Patterns of acoustic phenology in an anuran assemblage of the Yungas Andean forests of Argentina

Martín Boullhesen1-2,*, Marcos Vaira1, Rubén Marcos Barquez2, Mauricio Sebastián Akmentins1

1 Instituto de Ecorregiones Andinas (INECOA), CONICET – UNJu. San Salvador de Jujuy, Argentina
2 Argentina b Instituto de Investigaciones de Biodiversidad Argentina (PIDBA), Facultad de Ciencias Naturales e Instituto Miguel Lillo, San Miguel de Tucumán, Argentina

*Corresponding author. Email: m.boullhesen@conicet.gov.ar

Submitted on: 2022, 7th December; revised on: 2023, 21st April; accepted on: 2023, 27th April
Editor: Mattia Falaschi

Abstract. Breeding seasons in anurans are usually noticed by their advertisement calls, which stand as the main signal emitted by males during their adult life. These calls are species-specific signals with multiple information and can be used to monitor anuran populations over extended time periods. Applying a Passive Acoustic Monitoring method (PAM), we described the acoustic breeding phenology of an anuran assemblage along an altitudinal elevation range in the Yungas Andean forests of Argentina. In addition, we propose a new classification scheme for their acoustic phenological strategies, based on the male's calling records throughout an entire year. Also, we assessed the temporal and spectral niche overlap by the anuran species recorded. The assemblage was active throughout the entire year, with a higher concentration of calls recorded during the spring-summer season. We describe five distinct Acoustic Breeding Strategies based on the calling patterns of the recorded species. Temporal niche overlap was higher in the spring-summer season and in the lowest study site. The use of a PAM as a tool to monitor the advertisement calls in anurans communities could be a reliable technique to obtain different information about the species' acoustic phenology and the temporal use of the acoustic communication channel.

Keywords. Acoustic breeding strategies, acoustic phenology, advertisement calls, anurans, calling guilds, Passive Acoustic Monitoring.

INTRODUCTION

Advertisement calls are the main signals emitted by anurans during the breeding season (Wells, 2007). These species-specific features are mainly expressed by males, with some exceptions in females (e.g., the bullfrog *Lithobates catesbeianus*) of certain species, and voiceless males like *Rhinella gallardoi* (Emerson and Boyd, 1999; Carrizo, 1992). Advertisement calls are considered key factors for mate selection (Márquez and Verrell, 1991; Gerhardt and Huber, 2002; Wells and Schwartz, 2007) and are used by receivers according to their temporal and spectral characteristics, both for identification and location of their potential pairs, as well as to recognize the quality of the emitter (Gerhardt and Schwartz, 2001; Mason, 2007). Thus, these signals are used in mate choice behaviour, transmitting several messages simultaneously (Candolin, 2003). In addition, due to their “species-specific” nature, these are used as a taxonomic character for species description and identification (Köhler et al., 2017). Thus, advertisement calls have been widely used in long-term monitoring programs (Bridges and Dorcas, 2000; Dorcas et al., 2009; Llusia, 2013; Márquez et al., 2014; Measey et al., 2016). The surveying of advertisement calls has proven to be a useful technique for detection and monitoring of anuran species at large spatial scales (de Solla et
The breeding phenology of anurans, monitored through their advertisement calls, can be explored at different time scales, mainly in regions where seasonality shapes the extent, the start, and ending of their breeding activity (Weir and Mossman, 2005). Thereby, different species may have distinct daily and seasonal calling patterns (Cook et al., 2011; Yoo et al., 2012). Studying these calling patterns, can serve as a tool to define the core calling periods of the species recorded (Lemckert and Mahony, 2008), for which it is considered of utmost importance to increase the detection probability in anuran survey programs. The use of automated recording devices for passive acoustic monitoring techniques (PAM) can add valuable information such as the acoustical breeding patterns, as several species of anurans may breed simultaneously in different reproductive sites (Nelson and García, 2017; Duarte et al., 2019; Ulloa et al., 2019; Pérez-Granados et al., 2020).

Amphibians are suffering alterations in their phenomenological patterns because of the increasingly frequent extreme climatic events caused by the global climate crisis (Lanno and Stiles, 2020). This is particularly concerning for the species distributed throughout the biodiversity hotspot of the tropical Andes of South America (Myers et al., 2000), which are severely affected by the consequences of climate change (Burrowes, 2008). In addition, in times of the global diversity crisis, amphibians are considered the most affected groups among terrestrial vertebrates (Stuart et al., 2004; IUCN, 2023).

Based on the temporal pattern of their breeding phenology, anurans have been historically classified into two discrete groups, as proposed by Wells (1977): explosive breeders and prolonged breeders. Although there is a behavioural continuum between these two extremes (Wells, 1977; 2007). Several works focused on better describing the breeding acoustic strategies that can be found in anuran species (Chen et al., 2023; Donnelly and Guyer, 1994; Forti et al., 2022; Huang et al., 2001; Prado et al., 2005; Prasad et al., 2022; Bertoluci and Rodrigues, 2002) but there is still missing information mainly for Neotropical anurans. Continuous monitoring of anuran advertisement calls along different spatial-temporal scales, can provide valuable information about the different breeding strategies displayed by each species within an assemblage (Moreira et al., 2007).

The acoustic niche hypothesis (Krause, 1987) proposes that each individual elaborating messages through sound in each environment will present a partition in its spectral and temporal features, to avoid being masked by others (Krause, 1993). In species-rich assemblages from different clades, different strategies are expected to be present to avoid the competition in the acoustic communication channel (Bertoluci and Rodrigues, 2002; Herrick et al., 2018; Klump and Gerhardt, 1992). One way to minimize competition for the acoustic space is made effectively by segregating the niche in its temporal and spectral dimensions (Both and Grant, 2012; Sinsch et al., 2012; Guerra et al., 2020, Lima et al., 2019). The temporal segregation of the advertisement calls can be fundamental for the constitution of large anuran assemblages, mainly in breeding areas where several species vocalize simultaneously (Drewry and Rand, 1983; Schwartz and Wells, 1983; Bertoluci and Rodrigues, 2002; Duarte et al., 2019).

The Yungas ecoregion is one of the most biodiverse environments in Argentina (Brown et al., 2006) harbouring up to 40 species of anurans (modified from Lavilla and Heatwole, 2010). These subtropical montane forests are characterized by a steeped altitudinal gradient described by phytogeographic strata (Grau and Brown, 2000). The anuran assemblages that inhabit the subtropical montane forest of the Parque Nacional Calileguá (PNC) within the Yungas ecoregion, was reported to present a wide range of temporal and spatial breeding patterns ranging from opportunistic to prolonged breeders (Vaira, 2002). However, these records of breeding activity were obtained based on regular monthly surveys lasting from 3 to 5 days carried out through active searches by visual and acoustic sampling (Vaira, 2002). The anuran assemblage of the PNC is composed by a few endemic species to the Yungas ecoregion and by numerous species with a wide distribution in Argentina and other South American countries (Lavilla, 2001; Vaira et al., 2017). In addition, a low number of studies aimed to describe and understand the complete acoustic breeding phenology over an entire year in neotropical anurans (Bertoluci and Rodrigues, 2002; Prado et al., 2005; Saenz et al., 2006). Therefore, there are still wide gaps of information about the daily and annual patterns of calling activity and the breeding acoustic phenology of the anuran species that conforms different assemblages inhabiting the Yungas forests.

A recent study suggests the use of PAM as an effective technique to monitor the species of anurans in the Yungas forests (Boullhesen et al., 2021). In addition, with the implementation of a PAM program, researchers were able to discover hidden behavioural insights of phenomenological activity, such as the nocturnal calling activity of a
frog species inhabiting the Yungas forests, that was previously considered as a strictly diurnal species (Pereyra et al., 2016).

In this study, we describe the daily and annual patterns of calling activity of the anuran assemblages inhabiting the Yungas Andean Forests using PAM. Furthermore, we propose a new classification scheme for the acoustic breeding strategies used by different species of Neotropical anurans. Also, we aim to explore the occupation level of the acoustic communication channel and to determine the temporal overlap of the acoustic niche of this subtropical anuran assemblage.

**MATERIALS AND METHODS**

**Study sites and data collection**

A PAM was carried out for 12 months (from September 2017 to August 2018) along an altitudinal gradient in the PNC, being the most representative portion of the Yungas ecoregion in Jujuy province, Argentina. The PNC harbours the phytogeographic strata described for the Yungas forests as well as different environments where anurans breed (Vaira, 2002). The study was carried out in the three main forest types described for the ecoregion: Premontane Lowland Forests (400 - 700 m a.s.l.) which is a semideciduous environment with a marked seasonality, the understory consists of dense bushes, herbs, ferns and lianas; Lower Montane Forests (700 - 1300 m a.s.l.) dominated by evergreen trees such as *Juglans australis*, *Cedrella balansae* and *Enterolobium contortisiliquum*; the Upper Temperate Montane Forests (1500 - 2500 m a.s.l.) which is a primary old forest dominated by trees from Myrtaceae family (Grau and Brown, 2000). The two upland forest types have markedly more moisture than the lower forest. Three automated recording units Song Meter 4 (Wildlife Acoustics Inc., Concord, Massachusetts), one per site were installed and programmed to record 3 minutes per hour (24/7) (Shirose et al., 1997; Márquez et al., 2014). Recordings were done in MONO channel using in-built low noise microphones and stored in 32GB SDXC Flash Cards in WAV format. The recorders were placed at 1.5 m above ground in three sites: Premontane Forest (PF) (23°45'16.84"S; 64°50'59.35"W, 650 m a.s.l.), at the edge of a permanent pond with an approximate area of 1114 m². The Lower Montane Forest (LMF) (23°41’36.84"S; 64°52’5.04"W, 1125 m a.s.l.), at the edge of a permanent stream and in Upper Montane Forest (UMF) (23°40’28.56"S; 64°53’44.15"W, 1750 m a.s.l.), attached to a tree near temporary ponds. Recorders were visited monthly to data download and battery replace. These locations are representative to the breeding areas used by the anuran assemblage of the region (Vaira, 2002).

**Data Analysis**

13,485 recordings were listened corresponding to one day per week (= 224.75 hours) from the three study sites together throughout a year-round monitoring. Recordings were inspected manually by a trained specialist in anurans call recognition of Yungas forests (MB) in Raven Pro 1.5 (Bioacoustics Research Program, 2014) using a window type = Hann, DFT size = 512 samples, and overlap = 50%. For the general description of the acoustic phenology of the anuran species recorded, the monitored year was divided in two seasons (six months each) marked by the regional climate as follows: A) spring-summer season, corresponding to the period from September 2017 to March 2018. B) fall-winter season, corresponding to the period from March 2018 to September 2018.

To describe the annual acoustic phenology, we used the classification of core calling periods proposed by Lemckert and Mahony (2008). This considers the core calling period for each species as the time-period containing > 90 % of the calling events. For these classifications we considered the species with a total of ≥ 50 calling events records only. With the data of the calling events per-day recorded throughout the year-round study we conducted a bottom-up hierarchical cluster analysis using vegan and cluster R packages. For this analysis euclidean distance and complete method were employed after correlation checking of the cophenetic distance obtained with the original data used (>0.90). This analysis was implemented in free software R.

To describe the daily and annual vocal activity of each species we adapted the classification proposed by Bridges and Dorcas (2000) as follows: 0 = no male vocalizing; 1 = one male vocalizing; 2 = multiple males vocalizing with the possibility of occasionally distinguishing single calls; 3 = multiple males vocalizing but unable to distinguish single calls. We considered each advertisement call detected from a recording as a “calling event”, since we could not assign a distinct call to an individual. Circular statistics was employed to describe and analyse the daily calling patterns (Jammalamadaka et al., 2001; Pewsey et al., 2013). Rayleigh test was applied to explore whether the population of circular data, from which a sample is drawn, differs from randomness (Wilkie, 1983).

To explore temporal niche overlap in calling activity, we computed the Pianka (Pianka, 1973) and Czekanowski indices (Feinsinger et al., 1981) using the TimeOverlap program (Castro-Arellano et al., 2010; Guerra, 2020). The Czekanowski index or Proportional Similarity Index
varies between 1 (widest amplitude of the niche where the population exploits the resources in proportion to their availability) and 0 (where the population specializes in the rarest state of a resource and skip the other items). Whereas the Pianka index can return values less than zero (allowing for a coexistence between species) or greater than one (promoting a competition between species).

To characterize the use of the acoustic communication channel in anurans recorded, the methodology proposed by Emmrich et al. (2020) was used according to their acoustic calls features where:
- **Guild A** = unmodulated simple call
- **Guild B** = modulated simple call
- **Guild C** = unmodulated pulsed call
- **Guild D** = modulated pulsed call
- **Guild E** = unmodulated pulsed multi-note call
- **Guild F** = uniform modulated pulsed multi-note call
- **Guild G** = non-modulated different multi-notes call
- **Guild H** = modulated different multi-notes call

These guilds groups where previously visualized using clean calls from each species recorded with the see-wave package in R using a Hanning type windows, 75 % overlap and 1024 sample size (Fig. S1).

**RESULTS**

**Annual Calling Periods**

A total of 3318 calling events of anuran species were recorded in the three study sites within the Calilegua National Park (Fig. 1). We detected calling activity throughout the entire year (Fig. 1), with a minimum of one species vocalizing in the driest months of July and August and a maximum of 16 species vocalizing simultaneously during December (Fig. 1). Most of the calls were recorded during the hottest and rainy period (spring-summer seasons), with peaks of calling activity during November, December, and January (Tables 1 and 2).

**Boana riojana** was the only species recorded throughout the year-round monitored and presented high records in the fall-winter period (Fig. 1). Meanwhile, the rest of the species from Hylidae family were recorded calling over the spring-summer period (Fig. 1-2). Most of the species from Leptodactylidae family where recorded calling at spring-summer periods, with the summer period having the highest core calling periods. However, **Pleurodema borelli** presented peaks of calling activity in the fall-winter period (Fig. 1). The two toads of the genus **Rhinella** were registered calling during the early spring-summer period. Meanwhile **Melanophryniscus rubriventris** was recorded vocalising in the spring-summer, but was also detected in the early dry-cold fall-winter period (Fig. 1). The direct development-frogs from Strabomantidae family presented calling records concentrated in the spring-summer period, mainly during the summer season (Fig. 1-2). The only arboreal specie from Phyllomedusidae family (**P. boliviana**) presented calling records concentrated in the summer season (Fig. 1-2).

**Acoustic Breeding Strategies**

The cluster analysis revealed five different acoustic breeding strategies (Fig. 3): continuous breeders, prolonged non-seasonal breeders, prolonged seasonal breeders, prolonged non-regular breeders, and sporadic seasonal breeders.

A continuous breeding strategy was found in males of **Boana riojana**, who vocalized during all months of the year. A prolonged non-seasonal breeding pattern was observed in **Pleurodema borelli**, which started calling in spring-summer and continued through the fall-winter period. Prolonged seasonal breeders were clustered into two subgroups: prolonged-regular breeders called evenly spaced throughout the breeding season (spring - summer period), presenting many calling events records; prolonged non-regular breeders had a calling activity spaced throughout the summer season. Sporadic seasonal breeders were found in nine species belonging to five different families, which called sporadically during the spring-summer period only (Fig. 3).

**Daily calling patterns**

Species of the family Bufonidae presented highly contrasting daily calling patterns. Species of the genus **Rhinella** presented a mainly crepuscular and nocturnal calling pattern; **R. arenarum** presented a peak of activity at 20:00 h, while **R. diptycha** vocalised mainly between 20:00 h and 21.00 h (Rayleigh = 0.77; P = 0.0002; Rayleigh = 0.93; P = 0.0001) (Table 1, Fig. 4). Meanwhile, **Melanophryniscus rubriventris** was mainly a diurnal species, with peaks of vocal activity at 06:00 h and 18:00 h (Rayleigh = 0.52; P <0.0001 (Table 1, Fig. 4).

The species of the family Hylidae at the PF site recorded a mainly crepuscular-nocturnal vocal activity, although sporadic calling activity was detected during the day. **Boana riojana** was recorded at PF and at the LMF sites, at the PF site presented a mainly nocturnal calling pattern, with peaks of vocal activity at 01:00 h and 03:00 h; meanwhile, at the LMF site, with a higher number of records, presented a mainly nocturnal calling pattern,
but also with diurnal vocalizations (Table 1, Rayleigh = 0.8; P < 0.0001). Dendropsophus nanus presented a crepuscular-nocturnal vocal activity, with separated activity peaks at 20:00 h, 21:00 h, and 05:00 h (Rayleigh = 0.65; P < 0.0001). Scinax fuscovarius presented a calling pattern mainly crepuscular and nocturnal, with a peak of records at 22:00 h and 06:00 h, but also presented vocalizations during the day at 14:00 and 17:00 h (Rayleigh = 0.42, P < 0.0001). By the other hand, S. nasicus presented a more sporadic and nocturnal vocal activity, with peaks at 22:00 h and 23:00 h (Rayleigh = 0.8, P = 0.005). Trachycephalus typhonius had a mainly crepuscular activity, with a peak of activity at 20:00 h, and was also recorded calling during the day at 14:00 h (Rayleigh = 0.59, P = 0.007).
Fig. 3. Dendrogam showing the bottom-up hierarchical cluster analysis in the species recorded at the Parque Nacional Calilegua. Colours represent the five acoustic breeding strategies obtained.

Table 1. Circular statistics for the anuran species recorded in the study sites. Mu = trigonometric moment; rho = length; sd = standard deviation; cos = cosine; sin = sine; p = order; n = number of calling events; Rayleigh = Rayleigh uniformity test; P value = confidence value.

<table>
<thead>
<tr>
<th>Species</th>
<th>mu</th>
<th>sd</th>
<th>rho</th>
<th>cos</th>
<th>sin</th>
<th>P</th>
<th>n</th>
<th>Rayleigh test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boana riojana (PF)</td>
<td>6.08°</td>
<td>0.66°</td>
<td>0.38</td>
<td>0.38</td>
<td>-0.008</td>
<td>2</td>
<td>100</td>
<td>0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B. riojana (LMF)</td>
<td>6.25°</td>
<td>0.98°</td>
<td>0.15</td>
<td>0.15</td>
<td>-0.01</td>
<td>2</td>
<td>337</td>
<td>0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dendropsophus nanus</td>
<td>0.51°</td>
<td>0.92°</td>
<td>0.15</td>
<td>0.02</td>
<td>0.15</td>
<td>2</td>
<td>167</td>
<td>0.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptodactylus ap,numa</td>
<td>4.13°</td>
<td>0.74°</td>
<td>0.33</td>
<td>0.29</td>
<td>0.15</td>
<td>2</td>
<td>50</td>
<td>0.75</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L. macrosternum</td>
<td>5.14°</td>
<td>1.01°</td>
<td>0.17</td>
<td>0.17</td>
<td>0.02</td>
<td>2</td>
<td>127</td>
<td>0.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L. elenae</td>
<td>6.49°</td>
<td>0.86°</td>
<td>0.14</td>
<td>0.14</td>
<td>-0.01</td>
<td>2</td>
<td>130</td>
<td>0.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L. fuscus</td>
<td>8.89°</td>
<td>0.89°</td>
<td>0.21</td>
<td>0.15</td>
<td>-0.14</td>
<td>2</td>
<td>39</td>
<td>0.66</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L. gracilis</td>
<td>6.9°</td>
<td>0.85°</td>
<td>0.08</td>
<td>0.08</td>
<td>-0.02</td>
<td>2</td>
<td>13</td>
<td>0.69</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>L. latinasus</td>
<td>-0.4°</td>
<td>0.77°</td>
<td>0.74</td>
<td>-0.07</td>
<td>0.73</td>
<td>2</td>
<td>115</td>
<td>0.74</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Melanophryynisc rubriventeris</td>
<td>12.43°</td>
<td>1.13°</td>
<td>0.05</td>
<td>-0.006</td>
<td>-0.05</td>
<td>2</td>
<td>67</td>
<td>0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physalaemus cuqui</td>
<td>5.77°</td>
<td>0.98°</td>
<td>0.02</td>
<td>0.02</td>
<td>0.001</td>
<td>2</td>
<td>212</td>
<td>0.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phyllomedusa boliviana</td>
<td>5.52°</td>
<td>0.64°</td>
<td>0.4</td>
<td>0.39</td>
<td>0.04</td>
<td>2</td>
<td>107</td>
<td>0.81</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pleurodema borelli</td>
<td>10.6°</td>
<td>1.35°</td>
<td>0.089</td>
<td>0.031</td>
<td>-0.08</td>
<td>2</td>
<td>96</td>
<td>0.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oreoibates barituciensis</td>
<td>13.85°</td>
<td>1.16°</td>
<td>0.18</td>
<td>-0.08</td>
<td>-0.016</td>
<td>2</td>
<td>31</td>
<td>0.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>O. berdemenos</td>
<td>15.39°</td>
<td>1.53°</td>
<td>0.07</td>
<td>-0.05</td>
<td>-0.04</td>
<td>2</td>
<td>167</td>
<td>0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rhinella arenarum</td>
<td>4.22°</td>
<td>0.71°</td>
<td>0.43</td>
<td>0.39</td>
<td>0.19</td>
<td>2</td>
<td>12</td>
<td>0.77</td>
<td>0.0002</td>
</tr>
<tr>
<td>R. diptycha</td>
<td>5.93°</td>
<td>0.35°</td>
<td>0.77</td>
<td>0.77</td>
<td>0.01</td>
<td>2</td>
<td>6</td>
<td>0.93</td>
<td>0.001</td>
</tr>
<tr>
<td>Scinax fuscovarius</td>
<td>1.13°</td>
<td>1.3°</td>
<td>0.2</td>
<td>0.06</td>
<td>0.19</td>
<td>2</td>
<td>77</td>
<td>0.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S. nasicus</td>
<td>4.23°</td>
<td>0.65°</td>
<td>0.6</td>
<td>0.54</td>
<td>0.27</td>
<td>2</td>
<td>7</td>
<td>0.8</td>
<td>0.005</td>
</tr>
<tr>
<td>Trachycephalus typhonius</td>
<td>5.38°</td>
<td>1.01°</td>
<td>0.079</td>
<td>0.078</td>
<td>0.012</td>
<td>2</td>
<td>13</td>
<td>0.59</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Fig. 4. Rose diagrams showing daily calling activity of recorded species in the study sites. Dotted lines = Kernel data distribution.
The species of the family Strabomantidae presented a daily vocal pattern but was mainly crepuscular-nocturnal. *Oreobates barituensis* presented a sporadic pattern during the day with records of calls from 17:00 h to 20:00 h, with a peak of vocal activity at 05:00 h (Rayleigh = 0.5; P <0.0002) (Table 1, Fig. 4). *Oreobates berdemenos* presented markedly crepuscular-nocturnal vocal activity with a peak of vocal activity between 05:00 h and 06:00 h but was recorded vocalizing continuously during the 24 hs of the day (Rayleigh = 0.3; P <0.0001).

Species of the family Leptodactylidae presented a mostly crepuscular-nocturnal daily vocal pattern, but records of calling activity were also detected during the day for some species. *Leptodactylus apepyta* began to vocalize at 19:00 h in the evening and peaked at 20:00 h and at 21:00 h (Rayleigh = 0.75; P <0.0001). *Leptodactylus macrosternum* presented a mainly nocturnal calling activity with a peak between 24:00 and 01:00 h but also was recorded calling during the daytime, frequently between 06:00 h and 08:00 h (Rayleigh = 0.59; P <0.0001). *Leptodactylus elenae* called mainly at night, presenting peaks of calling activity at 22:00 h and at 01:00 h (Rayleigh = 0.68; P <0.0001). *Leptodactylus fuscus* presented a mainly nocturnal vocal activity, with sporadic peaks at 21:00 h and between 05:00 h and 06:00 h, with sporadic records during the daytime (Rayleigh = 0.66, P <0.0001). *Leptodactylus gracilis* was recorded even more sporadically, mainly at twilight, with a peak at 20:00 h (Rayleigh = 0.69; P = 0.0009). *Leptodactylus latinasus* presented a twilight-nocturnal vocal activity, beginning to call at 19:00 h, with extensive records during the last hours of the night and a peak of calling activity at 20:00 h (Rayleigh = 0.74; P <0.0001) (Table 1, Fig. 4). *Phylomedusa boliviana*, an arboreal species, presented a strictly nocturnal vocal activity, with records of calls at 21:00 h and at 02:00 h, and a peak of calling at 22:00 h (Rayleigh = 0.81; P <0.0001) (Table 1, Fig. 4).

**Temporal niche overlap**

The annual temporal niche overlap, and the spring-summer season overlap of the recorded species, was moderately high at PF site according to Pianka’s index. According to the Czekanowski’s index, the use of the temporal acoustic space was equitable (Czekanowski ~ 0.5) in the species assemblage recorded at the same site, both for the entire year and during the highest records of vocal activity in spring-summer season (Table 2). Meanwhile, the temporal niche overlap was lower in the LMF and UMF sites (Table 2), according to the Pianka’s index (Table 2). In addition, the time niche overlap during the fall-winter season was moderate at LMF, according to the Pianka’s index (Table 2). According to the Czekanowski’s index, a temporal niche overlap was found to be greater than that expected by chance (coincident activity) in the spring-summer period in the species that vocalize at the LMF site (Table 2).

**Calling guilds**

Six different calling guilds were recorded based on their advertisement calls features (Table 3; Fig. S1). *Leptodactylus apepyta*, was the only representative of the calling guild “A”; *L. elenae, L. fuscus, L. latinasus, Physalaeus cuqui* and *Trachycephalus typhonius*, were framed within the calling guild “B”. *Rhinella arenarum* and *R. dypticha* were part of the guild “D”. *Oreobates barituensis*

<table>
<thead>
<tr>
<th>Site</th>
<th>Period</th>
<th>Czekanowski</th>
<th>p-value</th>
<th>Pianka</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>Annual</td>
<td>0.51</td>
<td>&lt;0.001</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LMF</td>
<td>Annual</td>
<td>0.26</td>
<td>0.003</td>
<td>0.37</td>
<td>0.005</td>
</tr>
<tr>
<td>UMF</td>
<td>Annual</td>
<td>0.38</td>
<td>1.12</td>
<td>0.42</td>
<td>0.041</td>
</tr>
<tr>
<td>PF</td>
<td>Spring-Summer</td>
<td>0.51</td>
<td>&lt;0.001</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LMF</td>
<td>Spring-Summer</td>
<td>0.31</td>
<td>0.003</td>
<td>0.4</td>
<td>0.007</td>
</tr>
<tr>
<td>UMF</td>
<td>Spring-Summer</td>
<td>0.38</td>
<td>1.12</td>
<td>0.42</td>
<td>0.041</td>
</tr>
<tr>
<td>PF</td>
<td>Fall-Winter</td>
<td>0.12</td>
<td>0.13</td>
<td>0.21</td>
<td>0.1</td>
</tr>
<tr>
<td>LMF</td>
<td>Fall-Winter</td>
<td>0.42</td>
<td>0.051</td>
<td>0.59</td>
<td>0.054</td>
</tr>
<tr>
<td>UMF</td>
<td>Fall-Winter</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2. Temporal acoustic niche overlap recorded in the study sites. Czekanowski index (0-1); Pianka index (0-1).**

<table>
<thead>
<tr>
<th>Guilds</th>
<th>N° of recorded species</th>
<th>Percent</th>
<th>PF</th>
<th>LMF</th>
<th>UMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>5.26 %</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>26.31 %</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>10.52 %</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>5.26 %</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>42.10 %</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>10.52 %</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3. Calling guilds recorded along an altitude gradient within the Parque Nacional Calilegua, Argentina: permanent pond in the premontane forest (PF, 650 m a.s.l.), permanent stream in lower montane Forest (LMF, 1125 m a.s.l.), and upper montane forest (UMF, 1750 m a.s.l.). Total number of species recorded n = 19. Guild A = unmodulated simple call; Guild B = modulated simple call; Guild C = unmodulated pulsed call; Guild D = modulated pulsed call; Guild E = unmodulated pulsed multi-note call; Guild F = uniform modulated pulsed multi-note call; Guild G = non-modulated different multi-notes call; Guild H = modulated different multi-notes call.**
was the only representative of the calling guild “E”. *Boa-na riojana*, *Dendropsophus nanus*, *L. gracilis*, *Oreobates berdemenos*, *Phyllomedusa boliviana*, *Pleurodema borelli*, *Scinax fuscovarius*, *S. nasica* were part of the largest recorded group, the calling guild “F”. *Leptodactylus macrosternum* and *Melanophryniscus rubriventris* formed the calling guild “G”.

**DISCUSSION**

In this study, the calling phenology of anuran species was described at a fine timescale (hourly and daily) and quantitatively assessed along an altitudinal gradient in three Andean Montane Forest ecosystems of the Yungas ecoregion, within the PNC. This level of detail enabled us to propose a new classification scheme of acoustic breeding strategies according to the time spent by each species calling throughout a year.

Previous breeding strategies classification schemes were used to describe the breeding patterns of anuran species in the Parque Nacional Calilegua, following Duellman and Trueb (1986). Following this previous classification scheme only two breeding strategies were recorded for the entire species assemblage in this protected area (Vaira, 2002). Prado et al. (2005) described three reproductive activity patterns for a diverse anuran assemblage in a floodplain in the Pantanal region of Brazil. With the use of automatic recorders Saenz et al. (2006) were able to classify the anuran breeding activity patterns into: explosive breeders, winter breeders, summer breeders and continuous breeders depending on the core calling period recorded. Kopp et al. (2010) identified up to four distinct anuran reproductive patterns recorded by 13 visual encounter surveys in the Cerrado of Brazil. In this study, with the implementation of a PAM over an entire year we were able to identify five distinct breeding acoustic patterns and provide a novel, standardized classification framework, so that it can be tested in other ecosystems.

The great majority of the species recorded in this study showed a markedly seasonal breeding acoustic pattern, with their core calling periods centred during the spring-summer season in agreement with the warm and rainy season. These records were also reported by a previous study conducted on the same assemblage 20 years ago (Vaira, 2002). However, we now provide a detailed acoustic breeding phenology of the anuran species inhabiting three phytogeographical strata of the Yungas Andean Forest. The marked seasonal acoustic pattern might be related to the breeding behaviour present in these species that mainly use lentic waterbodies for their reproduction, with free-living tadpoles (Vaira et al., 2002; Pereyra et al., 2018; Boullhesen et al., 2019).

Strictly sporadic seasonal breeders were found in the families Bufonidae, Leptodactylidae, and Strabomantidae, with one to four calling events per month. The calling activity recorded for sporadic-breeding species in a tropical forest of Guyana lasted between one and three consecutive nights (Gottsberger and Gruber, 2004). The records of calls from explosive breeders were in coincidence with the warm and rainy period of the study. The calling activity of explosive breeders has been documented to correlate positively with temperature and rainfall cues in tropical anuran assemblages that use ephemeral ponds (Ulloa et al., 2019). Similarly, another study described the explosive breeders to call in the rainy season in the Pantanal of Brazil (Prado et al., 2005).

Prolonged seasonal breeders were the most representative groups in the study area. In another study along an altitudinal gradient of Brazil this reproductive pattern was also predominant among anuran species whose calling activity was recorded for 6 months (Arzabe, 1999). These temporal reproductive patterns agreed with others already reported for the Yungas forests (Vaira, 2002; Akmentins et al., 2015; Pereyra et al., 2018). The species of the family Leptodactylidae are mainly prolonged breeders, calling throughout the night and with sporadic calling activity during the day in representatives of the genus: *Leptodactylus, Physalaemus*, and *Pleurodema* (Vaira, 2002; Camurugi et al., 2017; Boullhesen et al., 2019).

However, it should be noted that certain species of the family such as *Leptodactylus fuscus* were characterized as mainly nocturnal in other ecoregions (Lucas et al., 2008; Guerra et al., 2020), although they showed an extended pattern of calling activity in daylight time recorded by a PAM in the Yungas ecoregion (Boullhesen et al., 2019). Our work provides novel information about the acoustic phenology patterns of a recently described species, *L. apepyta*, calling in the summer season showing a crepuscular-nocturnal activity where males vocalize outside subterranean nests that they built near the edge of temporary ponds (Schneider et al., 2019).

The tree frog *Boana riojana* was the only continuous breeder recorded in the study area calling throughout every month of the year. Duellman (1970) suggested that the hyliids of Central America reproduce continuously throughout the year if the environmental conditions are favourable. In addition, these records may, in turn, be linked to the characteristics of the monitored site. For example, the site LMF is characterized by abundant vegetation (evergreen forest) and a permanent mountain stream, providing optimal conditions for *B. riojana* to vocalise and breed throughout the entire year. In other
species of this genus, a continuous breeding strategy has been suggested to correlate with adult male gravid and post metamorphic females' abundance recorded throughout a year of survey (Hiert and Moura, 2010). Previous research suggested that the members of the family Hylidae have a crepuscular-nocturnal vocal activity (Guerra et al., 2020). However, our PAM study showed that B. riojana also calls during daylight, thus suggesting a more plastic calling behaviour in this species. There are reports of diurnal movement in species of the genus Boana in temperate forests of Brazil (de Oliveira et al., 2016). Therefore, it is interesting to explore the vocal repertoire of B. riojana, to determine if these daytime vocalizations correspond to advertisements calls or other types of vocalizations, such as territorial interactions or rain calls (Toledo et al., 2015).

The anuran assemblage recorded in this study presented a slight temporal acoustic niche overlap (time spent calling), mainly in the site with the highest diversity of species (PF). In addition, the temporal overlap of advertisement calls detected during the spring-summer period in the LMF site is remarkable. These results are in contrast with those reported for congeneric syntopic species that reproduce in permanent water bodies in the Cerrado savanna of Brazil (Guerra et al., 2020). The high diversity of species that compose the assemblages of the Yungas forests (Lavilla and Heatwole, 2010; Vaira et al., 2017), suggests that in places where several species reproduce simultaneously, the greatest diversity of calling patterns should be found to avoid being masked by others (Bertoluci and Rodrigues, 2002; Herrick et al., 2018; Klump and Gerhardt, 1992). The classification of advertisement calls of anurans, according to their spectral parameters in guilds, enables the comparison between groups of species to be faster and easier (Emmrich et al., 2020). This variety of acoustic strategies can be seen reflected in the calling guilds diversity detected in this study, mainly in the assemblage belonging to the PF site, with a total of five calling guilds recorded. Nevertheless, the calling guild with modulated pulsed multi-note calls was the most representative of the study, and unlike other guilds detected where there is a strong phylogenetic signal, this guild is made up of species from five different families. This observed pattern may conform with the Public Information Theory where anuran species with different phylogenetic relatedness elaborate advertisement calls with similar acoustics features (Danching et al., 2004; Goodale et al., 2010; Sugai et al., 2021). In this sense, the anuran species belonging to different lineages could be using the same venue for information to determine the optimal conditions to breed. Thus, enabling an inadvertent social information resource available and driving the species to use similar acoustic traits (Danching et al., 2004; Goodale et al., 2010).

The PAM employed in this study allowed us to describe in detail the acoustic reproductive patterns in an anuran assemblage in the mountain forests of the southern Yungas of north-western Argentina. This information made it possible to extend the previous knowledge regarding the calling activity of the species, contributing quantitatively to a better knowledge of a key aspect in the life history for each species recorded (calling activity), and to add new data for several secretive species.

In addition, it was possible to better characterize the variety of calling breeding strategies of the species recorded and, describe a standardized classification scheme to be tested in other ecosystems employing PAM method. In addition, we were able to access precise information on the temporal daily and seasonal acoustic distribution of the recorded anuran assemblage. This data is valuable and may be of great importance for implementing monitoring programs of anuran diversity in the Andean regions of South America in the current context of climate crisis.

ACKNOWLEDGMENTS

We thank Dirección Regional Noroeste of the Administración de Parques Nacionales (APN DRNOA) for providing MB with the research permits in the Calilegua National Park (118/2017 Rnv. 1). We thank The Rufford Foundation for awarding MB a Rufford Small Grant (Project ID- 22246-1). We thank CONICET for a full scholarship awarded to MB. The present work was partially supported by PIO CONICET 094 and PUE INECOA 22920170100027CO.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 14050.

REFERENCES


Paterns of acoustic phenology in anurans of the Yungas


