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Acoustic Repertoire and Calling Behavior of the Gliding Treefrog, *Agalychnis spurrelli* (Anura: Hylidae)

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Abstract. Acoustic communication is essential for reproductive success in frogs. Males produce different types of calls to attract females, advertise territoriality or location in a chorus, or communicate with heterospecifics. Quantitative descriptions of amphibian calls provide basic information for taxonomic, ecological, evolutionary, and conservation studies. Here, we describe the acoustic repertoire of *Agalychnis spurrelli* and discuss this species' reproductive behavior on the basis of observations made over the course of a breeding season in May–December 2007. Males produced one type of advertisement call and two types of aggressive calls (calls A and B) that differed in temporal and spectral frequency. The advertisement call was a single note. Aggressive call A was a long-pulsed single note, and call B was composed of more than two notes per call. Calling behavior was influenced by chorus size and male-to-male proximity, with males altering their advertisement calls with the increase of chorus size and eventually producing aggressive calls. These results suggest that *A. spurrelli* might exhibit graded aggressive signaling, helping males to delimit their calling site and reducing the number of agonistic encounters.

Keywords. Agonistic encounters; Chorus activity; Reproductive behavior; Vocalization.

INTRODUCTION

Acoustic communication is an important component of reproductive success and social interaction in anurans (Wells, 1988; Wells and Schwartz, 2006). Most anuran species produce different calls that can vary in their functions (Littlejohn, 1977; Wells, 1977, 1988, 2007; Toledo et al., 2015; Köhler et al., 2017); nonetheless, advertisement and aggressive calls are the most commonly heard (Wells and Schwartz, 2006). The advertisement call is mainly emitted to attract mates (Wells, 1977; Rand and Ryan, 1981; Wells and Greer, 1981; Wells and Schwartz, 1984a), although it can also advertise location and maintain spatial separation between males in a breeding chorus (Wells, 1977; Wells and Greer, 1981; Wells, 2007). Aggressive calls serve as a warning to other males in order to defend territories or calling sites (Wells and Schwartz, 1984b; Pröhl, 1997; Bastos and Haddad, 2002). Additionally, the properties of the advertisement call can be used by females to assess male mate quality, while the aggressive call can contain cues on the size or fighting ability of the emitter (Gerhardt and Huber, 2002; Gerhardt and Bee, 2006; Wells, 2007).

Calling behavior in anurans can be influenced by the acoustic environment including background noise, acous-

tic interference, and vocal responsiveness of nearby competitors (Schwartz and Wells, 1984; Schwartz and Wells, 1985; Schwartz et al., 2002; Schwartz and Bee, 2013). Consequently, males can alter their call rate, complexity, duration, and intensity during choruses (Wells, 2007). Therefore, it is important to identify and describe the differences in anuran call repertoires as they are key factors that potentially influence the evolution of acoustic signaling and sexual selection in anurans. Additionally, it is important to establish how such differences vary among species.

Most species of *Agalychnis* Cope, 1864 are prolonged breeders (*A. annae*, *A. callidryas*, *A. dacnicolor*, *A. granulososa*, *A. lemur*, *A. moreletii*: Duellman, 1970; Pyburn, 1970; Gomez-Mestre et al., 2008; Vilela et al., 2015), with the exception of *A. saltator* and *A. spurrelli*, which show explosive breeding with an unusual aggregation behavior (Scott and Starret, 1974; Roberts, 1994; Ortega-Andrade et al., 2011). Nonetheless, different breeding patterns have been reported among populations of *A. spurrelli* (Vargas et al., 2000). The acoustic repertoires of *Agalychnis* species consist of advertisement calls of a single note; however, some species of this genus present advertisement calls of more than one note repeated at different intervals of a few seconds to several minutes (Duellman,

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1970; Cannatella, 1980; Pimenta et al., 2007; Hertz and Lotzkat, 2010; Vilela et al., 2015). The vocal repertoire of *A. spurrelli* includes an advertisement call, described as a single, low-pitched groan (Duellman, 1970), and a second call, reported by Scott and Starret (1974). The description of Scott and Starret (1974) is limited to the call rate and provides no further details about any other acoustic parameter, leaving the functions and specific context of this call unknown. Most of the calling pattern of *A. spurrelli* is based on observations of few individuals (Duellman, 1970; Ortega-Andrade, 2008). Therefore, information about the calling behavior in this species is relatively limited. We describe the call properties and calling patterns of *A. spurrelli* in relation to its social behavior and compare them with those of other species of *Agalychnis*. We also assess the influence of environmental factors on the calling phenology of this species to determine possible correlations between calling males and abiotic variables.

MATERIALS AND METHODS

We conducted this study in May–December 2007 at Kingfisher pond (09°09'24.6"N, 79°51'28.4"W), a seasonal pond located on Barro Colorado Island (BCI). BCI is a 1,560-ha reserve and research station located in the Panama Canal that is operated by the Smithsonian Tropical Research Institute. The vegetation on BCI is characterized by a seasonal lowland moist forest. The island receives approximately 2,623 mm of precipitation during the rainy season (mid-May to mid-December), with a pronounced dry season from mid-December to mid-April (Leigh, 1999). Kingfisher pond has an oval shape and area of approximately 338 m², with a maximum depth of 0.9 m. The vegetation immediately surrounding the pond is formed mainly by *Elaeis oleifera* (HBK) Cortes (1897) a palm tree not exceeding 20 m in height, and shrubs, grass, and old-growth forest.

We visited Kingfisher pond monthly for 5–7 nights per visit. We sampled following the new moon lunar phase, as suggested by Kubicki (2004). At the beginning of our study, behavioral sampling was carried out from 19:00–06:00 or until calling activity diminished or ended. We observed the first males calling around 21:30, so we modified our sampling methods to start at this time. We recorded vocalizations from 35 calling males using a Panasonic digital recorder-RRUS395PS with a Sony ECM-C115 microphone at about 1 m from the individual. After recording, we marked each focal calling male by a unique toe-clip combination (up to three clips per frog) following Donnelly's scheme (Heyer et al., 2001), and all frogs were released at the same point of capture within 24 h.

Vocalizations were digitized at 22 kHz and 16 bit resolution and analyzed using the software Raven Pro version 1.5 (Bioacoustics Research Program, 2011)

through Fast Fourier Transformation at 512-point width, Hanning window type, and overlapped at 90%. We measured the following call variables: call duration (s), call repetition rate (calls/min), number of notes per call, note duration (s), pulses/note, and dominant frequency (Hz). Measurements are presented as mean \pm SE (range). We classified calls on the basis of the terminology proposed by Wells (2007) and Toledo et al. (2015). Additionally, we recorded behavioral observations of calling males during courtship and agonistic interactions to categorize the vocalizations emitted by *Agalychnis spurrelli* according to the social context. Some sound files were deposited in Fonoteca Zoológica (FZ 10052, 10053), Museo Nacional de Ciencias Naturales de Madrid.

Climate data such as humidity, temperature, and precipitation were taken for every day of recording from the meteorological station on BCI, called "El Claro," which is located 2.2 km from the study site. To determine whether the density of males increased the aggressive call rate, we divided the total number of aggressive calls emitted by all males by the total number of males for each night and compared this value with the total number of males in the pond. We used the Shapiro-Wilk test to check for normality. Due to violation of residual normality, we used Spearman's rank correlation. Data were analyzed using SPSS 21.0 (IBM Corporation, 2012), and statistical significance was considered at $P \leq 0.05$.

RESULTS

Vocalization

Three types of calls of *Agalychnis spurrelli* could be clearly differentiated in our recordings on the basis of differences in call structure and social context. Call parameters are shown in Table 1. Males produced an advertisement call (Fig. 1A) consisting of a single, simple note ($n = 96$ calls; 16 males) of 0.20 ± 0.01 s (0.16–0.30 s) duration and emitted at a rate of 3.1 calls/min. This call had a pulse rate of 81.9 ± 2.9 s (61–99 s) and a dominant frequency of 884.2 ± 25.6 Hz (750–1,054.7 Hz). Figure 1A shows the high energy at the beginning of the call, with less energy after the middle of the note.

The other two aggressive calls (calls A and B) were produced by males during agonistic encounters (Fig. 1B–C). Aggressive call A was emitted at rate of about 3.5 ± 0.6 calls/min ($n = 28$ calls; seven males), with a duration of 0.59 ± 0.03 s (0.47–0.69 s), and a pulse rate of 133.3 ± 9.4 s (44–241 s). This call was emitted at a dominant frequency of 904 ± 28.2 Hz (843–1,062.5 Hz). Aggressive call B was a multi-note call consisting of 2–22 notes ($n = 33$ calls; 12 males), with longer call duration (0.97 ± 0.2 s), and emitted at shorter call intervals (4.05 ± 0.7 s) compared to the advertisement call.

Call B was emitted at a frequency of 809 ± 11.5 Hz (750–847.7 Hz). Aggressive call A was produced singly, but occasionally in combination with call B (Fig. 2).

Calling behavior

The breeding season of *Agalychnis spurrelli* on BCI took place from mid-June to late October, but reproductive activity usually lasted 3–4 d. The highest number of

calling males at Kingfisher pond occurred in July–September; thereafter, the number of individuals decreased dramatically (Fig. 3). The number of males calling at Kingfisher pond per month was positively correlated with relative humidity ($r_s = 0.381$; $P = 0.034$; $n = 31$) and rainfall ($r_s = 0.378$; $P = 0.036$; $n = 31$), but not with temperature ($r_s = -0.275$; $P = 0.135$; $n = 31$).

Calling activity almost always began after 00:00 and continued until 06:00. Advertisement calls were emitted across the night or during nights with a lower number of

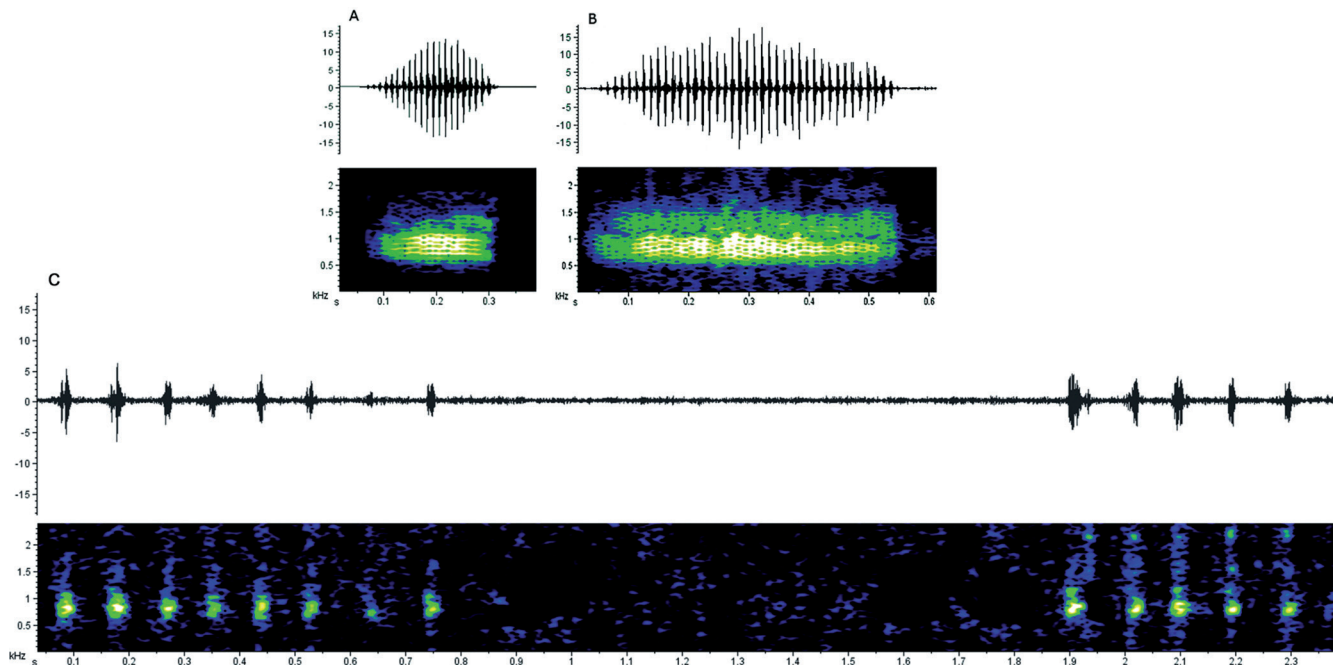


Figure 1. Waveform (top) and spectrogram (bottom) of calls produced by different males of *Agalychnis spurrelli*. (A) Advertisement call. (B) Aggressive call A. (C) Aggressive call B. Note the difference in call duration among calls. Spectrogram produced with Hanning window function, 512 point width; recordings made at 25°C and 24°C air temperature.

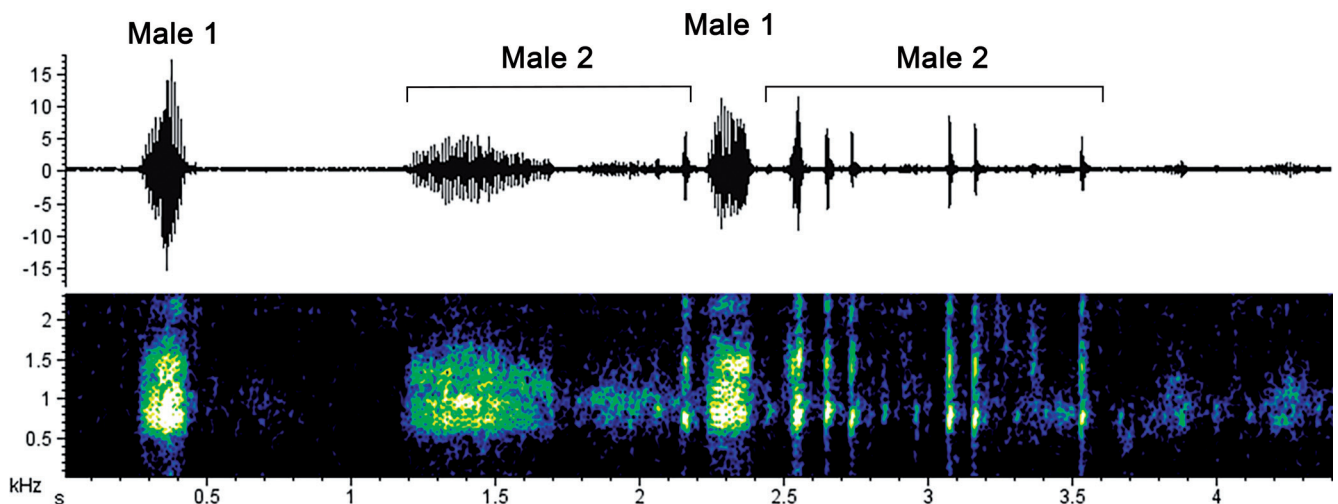
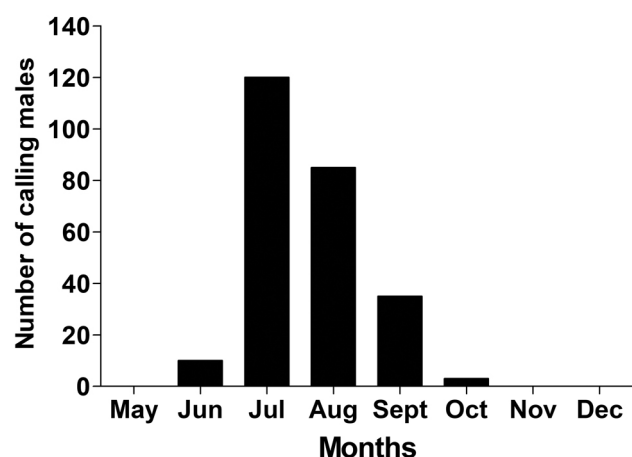


Figure 2. Agonistic interaction between two male *Agalychnis spurrelli* in Kingfisher pond: Male (1) and Male (2). Waveform (top) and spectrogram (bottom). Male 1 produced two advertisement calls, while male 2 emitted a combination of aggressive calls: call A followed by call B. Spectrogram produced with Hanning window function, 512 point width; recordings made at 25°C air temperature.

Table 1. Acoustic parameters of *Agalychnis spurrelli*. Values are presented as mean \pm SE.

	Advertisement call		Aggressive call A		Aggressive call B	
	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
Call/duration (s)	0.20 \pm 0.01	0.13–0.36	0.59 \pm 0.03	0.50–0.69	0.97 \pm 0.2	0.17–1.62
Call rate (min)	3.1 \pm 0.2	2–5	3.5 \pm 0.6	2–6	4.46 \pm 0.7	2–5
Call interval (s)	16.1 \pm 1.5	4–26.9	5.1 \pm 1.2	1.4–18.0	4.05 \pm 0.7	1.4–15.4
Notes duration (s)	---	---	---	---	0.018 \pm 0.001	0.012–0.027
Notes/call	1	---	1	---	9.3 \pm 0.8	2–22
Notes rate (s)	---	---	---	---	5.1 \pm 0.6	2–11
Pulse per note	17 \pm 1.3	14–30	45 \pm 2.4	28–65	14 \pm 0.9	8–26
Pulses rate (s)	81.9 \pm 2.9	61–99	133.3 \pm 9.4	44–241	58.3 \pm 7.5	21–100
Dominant frequency (Hz)	884.2 \pm 25.6	750–1054.7	904.3 \pm 28.2	781.2–1062.5	809.2 \pm 11.5	750–847.7

**Figure 3.** Number of males observed calling during 8 months at Kingfisher pond. Breeding season of *Agalychnis spurrelli* is from May to December 2007. The highest numbers of males calling were between July and September.

calling males. In contrast, males emitted aggressive calls on nights with high activity. The number of aggressive calls emitted per male increased with the number of calling males in the pond ($r_s = 0.846$, $P < 0.001$, $n = 31$). Males produced exclusively aggressive calls when facing other males in calling bouts, especially when they were close to each other (0.23–1.2 m; $n = 6$ observations), even without physical interaction between them. On one occasion, we observed a male in amplexus producing aggressive call B while other males in the surrounding area were also producing the same call. We observed only one fight between two males, which lasted few seconds. Both males were emitting combinations of aggressive calls A and B before the physical combat.

At the beginning of chorus activity, a few males produced advertisement calls, which were followed by the rest of the males throughout the night. As the number of calling males increased, we observed that males reduced the call rate per minute and produced aggressive call A before emitting call B. There were moments in which all males emitted only aggressive call B. No displacements of amplexant pairs were observed. We observed the presence

of amplexus after 01:30 ($n = 10$). Throughout the night, there were two peaks in calling activity, one at 02:30 and other at 04:30. The chorus began to decline after 04:30, when the only males calling were those close to a female. At 06:00, there were few if any males calling.

DISCUSSION

In this study, we describe the acoustic repertoire and calling behavior of *Agalychnis spurrelli*. We provide details about this species' advertisement call and describe two types of aggressive calls with information about the behavioral context in which they were displayed. The characteristics of the advertisement call reported here differ from those reported by Ortega-Andrade (2008) and Duellman (1970). We recorded advertisement calls with similar pulse rates but shorter note duration and higher dominant frequency than those described previously by Duellman (1970), who described the advertisement call of *A. spurrelli* as a single note with duration of 0.34–0.40 s, a pulse rate of about 60–90 s, and a dominant frequency of 435–750 Hz. These differences in note length and spectral frequency are likely related to the small sample size and regional and size differences between the individuals studied by Duellman (1970; $n = 2$ frogs, Panama), Ortega-Andrade (2008; $n = 1$ frog, Ecuador), and us ($n = 16$ frogs) in Panama.

Males produced two calls to which we have assigned aggressive functions. These calls differ from the advertisement call in acoustical properties and the behavioral context in which they were emitted. Scott and Starret (1974) described call B as a weak call emitted constantly, with note rate (150 notes/min) being the only acoustic parameter they mentioned. They interpreted this call as a bluff attempt by a male in the presence of another male. In contrast, Ibáñez et al. (1999) suggested it might be an aggressive call but added no detail. We also detected an additional aggressive call (call A, Fig. 1B). Males produced these calls only on nights with a high number of calling males, when males were in close proximity to each other or dur-

Table 2. Acoustic parameters of the advertisement call of frogs of the genus *Agalychnis*.

Species	Call duration (s)	Notes/call	Pulse per note	Pulse rate (s)	Dominant frequency (Hz)	Source
<i>A. annae</i> (Duellman, 1963)	0.16–0.44	1	6.0–17.0	38–50	1,044–1,295	Duellman (1970)
<i>A. aspera</i> (Peters, 1873)	0.014–0.05	1	3.0–4.0	-	1,679.59–2,110.00	Pimenta et al. (2007)
<i>A. callidryas</i> (Cope, 1862)	0.08–0.24	1.0–2.0	-	180–200	1,488–2,400	Duellman (1970); Lee (1996)
<i>A. dacnicolor</i> (Cope, 1864)	0.16–0.36	1	-	120–190	1,120–2,240	Duellman (1970)
<i>A. granulosa</i> (Cruz, 1988)	0.019–0.049	1	3.0–7.0	151–364	1,490–2,101	Vilela et al. (2015)
<i>A. lemur</i> (Boulenger, 1882)	0.32–0.40	1	-	39–41	950–1,000	Cannatella (1980)
<i>A. medinae</i> (Funkhouser, 1962)	0.67–0.72	5	-	-	2,092.7–2,506.6	Hertz and Lotzkat (2010)
<i>A. moreletii</i> (Duméril, 1853)	0.022–0.088	1	1.0–26.0	55–61	1,046–1,396	Duellman (1970); Briggs (2010)
<i>A. psilopygion</i> (Cannatella, 1980)	0.04–0.05	1	-	-	~1,900	Cannatella (1980)
<i>A. saltator</i> Taylor, 1955	0.08–0.12	1.0–2.0	-	105–110	1,844–1,890	Duellman (1970)
<i>A. spurrelli</i> Boulenger, 1913	0.13–0.36	1	14–30	61–99	750–1,051.7	This study

ing agonistic encounters. Males emitting combinations of calls A and B before a physical combat support the general assumption of aggressive call function described elsewhere (Wells, 2007; Toledo et al., 2015). In addition, our field observations suggest that these calls are likely used to maintain distance between males during agonistic interactions.

The calling behavior of *Agalychnis spurrelli* is similar to that of several species of *Agalychnis* (Table 2). Most species present an advertisement call composed of a single note, except for *A. callidryas*, *A. saltator*, and *A. medinae* (Duellman, 1970; Hertz and Lotzkat, 2010). The advertisement call of *A. spurrelli* has the highest value of pulses per note and lowest dominant frequency of the genus. Advertisement call duration in *A. spurrelli* is most similar to that of *A. annae* and *A. dacnicolor*.

Interestingly, only four species of *Agalychnis* (among the 11 described) present another type of call besides the advertisement call. Jungfer and Weygoldt (1994) described an encounter call in captive individuals of *A. lemur*, which they characterized as a short note with duration of 0.05–0.15 s, 2–4 pulses per note, and 800–2,750 Hz of dominant frequency. Similar to our observation in *A. spurrelli*, Jungfer and Weygoldt (1994) mentioned that this encounter call was emitted when a male was near another one. Similarly, *A. callidryas* emits a “chuckle” aggressive call that might reinforce the boundaries of calling territories (Pyburn, 1970; Caldwell et al., 2010). Caldwell et al. (2010) observed that male *A. callidryas* displayed vibrational signals (tremulations) and emitted aggressive calls during agonistic interactions, but they did not provide any information about the properties of this call. D’Orgeix (1996) also found that *A. callidryas*, besides chuckle calls and vibrations, produced a “soft tlock call.” He described the chuckle call as having 3–5 notes, with duration of 0.35 s, and dominant frequency of 1,285 Hz and the “soft tlock call” as a single note of 0.028 s duration and mean dominant frequency of 1,565 Hz. As in *A. callidryas*, males of *A. moreletii* use tremulation displays and aggressive chuckle calls during agonistic interactions that might be important to defend territories or

calling sites (Serrano et al., 2018). In *A. saltator*, Roberts (1994) reported that during breeding aggregations males produced a “soft squeaking and chuckling noise” that differed from the advertisement call. Despite the reports of different types of calls in *A. moreletii* and *A. saltator*, only the advertisement call has been described in these species (Duellman, 1970; Briggs, 2010).

We observed a male emitting aggressive call B while amplexing a female. Similar behavior has been observed in other phyllomedusine species. Pyburn (1970) observed that *Agalychnis callidryas* emitted chuckle calls when unattached males approached amplexant pairs, and Pyburn (1970) and Bagnara et al. (1986) observed that *A. dacnicolor* produced calls when unattached males approached amplexant pairs. A possible explanation is that males use this call to evaluate other males before engaging in escalated combats (see below). Among phyllomedusine, it is common for males in amplexus to emit aggressive calls when solitary males attempt to displace amplexing pairs (Martins et al., 1998; Abrunhosa and Wogel, 2004; Wogel et al., 2005; Venâncio and Melo-Sampaio, 2010; Oliveira et al., 2012; Dias et al., 2017).

When producing aggressive calls, males usually alter some parameters of the advertisement call, such as duration, pulse rate, and spectral frequency. It is well-known that males can alter some acoustic structures of the call in relation to chorus size in order to increase its relative attractiveness (Schwartz and Wells, 1985; Schwartz et al., 2002). Males can present an aggressive threshold relative to the call amplitude of neighbors that can be used to repel neighboring males from their immediate vicinity (Brenowitz, 1989). For instance, when an intruder male produces calls above the resident’s aggressive threshold, the resident male will produce aggressive calls. If the amplitude of the intruder male rises, the resident will increase the number of aggressive calls and finally repel the intruder or engage in physical combat (Rose and Brenowitz, 1991; Brenowitz and Rose, 1994).

Although playback experiments were not carried out in this study, we speculate that a similar pattern may ex-

plain the calling behavior of *Agalychnis spurrelli*. We observed that males increase their advertisement call rate when they are near one another. Thereafter, males produce call A, evoking an aggressive response, and finally switch to call B. However in this study, we observed that agonistic interactions are not constant in *A. spurrelli*. When a neighbor male stops the aggressive calls or begins emitting advertisement calls again the others return to the production of advertisement calls. This may suggest that *A. spurrelli* also presents an aggressive threshold and that males can adjust their aggressive behavior based on the calling intensity within the chorus. Playback experiments in males considering all call components and responses to the different call types of *A. spurrelli* are needed to test this hypothesis.

Despite *Agalychnis spurrelli* having been categorized as an explosive breeder with individuals that congregate in aquatic breeding sites for short periods of time (Scott and Starret, 1974; Wells, 2007), dissimilar breeding behaviors have been observed in populations from different places. Vargas et al. (2000) mentioned that this species has a prolonged breeding behavior on the basis of the year-round presence of adults and tadpoles in different larval stages in a population at Anchicaya, Colombia. In our study, the breeding behavior of *A. spurrelli* on BCI occurred over 4 months during the rainy season. At the end of the rainy season, no individuals of *A. spurrelli* were found at Kingfisher pond. This breeding behavior is similar to that report by Scott and Starrett (1974) in Costa Rica and Ortega-Andrade et al. (2011) in Ecuador. Based on the reproductive pattern observed on BCI, we support the assumption that this species is an opportunistic explosive breeder, as previously suggested for populations in Costa Rica and Ecuador (Gray, 1997; Ortega-Andrade et al., 2011). These variations among populations suggest that the reproductive pattern displayed by *A. spurrelli* might be influenced by ecological factors present at the breeding sites (Scott and Starret, 1974). It is well known that in the tropics ecological factors such as rainfall seasonality influence the reproductive behavior of anurans that depend on water for reproduction (Donnelly and Guyer, 1994; Bertoluci, 1998; Protázio et al., 2015). Therefore, *A. spurrelli* is likely able to combine both prolonged and explosive breeding patterns depending on whether weather conditions are favorable for reproduction and water availability is restricted for a short period (Gray, 1997). This opportunistic reproductive behavior suggests that in June sufficient water had accumulated in the pond to ensure the successful development of *A. spurrelli* tadpoles. In addition, frogs may decrease their reproductive effort at the end of the season to avoid the risk of larval mortality caused by the drying of the pond before completing their metamorphosis. Similar patterns have been observed in other hylids that breed in temporary ponds (Donnelly and Guyer, 1994).

In summary, we presented a detailed description of the vocalizations and calling behavior of *Agalychnis spurrelli*. Our results show that the acoustic signal and social interaction of this species are considerably more complex than those of other *Agalychnis* species. Males may have a graded aggressive signaling depending on the neighboring males' calling intensity. Further studies are required to determine if this behavior is advantageous for the reproductive success of males. Finally, this species presents a highly adapted reproductive behavior that is influenced by the ecological factors of its breeding localities.

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