

Variation in Bioacoustic Characteristics in *Eleutherodactylus coqui* Thomas, 1966 and *Eleutherodactylus antillensis* (Reinhardt and Lutken, 1863) (Anura: Eleutherodactylidae) in the Puerto Rico Bank¹

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Abstract: Bioacoustics is an interdisciplinary science that combines biology, acoustics, and mathematics. This discipline can be used to study population ecology and behavior. Furthermore, we can use this tool to assess a population and suggest if a species of interest may be in a transitional state of becoming a new species by allopatric speciation. Amphibians communicate via sound and the environment has a key role in metabolism and sound dispersion. By analyzing temporal and spectral properties of acoustical communication in anurans, we can understand better how these animals are evolving to cope with their ever-changing environment. We studied the variation in acoustic parameters among five populations each of the red-eye coqui, *Eleutherodactylus antillensis* (Reinhardt and Lutken, 1863) and the common coqui, *E. coqui* Thomas, 1966 across the Puerto Rico Bank. These species are changing their vocalizations. Some populations have higher sound frequencies than other conspecific populations; other nocturnal species have populations with different temporal patterns of sound production. We found strong variation among the five populations examined for each species. In *E. antillensis*, the size of the organism relates to temporal variation in sound production (i.e., inter-note interval and total call duration) and did not relate to spectral differentiation. In *E. coqui*, the population living at highest elevation above sea level assessed had a spectral footprint no other population shares, probably due to geographic isolation from other conspecific populations that live in lower elevations.

Keywords: Bioacoustics, *Eleutherodactylus*, *Eleutherodactylus coqui*, *Eleutherodactylus antillensis*, Puerto Rico, microevolution, communication, sound spectrogram, speciation,

Introduction

Natural History

Amphibians are important components of many ecosystems because their total number and biomass affect ecosystem function through complex trophic interactions (Dodd 2010). However, these vertebrates are not immune from the ravages that are happening on Earth. Herpetofaunas are susceptible to sudden environmental changes (Sala et al. 2000, Cushman 2006), and these changes can either diminish their populations or, in extreme cases, extinguish them. Some of

¹ Received on November 5, 2019. Accepted on November 19, 2019. Last revisions received on December 5, 2019.

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the factors that affect animal populations are land use, such as agriculture, recreational uses, construction of urban areas and military training sites; all leading to habitat fragmentation (Gibbons et al. 2000, Thrush et al. 2008, Ríos-Franceschi et al. 2016). In such cases, many amphibians are unable to escape from the area that has been fragmented or modified, mainly because of their low mobility (Vredeburg and Wake 2007).

Puerto Rico lies within the Caribbean biodiversity hotspot (Cox and Moore 2000). Puerto Rican diversity consists of approximately 26,410 species of plants, fungi and animals (Joglar 2005). These species, within the Puerto Rican Bank have been isolated, by physical barriers, such as by the rising sea levels after the last glacial maximum (Heatwole et al. 1981 and Figure 1, below) for approximately 8,000–10,000 years, creating different niches and augmenting the diversity in the archipelago. This can provide a privileged location to study evolution in a microscale for many different taxa, including amphibians.

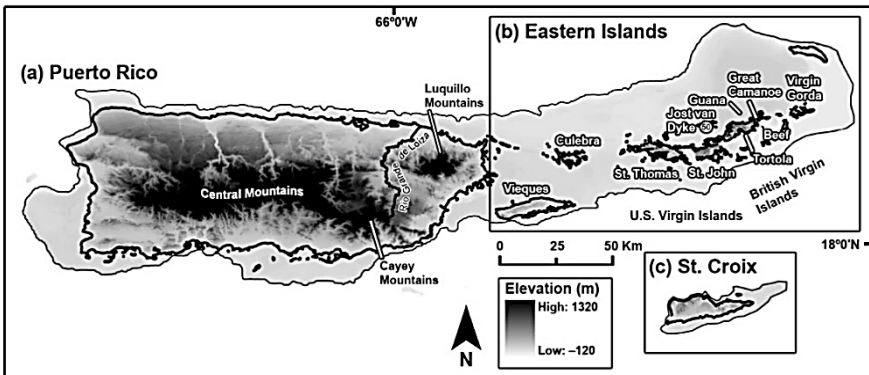


Figure 1. Map of the Puerto Rican Bank (a and b) and St. Croix (c) illustrating the topography of the islands and the approximate geographic location of the sampling localities of *Eleutherodactylus antillensis*. The outermost line in a, b and c indicates the approximate land configuration at maximum sea level (-120 m, Siddall et al. 2003), during the Last Glacial Maximum (circa 26.5-19 kya). The thicker line depicts the current extent of land area. Map was modified from Barker et al. 2012 by José R. Almodóvar.

Overall morphological similarity and intrapopulation variation of morphological features make some species identification challenging (Angulo and Reichle 2008, Bickford et al. 2007). Species descriptions for a variety of taxa have traditionally relied primarily on morphological traits (Mayden 1997, Angulo and Reichle 2008). Some cryptic species cannot be identified by morphological analysis alone and scientists need to use other characteristics. Acoustic signals are the main vehicle of communication in most anurans (Ryan 1988, Gerhardt and Huber 2002, Röhr and Juncá 2013). Herpetologists often use acoustic, or sonic, signals in species identification and diagnosis for taxa that use such signals to communicate (Angulo and Reichle 2008). For example, advertisement calls have

been used to resolve the systematics of several groups within the family Eleutherodactylidae (Heyer 1984, Angulo and Reichle 2008). Although most species may have more than one kind of vocalization, the advertisement call is the most widespread and it is the focus of most bioacoustics' studies of frogs (Gerhardt 1994, Gerhardt and Huber 2002, Röhr and Juncá 2013). Advertisement calls are conspicuous acoustic signals emitted by males of most species of anurans (Erdtmann et al. 2011). These calls generally have multiple purposes, such as female attraction, territory defense against conspecific males (Duellman and Trueb 1994, Wells 2007; Erdtmann et al. 2011, Papes and Ladich 2011, Röhr 2013, Velázquez et al. 2013) and informing about a male's physical condition. On the other hand, animal communication signals have an important role in species divergence, promoting reproductive isolation and speciation (Coyne and Orr 1998, Velázquez et al. 2013). Variation of behavioral characters across their geographical distribution has been the subject of studies aiming to establish the degree of divergence among populations (Coyne and Orr 1998; Stafford et al. 2001, Coyne and Orr 2004, Quispe et al. 2009, Velázquez et al. 2013).

Clinal variation of advertisement calls related to geographical distance between populations (Nevo and Capranica 1985; Wilczynski and Ryan 1999; Castellano et al. 2002; Giacoma and Castellano 2001; Bernal et al. 2005; Pröhl et al. 2006, 2007; Velázquez et al. 2013) or the relevance of adaptations to different environmental conditions (Hunter and Krebs 1979, Boughman 2002, van Dongen et al. 2010) has been reported for different anurans. These conditions have been affected by climate change and these effects force species to meet dynamic thermal environments and may jeopardize the performance of their basic physiological functions, especially in ectothermic organisms, like frogs (Pörtner and Knust 2007, Huey et al. 2009, Kearney et al. 2009, Duarte et al. 2011, Llusia et al. 2013). Predictions of the compensatory response of ectotherms to climate change, which is an urgent and challenging task in current biology, depend upon comprehending the ecological performance of key biological functions under different thermal environments (Pörtner and Knust 2007, Buckley 2008, Huey et al. 2009, Kearney et al. 2009), and the mechanisms that underlie the thermal adjustments of the species (Helmuth et al. 2005, Angilletta 2009, Llusia et al. 2013).

The effects of temperature on anuran vocalizations

Ectothermic animals are dependent on environmental heat sources and control their body temperature through external means (Papes and Ladich 2011). Compared to endothermic animals, they conserve relatively low metabolic rates. In general, the speed of all metabolic processes is influenced by the body temperature, which, for ectothermic organism, depends on the ambient temperature (Cossins et al. 1977, Salem and Omura 1998, Navarro 2002, Andersson 2003, Itoi et al. 2003). Ambient temperature affects various physiological processes, such as neuronal and muscular activities, including all

sensory systems of ectothermic animals (Ryan 1985, Stiebler and Narins 1990, Pires and Hoy 1992, Márquez and Bosch 1995). It is well known that temperature affects temporal acoustical parameters between anuran populations (Walker 1957, 1975; Lörcher 1969, Schneider 1974; Gerhardt and Mudry 1980; Gayou 1984; Ryan 1985; Stiebler and Narins 1990; Pires and Hoy 1992; Márquez and Bosch 1995; Llusia et al. 2013; Ríos-Franceschi et al. 2016). Thus, environmental temperatures beyond specific thermal thresholds may constrain physiological processes associated with sound production to such an extent that they inhibit calling behavior (Lörcher 1969, Schneider 1974, Walker 1975, Gayou 1984, Gerhardt and Huber 2002, Llusia et al. 2013). Prior to adaptation, ectotherms depend on complex thermal adjustment mechanisms to persist in new environments (Feder and Burggren 1992, Angilletta 2009, Llusia et al. 2013). Examining calling temperatures and their patterns of geographical and seasonal variation in dissimilar thermal environments (e.g., thermal extremes of species range) provides insights into the thermal breadth of calling activity of species, and the physiological and behavioral mechanisms that enable ectotherms to adjust to heterogeneous and changing thermal environments (Narins 2001, Llusia et al. 2013).

Studying contemporary populations may help reveal selective forces that have shaped and maintained, vocal traits (Gerhardt and Huber 2002), thereby providing a better understanding of inter- as well as intraspecific variation in anuran vocalization (Röhr 2013). Some of this variation may be attributed mainly to sexual selection in a strict sense (Wilbur et al. 1978, Gerhardt 1991, Márquez 1995, Rosso et al. 2006, Röhr and Juncá 2013) and some to environmental limitations (e.g., Ryan et al. 1990; Ryan and Wilczynski 1991, Kime et al. 2000, Castellano et al. 2003, Röhr and Juncá 2013). For example, the transmission of a sound signal can be affected by its acoustic characteristics (Morton 1975, Marten et al. 1977, Ryan and Sullivan 1989, Kime et al. 2000) as well as by a variety of other factors such as habitat and vegetation structure (Wiley and Richards 1978, Sorjonen 1986, Ryan et al. 1990, Endler 1992), ambient noise (Feng et al. 2006, Preininger et al. 2007), climatic conditions (Wiley and Richards 1978), and height of vocalization perch (Wiley and Richards 1978, Wilczynski et al. 1989, Kime et al. 2000, Papes 2011). Furthermore, ambient temperature affects hearing in invertebrates and ectothermic vertebrates (Narins 2001, Papes 2011). Such effects have been tested in amphibians (Benedix et al. 1994, Egert and Lewis 1995, Long et al. 1996, Van Dijk et al. 1997) and reptiles (Eatock and Manley 1981, Smolders and Klinke 1984, Papes 2011). For these reasons, we analyzed the variation in temporal and spectral properties of these populations and how these variation affects anuran communication by the means of sexual selection.

Methods

The sound patterns of two common species of eleutherodactylid frogs *Eleutherodactylus coqui* Thomas, 1966 and *Eleutherodactylus antillensis*

(Reinhardt and Lutken, 1863) (Joglar 1998, Rivero 1998) (Figure 2), were recorded across Puerto Rico during 2016-2018. A total of 731 minutes from ten different sites (approximately 73.0 minutes for each site) were recorded and analyzed (Figure 3).

Study localities

From these results, six major localities were selected and documented: (1) Mayagüez (University of Puerto Rico, Mayagüez Campus', Urban Forest. Elevation: 24.0 m), (2) Utuado (Río Abajo State Forest. Elevation: 426.0 m), (3) Arecibo (Islote, near Caño Tiburones Reserve. Elevation: 3.0 m), (4) Río Piedras (University of Puerto Rico, Río Piedras Campus. Elevation: 26.0 m), (5) Jayuya (Coabey, near Tres Picachos Reserve. Elevation: 996.0 m), and (6) Culebra Island (Mount Resaca. Elevation: 130.0 m). These sites were selected for their variation in elevation and climatic conditions. The other four sites were eliminated due to duplicity of data collection near two sites: Utuado (three sites) and Mayagüez (one site) area.

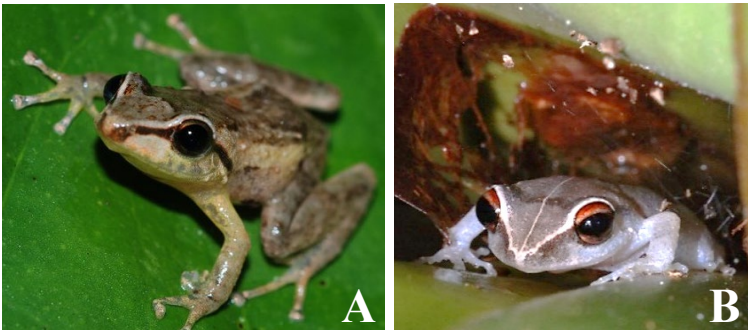


Figure 2. A. *Eleutherodactylus coqui* in Mount Guilarte (Adjuntas), Puerto Rico. B. *Eleutherodactylus antillensis* taking refuge in a bromeliad in Mount Resaca National Wildlife Refuge, Culebra Island. Photos by Alejandro Ríos-Franceschi.

Sonic data collection

We used in-site audio recordings (.WAV format). This technique allows the researcher to record a specific individual while calling, which adds specificity and precision to the research. For this task a hand-held recorder, H6 Sound Recorder from Zoom Laboratories® was used. Data was analyzed using Raven Pro Sound Analysis Software® from Cornell University's Ornithology Laboratory. This software processes the field data and extracts information such as spectrogram, frequency, call length, power (dB), energy (dB) and amplitude.

Morphometric measurements

Five morphometric measurements were taken from each frog recorded: snout-vent-length (SVL), head diameter (from eye socket to eye socket), tibio-

fibula length (TL), tympanic diameter (TYM) and weight. Measurements were taken using a digital caliper (Calipro®, 0.02 mm), for weight a spring scale (Pesola®, 100.00 g) and for body temperature a laser thermometer (Etekcity®, <math> < 100^{\circ}\text{C} \pm 2.0^{\circ}\text{C}</math>).

Statistical analyses of morphometric measurements

A Student’s t-test was used to observe differences in morphometry (SVL, TL, TYM, head diameter, and weight), of the collected individuals. Furthermore, to estimate if there were differences in acoustic variables across populations, we used ANOVA. For the latter, we used the Shapiro-Wilks normality test ($n = 10, p > 0.05$) and Q-Q plots to test whether our data was normally distributed, reducing “type 1” error (Figure 4). Moreover, to examine which populations had similarities between these variables, the Fisher’s Least Significant Difference (LSD) test (Meier 2006) was used to estimate these differences by comparing mean values of acoustic variables in the ANOVA such as duration of each note, inter-note interval, and frequencies. As a result, these populations can be clustered according to their vocal characteristics. These analyses provide information about the possible plasticity or microevolution occurring in the species.

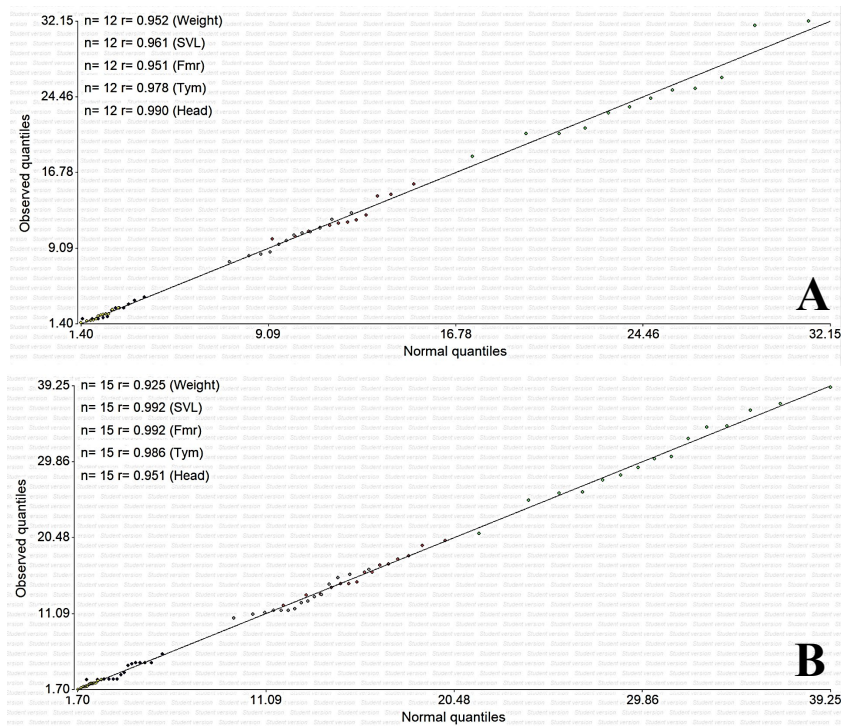


Figure 4. Q-Q plot for normal distribution data using morphometric variables. A, *Eleutherodactylus antillensis* and, B, *E. coqui*.

Sonic data collection

In each site, two species of the genus *Eleutherodactylus* were recorded between 20:00–02:00. After encountering a calling male, we recorded individuals calls with a shotgun microphone (Zoom® SGH-6) data from 1.0 m away (Figure 5) as proposed by Meenderink et al. (2010), Narins and Meenderink (2014).

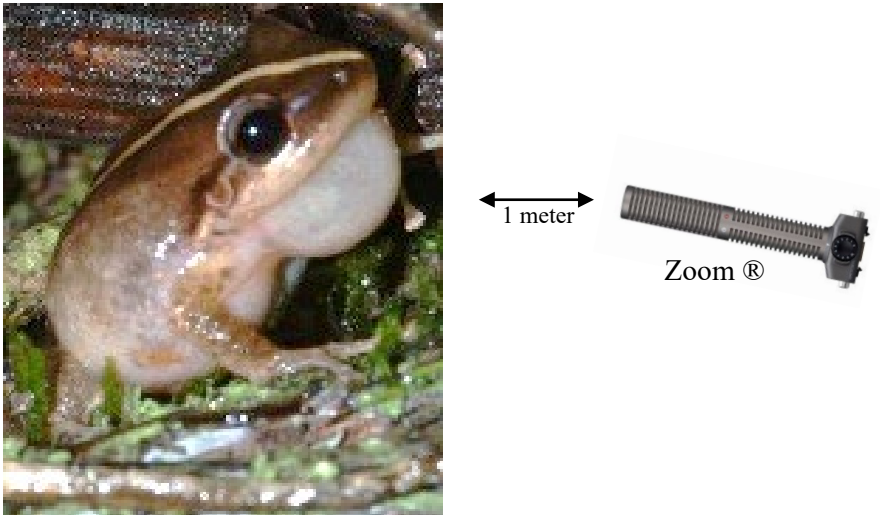


Figure 5. Recording distance between the shotgun microphone and the target amphibian (1.0 meter).

Every sample was analyzed using *Raven Pro*® software to extract the best recordings (e.g., highest signal-to-noise ratio). Each recording was an individual frog calling at the site. At that point, 30 recordings of one minute each within each site were selected and analyzed to determine the lowest frequency, highest frequency, power (dB), energy (dB) and call length of each individual note of the call of each species for a total of 150 recordings for *E. coqui* and 151 recordings for *E. antillensis*. Sound spectrographs were used in identifying the species courtship notes, which vary depending on the behavioral context of the caller (e.g., another male or female nearby) (Figure 6). Also, a rare form of a call was found during the analysis, this call was found only in one specimen of *E. coqui* in the Utuado area (Figure 7).

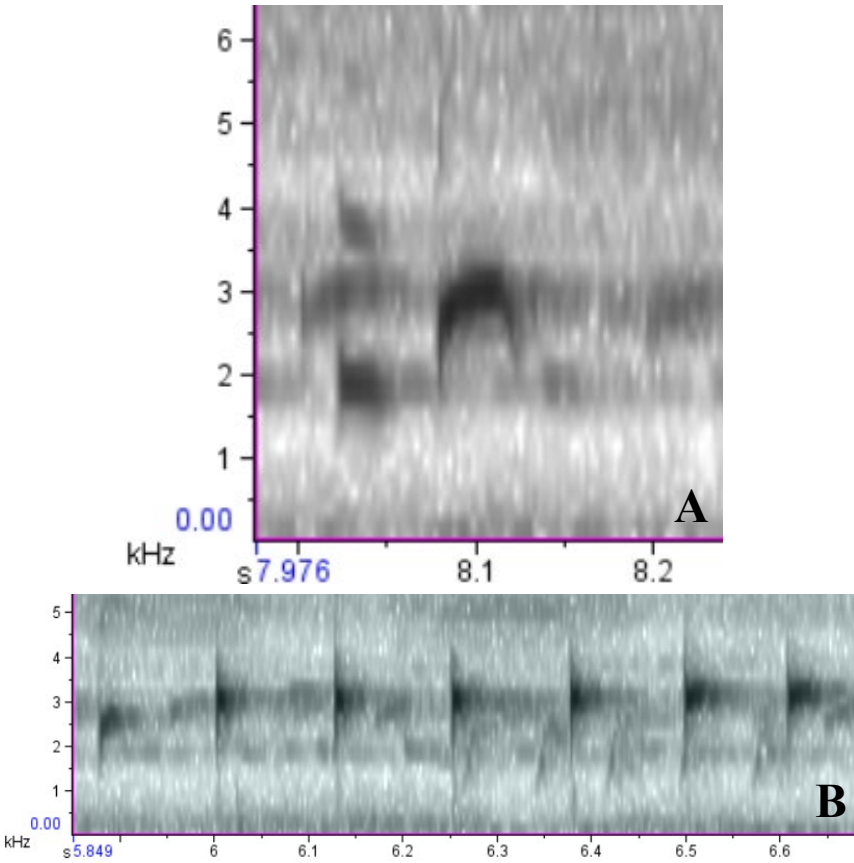


Figure 6. *Eleutherodactylus antillensis* sound spectrograph. A. Common call, “churi”, https://blaypublishers.files.wordpress.com/2019/11/figure5_a.wav. B. Aggressive trill call, “qui... qui... qui... qui”, https://blaypublishers.files.wordpress.com/2019/11/figure5_b.wav.

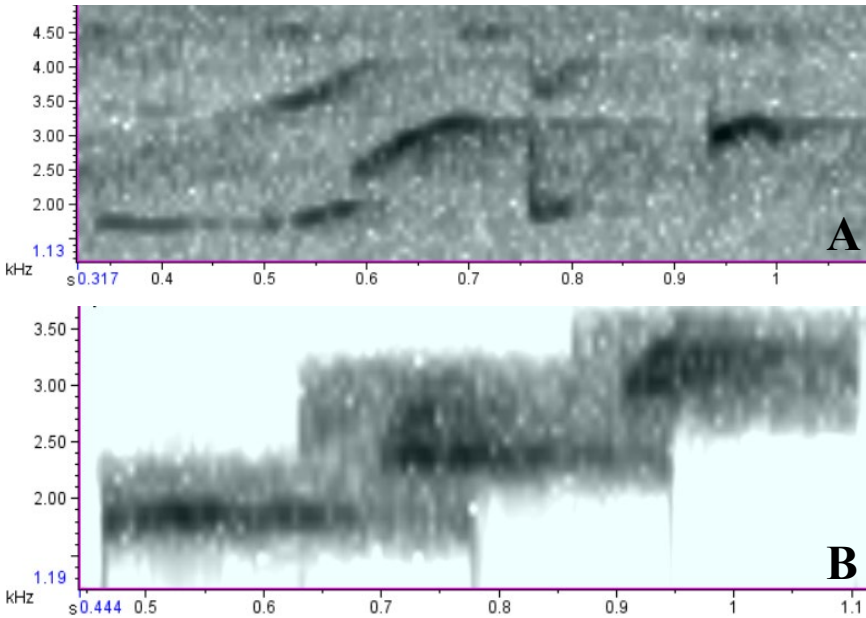


Figure 7. *Eleutherodactylus coqui* sound spectrograph. A, Aberrant call found in Utuado, Puerto Rico, https://blaypublishers.files.wordpress.com/2019/11/figure6_a.wav B, Territorial call, “co...co... qui”, https://blaypublishers.files.wordpress.com/2019/11/figure6_b.wav .

Statistical analyses of sonic data

The following statistical analyses were performed using (Info Stat®, 2012). An analysis of variance (ANOVA) was performed for *Eleutherodactylus antillensis* and for *E. coqui* to examine if the localities or different environmental factors investigated were associated to the differences in the variables of interest, such as *lowest frequency*, *highest frequency* and *call length* ($n = 150$ for *E. coqui* and $n = 151$ for *E. antillensis*, p -value < 0.0001 ; each value represents a recording from a male).

Furthermore, we wanted to test if the variables described above (“CO” “QUI” and “CHU” “RI”) were being affected in the same way in each locality. To do this, we used a Fisher’s LSD test with an $\alpha = 0.05$. This test compares each variable independently with each locality and groups them with similar variances. This test helps us estimate which populations are most related. The “coqui” and “churi” calls were divided into two distinct notes. These notes were selected as independent and were analyzed as follows: highest and lowest frequency, energy (d), power (dB), call length (s) and amplitude. Also, the entire call length and time between each note was measured for each call (Figure 8). Additionally, linear regressions were performed to establish relations between body morphology (SVL) and frequency.

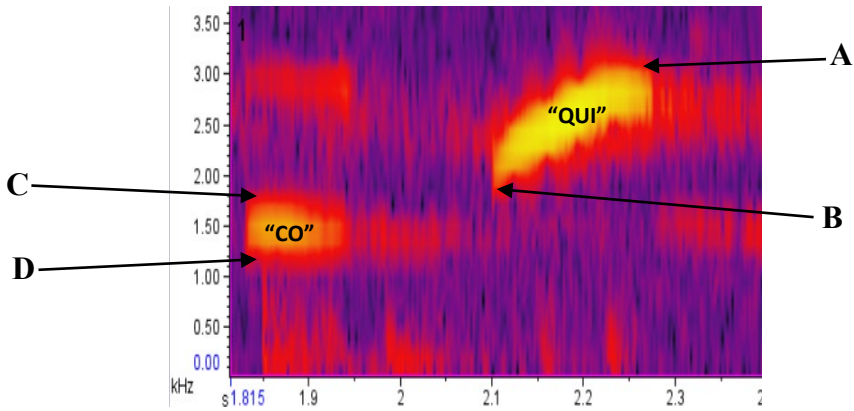


Figure 8. The image represents a spectrogram generated by Raven Pro®. The spectrogram shows the *Eleutherodactylus coqui* call and the arrows indicate the highest (A and C) and lowest (B and D) frequency of the “CO” and “QUI” note. This image is presented in color for it allows better visualization of the different spectral regions of the notes. The number “1” that appears on the upper left-hand corner of the panel represent the “click” counts that the software automatically places on the spectrograph. It is meaningless for the analyses.

Results

Morphology, body temperature, and acoustics of Eleutherodactylus coqui

Common organisms, such as *Eleutherodactylus coqui*, can be more amenable for research owing to its widespread distribution and environmental diversity that can be suited for comparative analysis. For this species, five localities were selected, from coastal areas to high elevation mountains. Morphological, body temperature, (Table 1), as well as acoustic data (Tables 2 and 3) were garnered. For acoustic data, ANOVA yielded significant differences between all populations across Puerto Rico ($n = 150$, p -value < 0.0001) for all variables, including a small difference for the highest frequency for the “CO” note ($n = 150$, p -value < 0.0004). Temperature data did not relate to any other variable.

By analyzing these notes individually, we can assess small fluctuations in sound that can affect intraspecific communication. Calling behavior plays a key role in frog reproduction and defense of territory. The “CO” note refers to a territorial call by which males defend their territory against other males. The “QUI” note functions as a call to attract females (Narins and Capranica 1978, Narins and Smith 1986). Additionally, as part of the analysis, total call duration was measured. Call duration (seconds), can be correlated to anuran metabolism. Individuals located in colder environments tend to have a slower call than its cohorts in higher temperatures due to changes in metabolic rate. Here, we measured the notes independently, “CO” and “QUI” and in their entirety, “CO QUI”.

Table 1. Mean and standard deviation for morphological traits and body temperature of *Eleutherodactylus coqui* for localities with n = 30.

Locality	SVL (mm)	TL (mm)	Head width (mm)	Tympanum diameter (mm)	Body temperature (°C)
Utuaado	28.35 sd = 2.40	15.25 sd = 1.85	11.62 sd = 3.02	2.25 sd = 0.33	21.21 sd = 1.01
Mayagüez	28.21 sd = 3.51	12.83 sd = 2.63	11.33 sd = 2.04	1.63 sd = 0.07	21.65 sd = 0.79
Jayuya	29.6 sd = 2.67	15.84 sd = 2.50	12.31 sd = 1.79	2.29 sd = 0.42	20.27 sd = 0.92
Arecibo	21.00 sd = 3.67	13.50 sd = 0.92	11.5 sd = 0.04	2.10 sd = 0.01	21.38 sd = 0.85
Río Piedras	26.10 sd = 2.20	12.10 sd = 2.25	11.20 sd = 0.64	2.30 sd = 0.46	21.56 sd = 0.95

We analyzed the “CO” and “QUI” notes in five localities which include: Mayagüez, Arecibo, Jayuya, Utuaado and Río Piedras. As shown in Figure 7, the note was analyzed by measuring highest and lowest frequency of the note in a spectrogram (96kHz, 24bit, 1 channel).

Advertisement “CO-QUI” note

Beginning with the lower part of the “CO” note, the Fisher’s LSD shows three distinct populations denoted by letters in Table 2. Jayuya had the lowest mean frequency of all populations, followed by Río Piedras and Utuaado (no significant differences) and finally Mayagüez and Arecibo with the highest frequency of the note (24.0 m and 3.0 m elevation respectively). Furthermore, the results of the upper part of the “CO” note also shows three distinct populations, although with less differentiation between them. Interestingly, Jayuya still has the lowest frequency but this time Río Piedras instead of Arecibo has the highest frequency.

Courtship “CO-QUI” note

For the lower part of the “QUI” note, the Fisher’s LSD shows four distinct populations, Jayuya being the lowest frequency and Río Piedras being the highest of the group. Also, the Fisher’s LSD shows that when we observe the upper part of the “QUI” note, Jayuya still has the lowest frequency and Río Piedras the highest (Table 2).

Table 2. Fisher's Least Significant Difference (LSD) for frequencies in <i>Eleutherodactylus coqui</i> in different localities in Puerto Rico. Dissimilar populations are denoted by different letters (n = 150).					
Note	Parameter	Locality	Mean (sec)	Populations	Comments
"CO"	Lowest Frequency	Jayuya	1.05	A	Alpha = 0.05, LSD = 0.09397, Error = 0.0339, df = 145
		Río Piedras	1.27	B	
		Utuaado	1.31	B	
		Mayagüez	1.48	C	
		Arecibo	1.54	C	
"QUI"	Lowest Frequency	Jayuya	1.83	A	Alpha = 0.05, LSD = 0.12460, Error = 0.0596, df = 145
		Utuaado	2.24	B	
		Mayagüez	2.33	B C	
		Arecibo	2.45	C D	
		Río Piedras	2.49	D	
"CO"	Highest Frequency	Jayuya	1.89	A	Alpha = 0.05, LSD = 0.11618, Error = 0.0518, df = 145
		Arecibo	2.01	B	
		Utuaado	2.03	B C	
		Mayagüez	2.11	B C	
		Río Piedras	2.14	C	
"QUI"	Highest Frequency	Jayuya	3.15	A	Alpha = 0.05, LSD = 0.12460, Error = 0.0596, df = 145
		Utuaado	3.44	B	
		Arecibo	3.52	B C	
		Mayagüez	3.62	C D	
		Río Piedras	3.69	D	

Table 3. Fisher's Least Significant Difference (LSD) for the length of independent notes an entire call duration in <i>Eleutherodactylus coqui</i> . Dissimilar populations are denoted by different letters (n = 150). Means with a common letter are not significantly different (p > 0.05).					
Note	Parameter	Locality	Mean (sec)	Populations	Comments
"CO"	Duration	Río Piedras	0.08	A	Alpha = 0.05, LSD = 0.01912, Error = 0.0014, df = 145
		Mayagüez	0.10	A	
		Arecibo	0.12	B	
		Jayuya	0.12	B	
		Utuaado	0.17	C	
"QUI"	Duration	Río Piedras	0.09	A	Alpha = 0.05, LSD = 0.01861, Error = 0.0013, df = 145
		Mayagüez	0.18	B	
		Arecibo	0.18	B C	
		Jayuya	0.19	B C	
		Utuaado	0.23	C	
"CO" "QUI"	Inter-note interval	Utuaado	0.09	A	Alpha = 0.05, LSD = 0.11618, Error = 0.0518, df = 145
		Arecibo	0.12	B	
		Mayagüez	0.12	B C	
		Río Piedras	0.13	B C	
		Jayuya	0.14	C	
"CO QUI"	Total duration of call	Utuaado	0.31	A	Alpha = 0.05, LSD = 0.04173, Error = 0.0067, df = 145
		Arecibo	0.39	B	
		Mayagüez	0.42	B C	
		Río Piedras	0.45	C D	
		Jayuya	0.48	D	

Call duration

The area with the shortest territorial note, “CO”, was the population located in Río Piedras followed by the populations in Mayagüez, Arecibo, Jayuya, and Utuado with the longest note as expected. The Fisher’s LSD separates this populations in three distinct groups (Table 3). The courtship note, “QUI”, had the exact Fisher’s LSD order as the territorial note, being Río Piedras the shortest note and Utuado the longest. Also, the inter-note interval between the “CO” and the “QUI” notes was measured (Table 3). Additionally, Utuado and Jayuya had the shortest and longest inter-note interval respectively, leaving the coastal populations with intermediate pauses between the first two. Finally, the entire call was measured to observe which population had the shortest and the lowest call of all the populations studied. As expected, lower elevation populations such as Río Piedras, Mayagüez and Arecibo, had shorter calls than the populations located in higher mountainous areas like Jayuya and Utuado (Narins 1995) (Table 3).

Morphology, body temperature, and acoustics of *Eleutherodactylus antillensis*

As *Eleutherodactylus coqui*, *E. antillensis* is also widespread in Puerto Rico but it was thought that it could be found more commonly in disturbed low elevations areas. These characteristics make it possible for researchers to study the species without negatively affecting their populations. As with *E. coqui*, we analyzed the *E. antillensis* two-note call (“CHU- RI”), for frequencies and body morphometry (Table 4) in different populations across the island of Puerto Rico. For this species, Río Piedras was substituted for Culebra Island due to the absence of the species in recordings.

Table 4. Mean and standard deviation for morphological traits and body temperature of *Eleutherodactylus antillensis* for localities with n = 30.

Locality	SVL (mm)	TL (mm)	Head width (mm)	Tympanum diameter (mm)	Body temperature (°C)
Utuado	26.52 sd = 1.01	12.47 sd = 0.66	9.88 sd = 1.07	2.01 sd = 0.06	24.70 1.12
Arecibo	27.66 sd = 0.89	10.03 sd = 0.33	10.75 sd = 0.54	3.0 sd = 0.78	24.66 sd = 0.45
Mayagüez	27.28 sd = 1.13	10.03 sd = 0.09	12.00 sd = 1.50	2.28 sd = 0.16	21.50 sd = 0.65
Culebra	23.45 sd = 1.42	11.17 sd = 1.89	9.47 sd = 1.26	1.73 sd = 0.56	22.65 sd = 0.66
Jayuya	22.8 sd = 1.34	10.30 sd = 1.37	8.70 sd = 1.99	2.40 sd = 0.22	22.18 sd = 0.89

“CHU-RI” note

Frequency measurements from the lower part of the “CHU” note spectrogram shows a lower frequency on the populations found in the mountainous region, being the Jayuya population the lowest. On the other hand, the Culebra population had the highest frequency of all (Table 5). Also, with the latest population, the Fisher’s LSD acknowledged this population as a separate group. Moreover, results from the upper part of the “CHU” note spectrogram shows an interesting pattern displaying the highest and lowest frequencies belong to the populations in high elevations (e.g., Jayuya and Utuado, respectively) and the intermediates to the lower elevation localities (e.g., Mayagüez, Culebra, and Arecibo).

“CHU-RI” note

Within the call, the “RI” note is something that this species can modulate to emit the unmistakable “QUI, QUI, QUI...” thrill call that Ovaska and Caldbeck (1997) described. The sound spectrogram presented similarities with the first note such as, frequency patterns with populations. For example, the high elevation populations still have the lowest frequency of all populations and as we go down in elevation, their frequencies get progressively higher (Table 5).

Table 5. Fisher's Least Significant Difference (LSD) for frequencies in *Eleutherodactylus antillensis*. Dissimilar populations are denoted by different letters (n =151). Means with a common letter are not significantly different (p > 0.05).

Note	Parameter	Locality	Mean (sec)	Populations	Comments
“CHU”	Lowest Frequency	Jayuya	1.26	A	Alpha = 0.05, LSD = 0.09305, Error = 0.0335, df = 146
		Utuado	1.31	A	
		Arecibo	1.54	B	
		Mayagüez	1.56	B	
		Culebra Is.	1.87	C	
“RI”	Lowest Frequency	Utuado	1.95	A	Alpha = 0.05, LSD = 0.12652, Error = 0.0619, df = 146
		Jayuya	1.95	A	
		Mayagüez	2.26	B	
		Arecibo	2.32	B	
		Culebra Is.	2.57	C	

		Jayuya	1.79	A	Alpha = 0.05, LSD = 0.16422, Error = 0.1042, df = 146
		Mayagüez	2.23	B	
“CHU”	Highest Frequency	Culebra Is.	2.26	B	
		Arecibo	2.26	B	
		Utuaado	2.58	C	
		Jayuya	2.93	A	Alpha = 0.05, LSD = 0.18152, Error = 0.1274, df = 146
		Mayagüez	3.14	B	
“RI”	Highest Frequency	Arecibo	3.20	B	
		Culebra Is.	3.32	B	
		Utuaado	3.53	C	

Call Duration

Examination of the duration of individual notes (CHU-RI), inter-note intervals and total call duration demonstrates that populations at lower elevations have shorter calls than populations at higher elevations. More importantly, populations such as Utuaado and Jayuya appear to be a separate group from the others (Table 6).

Table 6. Fisher's Least Significant Difference (LSD) for the length of independent notes an entire call duration in <i>Eleutherodactylus antillensis</i> . Dissimilar populations are denoted by different letters (n = 151). Means with a common letter are not significantly different (p > 0.05).					
Note	Parameter	Locality	Mean (sec)	Populations	Comments
		Mayagüez	0.04	A	Alpha = 0.05, LSD = 0.04370, Error = 0.0074, df = 146
		Culebra Is.	0.08	A B	
“CHU”	Duration	Arecibo	0.08	A B	
		Utuaado	0.10	B C	
		Jayuya	0.13	C	
		Mayagüez	0.07	A	Alpha = 0.05, LSD = 0.04954, Error = 0.0095, df = 146
		Arecibo	0.09	A B	
“RI”	Duration	Culebra Is.	0.10	A B	

		Utuaado	0.14	B C	
		Jayuya	0.22	C	
		Mayagüez	0.03	A	Alpha = 0.05 LSD = 0.05133 Error = 0.0102 df = 146
		Arecibo	0.04	A	
“CHU” — “RI”	Inter-note interval	Culebra Is.	0.04	A	
		Utuaado	0.04	A	
		Jayuya	0.15	B	
		Mayagüez	0.12	A	
		Arecibo	0.15	A	
“CHU RI”	Total duration of call	Culebra Is.	0.18	A	
		Utuaado	0.28	B	
		Jayuya	0.50	C	

Discussion

Vocalization evolves as the result of a variety of selection pressures in the environment (Drewry and Rand 1983). *Eleutherodactylus* is one of the few genera of anurans that can use the same notes to engage in courtship and advertisement calls rearranging the notes (Narins and Capranica 1978, Stewart and Rand 1991, Stewart and Bishop 1994). A study conducted on the genus *Eleutherodactylus* in the main island of Puerto Rico showed that *E. antillensis* has a high activity peak call during midnight and low activity at dusk and dawn (Drewry and Rand 1983). In Culebra Island, the pattern of activity was different as this species was more active during dusk and dawn (Ríos-Franceschi et al. 2016). These differences may function in defense of a calling territory to other males (Ovaska and Caldbeck 1997). In the case of Culebra Island, the frequency of calls by *E. antillensis* was broader in range compared to its conspecifics at El Yunque Rain Forest (Drewry and Rand 1983), being 1.05 kHz higher (Ríos-Franceschi et al. 2016). It is known that frogs can modify the amplitude of the call depending of the environment, depending on how open or closed is their habitat space and the intensity of interspecific competitors (Drewry and Rand 1983, Lopez et al. 1988). For visual reference, see Figures 9-10 and 12.

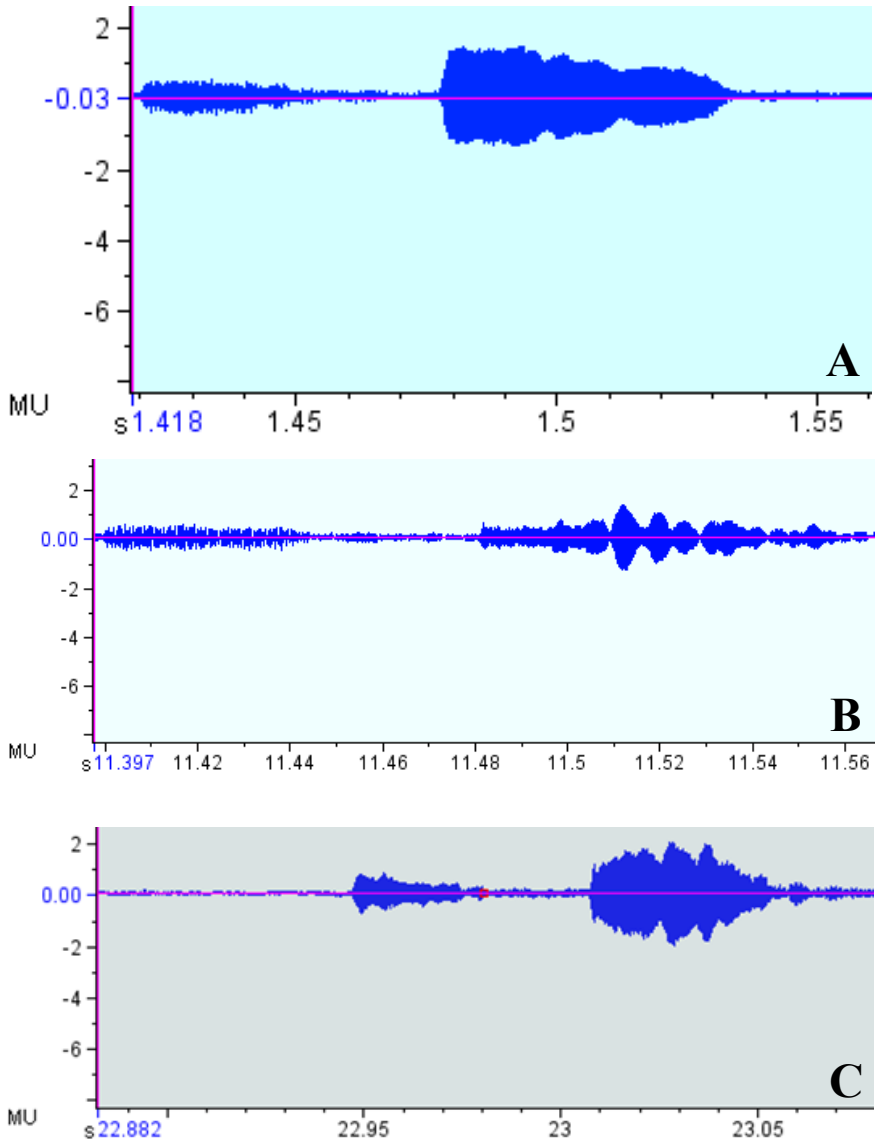


Figure 9. Representative waveforms (amplitude vs. time) of advertisement calls of *Eleutherodactylus antillensis* in four localities in Puerto Rico. A, Arecibo; B, Jayuya; and C, Río Piedras. Each plot was generated using Raven Pro1.5®, Cornell University.

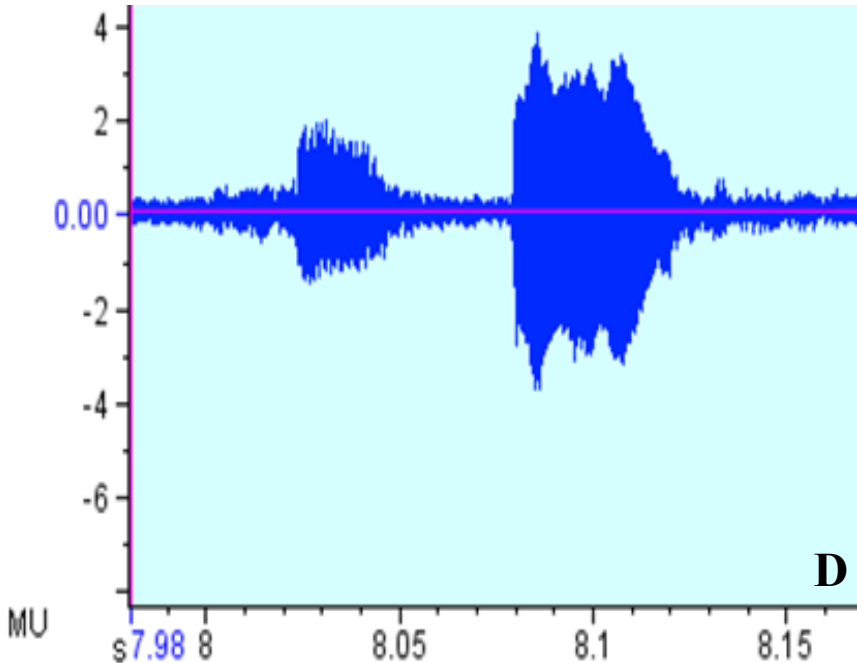


Figure 9 (continuation). Representative waveforms (amplitude vs. time) of advertisement calls of *Eleutherodactylus antillensis* in Puerto Rico. D, Utuado. Each plot was generated using Raven Pro1.5®, Cornell University.

Eleutherodactylus antillensis. Spectrographs showed that *Eleutherodactylus antillensis* frogs usually trill (aggressive calls), which are used in defense of a territory (Ovaska and Caldbeck 1997) when *E. coqui* is present. It is well known that these two species share the same habitat, thus they may compete for the same resources. Narins (1995) stated that frog calling assemblages can exhibit time-sharing on a millisecond time scale or on a diel time scale, restricting their calling to particular times of the day (Villanueva-Rivera 2014, Ospina et al. 2013). *Eleutherodactylus coqui* does not seem to be occurring in Culebra Island because both species were recorded calling at the same time in almost every single recording from Culebra Island. On the other hand, at the other data collection localities there seems to be evidence of time-sharing (Narins 1995) between *Eleutherodactylus antillensis* and *E. coqui*.

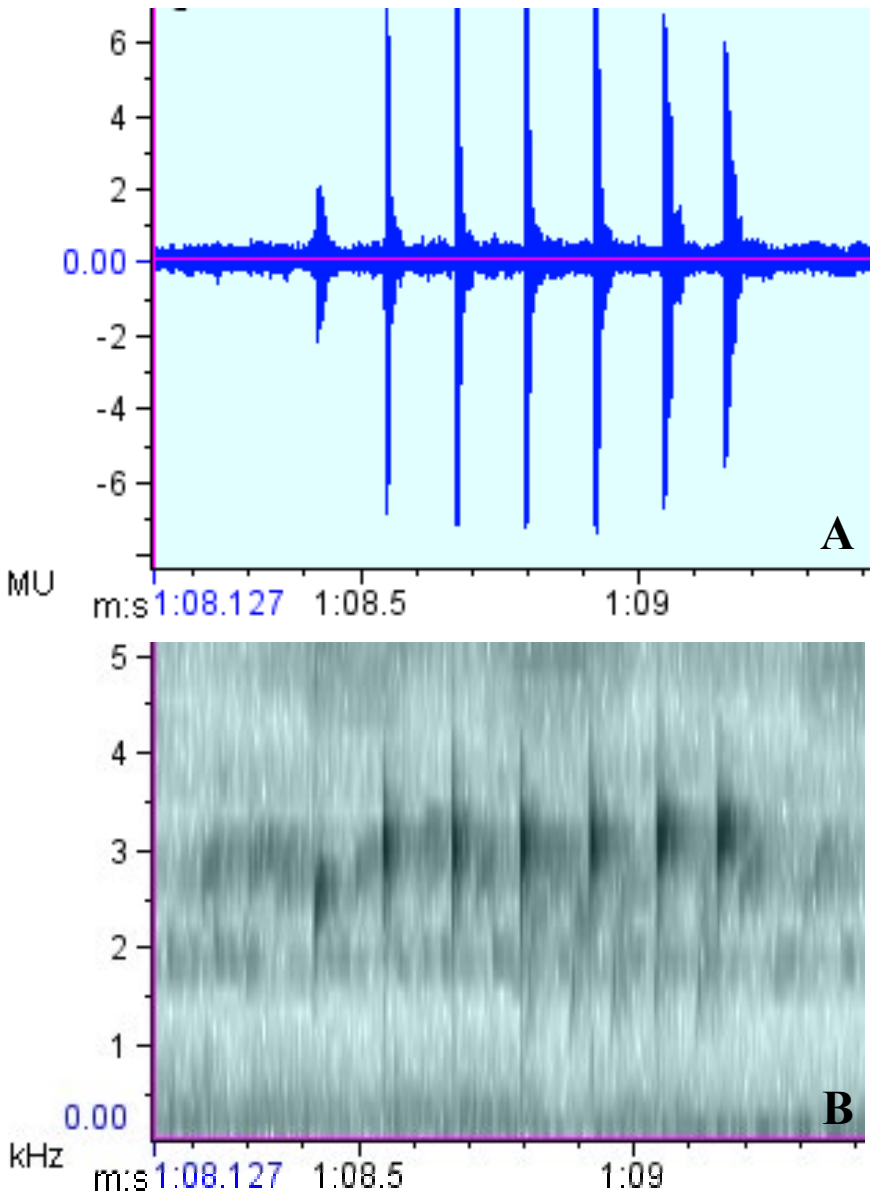


Figure 10. Representative waveform of *Eleutherodactylus antillensis* in Puerto Rico. A. Amplitude vs. time. B. Spectrograph of “thrill” call.

Environmental factors such as, humidity, temperature, elevation and body size are known to affect the call frequencies and rates with which a male can communicate. By examining populations across several localities (e.g., in this study, all secondary forests, Figure 15) using an elevation gradient, we can analyze these factors. For example, a recent study testing the hypotheses surrounding vicariance and dispersal using mtDNA sequences from *E. antillensis*, found support for a hypothesis of colonization of the islands east of Puerto Rico from sources located in eastern Puerto Rico during the penultimate and last glacial period. This occurred when a land bridge united the Puerto Rican Bank, thus dividing populations in different haplotypes (Barker et. al. 2012), probably contributing to speciation. Using linear regressions, we found that the size (SVL) of *E. antillensis* frogs tends to be negatively correlated to call frequency (kHz) although this trend is not statistically significant. High “CHU” and “RI” frequencies were significantly correlated with SVL, the larger the frog, the lower the frequency, but these correlations were not considered strong ($R^2 = 0.48$ and $R^2 = 0.52$, respectively, Figure 11). The reason could be to low frog collection in each locality ($n = 30$, per locality).

As is known, the larger the organism, the lower its call frequency, but in this case, Arecibo (Puerto Rico), is located nine meters above sea level and, as expected, their population had one of the highest sonic frequencies of all, but more importantly, it had the largest individuals of all populations (mean SVL = 27.66 mm, $n = 30$). Morphological features such as the size of the larynx may play a key role in frequency production in each population (Ríos-Franceschi, unpublished data). Also, for *E. antillensis* spectrogram analysis revealed highest energy (dB) in Jayuya, Puerto Rico with a mean dB = 132 and max dB = 170, $n = 10$.

If we combine the ethological and morphological data collected, we can suggest that each population of *E. antillensis* has a different accent, in such cases, some populations vary so much that we could speculate that sexual selection could be in play. This suggests that selection has likely favored the evolution of a specialized neural auditory mechanism for extracting time and frequency information from such calls (Hall 1994, Richards 2006).

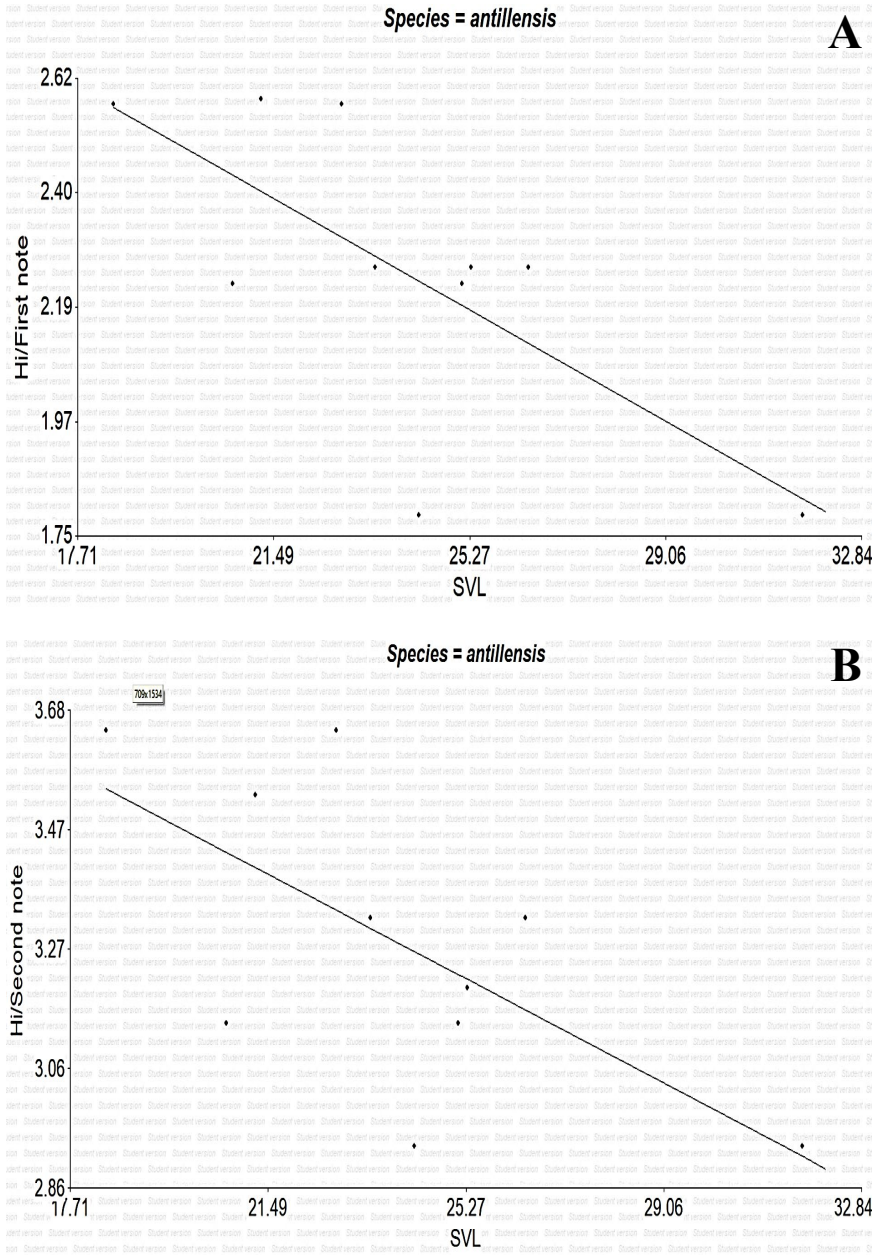


Figure 11. Linear regressions of *E. antillensis*. A, SVL* High frequency “CHU” note. B, SVL* High frequency “RI” note.

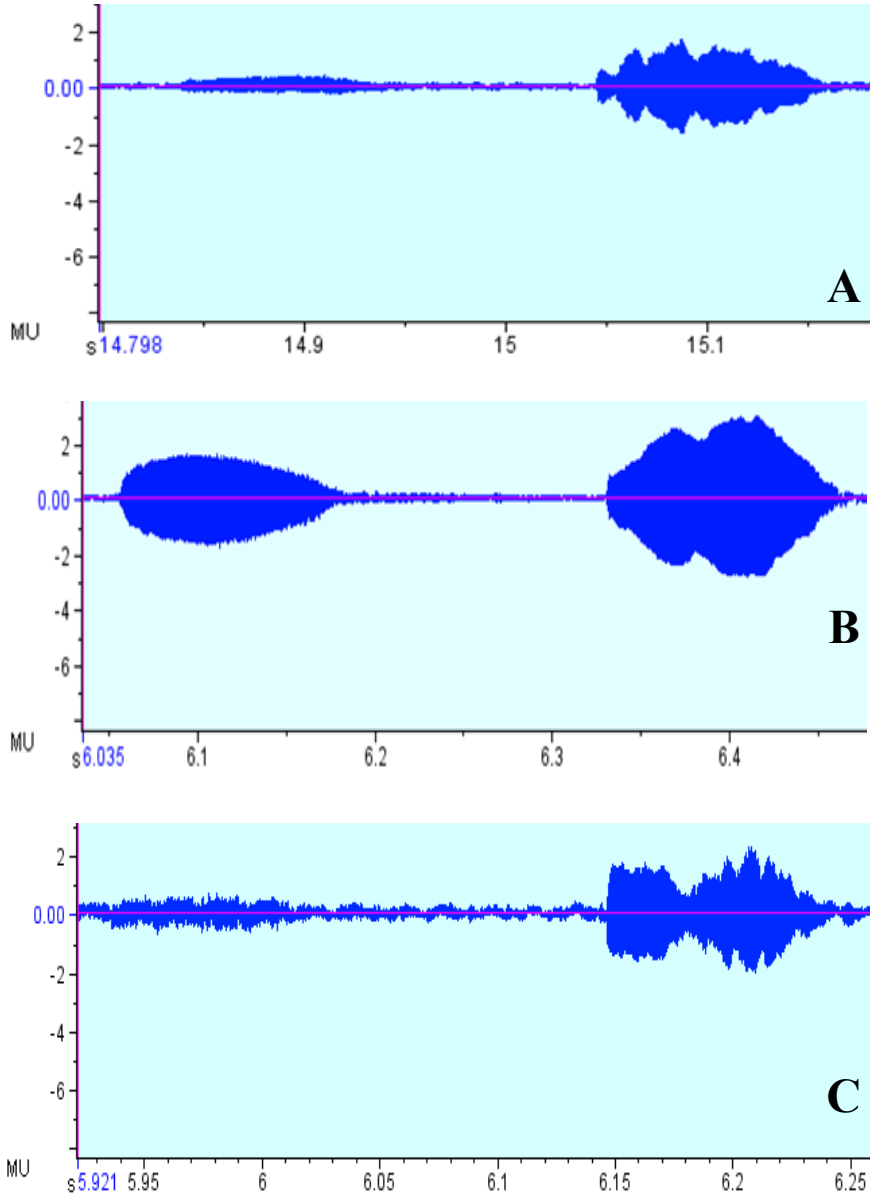


Figure 12. Representative waveforms (amplitude vs. time) of advertisement calls of *Eleutherodactylus coqui* in five localities in Puerto Rico. A, Arecibo and B, Jayuya. Each plot was generated using Raven Pro1.5[®], Cornell University.

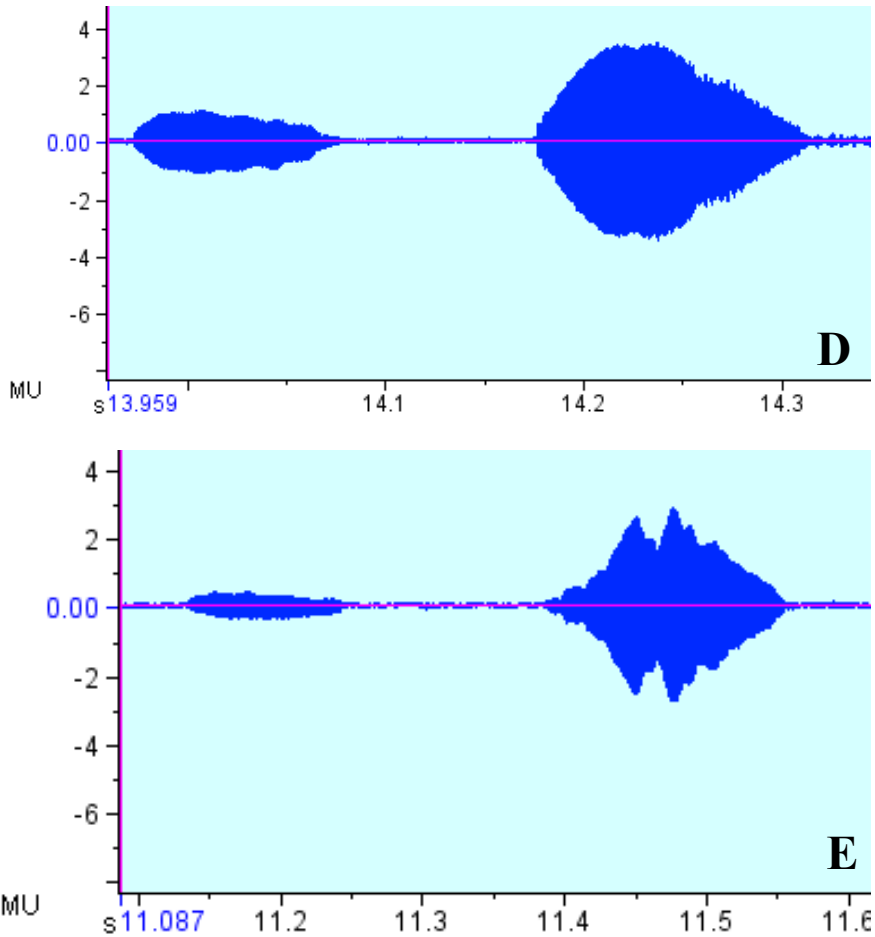


Figure 12 (continuation). Representative waveforms (amplitude vs. time) of advertisement calls of *Eleutherodactylus coqui* in Puerto Rico. C, Río Piedras; D, Utuado; and E, Mayagüez. Each plot was generated using Raven Pro1.5®, Cornell University.

If we compare the mountainous populations, Utuado and Jayuya, they can call at the same lower frequencies of both notes (e.g., CHU-RI), but the population located in Utuado has a higher frequency range. Furthermore, the Utuado population has shorter calls than the Jayuya population. We can assume that the Utuado and Jayuya populations can communicate using different accents. However, if we compare the populations at a lower elevation locality, such as Figure 11. Linear regressions of *E. antillensis*. A, SVL* High frequency “CHU” note. B, SVL* High frequency “RI” note.

Frogs from Culebra Island and Jayuya are so sonically dissimilar (Figure 13) that they may communicate in different spectral and temporal properties that they may not recognize each other if they are placed together, promoting sexual divergence and isolation, which could lead to speciation. Research on anuran papillae demonstrates spectral sensitivity to distinct frequencies (Meenderink et al. 2010); in other words, each isolated population varies in spectral and temporal properties in which case, the auditory papillae can be fixed to specific frequencies. This can be tested by doing playbacks in every population to observe intra-sexual and inter-sexual response.

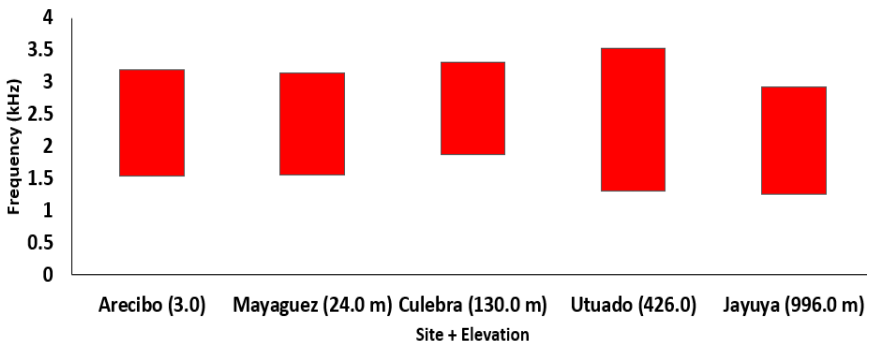


Figure 13. Total frequency range of *E. antillensis* at each locality (elevation, in meters, above sea level).

Eleutherodactylus coqui. For the call frequencies of *E. coqui*, the population in Jayuya, seems to emit territorial and courtship call at different frequencies that frogs at the other localities analyzed. This is important because this species has specific frequency sensitivity, so the probability that the population in Jayuya can communicate effectively with any other population analyzed in this research is low (Figure 14). This hypothesis has to be tested by playbacks recordings in each location.

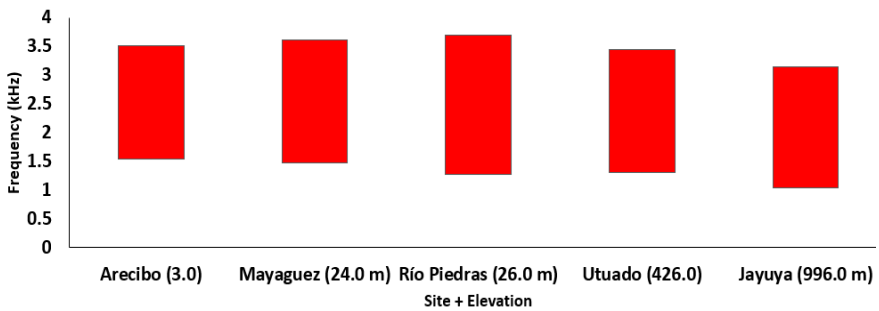


Figure 14. Total frequency range of *E. coqui* at each locality (elevation, in meters, above sea level).

For the acoustic communication to be successful, the individuals must be sensitive to spectral and temporal signals in the call (Gerhardt and Schwartz 2001, Meenderink et al. 2010). Spectral and temporal properties of a call can be affected by temperature and morphology, such as body size (Littlejohn 1977, Narins and Smith 1986). The call frequency in *E. coqui* varies with an altitudinal gradient, rather than temperature (a 6°C decrease every 1,000 meters, El Yunque National Forest), SVL was suggested to be the reason of these variations in calling frequencies within the altitudinal gradient in El Yunque National Forest (Meenderink et al. 2010). Linear regressions testing SVL and frequencies revealed a weak ($R^2 < 0.55$) negative correlation between the two variables, but none was statistically significant, probably caused by low data collection. Background noise such as the El Yunque National Forest can influence amphibian diel calling patterns and temporal properties of their calls in presence of sound interference (Narins 1982, Narins and Smith 1986). For them, to detect calls in noisy environments requires the appropriate sensitive mechanism (Ryan 1986, Meenderink et al. 2010). Also, for *E. coqui* spectrogram analysis revealed highest energy (dB) in Utuado, Puerto Rico with a mean dB = 127 and max dB = 155, n = 10.

Intraspecific divergence in mating signals, whether generated by sexual selection, pleiotropic effects, drift, or other evolutionary processes when correlated with divergence in female preference can lead to reproductive isolation and speciation (Richards 2006). Females often maintain this isolation through selection of male calls. Furthermore, anurans adapt to these environmental changes by modifying their behavior and possibly their inner ear anatomy to adjust to these changes in acoustical signals. Although ear anatomy is an important part of signal reception, this paper has focused on signal production.

Acknowledgments

Many thanks to the following undergraduate students: Fabio Lugo, Carlos Germosen, Bianca Arzuaga, Josué Maldonado, Eveyzkalis Méndez, and to Teodoro Torres, a resident of Culebra Island. Also, my thanks to the US Fish and Wildlife Service, specially to Ana Román for facilitating the permit process (OMB Control # 1018-0102). Many thanks to the Department of Environmental and Natural Resources (DNER), specially to Ramón L. Rivera for the permit (O-VS-PVS15-SJ-00977-12042018). We are grateful to Dr. Peter Narins (University of California, Los Angeles, California, USA) and seven anonymous reviewers for making this manuscript better.

Literature Cited

- Andersson, E., S. R. Kumar, J. Ackers, G. L. Taranger, and S. O. Stefansson. 2003. Photoperiod and temperature affects seasonal ovarian gene expression of p450 aromatase and gonadotropin receptors in Atlantic Salmon (*Salmo salar* L.) broodstock. *Fish Physiology and Biochemistry* 28(1-4):411. <https://doi.org/10.1023/B:FISH.0000030608.76252.22>
- Angilletta M. J. Jr. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press. Oxford, England, UK. 195 pp. <https://doi.org/10.1093/acprof:oso/9780198570875.001.1>
- Angulo, A. and S. Reichle. 2008. Acoustic signals, species diagnosis, and species concepts: the case of a new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia. *Zoological Journal of the Linnean Society* 152:59-77. <https://doi.org/10.1111/j.1096-3642.2007.00338.x>

- Barker, B., J. A. Rodríguez-Robles, V. S. Aran, and J. A. Cook. 2002. Phylogeography of the Puerto Rican Red-eyed Coqui, *Eleutherodactylus antillensis*. *Molecular Ecology* 21(24):6033-6052. <https://doi.org/10.1111/mec.12020>
- Benedix, J. H. Jr., M. Pedemonte, R. Velluti, R. and P. M. Narins. 1994. Temperature-dependence of two-tone rate suppression in the northern leopard frog *Rana pipiens pipiens*. *Journal of Acoustic Society of America* 96: 2738-2745. <https://doi.org/10.1121/1.411280>
- Bernal X., C. Guarnizo, and H. Lüddecke. 2005. Geographic variation in advertisement call and genetic structure of *Colostethus palmatus* (Anura: Dendrobatidae) from the Colombian Andes. *Herpetologica* 61: 395-408. <https://doi.org/10.1655/04-87.1>
- Bickford, D., D. J. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22:148–155. <https://doi.org/10.1016/j.tree.2006.11.004>
- Boughman J. 2002. How sensory drive can promote speciation. *Trends in Ecology and Evolution* 17: 571–577. [https://doi.org/10.1016/S0169-5347\(02\)02595-8](https://doi.org/10.1016/S0169-5347(02)02595-8)
- Buckley, L. B. 2005. Lizard habitat partitioning on islands: the interaction of local and landscape scales. *Journal of Biogeography* 32:2113-2121. <https://doi.org/10.1111/j.1365-2699.2005.01340.x>
- Castellano S., B. Cuatto, R. Rinella, A. Rosso, and C. Giacoma. 2002. The advertisement call of the European treefrogs (*Hyla arborea*): a multilevel study of variation. *Ethology* 108:75–89. <https://doi.org/10.1046/j.1439-0310.2002.00761.x>
- Cossins, A. R., M. J. Friedlander, and C. L. Prosser. 1977. Correlations between behavioral temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes. *Journal of Comparative Physiology* 120:109–121. <https://doi.org/10.1007/BF00619309>
- Coyne, J. A., and H. A. Orr. 1998. The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society of London* 353:287-305. <https://doi.org/10.1098/rstb.1998.0210>
- Cox, C. B. and P. D. Moore. 2000. *Biogeography: An Ecological and Evolutionary Approach*. Sixth Edition. Blackwell Science Ltd. Oxford, England, UK. 298 pp.
- Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128:231-240. <https://doi.org/10.1016/j.biocon.2005.09.031>
- de Queiroz, A. 2002. Contingent predictability in evolution: key traits and diversification. *Systematic Biology* 51:917–929. <https://doi.org/10.1080/10635150290102627>
- Dodd, C. K. 2010. *Amphibian Ecology and Conservation: A handbook of techniques*. Oxford, University Press. Oxford, England, UK and New York, NY, USA. 556 pp.
- Dominguez-Cristóbal, C. M. 2006. Presencia del tabonuco (*Dacryodes excelsa*) en el acontecer histórico de Puerto Rico. *Acta Científica* 20(1-3):33-37.
- van Dongen, W., K. Maldonado, P. Sabat, and R. A. Vásquez. 2010. Geographic variation in the repeatability of a personality trait. *Behavioral Ecology* 21:1243–1250. <https://doi.org/10.1093/beheco/arq145>
- Duarte H., M. Tejedo, G. E. Drewry, and A. S. Rand. 1983. Characteristics of an acoustic community: Puerto Rican frogs of the Genus *Eleutherodactylus*. *Copeia* 4:941-953. <http://dx.doi.org/10.2307/1445095>
- Duarte, H., M. Tejedo, and M. Katzenberger 2011. Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology* 18, 412–421. <https://doi.org/10.1111/j.1365-2486.2011.02518.x>
- Duellman, W. E. and L. Trueb 1994. *Biology of Amphibians*. Johns Hopkins University Press. Baltimore, Maryland, USA. 696 pp.
- Eatock, R. A. and G. A. Manley. 1981. Auditory nerve fiber activity in the Tokay Gecko: II. Temperature effect on tuning. *Comparative Physiology* 142:219–226. <https://doi.org/10.1007/BF00605740>
- Egert, D. and E. R. Lewis. 1995. Temperature-dependence of saccular nerve fiber response in the North American bullfrog. *Hearing Research* 84:72–80. [https://doi.org/10.1016/0378-5955\(95\)00019-Z](https://doi.org/10.1016/0378-5955(95)00019-Z)

- Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist* 139: S125–S153. <https://doi.org/10.1086/285308>
- Erdtmann, L. K., P. I. Simões, A. C. Mello, and A. P. Lima. 2011. Do natural differences in acoustic signals really interfere in conspecific recognition in the pan-Amazonian frog *Allobates femoralis*? *Behaviour* 148:485–500. <https://doi.org/10.1163/000579511X565754>
- Ewel J. J. and J. L. Whitmore 1973. *The Ecological Life Zones of Puerto Rico and the U. S. Virgin Islands*. Forest Service Research Paper ITF-18. Institute of Tropical Forestry Rio Piedras. Forest Service United States Department of Agriculture. San Juan, Puerto Rico. 72 pp.
- Feder, M. E. and W.W. Burggren. 1992. *Environmental Physiology of the Amphibians*. The University of Chicago Press. Chicago, Illinois, USA. 472 pp.
- Feng, A. S., P. M. Narins, C. Xu, W. Lin, Z. Yu, Q. Qiu, Z. Xu, and J. Shen 2006. Ultrasonic communication in frogs. *Nature* 404:333–336. <https://doi.org/10.1038/nature04416>
- Fouquette, M. J. 1975. Speciation in chorus frogs. I. Reproductive character displacement in the *Pseudacris nigrita* complex. *Systematic Zoology* 24:16–23. <https://doi.org/10.1093/sysbio/24.1.16>
- Gayou, D. C. 1984. Effects of temperature on the mating call of *Hyla versicolor*. *Copeia* 1984:733–738. <https://doi.org/10.2307/1445157>
- Gerhardt, H. C. and K. M. Mudry. 1980. Temperature effects on frequency preferences and mating call frequencies in the green treefrog, *Hyla cinerea* (Anura: Hylidae). *Journal of Comparative Physiology A* 137:1–6. <https://doi.org/10.1007/BF00656911>
- Gerhardt, H. C. 1991. Female choice in Tree frogs: static and dynamic acoustic criteria. *Animal Behavior* 42:615–635. [https://doi.org/10.1016/S0003-3472\(05\)80245-3](https://doi.org/10.1016/S0003-3472(05)80245-3)
- Gerhardt, H. C. 1994. The evolution of vocalization in frogs and toads. *Annual Review of Ecology and Systematics* 25:293–324. <https://doi.org/10.1146/annurev.es.25.110194.001453>
- Gerhardt, C. H., and F. Huber. 2002. *Acoustic communication in insect and anurans*. University of Chicago Press. Chicago, Illinois, USA. 542 pp.
- Giacoma, C. and S. Castellano 2001. Advertisement call variation and speciation in the *Bufo viridis* complex. pp. 205–219. In: Ryan M. (Editor). *Anuran Communication*. Smithsonian Institution Press. Washington, District of Columbia, USA. 252 pp.
- Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C.T. Winne. 2000. The global decline of reptiles, déjà-vu amphibians. *Bioscience* 50: 653–667. [https://doi.org/10.1641/0006-3568\(2000\)050\[0653:TGDORD\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2000)050[0653:TGDORD]2.0.CO;2)
- Hall, J. C. 1994. Central processing of communication sounds in the anuran auditory system. *American Zoology* 34: 670–684. <https://doi.org/10.1093/icb/34.6.670>
- Heatwole, H., R. Levins, and M. D. Byer. 1981. Biogeography of the Puerto Rican Bank. *Atoll Research Bulletin* 251:1–55. <https://doi.org/10.5479/si.00775630.251.1>
- Helmuth, B., J. G. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? *Annual Review of Physiology* 67:177–201. <https://doi.org/10.1146/annurev.physiol.67.040403.105027>
- Hertwig, I. 1987. Morphogenesis of the inner ear of *Rana temporaria* (Amphibia, Anura). *Zoomorphology* 107: 103–114. <https://doi.org/10.1007/BF00312120>
- Hertwig, I. and H. Schneider. 1999. Comparative light and electron microscope study of the auditory organ of two species of fishes (Pisces): *Hyphessobrycon simulans* (Ostariophysi) and *Poecilia reticulata* (Acanthopterygii). *European Journal of Science* 37: 17–28. <https://doi.org/10.1076/ejom.37.1.17.4765>
- Heyer W.R. 1984. The systematic status of *Adenomera griseigularis* Henle, with comments on systematic problems in the genus *Adenomera* (Amphibia: Leptodactylidae). *Amphibia-Reptilia* 5: 97–100. <https://doi.org/10.1163/156853884X-005-02-04>
- Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz 2005. Reinforcement drives rapid allopatric speciation. *Nature* 437:1353–1356. <https://doi.org/10.1038/nature04004>
- Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. Alvarez Pérez, and T. Garland, Jr. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society: Biological Sciences* 276:1939–1948. <https://doi.org/10.1098/rspb.2008.1957>

- Hunter, M. and J. Krebs 1979. Geographical variation in the song of the great tit (*Parus major*) in relation to ecological factors. *Journal of Animal Ecology* 48: 759–785. <https://doi.org/10.2307/4194>
- Itoi, S., S. Kawahara, H. Kondo, T. Sakai, and S. Watabe. 2003. Changes in mitochondrial fatty acid composition following temperature acclimation of carp and their possible effects on F0F1-ATPase activity. *Fish Physiology and Biochemistry* 29:237–244. <https://doi.org/10.1023/B:FISH.0000045721.26261.a4>
- Jagla, W., I. Hertwig, and H. Schneider. 1994. Vergleichende licht- und elektronenmikroskopische Untersuchungen an den Höropapillen ausgewählter Amphibia. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 87:247.
- Jagla, W. and H. Schneider 1999. Comparative surface morphology of the auditory papillae of *Paramesotriton caudopunctatus* (Hu, Djao and Liu, 1973), *Ambystoma mexicanum* (Shaw, 1789) (Amphibia: Caudata) and *Bombina orientalis* (Boulenger, 1890) (Amphibia: Anura). *Amphibia-Reptilia* 20:133–147. <https://doi.org/10.1163/156853899X00150>
- Joglar, R. L. 1998. *Los Coquiés de Puerto Rico: Su Historia Natural y Conservación*. Editorial de la Universidad de Puerto Rico. San Juan, Puerto Rico. 231 pp.
- Joglar, R. L. 2005. *Biodiversidad de Puerto Rico: Vertebrados Terrestres y Ecosistemas*. Editorial del Instituto de Cultura Puertorriqueña. San Juan, Puerto Rico. 563 pp.
- Katzenberger, M. 2011. Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology* 18:412–421. <https://doi.org/10.1111/j.1365-2486.2011.02518.x>
- Kearney, M., R. Shine, and W. P. Porter. 2009. The potential for behavioral thermoregulation to buffer cold-blooded animals against climate warming. *Proceedings of the National Academy of Sciences of the United States America* 106:3835–3840. <https://doi.org/10.1073/pnas.0808913106>
- Kime, N. M., W. R. Turner, and M. J. Ryan. 2000. The transmission of advertisement calls in Central American frogs. *Behavioral Ecology* 11:71–83. <https://doi.org/10.1093/beheco/11.1.71>
- Lewis, E. R. 1976. Surface morphology of the bullfrog amphibian papilla. *Brain Behavioral Evolution* 13:196–215. <https://doi.org/10.1159/000123810>
- Lewis, E. R. 1978. Comparative studies of the anuran auditory papillae. *Scanning Electron Microscopy* 2:633–642.
- Lewis, E. R. 1984. On the frog amphibian papilla. *Scanning Electron Microscopy* 4:1899–1913.
- Littlejohn, M. J. 1965. Premating isolation in the *Hyla ewingi* complex (Anura, Hylidae). *Evolution* 19:234–243. <https://doi.org/10.1111/j.1558-5646.1965.tb01709.x>
- Llusia, D., R. Márquez, J. F. Beltrán, M. Benítez, and J. P. Do-Amaral. 2013. Calling behavior under climate change: geographical and seasonal variation of calling temperatures in ectotherms. *Global Change Biology* 19:2655–2674. <https://doi.org/10.1111/gcb.12267>
- Loftus-Hills, J. J. and M. J. Littlejohn 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (Anura, Microhylidae) a reexamination. *Evolution* 46:896–906. <https://doi.org/10.1111/j.1558-5646.1992.tb00607.x>
- Long, G. R., P. V. Dijk, and H. P. Wit. 1996. Temperature dependence of spontaneous otoacoustic emissions in the edible frog (*Rana esculenta*). *Hearing Research* 98:22–28. [https://doi.org/10.1016/0378-5955\(96\)00057-3](https://doi.org/10.1016/0378-5955(96)00057-3)
- López, P. T., P. M. Narins, E. R. Lewis, and S. W. Moore. 1988. Acoustically-induced call modification in the white-lipped frog *Leptodactylus albilabris*. *Animal Behaviour* 36:1295–1308. [https://doi.org/10.1016/S0003-3472\(88\)80198-2](https://doi.org/10.1016/S0003-3472(88)80198-2)
- Lörcher, K. 1969. Vergleichende bio-akustische Untersuchungen an der Rot-und Gelbbauchunke, *Bombina bombina* (L.) und *Bombina v. variegata* (L.). *Oecologia* 3:84–124. <https://doi.org/10.1007/BF00344635>
- Márquez, R. 1995. Female choice in the Midwife Toads (*Alytes obstetricans* and *A. cisternasii*). *Behaviour* 132:151–161. <https://doi.org/10.1163/156853995X00342>
- Márquez, R. and J. Bosch. 1995. Advertisement calls of the midwife toads *Alytes* (Amphibia, Anura, Discoglossidae) in Spain. *Journal of Zoological Systematics and Evolutionary Research* 33:185–192. <https://doi.org/10.1111/j.1439-0469.1995.tb00971.x>

Marten, K., D. Quine, and P. Marler. 1977. Sound transmission and its significance for animal vocalization: II. Tropical forest habitats. *Behavioral Ecology and Sociobiology* 2:291–302. <https://doi.org/10.1007/BF00299741>

Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. pp. 381–424. In, Claridge, M. F., H. A. Dawah, and M. R. Wilson (Editors). *Species: The Units of Biodiversity*. Chapman and Hall. New York, New York, USA. 442 pp.

Meenderink, S. W. F., M. Kits, and P. M. Narins. 2010. Frequency matching of vocalizations to inner-ear sensitivity along an altitudinal gradient in the coqui frog. *Biology Letters* 6:278–281. <https://doi.org/10.1098/rsbl.2009.0763>

Meier, U. 2006. A note on the power of Fisher’s least significant difference procedure. *Pharmaceutical Statistics* 5(4):253–263. Doi:10.1002pst/210

Morton, E. S. 1975. Ecological sources of selection on avian sounds. *American Naturalist* 109:17–34. <https://doi.org/10.1086/282971>

Narins, P. M. and R. R. Capranica. 1978. Communicative significance of the two-note call of the treefrog *Eleutherodactylus coqui*. *Journal of Comparative Physiology* 127:1–9. <https://doi.org/10.1007/BF00611921>

Narins, P. M. and S. L. Smith. 1986. Clinal variation in anuran advertisement calls: basis for acoustic isolation? *Behavioral Ecology and Sociobiology* 19:135–141. <https://doi.org/10.1007/BF00299948>

Narins, P. M. 1995. Frog Communication. *Scientific American* 273(2):78–83. <https://doi.org/10.1038/scientificamerican0895-78>

Narins, P. M. and S. W. F. Meenderink. 2014. Climate change and frog calls: long-term correlations along a tropical altitudinal gradient. *Proceedings of the Royal Society* 281(1783):20140401. <https://doi.org/10.1098/rspb.2014.0401>

Navarro, I., P. Rojas, E. Capilla, A. Albalat, and J. Castillo. 2002. Insights into insulin and glucagon responses in fish. *Fish Physiological Biochemistry* 27: 205–216. <https://doi.org/10.1023/B:FISH.0000032726.78074.04>

Nevo, E. and R. Capranica. 1985. Evolutionary origin of ethological reproductive isolation in cricket frogs. *Acris. Evolutionary Biology* 19:147–214. https://doi.org/10.1007/978-1-4615-6980-0_4

Ospina, O. E., L. J. Villanueva-Rivera, C. J. Corrada-Bravo, and T. M. Aide. 2013. Variable response of anuran calling activity to daily precipitation and temperature: implications for climate change. *Ecosphere* 4(4):47. <http://dx.doi.org/10.1890/ES12-00258.1>

Ovaska, K. E. and J. Caldbeck. 1997. Vocal behavior of the frog *Eleutherodactylus antillensis* on the British Virgin Islands. *Animal Behaviour* 54:181–188. <http://dx.doi.org/10.1006/ambe.1996.0414>

Papes, S. and F. Ladich. 2011. Effects of Temperature on sound production and auditory abilities in the Striped Raphael Catfish *Platydoras armatulus* (Family Doradidae). *PlosOne* 6(10):1–10. <https://doi.org/10.1371/journal.pone.0026479>

Pellet, J. and B. R. Schimdt. 2004. Monitoring distributions using call surveys: estimating site occupancy, detection probabilities and inferring absence. *Biological Conservation* 123:27–35. <https://doi.org/10.1016/j.biocon.2004.10.005>

Pires, A., and R. R. Hoy 1992. Temperature coupling in cricket acoustic communication. *Journal of Comparative Physiology A* 171:79–92. <https://doi.org/10.1007/BF00195963>

Pörtner, H. O. and R. Knust. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* (American Association for the Advancement of Science, Washington, District of Columbia, USA) 315:95–97. <https://doi.org/10.1126/science.1135471>

Preininger, D., M. Böckle, and W. Hödl. 2007. Comparison of anuran acoustic communities of two habitat types in the Danum Valley Conservation Area, Sabah, Malaysia. *Salamandra* 43:129–138.

Pröhl, H., S. Hagemann, J. Karsch, and G. Höbel. 2007. Geographic variation in male sexual signals in strawberry poison frogs (*Dendrobates pumilio*). *Ethology* 113:825–837. <https://doi.org/10.1111/j.1439-0310.2007.01396.x>

Pröhl, H., R. Koshy, U. Mueller, S. Rand, and M. Ryan. 2006. Geographic variation of genetic and behavioral traits in northern and southern túngara frogs. *Evolution* 60:1669–1679. <https://doi.org/10.1111/j.0014-3820.2006.tb00511.x>

- Quispe, R., C. P. Villavicencio, A. Cortés, and R. A. Vásquez. 2009. Inter-population variation in hoarding behaviour in degus, *Octodon degus*. *Ethology* 115:465–474. <https://doi.org/10.1111/j.1439-0310.2009.01621.x>
- Richards, C. L. 2006. Has the evolution of complexity in the amphibian papilla influenced anuran speciation rates? *European Society for Evolutionary Biology* 19(4):1222-1230. <https://doi.org/10.1111/j.1420-9101.2006.01079.x>
- Ríos-Franceschi, A., J. G. García-Cancel, F. J. Bird-Picó, and L. D. Carrasquillo. 2016. Spatiotemporal changes of the herpetofaunal community in Mount Resaca and Luis Peña Cay, Culebra National Wildlife Refuge, Culebra, Puerto Rico. *Life: The Excitement of Biology* 3(4):254-289. <https://blaypublishers.files.wordpress.com/2016/02/rc3ados-franceschi-et-al-2016-2015-leb-34254-289.pdf>
- Rivero, J. A. 1998. *Los Anfibios y Reptiles de Puerto Rico*. Second Edition. Editorial de la Universidad de Puerto Rico. San Juan, Puerto Rico. 510 pp.
- Röhr, D. L., and F. A. Juncá. 2013. Micro-habitat influence on the advertisement call structure and sound propagation efficiency of *Hypsiboas crepitans* (Anura: Hylidae). *Journal of Herpetology* 47(4):549–554. <https://doi.org/10.1670/10-210>
- Rosso, A., S. Castellán, and C. Giacoma. 2006. Preferences for call spectral properties in *Hyla intermedia*. *Ethology* 112:599–607. <https://doi.org/10.1111/j.1439-0310.2005.01186.x>
- Ryan, M. J. 1985. *The Tungara frog*. University of Chicago Press Chicago, Illinois, USA. 246 pp.
- Ryan, M. J. 1986. Neuroanatomy influences speciation rates among anurans. *Proceedings of the National Academy of Sciences of the United States America* 83:1379–1382. <https://doi.org/10.1073/pnas.83.5.1379>
- Ryan, M. J. 1988. Constraints and patterns in the evolution of anuran acoustic communication. pp. 637–677. In, B. Fritzsche (Editor). *The Evolution of the Amphibian Auditory System*. John Wiley and Sons Inc. New York, NY, USA. 705 pp.
- Ryan, M. J. and B. K. Sullivan. 1989. Transmission effects of temporal structures in the advertisement calls of two toads, *Bufo woodhousii* and *Bufo valliceps*. *Ethology* 80:182–189. <https://doi.org/10.1111/j.1439-0310.1989.tb00738.x>
- Ryan, M. J., R. B. Cocroft, and W. Wilczynski 1990. The role of environmental selection in intraspecific divergence of mate recognition signals in the Cricket Frog, *Acris crepitans*. *Evolution* 44:1869–1872. <https://doi.org/10.1111/j.1558-5646.1990.tb05256.x>
- Ryan, M. J. and W. Wilczynski. 1991. Evolution of intraspecific variation in the mating call of a cricket frog (*Acris crepitans*, hylidae). *Biological Journal of the Linnean Society* 44:249–271. <https://doi.org/10.1111/j.1095-8312.1991.tb00619.x>
- Sala, O. E., F. S. I. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, and D. H. Wall. 2000. Global biodiversity scenarios for the year 2100. *Science* (American Association for the Advancement of Science, Washington, District of Columbia, USA) 287:1770-1090. <https://doi.org/10.1126/science.287.5459.1770>
- Salem, M. A., and Y. Omura. 1998. Embryonic development of the Inner Ear and Otolith of the Rainbow Trout *Oncorhynchus mykiss*. *Archives of Histology and Cytology* 61(2):179–187.
- Schneider, H. 1974. Structure of the mating calls and relationships of the European tree frogs (Hylidae, Anura). *Oecologia* 14:99–110. <https://doi.org/10.1007/BF00344901>
- Siddall, M., E. J. Rohling, and A. Almogi-Labin. 2003. Sea-level fluctuations during the last glacial cycle. *Nature* 423:853–858. <https://doi.org/10.1038/nature01690>
- Smolders, J. W. T. and R. Klinke. 1984. Effects of temperature on the properties of primary auditory fibres of the spectacled caiman, *Caiman crocodilus* (L.). *Journal of Comparative Physiology* 155:19–30. <https://doi.org/10.1007/BF00610927>
- Stiebler, L., and P. M. Narins. 1990. Temperature-dependence of auditory nerve response properties in the frog. *Hearing Research* 46: 63-82. [https://doi.org/10.1016/0378-5955\(90\)90140-K](https://doi.org/10.1016/0378-5955(90)90140-K)
- Sorjonen, J. 1986. Factors affecting the structure of song and singing behavior of some Northern European passerine birds. *Behavior* 98:286–304. <https://doi.org/10.1163/156853986X01017>
- Stafford, K., S. Nieuwkirk, and C. Fox. 2001. Geographic and seasonal variation of blue whale calls in the north pacific. *Journal of Cetacean Research and Management* 3:65–76.

- Stewart, M. M. 1985. Arboreal habitat use and parachuting in a subtropical forest frog. *Journal of Herpetology* 19:391–401. <https://doi.org/10.2307/1564267>
- Stewart, M. M. and S. Rand. 1983. Characteristics of an acoustic community: Puerto Rican frogs of the genus *Eleutherodactylus*. *Copeia* 4:941-953. <https://doi.org/10.2307/1445095>
- Stewart, M. M. and S. Rand. 1991. Vocalizations and the defense of retreat sites by male and female frogs, *Eleutherodactylus coqui*. *Copeia* 4: 1013-1024. <https://doi.org/10.2307/1446096>
- Stewart, M. M. and P. J. Bishop. 1994. Effects of increased sound level of advertisement calls on calling male frogs, *Eleutherodactylus coqui*. *Journal of Herpetology* 28(1):46-53. <http://dx.doi.org/10.2307/1564679>
- Thrush, S. F., J. Halliday, J. E. Hewitt, and A. M. Lohrer. 2008. The effects of habitat loss, fragmentation, and community homogenization on resilience in estuaries. *Ecological Applications* 18:12–21. <https://doi.org/10.1890/07-0436.1>
- Van Dijk, P., H. P. Wit, and J. M. Segenhout. 1997. Dissecting the frog inner ear with Gaussian noise. II. Temperature dependence of inner ear function. *Hearing Research* 114:243–251. [https://doi.org/10.1016/S0378-5955\(97\)00169-X](https://doi.org/10.1016/S0378-5955(97)00169-X)
- Velázquez, N. A., J. Marambio, E. Brunetti, M. A. Méndez, R. A. Vázquez, and M. Penna. 2013. Bioacoustic and genetic divergence in a frog with a wide geographical distribution. *The Linnean Society of London, Biological Journal of the Linnean Society* 110:142–155. <https://doi.org/10.1111/bij.12113>
- Villanueva-Rivera L. J. 2014. *Eleutherodactylus* frogs show frequency but no temporal partitioning: implications for the acoustic niche hypothesis. *PeerJ* 2:e496. 1-14pp. <https://doi.org/10.7717/peerj.496>
- Vreddeburg, V. T. and D. B. Wake. 2007. Global declines of amphibians. pp. 691-699. In, *Encyclopedia of Biodiversity*. University of California. Berkeley, California, USA. 5,504 pp. <https://doi.org/10.1016/B978-012226865-6/00578-X>
- Walker, T. J. 1957. Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Annals of the Entomological Society of America* 50:626–636. <https://doi.org/10.1093/aesa/50.6.626>
- Walker, T. J. 1975. Effects of temperature on rates in poikilotherm nervous systems: evidence from the calling songs of meadow katyids (Orthoptera: Tettigoniidae: Orchelimum) and reanalysis of published data. *Journal of Comparative Physiology* 101:57–69. <https://doi.org/10.1007/BF00660119>
- Wells, K. D. 2007. *The Ecology and Behavior of Amphibians*. University of Chicago Press. Chicago, Illinois, USA. 1,400 pp. <https://doi.org/10.7208/chicago/9780226893334.001.0001>
- Wilbur, H. M., D. I. Rubenstein, and L. Fairchild. 1978. Sexual selection in toads: the roles of female choice and male body size. *Evolution* 32:264–270. <https://doi.org/10.1111/j.1558-5646.1978.tb00642.x>
- Wilczynski, W., M. J. Ryan, and E. A. Brenowitz. 1989. The display of the Blue-black Grassquit: the acoustic advantage of getting high. *Ethology* 80:218–222. <https://doi.org/10.1111/j.1439-0310.1989.tb00741.x>
- Wilczynski, W., A. C. Keddyhector, and M. J. Ryan 1992. Call patterns and basilar papilla tuning in cricket frogs. Differences among populations and between the sexes. *Brain Behavioral Evolution* 39:229–237. <https://doi.org/10.1159/000114120>
- Wilczynski, W. and M. Ryan 1999. Geographic variation in animal communication system. pp. 234-261. In, Foster, S. and J. Endler (Editors). *Geographic Variation in Behavior*. New Oxford University Press. New York, NY, USA. 336 pp.
- Wiley, R.H. and D. G. Richards 1978. Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behavioral Ecology and Sociobiology* 3:69–94. <https://doi.org/10.1007/BF00300047>



Figure 15. An example of a secondary forest where this study was conducted. These photos depict one of the sites located in the Rio Abajo State Forest, Utuado, Puerto Rico. A. Before Hurricane Maria hit Puerto Rico (September 20, 2017). B. Same site after the vegetation was destroyed by the hurricane. More information on the devastation caused by Maria to Puerto Rican forests can be found here: *3-D Views of Puerto Rico's Forests After Hurricane Maria*, available in this link: <https://www.youtube.com/watch?v=QeGfaqwDY3s>