

CALLING ACTIVITY OF *ELEUTHERODACTYLUS* FROGS OF  
PUERTO RICO AND HABITAT DISTRIBUTION OF *E. RICHMONDI*

by

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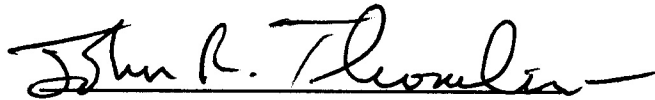
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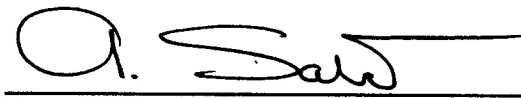
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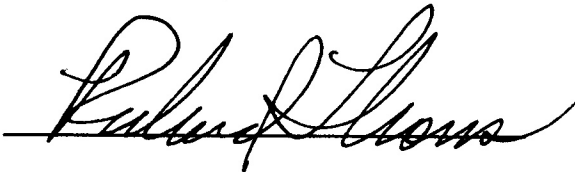
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A mi familia

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**CHAPTER 1: DIGITAL RECORDERS INCREASE THE DETECTED  
CALLING ACTIVITY LEVEL AND DETECTION PROBABILITY FOR  
ACOUSTICAL MONITORING OF *ELEUTHERODACTYLUS* FROGS**

## **Abstract**

Although there is evidence that many amphibians are declining, long-term projects are needed to accurately determine a decline. Acoustical surveys allow one to survey an area quickly, but the timing of the surveys may limit the data collected. The experience of the observers and the presence of loud species may limit the number of species detected at a site. I was able to detect more species and to determine the correct calling activity level of the species present by using digital recorders and computer software compared to just listening to the recordings. This technique can serve to increase species detection in areas with high anuran diversity and where loud species may interfere with the detection of other species.

## **Introduction**

There is much evidence that amphibian species are declining worldwide (Stuart et al. 2004). However, it is difficult to determine the extent or severity of many of these declines because long-term monitoring projects are needed to distinguish real population declines from temporary fluctuations (Pechmann et al. 1991) or source-sink metapopulation dynamics (Hecnar and M'Closkey 1996). To identify actual declines and determine possible causes, researchers need to improve current methods and develop new methods for large-scale and long-term monitoring projects.

Acoustical surveys allow researchers to quickly determine species presence and calling activity level (Lips et al. 2001; Zimmerman 1994). The North American Amphibian Monitoring Program uses acoustical surveys to monitor species in ponds and wetlands (United States Geological Survey 2004). In this program, volunteers survey sites and determine the species present and the calling activity level of each species. One of the limitations of this type of voluntary program is that the observers must be able to identify correctly all species present in the study area. In a temperate area the local species diversity could be low, but in a tropical area the diversity may be higher than a volunteer can correctly identify. For example, species diversity of anurans in the Neotropics (2135) is much greater than in the Nearctic (90) (Duellman 1999). Furthermore, abundant or loud species may cause interference and lower the detection probability of other species.

An assumption that needs to be tested in acoustical surveys is that detection is highly correlated with the species presence or activity. Without an appropriate correction for the detection probability, the resulting data will not reflect the true status of the species (MacKenzie et al. 2002). This will result in management decisions that do not respond to the real situation, wasting time, money, and other resources.

Digital recorders can be used as a standard method for the detection of species and the determination of the calling activity level in amphibian choruses in long-term monitoring projects. Digital recordings can be stored and transferred easily. This can allow experts to analyze the recordings at anytime, and they are not restricted to the reproductive period of the species. In addition, loud or abundant species can be filtered using computer software to increase the detection of other species.

In this paper, I tested the hypothesis that detection of the species presence and calling activity level of anuran choruses will be higher when recordings were analyzed with the help of computer software compared to only hearing the recordings. Computer software can filter loud calls from other species and the spectrogram facilitated the detection of the species presence. The resulting increase in detection should result in better population status data and reduce false negatives (i.e. when a species is not detected due to interference).

## Materials and Methods

Recordings of amphibian choruses were made at two sites in the Luquillo Experimental Forest, near the Tradewinds Trail (18.290°N, 65.798°W) and near the Mount Britton Tower (18.303°N, 65.795 °W), and at one site in the Carite State Forest, near road 7740 (18.103°N, 66.035°W). The Luquillo Experimental Forest is located in the northeast of Puerto Rico and the Carite State Forest is in the southeast (Fig. 1). The Tradewinds and Mount Britton sites were located in the Lower Montane Wet Forest lifezone and the Carite State Forest site was located in the Subtropical Wet Forest lifezone (Ewell and Whitmore 1973). The recordings were made using an Automated Digital Recording System (ADRS; Acevedo and Villanueva-Rivera In press). The ADRS used is described in detail in Villanueva-Rivera (this volume, chapter 2).

To test whether digital recordings can be used to increase detection in tropical frog choruses, I compared one minute recordings, made at 2000, 2200 and 0000 h on five consecutive days, for a total of fifteen recordings per site, using two methods. First, I listened to the recordings with headphones, and each species heard was given an Amphibian Calling Index value (ACI; United States Geological Survey 2004). The ACI has four possible values: 0 represents no animals calling; 1 a few animals calling without overlap; 2 some overlap; and 3 represents a full chorus of the species. Second, I analyzed the recordings using computer software to visually detect the signal of each species' call in the spectrum. There is little overlap in the call spectrum of the *Eleutherodactylus*

frogs in Puerto Rico, and thus it was possible to distinguish each species (Drewry and Rand 1983). Each recording was then filtered to remove the range of frequencies of the dominant species, *Eleutherodactylus coqui* (1 - 2.4 kHz), listened to again, and each species was given a new ACI value. Each pair of results, for each recording, by species, were compared using Wilcoxon signed ranks tests.

To evaluate the differences in the sound pressure of the species, which may explain why some species were not heard, I obtained the average pressure of sound by frequency using the values of decibels full scale (dBFS), which corresponds, on a logarithmic scale, to the signal in the digital file. On this scale the maximum is 0 dB and the minimum is -96 dB, which corresponds to the maximum and minimum levels, respectively, of sound that the digital file can store (Fries and Fries 2005). The recordings were played and analyzed using the program AUDITION (ver. 1.0, Adobe Systems, Inc., California, USA).

## Results

A total of eight species of *Eleutherodactylus* frogs were detected. Seven species were detected at the Tradewinds Trail site (Fig. 2a). There was no difference between the methods for *E. coqui* and *E. gryllus*. The ACI values of two species were higher on the computer-analyzed recordings: *E. hedricki* ( $P = 0.016$ ) and *E. portoricensis* ( $P = 0.022$ ). Three species were detected only when the signals of their calls were seen in the spectrogram and the recordings were

filtered: *E. locustus*, *E. unicolor*, and *E. wightmanae*. *E. locustus* and *E. unicolor* were detected in five recordings and *E. wightmanae* was detected in only three of the fifteen recordings. Two species had the highest sound pressures, *E. coqui* and *E. portoricensis* (Fig. 3a). The average sound pressure was -41.7 dB and -40.0 dB for the first and second notes of *E. coqui*, respectively, and -37.7 dB and -44.7 dB for the first and second notes, respectively, of *E. portoricensis*. The average maximum sound pressure for *E. locustus* was -50.7 dB, 13.0 dB less than the average of the loudest species, *E. portoricensis*. The average maximum sound pressure of *E. unicolor* was -44.5 dB and for *E. wightmanae* was -43.3 dB; these values were 6.8 dB and 5.6 dB lower than the loudest species, respectively.

There were four species at the Mount Britton Tower site: *E. coqui*, *E. gryllus*, *E. portoricensis*, and *E. unicolor*. There was no significant difference between methods for the four species (Fig. 2b).

I detected three species at the Carite State Forest site (Fig. 2c). There was no difference between methods for *E. coqui*, but the other two species had higher ACI values when analyzed in the computer: *E. richmondi* ( $P = 0.004$ ) and *E. wightmanae* ( $P = 0.002$ ). The species *E. coqui* had the highest sound pressure with -30.1 dB and -28.4 dB for the first and second notes, respectively (Fig. 3c). The average sound pressure for *E. richmondi* was -58.8 dB, 30.4 dB less than *E. coqui*, and for *E. wightmanae* it was -46.3 dB, 17.9 dB less than the loudest species.

## Discussion

The loudest species at the study sites, *E. coqui* and *E. portoricensis*, had a continuous chorus. In addition, the sound pressure of these species was higher than the species that could not be detected, up to 30 dB louder (Fig. 3). The continuous loud chorus of *E. coqui* and *E. portoricensis* caused interference in the detection of the other species, either reducing the detected calling activity level or by reducing the probability of detecting the species, particularly in the cases of the small populations of *E. locustus*, *E. unicolor* and *E. wightmanae* at the Tradewinds site.

The detection and level of activity measured using the ACI increased at two of three sites when recordings were filtered and the spectrogram was evaluated in the computer compared to only listening to the recordings. Of particular importance was the detection of three species, *E. locustus*, *E. unicolor*, and *E. wightmanae* at one of the sites only after filtering. These three species appear to be threatened or endangered since their range is restricted and few populations are known (Burrowes et al. 2004; pers. obs.).

The ACI of four species was higher when the recordings were filtered to remove the common and loud *E. coqui*. The use of computer software allowed determination of the correct index value for these species, instead of the perceived value. Two of these four species, *E. hedricki* and *E. portoricensis*, have limited distribution in the highlands of Puerto Rico, and the two other species, *E. richmondi* and *E. wightmanae*, are thought to have only a few small



populations (Burrowes et al. 2004; Joglar 1998). Digital recordings, and their analysis using software, can be used as a tool to search populations in areas where limited herpetological work has been conducted and where loud species interfere with the detection of others.

Populations of species declared extinct could be cases of missed detection due to interference by other sounds or due to their reproductive phenology. For example, Bridges and Dorcas (2000) found that *Rana sphenocephala*, a species that was thought to be breeding only during the spring and fall, was also calling during the summer between 0200 and 0500 h. This finding, made with a cassette-based automated recording system, indicated a calling period that was missed by most herpetological work done in the area (Bridges and Dorcas 2000).

The use of digital recordings has important implications for the current discussion of amphibian declines and the need for long-term monitoring projects. In a study that tested the use of recorders to detect species of birds, several experts agreed on the identifications of the species present in the recordings, so the method was recommended as an effective avian monitoring method (Rempel et al. 2005). In another study, the number of species identified with recordings made with an ADRS was higher when compared to point counts for birds and transects for amphibians (Acevedo and Villanueva-Rivera In press). For amphibian monitoring projects, technicians can deploy ADRS and record choruses to be analyzed by experts in the laboratory at another time. This can

reduce personnel costs, especially for areas with a small temporal window of amphibian reproductive activity, areas that have high frog diversity, species that do not congregate, and species with low detection probabilities. Another important advantage is that a recording is a permanent record of the presence of a species. Another advantage of digital recorders is that the use of computer software may help detect rare species or calls that are difficult to detect due to interference.

Monitoring projects directed at detecting declines in amphibians should yield results that accurately indicate a decline and not a short-term fluctuation. Amphibians may have fluctuating recruitment (Pechmann et al. 1991), in which a low recruitment or decline during a few years may not indicate a threatening decline, but a normal short-term fluctuation. Several methods have been tested, and the best method to identify a decline correctly is using mark-recapture methods (Alford and Richards 1999; Funk et al. 2003). However, there is no accurate method for non-congregating and non-migrating species (Alford and Richards 1999), like *Eleutherodactylus* species, in which a mark-recapture project may indicate a local decline but not a generalized trend for the species. A five year study with *Eleutherodactylus* frogs in Ecuador that compared methods to detect a decline found that a mark-recapture study was the best method, but the power was low (Funk et al. 2003). It would require a larger sample size to increase power, which is usually not feasible. Probably the only cost-effective method for these species is using presence/absence data, corrected for

detection probabilities (MacKenzie et al. 2002), in combination with metapopulation dynamics studies to evaluate the health of the species at a large scale. Deployment of ADRS monitors would allow this intensive, large-scale effort.

## Literature cited

- Acevedo, M. A. and L. J. Villanueva-Rivera. In press. Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians. *Wildlife Society Bulletin*.
- Alford, R. A. and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* 30:133-165.
- Bridges, A. S. and M. E. Dorcas. 2000. Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. *Copeia* 2000: 587-592.
- Burrowes, P. A., R. L. Joglar, and D. E. Green. 2004. Potential causes for amphibian declines in Puerto Rico. *Herpetologica* 60:141-154.
- Drewry, G. E. and A. S. Rand. 1983. Characteristics of an acoustic community: Puerto Rican frogs of the genus *Eleutherodactylus*. *Copeia* 1983:941-953.
- Duellman, W. E. 1999. Global distribution of amphibians: patterns, conservation, and future challenges. *In* W. E. Duellman (ed.), *Patterns of distributions of amphibians: a global perspective*, pp. 1-30. Johns Hopkins University Press, Baltimore, Maryland.
- Ewell, 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, U. S. Forest Service, Río Piedras, Puerto Rico.
- Fries, B. and M. Fries. 2005. *Digital Audio Essentials*. O'Reilly Media, Inc.,

- Sebastopol, California. 357 pp.
- Funk, W. C., D. Almeida-Reinoso, F. Nogales-Sornosa, and M. R. Bustamante. 2003. Monitoring population trends of *Eleutherodactylus* frogs. *Journal of Herpetology* 37:245-256.
- Hecnar S. J. and R. T. M'Closkey. 1996. Regional dynamics and the status of amphibians. *Ecology* 77:2091-2097.
- Joglar, R. L. 1998. Los coquíes de Puerto Rico: su historia natural y conservación. Editorial de la Universidad de Puerto Rico, San Juan. 232 pp.
- Lips, K. R., J. K. Reaser, B. E. Young, and R. Ibáñez. 2001. Amphibian monitoring in Latin America: a protocol manual. Society for the Study of Amphibians and Reptiles, New Haven, Connecticut. 115 pp.
- MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-2255.
- Pechmann, J. H. K., D. E. Scott, R. D. Semlitsch, J. P. Caldwell, L. J. Vitt, and J. W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* 253:892-895.
- Rempel, R. S., K. A. Hobson, G. Holborn, S. L. Van Wilgenburg, and J. Elliott. 2005. Bioacoustic monitoring of forest songbirds: interpreter variability and effects of configuration and digital processing methods in the laboratory. *Journal of Field Ornithology* 76:1-11.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L.

Fischman, and R. W. Waller. 2004. Status and Trends of Amphibian

Declines and Extinctions Worldwide. *Science* 306:1783 – 1786.

United States Geological Survey. 2004. North American Amphibian Monitoring

Program. Accesible at <http://www.pwrc.usgs.gov/naamp/index.html>.

Zimmerman, B. L. 1994. Audio Strip Transects. *In* W. R. Heyer, M. A. Donnelly,

R. W. McDiarmid, L. C. Hayek and M. S. Foster (eds.), *Measuring and*

*monitoring biological diversity: Standard methods for amphibians*, pp. 92-

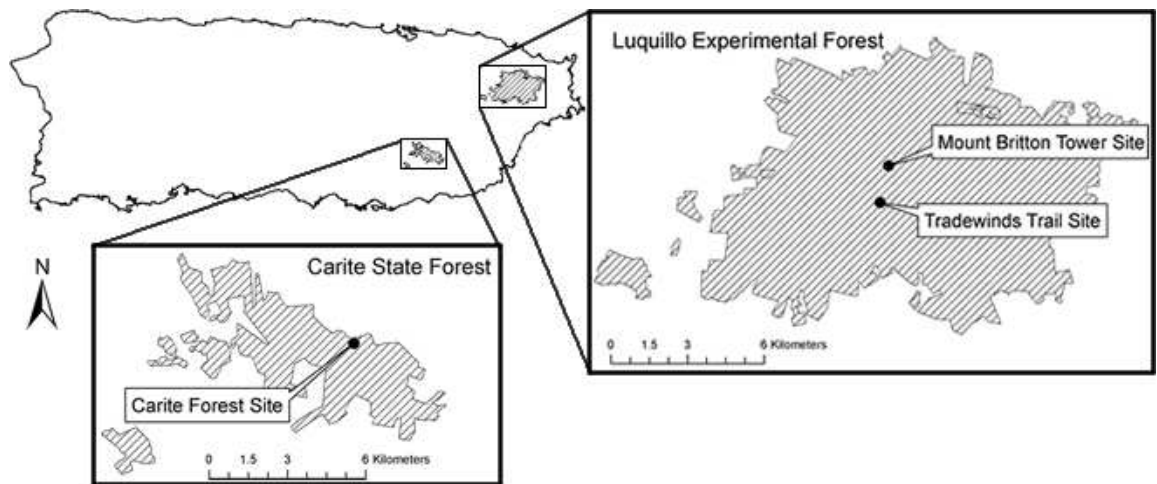
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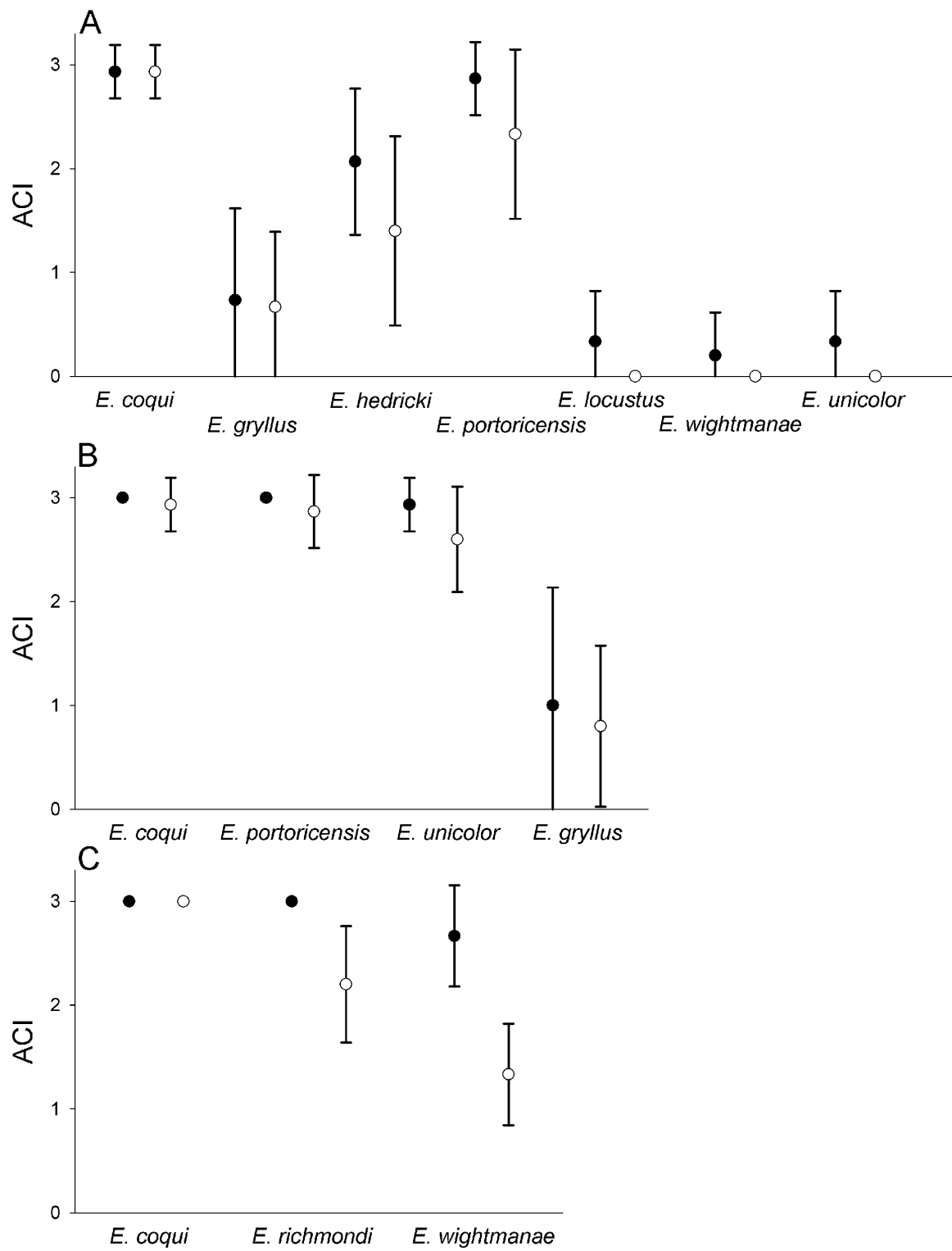
Fig. 1. Location of the three study sites in Puerto Rico.

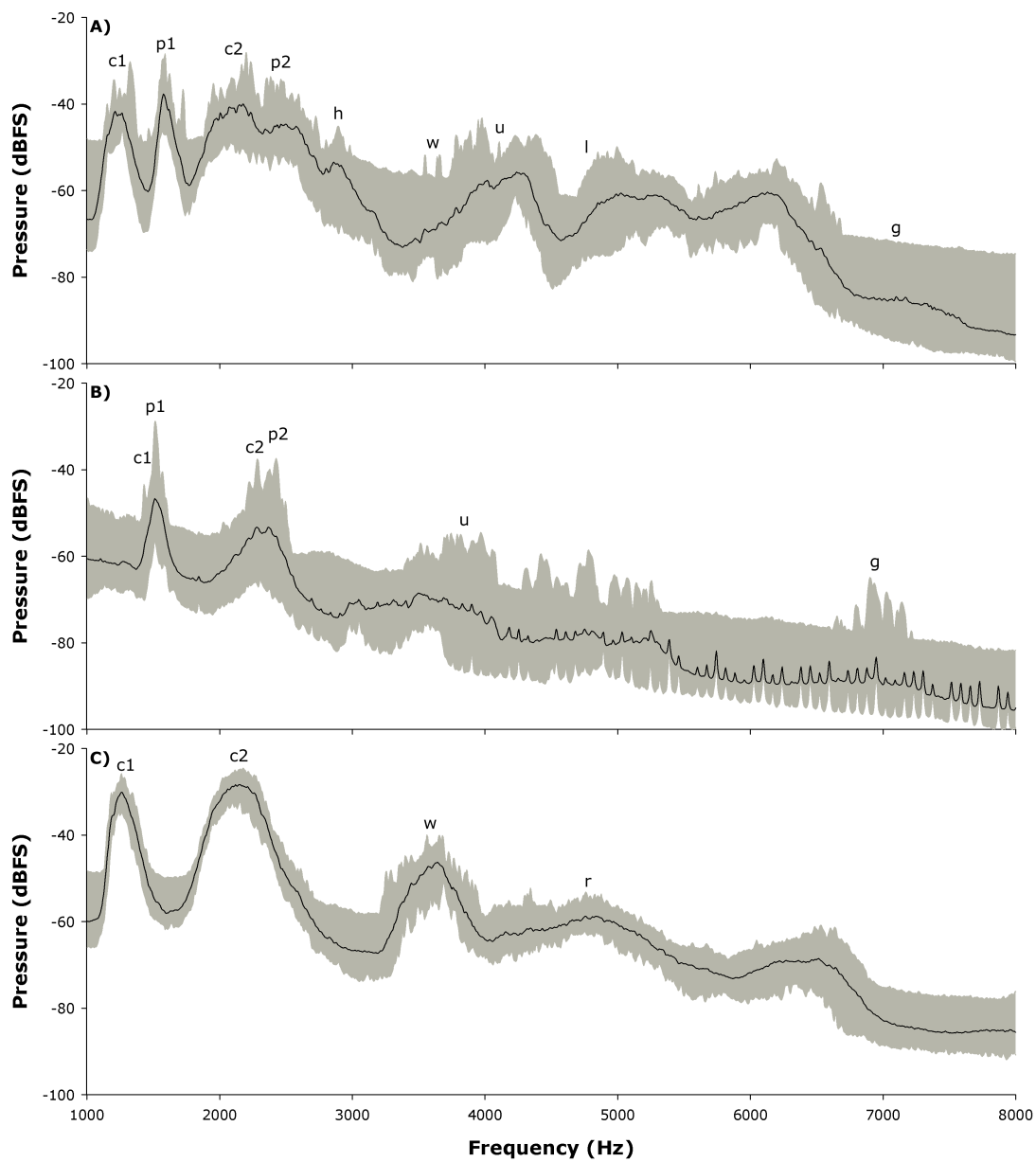
Fig. 2. Average and one standard deviation values of the ACI for each species using two methods at (A) Tradewinds Trail site, (B) Mount Britton Tower site, and (C) Carite Forest site. The filled circles represent the values for the method of analyzing the recordings using computer software and the open circles represent the values for the method of listening to the recordings.

Fig. 3. Sound pressure by frequency of species of *Eleutherodactylus* frogs at the (A) Tradewinds Trail site, (B) Mount Britton Tower site, and (C) Carite Forest site. Sound pressure is measured in decibels full scale (dBFS). The line represents the average for the fifteen recordings at each site and the shaded area encloses the range of values for each frequency. Letters above the graphs represent the average dominant frequency for the species. c1 = first note of *E. coqui*; c2 = second note of *E. coqui*; p1 = first note of *E. portoricensis*; p2 = second note of *E. portoricensis*; h = *E. hedricki*; w = *E. wightmanae*; u = *E. unicolor*; l = *E. locustus*; r = *E. richmondi*; g = *E. gryllus*. Other peaks represent sounds made by insects.









**CHAPTER 2: CALLING ACTIVITY OF HIGHLAND  
*ELEUTHERODACTYLUS* FROGS OF PUERTO RICO:  
IMPLICATIONS FOR MONITORING PROJECTS**

## **Abstract**

Amphibians have received a lot of interest from the apparent worldwide decline of populations. This decline has promoted a call for monitoring and research, particularly in the tropics due to their high amphibian diversity. Acoustical surveys can be used as a fast method to determine species distribution in a large-scale and long-term monitoring project. I used an Automated Digital Recording System to determine the calling activity during the night of eight Puerto Rican species, most of which seem to be declining. The patterns obtained revealed that five species should be monitored between sunset and midnight. One species, *Eleutherodactylus gryllus*, had a small peak of activity during the first two hours of the night. Two species had no significant difference during the night. These patterns should help to find and monitor populations of these species to determine their current status.

## Introduction

Amphibians have received a lot of interest since 1989, when an apparent worldwide decline of populations promoted a call for monitoring and research, particularly in areas with high amphibian diversity (Wake 1991). Rapid declines have been documented since (e.g. Drost and Fellers 1996; Houlahan et al. 2000; Lips 1999; Richards et al. 1993; Woolbright 1997), with populations disappearing from areas with little, if any, human alteration.

Species in the Neotropics seem to be declining more than in other regions of the world (Stuart et al. 2004). In addition, the high biodiversity and small numbers of workers in the region make it imperative to establish simple but effective monitoring methods.

While there is some evidence that most Puerto Rican frogs seem to be either endangered or threatened (Burrowes et al. 2004), the evidence is very limited in range and island-wide application. Two studies have reported declines in populations of Puerto Rican frogs, including the widespread *Eleutherodactylus coqui*, but both studies were done in single transects at or near trails in the Luquillo Experimental Forest (LEF) (Burrowes et al. 2004; Stewart 1995). The longest population data series (two years) available from other forests outside the LEF are from the Maricao and Toro Negro State Forests (Fogarty and Vilella 2002). Anecdotal reports of populations that have disappeared seem to indicate the extinction of populations from several forests (Joglar and Burrowes 1996; Woolbright 1997), but the reports are limited to areas near roads and trails, and

there is little information from areas such as the Cordillera Central mountain range and the forest surrounding El Toro peak in the LEF.

Mark-recapture studies seem to be the best way to detect a decline in a monitoring project (Alford and Richards 1999), including for *Eleutherodactylus* species (Funk et al. 2003). However, this type of study had low power to detect a fast decline in *Eleutherodactylus* species (Funk et al. 2003), which comprise the majority of the native amphibian fauna of Puerto Rico. Many replicates would be needed to increase the power, which is difficult due to economic and personnel limitations.

Acoustical surveys can be used as a quick method in a large-scale and long-term monitoring project (Zimmerman 1994). If the acoustical survey data are coupled with population parameters such as population size, sex ratio, and density, in addition to recently developed models that correct detection probabilities from repeated surveys at a site (MacKenzie et al. 2002) and determine abundance categories from a calling activity index (Royle and Link 2005), we can obtain better quality data to determine the status of a species at a large scale. Small-scale population-level studies can provide more information that can allow us to extrapolate a decline in calling activity to a decline in a population.

To establish a proper long-term and large-scale acoustic monitoring project, it is important to determine the best times to sample populations. False negatives (i.e. the absence of a species because it was not calling) may mislead

researchers into concluding that there is a decline or extinction in a population when it was not detected, simply because it was sampled at a time when the males do not call.

Peterson and Dorcas (1994) described an automated recording system (ARS) that can sample a chorus in the wild at desired intervals using a cassette recorder and an electric timer. The ARS allows recording of the amphibian community at a site more frequently and for a longer time than researchers can in person. In addition, the recordings can serve as a permanent record and they can be analyzed with the help of computer software. I designed an Automated Digital Recording System (ADRS) that replaced the tape recorder with a digital recorder and the solid-state electric timers with a microcontroller (Acevedo and Villanueva-Rivera In Press). These modifications allow a smaller drift in time in the recording periods and easy transfer of the recordings to a computer for analysis, archive, and dissemination.

Temporal calling patterns for fourteen Puerto Rican species have been described (Drewry 1970; Drewry and Rand 1983). However, these patterns were generated systematically for five species and subjectively for the other nine species. It is not clear if these patterns are accurate enough to determine the peak of activity for each species for a long-term project. The objective of this study was to describe the calling activity during the night of highland species of *Eleutherodactylus* from Puerto Rico using ADRS to determine the best time of night to conduct an acoustic monitoring project. The accurate patterns will help

develop an effective long-term and large-scale monitoring project. This methodology can also be used in other tropical areas, where limited visibility or high diversity may yield underestimates of species diversity or indicate misidentified declines. In addition, recorders can help to find populations of species at sites where they were previously unknown.

## **Materials and Methods**

Historical and known population sites of highland Puerto Rican *Eleutherodactylus* frogs were sampled with ADRS to determine the calling activity for each species between 2003 and 2004. Other areas evaluated in preliminary surveys and deemed appropriate by the author were also sampled (Appendix 1).

The ADRS consisted of a Nomad Jukebox 3 digital player and recorder (Model DAP-HD0003, Creative Labs, Inc, California) with a custom-made controller. The recorder was set to record in 16-bit wave files with a sampling rate of 48 kHz. Each recording period was stored in a separate file in an internal hard drive with a date and time stamp. The controller was based on the MSP430 microcontroller (Texas Instruments, Inc., Texas) mounted on a MSP430-P-1121M board (Olimex Ltd., Plovdiv, Bulgaria). The controller was programmed using C language with the KICKSTART software (version 1.26a), obtained from the Texas Instruments website (<http://www.ti.com>). The program in the controller (Appendix 2) activated relays that closed circuits in the remote jack of the



recorder with resistance values that simulated commands received from a wired remote control: A: to start recording with 11.5 kOhm for two seconds; and, B: to stop with 60.1 kOhm for one second (Fig. 1). The ADRS recorded 1 minute every 30 minutes. Each ADRS had an electret condenser microphone (Model ECM-MS908C, Sony Electronics Inc, California) that was placed at approximately one meter from the ground, inside a plastic pipe, open on the sides and below, to protect it from the rain. Since the recorder had no microphone input, only a line level input, the microphone was connected to a portable preamplifier (Model SP-PREAMP, The Sound Professionals, Inc., New Jersey) via a waterproof plug on the side of a plastic sealed box that protected the recorder, controller, preamplifier, and batteries from the rain and humidity of the forest. The controller and recorder were powered by two lead acid batteries from uninterruptible power supply units (Model Back-UPS ES 500, American Power Conversion, Inc., West Kingston, Rhode Island). This system recorded during six days before the batteries had to be recharged. After pickup, the recordings were transferred to a computer using an IEEE 1394 (*i.e.* Firewire) cable and recorded on optical disks for archive.

Each recording was listened to with headphones to determine the species calling and their respective activity level. The recordings were loaded to the AUDITION software (ver. 1.0; Adobe Systems, Inc., California, USA) to visualize the species' calls in the spectrogram that were not audible due to interference by other loud species, usually by *Eleutherodactylus coqui* (Villanueva-Rivera this

volume, chapter 1). Each species has a different range of frequencies, with little overlap, and their calls have been described (Drewry and Rand 1983). After listening to a recording once, if the spectrogram indicated the possibility of another species that was not heard, the recording was filtered to remove the range of frequencies of other species and listened to again. The activity level of each species was identified using the Amphibian Calling Index (ACI; United States Geological Survey 2004). The ACI can have four values: 0 represents no individuals calling; 1 a few individuals calling with no overlap between the calls; 2 there is some overlap; and 3 a full chorus.

The ACI values for each species were analyzed using a Kruskal-Wallis test to determine if the level of calling activity differed during the night (1800 – 0600 h). The noise from rain or wind in several recordings made it difficult to determine the ACI for the species. These recordings were not included in the analysis, which resulted in dissimilar sample size between the 25 periods during the night. In some sites, the average ACI of some species during the night was always lower than 1. When this was the case, the data for the species at the site was not included in the analysis.

## Results

At the 11 sites surveyed, a total of 10 species of frogs were detected in 1546 one-minute recordings. Two species, *Leptodactylus albilabris* and *Eleutherodactylus antillensis*, with a widespread distribution on the island

(Rivero, 1998), were heard occasionally at some sites and were not included in the analysis. One species, *Eleutherodactylus coqui*, a generalist species, was present at all sites (Appendix 1).

The pattern of calling activity during the night for eight species was determined (Fig. 2). Five species had their peak of activity between sunset and midnight: *E. coqui*, *E. hedricki*, *E. portoricensis*, *E. richmondi*, and *E. wightmanae*. Their calling activity declined steadily after midnight. *E. gryllus* had a short peak of activity between 1900 to about 2100 h. *E. locustus* and *E. unicolor* showed no significant difference in their activity during the night.

Three species had a small peak of activity during the last hours of the night. *E. gryllus*, *E. portoricensis*, and *E. wightmanae* increased their activity two hours before sunrise from the declined activity level of the hours after midnight. These peaks were always smaller than the main peak before midnight.

New populations of *E. locustus* and *E. wightmanae* were detected with the ADRS in the LEF. At Pico del Este, I detected a new population of *E. locustus*. Near the Tradewinds Trail I found populations of *E. locustus* and *E. wightmanae*. Next to road 191, near km 9.3 I found another population of *E. wightmanae*. In addition, I found another population of *E. wightmanae* at the Toro Negro State Forest. To my knowledge, these populations had not been reported in the literature before.

## Discussion

The calling activity during the night was determined for eight highland *Eleutherodactylus* species from Puerto Rico. The level of activity of six of these species was not uniform during the night, which indicates that the time when the species are monitored acoustically is important. Most species called from sunset to midnight, and their activities declined afterwards.

The activity periods of six species were similar to those reported by Drewry and Rand (1983). However, two species, *E. portoricensis* and *E. richmondi*, had a pattern that differed from their description. The activity of *E. portoricensis* had a peak earlier in the night. Drewry and Rand (1983) described the pattern for *E. richmondi* as increasing during the night with a peak after midnight, while in this study there was a peak of activity before midnight and a decline afterwards (Fig. 2).

It was expected that species should limit their calling activity to a period when its benefits (attracting females) are outweighed by its costs (reduced foraging, energy expenditure, and predation risk). Males of *E. coqui* reduce the number of prey they consume while calling (Woolbright and Stewart 1987). There are few studies that compare the energetic cost of calling, but it appears that the metabolic rates increase ten times, or more, from the resting state in some species (Wells 2001). Predation on *Eleutherodactylus* by owls (*Megascops nudipes*) has been reported (Waide 1996), so it is possible that these predators may sometimes use the call of the males to hunt them.

Results from this study suggest that *Eleutherodactylus* species acoustical monitoring should take place from sunset to midnight, when most of the species are highly active. The data collected from surveys conducted after midnight should be taken with caution since a negative or low calling activity level value could be due to the time and not a local extinction or a declining population.

Seasonal variations in activity of the species must also be considered in monitoring projects. Previous studies have found that *E. coqui* had a higher period of reproductive activity, measured as both number of calling males and number of egg clutches deposited, during May and June (Townsend and Stewart 1994), and between June and December for overall frog numbers (Stewart 1995). The ground-dwelling species *E. unicolor* has its peak of calling activity between January and June, apparently negatively correlated with temperature (Joglar 1998). These seasonal variations must be taken into account, in addition to the pattern of activity during the night, for the monitoring of these species.

New populations of two species, *E. locustus* and *E. wightmanae*, were detected using the ADRS. The high sensitivity of microphones and recorders in an ADRS can help determine if a species is present or absent in an area with reasonable confidence, since the system can take many samples during several days or weeks. By analyzing the recordings with computer software, it is possible to detect species that are difficult to hear in acoustical surveys (Villanueva-Rivera this volume, chapter 1), which will help to find extant populations, in particular of species that have limited distributions.

There was a recent report of population declines of some *Eleutherodactylus* species in the LEF from data collected in two transects during 10 years (Burrowes et al. 2004). One of the species that was declining, *E. gryllus*, was sampled with acoustical surveys only. Unfortunately, the time when the surveys were made was not provided. The results of this study call into question their conclusion that there was a population decline of *E. gryllus*, because the period of activity is limited to the first two hours of the night. If the surveys were not always done at the same hour in the night, the apparent decline in that population may be a methodological problem. If the surveys were conducted at a later time during the last years of the study, the correlation would have had a negative value due to the reduced calling activity of the species and not due to a decline in the population. Since the species was still abundant in the area during this study, 6 years after the end of the Burrowes et al. (2004) study, and declines are usually very fast, taking from a few months (Laurance et al. 1996) to three years of constant decline until population extinction (Lips 1998; Pounds and Crump 1994), it is probable that the species was not declining. These conclusions indicate the importance of testing the methodology to be used in long-term studies. Erroneously declared declines could put other species in jeopardy by focusing funds and efforts on species that do not have as a great a need.

## Literature Cited

- Acevedo, M. A. and L. J. Villanueva-Rivera. In press. Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians. *Wildlife Society Bulletin*.
- Alford, R. A. and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* 30:133-165.
- Burrowes, P. A., R. L. Joglar, and D. E. Green. 2004. Potential causes for amphibian declines in Puerto Rico. *Herpetologica* 60:141-154.
- Drewry, G. E. 1970. Factors affecting activity of rain forest frog populations as measured by electrical recording of sound pressure levels. *In* H. T. Odum and R. F. Pigeon (eds.), *A Tropical Rain Forest*, pp. E55-E68. Division of technical information. U.S. Atomic Energy Commission.
- Drewry, G. E. and A. S. Rand. 1983. Characteristics of an acoustic community: Puerto Rican frogs of the genus *Eleutherodactylus*. *Copeia* 1983:941-953.
- Drost, C. A. and G. M. Fellers. 1996. Collapse of a regional frog fauna in Yosemite area of the California Sierra Nevada, USA. *Conservation Biology* 10:414 - 425.
- Fogarty, J. H. and F. J. Vilella. 2002. Population dynamics of *Eleutherodactylus coqui* in Cordillera Forest Reserves in Puerto Rico. *Journal of Herpetology* 36:193-201.
- Funk, W. C., D. Almeida-Reinoso, F. Nogales-Sornosa, and M. R. Bustamante. 2003. Monitoring population trends of *Eleutherodactylus* frogs. *Journal of*

- Herpetology 37:245-256.
- Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian populations declines. *Nature* 404:752-755.
- Joglar, R. L. 1998. Los Coquíes de Puerto Rico, su historia natural y conservación. Editorial de la Universidad de Puerto Rico, San Juan, Puerto Rico. 232 pp.
- Joglar, R. L. and P. A. Burrowes. 1996. Declining amphibian populations in Puerto Rico. *In* R. Powell, and W. Henderson (eds.), *Contributions to West Indian Herpetology: A Tribute to Albert Schwartz*, pp. 371-380. Society for the Study of Amphibians and Reptiles, Ithaca, New York.
- Laurance, W. F., K. R. McDonald, and R. Speare. 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conservation Biology* 10:406-413.
- Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. *Conservation Biology* 12:106-117.
- Lips, K. R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* 13:117-125.
- MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-2255.
- Peterson, C. R. and M. E. Dorcas. 1994. Automated data acquisition. *In* Heyer,



- W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*, pp 47-57. Smithsonian Institution Press, Washington, D.C.
- Pounds, J. A. and M. L. Crump. 1994. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. *Conservation Biology* 8:72-85.
- Richards, S. J., K. R. McDonald, and R. A. Alford. 1993. Declines in populations of Australia's endemic tropical rainforest frogs. *Pacific Conservation Biology* 1:66-77.
- Rivero, J. A. 1998. *Los Anfibios y Reptiles de Puerto Rico*. Editorial de la Universidad de Puerto Rico, San Juan, Puerto Rico. 2nd edition. 510 pp.
- Royle, J. A. and W. A. Link. 2005. A general class of multinomial mixture models for anuran calling survey data. *Ecology* 86:2505-2512.
- Stewart, M. M. 1995. Climate driven population fluctuations in rain forest frogs. *Journal of Herpetology* 29:437-446.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodriguez, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783-1786.
- Townsend, D. S. and M. M. Stewart. 1994. Reproductive ecology of the Puerto Rican frog *Eleutherodactylus coqui*. *Journal of Herpetology* 28:34-40.
- United States Geological Survey. 2004. *North American Amphibian Monitoring*

- Program. Accesible at <http://www.pwrc.usgs.gov/naamp/index.html>.
- Waide, R. B. 1996. Birds. *In* D. P. Reagan and R. B. Waide (eds.), *The Food Web of a Tropical Rain Forest*, pp. 364-398. University of Chicago Press, Chicago.
- Wake, D. B. 1991. Declining amphibian populations. *Science* 253:860.
- Wells, K. D. 2001. The energetics of calling in frogs. *In* M. J. Ryan (ed), *Anuran Communication*, pp. 45-60. Smithsonian Institution Press, Washington.
- Woolbright, L. L. 1997. Local extinctions of anuran amphibians in the Luquillo Experimental Forest of northeastern Puerto Rico. *Journal of Herpetology* 31:572-576.
- Woolbright, L. L. and M. M. Stewart. 1987. Foraging success of the tropical frog, *Eleutherodactylus coqui*: the cost of calling. *Copeia* 1987:69-75.
- Zimmerman, B. L. 1994. Audio strip transects. *In* Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek and M. S. Foster (eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*, pp. 92-97. Smithsonian Institution Press, Washington, D.C.

## Appendix 1. List of sites sampled with ADRS and frog species present.

Site and coordinates	<i>E. antillensis</i>	<i>E. coqui</i>	<i>E. gryllus</i>	<i>E. hedricki</i>	<i>E. locustus</i>	<i>E. portoricensis</i>	<i>E. richmondi</i>	<i>E. unicolor</i>	<i>E. wightmanae</i>	<i>L. albilabris</i>
LEF – Road km 9.1 18.31278 °N / 65.77290°W		X							X <sup>1</sup>	
LEF – Road 191, km 9.3 18.31189°N / 65.77364°W	X <sup>2</sup>	X							X <sup>2</sup>	
LEF – Near Mt. Britton Tower 18.30159°N / 65.79397°W		X	X			X		X <sup>2</sup>		
LEF – Palo Colorado 18.29988°N / 65.77840°W		X		X						
LEF – Pico del Este 18.27988°N / 65.76656°W		X	X <sup>2</sup>		X <sup>1,2</sup>	X		X		X <sup>2</sup>
LEF – Pico del Este 18.27741°N / 65.76386°W		X	X		X			X <sup>2</sup>		X <sup>2</sup>
LEF – Tradewinds Trail 18.29029°N / 65.79667°W		X	X	X	X <sup>1</sup>	X		X		
LEF – Tradewinds Trail 18.28985°N / 65.79849°W		X	X	X	X <sup>1,2</sup>	X		X	X <sup>1,2</sup>	X <sup>2</sup>
LEF – Mt. Britton Spur 18.30295°N / 65.79498°W		X	X			X		X		
Carite State Forest 18.10278°N / 66.03488°W		X					X		X	
Guajataca State Forest 18.42022°N / 66.96347°W	X <sup>2</sup>	X					X			
Maricao State Forest 18.16140°N / 66.99812°W		X					X <sup>2</sup>			
Toro Negro State Forest 18.16029°N / 66.56764°W		X	X						X <sup>1</sup>	
Lago Guineo 18.15944°N / 66.53047°W		X				X <sup>2</sup>			X <sup>2</sup>	X <sup>2</sup>

1. Detected for the first time with ADRS.

2. The data for this species at this site was not used for analysis (see Materials and Methods).

## **Appendix 2.** Program for the MSP430 microcontroller that controlled the Nomad 3 recorder.

```
#include <msp430x11x1.h>

#define ONE_SECOND    1365

#define PARTIAL_SECOND  500

void delay_seconds(unsigned int num_of_seconds);

void delay_minutes(unsigned int num_of_minutes);

void delay_partial(unsigned int partial_sec_value);

void main(void)
{
    unsigned int index;

    WDTCTL = WDTPW + WDTCTL;

    P1DIR |= 0x0F;

    P1OUT = 0x00;

    BCSCTL2 = 0xF0;

    for (index=0; index<5000; index++);

    delay_seconds((unsigned int) 2);

    for (;;)
    {
        P1OUT = 0x01;

        delay_seconds((unsigned int) 3);

        P1OUT = 0x00;

        delay_seconds((unsigned int) 62);
```

```

    P1OUT = 0x02;

    delay_seconds((unsigned int) 1);

    P1OUT = 0x00;

    delay_minutes((unsigned int) 28);

    delay_seconds((unsigned int) 50);

    delay_partial((unsigned int) PARTIAL_SECOND);
}
}

void delay_partial(unsigned int partial_sec_value)
{
    unsigned int par_loop_val;

    par_loop_val = partial_sec_value;

    do

        (par_loop_val--);

    while (par_loop_val != 0);
}

void delay_seconds(unsigned int num_of_seconds)
{
    unsigned int one_second_val;

    unsigned int sec_loop_val;

    for(sec_loop_val = 0; sec_loop_val < num_of_seconds;

        sec_loop_val++)

    {

```

```

    one_second_val = ONE_SECOND;

    do

        (one_second_val--);

        while (one_second_val != 0);

    }

}

void delay_minutes(unsigned int num_of_minutes)
{
    unsigned int min_loop_val;

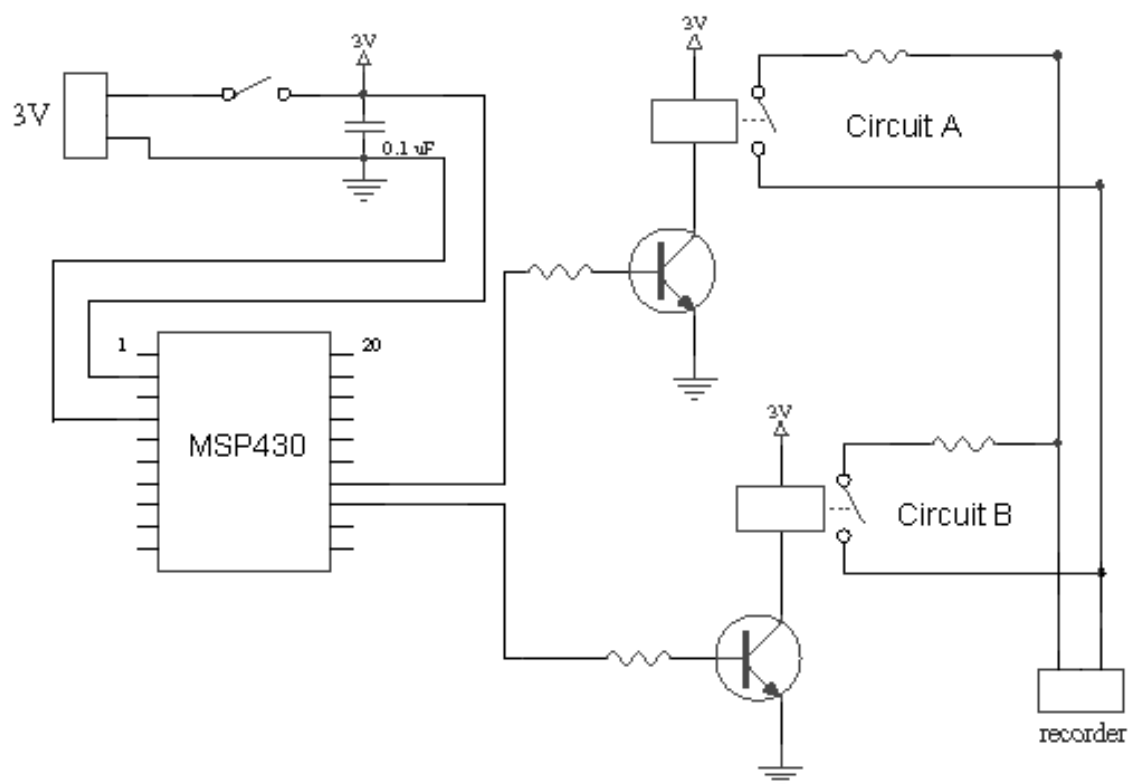
    for(min_loop_val = 0; min_loop_val < num_of_minutes;
        min_loop_val++)
    {
        delay_seconds((unsigned int) 60);
    }
}

```

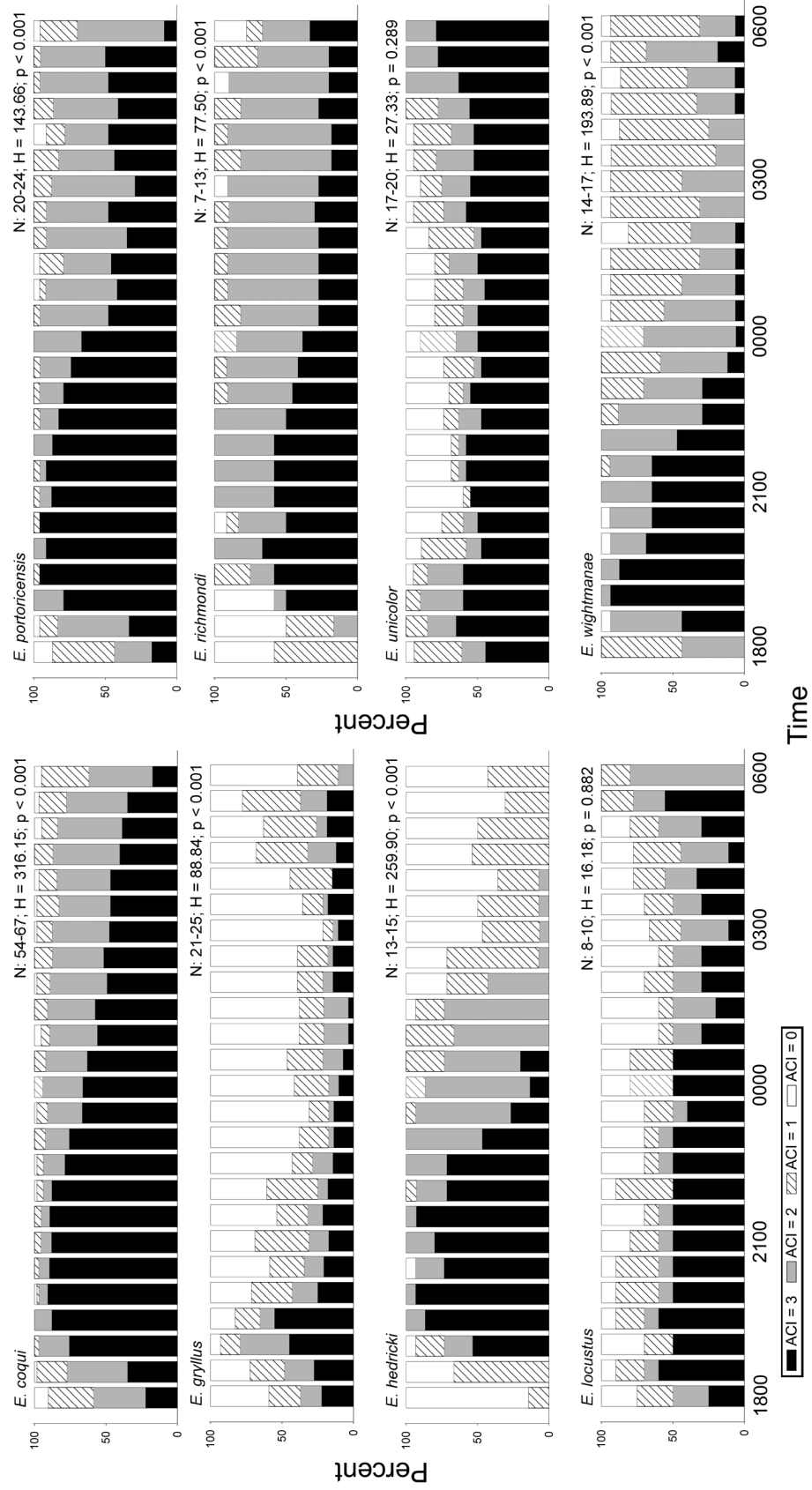
## List of Figures

Fig. 1. Schematic drawing of the controller circuit for the ADRS using a Nomad 3 recorder. The microcontroller (MSP430) triggers the relays at the programmed times.

Fig. 2. Percentage of calling activity level measured as Amphibian Calling Index (ACI) for eight species of *Eleutherodactylus* frogs from Puerto Rico.







**CHAPTER 3: HABITAT DISTRIBUTION MAP FOR THE VULNERABLE  
FROG *ELEUTHERODACTYLUS RICHMONDI* FROM PUERTO RICO**

## **Abstract**

The majority of the amphibians of Puerto Rico seem to be endangered, and some populations have disappeared in recent years. One species, *Eleutherodactylus richmondi*, has disappeared from all but one location in the Luquillo Experimental Forest, and it is critical to find new populations to monitor and to protect. I used geographic information layers to create an islandwide potential habitat distribution map using the locations of known populations of *E. richmondi*. The map identified several sites in the Cordillera Central, Carite State Forest and the karst region of the island as potential habitat for the species. These areas should be searched with more intensity in order to determine the actual status of *E. richmondi*.

## **Introduction**

Several reports point to the possibility that the majority of Puerto Rican amphibians are threatened or endangered and that populations have gone extinct, coinciding with the worldwide Declining Amphibian Populations (DAP) phenomenon (Stuart et al. 2004). Populations in the Luquillo Experimental Forest (LEF) have been reported as going extinct (Burrowes et al. 2004; Woolbright 1997). Some of these species have small populations which may reduce their survival probabilities. This situation, and the pressure from urban sprawl (Lugo et al. 2001), make imperative a landscape-level analysis to find populations of these species at sites where they were not previously known, and to help determine areas that need to be protected.

Some suggested causes of the DAP are habitat loss, modification and fragmentation, climate change, pollution, increased UV-B radiation levels, introduction of exotic species, a pathogenic fungus, or a combination of several factors (Blaustein et al. 1995; Davidson et al. 2001; Laurance 1996; Lips 1999). However, the most common cause for amphibian decline, as with most species, has been habitat destruction (Alford and Richards 1999).

One limitation in the protection and study of Puerto Rican amphibians is that most monitoring and other research efforts have been limited to roads and trails within the LEF (Burrowes et al. 2004; Joglar 1998; Joglar and Burrowes 1996; Moreno 1991; Stewart 1995; Stewart and Woolbright 1996). To understand the causes and extent of amphibian declines, we need to explore

new areas to determine the complete distribution of populations of each species. This will help to establish better conservation strategies when the refined distribution data are combined with information on historical land use, population dynamics and genetics, and forest connectivity.

Out of the 18 native amphibians of Puerto Rico, one, *Bufo lemur*, needs standing water in temporal ponds to reproduce (Rivero 1998), which may act as a limiting factor in the species' distribution. The other species that has a tadpole stage, *Leptodactylus albilabris*, uses small temporary water bodies in ditches and flat areas (Rivero 1998), so this does not limit their distribution. The other 16 species, all in the genus *Eleutherodactylus*, have direct development and deposit their eggs in the vegetation or underground (Joglar et al. 1996; Joglar et al. 2005; Ovaska and Estrada 2003; Townsend and Stewart 1986; Vega-Castillo 2000; Villanueva-Rivera and Joglar 2001). However, only generalized habitat descriptions are available for these species (Joglar 1998; Rivero 1998).

*Eleutherodactylus richmondi* is an understory, endemic frog of Puerto Rico (Rivero 1998) which has been classified as a vulnerable species (Departamento de Recursos Naturales y Ambientales 2004). Some populations of *E. richmondi* have been reported as going extinct during the last few decades and the few remnant populations are small (Table 1).

*E. richmondi* was first described in 1904 from several individuals that were collected in the Catalina Plantation in El Yunque by Stejneger (1904). In

the Scientific Survey of Porto Rico and the Virgin Islands, Schmidt (1928) mentioned that *E. richmondi* seemed to be limited in distribution to El Yunque. The species range in the literature includes the Cordillera Central, the LEF, some populations in Arecibo, the Sierra de Pandura and the Guajataca and Susúa State Forests (Joglar 1998; Rivero 1998).

It is imperative to find more populations to protect and study in order to determine whether the population extinctions are localized events or if there is an island-wide threat to the species. The objective of this study was to develop an island-wide habitat distribution map for *E. richmondi* using geographic information layers for land cover, soil type, elevation, temperature, and precipitation. The areas identified in the map can be used to direct search efforts to find previously unknown populations of the species by identifying the most appropriate areas.

## **Materials and Methods**

Historical populations were gathered from the literature when enough details were available for georeferencing the location of the population. One population reported in the literature at the Lago Guineo area (Joglar 1998), in the municipality of Orocovi, was not included because that report was unconfirmed (R. Joglar pers. com.). Local biologists were consulted to find extant populations of *Eleutherodactylus richmondi*.

The location of each population used (Table 2) was imported into a

geographical information system (ArcMap 9.1, Environmental Systems Research Institute, California) with six layers (Table 3) that represent environmental and habitat characteristics that may limit the distribution of a direct-developing frog. Temperature and precipitation appear to determine population fluctuations and reproductive activity in *E. coqui* (Stewart 1995; Townsend and Stewart 1994). Since *E. richmondi* populations have been reported only in forested areas, the type of land use may limit its distribution. Soil type may also be a limiting factor, because individuals have usually been found on the ground and in low vegetation (Rivero 1998), and the only report of an egg clutch was observed under a rotten log on the ground (H. Heatwole, unpublished data cited by Joglar 1998).

The layers were projected to the Puerto Rico State Plane, using the North American Datum of 1927, with a resolution of 30 x 30 m per pixel. Each map was reclassified to a binary variable with a value of one given to each cell that passed the following decision rules, and zero given to each cell that failed. The decision rules were applied to each cell as follows: for land cover and soil type, did the cell match one of the values observed in cells with a known population; for elevation, was the cell within the range of minimum and maximum values of cells with a known population; for February temperature and rainfall, was the cell value greater than or equal to the minimum value observed in cells with a known population; and for August temperature, was the cell value less than or equal to the maximum value observed in cells with a known population. The binary maps were then multiplied together to derive a binary suitability map,

in which a value of one indicated that values of all variables were within the range in which populations were known to exist.

To test the accuracy of the map, areas identified on the map in the LEF and the Carite State Forest were searched using Automated Digital Recording Systems (ADRS; Acevedo and Villanueva-Rivera In press) to determine the species present at each site. In addition, extinct population sites were surveyed using ADRS to determine if the species had been overlooked or if it had recolonized the area.

## Results

Six layers for the island of Puerto Rico were combined to extract the range for each layer that included known populations of *E. richmondi* (Fig. 1). The lowest minimum temperature of the coldest month, February, was 14 °C, while the highest maximum temperature for the hottest month, August, was 32 °C. The site with the lowest amount of precipitation had 1640 millimeters of rain on average for the month with the least amount of precipitation, February.

The locations of the populations included seven types of land cover (Table 4) and were located in limestone, volcaniclastic, plutonic, and serpentinite soil types. The elevation of the populations ranged from 160 – 1170 m above sea level.

The six layers were multiplied to obtain a binary habitat distribution map (Fig. 2) with a total area of suitable habitat of 1265 km<sup>2</sup>. This map shows



that the appropriate habitat for *E. richmondi* is distributed in the LEF, the Carite State Forest, some areas in the Sierra de Pandura range, several areas in and near the Toro Negro and Maricao State Forests, in the Cordillera Central, and along the karst region in the north of the island.

Twenty sites in areas identified in the map were searched using ADRS (Table 5). No new populations of *E. richmondi* were found. The sites of the historical localities where the species has been declared extinct were searched and the species was not found (Table 5).

## **Discussion**

Known and historical populations of *E. richmondi* were integrated in a GIS to determine similar habitats in Puerto Rico. These areas should have priority in the search for new populations of the species. The map generated (Fig. 2) revealed that an important area for *E. richmondi* that has little protection today is the karst region, in the northern area of the island. This region has been and is under pressure for construction projects (Lugo et al. 2001) and needs to receive protection. A study found 51 species of amphibians and reptiles in the karst region (Lugo et al. 2001), including *E. richmondi*, making it one of the most important areas for the herpetofaunal diversity of the island.

*Eleutherodactylus richmondi* seems to be one of the most endangered species of amphibian in Puerto Rico. Few populations are known and most of the known populations have gone extinct (Table 2). Unfortunately, most

searches have been limited to easy access areas near roads and trails, so it is possible that there are more populations that could be in better condition.

Although I did not find other populations of *E. richmondi*, it is possible that the species still has other extant populations. The known populations are small and the louder *E. coqui* can interfere with the detection of their calls (Villanueva-Rivera, this volume, chapter 1). Furthermore, the unexplored area of potential habitat is very large. For example, suitable habitat in the Carite State Forest covers 24.4 km<sup>2</sup>, while the known population only occupies an area of less than 1 hectare (pers. obs.).

The island of Puerto Rico was deforested extensively, with up to 88% of the island deforested for agriculture during the first half of the 20<sup>th</sup> century (Birdsey and Weaver 1987). This extensive deforestation left a few pockets of native forest at the highest elevations (Birdsey and Weaver 1987) that could have hosted small populations of the currently endangered frogs. Most of the extant populations of threatened and endangered anurans in Puerto Rico occur in high elevations (Joglar and Burrowes 1996). With the change in the island's economy from agriculture- to industry-based, the agricultural lands were abandoned (Grau et al. 2003) and by 1991, 42% of the island was covered by forests (Helmer et al. 2002). This increase in forest cover could provide species with new habitat to colonize. We need to establish a cooperative monitoring and search project on the island to cover these old and young forests to determine the species distribution. With a large-scale project we can start to generate

robust distribution models for the species and determine the real status of the island's herpetofauna. In addition, the secondary forest areas may be used to re-introduce species to suitable habitats. Local captive breeding programs can generate individuals to try these re-introductions as a conservation measure.

## Literature Cited

- Acevedo, M. A. and L. J. Villanueva-Rivera. In press. Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians. *Wildlife Society Bulletin*.
- Alford, R. A. and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* 30: 133-165.
- Birdsey, R. A. and P. L. Weaver. 1987. Forest area trends in Puerto Rico. U.S. Department of Agriculture. Forest Service Research Note SO-331. San Juan, Puerto Rico.
- Blaustein, A. R., B. Edmond, J. M. Kiesecker, J. J. Beatty, and D. G. Hokit. 1995. Ambient ultraviolet radiation causes mortality in salamander eggs. *Ecological Applications* 5:740-743.
- Briggs, R. P. and J. P. Akers. 1965. Hydrogeologic map of Puerto Rico and adjacent islands. *Hydrologic Investigations Atlas, Map HA-197*. Dept. of the Interior, U.S. Geological Survey, Washington, DC, USA.
- Burrowes, P. A., R. L. Joglar, and D. E. Green. 2004. Potential causes for amphibian declines in Puerto Rico. *Herpetologica* 60: 141-154.
- Daly, C., E. H. Helmer, and M. Quiñones. 2003. Mapping the climate of Puerto Rico, Vieques and Culebra. *International Journal of Climatology* 23:1359-1381.
- Davidson, C., H. B. Shaffer, and M. R. Jennings. 2001. Declines of the California red-legged frog: climate, UV-B, habitat, and pesticides hypotheses.

Ecological Applications 11:464-479.

Departamento de Recursos Naturales y Ambientales. 2004. Reglamento para  
Regir las Especies Vulnerables y en Peligro de Extinción en el Estado  
Libre Asociado de Puerto Rico. San Juan, Puerto Rico.

Drewry, G. E. 1970. Factors affecting activity of rain forest frog populations as  
measured by electrical recording of sound pressure levels. *In* H. T. Odum,  
and R. F. Pigeon (eds.), A tropical rain forest. A study of irradiation and  
ecology at El Verde, Puerto Rico, pp E55-E68. U. S. Atomic Energy  
Commission.

Drewry, G. E. and S. Rand. 1983. Characteristics of an acoustic community:  
Puerto Rican frogs of the genus *Eleutherodactylus*. *Copeia* 1983:941-953.

Grau, H. R., T. M. Aide, J. K. Zimmerman, J. R. Thomlinson, E. Helmer, and X.  
Zou. 2003. The Ecological Consequences of Socioeconomic and Land-  
Use Changes in Postagriculture Puerto Rico. *BioScience* 53: 1159-1168.

Helmer, E. H., O. Ramos, T. del M. López, M. Quiñonez, and W. Díaz. 2002.  
Mapping the forest type and land cover of Puerto Rico, a component of  
the Caribbean biodiversity hotspot. *Caribbean Journal of Science* 38: 165–  
183.

Joglar, R. L. 1998. Los coquíes de Puerto Rico: su historia natural y  
conservación. Editorial de la Universidad de Puerto Rico. San Juan,  
Puerto Rico.

Joglar, R. L. and P. A. Burrowes. 1996. Declining amphibian populations in

- Puerto Rico. Pages 371-380 in R. Powell, and W. Henderson, editors.  
Contributions to West Indian herpetology: A tribute to Albert Schwartz.  
Society for the Study of Amphibians and Reptiles, Ithaca, New York.
- Joglar, R. L., P. A. Burrowes, and N. Ríos. 1996. Biology of the Puerto Rican cave-dwelling frog, *Eleutherodactylus cooki*, and some recommendations for conservation. Pages 251-258 in R. a. H. Powell, R. W., editor.  
Contributions to West Indian Herpetology: A Tribute to Albert Schwartz.  
Society for the Study of Amphibians and Reptiles, Ithaca (New York).
- Joglar, R. L., P. A. Burrowes, D. Dávila, A. Rodríguez, A. López, A. Longo, and P. Medina. 2005. *Eleutherodactylus wightmanae* (Coquí melodioso).  
Reproduction. Herpetological Review 36:433-434.
- Laurance, W. F. 1996. Catastrophic declines of australian rainforest frogs: is unusual weather responsible? Biological Conservation 77:203-212.
- Lips, K. R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 13:117-125.
- Lugo, A. E., L. M. Castro, A. Vale, T. del Mar López, E. Hernández Prieto, A. García Martínó, A. R. Puente Rolón, A. G. Tossas, D. A. McFarlane, T. Miller, A. Rodríguez, J. Lundberg, J. Thomlinson, J. Colón, J. H. Schellekens, O. Ramos, and E. Helmer. 2001. Puerto Rican karst – a vital resource. General Technical Report WO-65. United States Department of Agriculture, Forest Service. 100 pp.
- Moreno, J. A. 1991. Status survey of the golden coquí *Eleutherodactylus jasperi*.

- In* J. A. Moreno (*ed.*). Status y distribución de los reptiles y anfibios de la región de Puerto Rico, pp 37-41. Departamento de Recursos Naturales de Puerto Rico. Publicación Científica Miscelánea No. 1.
- Ovaska, K. and A. R. Estrada. 2003. *Eleutherodactylus antillensis* (Coquí Churí). Reproduction. Herpetological Review 34:229.
- Rivero, J. A. 1998. Los anfibios y reptiles de Puerto Rico. The amphibians and reptiles of Puerto Rico. Editorial de la Universidad de Puerto Rico, San Juan, Puerto Rico. 510 pp.
- Schmidt, K. P. 1928. Scientific survey of Porto Rico and the Virgin Islands, vol. X-Part 1: Amphibians and land reptiles of Porto Rico, with a list of those reported from the Virgin Islands. New York Academy of Sciences. New York.
- Stejneger, L. 1904. The herpetology of Porto Rico. Report of the United States National Museum for 1902: 548-724.
- Stewart, M. M. 1995. Climate driven population fluctuations in rain forest frogs. Journal of Herpetology 29:437-446.
- Stewart, M. M. and L. L. Woolbright. 1996. Amphibians. *In* D. P. Reagan and R. B. Waide (*eds.*). The food web of a tropical rain forest. University of Chicago Press. pp. 273-320.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodriguez, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.

- Townsend, D. S. and M. M. Stewart. 1986. Courtship and mating behavior of a Puerto Rican frog, *Eleutherodactylus coqui*. *Herpetologica* 42:165-170.
- Townsend, D. S. and M. M. Stewart. 1994. Reproductive ecology of the Puerto Rican frog *Eleutherodactylus coqui*. *Journal of Herpetology* 28:34-40.
- United States Geological Survey. 2002. Shuttle Radar Topography Mission (SRTM) Elevation Data Set. Available from <http://seamless.usgs.gov>.
- Vega-Castillo, S. I. 2000. Habitat description and comparison of the Puerto Rican Demon or Guajón, (*Eleutherodactylus cooki*) at two localities in the eastern part of Puerto Rico. Page 46. University of Puerto Rico, Mayagüez Campus. Unpublished M. S. Thesis.
- Villanueva-Rivera, L. J. and R. L. Joglar. 2001. *Eleutherodactylus cochranae* (Coquí Pitito). Reproduction. *Herpetological Review* 32:182.
- Woolbright, L. L. 1997. Local extinctions of anuran amphibians in the Luquillo Experimental Forest of northeastern Puerto Rico. *Journal of Herpetology* 31:572-576.



Table 1. Populations of *E. richmondi* reported in the literature.

Location of the population	Reference	Notes
LEF – Catalina Plantation	Stejneger, 1904	Type location
LEF – El Yunque Peak	Schmidt, 1928	From 400 m to the peak
LEF – El Yunque Rock	Drewry & Rand, 1983	
LEF – Mt. Britton	Joglar & Burrowes, 1996	Extinct in 1980
LEF – Near UPR Field Station in El Yunque	Joglar & Burrowes, 1996	Extinct before 1986
LEF – Near El Verde Field Station	Drewry, 1970; Drewry & Rand, 1983; Stewart, 1995; Woolbright, 1997	Heard for the last time in 1989
LEF – Elfin Forest in El Toro peak	Joglar, 1998	
Carite Forest	Joglar, 1998; Rivero, 1998	
Sierra de Pandura	Joglar, 1998	Not found in recent years
Maricao Forest	Joglar, 1998; Rivero, 1998	
Guajataca Forest	Rivero, 1998	
Arecibo	Rivero, 1998; Lugo <i>et al.</i> , 2001	
Guayama, Highway 15	Drewry & Rand, 1983	
Ciales	Lugo <i>et al.</i> , 2001	
Toro Negro Forest	Joglar, 1998; Rivero, 1998	

Table 2. Populations of *E. richmondi* used in this study.

<b>Location of the population</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Source</b>
LEF - El Verde	18.32310 N	65.81640 W	Woolbright, 1997
LEF - El Toro	18.27210 N	65.82980 W	V. Cuevas (pers. com.)
LEF - El Yunque Rock	18.31055 N	65.79292 W	Drewry & Rand, 1983
LEF - Mt. Britton	18.30099 N	65.79312 W	Joglar, 1998
LEF – Road 191, km 9.3	18.31189 N	65.77364 W	N. Ríos (pers. com.)
LEF - University of Puerto Rico	18.29915 N	65.78035 W	Joglar, 1998
Rico Field Station			
Carite State Forest	18.10278 N	66.03488 W	
Cerro Punta	18.16956 N	66.58781 W	Joglar, 1998
Ciales Municipality	18.30913 N	66.54349 W	A. Puente (pers. com.)
Finca Tallonales	18.40800 N	66.73173 W	A. Puente (pers. com.)
Guajataca State Forest	18.42022 N	66.96347 W	J. Delgado (pers. com.)
Maricao State Forest	18.16140 N	66.99812 W	
Maricao State Forest –	18.15005 N	66.99274 W	Joglar, 1998
Casa de Piedra			
Toro Negro Pool Area	18.17145 N	66.48804 W	Joglar, 1998

Table 3. Layers used in the analysis of the habitat for the species and their source.

<b>Layer</b>	<b>Source</b>
Landcover type	Helmer et al. 2002
Soil type	Briggs and Akers 1965
Elevation	United States Geological Survey 2002
Average minimum temperature of coldest month (February)	Daly et al. 2003
Average maximum temperature of hottest month (August)	Daly et al. 2003
Average precipitation of driest month (February)	Daly et al. 2003

Table 4. Landcover types identified in the populations of *E. richmondi*. The landcover types were from Helmer and collaborators (2002).

<b>Landcover type</b>
Lowland moist seasonal evergreen and semi-deciduous forest
Lowland moist seasonal evergreen and semi-deciduous forest/shrub
Submontane and lower montane wet evergreen sclerophyllous forest
Submontane wet evergreen forest
Submontane and lower montane wet evergreen forest/shrub and active/abandoned shade coffee
Lower montane wet evergreen forest - tall cloud forest
Lower montane wet evergreen forest - mixed palm and elfin cloud forest

Table 5. Locations where the species *E. richmondi* was searched for using ADRS, and the species detected at each site.

Site and coordinates	<i>E. antillensis</i>	<i>E. brittoni</i>	<i>E. coqui</i>	<i>E. gryllus</i>	<i>E. hedricki</i>	<i>E. locustus</i>	<i>E. portoricensis</i>	<i>E. richmondi</i>	<i>E. unicolor</i>	<i>E. wightmanae</i>	<i>L. albilabris</i>
LEF – University of Puerto Rico Field House 18. 29919 °N / 65.78035 °W			X		X					X	
LEF – Road 191, km. 9.1 18.31278 °N / 65.77290 °W			X							X	
LEF – Road 191, km 9.3 18.31189 °N / 65.77364 °W	X		X							X	
LEF – Near Mt. Britton Tower 18.30159 °N / 65.79397 °W			X	X			X		X		
LEF – Palo Colorado 18.29988 °N / 65.77840 °W			X		X						
LEF – Pico del Este 18.27988 °N / 65.76656 °W			X	X		X	X		X		X
LEF – Tradewinds Trail 18.29029 °N / 65.79667 °W			X	X	X	X	X		X		
LEF – Tradewinds Trail 18.28985 °N / 65.79849 °W			X	X	X	X	X		X	X	X
LEF – Mt. Britton Spur 18.30295 °N / 65.79498 °W			X	X			X		X		
LEF- Pico del Este 18.27741 °N / 65.76386 °W			X	X		X			X		X
LEF – La Roca del Yunque 18.31055 °N / 65.79292 °W			X	X			X		X		
Carite State Forest 18.10278 °N / 66.03488 °W			X					X		X	
Near Cerro de la Tabla 18.05573 °N / 66.11861 °W		X	X				X			X	
Carite State Forest – Near Road 7741 18.05711 °N / 66.11283 °W		X	X								

Site and coordinates	<i>E. antillensis</i>	<i>E. brittoni</i>	<i>E. coqui</i>	<i>E. gryllus</i>	<i>E. hedricki</i>	<i>E. locustus</i>	<i>E. portoricensis</i>	<i>E. richmondi</i>	<i>E. unicolor</i>	<i>E. wightmanae</i>	<i>L. albilabris</i>
Guajataca State Forest 18.42022 °N / 66.96347 °W	X		X					X			
Maricao State Forest 18.16140 °N / 66.99812 °W			X					X			
Toro Negro State Forest 18.16029 °N / 66.56764 °W			X	X						X	
Toro Negro State Forest – Near Cerro Maravilla 18.15668 °N / 66.56146 °W			X								
Cerro Punta 18.17200 °N / 66.58910 °W			X								
Lago Guineo 18.15944 °N / 66.53047 °W			X				X			X	X

## List of Figures

Fig. 1. Locations of the populations of *E. richmondi* used to generate the habitat distribution map. Open circles represent extinct populations and filled circles represent extant populations.

Fig. 2. Binary map of the habitat distribution for *E. richmondi*. Dark areas represent potentially suitable habitat.

