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***Eleutherodactylus* frogs show frequency but no temporal partitioning: implications for the acoustic niche hypothesis**

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ABSTRACT

Individuals in acoustic communities compete for the use of the sound resource for communication, a problem that can be studied as niche competition. The acoustic niche hypothesis presents a way to study the partitioning of the resource, but the studies have to take into account the three dimensions of this niche: time, acoustic frequency, and space. I used an Automated Digital Recording System to determine the partitioning of time and acoustic frequency of eight frogs of the genus *Eleutherodactylus* from Puerto Rico. The calling activity was measured using a calling index. The community exhibited no temporal partitioning since most species called at the same time, between sunset and midnight. The species partitioned the acoustic frequency of their signals, which, in addition to the microhabitat partitioning, can provide some insight into how these species deal with the problem. This data also suggest that monitoring projects with this group should take place only before midnight to avoid false negatives.

Keywords: Acoustic niche hypothesis, *Eleutherodactylus*, Puerto Rico, community, bioacoustics

INTRODUCTION

- 1 The problem of how species in acoustic communities deal with the limited bandwidth of the acoustic resource
- 2 can be studied as niche competition. In this case the resource is a single medium of communication: air for
- 3 terrestrial communities and water for aquatic and marine systems. In particular, there are three dimensions in
- 4 which communities can partition this niche: in time, acoustic frequency and space (Garcia-Rutledge and Narins,
- 5 2001; Wells, 2007).

6 Partitioning of the acoustic frequency and timing of the signals has been subject of study in anuran communities
7 for decades (Littlejohn, 1965; Chek et al., 2003; Steelman and Dorcas, 2010). This partitioning, formally posited
8 as the acoustic niche hypothesis, may help explain community structure when each community assembles itself in
9 ways to reduce competition for the sound (Krause, 1993; Farina et al., 2011).

10 Most studies on anuran acoustic communities have been limited to a single dimension, making generalizations
11 very hard to reach (Wells, 2007). An assumption that is often made is that the whole community is stable, where
12 the partitioning is caused by competition and displacement of the acoustic frequency of their calls (Wells, 2007).
13 However, without data on the temporal partitioning it is not possible to determine that the competition pressures are
14 enough to drive a change. This is particularly difficult in assemblages at a same study site that are highly variable
15 (Guyer and Donnelly, 2005; Wells, 2007).

16 The acoustic community of the Puerto Rican *Eleutherodactylus* frog species was described as having both tem-
17 poral and acoustic partitioning (Drewry, 1970; Drewry and Rand, 1983). However, these patterns were generated
18 systematically for five species and subjectively, from field notes, for the other nine species. It is not clear if these
19 patterns are accurate enough to determine the peak of activity for each species and they did not seem to match
20 observations in the field (pers obs).

21 The objective of this study was to test the acoustic niche hypothesis in anurans by determining if there is
22 temporal and acoustic frequency partitioning in the calling activity of highland species of *Eleutherodactylus* frogs
23 from Puerto Rico. These patterns were determined using Automated Digital Recording System (ADRS (Acevedo
24 and Villanueva-Rivera, 2006)). This community exhibits microhabitat partitioning but this dimension was not
25 included in this study (Stewart and Woolbright, 1996).

26 METHODS

27 Populations of highland Puerto Rican *Eleutherodactylus* frogs were sampled at 14 sites with ADRS to determine
28 the calling activity for each species between 2003 and 2004 (Fig. 1). The ADRS consisted of a Nomad Jukebox 3
29 digital player and recorder (Model DAP-HD0003, Creative Labs, Inc, California), a portable preamplifier (Model
30 SP-PREAMP, The Sound Professionals, Inc., New Jersey) and an electret condenser microphone (Model ECM-
31 MS908C, Sony Electronics Inc, California). The microphone was placed at approximately one meter from the
32 ground. The system was controlled with a microcontroller (model MSP430-P- 1121M, Olimex Ltd., Plovdiv,
33 Bulgaria), which triggered commands to the recorder to record 1 minute every 30 minutes. Each sample was
34 stored as a .wav file using a sampling rate of 48 kHz.

35 Each recording was listened to with headphones to determine the species calling and their respective activity
36 level. The recordings were loaded to the AUDITION software (ver. 1.0; Adobe Systems, Inc., California, USA)
37 to visualize the species' calls in the spectrogram that were not audible due to interference by other loud species,
38 usually by *Eleutherodactylus coqui* (Villanueva-Rivera, 2007). Each species has a distinct call, which have been
39 described and published (Rivero, 1998). After listening to a recording once, if the spectrogram indicated the
40 possibility of another species that was not heard, the recording was filtered to remove the range of frequencies of
41 other species and listened to again. The activity level of each species was categorized using the Amphibian Calling
42 Index (ACI). The ACI can have four values: (0) represents no individuals calling; (1) a few individuals calling with
43 no overlap between the calls; (2) there is some overlap; and (3) a full chorus (Nelson and Graves, 2004).

44 To determine temporal partitioning, the ACI values for each species were analyzed using a Kruskal-Wallis test
45 with the null hypothesis that the calling activity was uniform during the night, between 1800 and 0600 h (Sokal
46 and Rohlf, 1995). The noise from rain or wind in several recordings made it difficult to determine the ACI for the
47 species. These recordings were not included in the analysis, which resulted in dissimilar sample size between the
48 25 periods during the night. In some sites, the average ACI of some species was lower than 1. When this was the
49 case, the data for the species at the site was not included in the analysis.

50 The recordings made at the time with the peak of calling activity for most species, 2000 h, were used to
51 determine acoustic partitioning. The frequency range of the *Eleutherodactylus* species present in these recordings
52 was measured using the software Pumilio (Villanueva-Rivera and Pijanowski, 2012).

53 Statistical analyses were performed using R (v. 2.5.1, R Development Core Team, Vienna, Austria) and
54 $\alpha = 0.05$. All the recordings and data tables are stored in Figshare (doi: 10.6084/m9.figshare.806302).

55 This research was conducted in the state forests under the authorization of the Department of Natural and
56 Environmental Resources of Puerto Rico (02-IC-068) and at the El Yunque National Forest under the authorization
57 of the United States Forest Service (CNF-2038). Since there was no collection or manipulation of individuals,
58 approval by the Institutional Animal Care and Use Committee was not required.

59 RESULTS

60 I detected 10 species of frogs in 1550 audio recordings at the 14 sites surveyed (Table 1). Two species, *Leptodactylus*
61 *albilabris* and *Eleutherodactylus antillensis*, with a widespread distribution on the island (Rivero, 1998), were
62 heard occasionally at some sites and were not included in the analysis. One species, *Eleutherodactylus coqui*, a
63 generalist species, was present at all sites.

64 **Temporal partitioning**

65 The pattern of calling activity was estimated using the Amphibian Calling Index (ACI) (Nelson and Graves, 2004).
66 The pattern during the night for six of the eight species was significantly different at some part of the night (Table
67 2). Five species had their peak of activity between sunset and midnight: *E. coqui*, *E. hedricki*, *E. portoricensis*, *E.*
68 *richmondi*, and *E. wightmanae*, measured as a higher proportion of samples with ACI values of 2 or 3 (Fig. 2). The
69 calling activity of these five species was highest between 1900 and 2100 h and declined steadily after midnight.
70 In the case of *E. gryllus*, the species had a short peak of activity between sunset to about 2000 h. Two species, *E.*
71 *locustus* and *E. unicolor*, showed no significant difference in their activity during the night.

72 Three species had a small peak of activity during the last hours of the night (Fig. 2). The species *E. gryllus*, *E.*
73 *portoricensis*, and *E. wightmanae* increased their activity two hours before sunrise from the declined activity level
74 of the hours after midnight. These peaks were smaller than the main peak before midnight.

75 **Acoustic frequency partitioning**

76 From the whole dataset, the 64 recordings made at 2000h were analyzed to determine acoustic partitioning. In all
77 sites with more than one species, most species exhibited frequency partitioning (Fig 3), where the frequency range
78 of their signals did not overlap. Only between *E. coqui* and *E. portoricensis* there was a large overlap in one of
79 the notes. Their first note, "co", did not overlap. Their second note, "qui", showed an overlap that averaged 63.9%
80 (54.5-85.9%) of the frequency range of the note of *E. coqui* and 57.8% (45.2-80.3%) of the frequency range of the
81 note of *E. portoricensis* ($n = 6$).

82 Two other cases showed some overlap to a lesser degree. The calls of between *E. wightmanae* and *E. richmondi*
83 at the Carite State Forest (Site 14 in Table 1) overlapped 42.0% (10.3-83.0) and 11.5% (4.2-21.0) respectively
84 ($n = 5$). At the El Yunque National Forest, the call of *E. hedricki* and the "qui" note of *E. portoricensis* showed
85 some overlap at the Tradewinds Trail sites (Sites 6 and 7 in Table 1). The overlap in the call of *E. hedricki* was
86 9.7% (0-17.9) and for the "qui" note of *E. portoricensis* was 7.0% (0-10.9).

87 **DISCUSSION**

88 **Temporal partitioning**

89 The results from this study indicated that the level of activity of six of eight highland *Eleutherodactylus* species
90 studied was not uniform during the night. Most species called from sunset to midnight, with a peak around 2000h.
91 These results suggest that this anuran community does not have temporal partitioning of their calling activity during

92 the night. Seasonal differences will need to be studied using long-term datasets.

93 A previous study found some temporal partitioning in several species at El Yunque National Forest (Drewry
94 and Rand, 1983). In particular, two species in that study, *E. portoricensis* and *E. richmondi*, were calling later than
95 the other species in the night. However, the data used in that study were collected using different methods and from
96 field notes, not from a systematic survey during the night. Another study using ADRS in a palustrine herbaceous
97 wetland in Puerto Rico also found temporal clustering in four *Eleutherodactylus*, among them the generalist *E.*
98 *coqui*. The species exhibited a peak of calling activity also at 2000h (Ríos-López and Villanueva-Rivera, 2013).
99 The sun sets between 1800 and 1900h (Ríos-López and Villanueva-Rivera, 2013).

100 It was expected that species should limit their calling activity to a period when its benefits (attracting females)
101 are outweighed by its costs (energy expenditure, reduced foraging, and predation risk). Reproductive success of
102 *E. coqui* is determined only by calling effort (Townsend and Stewart, 1994). In several studies that compare the
103 energetic cost of calling, the metabolic rate increases up to ten times, making it a very energetically expensive
104 activity (Gerhardt, 1994; Wells, 2001). In *E. coqui*, males reduce the number of prey they consume while calling
105 (Woolbright and Stewart, 1987) and the energy requirements of calling stop their growth (Woolbright, 1989). Pre-
106 dation on *Eleutherodactylus* by owls (*Megascops nudipes*) has been reported (Zelick and Narins, 1982), so it is
107 possible that these predators may sometimes use the call of the males to hunt them.

108 Acoustic surveys are a standard method for anurans (Zimmerman, 1994; Rödel and Ernst, 2004; Dorcas et al.,
109 2009), but communities with temporal clustering present some problems. Very loud species, like *E. coqui*, can
110 mask other species present at the sites (Villanueva-Rivera, 2007). Audio recordings can be a better method for
111 monitoring these species, in particular when combined with automated identification (Aide et al., 2013). Results
112 from this study suggest that acoustical monitoring of Puerto Rican *Eleutherodactylus* species should take place
113 from sunset to midnight, when most of the species are highly active, with recorders to reduce false negatives due
114 to masking. Furthermore, surveys conducted after midnight should be avoided as low calling activity levels could
115 be due to the time and not a local extinction or a declining population. Special attention should be given to cases
116 like *E. gryllus*, with a very short peak of calling activity limited to the first two hours of the night.

117 **Acoustic frequency partitioning**

118 The acoustic community of *Eleutherodactylus* species exhibited partitioning in the acoustic frequency of their
119 sound signals. The calls of *E. wightmanae* and *E. richmondi* showed some overlap, however the calls are very
120 different, which could reduce the pressure for frequency displacement. The call of *E. wightmanae* is a repetition

121 series of a note while the call of *E. richmondi* is a short click with a very broad range in frequency (Rivero, 1998).
122 In the other case of overlap, the species have spatial partitioning. *Eleutherodactylus portoricensis* is found in the
123 understory up to 3m of the ground, while *E. hedricki* only calls from holes in old branches near the canopy (Stewart
124 and Woolbright, 1996).

125 The large overlap in the second note, "qui", of the call of *Eleutherodactylus coqui* and *E. portoricensis* found in
126 this study deserves further analysis, in particular because the species do not show temporal or spatial partitioning.
127 Both species partition the frequency space of their "co" note. In invasive *E. coqui* populations in Hawaii, the "co"
128 has very little inter-individual variation, while the "qui" note seems to be more variable (Benevides and Mautz,
129 2013). This high variability and overlap between sympatric *E. coqui* and *E. portoricensis* could indicate that this
130 is not an important signal for distance communication since evolution has not separated this signal as the others.
131 The two-note call of *E. coqui* has been studied in some detail. The first note, "co", seems to be important to
132 maintain distance between calling males and to establish their territory, while the second one, "qui", is used to
133 attract females (Narins and Capranica, 1976, 1978; Zelick and Narins, 1982).

134 A study that tested the effect of two levels of density of sympatric *E. portoricensis* on the dominant frequency
135 of the notes of *E. coqui* found that the "co" did not change, while the acoustic frequency of the "qui" was lower
136 in sites with high densities of *E. portoricensis* (Luther et al., 2012). *E. coqui* might be trying to avoid masking
137 of the noise or the higher densities of *E. portoricensis* could be triggering a suppression of the call in individuals
138 with higher overlap in the frequency range (Zelick and Narins, 1982). The mechanism that is driving this effect
139 of lower frequency could be studied by determining whether the females select males with lower frequency due
140 to masking or if males that call at higher frequencies have less reproductive success because they are suppressed
141 by the heterospecific call. In *Hyla cinerea*, the presence of *H. gratiosa* was related to displacement in female
142 preference and in the advertisement call of the males (Höbel and Gerhardt, 2003). However, a study with sympatric
143 populations of two *Pseudacris* species showed that the character displacement was variable among sites (Lemmon,
144 2009).

145 Several studies seem to indicate that acoustic frequency partitioning in anuran communities is not common. In
146 a review of 11 assemblages, only 3 showed acoustic partitioning (Chek et al., 2003). However, there was no data
147 on temporal or spatial partitioning in most assemblages and some included data from a large geographical region.
148 Other factors can make it harder to study, including separation of the acoustic frequency due to factors other than
149 competition for the acoustic resource (Gerhardt and Schwartz, 1995).

150 A null model of a 7-species community in a pond in Costa Rica did not find differences in the frequency parti-

151 tioning with random assemblages (Guyer and Donnelly, 2005). However, this study documented 28 combinations
152 of up to 6 species during 47 sampling nights. This large variability of species may not be enough selective pressure
153 to induce displacement of the acoustic frequencies (Pfennig and Pfennig, 2009).

154 In a community of five anurans in the Andes of Colombia, three of them *Eleutherodactylus*, the four species
155 that were nocturnal had their peak of calling activity between one and two hours after sunset (Lüddecke et al.,
156 2000). The species partitioned both the calling sites used and the acoustic frequency range (Lüddecke et al., 2000).
157 In a Thailand assemblage of 11 species in 3 families, a study found partitioning in acoustic frequency, timing and
158 space (Garcia-Rutledge and Narins, 2001).

159 In a community of 13 species in Brazil studied in permanent and temporary ponds and swamps, species that
160 did not partition in space, partitioned their acoustic signals (Santos and Rossa-Feres, 2007). In turn, species that
161 had similar calls partitioned their use of space (Santos and Rossa-Feres, 2007).

162 **Conclusions**

163 This study provided support for the acoustic niche hypothesis in anurans. However, it seems the partitioning of the
164 acoustic resource of anurans is not a simple phenomenon to study and previous studies have been too limited to
165 provide evidence for or against it. Although there have been suggestions for the study of this problem using null
166 models and by comparing sympatric and allopatric communities (Gerhardt, 1994), few studies have used this type
167 of comparison and the ones that have do not present a clear consistent result in anuran acoustic communities (Chek
168 et al., 2003; Lemmon, 2009; Luther et al., 2012). Confounding factors, like evolutionary history, in particular when
169 dealing with several families of anurans that congregate at the same site, and diversity of reproductive strategies
170 should be taken into account in future studies to determine which are the determining factors in these acoustic
171 community assemblages. Conservation efforts should also take these sources of competition into consideration
172 when selecting sites for re-introduction.

173 The acoustic niche hypothesis will need to be studied in all its dimensions, time, acoustic frequency and space.
174 In this study the species exhibited no partitioning in the time dimension but partitioning in the acoustic frequency
175 dimension. The study of the spatial dimension will depend on what is known of the behavior of the species. For
176 either of the other two dimensions, acoustic frequency (Chek et al., 2003) or time (Steelman and Dorcas, 2010),
177 may not provide enough data, or the conclusions might not be generalizable due to the unknown influence of the
178 other dimensions.

179 This acoustic community of *Eleutherodactylus* frogs from Puerto Rico present a good opportunity to study the

180 acoustic niche hypothesis and the evolution of call displacement. All the species of Puerto Rico are closely related
181 (Heinicke et al., 2007; Hedges et al., 2008), most call at the same period of the night, and they are the majority
182 of the anuran fauna in the island. These qualities reduce the added complexity of previous studies that compared
183 communities comprised of several families (Gerhardt and Schwartz, 1995; Chek et al., 2003).

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TABLES

Site No.	Site*	Dates	Species not analyzed
1	EYNF Road 191, km 9.3	30/Oct/03 - 1/Nov/03	<i>E. antillensis</i>
			<i>E. wightmanae</i>
2	EYNF Road 191, km 9.1	28/Apr/04 - 3/May/04	<i>E. wightmanae</i>
3	EYNF Mt. Britton Spur	28/Apr/04 - 3/May/04	
4	EYNF Mt. Britton Tower	30/Oct/03 - 3/Nov/03	<i>E. unicolor</i>
5	EYNF Palo Colorado	11/Apr/04 - 16/Apr/04	
6	EYNF Tradewinds Trail	15/Jul/04 - 20/Jul/04	<i>E. locustus</i>
			<i>E. wightmanae</i>
			<i>Leptodactylus albilabris</i>
7	EYNF Tradewinds Trail	11/Apr/04 - 16/Apr/04	<i>E. locustus</i>
8	EYNF Pico del Este	28/Apr/04 - 3/May/04	<i>E. gryllus</i>
			<i>E. locustus</i>
			<i>L. albilabris</i>
9	EYNF Pico del Este	12/Aug/04 - 17/Aug/04	<i>E. unicolor</i>
			<i>L. albilabris</i>
10	Maricao State Forest	7/Feb/04 - 11/Feb/04	<i>E. richmondi</i>
11	Guajataca State Forest	19/Apr/04 - 24/Apr/04	<i>E. antillensis</i>
12	TNSF	16/Jun/04 - 21/Jun/04	
13	TNSF - Lago Guineo	19/Feb/04 - 24/Feb/04	<i>E. portoricensis</i>
			<i>E. wightmanae</i>
			<i>L. albilabris</i>
14	Carite State Forest	19/Mar/04 - 21/Mar/04 30/Mar/04 - 4/Apr/04	

* EYNF = El Yunque National Forest; TNSF = Toro Negro State Forest.

Table 1. Sites, dates sampled, and species detected but not used for the analysis in this study.

Species	H value	Range of <i>n</i>
<i>E. coqui</i>	319.4*	54 - 67
<i>E. gryllus</i>	94.7*	21 - 29
<i>E. hedricki</i>	268.9*	12 - 15
<i>E. locustus</i>	15.3	8 - 10
<i>E. portoricensis</i>	154.8*	19 - 24
<i>E. richmondi</i>	77.6*	7 - 12
<i>E. unicolor</i>	26.4	17 - 20
<i>E. wightmanae</i>	190.6*	14 - 17

* $p < 0.001$

Table 2. Results of the Kruskal-Wallis test on the uniformity of the calling activity during the night for each *Eleutherodactylus* species in this study. Some samples had noise from rain or wind and were not included, which resulted in different sample sizes for some time periods.

FIGURE LEGENDS

Figure 1. Locations surveyed acoustically for frogs in Puerto Rico. Shaded areas represent protected areas of the island. The numbers represent the locations as listed in Table 1.

Figure 2. Percentage of calling activity level measured as Amphibian Calling Index (ACI) for eight species of Eleutherodactylus frogs from Puerto Rico. White bars represent ACI = 0 (no individuals calling), diagonal lines represent ACI = 1 (a few individuals calling with no overlap), gray bars represent ACI = 2 (there is some overlap), and black bars represent the percentage of samples with ACI = 3 (full chorus).

Figure 3. Frequency range occupied by each species at each site. Both *E. coqui* and *E. portoricensis* have two notes, known as "co" and "qui", that were measured separately.





