability of occupancy and significant site specific covariates can be seen in (Figure 8).

Table 6. Estimates for the most parsimonious occupancy model of *E. richmondi*. Estimates are shown on the logit-link scale.

	Estimate	SE	z	P(> z)
Occupancy(ψ)				
Intercept	-7.6798	3.2316	-2.38	0.0175
Canopy Cover	0.0889	0.0396	2.24	0.0250
Slope	0.1872	0.0777	2.41	0.0159
Detection(p)				
Intercept	19.421	11.925	1.63	0.1030
Humidity	-0.168	0.118	-1.42	0.1550

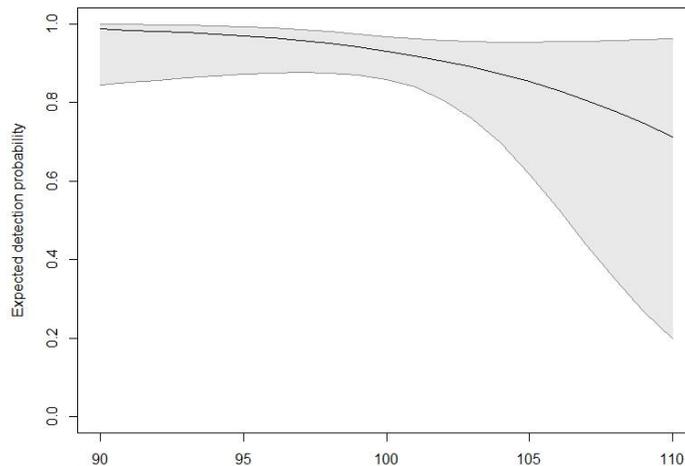


Figure 7. Predicted relationship between detection probability and relative humidity. Predicted values were produced using the most parsimonious model. Shaded area represents 95% confidence intervals.

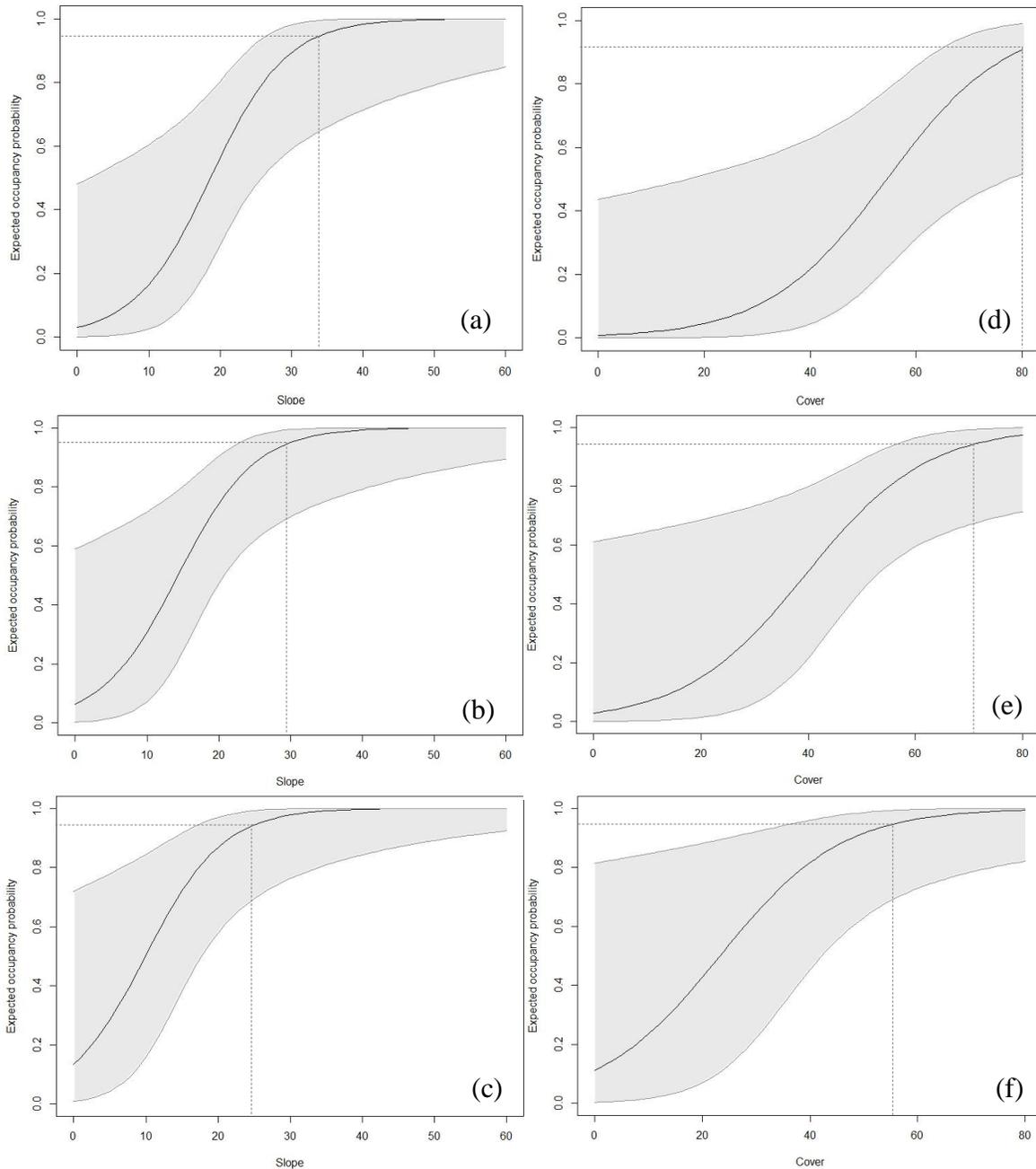


Figure 8. Predicted relationship between probability of occupancy and the covariates. Shaded area is the 95% confidence interval. The relationship between the probability of occupancy and the slope of the terrain is shown at different levels of canopy cover: 47% (a), 56% (b), 66% (c). The relationship between the probability of occupancy and the % canopy cover is shown at different levels of the slope of the terrain: 15° (d), 22° (e), 30° (f).

As seen in Figure 8, a site increased its probability of being occupied as the slope of the terrain in said site increases. At a 47% canopy cover, the probability of occupancy is 0.95 when the slope of the terrain is around 35° (Figure 8a). At a site with 56% canopy cover, the probability

of occupancy is 0.95 when the slope is slightly under 30° (Figure 8b). At 66% canopy cover, this probability is 0.95 when the slope is approximately 25° (Figure 8c). When the slope of the terrain is 15°, the probability of occupancy is 0.90 when the site has 80% canopy cover (Figure 8d). At a 22° slope, the probability of occupancy is 0.95 at over 70% canopy cover (Figure 8e). And when there is a 30° slope, the probability of occupancy is 0.95 at over 50% canopy cover (Figure 8f). The pattern that can be seen in this covariate effects is that the probability of occupancy could stay the same with a percent canopy cover decrease, as long as there is a steep slope in the terrain.

Diurnal calling activity of *E. richmondi*

The diel calling activity of *Eleutherodactylus richmondi* in the southwest portion of the Carite State Forest can be seen in Figure 9. This figure represents the average detection frequency of *E. richmondi* per hour over all the sites in which it was present (n=15). During the day hours (6am to 5pm) *richmondi* had an average detection frequency of over 23% at all hours, going up to 57% at 6am. From 4 to 5pm it lowers its calling activity from 42% to 25%. From 5p to 8pm it almost quadruples its activity, and from 8pm it continues to appear in over 93% of the recordings throughout the night hours. The hour with the highest calling activity of *E. richmondi* was 4am with 98.6% detection frequency.

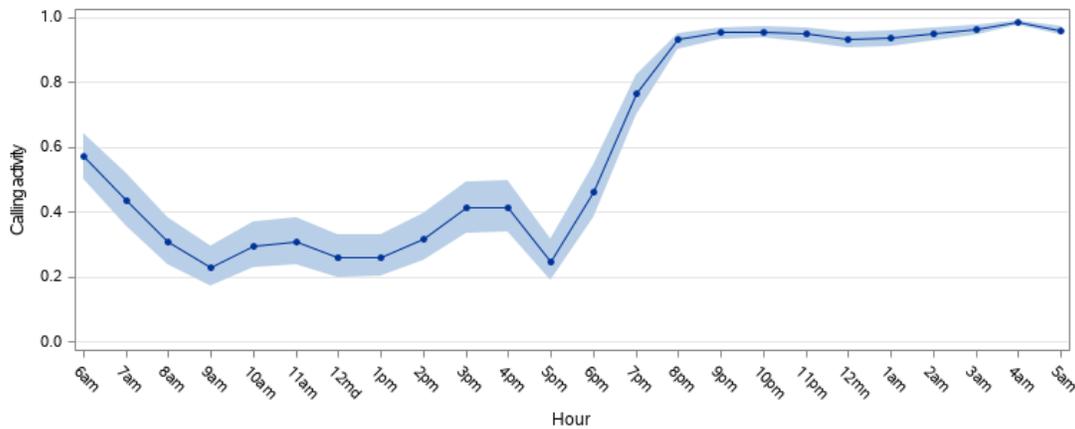


Figure 9. Average diel calling activity of *E. richmondi*. 95% confidence intervals are shown as the shaded area.

Diurnal calling activity of *E. richmondi* was significantly lower than nocturnal activity, with an average detection frequency of 37% and 85% respectively (F: 1086.18; $p < 0.001$). In all of the 15 sites there was diurnal activity in the hours between 5am and 5pm. The diurnal and nocturnal calling activity of *E. richmondi* varied per site (Figure). Five of the 15 sites had high calling activity ($>60\%$). One of the sites (OC25) showed no significant difference between the diurnal and nocturnal calling activity of this species (t-val: 0.78; p-value= 0.4371). Site OC27 was the only site that had significantly higher calling activity during the day, in comparison to its nightly activity (t-val: 5.45; $p < .0001$). In this site *E. richmondi* had a diurnal detection frequency of 0.93, and a nocturnal detection frequency of 0.80.

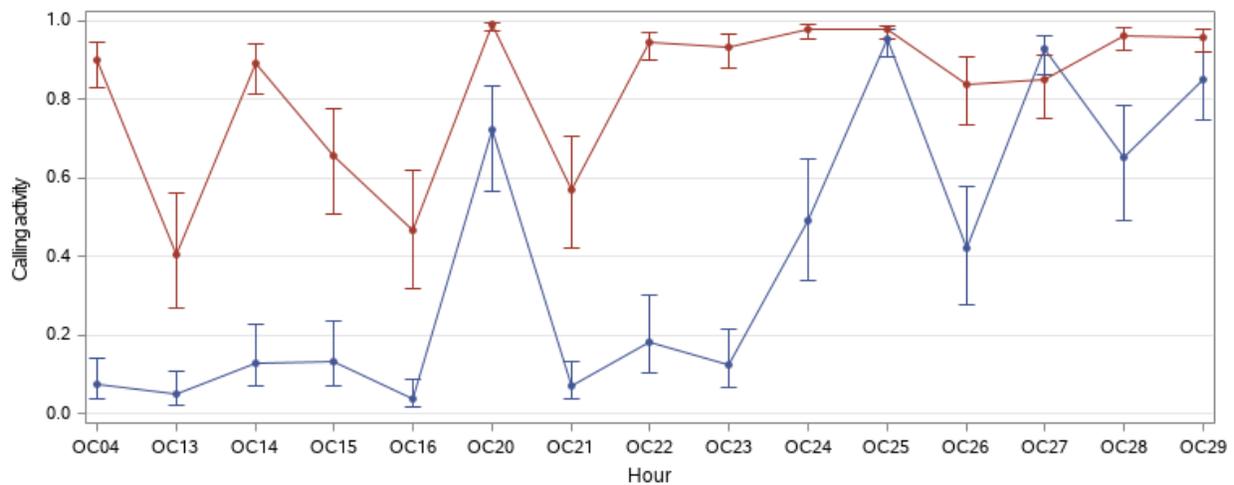


Figure 10. Model based average 24h calling activity of *E. richmondi* per site. Diurnal activity (from 5am to 5pm) is marked as a blue line and nocturnal activity (6pm to 5am) is shown as a red line. 95% confidence intervals are shown.

After the analysis of variance between sites for each of the habitat covariates (temperature, humidity, % canopy cover, horizontal cover, leaf litter depth, and slope), no significant pattern was found to possibly explain the differences in diurnal activity shown in Figure 10.

DISCUSSION

Most of the studies regarding populations of *Eleutherodactylus richmondi* have reported a decrease in abundance or localized extinctions (Joglar & Burrowes, 1996; Hedges, 1993; Woolbright, 1991; Burrowes, et al., 2004; Vilella & Fogarty, 2005). The survey area of this study was based at the southeast portion of the Carite State Forest, an area that falls under suitable habitat for this species according to the report of Villanueva-Rivera (2006). The results from this study prove that assumption after finding that the majority of the sites sampled were occupied by the species. Accounting for all of the sites where *E. richmondi* was present and the approximate size of each site, an area of 6.50 hectares of an active population of *E. richmondi* was found. The only population density estimates of this species come from the report of Stewart & Woolbright (1996) at the LEF. They report that the estimated density of *E. richmondi* was 100 individuals per hectare. Although it is not the same population or the same location, an inference can be made using those estimates; an approximate maximum population of 650 individuals could be found in the occupied area surveyed in this study.

In this study I used passive acoustic monitoring and occupancy modelling to identify habitat features associated with site selection of *E. richmondi*. Because of its floor-dwelling nature, *Eleutherodactylus richmondi* depends on the microclimatic of conditions of the topsoil it inhabits. Results showed that among the models that fitted well, the most parsimonious model was the one that included humidity to explain detection, and canopy cover and terrain slope to explain occupancy probability. Although the relationship between humidity and detection was not significant, the relationship that can be seen is negative; with a high calling activity in high humidity but not over saturation. An environment with over 100% relative humidity lead to a lower detection probability. No similar results were found and humidity thresholds are not available for

this species to make possible assumptions to explain this result. To better understand the relationship between detection and humidity, long term future studies should be performed on a larger number on sites and months of the year.

Percent of canopy cover and slope of the terrain were both significant and have a positive relationship with the site occupancy probability of *E. richmondi*. This means that keeping slope constant, the probability of occupancy of a site increases with a higher percentage canopy cover. And vice versa, keeping the percentage canopy cover constant, the probability that *Eleutherodactylus richmondi* occupies a site increases with a steeper slope. In other words, the probability of occupancy could stay the same with a percent canopy cover decrease, as long as there is a steep slope in the terrain. Percent canopy cover and terrain slope are both related to the amount of moisture in the topsoil (Baumann, et al., 2008; Tanner, et al., 1991). A decrease in percent canopy cover has been proved to increase the amount of moisture in the soil (Tanner, et al., 1991). Texture, drainage conditions, and slope gradients of the soil are key factors in runoff control and rainfall accumulations (Baumann, et al., 2008). The soil in this pocket of forest is a moderately permeable silty clay loam, with high available water capacity and susceptibility to erosion (USDA, 1977). These soil characteristics in addition to a low slope could cause the top soil to not drain water while retaining rainfall and moisture. Terrestrial modes of reproduction, like the one of *E. richmondi* have been strongly correlated with steep terrain and low availability of accumulated water sources (Liedtke, et al., 2017). The excess moisture in the soil could cause the microclimate of this species to become over saturated, which in turn could cause the individuals to look for steeper terrain that drains accumulated water. Furthermore, a steep slope in addition to erosion-prone soil causes the runoff to uncover weathered volcanic rocks. Crevices under these

low boulders have been reported as one of the preferred habitats for this species (Stewart & Woolbright, 1996; Woolbright, 1997; Rivero, 1998).

Results of the diel calling activity of *E. richmondi* show a pattern of exponential increase after 5pm, keeping a steady high calling activity up to 4am where a slight but significant increase occurs. These results contrast with the high peak of activity before midnight of the Maricao population reported by Villanueva-Rivera (2006). Instead, it agrees more with Drewry & Rand's report (1983), who measured the highest activity after midnight. Contrary to both of those studies, calling activity of this population was 2 hours before dawn. Differences in these results could come from the definition of the calling activity variable. Villanueva-Rivera (2006) defined calling activity by using qualitative values of ACI (amphibian calling index) and Drewry and Rand (1983) used the total number of calls per unit time, while the definition used in this study was the total number of recordings in which the species vocalized divided by the total amount of recordings per unit time. Another possible explanation to the difference in highest activity peak can come from the location of each of these studies. It is possible that because of altitude differences, or type of forest, the pattern of calling activity differs. Reports of calling activity of this species have been focused on nocturnal activity, but the results of 24 surveys of this population show that, in most of the sites in which it was present, it had diurnal activity of at least 10%. Distinct diurnal calling activity at different sites indicates that site-specific habitat variables are responsible of this discrepancy in diurnal calling activity. Because only two sites showed a high pattern of diurnal calling activity, and given that this is too small of a sample size, inferences could not be made on the specific reasons this could have happened.

An important point to be taken into account when seeing the results of this study is that the survey was done 10 months after Hurricane María made landfall on the Island. This hurricane almost completely defoliated the canopy of Puerto Rican forests (NASA/Goddard, 2018). This modifies the microclimate by restructuring the vegetation, which increases the light and temperature of the understory, adds variation in habitat heterogeneity and changes food supply (Waide, 1991), all factors that could affect the behavior of *Eleutherodactylus richmondi*. This study works then as a photograph of how this species was behaving 10 months after the hurricane and ultimately the results could explain how this species might answer to the effects of a disturbance. Canopy cover is variable that was proved to be important in the site selection criteria of *E. richmondi*, and its removal is one of the most affected by hurricanes. This combination could be then the possible trigger for *E. richmondi* to prefer sites with a steeper slope. Canopy loss increases the amount of biomass on the understory by the addition of leaf litter, fallen branches and organic debris. This increase of habitat heterogeneity could give *E. richmondi* a more diverse shadowed area to inhabit, which could also explain its diurnal calling activity.

GENERAL CONCLUSION AND RECOMMENDATIONS

This study quantified the effect that Hurricane María had on the *Eleutherodactylus* community in and around Carite State Forest, it identified habitat features associated with site selection of *E. richmondi*, and it described the diurnal calling activity shown by *E. richmondi*. The first chapter of this study showed that large scale disturbances not only affect the physical environment, but also the acoustic environment of a forest. The *Eleutherodactylus* species surveyed did not all react in the same way to the pressure produced by a hurricane, results that are confirmed by past hurricane-related studies (Woolbright, 1991; Vilella & Fogarty, 2005). This variation in response can be attributed to their differences in abilities and habitat selection, consequently giving large-scale disturbances a major role in natural selection. These evolutionary responses provide a key to understanding the structure of the communities that are chronically disrupted (Waide, 1991). The time after the hurricane when the area was surveyed could have caused some of the contrasting results with other studies in how specific *Eleutherodactylus* species have reacted differently to other hurricanes. Long term studies of these populations are necessary to give a better image of the chronosequence of succession of the *Eleutherodactylus* assemblages and how this reaction changes in accordance to vegetation restructuring. Manual mimicking of hurricane effects on predetermined plots as done by Klawinski et al. (2014), should also be done for other species of *Eleutherodactylus*. These types of study would allow for the inference of the specific changes in habitat that affect the variable response of each species. In future studies, special attention should be given to *E. gryllus* because of its low occurrence and lack of information on how it deals with disturbances.

The second chapter proved the assumptions made by Villanueva-Rivera (2006) about the possible healthy populations of *E. richmondi* on the Carite State Forest, by reporting and quantifying a new population of *Eleutherodactylus richmondi* on the southeast part of this forest. The slope of the terrain had been used as an anecdotal way to describe locations of this population, and this study gives empirical proof of this species preference to it. Slope should then be added as part of the habitat suitability models for this species. The extension of this population could be larger than previously measured due to the considerable size of suitable area. To measure the actual total extension of this population, and possibly find new ones, a completely random site selection should be done over the whole extension of this pocket of State Forest. Also, yearly repeated visits and an analysis using multi season occupancy modelling could provide support to the hypothesis that populations of *E. richmondi* are metapopulations (Zegarra-Vila, 2007). Overall, this study aids in the understanding of how species react to disturbances and adds necessary information on the site occupancy of the highly understudied species *Eleutherodactylus richmondi*.

LITERATURE CITED

- Acevedo, M. A., & Villanueva-Rivera, L. J. (2006). Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians. *Wildlife Society Bulletin*, 34(1), 211-214.
- Aide, T. M., Campos-Cerqueira, M., & Acevedo-Charry, O. (2017). *The search for the Golden Coqui and the Elfin Woods Warbler in the Sierra de Cayey, Puerto Rico*. US Fish and Wildlife Service. Rio Piedras, Puerto Rico: Sieve Analytics for global solutions.
- Aide, T. M., Corrada-Bravo, C., Campos-Cerqueira, M., Milan, C., Vega, G., & Alvarez, R. (2013). Real-time bioacoustics monitoring and automated species identification. *PeerJ*, 1, e103, 1-19.
- Angulo, A. (2008). *Eleutherodactylus richmondi*. *The IUCN Red List of Threatened Species*.
- Bailey, L. L., Simons, T. R., & Pollock, K. H. (2004). Estimating Site Occupancy and Species Detection Probability Parameters for Terrestrial Salamanders. *Ecological Applications*, 14(3), 692-702.
- Bailey, L., & Adams, M. (2005). *Occupancy Models to Study Wildlife*. U.S. Department of Interior. U.S. Geological Survey.
- Barker, B. S., & Rios-Franceschi, A. (2014). Population declines of mountain coqui (*Eleutherodactylus portoricensis*) in the Cordillera Central of Puerto Rico. *Herpetological Conservation and Biology*, 9(3), 578-589.
- Baumann, J., Rodriguez Morales, J. A., & Arellano Monterrosas, J. L. (2008). The effect of rainfall, slope gradient and soil texture on hydrological processes in a tropical watershed. *15th International Congress of the International Soil Conservation Organization-ISCO*. Budapest, Hungary.
- Beard, K. H., Vogt, K. A., Vogt, D. J., Scatena, F. N., Covich, A. P., Sigurdardottir, R., Siccama, T. G., Crowl, T. A. (2005). Structural and Functional Responses of a subtropical forest to 10 years of hurricanes and droughts. *Ecological Monographs*, 75(3), 345-361.
- Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference*. New York, USA: Springer-Verlag New York, Inc.
- Burrowes, P. A., Joglar, R. L., & Green, D. E. (2004). Potential Causes for Amphibian Declines in Puerto Rico. *Herpetologica*, 60(2), 141-154.
- Campos-Cerqueira, M., & Aide, M. (2016). Improving distribution data of threatened species by combining acoustic monitoring and occupancy modelling. *Methods in Ecology and Evolution*, 7(11), 1-9.

- Campos-Cerqueira, M., & Aide, M. T. (2017). Changes in the acoustic structure and composition along a tropical elevational gradient. *Journal of Ecoacoustics*, 1, pnc07i, 1-13.
- Campos-Cerqueira, M., & Aide, T. M. (2017). Lowland extirpation of anuran populations on a tropical mountain. *PeerJ*, 5, e4059, 1-21.
- Cangialosi, J. P., Latta, A. S., & Berg, R. (2018). *Tropical Cyclone Report: Hurricane Irma*. National Hurricane Center.
- Connell, J. H. (1989). Some processes affecting the species composition in forest gaps. *Ecology*, 70(3), 560-562.
- Crossland, M. R., Mackenzie, D. I., & Holzapfel, S. (2005). *Assessment of site-occupancy modeling as a technique to monitor Hochstetter's frog (Leiopelma hochstetteri) populations*. Department of Conservation. Wellington, New Zealand: Science & Technical Publishing.
- Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation*, 128(2), 231-240.
- Dale, V. H., Joyce, L. A., McNulty, S., Neilson, M. P., Flannigan, M. D., Hanson, P. J., Irland, L.C., Lugo, A. E., Peterson, C. J., Simberloff, D., Swanson, F. J., Stocks, B. J., Wotton, B. M. (2001). Climate Change and Forest Disturbances. *BioScience*, 51(9), 723-734.
- Deichmann, J. L., Hernández-Serna, A., Delgado C., J. A., Campos-Cerqueira, M., & Aide, T. M. (2017). Soundscape analysis and acoustic monitoring document impacts of natural gas exploration on biodiversity in a tropical forest. *Ecological Indicators*, 74, 39-48.
- Depraetere, M., Pavoine, S., Jiguet, F., Gasc, A., Duvail, S., & Sueur, J. (2012). Monitoring animal diversity using acoustic indices: Implementation in a temperate woodland. *Ecological Indicators*, 13(1), 46-54.
- Drewry, G. E., & Rand, S. (1983). Characteristics of an Acoustic Community: Puerto Rican Frogs of the Genus *Eleutherodactylus*. *Copeia*, 4, 941-953.
- DRNA. (2016). *Reglamento para regir especies vulnerables y en peligro de extincion en el estado libre asociado de Puerto Rico*. Puerto Rico: Departamento de Recursos Naturales y Ambientales.
- Ernstes, R., & Quinn, J. E. (2016). Variation in Bird Vocalizations across a Gradient of Traffic Noise as a Measure of an Altered Urban Soundscape. *Cities and the Environment*, 8(1), 1-13.
- Farina, A., & James, P. (2016). The acoustic communities: Definition, description and ecological role. *BioSystems*, 147, 11-20.

- Farina, A., & Pieretti, N. (2012). The soundscape ecology: A new frontier of landscape research and its application to islands and coastal systems. *Journal of Marine and Island Cultures*, 1(1), 21-26.
- Farina, A., Buscaino, G., Ceraulo, M., & Pieretti, N. (2014). The Soundscape Approach for the Assessment and Conservation of Mediterranean Landscapes: Principles and Case Studies. *Journal of Landscape Ecology*, 7(1), 10-22.
- Feng, Y., Negron-Juarez, R. I., Patricola, C. M., Collins, W. D., Uriarte, M., Hall, J. S., Clinton, N., Chambers, J. Q. (2018). Rapid remote sensing assessment of impacts from Hurricane Maria on forests of Puerto Rico. *PeerJ Preprints*.
- Ficetola, G. F., Rondinini, C., Bonardi, A., Baisero, D., & Paola-Schioppa, E. (2014). Habitat availability for amphibians and extinction threat: a global analysis. *Diversity and Distributions*, 21(3), 1-10.
- Ficetola, G. F., Rondinini, C., Bonardi, A., Katariya, V., Padoa-Schioppa, E., & Angulo, A. (2013). An evaluation of the robustness of global amphibian range maps. *Journal of Biogeography*, 41(2), 1-11.
- Fiske, I. J., & Chandler, R. B. (2011). unmarked: An R Package for Fitting Hierarchical Models of Wildlife Occurrence and Abundance. *Journal of Statistical Software*, 43(10), 1-23.
- Gannon, M. R., Kurta, A., Rodríguez, D. A., & Willig, M. R. (2006). Bats of Puerto Rico An Island Focus and A Caribbean Perspective. *Journal of Mammalogy*, 87, 635-635.
- Gotelli, N. J., & Colwell, K. R. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, 4, 379-391.
- Gould, W. A., Alarcón, C., Fevold, B., Jiménez, M. E., Martinuzzi, S., Potts, G., . . . Ventosa, E. (2008). *The Puerto Rico Gap Analysis Project*. International Institute of Tropical Forestry, U.S. Department of Agriculture Forest Service. Rio Piedras, Puerto Rico: International Institute of Tropical Forestry.
- Greenhawk, N., Zlotnik, S., Billy, L. M., Boas, S., & Gabel, S. (2017). Baseline amphibian survey and sampling of *Batrachochytrium dendrobatidis* in the Icaco and Hormiga valleys, Patillas, Puerto Rico. *Phyllomedusa*, 16(1), 63-69.
- Halliday, T. R. (2008). Why amphibians are important. *International Zoo Yearbook*, 42, 7-14.
- Heatwole, H., Mercado, N., & Ortiz, E. (1965). Comparison of critical thermal maxima of two species of Puerto Rican frogs of the genus *Eleutherodactylus*. *Physiological Zoology*, 38(1), 1-8.
- Hedges, B. S. (1993). Global amphibian declines: a perspective from the Caribbean. *Biodiversity and Conservation*, 2(3), 290-303.

- Hernández-Ordóñez, O., Urbina-Cardona, J. N., & Martínez-Ramos, M. (2015). Recovery of Amphibian and reptile assemblages during old-field succession of tropical rain forests. *Biotropica*, 47(3), 377-388.
- Herrera-Montes, M. I. (2018). Protected Area Zoning as a Strategy to Preserve Natural Soundscapes, Reduce Anthropogenic Noise Intrusion, and Conserve Biodiversity. *Tropical Conservation Science*, 11, 1-15.
- Herrera-Montes, M. I., & Aide, T. M. (2011). Impacts of traffic noise on anuran and bird communities. *Urban Ecosystem*. 14(3), 415-427.
- Hocking, D. J., & Babbitt, K. J. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, 9(1), 1-17.
- Horn, H. S. (1974). The Ecology of Secondary Succession. *Annu. Rev. Ecol. Syst.*, 5, 25-37.
- Joglar, R. L. (1998). *Los coquíes de Puerto Rico: su historia natural y conservación*. Editorial. San Juan, Puerto Rico: Editorial de la Universidad de Puerto Rico.
- Joglar, R. L., & Burrowes, P. A. (1996). *Declining amphibian populations in Puerto Rico*. Society for the Study of Amphibians and reptiles. New York: Contributions to West Indian Herpetology: A tribute to Albert Schwartz, Publisher: Society for the Study of Amphibians and Reptiles, Ithaca (New York), Editors: R. Powell, R. W. Henderson.
- Klawinski, P. D., Dalton, B., & Shiels, A. B. (2014). Coqui frog populations are negatively affected by canopy opening but not detritus deposition following an experimental hurricane in a tropical rainforest. *Forest Ecology and Management*, 332, 118-123.
- Klotzbach, P., & Bell, M. M. (2017). *Summary of 2017 Tropical Cyclone Activity and Verification of Authors' Seasonal and Two-week Forecasts*. Colorado State University, Department of Atmospheric Science.
- Liedtke, H. C., Müller, H., Hafner, J., Penner, J., Gower, D. J., Mazuch, T., Loader, S. P. (2017). Terrestrial reproduction as an adaptation. *Proc. R. Soc. B*, 284(1851), 1-9.
- Lugo, A. E. (2008). Visible and invisible effects of hurricanes on forest ecosystems: an international review. *Austral Ecology*, 33(4), 368-398.
- MacKenzie, D. I., & Bailey, L. (2004). Assessing the Fit of Site-Occupancy Models. *Journal of Agricultural, Biological, and Environmental Statistics*, 9(3), 300-318.
- Mackenzie, D., Nichols, J. D., Royle, A. J., Pollock, K. H., Bailey, L. L., & Hines, J. E. (2006). *Occupancy Estimation and Modeling Inferring Patterns and Dynamics of Species Occurrence*. Elsevier Academic Press publications.
- Monroe, K. D., Collazo, J. A., Pacifici, K., Reich, B. J., Puente-Rolón, A. R., & Terando, A. J. (2017). Occupancy and Index of Abundance of *Eleutherodactylus wightmanae* and *E.*

- brittoni along Elevational Gradients in West- Central Puerto Rico. *Caribbean Naturalist*, 40, 1-18.
- NASA/Goddard, S. F. (2018, July 11). *NASA surveys hurricane damage to Puerto Rico's forests*. Retrieved from ScienceDaily: www.sciencedaily.com/releases/2018/07/180711182759.htm
- National Hurricane Center. (2015). *Tropical Cyclone Climatology*. Retrieved from National Oceanic and Atmospheric Administration: <https://www.nhc.noaa.gov/climo/>
- Nudds, T. D. (1977). Quantifying the vegetative structure of wildlife cover. *Wildlife Society Bulletin*, 5(3), 113-117.
- Ospina, O. E., Villanueva-Rivera, L. J., Corrada-Bravo, C. J., & Aide, T. M. (2013). Variable response of anuran calling activity to daily precipitation and temperature: implications for climate change. *Ecosphere*, 4(4), 1-12.
- Pasch, R. J., Penny, A. B., & Berg, R. (2018). *National Hurricane Center tropical cyclone report: Hurricane Maria (AL152017)*. National Ocean and Atmospheric Administration. National Hurricane Center.
- Pellet, J., & Schmidt, B. (2005). Monitoring distributions using call surveys: estimating site occupancy, detection probabilities and inferring absence. *Biological Conservation*, 123(1), 27-35.
- Ríos-López, N., & Aide, T. M. (2007). Herpetofaunal Dynamics During Secondary Succession. *Herpetologica*, 63(1), 35-50.
- Ríos-López, N., Flores-Rodríguez, Y. M., Agosto-Torres, E., Vicens-López, C., & Hernández-Muñiz, R. M. (2015). Life History Observations on the Melodious Coqui, *Eleutherodactylus wightmanae* (Anura: *Eleutherodactylidae*) from Puerto Rico: Double Clutches and Adult Predation by the Yellow-Chinned Anole, *Anolis gundlachi* (Squamata: *Dactyloidae*). *Life: The Excitement of Biology*, 3(2), 137-148.
- Rivero, J. A. (1998). *Los anfibios y reptiles de Puerto Rico*. San Juan, Puerto Rico: Editorial de la Universidad de Puerto Rico.
- Roznik, E. A., Sapsford, S. J., Pike, D. A., Schwarzkopf, L., & Alford, R. A. (2015). Natural disturbance reduces disease risk in endangered rainforest frog populations. *Nature: Scientific Reports*, 5, 1-11.
- Rykiel, E. J. (1985). Towards a definition of ecological disturbance. *Australian Journal of Ecology*, 10, 361-365.

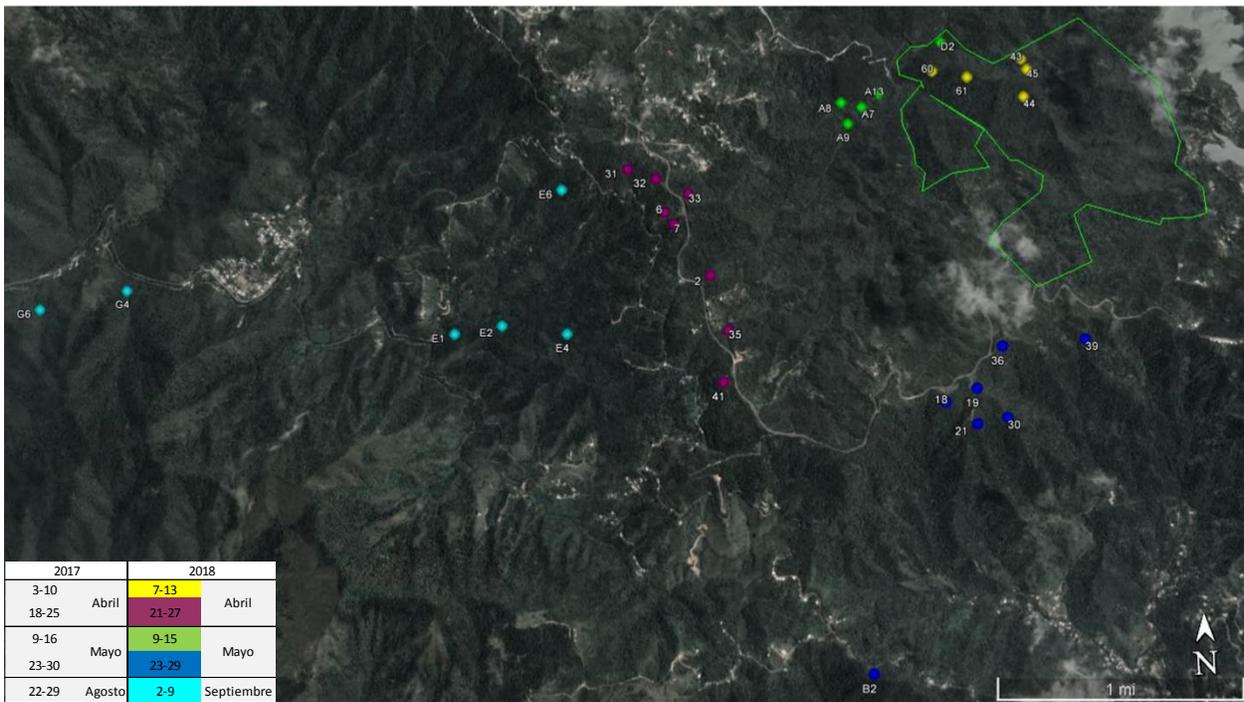
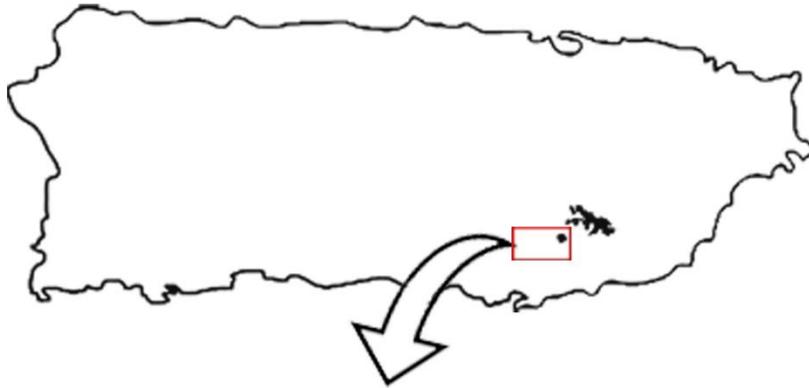
- Schwartz, A., & Henderson, R. (1991). *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. Gainesville, Florida, USA: University of Florida Press.
- Semlitsch, R. D. (2003). General Threats to Amphibians. In R. D. Semlitsch (ed), *Amphibian Conservation* (pp. 1-7). Smithsonian Institution.
- Sousa, W. P. (1984). The Role of Disturbance in Natural Communities. *Ann. Rev. Ecol. Syst.*, 15, 353-391.
- Stallard, R. F. (2001). Possible Environmental Factors Underlying Amphibian Decline in Eastern Puerto Rico: Analysis of U.S. Government Data Archives. *Conservation Biology*, 15(4), 943-953.
- Stewart, M. M. (1995, September). Climate Driven Population Fluctuations in Rain Forest Frogs. *Journal of Herpetology*, 29(3), 437-446.
- Stewart, M. M., & Woolbright, L. (1996). Amphibians. In P. Reagen, & R. B. Waide, *The Food web of a tropical rain forest* (pp. 273-320). Chicago: The University of Chicago Press.
- Suazo-Ortuño, I., Urbina-Cardona, J. N., Lara-Urbe, N., Marroquín-Páramo, J., Soto-Sandoval, Y., Rangel-Orozco, J., Lopez-Toledo, L., Benitez-Malvido, J. & Alvarado-Díaz, J. (2018). Impact of a hurricane on the herpetofaunal assemblages of a successional chronosequence in a tropical dry forest. *Biotropica*, 50(4), 1-15.
- Suzuki, R., & Shimodaira, H. (2006). Pvcust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics*, 22(12), 1540-1542.
- Tanner, E. V., Kapos, V., & Healey, R. J. (1991). Hurricane Effects on Forest Ecosystems in the Caribbean. *Biotropica*, 23(4a), 513-521.
- USDA. (1977). *Soil Survey of Humacao Area of Eastern Puerto Rico*. Soil Conservation Service.
- USGS. (1999). *Fact Sheet 040-99 Hurricane Georges*. Guaynabo, PR : U.S. Department of the Interior.
- Vilella, F. J., & Fogarty, J. H. (2005). Diversity and Abundance of Forest Frogs (Anura: Leptodactylidae) before and after Hurricane Georges in the Cordillera Central of Puerto Rico. *Caribbean Journal of Science*, 41(1), 157-162.
- Villanueva-Rivera, L. J. (2006). *Calling activity of Eleutherodactylus frogs of Puerto Rico and habitat distribution of E. richmondi*. Master's Thesis, University of Puerto Rico, Department of Biology, Rio Piedras.
- Waide, R. B. (1991). Summary of the Response of Animal Populations to Hurricanes in the Caribbean. *Biotropica*, 23(4), 508-512.

- Walker, L. R., Lodge, D. J., Brokaw, N. V., & Waide, R. B. (1991, December). An Introduction to Hurricanes in the Caribbean. *Biotropica*, 23(4), 313-316.
- Walker, L. R., Zarin, D. J., Fetcher, N., Myster, R. W., & Johnson, A. H. (1996). Ecosystem development and plant succession on landslides in the Caribbean. *Biotropica*, 28(4), 566-576.
- Whiles, M. R., Lips, K. R., Pringle, C. M., Kilham, S. S., Bixby, R. J., Brenes, R., Connelly, S., Colon-Gaud, J.C., Hunte-Brown, M., Hurn, A. D., Montgomery, C., & Scot, P. (2006). The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology and the Environment*, 4(1), 27-34.
- White, P. S., & Jentsch, A. (2001). The Search for Generality in Studies of Disturbance and Ecosystem Dynamics. *Progress in Botany*, 62, 399-450.
- Woolbright, L. L. (1991, December). The Impact of Hurricane Hugo on Forest Frogs in Puerto Rico. *Biotropica*, 23(4), 462-467.
- Woolbright, L. L. (1997). Local extinctions of anuran amphibians in the Luquillo Experimental Forest of northeastern Puerto Rico. *Journal of Herpetology*, 31(4), 572-576.
- Wunderle, J. M. (1999). Pre- and Post-Hurricane fruit availability: Implications for Puerto Rican parrots in the Luquillo Mountains. *Caribbean Journal of Science*, 35(3-4), 249-264.
- Zegarra-Vila, J. P. (2007). *Assessment of Eleutherodactylus richmondi populations at the Maricao State Forest, Puerto Rico*. Master's Thesis, University of Puerto Rico, Department of Biology, Mayaguez.
- Zimmerman, J. K., Willig, M. R., Walker, L. R., & Silver, W. L. (1996). Introduction: Disturbance and Caribbean Ecosystem. *Biotropica*, 28(4a), 414-423.

APPENDIXES

Appendix A. Coordinates of sites used for 2017-2018 comparisons.

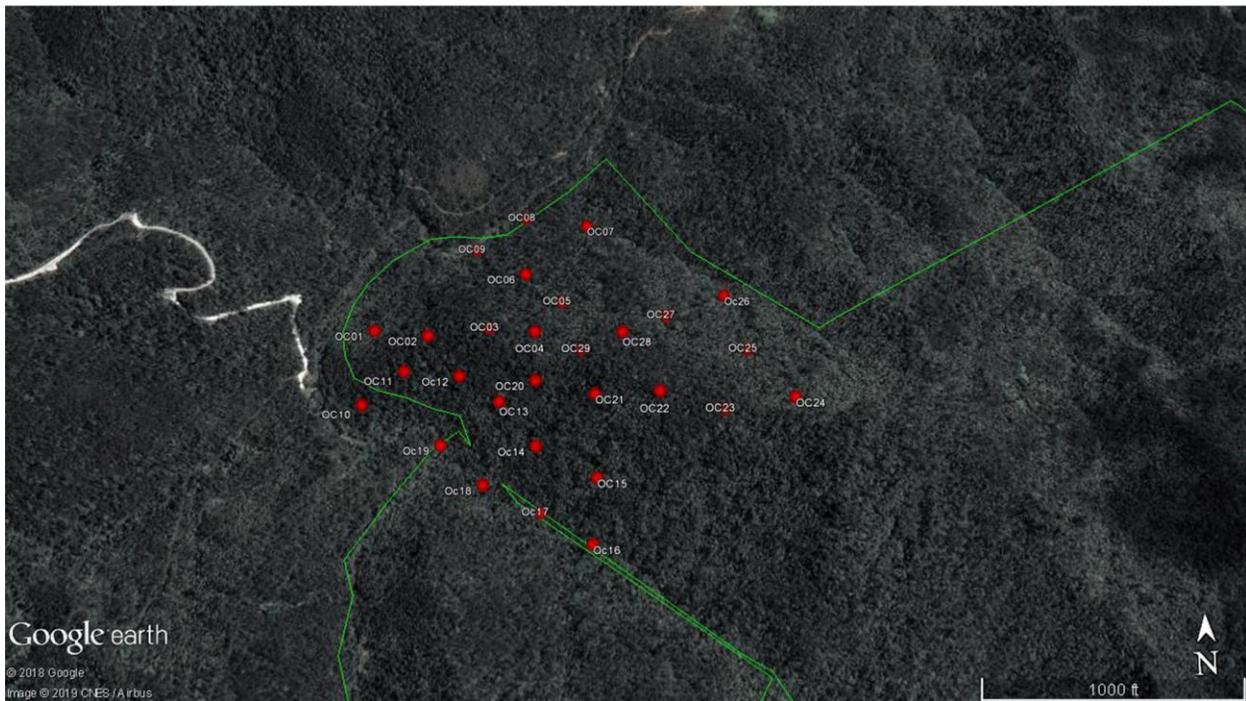
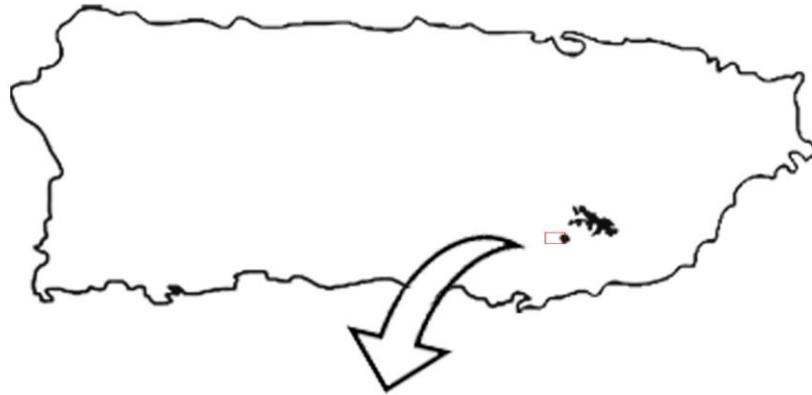
Site Name	Latitude	Longitude	Altitude
61	18.070430°	-66.117657°	600
60	18.070939°	-66.119713°	600
44	18.068983°	-66.114428°	600
45	18.070608°	-66.114059°	600
43	18.071256°	-66.114332°	600
41	18.053240°	-66.133980°	700
35	18.056050°	-66.133500°	700
33	18.063840°	-66.135280°	807
32	18.064820°	-66.137140°	700
31	18.065510°	-66.138740°	700
2	18.059160°	-66.134310°	825
7	18.062220°	-66.136230°	700
6	18.062940°	-66.136760°	700
D2	18.072863°	-66.118876°	600
A13	18.069860°	-66.123000°	600
A9	18.068020°	-66.125170°	600
A8	18.069360°	-66.125440°	600
A7	18.069050°	-66.124180°	600
39	18.054613°	-66.112618°	600
B2	18.037193°	-66.126438°	600
36	18.054313°	-66.117968°	600
30	18.050513°	-66.117598°	700
21	18.050243°	-66.119468°	600
19	18.052133°	-66.119538°	600
18	18.051463°	-66.121388°	600
G4	18.061069°	-66.170147°	325
E6	18.064950°	-66.142751°	500
E4	18.056763°	-66.142889°	500
E2	18.057563°	-66.146850°	500
E1	18.057279°	-66.149794°	500



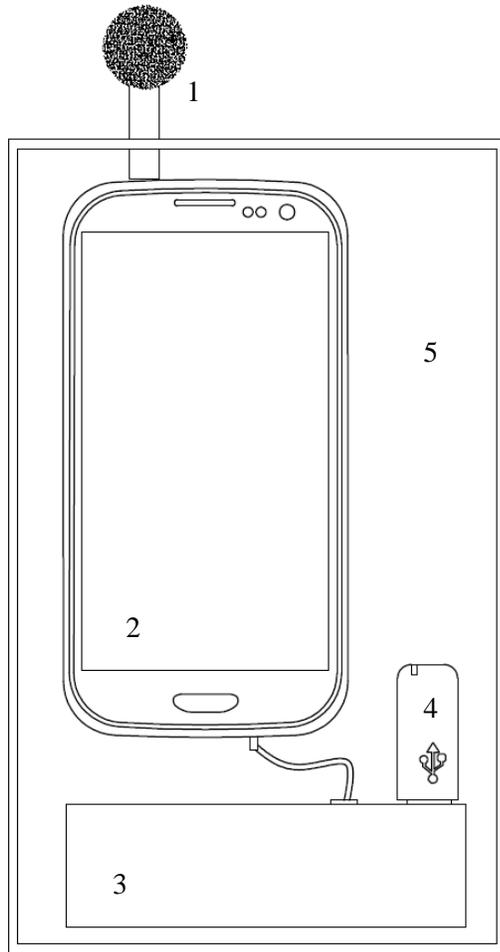
Appendix B. Location of the sites used for the pre and post-hurricane comparisons. Color coded by the dates of survey. The Carite State Forest periphery is drawn with a green line.

Appendix C. Coordinates of sites used for occupancy modelling and day activity description of *E. richmondi*. (*) Not used for analysis.

Site Name	Latitude	Longitude	Altitude
OC01*	18.071506°	-66.121396°	587
OC02	18.071363°	-66.120708°	608
OC03	18.071392°	-66.119948°	614
OC04	18.071341°	-66.119409°	613
OC05	18.071729°	-66.119071°	592
OC06	18.072111°	-66.119513°	581
OC07	18.072761°	-66.118724°	560
OC08	18.072902°	-66.119492°	557
OC09	18.072528°	-66.120146°	552
OC10	18.070665°	-66.121593°	537
OC11	18.070997°	-66.121036°	591
OC12	18.070900°	-66.120359°	603
OC13	18.070566°	-66.119882°	607
OC14	18.070032°	-66.119460°	589
OC15	18.069627°	-66.118745°	594
OC16	18.068907°	-66.118839°	591
OC17	18.069293°	-66.119445°	579
OC18	18.069654°	-66.120139°	577
OC19	18.070149°	-66.120648°	573
OC20	18.070545°	-66.118716°	614
OC21	18.070759°	-66.119423°	605
OC22	18.070507°	-66.117953°	636
OC23	18.070339°	-66.116389°	633
OC24	18.070260°	-66.117200°	645
OC25	18.071714°	-66.117057°	600
OC26	18.070980°	-66.116851°	612
OC27	18.071473°	-66.117800°	599
OC28	18.071262°	-66.118350°	618
OC29	18.071061°	-66.118853°	619



Appendix D. Location of the sites surveyed for occupancy modelling of *E. richmondi*. The green line represents the periphery of the Carite State Forest.



1. Condenser microphone (Monoprice – Model 600200)
2. Cellular phone (BLU A4)
3. External battery pack (20,000mAh),
4. Usb stick with a red LED light
5. Weather-proof box.

Appendix E. Diagram of automatic microphones.