ABSTRACT

RIVERA-BURGOS, ANA-CECILIA. Habitat Suitability for *Eleutherodactylus* Frogs in Puerto Rico: Indexing Occupancy, Abundance and Reproduction to Climatic and Habitat Characteristics. (Under the direction of Jaime A. Collazo).

What makes a habitat "suitable" for *Eleutherodactylus* frogs and can we predict the consequences of climate change on this taxonomic group in Puerto Rico? These questions stem by the fact that amphibian populations in Puerto Rico are declining, a trend also observed in other amphibians elsewhere in the world, and that one of the major drivers behind the negative trends is global warming. To help answer the aforementioned questions, I characterized the relationship between environmental and habitat variables, measured at the local/site level, and occupancy and the probability of detecting a chorus (≥ 4 individuals) of *Eleutherodactylus wightmanae*, *E. brittoni*, and *E. antillensis*, 3 species of differing size, distribution and habitat affinities. This understanding is important because shifts in abundance and distribution at larger scales begin with responses at local scales. Additionally, I determined how the same variables influenced nesting activity of *E. coqui*, a common and widely distributed species. Finally, I established critical thermal maximum for all 4 species as a means to gain insights on how they might respond to environmental change. Occupancy of *E. wightmanae* and *E. brittoni* was affected positively by physical/environmental covariates (e.g., relative humidity, elevation), while *E. antillensis* was not. Physical/environmental covariates also had a positive influence on detecting a chorus of *E. wightmanae* and *E. brittoni,* but negative on *E. antillensis*. Annual variation of macrohabitat covariates (e.g., ground cover) had a positive influence on *E. brittoni* and *E. antillensis*. The passing of Hurricane Maria on September 20, 2017 did not have a devastating effect on local population abundance, at least the sites that were accessible for monitoring. Relative humidity and temperature exerted the strongest influence on the probability

of encountering active nests of *E. coqui*, where sites with ≤ 24 °C and ≥ 90% relative humidity had > 50% chance of having an active nest. *E. brittoni* (34.43 ± 0.9 ˚C) and *E. wightmanae* (38.72 ± 1.62 °C) had statistically similar CT_{Max} values. In contrast, CT_{Max} for *E. coqui and E. antillensis* were 10-18 ˚C higher. My findings help develop criteria to index habitat quality to facilitate managed translocations or *in-situ* habitat enhancement. My CT_{Max} results suggest that it might be possible to group species according to their responses to ambient conditions, but also why the species does not occur below 200 m. The positive response to physical/environmental covariates by *E. wightmanae* and *E. brittoni* as compared to *E. antillensis* lend support to such a possibility. Taken together, my findings provide a stronger ecological basis to postulate hypotheses that more accurately test the potential linkages between environmental conditions and physiological, and ultimately, demographic responses of *Eleutherodactylus* frogs. Many conservation decisions, which depend on the organism's ability to survive and reproduce, should be informed by a sound understanding of the species' thermal window of tolerance and demographic responses to varying climatic conditions at local levels.

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Habitat Suitability for *Eleutherodactylus* Frogs in Puerto Rico: Indexing Occupancy, Abundance and Reproduction to Climatic and Habitat Characteristics

by
Ana Cecilia Rivera-Burgos

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APPROVED BY:

_____	_____
Dr. Jaime A. Collazo Committee Chair	Dr. J. Krishna Pacifici
_____	
Dr. Adam J. Terando	

DEDICATION

I dedicate this work to my parents, Ferdinand and Consuelo, who have supported me through this journey from the very beginning. You taught me to dream big and to never give up on what I love.

To my sister, Viviana, who I lean on, more times than I can count. I can't imagine my life without you.

Finally, to Rafael, the love of my life. Thank you for joining me during this adventure, for all your hard work each field season, for being patient and understanding. There is nothing we can't achieve together.

Thank you, all of you. You gave me strength, courage, and support during the most difficult time of my life. This is for you, with all my love.

BIOGRAPHY

I was raised on the west coast of Puerto Rico, where I spent my childhood running along the beach, looking at birds and searching for manatees. After a few years on the coast, my parents decided to restore my great-grandparents' farm, so we moved from the beach up to the mountains. Coming from a long line of agronomists, I decided to study Animal Science at the University of Puerto Rico, Mayaguez Campus, and that is where I fell even more in love with animals. After volunteering for the U.S. Fish and Wildlife Service for many summers, in 2012 I began an official internship. I had always admired what they did, and during that summer, I grew fond of fieldwork. In 2015 I started my master's degree at North Carolina State University, in the field of Animal Science. After a semester I found myself searching for something I felt more passionate about. With this in mind, I walked into David Clark Labs to see if I could enroll in a wildlife elective class, something I had always dreamt about, but unfortunately never had been able to take. There I met incredible people, with incredible jobs. It reminded me of that internship I had loved so much. I knew I had found that passionate field I was searching for, so I made the jump from an Animal Science program to the Zoology program in the Applied Ecology Department.

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INTRODUCTION

Global warming has become a driver of biodiversity decline and species extinction (Stuart et al. 2004, Navas 2006, D'Amen et al. 2011, Alcala et al. 2012, Bestion et al. 2015). Amphibians are among the many vertebrates affected by climatological changes, but they are poorly represented in conservation studies in comparison to other taxa (D'Amen et al. 2011, Dowdy 2016). This underrepresentation is of great concern since small decreases in annual rainfall and a small increase in temperature have been attributed to amphibian population declines worldwide (Burrowes et al. 2004, Delgado-Acevedo and Restrepo 2008). Puerto Rico is no exception, as model projections suggest warmer and drier conditions (Khalyani et al. 2016). The effects of climatological changes on amphibians are of more concern in tropical regions, as these areas harbor more species of plants and animals that would be vulnerable to change (Burrowes et al. 2004, von May et al. 2017).

Puerto Rico is located in the Caribbean Basin and considered a tropical island. It consists of 3 different physiographic regions; the karst, coastal plains and valleys, and the mountainous interior. The mountainous interior is the largest region of the island, and includes the Sierra de Luquillo and the Cordillera Central (Soto and Pintó 2010, Joglar et al. 2011). Trade winds, coming from the East and Northeast of the island, meet the mountain ranges resulting in higher humidity and wet forests. The south and southwest part of the island is protected by the Cordillera Central and Sierra Bermeja, resulting in dry xerophytic vegetation with higher temperatures and low precipitation (Soto and Pintó 2010).

There are 25 known species of amphibians in Puerto Rico, of which 19 are native. Puerto Rico only has 2 families of native amphibians (Leptodactylidae and Bofonidae), and 3 genera (*Eleutherodactylus, Leptodactylus,* and *Peltophryne*); 89.5% of the individuals belong to the

genus *Eleutherodactylus,* and 94.7% belong to the family Leptodactylidae (Joglar et al. 2011). There are 17 native species of *Eleutherodactylus* in Puerto Rico, all inhabiting distinct microhabitats found on the island (Joglar 1998, Nowakowski et al. 2016). This is possible due to the climatic and geographic diversity of Puerto Rico (Joglar 1998). The most commonly found *Eleutherodactylus* species is the common coqui (*Eleutherodactylus coqui*), which is considered a national symbol (Joglar 1998), and is even represented by the Taíno Indians in their petroglyphs (Hayward et al. 2009). The coqui's mating call is loudly heard during the nights and has been the inspiration for numerous children's books, songs, and legends told for generations by the Puerto Rican people. Other *Eleutherodactylus* species are not as commonly found, and are limited to specific habitats and microclimates (Rivero 1978, Joglar 1998).

Out of the 17 *Eleutherodactylus* species found in Puerto Rico, 3 are federally listed as endangered or threatened (*E. cooki, E. jasperi, E. juanariveroi*), and the remaining 14 are considered species at risk by the U.S. Fish and Wildlife Service (Burrowes et al. 2004, Barker and Ríos-Franceschi 2014, Dowdy 2016). The U.S. Fish and Wildlife Service is interested in determining the environmental and habitat features that define suitable habitat to inform management decisions aimed at minimizing listing species at risk (pre-listing conservation), and identifying areas of conservation interest to assist in the recovery of species already listed (e.g., *E. juanariveroi*).

Parameters that characterize suitable habitat serve to guide pre-listing and recovery efforts if they impinge on demographic rates (Morrison et al. 2012). Information indexing demographic parameters to habitat correlates is scant in Puerto Rico, but available information points at the close relationship between environmental conditions and demography. For example, Burrowes et al. (2004) and Ríos-López et al. (2015) reported the influence of

environmental conditions on various sources of mortality, in some cases, mediated by weather-influenced disease. Dowdy (2016) reported that long-term average precipitation, coupled with canopy and ground cover, influenced patterns of occupancy for *E. wightmanae* and *E. brittoni* along elevational gradients on the island. These conditions also influenced the probability of detecting 1 or ≥ 2 individuals at survey stations, a proxy of abundance.

Herein, I build upon the foundational knowledge laid by Dowdy (2016) by addressing the following 3 objectives. First, abundance patterns of *E. brittoni*, *E. wightmanae* and *E. antillensis* were characterized as a function of environmental (weather) and habitat characteristics using a more appropriate abundance index (i.e., few or chorus). Acoustic monitoring (spectrogram) allows discerning 1-3 individuals without ambiguity; however, in most instances, species will occur in a chorus (≥ 4 individuals). The latter case is recognized when there are overlapping bands on the spectrogram (M. Cerqueria-Campos, Sieve Analytics, pers. Comm). Second, reproductive activity (e.g., nesting attempts) of *Eleutherodactylus* species were monitored using artificial nest structures to ascertain environmental (weather) and habitat correlates. Lastly, the critical thermal maximum (CT_{Max}) of *E. brittoni*, *E. wightmanae*, *E. coqui*, and *E. antillensis* was determined to better understand patterns of occupancy and abundance under varying environmental conditions, and the vulnerability of species to projected climatic changes in Puerto Rico.

I addressed or tested the following questions and predictions. First, given that CT_{Max} results indicated that *E. wightmanae* and *E. brittoni* had significantly lower values at the same elevation than *E. coqui* and *E. antillensis*, I predicted that their response (i.e., occupancy, abundance) to environmental covariates would be more similar for *E. wightmanae* and *E. brittoni* than for *E. antillensis*. Reproduction in amphibians is affected by climate change components,

such as temperature and moisture (Carey and Alexander 2003). In the genus *Eleutherodactylus*, breeding activity is affected by climatic conditions and by number of calling males (Townsend and Stewart 1994, Villanueva-Rivera 2006, Ríos-López et al. 2014). Accordingly, I predicted that breeding activity, defined as nesting attempt with a clutch of eggs, would be positively correlated with covariates tied to the micro-habitat and micro-environmental conditions of surveyed habitat (e.g., relative humidity, soil moisture, litter depth and leaf layer).

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CHAPTER 1

Critical Thermal Maximum of Four Species of *Eleutherodactylus* Frogs in West-Central Puerto Rico.

ABSTRACT

As ectotherms, ambient conditions (e.g., temperature, relative humidity) mediate important biological processes of amphibians that include respiration, osmoregulation, predator avoidance, and reproduction. Understanding the physiological thresholds that foster these processes is of utmost importance because they impinge on survival and reproduction, hence, abundance and distribution. Physiological thresholds are limits after which organisms exhibit signs detrimental to their survival. Herein I determined the critical thermal maximum (CT_{Max}) of *E. coqui, E. wightmanae, E. brittoni, and E. antillensis*. CT_{Max} was defined as the temperature that triggered spasms and erratic behavior that impairs predator avoidance. *E. brittoni* (34.43 ± 0.9 ˚C) and *E. wightmanae* (38.72 ± 1.62 ˚C) had statistically similar CT_{Max} values. In contrast, CT_{Max} for *E. coqui and E. antillensis* were 10-18 ˚C higher. My results point at least 3 important conservation implications. First, annual maximum ambient temperatures between July and September at < 400 m are within 1-7 ˚C of the thermal limit of *E. wightmanae* and *E. brittoni*, providing an ecophysiological basis for their high elevation distribution. Second, results suggest that it might be possible to group species according to their thermal limits with the expectation that species sharing similar CT_{Max} values would respond in similar fashion to ambient conditions. Lastly, groupings would provide a stronger ecological basis to postulate hypotheses that more accurately test the potential linkages between environmental conditions and physiological, and ultimately, demographic responses of *Eleutherodactylus* frogs. Many conservation decisions,

which depend on the organism's ability to survive and reproduce, should be based on a sound understanding of the species' thermal window of tolerance.

INTRODUCTION

Population declines have been reported in at least 4 endemic amphibians in Puerto Rico, *Peltophryne* (*Bufo) lemur*, and 3 *Eleutherodactylus* species (*E. karlschmidti, E. jasperi, and E. eneidae*) (Joglar 1998, Burrowes et al. 2004, Joglar et al. 2011). *Peltophryne lemur* is still extant on the island thanks to recovery activities that protect natural breeding areas and supplement population in concert with a captive breeding program (Joglar et al. 2011). However, *E. karlschmidti, E. jasperi, and E. eneidae* have not been found since 1976, 1981, and 1990, respectively (Joglar 1998, Burrowes et al. 2004). Among the factors that have contributed to the rare and endangered status of the aforementioned species is climate change (Burrowes et al. 2004, Joglar et al. 2011, Scheffers et al. 2013, Barker and Ríos-Franceschi 2014, Brusch et al. 2016). Climate change encompasses global warming, which is now considered a dominant threat to biodiversity (Bernardo et al. 2007) either because species cannot compensate physiologically, or because they are unable to shift geographical ranges (Calosi et al. 2008).

An ectotherm's body temperature is determined through ambient temperature and behavior modifications. Their body temperature regulates physiological, cellular, and biochemical rates such as O_2 transport, metabolization of food, respiration, reproduction, and predator avoidance (Carey and Alexander 2003, Hillman et al. 2009, Scheffers et al. 2013, Frishkoff et al. 2015). Amphibians need to maintain their body temperature within its range of tolerance to avoid suffering from impaired functions (Hillman et al. 2009). Therefore, there is a growing interest in ascertaining the physiological thresholds (e.g., thermal), as well as the upper limits that could lead to a fatal physiological condition (Hillman et al. 2009, Scheffers et al. 2013). This knowledge is important to gain insights about the current physiological state of amphibian species, and how they might respond in the face of global warming.

Physiological thresholds are broadly defined as those limits where signs of detriment in the organism's physiology are observed. In anurans and many ectotherms, multiple traits can be used to establish these thresholds, ranging from monitoring physiological traits at sub-lethal levels (Wheeler et al. 2015) to establishing the critical (i.e. lethal to organism) thermal limits (Cortes et al. 2016). Herein I focused on establishing the critical (i.e. lethal to organism) thermal limits (Cortes et al. 2016) of 4 species of *Eleutherodactylus*, namely, *E. coqui, E. wightmanae, E. brittoni, and E. antillensis*. Determining these values is important because animals exposed to temperatures around the upper zone of resistance, could lose normal motor functions, and therefore, considered ecologically dead (Hillman et al. 2009, Scheffers et al. 2013). Moreover, information of physiological limits is needed because observational studies alone will not be able to discern triggers driving local and landscape level shifts in distribution and abundance. Lastly, data on thermal limits provide another basis upon which to formulate hypotheses to predict plausible responses to environmental conditions.

To date, only 2 previous studies have estimated CT_{Max} in *Eleutherodactylus* frogs in Puerto Rico. Heatwole et al. (1965) determined CT_{Max} for *E. portoricensis* and *E. richmondi*, both distributed across wet montane rainforests (Joglar 1998, Barker and Ríos-Franceschi 2014). However, inferences about these values were obscured by the fact that what was thought to be only *E. portoricensis* was in fact 2 species: *E. coqui* and *E. portoricensis.* This taxonomic shortcoming was later corrected by Christian et al. (1988), who determined the CT_{Max} and CT_{Min} of *E. coqui* and *E. portoricensis*.

I believe that information on CT_{Max} obtained from my focal species will be insightful because species vary by size, and exhibit a sharp contrast in distribution, particularly along elevation, and habitat preferences. *E. coqui* (35 mm SVL) and *E. antillensis* (30 mm SVL) are

both widely distributed across the island, although *E. antillensis* is primarily found at low elevations (Rivero 1978, Joglar 1998). *E. coqui* can be found both on the ground and up to 30 m from the ground, while *E. antillensis* prefers low vegetation (Joglar 1998). *E. brittoni* (16 mm SVL can be found from 183 m to 640 m of elevation, but Monroe et al. (2017) reported it at higher elevations, and is commonly seen in grasses, hence, its common name 'grass coqui' (Rivero 1978, Joglar 1998). Lastly, *E. wightmanae* (19 mm SVL) is found in the mountainous interior of the island, from 150 m to 1,189 m of elevation, and prefers the leaf litter, and ground floor (Joglar 1998, Ríos-López and Dávila-Casanova 2014, Monroe et al. 2017). I discuss the implications of my findings for conservation of *Eleutherodactylus* frogs in Puerto Rico and elsewhere, and outline recommendations for future research.

METHODS

Collection

I collected 12 individuals of each of 4 species of *Eleutherodactylus* frogs to ascertain their critical thermal limits (i.e., critical thermal maximum or CT_{Max}). The species were *Eleutherodactylus coqui*, *E. wightmanae*, *E. brittoni,* and *E. antillensis*. Collection sites were identified with the aid of acoustic data (e.g., sites where chorus was detected, Chapter 2). Most collections (60%) occurred at 526 m of elevation, with an additional 13 at 890 m, 5 at 232 m and 1 at 384 m. The 5 individuals at 232 m were *E. coqui*. I controlled as much as possible for elevation because it has been shown that it may influence variability in CT_{Max} as compared to adjusting for body mass (g) or length (SVL; von May et al. 2017).

Upon arriving at a collection site, I recorded temperature and relative humidity utilizing a pocket weather meter (Kestrel® 3500 Pocket Weather Meter; accuracy = ±1 °C, ±3%, respectively). Individuals were collected utilizing food grade plastic cups to avoid contact with

the specimen. I recorded the frog's *in-situ* temperature (spot where they were located) with the use of an infrared thermometer (Craftsman Laser Infrared thermometer; accuracy = ±1 °C). If frogs were calling when found, they were classified as male; if they were silent, frogs were classified as unknown. The weight (g) of each individual was measured using a portable top loading balance (VWR-123P). Each cup, containing an individual, was placed inside a food grade foam cooler with ventilation where they were transported to the laboratory.

Laboratory Protocols

Each frog was transferred to an individual 16 qt (4 gal) housing unit (Ziploc weather shield box), which was modified with a water atomizer on the lid and an 8 cm mesh ventilation hole in the front facing wall. Each box was lined with 2 sterile paper napkins, misted with distilled water (EMD MilliporeTM ElixTM Essential 5 unit), with napkins being changed and misted every other day. Once each individual was placed in housing, I offered 2 small crickets (acquired at PetSmart). Individuals were fed 2 crickets daily. If they had ingested only 1 cricket, the missing one was replaced; if none were ingested, they were left in the box. If a dead cricket was found, it was replaced with a live one. Frogs were misted daily for 1 minute, with approximately 18 mL of water. Crickets were housed in a large cricket box (All Living Things Kricket Keeper) and offered Flukers food (Flukers High Calcium Criket Food) and Flukers water (Flukers Cricket Quencher Calcium Fortified).

Frogs were kept between 7-14 days in the laboratory to acclimate to their new housing units before performing the experiment. Two days prior to CT_{Max} experiments, individuals were placed on a fasting protocol. That is, feeding ceased and live crickets were removed from their housing. The day of the experiment, the individual was removed from its housing unit, and weighed using the same top loading balance (VWR-123P) to note its weight change from the

date of capture to the date of the experiment. Individuals were then placed inside the critical thermal maximum chamber whose design was conceived in collaboration with Dr. Eloy Martinez Rivera (Assistant Professor at Eastern Illinois University). The glass chamber box with dimensions 0.3 m x 0.18 m x 0.15 m had 3 of the sides insulated on the exterior with a 1.27 cm polypropylene plastic board. The box had a wet paper napkin at the bottom to help lower the temperature to 25°C and maintain relative humidity. The napkin was changed at the beginning of every experiment to avoid cross-contamination.

Prior to initiating the experiment, each frog had a "soaking" period of 20 minutes, where temperature was kept constant at 25°C to ensure the same baseline temperature before commencing the experiment. After the "soaking period," a temperature increase (ramp) of 0.4°C per minute was initiated. The specimens were continuously observed until muscle spasms or loss of coordination were noted; probe value temperatures and time elapsed between the start and finish of the experiment were also recorded. I also recorded the system value or temperature at the controller device that gauged the chamber temperature to keep the increase (ramp) at 0.4°C per minute. After the experiment was completed, the specimen was removed from the chamber utilizing a new (different) food grade cup, then placed into its housing unit, and misted for 1 minute. Two crickets were offered to the individual, who was kept overnight to ensure the survival of the experiment. Each individual was released during early morning hours to ensure cool temperatures and high humidity.

RESULTS

With the exception of *E. coqui* (unknown sex), the majority of *E. wightmanae*, *E. antillensis*, and *E. brittoni* were males (35). Average ambient temperature at collection sites was similar for *E. antillensis, E. wightmanae*, and *E. brittoni*, and 2-4 degrees warmer for *E. coqui*

(Table 1). Similarly, the *in-situ* (capture location) temperature was similar among species except *E. coqui*, which was much higher. The *in-situ* temperature was significantly and positively correlated to ambient temperature (0.51, Spearman's Prob > rho = 0.001). The average arrival weight of frogs at the laboratory underscored average body-size differences among species. *E. antillensis* (1.55 g) and *E. coqui* (2.68 g) are larger coquis, whereas *E. wightmanae* (0.59 g) and *E. brittoni* (0.46 g) are smaller. All species had gained some weight by the time the experiments commenced, with the exception of *E. brittoni* that exhibited a minor loss (0.04 g; Table 2).

Average probe temperature at time-of-reaction (e.g., spasms) ranged from 34.43 ˚C (*E. brittoni*) to 52.17 ˚C (*E. antillensis*; Table 2). Time-to-reaction was markedly lower for *E. brittoni* and *E. wightmanae* as compared to *E. coqui* and *E. antillensis*. Excluding 6 *E. coqui* and 1 *E. brittoni* captured below 500 m, differences in average probe temperature at time-of-reaction showed that *E. wightmanae* (38.72 ± 1.62) and *E. brittoni* (33.98 ± 0.88) were similar, but statistically different from *E. coqui* (46.85 ± 1.54) and *E. antillensis* (52.17 ± 1.44; $F = 38.82$, d.f. = 3, 37, $P < 0.001$). The average probe temperature at time-of-reaction for *E. coqui,* collected at 526 m (N = 5), was 46.85 ± 1.54. This value was not statistically different from those *E. coqui* collected at 232 m (49.04 ± 3.15; N = 6; $P > 0.05$).

There was a strong negative relationship between annual maxima temperature recorded between July and September, the months of highest maximum temperatures between 1970 and 2000 (WorldClim; Hijmans et al. 2005), and elevation ($F = 2495.51$, d.f. = 1, 247; $P < 0.001$). Average maxima < 400 m ranged from 31.7 to 30.7 ºC. These averages are 1-7 ˚C from the thermal limits of *E. brittoni* and *E. wightmanae*, much closer if we consider the lower 95% confidence intervals for both species. Average maxima temperature recorded during the same

years within 7 coastal protected areas (e.g., Guanica Dry Forest) was 32.53 ± 0.08, which does overlap the lower 95%CI for *E. brittoni*.

DISCUSSION

I determined CT_{Max} for 4 species of *Eleutherodactylus* that varied in size and distribution. Two species were clearly on the lower range of values among all species. CT_{Max} for *E. brittoni* was 34.29 ± 0.88 ˚C and 38.66 ± 1.62 ˚C for *E. wightmanae*. In contrast, CT_{Max} for the other 2 species (*E. coqui, E. antillensis*) was 10-18 ˚C higher. I note that there were marked differences in CT_{Max} for *E. coqui* between those reported by Christian et al. (1988) and this work. However, results from this study cannot be compared to those published by Christian et al (1988). First, Christian et al. (1988) employed a rather rudimentary approach with a non-automated ramping method that likely underestimated CT_{Max}. The means for thermal transfer relied on submerging the specimen bodies in water, which may also bias the frog's response since thermal stress might be accompanied by hormonally-driven changes in water uptake, causing osmotic rather than thermal stress. The rigorous, automated (water supply and thermal ramp) standardization of pre-experimental and experimental conditions in this study provides a more reliable CT_{Max} for every specimen. Christian et al. (1988) also employed a prodding response as a CT_{Max} indicator, a response that has very limited relevance to the specimen survival. Studies such as Von May (2019) used righting response as a better indicator, for example, because frogs use their righting ability for predator avoidance/escape. In this study, I sought the onset of spasms, and erratic behavior as a general proxy for neurological malfunction that also redound in lack of coordination and a reduced ability to avoid a predator. Behavioral responses were triggered and exhibited by the experimental animals themselves in direct response to rising temperatures, not

instigated by an external stimulus, which might also be confounded with some degree of habituation.

My results point at least 3 important conservation implications. First, maximum annual temperatures across the study area (Chapter 2) and at 7 locations within coastal protected areas (e.g., Guanica Dry Forest) were recorded between July and September between 1970 and 2000. Averages at < 400 m were within 1-7 °C of the critical thermal limit of *E. brittoni* and *E. wightmanae*. These results imply that extended exposure of either species to such temperatures could result in sub-optimal performance of activities that impinge on survival (e.g., predator avoidance; Hillman et al. 2009). It may also have adverse effects on embryo development as shown by Scheffers et al. (2013) for amphibians in the Phillippines. Although CT_{Max} may vary with elevation (von May et al. 2017), the proximity between CT_{Max} values for *E. brittoni* and *E. wightmanae* and annual maximum temperature may account for much of the variation explaining their affinity to high elevation (Chapter 2, Joglar 1998). Certainly, the probability of either species occupying lower elevation sites (e.g., < 400) will likely be nil if ambient temperatures increase as projected by 2040-2071 (6-8 °C; Hanareh-Khalyani et al. 2016).

Second, findings suggested that it might be possible to group species according to their thermal limits with the expectation that species sharing similar CT_{Max} values would respond in similar fashion to climatic changes. Although information on other species, sex-specific responses, and perhaps on other ecophysiological performance criteria might be needed, the idea would be to create functional groups much like avian foraging guilds (e.g., Root 1967) or *Anolis* ecomorphs (e.g., Losos et al. 1990). Second and if possible, such groupings would provide a stronger ecological basis to postulate hypotheses that more accurately test the potential linkages between environmental conditions and responses, physiological and ultimately demographic, of

Eleutherodactylus frogs (e.g., Rogowitz and Sanchez-Rivoleda 1999, Rogowitz 2003). An example of such an application was used in Chapter 2, where I predicted that *E. wightmanae* and *E. brittoni* would respond to environmental conditions along an elevation gradient in a similar fashion as compared to *E. antillensis*.

I conclude by stressing the potential of using thermal limits and ecophysiological performance to better predict potential shifts in distribution and abundance in the advent of climate change. My work begins to lay the foundation for such understanding for *Eleutherodactylus* species in Puerto Rico. The value of my work could be strengthened by additional research in 2 topics. In this work, *E. coqui* exhibited a ~2 $^{\circ}$C difference between individuals at 232 m and those from 526 m, albeit not statistically significant. Von May (2017) cautioned that CT_{Max} should not be viewed as a fixed value, and that it might be correlated with elevation and other factors. Therefore, it is important to account for such sources of variation (e.g., elevation), which might also reflect local adaptations. Second, my work explored thermal limits, but it is just as important to explore physiological performance across sublethal conditions. Physiological performance of ectotherms as a function of their environmental temperature are often described as a bi-phasic curve, where an optimal range lies normally around the most frequent temperature experienced by the organism (Angilletta 2009). However, this observation falls short of describing physiological performance in progressively warming scenarios, mainly due to the organism's inability to further compensate to the elevated temperatures (Martinez et al. 2016). Consequently, most management and conservation decisions, which depend on the organism's ability to survive and reproduce, should be based on a sound understanding of the species' thermal window of tolerance.

Table 1. Collection site elevation (m), relative humidity (%), ambient and in-situ or capture spot temperature (°C), and arrival body weight (g) of 4 *Eleutherodactylus* species at the time of capture in west-central Puerto Rico, 2018. I report ± 1 standard error.

Species	Elevation	Relative Hum	Ambient Temp	*In-situ* Temp	Arrival Wgt
E. antillensis	526 (0)	80.3 (0.62)	23.58 (0.47)	21.34 (0.45)	1.55 (0.15)
E. brittoni	514.16 (11.83)	88.72 (0.23)	23.73 (0.21)	20.97 (0.45)	0.46 (0.01)
E. coqui	392.36 (46.29)	58.05 (0.41)	27.30 (0.07)	28.87 (1.10)	2.68 (0.22)
E. wightmanae	890(0)	81.85 (0.62)	25.81 (0.56)	20.11 (0.28)	0.59 (0.01)

Table 2. Summary of critical thermal maximum experiments of 4 *Eleutherodactylus* species conducted in west-central Puerto Rico, 2018. Parameters were individual frog weight on day of experiment (g), weight gain or loss from capture to experiment day (g), probe value (temperature, °C) at reaction time, system value at reaction time (temperature, °C), and total duration of experiment (minutes). I report ± 1 standard error.

Species	Weight Exp. Day	Weight Gain/Loss	Probe value at reaction	System value at reaction	Total Duration
E. antillensis	1.75 (0.17)	0.24 (0.09)	52.17 (1.44))	52.32 (1.48)	67.66 (3.58)
E. brittoni	0.42 (0.02)	-0.04 (0.02)	34.43 (0.92)	34.29 (0.92)	19.66 (2.23)
E. coqui	3.76 (0.39)	1.08 (0.24)	47.84 (1.60)	48.02 (1.56)	59.72 (3.87)
E. wightmanae	0.59 (0.02)	0.003 (0.01)	38.72 (1.62)	38.66 (1.62)	35.5 (4.15)

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CHAPTER 2

Improving our Understanding of the Environmental and Habitat Covariates that Influence Local Occupancy, Abundance, and Reproduction of *Eleutherodactylus* Frogs.

ABSTRACT

Amphibian populations are declining worldwide, and global warming is a factor driving trends. Thus, understanding the relationships between climatic conditions and demographic processes is essential to formulate conservation strategies. I determined the effect of environmental/habitat features at local scales on occupancy and the probability of detecting a chorus (≥ 4 individuals) of *Eleutherodactylus wightmanae*, *E. brittoni*, and *E. antillensis*. I also assessed factors influencing reproduction of *E. coqui*. Occupancy of *E. wightmanae* and *E. brittoni* was affected positively by physical/environmental covariates (e.g., relative humidity), while *E. antillensis* was not. Physical/environmental covariates also had a positive influence on detecting a chorus for *E. wightmanae* and *E. brittoni,* but negative for *E. antillensis*. Annual variation on macrohabitat covariates (e.g., ground cover), attributed to the passing of Hurricane Maria on September 20, 2017, had a positive influence on *E. brittoni* and *E. antillensis*. Relative humidity (≥ 90%) and temperature (≤ 24 °C) exerted the strongest influence on the probability of encountering active nests of *E. coqui* (> 50%). My findings help develop criteria to index habitat quality to facilitate managed translocations or *in-situ* habitat enhancement. Insights about species responses to climatic conditions benefited from establishing critical thermal limits. The positive response to physical/environmental covariates by *E. wightmanae* and *E. brittoni* as compared to *E. antillensis* (10-18 ˚C higher) conformed to their critical thermal limits. CT_{Max} might be a criterion to group *Eleutherodactylus* species according to their tolerance limits, and

thereby, provide a basis to formulate testable hypotheses and more accurately predict impacts of climate change on their abundance and distribution.

INTRODUCTION

Amphibian populations have been declining worldwide, and in the case of Puerto Rico, western United States and northeastern Australia, the trend has been documented since the 1970s (Stuart et al. 2004, Alcala et al. 2012). Habitat destruction, diseases (i.e., pathogens like the chytrid fungus *Batrachochytrium dendrobatidis*), parasites, predation by introduced species, and climate change are among the factors precipitating the declines (Alford and Richards 1999, Alcala et al. 2012, Barker and Ríos-Franceschi 2014, Bower et al. 2017, Scheele et al. 2019). The latter, climate change, is of particular interest because, as ectotherms, amphibian demographic processes are tightly related to macro and micro-climatic conditions.

The mechanisms by which climate change can impinge on amphibian demography are a myriad, and include but are not limited to metabolism, muscle contraction, body size, growth, O_2 transport, disease, and reproduction (Carey and Alexander 2003, Hillman et al. 2009, Scheffers et al. 2013, Greenberg and Palen 2019, Scheele et al. 2019). At broad scales, for example, amphibians' body temperature is usually similar to ambient temperature (Hillman et al. 2009). In this context, rising temperatures are believed to be a greater threat to tropical ectotherms than temperate ones because the latter are more likely to benefit from warmer temperatures than the other way around (Bestion et al. 2015). This is because tropical ectotherms are already exposed to temperatures near their upper thermal limits, making them more sensitive to climate warming, including fatal physiological responses (Scheffers et al. 2013, Nowakowski et al. 2016).

Reproduction, and other amphibian biological traits, are also strongly affected by climatic conditions (Carey and Alexander 2003). *Eleutherodactylus* breeding activity occurs during every month of the year, but is affected by climatic conditions and by number of calling males (Townsend and Stewart 1994, Villanueva-Rivera 2006, Ríos-López et al. 2014). For example,

the calling activity of *E. coqui* and *E. cochranae* is positively correlated to increases in temperature, while precipitation does not seem to be an important driver. However, the same activities are negatively affected by increasing temperature and precipitation in *E. brittoni* and *E. juanariveroi*. With respect to reproduction, *Eleutherodactylus* egg development can be affected by changes in temperature. Egg vulnerability stems from the fact that most *Eleutherodactylus* frogs are terrestrial breeders, and except for the ovoviviparous *Eleutherodactylus jasperi* (golden coqui), all exhibit direct development (i.e., bypass the tadpole stage; Hedges et al. 2008). If the ambient temperature is too low, it can cause delays in development; if the temperature is too high, it can cause abnormalities (Townsend and Stewart 1986). For example, Townsend and Stewart (1986) reported that the average ambient temperature during the embryo developmental period for *E. coqui* was between 21.1-25.1 ºC. They also reported an increase of 2.5 days of developmental period for every 1 ºC dropped, resulting in the development of eggs taking 1.57 times longer to hatch in January-February, as in May-June.

Recent papers of *Eleutherodactylus* frogs in Puerto Rico have relied on macro-ecological covariates to characterize the probability of occupancy and 2 states or classes of abundance (i.e., few, many; Barker and Ríos-Franceschi 2014, Campos Cerqueira and Aide 2017, Monroe et al. 2017). These studies highlighted the importance of region, forest community types, and elevation, which coupled with climatogical covariates derived from long-term weather station datasets (e.g., WorldClim; Hijmans et al. 2005), have led to a greater understanding of factors governing landscape-level distribution patterns on the island. The same is true elsewhere in tropical and temperate regions, where similar covariates have been modeled to discern distribution patterns (Semlitsch and Bodie 2003, Trenham and Shaffer 2005, Ficetola et al. 2008,

Harper et al. 2008, Furlani et al. 2009, Hilje and Aide 2012, Frishkoff et al. 2015, Escalera-Vázquez et al. 2018).

The aforementioned studies have partitioned the variance explaining distribution patterns across landscapes. In this work, I set out to partition variance at "local" or "site-specific" scales along an altitudinal gradient. Emphasis on local factors is necessary because ecological processes operate at different spatial scales (Levin 1992). Local demographic processes are a contributing factor driving shifts in distribution at landscape levels (Miguet et al. 2016). Therefore, my objective was to determine the effect of local environmental/habitat features on occupancy probability and the probability of detecting a few or many (i.e., chorus) individuals of *E. wightmanae*, *E. brittoni*, and *E. antillensis*. I characterized sites not only by recording well-known environmental parameters (e.g., temperature, relative humidity, precipitation), but also other microhabitat features (e.g., soil moisture, litter depth, leaf layers, horizontal cover). The latter influence the quality of food and shelter used to carry out calling and reproductive activities (Tews et al. 2004, Ospina et al. 2013, Huang et al. 2014, Whitfield et al. 2014).

I used a multi-season, multi-state occupancy modeling framework (Nichols et al. 2007) to estimate both parameters using program PRESENCE (Hines 2006). I created a candidate set of models to assess their support in the data, paying particular attention to models testing predictions concerning species responses to environmental and habitat covariates (Table 1). Specifically, I predicted that occupancy would be positively and strongly affected by macro-habitat features (e.g., canopy and ground cover) as site suitability for colonization and occupancy likely begins with such features (Klawinski et al. 2014). Abundance (i.e., probability of detecting a chorus) of *E. wightmanae* and *E. brittoni*, 2 high elevation specialists (Joglar 1998),

would be strongly and positively influenced by micro-habitat features as compared to *E. antillensis*, a generalist, widespread species on the island.

Additional insights on the influence of site-specific covariates on local demographic processes were gained through 2 other means. First, I laid out artificial nest structures to determine the microhabitat characteristics influencing the probability that a survey site would be used for reproduction using ordinal logistic regression. Given the sensitivity of egg development of *Eleutherodactylus* species to changes in temperature (Townsend and Stewart 1986), I predicted that the probability of a site being used for reproduction by *E. coqui* would be positively and strongly influenced by microclimatic conditions (e.g., temperature, relative humidity, soil moisture) and the structure of the microhabitat (e.g., litter depth, leaf layers; Table 1). Second, I completed critical thermal maximum (CT_{Max}) experiments on the aforementioned 3 frog species and *E. coqui* as a first step to define their thermal limits and ecophysiological performance (Chapter 3). Findings indicated that *E. wightmanae* and *E. brittoni* had statistically similar average CT_{Max} values as compared to *E. antillensis* and *E. coqui*, arguably the 2 species with the widest distribution in Puerto Rico (Joglar 1998, Beard et al. 2003, Barker et al. 2012). Therefore, I predicted that occupancy and abundance of *E. wightmanae* and *E. brittoni* would respond similarly to site-specific weather covariates as compared to *E. antillensis* (Table 1). I believe that the joint findings of this work, and previous studies at landscape levels, will help identify local and regional indicators needed to guide habitat conservation, project potential changes in abundance and distribution at landscape levels, while at the same time, evaluate site quality for local conservation actions (e.g., managed translocations; McDonald-Madden et al. 2011, Miguet et al. 2016).

METHODS

Study Area

Forty-eight survey sites were randomly selected between the townships of Adjuntas (18°10'58.27"N; 66°40'29.91"W) and Maricao (18° 8'37.56"N; 66°58'47.87"W; datum = NAD83; Figure 1) in 2017. Twenty-four western sites were located in the municipalities of Mayaguez, Las Marías, Maricao and Sabana Grande, and the other 24 sites were found in the central municipalities of Adjuntas, Jayuya, and Ponce. I allocated these survey sites equally along 3 elevation gradients: 8 stations in low elevation (0-299 m), 8 stations in mid elevation (300-599 m) and 8 stations in high elevation (600 m-up). Survey sites were placed 100 m from the road and were at least 500 m apart from each other.

Study sites located in the central municipalities (≥ 600 m) were dominated by *Syzygium jambos (rose apple tree), Prestoea montana* (sierran palm)*, Alsophila portoricensis* also known as *Cyathea portoricensis* (Puerto Rico alsophila)*, Cordia sulcata* (mucilage manjack)*, Guarea guidonia* (American muckwood)*, Coffea spp.* (coffee)*, Hirtella rugosa* ("teta de burra cinarron")*, Inga vera* (river koko)*,* and *Cordia alliodora* (Spanish elm). Vegetation between 300-599 m was dominated by *Guarea guidonia* (American muckwood), *Syzygium jambos* (rose apple tree), *Spathodea campanulata* (African tuliptree), *Coffea spp.* (coffee), *Nectandra turbacensis* ("laurel amarillo"), *Cissus sicyoides* (seasonvine), *Dendropanax arboreus* (angelica tree), *Myrciaria floribunda* (guavaberry), *Andira inermis* (cabbagebark tree), and *Cecropia schreberiana* (pumpwood). Dominant vegetation between 16-299 m include *Guarea guidonia* (American muckwood), *Spathodea campanulata* (African tuliptree), *Inga fagifolia* (sacky sac bean), *Citrus reticulata* (mandarin orange), *Casearia guianensis* (Guayanese wild coffee), and *Siphoneugena densiflora* ("hoja menuda").

Dominant vegetation in the western municipalities (≥ 600 m) were *Pinus caribaea* (Caribbean pine), *Clusia rosea* (Scotch attorney), *Ocotea floribunda* ("laurel espada"), *Eucalyptus robusta* (swamp mahogany), and *Cestrum diurnum* (day jessamine). At intermediate elevation, vegetation was dominated by *Hymenaea courbaril* (stinkingtoe), *Eugenia biflora* (blackrodwood), *Genipa Americana* (jagua), *Mangifera indica* (mango), *Licaria parvifolia* (Puerto Rico cinnamon), *Myrcia citrifolia* (red rodwood), *Myrciaria floribunda* (guavaberry), *Eucalyptus robusta* (swamp mahogany), *Casearia arborea* (gia verde), *Guarea guidonia* (American muckwood), *Cupania americana* (wild ackee), *Spathodea campanulata* (African tuliptree), *Cecropia schreberiana* (pumpwood), and *Psychotria grandiflora* (largeflower wild coffee). Lastly, at low elevation (16-299 m) dominant vegetation was made up of *Eucalyptus robusta* (swamp mahogany), *Comocladia dodonaea* (poison ash), *Comocladia glabra* ("carrasco"), *Leucothrinax morrisii* (key thatch palm), *Swietenia mahagoni* (West Indian mahogany), *Syzygium jambos (rose apple tree), Artocarpus altilis* (breadfruit), *Coffea spp.* (coffee), and *Mangifera indica* (mango).

Data Collection

My sampling scheme was structured after Pollock's Robust Design (Pollock 1982). In this scheme, there were 2 (2017) and 3 (2018) primary seasons or sampling periods, each having 3 secondary sampling occasions. Primary periods in 2017 were April-May, July-August. Primary periods in 2018 were February-March, April-May, and July-August. Puerto Rico was hit by Hurricane Maria on September 20th, 2017. Only 18 out of the original 48 survey sites were accessible in the aftermath of the hurricane. Within this constraint, I established 17 additional survey sites within relatively close proximity to those that remained from 2017 (at least 500 m) for a total of 35 survey sites in 2018.

Vegetation and environmental data were invariably collected during the first secondary period of every season. I placed a HOBO data logger (Onset's HOBO U23 Pro v2 Temperature/Relative Humidity Data Logger U23-001; accuracy = ± 0.21 °C, ± 2.5 %) at every survey site to record temperature (°C, T) and relative humidity (%, RH). Data were recorded every 30 min until removed at the end of each primary sampling period. Data were periodically downloaded to a computer and analyzed using the software application HOBOware and averaged for each primary sampling period. To facilitate recording other covariates, I established a 10 x 10 m grid around a tree located in the approximate center of the survey site. Four flags were placed 5 m from the center of the grid, each marking the cardinal directions (North, East, South, West), and the grid was sectioned into 4 quadrants: Northeast (NE), Southeast (SE), Southwest (SW) and Northwest (NW) (Figure 2). Thirty-six flags were placed inside the grid, 9 per quadrant, to mark the placement of a reproduction artificial nesting structures (see below). Flags were placed at a distance of 1 m, 3 m, and 5 m from the center. Each flag was numbered and lettered to avoid confusion during reproduction surveys, and each flag was spaced 2 m apart to avoid stepping on one of the nesting structures during them as well.

I measured canopy cover (CC, %) using a densiometer in the center of the survey site (grid) and at the 4 cardinal directions, 5 m from the center tree (Figure 2). Horizontal cover (HC, %) was measured using a density pole 50.8 mm wide and 1.52 m tall, segmented into 3 sections, each measuring 0.46 m in length (Nudds 1977). Measurements were made 5 m away from the pole located at the 4 cardinal directions, and the average was expressed in percent cover. Soil moisture (%, SM), ground cover (%, GC), leaf layer (no. leaves, LL), and litter depth (mm, LD) were all measured at the center of the survey site, 2 m from the center of the grid, and 4 m from the center (Figure 2). These covariates describe the conditions of the ground floor in which

several species of *Eleutherodactylus* frogs are commonly found, feed and nest (Rivero 1978, Joglar 1998, Ríos-López et al. 2016). Soil moisture (SM) was measured 36 times per site utilizing a soil moisture sensor (Delta-T Devices SM300; accuracy = ± 2.5%, depth of 50.8 mm) and a soil moisture meter (Delta-T Devices HH2 Moisture Meter); the average was expressed as a percent. I measured GC using a 0.3 m x 0.3 m quadrat frame. I measured the average number of leaves (LL) in each survey site by utilizing a 0.25 m bamboo skewer. The skewer pierced the accumulation of leaves on the ground, leaving the soil beneath the layers unexposed. The layers were counted 12 times per site (Fehmi 2010). Litter depth was measured with the use of a ruler and a digital caliper (Husky 6 in 3-Mode Digital Fractional Caliper; accuracy ± 0.001 in ±0.02 mm). In order to measure leaf litter depth with accuracy, a ruler was used, rather than the sharp end of the caliper, making sure not to pierce the soil. (The ruler was used as a tool rather than a measurement instrument.) The ruler would touch the soil, and the top leaf layer was marked with an erasable marker, then measured with the caliper. The depth was measured in mm 12 times per site; the average was used as a covariate.

The daily rainfall rate (mm day^{-1}) was estimated for 5 multi-month periods over 2017 and 2018: April-June, and July-September in 2017 and February-March, April-June, and July-September 2018. No high-resolution gridded meteorological dataset exists that could resolve the influence of the island's complex topography on daily precipitation. However, there is a dense network of 147 long-term recording stations of daily precipitation, which can be used to estimate rainfall rates at the sampling locations. Rather than performing a simple averaging of nearby stations to obtain a daily rainfall rate, which could mask the spatial variability in rainfall, 3 criteria were used to choose the representative weather station for each sampling location: 1) minimize distance to sampling location, 2) minimize elevation difference, and 3) minimize the

amount of missing observations. For the first 2 criteria, weights were assigned to each weather station relative to each sampling location. The weights (ranging from 0 to 1) were calculated based on sigmoidal functions with parameter values set so that a weight of 0.5 (i.e. half) or less applied at distances of 25 km or more for the first criteria, and at elevational differences of 500 m of more for the second criteria. The 2 weights were summed (i.e. each criterion is valued equally) and then the station with the highest combined weight and less than 50% missing observations was used to represent conditions at that site.

Reproduction Surveys

I placed 36 artificial nest structures at each survey site. Structures were placed within the 10 x 10 m grid, 9 within each of 4 quadrants (Figure 2). Artificial nest structures or tubes were made of white shower rod plastic covers. Each structure (tube) had a diameter of 25.4 mm and a length of 0.20 m, placed on the surface of the ground following Ríos-López et al. (2016). Prior to Hurricane Maria, 19 study sites, 7 in the upper elevation gradient (600 m and up), 6 in mid elevation gradient (300 to 600 m), and 6 in the lower elevation gradient (0 to 200 m), also had nest structures placed in robust trees with zip ties and rope. Due to the great number of trees fallen in my study sites after hurricanes Irma and Maria, only 7 sites in the upper elevation gradient, and 5 in the mid elevation gradient had tubes tied to trees. Nest structures were monitored on every secondary sampling occasion. The presence of *Eleutherodactylus* frogs was noted, as well as the presence of eggs. If egg clutches were present, it was designated as active. I recorded egg condition, number of eggs, number of clutches, and number of hatchlings.

Acoustic Sampling

Acoustic surveys followed Pollock's Robust Design (Pollock 1982), as described above. Acoustic data were recorded using ARBIMON (Automated Remote Biodiversity Monitoring

Network) portable recorders. Each recording device is composed of an LG smartphone (with the program ARBIMON II) inside a modified waterproof Otterbox case (model 2000) with a Monoprice microphone connected to the exterior (Aide et al. 2013, Campos Cerqueira and Aide 2016, 2017a). Recorders were tied to the center tree of each study plot at a height of 1.5 m. Recording devices surveyed 3 times during a 21-day period, for 2 consecutive nights each time. Each device was programed to record every minute on the hour, from 1800 h to 0600 h. Each sampling occasion had a total of 24 recordings per site.

Acoustic data were downloaded from each portable recorder to a computer and visually and acoustically inspected to remove damaged ones (e.g., equipment malfunction, environmental disruptions) before recordings were uploaded to the ARBIMON II project website (https://arbimon.sieve-analytics.com/project/ncsu-coqui/dashboard) and made available for analyses by the Sieve Analytics team. A total of 13,561 recordings were analyzed to determine the presence, absence (non-presence), and 2 abundance classes (i.e., few, chorus) of *E. wightmanae*, *E. antillensis* and *E. brittoni*. Recordings containing at least 1 or a few vocalizations of the focal species (≤ 3 individuals) were designated as "state 1", indicating low abundance. Recordings containing overlapping vocalizations (≥ 4 individuals) were designated as "state 2," indicating high abundance. The latter recognized by the presence of a dark band within a focal species' vocalization frequency range. Recordings without the species (non-presence) were classified as "state 0." These codes were used to create an encounter history for each sampling period for analysis in program PRESENCE (Hines 2006). As noted above, each sampling occasion was made up of 24 recordings per site. Encounter histories were constructed considering all recordings per secondary sampling period. For example, I adjudicated a presence (state 1) to a secondary sampling occasion if there was at least one detection on a given site over

the 24 recordings. Similarly, I adjudicated a chorus (state 2) to a secondary sampling occasion if there was at least a chorus detected on a given site over the 24 recordings.

DATA ANALYSIS

I estimated the mean (SE) of 9 environmental (weather) and habitat covariates per season and year. Covariates were precipitation (mm), temperature (˚C), relative humidity (%), soil moisture (%), canopy cover (%), horizontal cover (%), ground cover (%), litter depth (mm) and leaf layer (number of leaves on the ground). I determined if there was a seasonal or annual difference between estimates of each covariate using ANOVA. Model terms were season and year with no interaction term because there were only 2 seasons in 2017. To address the possibility of a year by season interaction, I created a dataset using only seasons 2 and 3 of each year.

Weather covariates (e.g., precipitation, temperature, relative humidity, soil moisture) were highly correlated with elevation. This was problematic for modeling for 2 reasons. First, correlated variables in the same model increase the likelihood of Type I errors. Second, it constrained the possibility ascertaining the relative importance of other micro-climatic covariates, largely because models with elevation alone received greater support than models with any other covariate by itself or in combination with others. Analyses under such constraints yield insights on WHERE amphibian species occurred along the altitudinal gradient (e.g., Monroe et al. 2017, Campos-Cerqueira and Aide 2017b), but give limited insights on WHY they occurred at a given location. For these reasons, I converted the dataset into a set of linearly uncorrelated variables using Principal Component Analysis (Johnson and Wichern 1982). This analysis decomposes the variance-covariance structure in the data, thereby, providing a means for data reduction. Fewer linear combinations or components now represent almost as much

variability as there was in the full suite of original variables and provide a basis to replace the original variables with component scores without much loss of information (Johnson and Wichern 1982). Moreover, principal component analyses often reveal relationships that were not previously suspected, thereby, facilitating interpretation of complex set of variables. The first principal component captures the largest possible variance, and each succeeding component in turn captures the highest variance possible under the constraint that it is uncorrelated (orthogonal) to the preceding components. Three terms are important to interpret components. The first is eigenvectors, which determine the directions of the new feature space. The second are eigenvalues that determine their magnitude, and lastly, component scores, the transformed variable values corresponding to a particular data point of the original dataset. Because the principal components depend on the correlation matrix (used in this work), there is no requirement for a multivariate normal assumption. I used a correlation matrix (standardized variables) because covariates in the model were measured in different and varying scales, and it is easier to interpret (i.e., eigenvectors range from 0 to 1).

I used principal component analysis in 2 cases. First, with the acoustic data, and second, with the reproductive data. Before running the PCA for the acoustic data, I averaged 8 environmental/habitat covariates (elevation excluded) across 2 (2017) and 3 (2018) seasonal primary sampling periods for every sampled site. I summarized data in this fashion because only 2 covariates differed between years (i.e., canopy cover, ground cover). Moreover, there were no differences between seasons 2 and 3 in either year, only between season 1 (Feb-Mar), and seasons 2 and 3 in 2018 for relative humidity, soil moisture and precipitation. The summarization does not mask the interannual variation in canopy and ground cover, and differences in seasonal averages between years are attributable to season 1 in 2018. Therefore, I

used elevation, year (2017, 2018), and the 8 seasonal covariates to run the PCA. The PCA for the reproductive data focused only on seasons 2 and 3 (average) because they encompassed 98% of the observed reproductive activity. I report the first 3 eigenvalues (first 3 components) and corresponding eigenvectors for each analysis. I also generated the scores for each component as the transformed site-specific covariate values for modeling.

A multi-season, multi-state occupancy modeling framework (Nichols et al. 2007) was used to obtain estimates of occupancy (Psi), index of abundance (R), detection (*p*), and delta (dlta) using program PRESENCE (Hines 2006). Occupancy is defined as the probability that a site is occupied, given that individuals of *Eleutherodactylus* spp. are available to be detected. Detection (*p*) is defined as the probability of detection in a survey station given that the station is occupied. Parameter R is the probability that a chorus of *Eleutherodactylus* spp. (> 4 individuals) was present, given that the survey station was occupied. Delta is defined as the probability that state 2 (chorus) was true, given that the survey station was occupied. I developed a suite of candidate models that included models to test specific predictions (Table 1). Models were ran using the set of 83 sites surveyed in 2017 (48) and 2018 (35). Acoustic data were collected over 2 seasons in 2017 (May-June, July-August) and 3 seasons in 2018 (Feb-Mar, May-June, July-August). I first modelled the detection process, determining whether detection was constant (.) or varied by season (S). The model with highest support (AIC_{wt}) was used to determine which covariate (PC1, PC2, or PC3) explained most of the variance in detection probability. The model with highest support (AIC_{wt}) was used to determine if occupancy (Psi) and the index of abundance (R) were constant (.), varied by the initial season as compared to the other 2 seasons (Init), or was season-specific (S). I note that initial occupancy (Psi0) is the proportion of occupied sites on the landscape, followed by the probability that a survey site is

occupied in seasons 2 and 3 (Psi23) regardless of whether the survey site was occupied or not in the initial season. A seasonal term meant that occupancy in season 2 (Psi2) was conditioned on a survey site being occupied or not during the initial season (Psi0). Occupancy in season 3 (Psi3) is the probability of a survey site being occupied given that it was occupied or not during season 2 (Psi2). I note that when modeling Psi23 or Psi2 and Ps3 I could not discern the "previous occupancy state" of sites because models were overparameterized. The same logic follows for parameter R or index of abundance. For example, seasonal term for season 3 (R3) is interpreted as the probability that a survey site occupied by few or chorus in season 2 (R2) will be occupied by a chorus in season 3. Once the best supported model was picked, I modeled parameters using single or combination of principal components (covariates) including models that described my predictions (Table 1).

Akaike's Information Criterion (AIC) was used to evaluate the support in the data for models in the candidate sets and the strength of each covariates effect on *Eleutherodactylus* species occupancy (Burnham and Anderson 2002). Models with a $\Delta AIC \leq 2$ were considered to have substantial support in the data. The effect of covariates (i.e., $\hat{\beta}$ coefficient) on occupancy was considered to be strong if the 95% confidence interval (CI) did not overlap 0, and weak otherwise. For models that had more than 1 covariate per parameter, I graphed parameter effects of 1 parameter while keeping the other parameter constant (e.g., median value). Parameter estimates ± SE are reported, except for models where I had to control one variable to depict the effect of the other.

Careful consideration of model assumptions was important for the interpretation of model results. Multi-season, multi-state occupancy models assume that: 1) sites are "closed" to changes in occupancy within primary sampling periods; 2) there are multiple site visits within

secondary periods; 3) that there are no false detections; and 4) detection across sites are independent (MacKenzie et al. 2002). I believe I met assumptions for the following reasons. Surveys made within primary periods were conducted over a short period of time (21 days) relative to life history events (e.g., reproduction; assumption 1). Each survey site was acoustically sampled 3 times (secondary periods) during each primary season (assumption 2). Encounter histories were derived from acoustic recording devices and analyzed as described by (Campos-Cerqueira and Aide 2016, 2017a), which provide a means to detect false positives (assumption 3). Survey stations were separated by > 500 m, greater than the longest known distance (100 m) from which frogs returned to their site of captured when translocated (Gonser and Woodbright 1995; assumption 4). The model defines parameter delta (dlta) as the probability of correctly identifying state 2, given it is in state 2 (MacKenzie et al. 2006). I assumed that was the case because call patterns (few vs chorus) could be visually identified using program ARBIMON's spectrogram (Aide et al. 2013, Monroe et al. 2017). However, because there is a chance that the spectrogram may not have registered instances of a chorus, thus not visible, I let PRESENCE estimate delta.

I used ordinal logistic regression to assess the probability of encountering active nests (i.e., at least 1 egg) on any of the 83 sites surveyed during this study. I ran 2 models with 2 types of response variables. The first classified reproductive activity data in a binary fashion (0 (no nest) or 1 (at least 1 nest with eggs)); the second, classified sites as those having ≥ 2 active nests or not. The latter implied that conditions on survey sites had to be better than just a site with 1 nest or none. Model terms were the ordinal response variables and covariates or 3 principal components described above. Final model covariates were selected interactively (adding or removing covariates). I used an alpha of 0.05 to keep or remove covariates.

RESULTS

Between Years and Among Seasons Comparisons

I surveyed 48 sites in 2017 and 35 in 2018. Environmental and vegetation characteristics at these sites differed only in terms of canopy and ground cover between 2017 and 2018 (Table 2). Canopy cover decreased by ~9%, whereas ground cover increased by ~5%. Precipitation, relative humidity, and soil moisture differed between Feb-Mar and the last 2 seasons (April-May, July-August) in 2018 (Table 2). Results underscore the relative dryness (e.g., lower precipitation) of the Feb-Mar season as compared to late spring and summer seasons. There were no significant differences for any covariate between April-May and July-August in either 2017 or 2018.

Principal Component Analysis

I conducted 2 principal component analyses. The first was used to conduct occupancy/abundance modeling. The second analysis focused on data from seasons 2 (April-May) and 3 (July-August) to run logistic regressions designed to identify factors (i.e., components) influencing the probability that I would encounter an active nest (≥ 1 egg/nest) at each surveyed site. Both analyses used 83 sites.

The first 3 components of the first analysis accounted for 63.9% (Table 3). Components were environmentally and biologically interpretable and used to label each component. The largest eigenvectors (magnitude) and their direction (+/-) of the first component were relative humidity (+) and temperature (-), followed closely by elevation (+), soil moisture (+), and precipitation (+) (Table 3). I termed this component the physical/environmental component (PC1) as it represented the variance in the data associated with the physical environment (elevation) and its micro-meteorological characteristics (e.g., relative humidity). The largest

eigenvectors of the second component were year (+) and ground cover (+), followed closely by canopy cover (+) and elevation (-). I termed this component (PC2) as the interannual/macrohabitat component as it represented the variance due to year and major vegetation features characterizing a site's vegetation (e.g., canopy cover). Lastly, the largest eigenvectors of the third component were horizontal cover (-), leaf layer (+), and litter depth (+). I termed this component (PC3) the microhabitat component as it represented the variation due to horizontal cover (ground up to ~0.5 m) and the structure of the ground cover.

Results of the second principal component analysis (83 sites, 2 seasons) resembled the results of the full analysis (83, 3 seasons) with minor differences. The first 3 components accounted for 60.43% of the variance (Table 4). The largest eigenvectors (magnitude) and their direction (+/-) of the first component (PC1) were relative humidity (+), temperature (-), and elevation (+), followed by soil moisture (+) and precipitation (+) (Table 4). The second component (PC2) highlighted the strength of year (+) and canopy cover (+), underscoring the macrohabitat nature of the component. The third component (PC3) highlighted the microhabitat components of each site.

Occupancy and Abundance (probability of detecting a chorus)

E. wightmanae: Variation in initial occupancy (Psi0) was best explained by a model that featured a positive, but weak influence from the physical/environmental component (i.e., PC1; Beta = 1.52 ± 1.32) and a negative but weak influence from the microhabitat component (i.e., PC3; Beta = -1.19 ± 0.92; AIC_{wt} = 0.90; Table 5). Occupancy in seasons 2 and 3 (Psi23) were strongly and positively influenced by the physical/environmental component (PC1; Beta = 0.58 ± 0.13) and strongly and negatively by microhabitat component (PC3; Beta = -0.91 ± 0.20). Figure 3 plots the response in occupancy for the initial season (Psi0), and seasons 2 and 3 (Psi23), as a

function of relative humidity. It is noteworthy that while occupancy and abundance increased with relative humidity, the probability of detecting a chorus on a surveyed site was slightly below occupancy after ~83% relative humidity. Figure 4 depicts the relationship as a function of horizontal cover (PC3), highlighting that sites not only rely on relative humidity to transition from one state (e.g., vacant) to another (e.g., chorus; Ps23), but also on increasing horizontal cover. The probability of detecting a "chorus" (R0) was strongly and positively influenced by the physical/environmental component (PC1; Beta = 0.74 ± 0.37) and negatively, but weakly, by the year/macrohabitat component (PC2; Beta = -0.58 ± 0.30). Detection probability was positively and strongly influenced by the physical/environmental component (PC1; Beta = 0.45 ± 0.13). The probability that state 2 (chorus) was true (delta), given that the survey station was occupied, ranged from 0.26 ± 0.04 to 0.82 ± 0.07.

E. brittoni: Variation in occupancy was invariant (constant) throughout all seasons. It was best explained by a model that featured a positive and strong influence by the physical/environmental component (i.e., PC1; Beta = 1.15 ± 0.53; AIC_{wt} = 0.69; Table 6). The probability of detecting a "chorus" (R0) was influenced positively and strongly by the physical/environmental component (PC1; Beta = 0.52 ± 0.23), and negatively and strongly by the year/macrohabitat component (PC2; Beta = -0.48 ± 0.18). Figures 5 and 6 depict the relationships between initial occupancy (Psi0), occupancy for seasons 2 and 3 (Psi23), and the probability of detecting a chorus (R0) as a function of relative humidity and ground cover. Relative to *E. wightmanae*, *E. brittoni* requires higher relative humidity for occupancy and abundance to increase markedly (~>80%), and up to ~90% for it to exceed 60% probability. It was noteworthy that transitioning from one state (e.g., occupied) to another (e.g. chorus) occurred at lower relative humidity levels. Likewise, the probability of detecting a "chorus" was greater in 2018 (post-Maria) than in 2017. Detection

probability was negatively and strongly influenced by the year/macrohabitat component (PC2; Beta = -1.00 ± 0.14). The probability that state 2 (chorus) was true (delta), given that the survey station was occupied, ranged from 0.09 ± 0.04 to 0.62 ± 0.06 in 2017, and from 0.58 ± 0.06 to 0.98 ± 0.01 in 2018.

E. antillensis: Variation in initial occupancy (Psi0) was best explained by a model that featured a positive, but weak influence from the physical/environmental component (i.e., PC1; Beta = 0.20 ± 0.24) and a positive but weak influence from the year/macrohabitat component (i.e., PC2; Beta = 0.25 ± 0.52; AIC_{wt} = 0.96; Table 7). Occupancy in seasons 2 and 3 (Psi23) were strongly and negatively influenced by the physical/environmental component (PC1; Beta = -0.66 ± 0.15), and strongly and positively by the year/macrohabitat component (PC2; Beta = 0.58 ± 0.14). The probability of detecting a "chorus" (R0) was influenced negatively and strongly by the physical/environmental component (PC1; Beta = -0.45 ± 0.17), and negatively and strongly by the year/macrohabitat component (PC2; Beta = -0.75 ± 0.22). Figures 7 and 8 depict the relationship between occupancy in seasons 2 and 3 (Psi23) and abundance (probability of detecting a chorus), and relative humidity and ground cover. Of note is the increasing probability of transitioning from one state (e.g., unoccupied) to another (e.g., chorus) with decreasing relative humidity. Unlike the previous 2 species, *E. antillensis* exhibits higher probability of occupying sites over time (Psi23) and of detecting a "chorus" at lower values of relative humidity (< 75%) than *E. brittoni* and *E. wightmanae*. Detection probability was negatively and strongly influenced by the mid-story/microhabitat component (PC3; Beta = -0.22 ± 0.10). The probability that state 2 (chorus) was true (delta), given that the survey station was occupied, ranged from 0.52 ± 0.06 to 0.74 ± 0.07.

Reproduction

The total number of *E. coqui* active nests (≥ 1 eggs) in 2017 was 51 and 82 in 2018. Active nests (≥ 1 eggs)/survey site was 1.04 (2017) and 2.41 (2018; Table 8). Hatching success was 0.23 (2017) and 0.10 (2018). Reproductive activity for the other 2 species of *Eleutherodactylus* encountered during the study was low (Table 8). Despite recording 35 nests occupied by adult *E. antillensis* in 2017 and 12 in 2018, only 2 were active in 2018. Likewise, I only found 2 active nests of *E. wightmanae* in 2017 but none in 2018.

Average (SE) values for elevation, temperature and relative humidity for used and non-used sites for reproductive activity are listed in Table 9. The probability of encountering an active nest in survey sites (83) increased with features that characterized the physical/environmental component (PC1) of surveyed sites (Chisq = 28.99; Prob>ChiSq = < 0.0001; $R^2 = 0.28$). Figure 9 illustrates the relationship between the probability of encountering ≥ 1 active nest and temperature and relative humidity. To attain a ≥ 50% probability of reproductive activity, on site temperature needs to be ≤ 24 ˚C and relative humidity needs to be ~90%. These were conditions recorded at elevations exceeding 700 m along my elevation gradient. Notice that the 2 active nests of *E. wightmanae* were recorded at approximately the same temperature and elevation (red "W" in figures). Similarly, the probability of encountering ≥ 2 active nests in surveyed sites also increased with PC1 at surveyed sites (Chisq = 24.84; Prob>ChiSq = < 0.0001; $R^2 = 0.26$). Figure 10 illustrates the relationship between the probability of encountering ≥ 2 active nests and temperature and relative humidity. The relationship is similar to the one for the probability of encountering ≥ 1 active nest, but as expected, values for relative humidity are slightly higher. There was no support, in either model, for the inclusion of the second (year/macrohabitat) or third (microhabitat) components.

DISCUSSION

My work examined the potential effects of local physical/environmental covariates on occupancy, abundance (i.e., probability of detecting a chorus) and reproduction of 4 species of *Eleutherodactylus* frogs in west-central Puerto Rico. My findings were in concert with Monroe et al. (2017) in that patterns of occupancy and abundance of *E. wightmanae* and *E. brittoni* along an altitudinal gradient increased with elevation/environmental covariates (PC1), but gradually tapering off toward the upper values of either elevation or relative humidity. I also predicted that *E. wightmanae* and *E. brittoni* would exhibit similar responses to physical/environmental covariates (PC1) because both species exhibited statistically similar CT_{Max} values (Chapter 1). Support for my prediction was strong, suggesting that it is plausible that both species share similar ecophysiological responses to environmental conditions. *E. wightmanae* and *E. brittoni* responded positively to PC1 as compared to *E. antillensis*, which responded negatively. The literature is clear about categorizing *E. wightmanae* as a high elevation specialist (Joglar 1998). This work places *E. brittoni* in a similar category inferred by their response to PC1. This inference is in concert with the fact that their CT_{Max} was just within 1-7 °C of average thermal maxima between July and September at lower elevations (e.g., < 400 m, Chapter 1).

Research on *E. antillensis* is scarce, presumably because it is a common and widespread species (Joglar 1998, Gould et al. 2007). In this sense, it parallels *E. coqui*, a species that is also common and widespread, but studied extensively (Lopez and Narins 1991, Stewart and Rand 1991, Townsend and Stewart 1994, Gonser and Woolbright 1995, Buckley et al. 2005, Velo-Antón et al. 2007, Kerney et al. 2010, Kulkarni et al. 2010, O'Neill and Beard 2010, Karadge and Elinson 2011, Ten Eyck and Regen 2014, Jennings et al. 2015). My work expands knowledge about *E. antillensis* in 2 ways and provides a plausible explanation for similarities in

distribution with *E. coqui*. First, thermal limits of both species were statistically similar, but about 10-18 ˚C higher than for *E. wightmanae* and *E. brittoni* (Chapter 3). Second, *E. antillensis* occupancy or abundance (i.e., probability of detecting a chorus) patterns did not resemble patterns of high elevation specialists like *E. wightmanae* or *E. brittoni*. While *E. antillensis* can occupy high elevation portions of an altitudinal gradient, it exhibits higher levels of occupancy and abundance at lower elevation where temperature is higher and relative humidity lower. *E. antillensis* is likely an example of a species with relatively low vulnerability as compared to *E. wightmanae* and *E. brittoni* to projected increases in temperature and lower precipitation. Indeed, while these changes are ensuing, there is potential for the species to exhibit increases in abundance across high elevation reaches based on its relationship to relative humidity and temperature (sensu Bestion et al. 2015).

Puerto Rico was affected by 2 major hurricanes in September 2017 (i.e., Irma and Maria). Of the 2, Hurricane Maria made landfall on the island packing winds of ~250 km/hr (155 mph). Although hurricanes are a natural and common occurrence in Puerto Rico, they inflict a lot of damage to human infrastructure and transform the environment. There is a vast body of literature on the impacts of hurricanes on fauna and flora of Puerto Rico (Tanner et al. 1991, Waide et al. 1991, Wiley and Wunderle 1992, Lloyd et al. 2019), including amphibians (Woolbright 1991, Klawinski et al. 2014). Certainly, my work gave us an opportunity to assess potential post-hurricane impacts as I surveyed 48 sites for 2 seasons in 2017 prior to Hurricane Maria, and then 35 sites over 3 seasons in 2018. Regarding environmental and structural components, I found that only canopy and ground cover exhibited statistically significant changes between 2017 and 2018. Canopy cover decreased about 9%, on average, among all surveyed sites, and ground cover increased about 5%, on average.

My findings indicated that there were annual differences in abundance (i.e., probability of detecting a chorus) associated with changes in canopy and ground cover for *E. brittoni* and *E. antillensis*, but not *E. wightmanae*. Although one could infer that such changes were caused by hurricane damage, the fact is that the probability of detecting a "chorus" of frogs for either species was higher in 2018 than in 2017. This finding was consistent with other studies in Puerto Rico and elsewhere. Woolbright (1991) studied the effects of Hurricane Hugo (September 1989) on *E. coqui*. A year post-hurricane, he reported that the estimated population of the species was 4 times what it was prior to the hurricane. He also reported an increase in *E. hedricki* (14%), but a decrease in *E. wightmanae* (31%), *E. portoricensis* (45%), and *E. richmondi* (83%). Elsewhere, Whitfield et al. (2014) wrote about the positive effect 4 hurricanes had on Florida's toad and frog population in 2004.

Although my findings were in accord with Woolbright (1991), Klawinski et al. (2014) reported that while increasing amounts of leaf litter had no significant effect on *E. coqui* density, a decrease in canopy cover did. The question is how much? My work suggested that a loss of < 10% was not detrimental to 2 species with very contrasting environmental requirements. However, I acknowledge the possibility of missing negative effects because I did not sample 14 sites that were rendered "inaccessible" in 2018 due to the high amount of fallen debris. I can suggest, however, that surveyed sites post-Maria were occupied and harbored varying levels of abundance (i.e., probability of detecting a chorus), and that sites in similar conditions across the landscape could have served as refuges for displaced frogs.

Oseen and Wassersug (2002) and Scheffers et al. (2013) reported that air and water temperature, rainfall, barometric pressure, relative humidity, and wind velocity affect anuran reproduction. Accordingly, I predicted that physical/environmental conditions (PC1) would

strongly and positively influence the probability of encountering an active nest. I also predicted that micro-habitat structure (PC3), where species build most nests, would be an important predictor of nesting activity. My findings showed that only PC1 or the physical/environmental covariate was needed to account for observed nesting patterns. I surmised that the range of microhabitat features measured during this study were adequate for reproduction. It was striking that *E. coqui* was the only species that used artificial nest structures readily. In light of occupancy and abundance patterns, I believe that artificial nest structures (tubes) were not suited to gauge reproductive output of all species rather than suggesting that sites were of poor quality for reproduction. Nonetheless, insights from *E. coqui* were valuable. High probability of encountering active nests was associated with sites characterized by lower temperatures (≤ 24 °C) and high relative humidity (≥ 90%). This is in concert with the tight relationship between environmental conditions and egg development reported by Townsend and Stewart (1986). Because *E. coqui* resembled *E. antillensis* in terms of CT_{Max}, I suggest that sites with a high probability of use for nesting by *E. coqui* were also suitable for *E. antillensis*. I note that the 2 active nests of *E. wightmanae*, considered a high elevation specialist, occurred in the upper range of conditions used by *E. coqui*, hinting at the need to investigate whether *E. wightmanae* has narrower requirements than *E. coqui*.

The need to predict demographic responses and set in motion conservation strategies for amphibians has gained impetus in recent years due to projected global climate change and the rapid pace at which landscapes are being modified by human activities (Hayes et al. 2006, Rohr et al. 2008, Becker et al. 2016, Eterovick et al. 2016, Grant et al. 2016, Nowakowski et al. 2018, Cohen et al. 2019, Greenberg and Palen 2019). Ecological processes (e.g., demographic) occur at multiple scales (Levin 1992), therefore, it is important to investigate these processes at local

and landscape levels to discern the ecological basis of shifts in distribution and abundance. In this study, I focused my attention on local demographic parameters, specifically, occupancy, abundance and reproduction. By determining the effects of physical/environmental and habitat structure on the aforementioned parameters, I laid a foundation to better understand how species might respond to climate or habitat modifications. This understanding is important because shifts in abundance and distribution at larger scales begin with responses at local scales. My work is also valuable because it helps to develop criteria to index habitat quality required to engage on managed translocations or *in-situ* habitat enhancement. Lastly, insights about potential responses to physical/environmental variables benefited from establishing critical thermal limits of the focal species. Support for my predictions regarding *E. wightmanae* and *E. brittoni* suggests that thermal limits could be used to group species according to their vulnerability to climate change, and hence, a basis to generate hypotheses and test predictions for stronger inferences about potential impact of projected climate change.

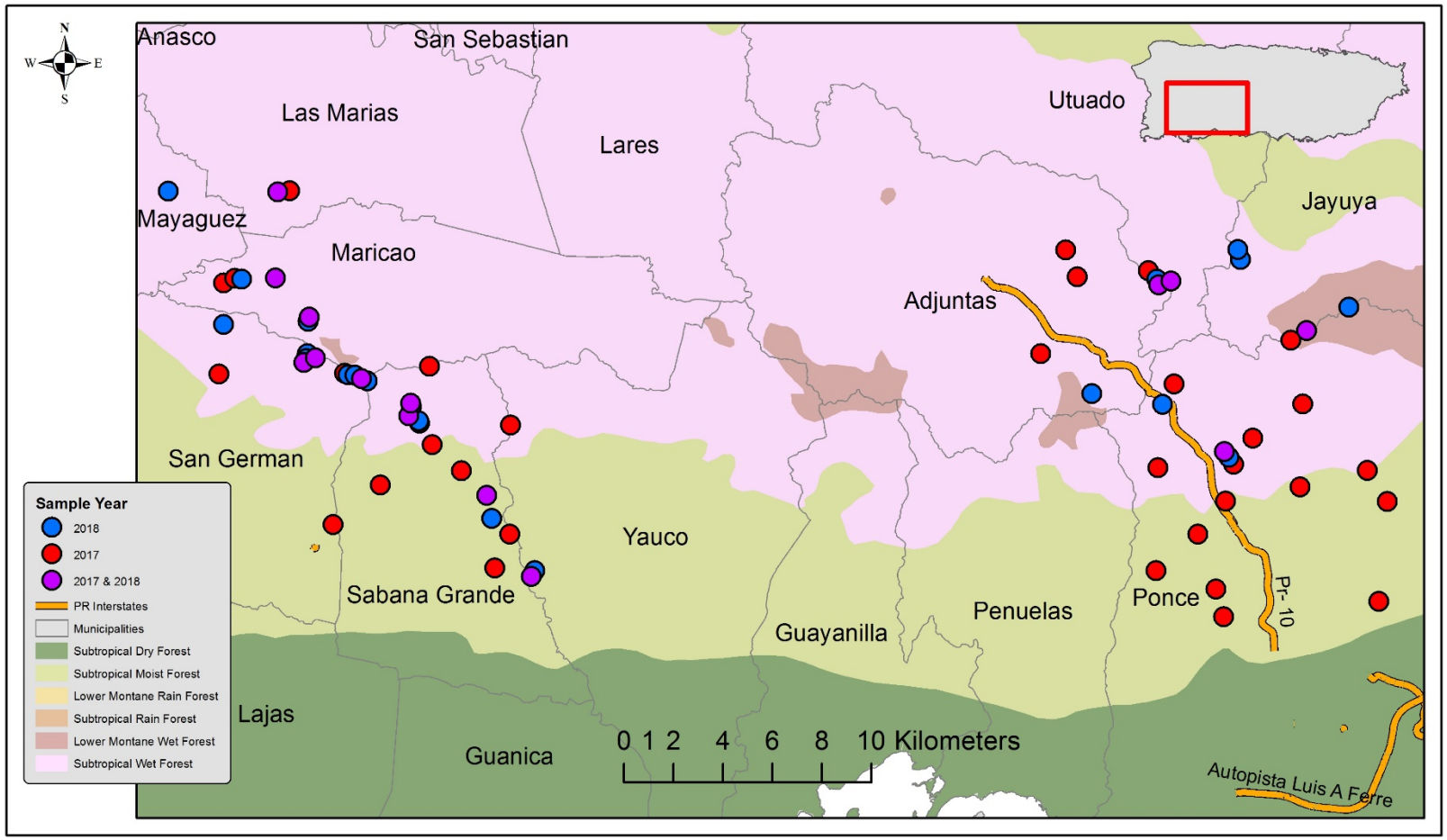

Figure 1. Map of Puerto Rico (inset) showing the west-central portion of the island. The map depicts life zones, municipalities and the location of 83 sites used to conduct acoustic surveys and monitor reproductive activity of *Eleutherodactylus wightmanae*, *E. brittoni*, *E. antillensis*, and *E. coqui*, 2017-2018.

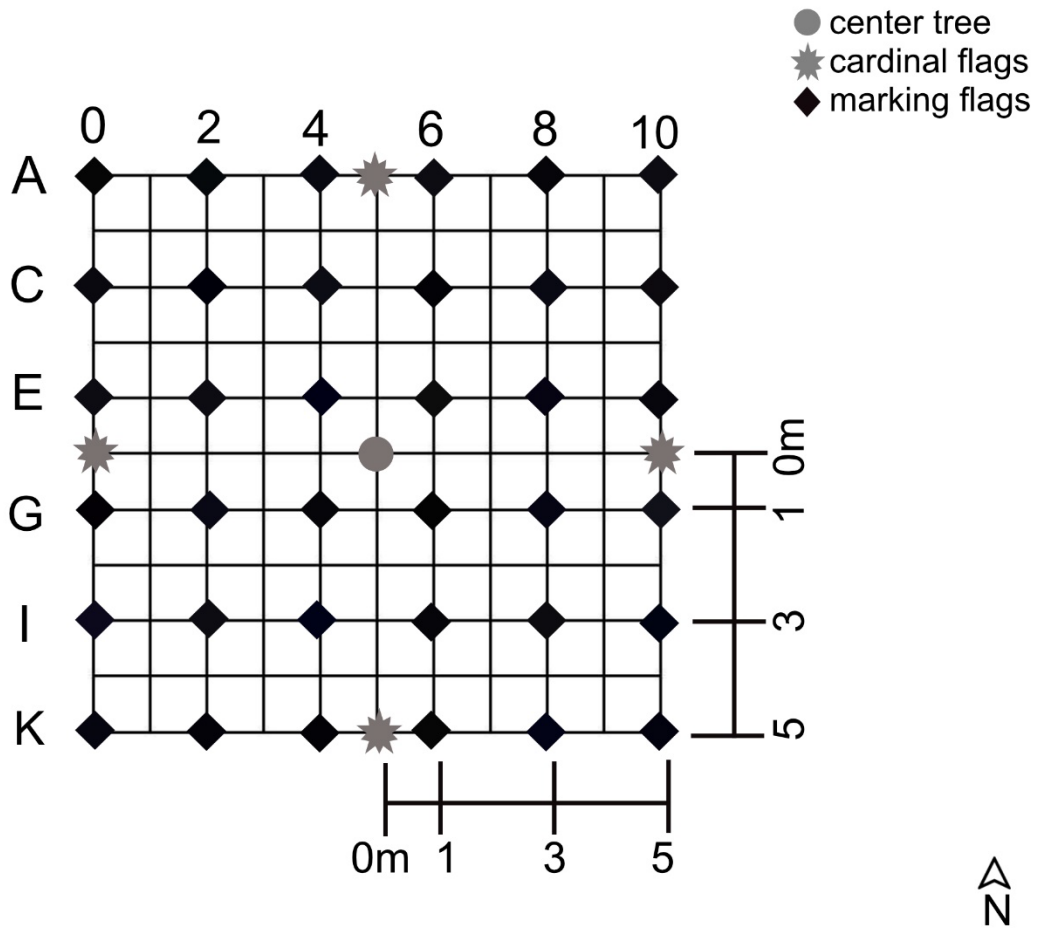

Figure 2. Layout of the plot used to record environmental and habitat covariates at each survey site in the west-central mountains of Puerto Rico, 2017-2018. Descriptions of the specific types of data collected are described in the text.

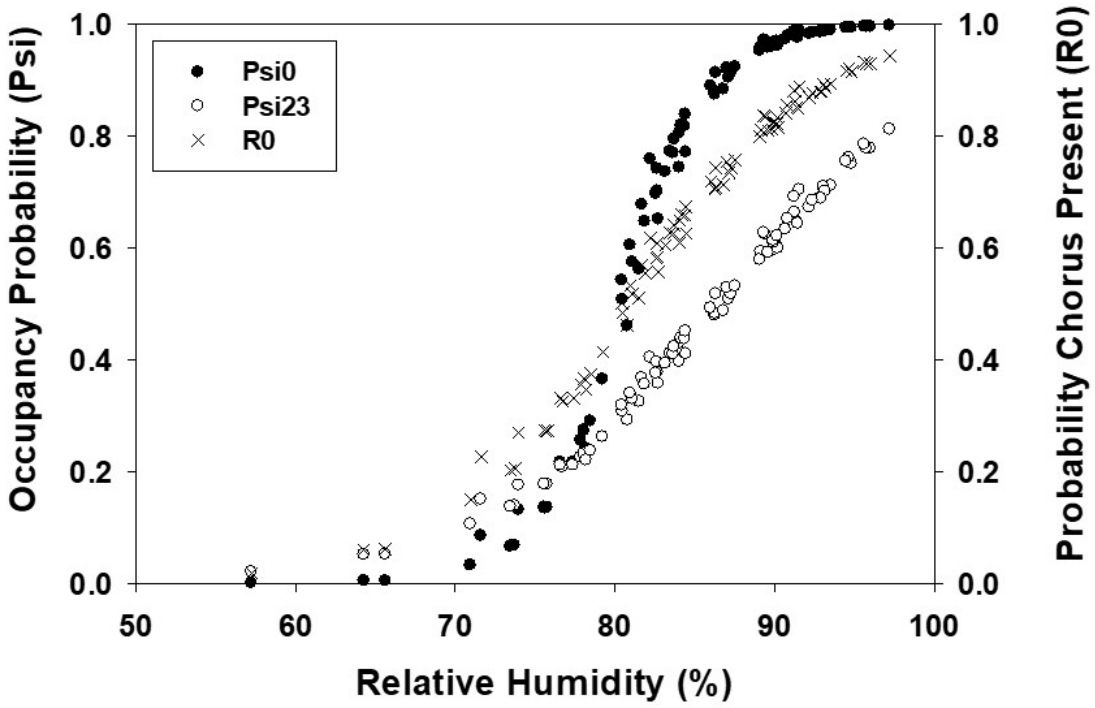

Figure 3. Occupancy probability for the initial season (Psi0), seasons 2 and 3 (Psi23), and the probability of detecting a chorus (R) of *Eleutherodactylus wightmanae* as a function of relative humidity (%) (holding annual/macrohabitat constant) in west-central Puerto Rico, 2017-2018.

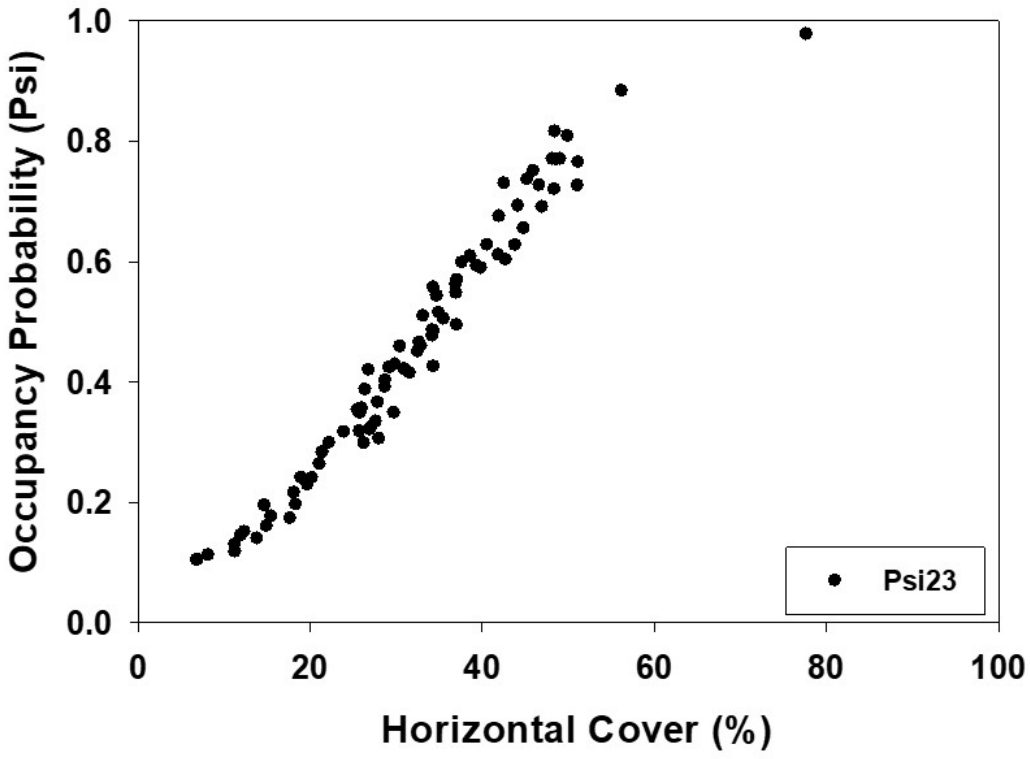

Figure 4. Occupancy probability for seasons 2 and 3 (Psi23) of *Eleutherodactylus wightmanae* as a function of horizontal cover (%) (holding physical/environmental covariate constant) in west-central Puerto Rico, 2017-2018.

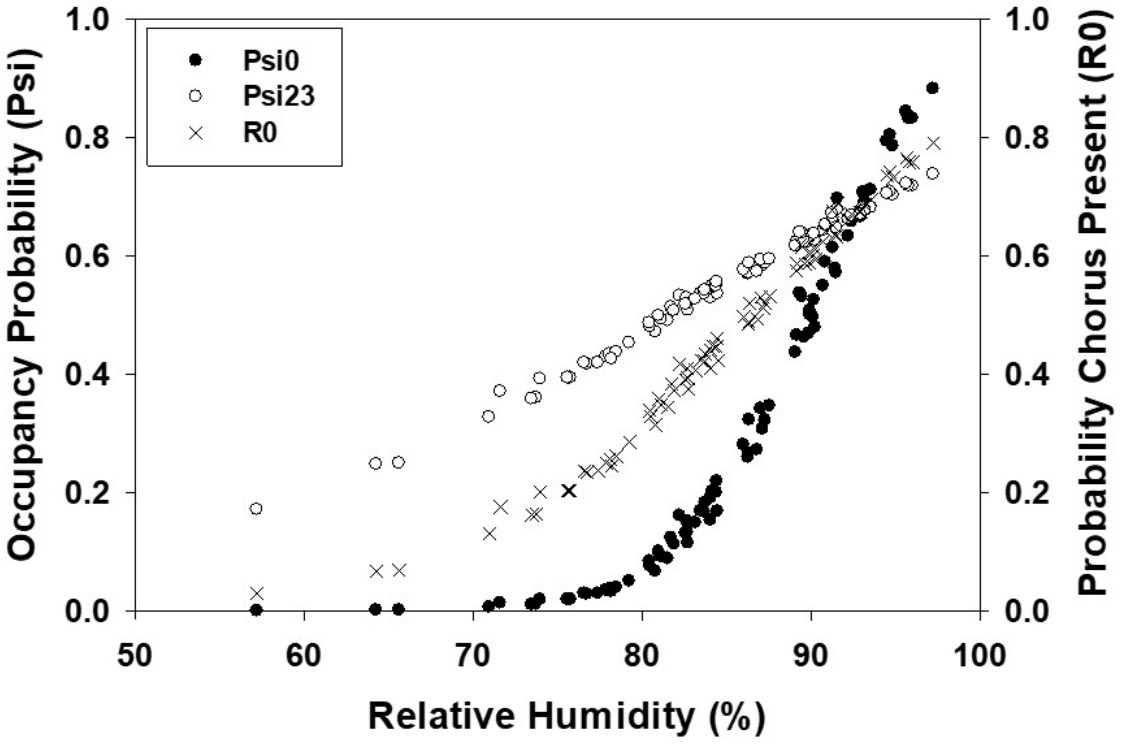

Figure 5. Occupancy for the initial season (Psi0), seasons 2 and 3 (Psi23), and the probability of detecting a chorus (R) of *Eleutherodactylus brittoni* as a function of relative humidity (%) in west-central Puerto Rico, 2017-2018. The relationship for R0 holds annual/macrohabitat covariate constant. Initial season (Psi0) standard errors ranged from 0.01 to 0.20.

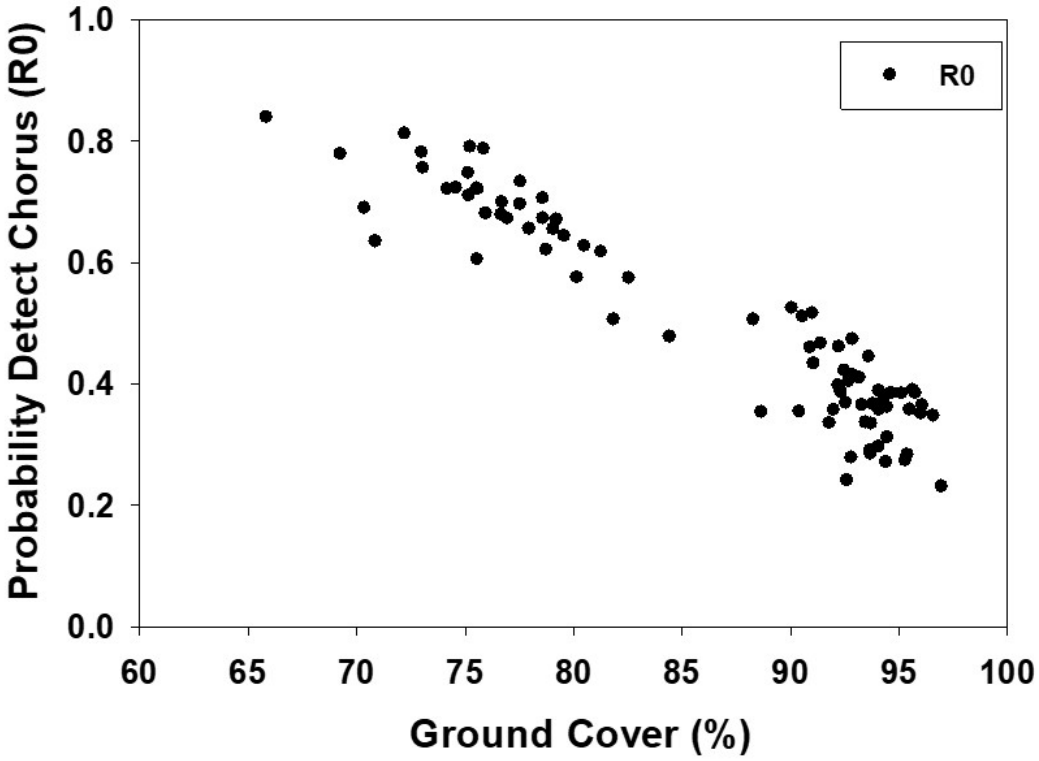

Figure 6. Probability of detecting a chorus (R0) of *Eleutherodactylus brittoni* as a function of ground cover (%) (holding physical/environmental covariate constant) in west-central Puerto Rico, 2017-2018.

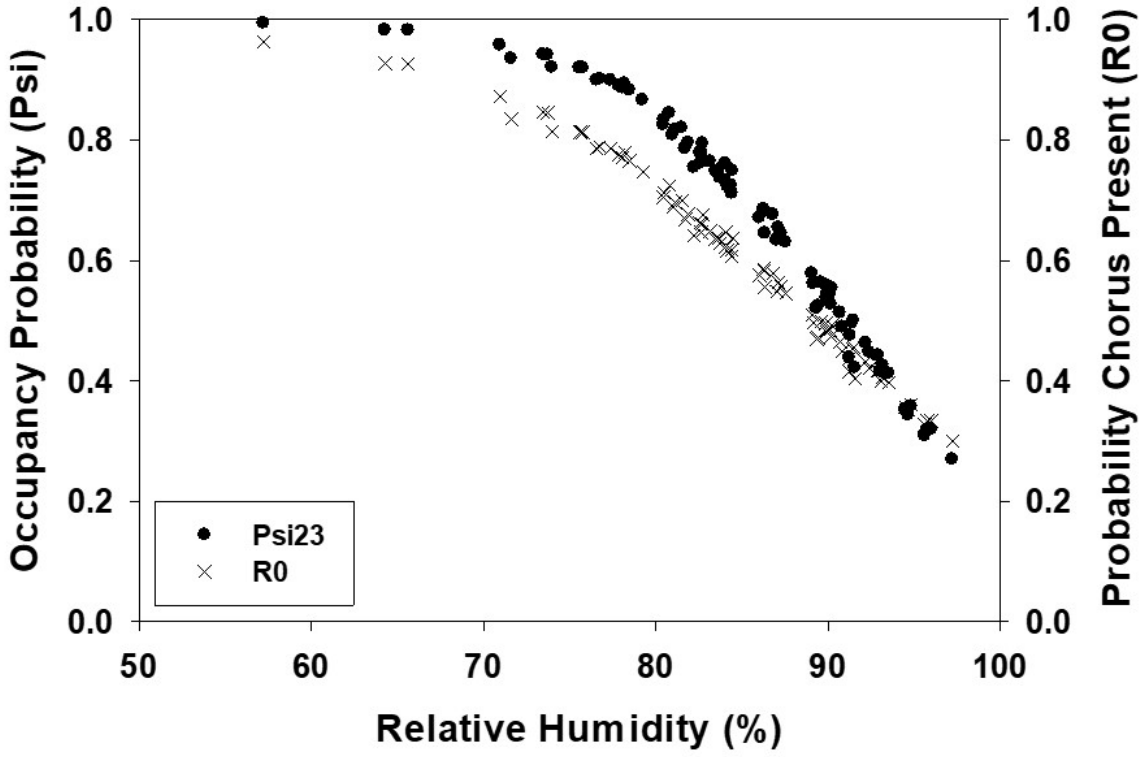

Figure 7. Occupancy probability for seasons 2 and 3 (Psi23) and the probability of detecting a chorus (R) of *Eleutherodactylus antillensis* as a function of relative humidity (%) (holding annual/macrohabitat covariate constant) in west-central Puerto Rico, 2017-2018.

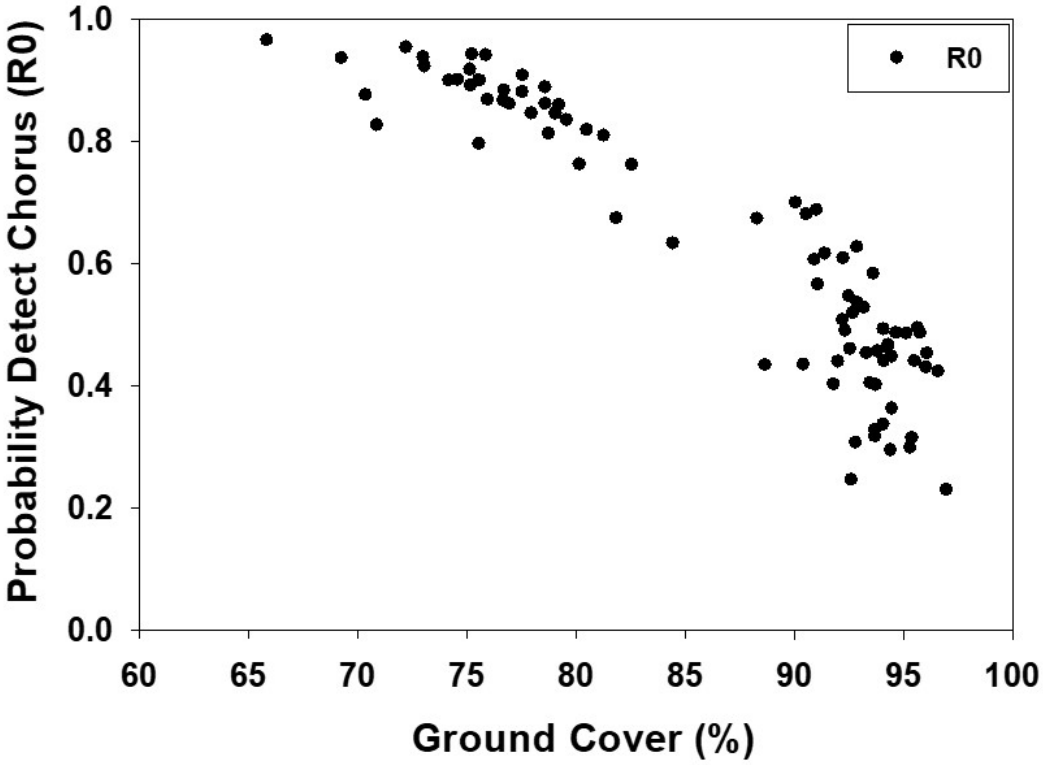

Figure 8. Probability of detecting a chorus (R0) of *Eleutherodactylus antillensis* as a function of ground cover (%) (holding physical/environmental covariate constant) in west-central Puerto Rico, 2017-2018.

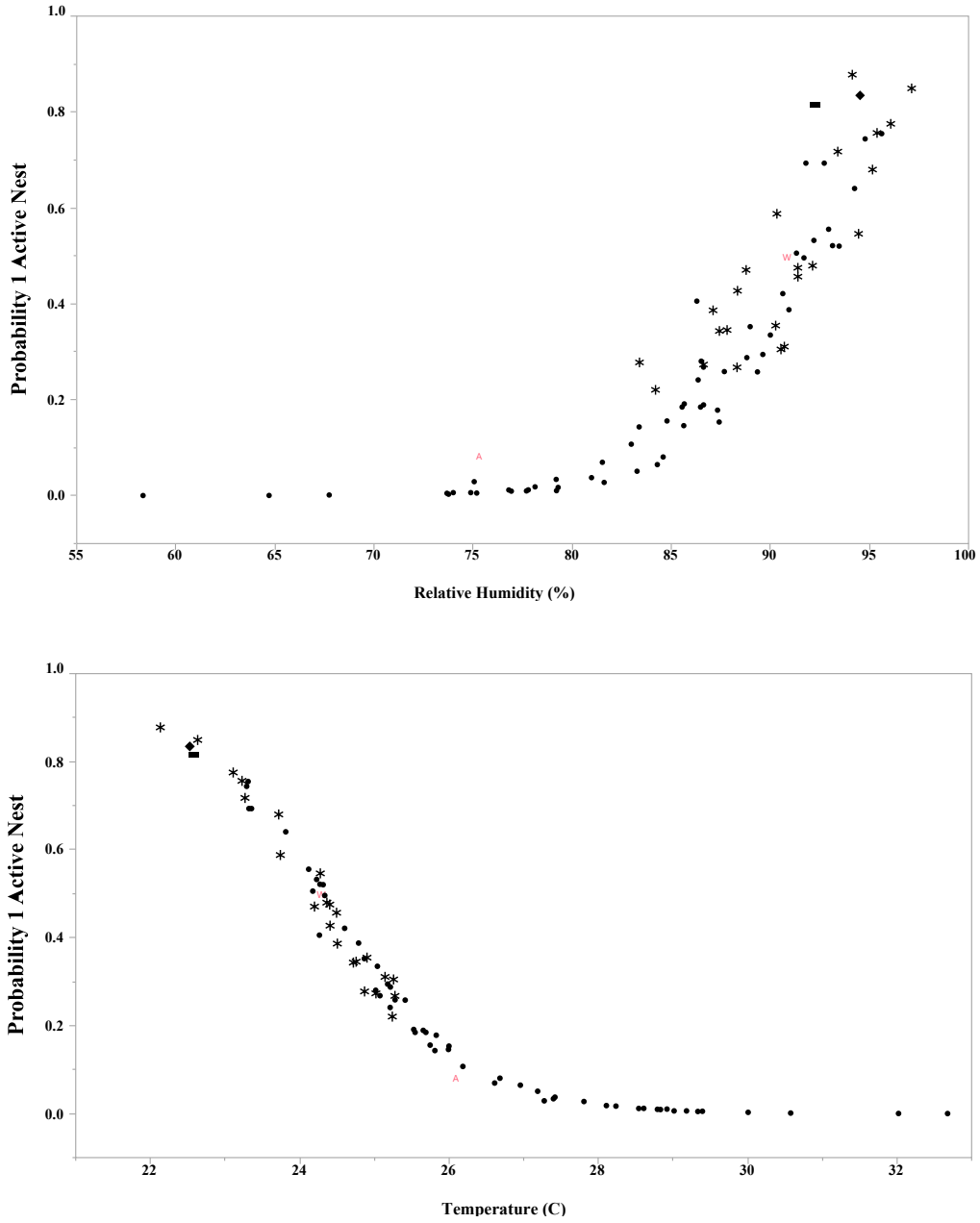

Figure 9. Probability of detecting ≥ 1 active nest of *Eleutherodactylus coqui* at a survey site (83 sites) as a function of relative humidity (%) and temperature (°C) in west-central Puerto Rico, 2017-2018. Red "W" indicates where 2 active nests of *E. wightmanae* were found.

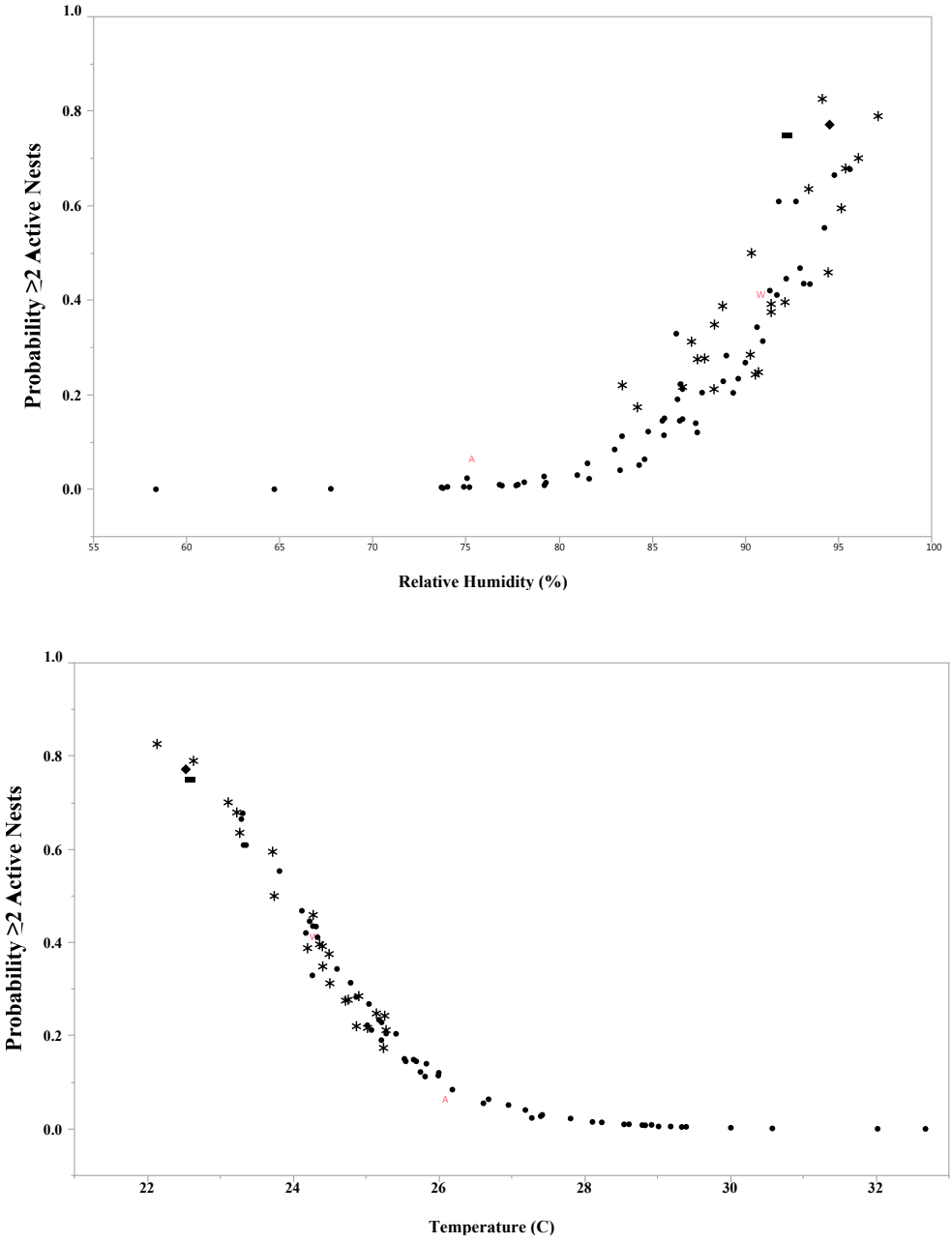

Figure 10. Probability of detecting ≥ 2 active nest of *Eleutherodactylus coqui* at a survey site (83 sites) as a function of relative humidity (%) and temperature (°C) in west-central Puerto Rico, 2017-2018. Red "W" indicates where 2 active nests of *E. wightmanae* were found.

Table 1. Hypothesis and predictions about the influence of environmental and habitat covariates on local occupancy (Psi) and categorical abundance (R; few or chorus) of *E. wightmanae*, *E. brittoni*, and *E. antillensis* in the leeward side of mountains in central and west-central Puerto Rico, 2017-18. I also list the hypothesis and prediction based on the same covariates on the probability of *E. coqui* using a survey site for reproduction. PC1 (eigenvectors) highlights the importance of elevation along with relative humidity and temperature in characterizing the physical environment. PC2 highlights macro-habitat features represented by canopy and ground cover by year; PC3 highlights micro-habitat features represented by horizontal cover, litter depth, leaf layering. The text contains a detailed description of covariates and principal components.

Hypothesis	Principal Component largest and direction (+/-) eigenvectors	Predictions Beta effect -- Direction (+/-) and strength (Strong/Weak)
1) *Eleutherodactylus* species are adapted to exploit high elevation, mesic environments (Joglar 1998), and long-term climatic conditions associated with high elevation (Joglar 1998; Burrowes et al. 2004). 2) Survey site vegetation structure influence quality and quantity of shelter, food, and microclimatic conditions (Huang et al. 2014, Klawinski et al. 2014; Whitfield et al. 2014). 3) CT_{Max} can influence abundance and distribution by defining tolerance limits to environmental change (von May et al. 2017, 2019).	PC1 Elevation (+) Temperature (-) Humidity (+) PC2 Ground Cover (+) Canopy Cover (+) Year (+) PC3 Litter Depth (+) Leaf Layer (+) Horiz. Cover (-)	1) Occupancy of all species will be influenced by the physical/microenvironmental covariates (PC1), but betas for *E. wightmanae* and *E. brittoni* will be positive and strong as compared to the more widely distributed *E. antillensis* (positive but weak). 2) Macro-habitat features (canopy and ground cover) represented by PC2 will have a stronger influence on occupancy than micro-habitat features represented by PC3 as the former promote site-level microclimatic conditions. 3) If a site is occupied, abundance of *E. wightmanae* and *E. brittoni* will be positively and strongly influenced PC1 and microhabitat features (PC3) as compared to *E. antillensis* (weak).

Table 1 (continued).

Survey site microclimatic and microhabitat conditions influence the quality of habitat for reproduction (Oseen and Wassersug 2002; Huang et al. 2014, Whitfield et al. 2014).		4) Physical/microenvironmental covariates (PC1) and the microhabitat features (PC3) will positively and strongly influence the probability that a site is used by *E. coqui* for reproduction.

Table 2. Average (± SE) of environmental and habitat covariates at 83 survey sites in west-central Puerto Rico. Year of sampling (2017, 2018) is indicated by the covariate code suffix. Covariates were canopy cover (CC), ground cover (GC), horizontal cover (HC), litter depth (LD), leaf layer (LL), relative humidity (RH), soil moisture (SM), and precipitation (precip). Superscript "S" indicates seasonal differences; "Y" indicates between-year differences ($P < 0.05$).

Covariate	Season 1	Season 2	Season 3
CC-17^{Y}	----------	97.64 ± 0.22	98.48 ± 0.21
CC-18	89.82 ± 1.61	89.42 ± 1.68	89.60 ± 1.66
GC-17^{Y}	----------	92.99 ± 1.18	92.23 ± 1.26
GC-18	97.43 ± 1.04	98.38 ± 2.68	97.07 ± 1.09
HC-17	----------	35.08 ± 2.56	34.41 ± 2.97
HC-18	26.83 ± 3.14	27.96 ± 3.09	31.79 ± 3.58
LD-17	----------	45.97 ± 2.73	36.78 ± 2.46
LD-18	43.85 ± 3.04	41.16 ± 3.09	40.80 ± 2.31
LL-17^{Y}	----------	4.03 ± 0.30	3.59 ± 0.20
LL-18	4.84 ± 0.33	5.12 ± 0.41	4.19 ± 0.28

Table 2. (continued).

RH-17	----------	85.57 ± 1.26	88.22 ± 1.34
RH-18^{S}	75.50 ± 1.63	83.31 ± 1.62	86.72 ± 2.25
SM-17	----------	32.25 ± 1.27	35.28 ± 1.60
SM-18^{S}	25.15 ± 1.56	31.81 ± 2.29	33.81 ± 1.53
Precip-17	----------	6.32 ± 0.38	7.66 ± 0.57
Precip-18^{S}	3.88 ± 0.38	6.22 ± 0.52	6.80 ± 0.54

Table 3. Principal component analysis based on 5 physical/environmental covariates, 5 habitat covariates, 3 seasons, and year. Data were collected in 83 sites in 2017 and 2018 in west-central Puerto Rico. I report eigenvalues and percent variance explained by each component, and the largest eigenvectors (bold) for each component.

PC	Eigenvalue	% Variance
1	3.24	29.46
2	2.41	21.91
3	1.38	12.59

	Prin1	Prin2	Prin3
Year (2017, 2018)	0.14832	**0.57763**	-0.14730
Elevation (m)	0.37486	-0.36831	-0.20515
Precipitation (mm)	0.35796	0.02777	-0.15963
Temperature (C)	**-0.45863**	0.29056	-0.04405
Rel. Humidity (%)	**0.49124**	0.03869	0.02500
Soil Moisture (%)	0.37243	-0.00048	-0.03462
Ground Cover (%)	0.15289	**0.50157**	-0.04321
Leaf Layer (count)	-0.09911	-0.13817	**0.45688**
Litter Depth (cm)	0.22818	-0.02703	**0.44120**
Horizontal Cover (%)	-0.03293	0.05325	**-0.60092**
Canopy Cover (%)	0.18063	**0.41202**	0.37497

Table 4. Principal component analysis based on 5 physical/environmental covariates, 5 habitat covariates, 2 seasons, and year. Data were collected in 83 sites in 2017 and 2018 in west-central Puerto Rico. I report eigenvalues and percent variance explained by each component, and largest (bold) eigenvectors for each component.

PC	Eigenvalue	% Variance
1	3.25	29.61
2	1.93	17.56
3	1.45	13.26

	Prin1	Prin2	Prin3
Year (2017, 2018)	-0.05201	**0.60619**	-0.12004
Elevetion (m)	**0.45104**	-0.21434	-0.21990
Precipitation (mm)	0.34759	0.04105	-0.17298
Rel Humidity (%)	**0.46599**	0.15032	0.04846
Soil Moisture (%)	0.34027	0.14222	-0.09135
Canopy Cover (%)	0.05357	**0.56265**	0.33708
Ground Cover (%)	0.09067	**-0.39433**	0.28743
Leaf Layer (count)	-0.00586	-0.22786	**0.52757**
Litter Depth (cm)	0.24706	0.07579	**0.40191**
Horizontal Cover (%)	-0.04215	-0.10159	**-0.51040**
Temperature (C)	**-0.51593**	0.04504	0.00053

Table 5. Model selection table for multi-season, multi-state occupancy models for *E. wightmanae* in west-central Puerto Rico. Acoustic surveys were conducted in April-May and July-August of 2017 and 2018; surveys were also conducted in February-March 2018. Model parameters were local occupancy (Psi), probability of encountering a chorus of individuals (≥ 4 individuals, R), probability of correctly classifying a survey site as harboring a chorus (dlta), and detection probability (p). Parameters were modeled as constant over time (.), season-specific (S), and initial season vs remaining 2 (Init). Covariates were 3 principal components describing the physical/environmental conditions (PC1), macro-habitat/annual structure (PC2), and microhabitat structure (PC3). The principal component analysis was based on 5 physical/environmental covariates, 5 habitat covariates, 3 seasons, and year.

Model	AIC	delta AIC	AIC wgt	no. Par.	-2*LogLike
Psi(Init+PC1+PC2),R(PC2+PC1),dlta(PC1),p(PC3)	699.89	0	0.9605	13	673.89
Psi(Init+PC1+PC2),R(PC2),dlta(PC1),p(PC3)	706.38	6.49	0.0374	12	682.38
Psi(Init+PC1+PC2),R(PC1),dlta(PC1),p(PC3)	712.23	12.34	0.002	12	688.23
Psi(Init+PC1),R(PC2),dlta(PC1),p(PC3)	722.38	22.49	0	10	702.38
Psi(Init+PC2),R(PC2+PC1),dlta(PC1),p(PC3)	726.13	26.24	0	11	704.13
Psi(Init+PC1),R(PC1),dlta(PC1),p(PC3)	728.27	28.38	0	10	708.27
Psi(Init+PC1),R(PC2+PC1),dlta(PC1),p(PC3)	728.27	28.38	0	10	708.27
Psi(Init+PC2),R(PC2),dlta(PC1),p(PC3)	732.43	32.54	0	10	712.43
Psi(Init+PC2),R(PC1),dlta(PC1),p(PC3)	738.24	38.35	0	10	718.24
Psi(Init),R(Init),dlta(PC3),p(PC3)	752.19	52.3	0	7	738.19
Psi(Init),R(Init),dlta(PC1),p(PC1)	753.4	53.51	0	7	739.4
Psi(Init),R(Init),dlta(.),p(.,.)	754.17	54.28	0	6	742.17
Psi(.),R(.),dlta(PC3),p(PC3)	754.75	54.86	0	6	742.75
Psi(Init),R(Init),dlta(PC2),p(PC2)	755.25	55.36	0	7	741.25
Psi(S),R(S),dlta(PC1),p(PC3)	755.68	55.79	0	10	735.68
Psi(Init+PC3),R(PC3),dlta(PC1),p(PC3)	756.33	56.44	0	10	736.33
Psi(Init),R(Init),dlta(S),p(S)	785.05	85.16	0	6	773.05

Table 6. Model selection table for multi-season, multi-state occupancy models for *E. brittoni* in west-central Puerto Rico. Acoustic surveys were conducted in April-May and July-August of 2017 and 2018; surveys were also conducted in February-March 2018. Model parameters were local occupancy (Psi), probability of encountering a chorus of individuals (≥ 4 individuals, R), probability of correctly classifying a survey site as harboring a chorus (dlta), and detection probability (p). Parameters were modeled as constant over time (.), season-specific (S), and initial season vs remaining 2 (Init). Covariates were 3 principal components describing the physical/environmental conditions (PC1), macro-habitat/annual structure (PC2), and microhabitat structure (PC3). The principal component analysis was based on 5 physical/environmental covariates, 5 habitat covariates, 3 seasons, and year.

Model	AIC	delta AIC	AIC wgt	no.Par.	-2*LogLike
Psi(Init+PC1),R(PC1+PC2),dlta(PC2),p(PC2)	513.45	0	0.69	11	491.45
Psi(Init+PC1+PC2),R(PC1+PC2),dlta(PC2),p(PC2)	517.18	3.73	0.11	13	491.18
Psi(Init+PC1),R(PC2),dlta(PC2),p(PC2)	517.27	3.82	0.10	10	497.27
Psi(Init+PC1),R(PC1),dlta(PC2),p(PC2)	518.27	4.82	0.062	10	498.27
Psi(Init+PC1),R(PC1+PC3),dlta(PC2),p(PC2)	520.12	6.67	0.02	11	498.12
Psi(Init+PC1+PC2),R(PC2),dlta(PC2),p(PC2)	521.02	7.57	0.01	12	497.02
Psi(Init+PC3),R(PC1+PC2),dlta(PC2),p(PC2)	524.53	11.08	0.003	11	502.53
Psi(Init+PC2),R(PC1),dlta(PC2),p(PC2)	527.96	14.51	0.0005	10	507.96
Psi(Init+PC2),R(PC2),dlta(PC2),p(PC2)	533.58	20.13	0	10	513.58
Psi(Init),R(.),dlta(PC2),p(PC2)	537.92	24.47	0	7	523.92
Psi(S),R(.),dlta(PC2),p(PC2)	539.91	26.46	0	8	523.91
Psi(Init+PC3),R(PC3),dlta(PC2),p(PC2)	542.52	29.07	0	10	522.52
Psi(S),R(S),dlta(PC2),p(PC2)	543.44	29.99	0	10	523.44
Psi(.),R(.),dlta(PC2),p(PC2)	545.73	32.28	0	6	533.73
Psi(Init),R(.),dlta(.),p(.,.)	563.62	50.17	0	6	551.62
Psi(Init),R(.),dlta(PC3),p(PC3)	565.36	51.91	0	7	551.36
Psi(Init),R(.),dlta(PC1),p(PC1)	565.61	52.16	0	7	551.61
Psi(Init),R(.),dlta(S),p(S)	605.84	92.39	0	6	593.84

Table 7. Model selection table for multi-season, multi-state occupancy models for *E. antillensis* in west-central Puerto Rico. Acoustic surveys were conducted in April-May and July-August of 2017 and 2018; surveys were also conducted in February-March 2018. Model parameters were local occupancy (Psi), probability of encountering a chorus of individuals (≥ 4 individuals, R), probability of correctly classifying a survey site as harboring a chorus (dlta), and detection probability (p). Parameters were modeled as constant over time (.), season-specific (S), and initial season vs remaining 2. Covariates were 3 principal components describing the physical/environmental conditions (PC1), macro-habitat/annual structure (PC2), and microhabitat structure (PC3). The principal component analysis was based on 5 physical/environmental covariates, 5 habitat covariates, 3 seasons, and year.

Model	AIC	delta AIC	AIC wgt	no. Par.	-2 * LogLike
Psi(Init+PC1+PC2),R(PC2+PC1),dlta(PC1),p(PC3)	699.89	0	0.9605	13	673.89
Psi(Init+PC1+PC2),R(PC2),dlta(PC1),p(PC3)	706.38	6.49	0.0374	12	682.38
Psi(Init+PC1+PC2),R(PC1),dlta(PC1),p(PC3)	712.23	12.34	0.002	12	688.23
Psi(Init+PC1),R(PC2),dlta(PC1),p(PC3)	722.38	22.49	0	10	702.38
Psi(Init+PC2),R(PC2+PC1),dlta(PC1),p(PC3)	726.13	26.24	0	11	704.13
Psi(Init+PC1),R(PC1),dlta(PC1),p(PC3)	728.27	28.38	0	10	708.27
Psi(Init+PC1),R(PC2+PC1),dlta(PC1),p(PC3)	728.27	28.38	0	10	708.27
Psi(Init+PC2),R(PC2),dlta(PC1),p(PC3)	732.43	32.54	0	10	712.43
Psi(Init+PC2),R(PC1),dlta(PC1),p(PC3)	738.24	38.35	0	10	718.24
Psi(Init),R(Init),dlta(PC3),p(PC3)	752.19	52.3	0	7	738.19
Psi(Init),R(Init),dlta(PC1),p(PC1)	753.4	53.51	0	7	739.4
Psi(Init),R(Init),dlta(.),p(.,.)	754.17	54.28	0	6	742.17
Psi(.),R(.),dlta(PC3),p(PC3)	754.75	54.86	0	6	742.75
Psi(Init),R(Init),dlta(PC2),p(PC2)	755.25	55.36	0	7	741.25
Psi(S),R(S),dlta(PC1),p(PC3)	755.68	55.79	0	10	735.68
Psi(Init+PC3),R(PC3),dlta(PC1),p(PC3)	756.33	56.44	0	10	736.33
Psi(Init),R(Init),dlta(S),p(S)	785.05	85.16	0	6	773.05

Table 8. Summary of reproductive effort of 3 species of *Eleutherodactylus* frogs using artificial nest structures in west-central PR. Nest structures were set in 48 (2017) and 35 (2018) survey sites. Each site had a grid (10x10) divided into 4 quadrants, where 9 artificial nest structures were placed per quadrant (N=36/site).

Species/Parameter	2017	2018
E. coqui		
Number of Survey Sites w/ Occupied Nests	16	15
Number of Occupied Nests/Survey Site	1.34	3.02
Average number of eggs/Active nest	24.6 (2.55)	46.21 (6.14)
Number of Active Nests/Survey Site	1.04	2.41
Average hatching success/Active nest	0.23 (0.10)	0.10 (0.04)
E. antillensis		
Number of Survey Sites w/ Occupied Nests	12	6
Number of Occupied Nests/Survey Site	0.71	0.35
Average number of eggs/Active nest	0	15 (0.0)
Number of Active Nests/Survey Site	0	0.06
Average hatching success/Active nest	0	0
E. wightmanae		
Number of Survey Sites w/ Occupied Nests	1	0
Number of Occupied Nests/Survey Site	0.04	0
Average number of eggs/Active nest	3.75 (0.70)	0
Number of Active Nests/Survey Site	0.04	0
Average hatching success/Active nest	1.06	0

Table 9. Average (± SE; range) elevation and environmental characteristics of used and non-used sites for reproduction by three species of *Eleutherodactylus* frogs recorded in west-central Puerto Rico, 2017 and 2018. Used sites were defined as those where at least 1 artificial nest structure contained eggs. Nest structures were set in 48 (2017) and 35 (2018) survey sites. Each site had a grid (10x10) divided into 4 quadrants, where 9 artificial nest structures were placed per quadrant (N=36/site). * means that averages were significantly different ($P < 0.05$).

	Not Used	Used
Elevation (m)	447.40 (27.97; 16-890)	740.46 (28.38; 396-1083)*
Temperature (°C)	26.32 (0.33; 22.22-34.98)	24.33 (0.23; 21.67-26.30)*
Relative Humidity (%)	84.67 (1.28; 52.27-97.67)	88.88 (1.02; 80.27-97.28)*

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