

Variable response of anuran calling activity to daily precipitation and temperature: implications for climate change

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Abstract. Long-term monitoring of frog populations is needed to understand the effects of global change. To better understand the relationships between climate variation and calling activity, we monitored an anuran assemblage in a Puerto Rican wetland by sampling the acoustic environment for one minute every 10 minutes, for 41 months. By automating data collection using passive acoustic monitoring hardware, we collected more than 110,000 recordings. These recordings were analyzed using species-specific identification algorithms of four *Eleutherodactylus* species. The peak calling activity of *E. coqui* (>0.3 detection frequency) and *E. cochranae* (>0.2) occurred between April and September, and there was a clear decline in activity during the dry months of January–March. There was no clear annual pattern in *E. brittoni* or *E. juanariveroi*, but *E. juanariveroi* did show a significant decline in calling activity over the 41-month study (~0.5 to ~0.35). Calling activity of *E. coqui* and *E. cochranae* was positively correlated with temperature, while *E. brittoni* and *E. juanariveroi* responded negatively to temperature and precipitation. This difference in response to temperature and precipitation could be related to differences in body size and the location of calling sites among the four species. For example, *E. brittoni* and *E. juanariveroi* are small species that call from the tips of the vegetation. High temperatures and intense precipitation may cause them to retreat into the vegetation, and reduce calling activity. In contrast, *E. coqui* and *E. cochranae* call lower in the vegetation and from leaf axils where they are more protected. Based on these findings, future scenarios of climate change could pose a threat for the survival of the populations of these four species. New climate regimes could negatively affect the calling activity, and thus diminish reproductive events.

Key words: automated species identification; declining amphibian populations; *Eleutherodactylus*; long-term monitoring; passive acoustic monitoring; precipitation; Puerto Rico; temperature.

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INTRODUCTION

The overall response of amphibians to climate change, e.g., increasing temperature and drier conditions, has been negative (e.g., Pounds et al. 1999, Araujo et al. 2006, Duarte et al. 2012).

Population declines have been associated with an increase of only 0.5°C in temperature (Pounds and Crump 1994), and lower rainfall (i.e., prolonged periods with daily rainfall <3 mm) has been associated with population declines up to 80% (Stewart 1995). Most studies on the effect

of climate on amphibians have been based on data collected using the Visual Encounter Survey methodology (VES, Crump and Scott 1994). In VES, an observer searches for amphibians within a given area (e.g., transect) and for a given time. This methodology is good for determining the presence of species, particularly in homogeneous and open habitats where it is easy to see individuals, but it is time consuming, making it difficult to frequently sample a site. In most studies, sampling occurs over short time spans (e.g., intense sampling for 2–4 weeks) (e.g., Aichinger 1987, Navas 1996) or when sampling is done for more than a year, the sampling frequency is usually monthly (e.g., Hatano et al. 2002, Prado et al. 2005). As a consequence, VES has rarely been implemented for long time spans (multiple years) with frequent sampling (e.g., daily or hourly); nevertheless, accurate assessments of the effects of climate change on frogs require long-term and detailed monitoring (Parmesan and Yohe 2003). An alternative methodology, Passive Acoustic Monitoring (PAM), could help to produce long-term datasets with hourly or sub-hourly monitoring of frog populations and assemblages (e.g., Bridges and Dorcas 2000, Dorcas et al. 2009, Steelman and Dorcas 2010).

The use of long-term and continuous PAM is particularly important since amphibians are becoming extinct 200 times faster than the background rate (McCallum 2007, Roelants et al. 2007), and the populations of more than 40% of amphibian species are declining (Alford and Richards 1999, Stuart et al. 2004). In the particular case of anurans (i.e., frogs and toads), approximately 400 species have been classified in risk of extinction since 1980 (Bielby et al. 2008). In response to these high levels of decline and extinction, international initiatives (e.g., Amphibian Conservation Action Plan [ACAP], Gascon et al. 2007; Global Amphibian Assessment [GAA], Stuart et al. 2004) have called for the implementation of long-term monitoring and assessment around the world (Blaustein et al. 1994) to determine the factors causing these declines. This increasing concern for anuran extinctions is not surprising because the loss of anuran populations and species could disrupt important ecosystem processes such as energy flow and food web dynamics (e.g., Ranvestel et al. 2004, Gibbons et al. 2006, Whiles et al. 2006). More-

over, anurans are considered good bioindicators, and the condition of their populations is a warning to us about the status of ecosystems (Hayes et al. 2002, Halliday 2008).

A number of causes have been proposed to explain population declines and species extinctions. However, the anuran crisis cannot be attributed to a single cause; there are many factors having synergistic effects. Previous studies have showed synergies between extreme pH levels (Pahkala et al. 2002) or depth of breeding ponds (Kiesecker et al. 2001) and excessive ultraviolet exposure, and between the use of pesticides and the presence of predators (Relyea and Mills 2001). Additionally, habitat loss is a major driver of population declines (Beebee and Griffiths 2005, Cushman 2006, Halliday 2008, Hillers et al. 2008, Angelone and Holderegger 2009), and the fungus, *Batrachochytrium dendrobatidis*, has been identified as cause of population declines and extinctions of many species worldwide (e.g., Rohr and Raffel 2010). On the other hand, the effects of climate as a direct driver of extinctions are less clear (e.g., Carey and Alexander 2003, Minter 2011). In Puerto Rico, an extended period of low rainfall between 1984 and 1989 was associated with a 60% decrease in population densities of *Eleutherodactylus coqui* in comparison with previous years (Stewart 1995). Similarly, in Costa Rica, extreme droughts were associated with major declines in population densities and shifts in male/female ratios (Pounds and Crump 1994, Pounds et al. 1999).

These changes in climate can directly affect calling activity, and thus the frequency and success of reproductive events. For instance, variation in temperature (5 to 20°C) was positively correlated with the number of *Hyloxalus subpunctatus* individuals calling (Navas 1996), and during the dry season in Puerto Rico calling activity of *E. coqui* decreases by approximately 60% (Woolbright 1985). Nevertheless, species do not always respond in a similar way. Two closely related species of *Pseudacris* responded differently to temperature changes (Stelman and Dorcas 2010). *Pseudacris crucifer* exhibited its peak calling activity between 15 and 20°C, but *P. feriarum* sang preferentially between 10 and 15°C. Temperatures above 30°C completely inhibited *P. feriarum* from calling, but not *P. crucifer* (Kirlin et al. 2006). In *Anaxyrus americanus*, calling activity was

positively correlated with water temperature, but in *Lithobates catesbeianus* and *L. clamitans* there was a negative correlation (Oseen and Wassersug 2002). Calling activity in other frog species did not respond to seasonal variation in precipitation and temperature. During a 3-year survey in a Brazilian floodplain, most species called either during the dry or wet season; however, three species called throughout the years (Prado et al. 2005). These studies demonstrate the high variability in the response of anurans to climate, making it difficult to predict how the group will respond to climate change.

To better understand the relationships between short-term variation in temperature and precipitation and calling activity, and the long-term consequences of climate change, we monitored the calling activity of four *Eleutherodactylus* species, including the recently described *E. juanariveroi*, in a Puerto Rican wetland. Specifically, we used a PAM approach to collect data at 10-minute intervals between November 2008 and March 2012.

METHODS

Study area and field data collection

The study was conducted in a wetland near the Caribbean Primate Research Center (CPRC) in Toa Baja, Puerto Rico (18°25'56.01" N and 66°11'45.62" W, 1 m asl). The dominant plant species is *Typha dominguensis* (cattail). Daily total precipitation and daily mean temperature between March 2008 and February 2012 were obtained for Levittown (approximately 2 km from the study area), and were provided by the National Oceanographic and Atmospheric Administration (NOAA, <http://www7.ncdc.noaa.gov/IPs/coop/coop.html>).

The amphibian community at this site includes five endemic, direct-development frog species (i.e., no tadpole stage, Hedges et al. 2008). Direct-development frogs are less dependent on water bodies than other frogs (Gonser and Woolbright 1995), and provide an opportunity to test the effects of temperature and precipitation without having to incorporate the location or presence/absence of breeding ponds. The five direct-development frogs in the wetland include: *Eleutherodactylus coqui* (Common Coquí), *E. cochranae* (Whistling Coquí), *E. brittoni* (Grass

Coquí), *E. antillensis* (Red-eyed Coquí), and the recently discovered *E. juanariveroi* (Plains Coquí), which is only known from this locality (Rios-López and Thomas 2007). *Eleutherodactylus antillensis* is rare in this site, and was excluded from the analyses.

Acoustical characteristics of the four *Eleutherodactylus* species

Each of the four *Eleutherodactylus* species emits sounds in a different range of frequencies (Fig. 1A). *Eleutherodactylus coqui* produces two notes ("CO" and "QUI"; Townsend et al. 1984, Stewart and Rand 1991, Rivero 1998): The "CO" occurs between 1.5 and 1.7 kHz, and the "QUI" from 1.9 to 4.0 kHz. At a slightly higher frequency range (3.3–4.2 kHz, Ovaska and Caldbeck 1997), a long note (0.1 to 0.4 s) similar to a whistle is emitted by *E. cochranae*, rarely followed by one or more clicks. The other two species, *E. brittoni* and *E. juanariveroi* are more similar in their call structure (i.e., repeated "chips"), but differ in their frequency ranges (*E. brittoni*: 4.5–6.0 kHz and *E. juanariveroi*: 6.0–9.0 kHz, Drewry and Rand 1983, Rios-López and Thomas 2007). The acoustical characteristics of each species determined the parameters used to create and train the species-specific identification models.

Automated monitoring

The daily and annual activity patterns of the *Eleutherodactylus* species were monitored as part of the Automated Remote Biodiversity Monitoring Network (ARBIMON) at the University of Puerto Rico, Rio Piedras Campus (UPRRP). Recording hardware consisted of a T-shaped microphone (model SP-SPSM-1, The Sound Professionals, Hainesport, New Jersey, USA; sensitivity = -42dB, flat response from 20 Hz to 20 kHz), installed at 1 m height, and approximately 30 m away from the access road. The microphone was connected to a laptop, which was programmed to record 1 minute of audio every 10 minutes in uncompressed files (wav format) and using a sampling rate of 44.1 kHz. This resulted in 144, 1-minute recordings per day. Solar panels provided electricity to the laptop and a directional antenna sent the audio files to the base station in the CPRC. The recordings were stored locally and automatically sent via Internet to the servers at UPRRP where they were

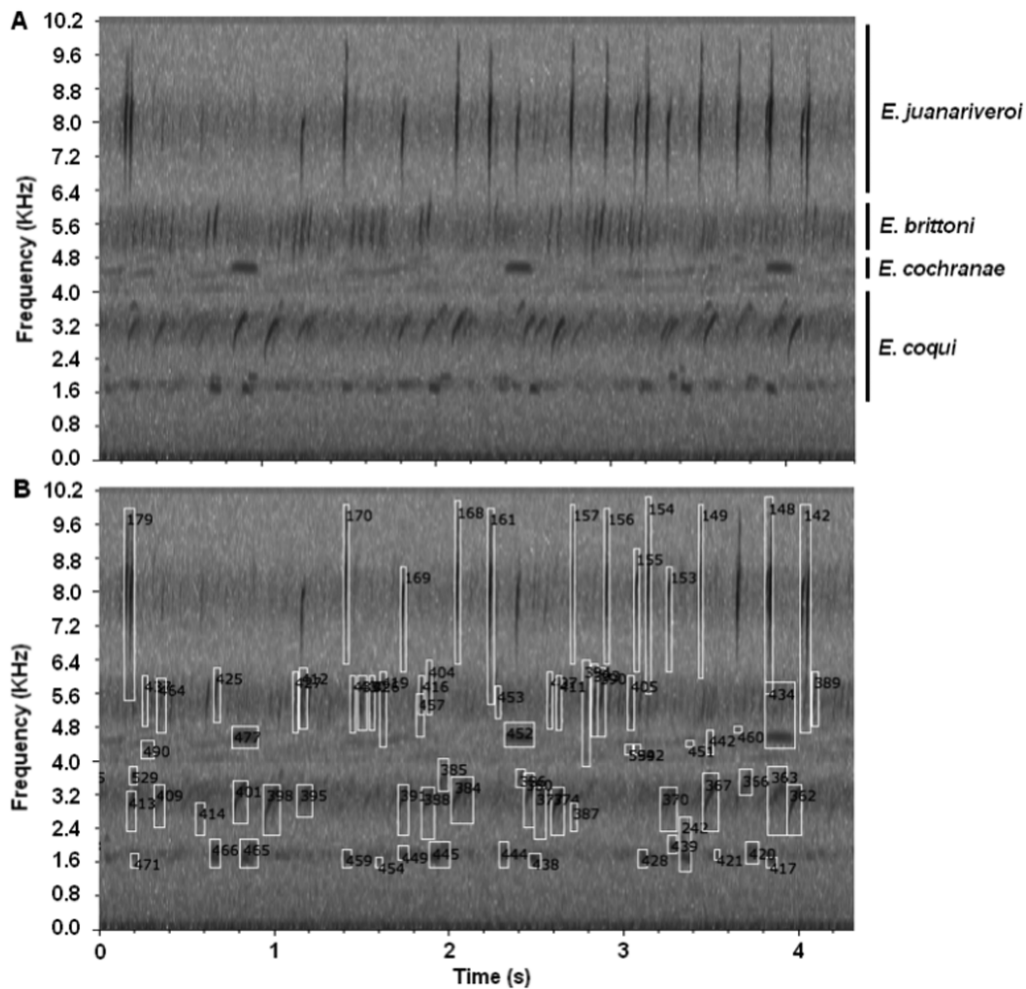


Fig. 1. Spectrogram from the Sabana Seca wetland as shown in the ARBIMON-acoustic visualizer. (A) Spectrogram showing the calls of the four species of *Eleutherodactylus*. (B) The same spectrogram marking the Regions of Interest (ROIs), with their identification numbers used to train and develop the automatic identification models.

processed and uploaded to the project webpage (<http://www.arbimon.net/>). For this analysis, we used 110,948 recordings that were collected between November 6, 2008 and March 31, 2012.

Species identification modeling

The presence/absence of each species in each 1-minute recordings was based on species-specific algorithms developed and implemented using the ARBIMON acoustic software. This process starts by identifying the regions of interest (ROIs) in each recording. This is done by analyzing the frequency-time matrix (i.e., spectrogram) and calculating the level of background noise within

each frequency band (every 86 Hz). This information was used to define threshold values of power that input signals must surpass to be considered as part of an acoustic event (e.g., a frog call). The regions defined as acoustic events were stored in a compressed sparse matrix (CSR) representation of the spectrogram. We analyzed the CSR using a depth-first search to determine which neighboring areas should be combined into a single acoustic event. The time and frequency variables that describe the bounding box of the resulting ROIs (Fig. 1B) are the data used in creating the automated species identification algorithms.

The ROIs that marked the calls were selected by an expert and used for training the species identification models. The models for *E. cochranæ*, *E. brittoni* and *E. juanariveroi* were designed to recognize the same note twice within the specified frequency range, with different times of duration and silence between ROIs. The model of *E. coqui* detected two notes, the “CO” and “QUI”. The models are based on the maximum and minimum frequencies, bandwidth, duration and silence between notes (or ROIs) in the calls. In addition, 221 randomly selected recordings were used for validating the species-specific models. In each of these recordings, an expert identified the presence or absence of each of the focal species. Precision and accuracy was estimated by applying each model to the 221 validation recordings and the decision (i.e., detected “D”, not detected “ND”) was compared with the expert’s evaluation (i.e., present “P”/absent “A”). Precision and accuracy were calculated as:

$$\text{Precision} = \frac{\text{D and P}}{\text{D}}$$

$$\text{Accuracy} = \frac{(\text{D and P}) + (\text{ND and NP})}{\text{total}}$$

Statistical analysis

Each species-specific model was applied to the 110,948 recordings. For all analyses we calculated the detection frequency (number of recordings where a species was present/total number of recordings) as a measure of a species calling activity for a given period of time.

To determine if calling activity had a positive or negative trend, we compared the calling frequencies during the same three-month period (January–March, April–June, July–September, and October–December) across the 41-month period using linear regression. We divided the analysis in these four periods to detect population trends while avoiding seasonal effects on the calling activity.

Multiple regression analysis was used to determine the impact of daily average temperature or daily total precipitation on calling activity. The results from the regression analysis were also supported by an ANOVA to estimate the significance of each variable in the species

identification models. Normal probability plots were produced to assess if the undetermined errors of the variables were normally distributed. We performed data analysis by using the R Statistical Package (R Development Core Team 2011).

RESULTS

Weather variation

Monthly average temperature between October 2008 and March 2012 was 26.6°C (±1.47) (Fig. 2). The coldest months were January to March (25.2°C, ±0.9), and the warmest months were June to September (27.7°C, ±1.0). Total monthly precipitation varied from 51.8 mm in

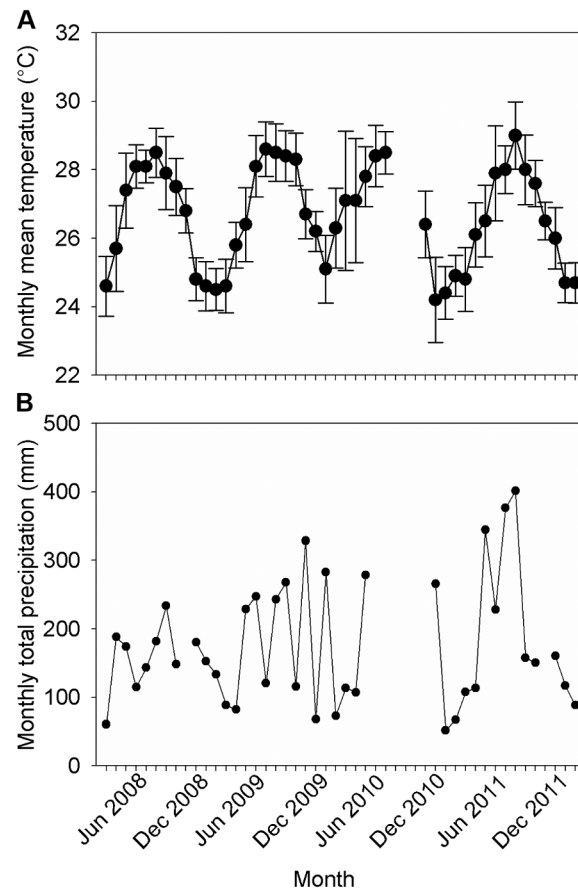


Fig. 2. Monthly mean temperature (A) and total precipitation (B) from March 2008 to February 2012. Data were downloaded from the National Oceanographic and Atmospheric Administration (NOAA, <http://www7.ncdc.noaa.gov/IPSCoop/coop.html>).

Table 1. Parameters used in the automated detection models for the four *Eleutherodactylus* species.

Measurement	<i>E. coqui</i>		<i>E. cochranæ</i>	<i>E. brittoni</i>	<i>E. juanariveroi</i>
	1	2	1 (2 ROIs)†	1 (2 ROIs)†	1 (2 ROIs)†
Max. frequency (Hz)	1864–3050	3358–3965	4300–5000	5743–6101	7800–10000
Min. frequency (Hz)	1536–2155	1980–3362	3630–4550	4281–4984	5100–7000
Bandwidth (Hz)	80–950	430–1625	100–1100	751–1528	1410–4000
Duration (s)	0.050–0.120	0.050–0.210	0.100–0.381	0.040–0.069	0.020–0.100
Silence between notes (s)	0.117–0.600		2.049–17.317	0.032–0.042	0.100–0.600

† The same note was repeated, each representing a ROI (region of interest) in the system.

January 2011 to 401.6 mm in August 2011 (Fig. 2). In general, March was the month with the lowest precipitation and September and October were the months with the highest precipitation.

Annual and daily calling activity

Automatic identification models, based on species-specific parameters (Table 1), were used to determine the patterns of annual and daily calling activity. The overall precision (82–100%) and accuracy (58–92%) varied among species-specific models, and they also varied within a model depending on the time of day (Table 2). In general, the models were more precise and accurate between 18:00 and 06:00 hours, the period of the greatest activity (Table 2).

The calling activity described by each identification model varied considerably among species and across years (Fig. 3). Annually, the calling activity patterns of *E. coqui* and *E. cochranæ* were cyclical (Fig. 3A and B): the main activity peak

occurred during the months of April to September (*E. coqui* detected in more than 50% of recordings and *E. cochranæ* in more than 30%), and December to March was the period of low activity (*E. coqui* detected in less than 50% of recordings and *E. cochranæ* in less than 20%). Calling activity in *E. brittoni* and *E. juanariveroi* was more irregular across each year in comparison to the other species (Fig. 3C and D). The average detection frequency of *E. brittoni* during the study was 50% (± 10), and June 2011 was the month with the lowest detection frequency (27%). Although all the species showed a decline in at least one of the four three-month-period comparisons, *E. juanariveroi* was the only species that had a significant decline in calling activity in three of the four three-month comparisons (Table 3). January to March was the only period that did not show a significant decline in calling activity. In January 2009, detection frequency for *E. juanariveroi* was 0.5, and in July 2011 the detection frequency was 0.2 (Fig. 3D).

Diel calling activity of the four species can be generally described by three distinctive periods (Fig. 4). The first period includes the main activity peak, which was observed during the night, between 18:00 (sunset) and 20:00 (detection >80%). In *E. coqui* and *E. cochranæ*, this peak was reached at 19:00 and 20:00, respectively. For *E. brittoni*, the peak was observed at 19:00, and for *E. juanariveroi* at 18:00 (the earliest among the species). The night peak was narrower in *E. juanariveroi* than in the other species. All species, except *E. cochranæ*, were detected in at least 30% of recordings from 00:00 to 04:00. A lower activity peak (in comparison to the night peak) was detected in the second period between 05:00 and 06:00 (sunrise) with detections in up to 60% of the recordings, which can be defined as a “dawn chorus”. In this dawn chorus, *E. cochranæ* was the species with the lowest detection

Table 2. Accuracy and precision (%) of species identification models (ARBIMON) for the four *Eleutherodactylus* species at different time intervals based on a validation dataset consisting of 221 randomly chosen recordings.

Period	<i>E. coqui</i>	<i>E. cochranæ</i>	<i>E. brittoni</i>	<i>E. juanariveroi</i>
Accuracy				
Total	79	92	58	74
00:00–06:00	64	88	32	66
06:00–12:00	90	98	71	78
12:00–18:00	77	96	83	69
18:00–00:00	85	87	48	83
Precision				
Total	97	94	100	82
00:00–06:00	100	90	100	97
06:00–12:00	100	†	100	63
12:00–18:00	73	†	100	45
18:00–00:00	100	97	100	100

† Precision was not calculated because there were no observations during these time periods.

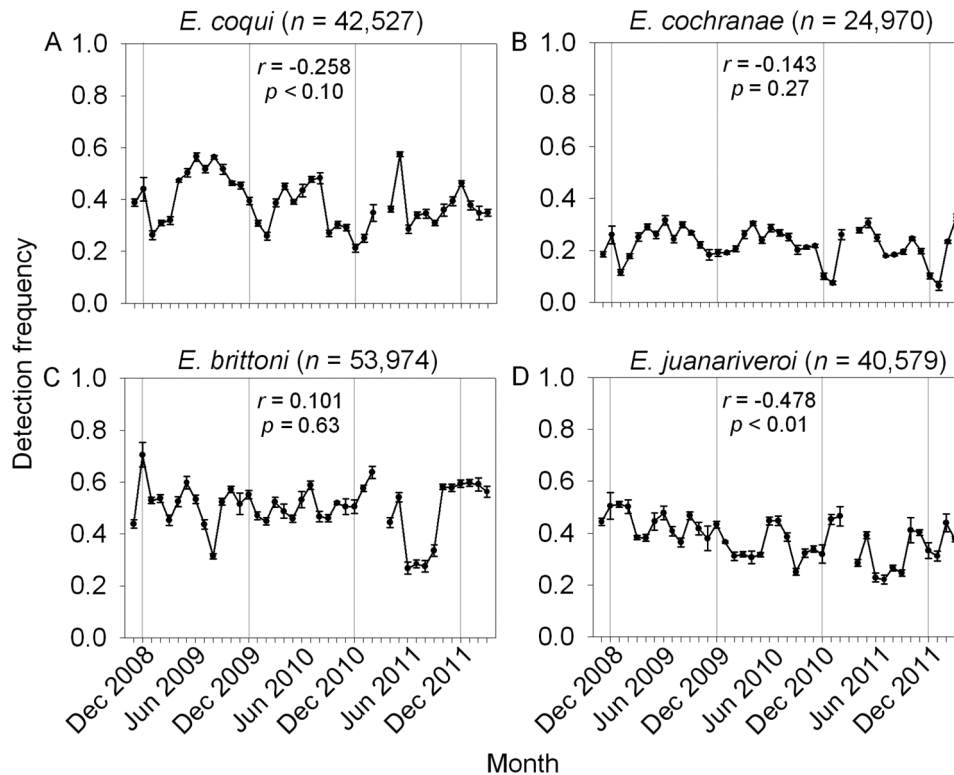


Fig. 3. Annual calling activity in terms of detection frequency in recordings from November 2008 to March 2012 for the four *Eleutherodactylus* species. The numbers in parentheses represents the total number of detections using the species-specific model in the 110,948 recordings. Standard deviation is showed for each point. *Eleutherodactylus juanariveroi* had a significant decline in calling activity during the 41-month study.

Table 3. Comparisons of the calling activity of four *Eleutherodactylus* species during three-month periods, over the 41-month study. The analysis was based on a linear regression.

Period	Spearman <i>r</i>	<i>F</i>	<i>p</i>
<i>E. coqui</i>			
January–March	0.417	2.64	0.17
April–June	−0.474	2.18	0.18
July–September	−0.791	12.86	<0.01**
October–December	−0.192	0.67	0.43
<i>E. cochranæ</i>			
January–March	0.108	0.03	0.88
April–June	−0.211	0.24	0.64
July–September	−0.791	16.77	<0.01**
October–December	−0.127	0.89	0.37
<i>E. brittoni</i>			
January–March	0.679	7.32	<0.05*
April–June	−0.527	4.23	0.08
July–September	−0.474	2.21	0.18
October–December	0.281	0.02	0.90
<i>E. juanariveroi</i>			
January–March	−0.323	0.68	0.43
April–June	−0.632	5.99	<0.05*
July–September	−0.738	10.43	<0.05*
October–December	−0.633	5.82	<0.05*

p* < 0.05, *p* < 0.01.

frequency (approximately 14%). During this period, *E. brittoni* had the most prolonged activity peak. Finally, a highly variable “daylight period” was observed from 07:00 to 16:00, with detections in up to 40% of the recordings. The species with the lowest calling activity during this period were *E. coqui* (<10%) and *E. cochranæ* (≈0%). On the other hand, during the daylight period *E. brittoni* and *E. juanariveroi* called in up to 40% of the recordings.

Response to weather variables

The four *Eleutherodactylus* species responded differently to temperature and precipitation (Table 4); two showed a significant relationship only with temperature (*E. coqui* and *E. cochranæ*) while the other two significantly responded to both temperature and precipitation (*E. brittoni* and *E. juanariveroi*). The calling activities of *E. coqui* and *E. cochranæ* were positively related

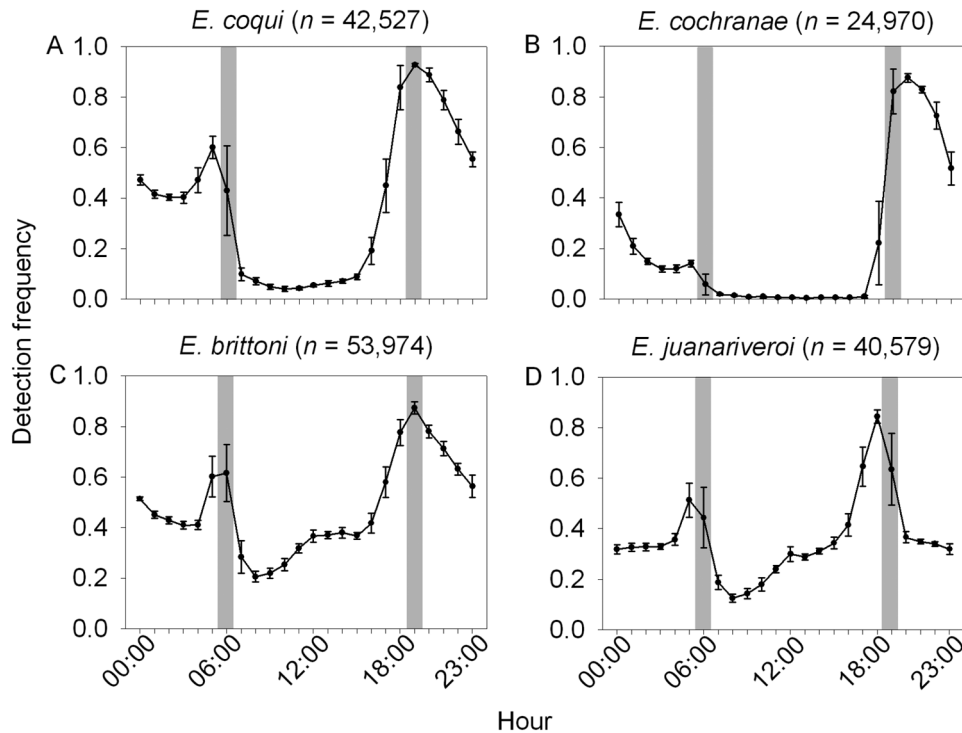


Fig. 4. Diel calling activity for the four *Eleutherodactylus* species. The numbers in parentheses represents the total number of detections using the species-specific model in the 110,948 recordings. Standard deviation is showed for each point. The gray areas show the variation in sunset and sunrise.

with daily average temperature (Spearman $r = 0.321$ and 0.228 respectively), but were not significantly related with daily total precipitation. At temperatures below 24°C , the average detection frequency of *E. coqui* was 31.5% (± 0.18)

and 19.7% (± 0.14) for *E. cochraeae*, but when daily temperature was above 29°C , detection frequency increased to 43.8% (± 0.17) and 23.6% (± 0.10), respectively.

Table 4. The relationship between detection frequency of the calls of the *Eleutherodactylus* species and climatic variables (i.e., daily mean temperature and daily total precipitation) using a multiple regression analysis ($n = 783$).

Variable	Spearman r	F	p
<i>E. coqui</i>			
Temperature	0.321	73.25	<0.001***
Precipitation	0.017	1.10	0.296
<i>E. cochraeae</i>			
Temperature	0.228	39.45	<0.001***
Precipitation	0.006	0.31	0.579
<i>E. brittoni</i>			
Temperature	-0.223	40.17	<0.001***
Precipitation	-0.034	11.35	<0.001***
<i>E. juanariveroi</i>			
Temperature	-0.133	10.73	<0.01**
Precipitation	-0.094	21.90	<0.001***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

In the cases of *E. brittoni* and *E. juanariveroi*, both temperature and precipitation had significant effects on their calling activity (Table 4). Calling activity was negatively correlated with daily temperature (Spearman $r = -0.223$ in *E. brittoni* and -0.133 in *E. juanariveroi*). In days with temperature below 24°C , detection frequency of *E. brittoni* and *E. juanariveroi* was 54.8% (± 0.17) and 40.8% (± 0.16), respectively, but when daily temperature was above 29°C , *E. brittoni* was detected in 41.1% (± 0.18) of the recordings, and *E. juanariveroi* in 35.1% (± 0.15). Detection of calls was also negatively correlated with total daily precipitation in *E. brittoni* and *E. juanariveroi* (Table 4, Spearman $r = -0.034$ and -0.094 , respectively). During days with no precipitation (0 mm), detection of *E. brittoni* was 47.5% (± 0.15) and 37.3% (± 0.14) in *E. juanariveroi*. Conversely, when total daily precipitation

was above 17 mm, the detection frequency of *E. brittoni* decreased to 41.6% (± 0.17), and to 30.5% (± 0.14) in *E. juanariveroi*.

DISCUSSION

Our results show that the calling activity of the four *Eleutherodactylus* species differed in their annual patterns and in their responses to daily temperature and precipitation. These patterns are based on more than 24,000 observations per species, over 41 months and sampling one minute every 10 minutes. This approach was possible due to the use of passive acoustic monitoring and automated species identification algorithms, and they allowed us to evaluate in detail the effects of variation in temperature and precipitation on calling activity. Although previous long-term studies relating climate variation to frog calling activity (e.g., Stewart 1995, Burrowes et al. 2004, Prado et al. 2005) have helped to meet the urgent need of assessing the status of frog populations, our approach can help understand the interactions between climate and calling activity at a finer time scale (e.g., daily, hourly). Due to the high variability in amphibian populations, this kind of high resolution data can help us better determine the population trends (Pechmann et al. 1991, Alford and Richards 1999).

Diel calling activity of the four species can be divided into three major periods. Most calling activity occurred in the first part of the night (17:00 to 01:00), which is common for species in the family Eleutherodactylidae (e.g., Drewry and Rand 1983, Navas 1996). From 01:00 to 06:00, calling activity is lower, but there is another peak at 05:00. This peak in activity the morning (dawn chorus) was present in all species, suggesting that it has an important function. Woolbright (1985) noted that during this dawn period individuals of *E. coqui* were moving from calling to retreat sites. Stewart and Rand (1991, 1992) complemented this observation, by arguing that *E. coqui* emitted aggressive calls (i.e., multinote calls) during this dawn period to defend retreat sites and avoid intraspecific encounters. Our results show that this behavior is very common in *E. coqui*, *E. brittoni* and *E. juanariveroi* (Fig. 4A, C and D). Although *E. cochranæ* only showed a small peak of activity in the dawn chorus, this

behavior has also been reported in experiments with captive individuals (Michael 1997). During the day (6:00–18:00), *E. coqui* and *E. cochranæ* rarely call. In contrast, *E. brittoni* and *E. juanariveroi* maintained low levels of calling throughout the day and the “nighttime chorus” often beginning around 16:00. Rios-López and Thomas (2007) reported daytime calling for *E. juanariveroi* in their description of the species, but our results are the first to show consistent evidence of acoustic activity during the day in *E. brittoni*.

Our identification models automatically detected the presence or absence of each species in each recording. Although we did not measure calling male density to compare it to the detection of calls, Fogarty and Vilella (2001) found that calling counts from point counts were correlated with density of *E. coqui* calling males. In addition, *E. coqui* has been found that they are very sedentary, moving in average 4.5 m per night (Woolbright 1985). Further studies should take this limitation into account or try to determine if the calling rate is correlated with the density of calling males.

Calling activity and species responses to weather variables

In contrast to other studies of *Eleutherodactylus* (Bertoluci and Rodrigues 2002, Burrowes et al. 2004) and other anuran species (Kirlin et al. 2006), our results did not show precipitation to be an important driver of calling activity for *E. coqui* or *E. cochranæ*. Standing water in the wetland may be providing all the necessary water during the drier parts of the year. On the other hand, calling activity in *E. brittoni* and *E. juanariveroi* responded negatively to precipitation. This difference between these species may be related to difference in their calling sites. In this wetland, *E. coqui* and *E. cochranæ* call at lower levels within the vegetation. In contrast, *E. brittoni* and *E. juanariveroi* call from the tips of the vegetation in the wetland (N. Rios-Lopez, *personal communication*). The preference for this calling site is likely related to their high frequency calls (>4.5 kHz), which would not transmit well from within the dense vegetation (Embleton et al. 1983). These species are small (*E. brittoni* 14–19 mm; *E. juanariveroi* 12–17 mm), and it is possible that the impact of raindrops or the noise during period of high rainfall may forced them to move to lower strata within the vegeta-

tion, and reduce or stop calling activity. In contrast, the calls of *E. coqui* or *E. cochranae* are emitted at lower frequencies (<4.0 kHz), and thus the call can propagate from within the vegetation.

The four *Eleutherodactylus* species responded differently to variation in daily temperature (Table 4). Calling activity in *E. coqui* and *E. cochranae* responded positively to increase in temperature, as is predicted for ectotherms (Navas and Bevier 2001), but *E. brittoni* and *E. juanariveroi* responded negatively. The size of frogs is an important determinant of water loss when temperatures are high (Van Berkum et al. 1982, Tracy et al. 2010), and we believe that the smaller *E. brittoni* and *E. juanariveroi* are more prone to dehydration during hot days, than the larger *E. coqui* (25–39 mm) and *E. cochranae* (18–24 mm). In addition, if these two species call from more exposed sites, the water loss will be increased. Frogs with high levels of dehydration will stop calling and retreat to areas where they can absorb water or reduce water loss by adopting a water-conservation position (Pough et al. 1983).

Future scenarios

Temperature in Puerto Rico is expected to increase by 1.1°C during the next 100 years (Ramirez-Beltran et al. 2007), and sites like Sabana Seca will probably become dryer during the dry season and wetter in the rainy season (Harmsen et al. 2009). If these predictions are correct, we expect that the populations of *E. brittoni* and *E. juanariveroi* will decline. This would occur because the hotter dry season and wetter wet season would lead to less calling and fewer reproductive events. Although the data used in this study were limited to calling males, calling males are good predictors of population size of *E. coqui*, even when compared with point-count estimates (Fogarty and Vilella 2001). If the decline in calling frequency of *E. juanariveroi* detected in this study reflects a decline in the population, and if this trend continues, this could lead to the extinction of *E. juanariveroi*, given that its distribution is restricted to the wetland where our study was conducted.

Concluding remarks and future directions

The response of calling activity of the four *Eleutherodactylus* species to variation in daily and seasonal variation in temperature and precipita-

tion varied greatly. *Eleutherodactylus. coqui* and *E. cochranae* were similar in their responses; they did not respond to variation in precipitation and calling activity was positively related to temperature. These responses suggest that these species may be less vulnerable to future climate change. In contrast, *E. brittoni* and *E. juanariveroi* responded negatively to increases in precipitation and temperatures, which are the future trends predicted for Puerto Rico (Ramirez-Beltran et al. 2007, Harmsen et al. 2009). This is particularly alarming for *E. juanariveroi*, which is already endangered due to its restricted distribution and rapid urban expansion around the wetland (Fish and Wildlife Service 2012). We believe that these differential responses are related to differences in body size and microhabitat used.

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