

Changes in call properties of *Boana pulchella* (Anura, Hylidae) in response to different noise conditions

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Abstract

The increasing expansion of urban areas leads to the emergence of new noisy environments that can affect animal communication. Calls play a crucial role in the mating displays of anurans, and the negative impact of anthropogenic noise-induced auditory masking has been reported in several species. We investigated the acoustic variation in 96 males ($n = 971$ calls) of the treefrog *Boana pulchella* across acoustically undisturbed sites and different noise conditions, associated with urban areas (URBAN) and roads (ROAD), in Central Argentina. We analyzed the effect of anthropogenic noise conditions on 6 temporal (call duration [CD], intercall interval, first and second note duration, internote interval, and call rate) and 3 spectral (dominant frequency of first and second note, frequency difference between them) call properties. The effects of temperature and size on acoustical variables were controlled. We observed differences in all call attributes among the noise conditions, except for intercall interval. Males exposed to URBAN and ROAD exhibited significant changes in CD, dominant frequency of the second note, and frequency difference between them. URBAN males had longest internote interval, while ROAD individuals displayed increased first and second note duration and call rates. Interestingly, ROAD males exhibited immediate changes in call rate in direct response to passing heavy vehicles. Our study emphasizes the impact of anthropogenic noise on the acoustic characteristics of *B. pulchella* calls. Understanding how animals adapt to noisy environments is crucial in mitigating the adverse effects of urbanization on their communication systems. Future investigations should explore whether the observed call adjustments are effective in avoiding or mitigating the negative consequences of anthropogenic noise on reproductive success.

Key words: acoustic communication, advertisement call, auditory masking, noise pollution, road traffic noise.

Rapid environmental changes induced by human activities are increasing globally, generating effects at the individual and population levels on wildlife (e.g., Sih et al. 2011; Shannon et al. 2016; Murray et al. 2019). In this context, human-generated noise associated with the urbanization process and infrastructure development has created environmental pressures that directly affect communication and negatively impact animal populations (Brumm and Slabbekoorn 2005; Shannon et al. 2016). Reported effects of noise include behavioral changes, physiological stress, and the masking of communication signals (Brumm and Slabbekoorn 2005; Barber et al. 2010; Tennessen and Langkilde 2016; Simmons and Narins 2018). In most anuran species, acoustic communication plays a critical role in reproductive success, and adverse effects of auditory masking produced by natural abiotic and biotic sources of noise are known in this group (e.g., Schwartz et al. 2001; Sun and Narins 2005; Bee and Swanson 2007; Cunningham and Fahrig 2010; Vargas-Salinas et al. 2014; Röhr et al. 2016). In noisy conditions, senders

must adapt their acoustic signals to improve the transfer of information to receivers, which often reduces its effectiveness (Warren et al. 2006). Recent evidence suggests that several anuran species adjust their calls to compensate for anthropogenic noise (Lengagne 2008; Parris et al. 2009; Cunningham and Fahrig 2010; Roca et al. 2016; Zaffaroni Caorsi et al. 2017; León et al. 2019; Grenat et al. 2019). While some species can adjust the properties of the acoustic signal according to their phenotypic flexibility (Ziegler et al. 2011), others reduce the masking of acoustic signals by means of long-term changes resulting in evolutionary adaptations of signaling behavior, driven by selective pressures imposed by noise sources (Zaffaroni-Caorsi et al. 2022). Furthermore, the type of noise, varying in amplitude, spectral and temporal pattern, and periodicity (i.e., chronic or punctual), could also alter noise sensitivity (Barber et al. 2010; Roca et al. 2016).

The impacts of human-generated noise on anuran amphibians are still widely understudied (Zaffaroni-Caorsi et al. 2022), and since different species show diverse responses

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to noisy conditions, it is important to describe and quantify these effects, allowing a better understanding of mitigation and management measures necessary to conserve vocal species in anthropogenic soundscapes. Furthermore, despite the worldwide distribution of anthropogenic noise, most studies on anurans were carried out in Europe and the United States (Blickley and Patricelli 2010; Pinto 2020; Zaffaroni-Caorsi et al. 2022). This geographic bias in research could limit the application of knowledge to certain regions, since the impacts may differ among habitats and species (Shannon et al. 2016; Zaffaroni Caorsi et al. 2022).

Our study aimed to test the prediction that males could differentially change their vocal behavior or call structure, as proposed by the Acoustic Adaptation Hypothesis (Morton 1975; Rabin et al. 2003), in response to different anthropogenic noise conditions. Therefore, we compared the calls of vocally active individuals of the tree frog *Boana pulchella* (Hylidae) from acoustically disturbed and undisturbed scenarios, identifying the call traits that varied in each noise situation. Populations of this species are widely distributed in the study area and occupy a wide range of environments, making them an excellent model to evaluate vocal responses in different anthropogenic noise conditions.

Material and Methods

Focal species and selection of sampling sites

The reproductive period of *B. pulchella* ranged between September and May, with daily reproductive activity mainly during crepuscular and nocturnal hours (Baraquet et al. 2007, 2013). The call of *B. pulchella* consists of 2 tonal notes, with the first note being shorter than the second (Figure 1).

Series of male advertisement calls were recorded at 5 sites in the center-south Córdoba province, Argentina, during the periods 2010–2012 and 2015–2018. We characterized the noise levels (SPL) at each site by taking measurements at 1-min intervals over a 10-min period in each pond, on different days and stable weather (no rain or high winds), using a digital sound level meter at a height of 1 m above the ground surface. The microphone was directed vertically upwards to avoid directionality and characterize the total ambient noise for each site. Based on the average noise data (Table 1), the sites were categorized following a gradient from lowest to highest noise level, under 3 exposure conditions: AUS, urban environment (URBAN), and road-associated (ROAD). Figure 2 shows the characteristic background noise for each noise condition. The sites included in each noise condition are detailed below: 1) AUS—include 2 reproductive sites belonging to the natural reserve of Alejandro Roca and the rural area of Las Acequias. These towns are located 60 km and 35 km southeast of the city of Río Cuarto, respectively. These sites are not associated with roads or large urban settlements; 2) URBAN—corresponds to semi-permanent water body located in the peri-urban area of the city of Río Cuarto, approximately 500 m from National Road N°36, within the campus of the National University of Río Cuarto. This water body represents the only known site where this species reproduces within the city; and 3) ROAD—include 2 sampling sites associated with Provincial Road N°30, near Río Cuarto city (33°07'38"S—64°20'47"W), were selected as sites with regular road traffic noise pollution (ROAD-DS). In both cases, the annual average daily traffic exceeds 6,000 vehicles, with a significant increase in vehicular flow during the summer months, as it is the main road to tourist towns in the south of Córdoba (ONDaT 2016; Vialidad Nacional 2021). The

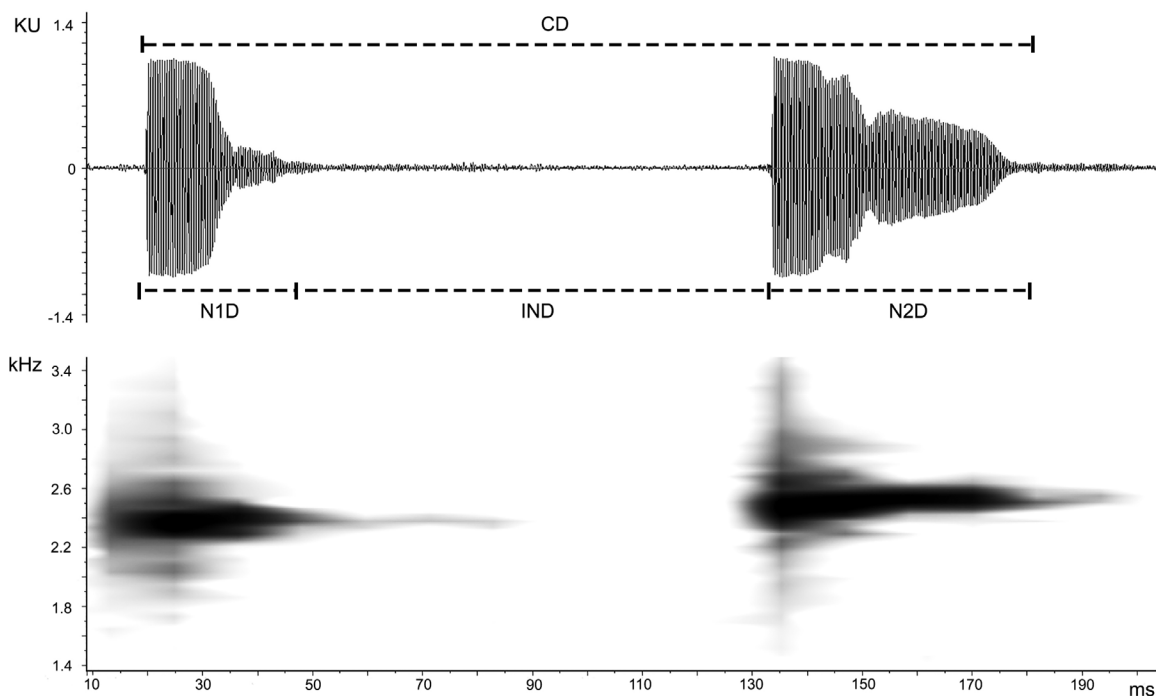


Figure 1 Oscillogram and spectrogram of a representative advertisement call of *B. pulchella*. SVL: 47 mm; temperature: 17.5 °C. Examples of measurements of temporal properties are shown in the figure. CD: call duration; N1D: first note duration; N2D: second note duration; and INI: internote interval.

Table 1 Summary of noise levels and temperature- and size-adjusted measurements (mean and standard deviation) of acoustic properties of *B. pulchella* call series from sites with different noise conditions

Sites	Noise level dB(C) leq	N	Acoustical parameters								
			CD (ms)	N1D (ms)	INI (ms)	N2D (ms)	ICI (ms)	Call rate (calls/min)	DFN1 (Hz)	DFN2 (Hz)	DFN2- DFN1 (Hz)
AUS	34.74 ± 5.53	31	119.1 ± 13.9	14.3 ± 3.2	67.8 ± 13.6	36.9 ± 5.2	548.4 ± 235.2	101.2 ± 34.3	2257.8 ± 177.7	2461.8 ± 160.4	203.9 ± 83.5
URBAN	52.62 ± 4.43	32	143.58 ± 25.9	18.9 ± 5.1	82.8 ± 26.4	41.6 ± 11.1	520.9 ± 261.7	89.1 ± 34.3	2199.9 ± 145.9	2322.2 ± 125.2	122.2 ± 37.6
ROAD	73.11 ± 4.57	29	147.9 ± 33.6	38.5 ± 18.2	66.9 ± 19.9	56.4 ± 15.4	460.1 ± 190.1	121.9 ± 50.6	2164.1 ± 97.9	2256.1 ± 112.5	92.1 ± 38.7

Parameters abbreviations: CD: call duration; N1D: first note duration; INI: internote interval; N2D: second note duration; ICI: intercall interval; DFN1: dominant frequency of first note; and DFN2: dominant frequency of second note.

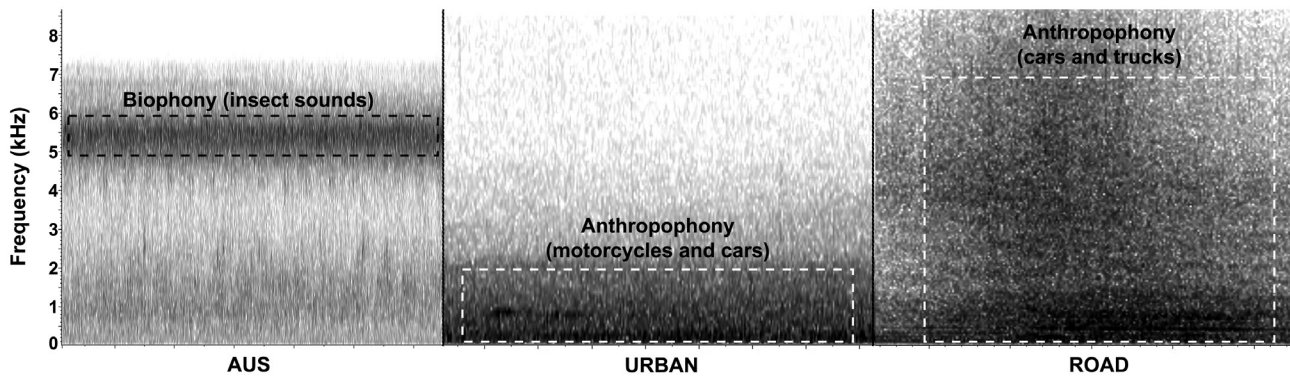


Figure 2 Spectrograms showing 10-s sound samples with the characteristic background noise for each noise condition. AUS: Center frequency = 1.12 kHz; Urban environment (URBAN): Center frequency = 0.49 kHz; Sites associated with roads (ROAD): Center frequency = 0.40 kHz. The dotted rectangles indicate the main sources of noise for each noise condition.

distance from the sampling sites to the noise sources range from 20 to 35 m.

All the sites were visited between 2 and 5 times during both time periods, except for ROAD sites, which were sampled only between 2015 and 2018. All sampling sites were located at least 3 km apart to avoid potential pseudoreplication (Cunnington and Fahrig 2010). The maximum distance between individual sampling sites was less than 60 km, minimizing the potential effect of differences in habitats associated with latitudinal or altitudinal variation.

Recording and analysis of advertisement calls

Vocal series of at least 10 calls per individual were recorded from 96 *B. pulchella* males (971 calls) using a Tascam™ DR-100 MKII Digital Recorder with a shotgun microphone Rode™ NTG2. Throughout each recording, the microphone was held at around 0.5–1 m from the focal calling male at least for 3 min. Immediately after the acoustic recording, the air temperature at level of the call site (to the nearest 0.1 °C) was registered and the snout-vent length (SVL) of each individual was measured using a digital caliper (± 0.01 mm). We used air temperature because males called exclusively from emergent vegetation. Acoustic signals of 31 individuals from acoustically undisturbed sites (AUS) sites and 65 individuals from acoustically disturbed sites (URBAN = 3; and ROAD = 32) were digitized and analyzed employing oscillograms, spectrograms, and power spectra using the software Raven Pro 1.6 (sampling rate: 44.1 KHz; bit depth: 16 bit; window function:

hamming; frame length: 512 points; grid resolution: 128 points; FFT size: 1024 points; and amplitude logarithmic). Five to 10 calls of a single series per individual were characterized by 5 temporal (call duration [CD], intercall interval [ICI], first note duration [N1D], second note duration [N2D], and internote interval [INI]; Figure 1), 2 spectral (dominant frequency of first [DFN1] and second note [DFN2]), and 2 calculated ([DFN2-DFN1] and call rate [calls/minute]).

Statistical analyses

The arithmetic means of all call parameters were calculated for each individual and used for further analyses. Acoustic data were shown to be normally distributed using the Shapiro–Wilk test for normality ($P < 0.05$). Linear regressions between call properties and water temperature were performed to assess the effect of this environmental variable on acoustic traits. Since temperature can affect the call properties of anurans (Gerhardt and Huber 2002), we corrected the acoustical properties significantly correlated with temperature by adjusting their values to 18 °C (mean of registered temperatures) following Heyer and Reid (2003). We also analyzed the relationship between size and spectral call traits, because of significant differences in SVL were found among noise conditions (ANOVA, $F = 16.8$; and $P < 0.0001$), with ROAD individuals (average SVL = 46.96 mm) being significantly larger than individuals from the 2 remaining sites (AUS SVL = 43.49 mm; URBAN SVL = 43.27; and Bonferroni's test; $P < 0.05$). The regression analysis showed a

significant negative relation between spectral parameters and SVL (DFN1: $r = -0.2449$; $P < 0.05$; DFN2: $r = -0.5477$; and $P < 0.0001$), as has been described in many anuran species (e.g., [Gingras et al. 2013](#)), for which the spectral parameters were size-corrected following [Velásquez et al. \(2018\)](#). These corrected values of the acoustic variables were used for all subsequent analyses.

We examined variation in call properties using generalized linear models (GLMs), with call properties as response variables, noise condition as fixed factor, and sampling sites as a random effect. We used akaike's information criterion (AIC) to determine the model that better fits the data. For significant effect interactions, post hoc tests (Fisher LSD) were Bonferroni-corrected to examine pairwise differences between noise conditions.

A multivariate approach based on principal component analysis (PCA) was performed to identify the acoustical properties that best accounted for variation among noise conditions. All analyses were conducted using InfoStat ([Di Rienzo et al. 2017](#)), excepting GLMs performed with R 3.3.2 ([R Core Team 2017](#)).

Results

Table 1 shows means and standard deviations for temperature- and size-adjusted acoustic properties of advertisement calls of individuals from different noise conditions. GLMs (**Table 2**) showed differences among noise conditions for all call attributes ($P < 0.05$), except for ICI (GLM, $P = 0.7452$). **Figure 3** shows that both noisy conditions had a statistically significant effect on CD, DFN2, and DFN2-DFN1 with higher values for the first parameter and lower values for the spectral properties in comparison with AUS males ($P < 0.01$, Fisher's LSD test). A significant increase in INI ($P < 0.01$, Fisher's LSD test) was found in URBAN individuals (**Table 2**, **Figure 3**) while ROAD individuals showed significantly highest N1D and ND2 and lowest DFN1 and DFN2. Significant differences were found between noise conditions in call rate (GLM, $P < 0.01$), with ROAD individuals showing the highest values (**Figure 3**). Furthermore, on several occasions, it was possible to observe immediate

changes in the call rate of the individuals associated with road sites, in direct response to the traffic noise, mainly of large vehicles (e.g., heavy trucks and trailers; **Figure 4**). Consequently, the highest dispersion of data was found for call rate for this noise condition (coefficient of variation $CV = 41.53\%$).

The relative contributions of the different acoustic parameters to PC1 and PC2 are illustrated in the PCA biplot (**Figure 5**). PC1 (77.1%) separated males calling from the 3 noise conditions, mainly ROAD and AUS individuals, and was positively associated mainly with N1D and N2D and negatively with ICI (0.38). PC2 had high positive loading for INI (0.66) and negative for call rate (0.44) and separated males from URBAN and ROAD conditions.

Discussion

In the present study, the impact of anthropogenic noise on *B. pulchella* was studied in its natural habitats. Our results demonstrate that males modify their calls in noisy situations, and that there is variation in certain acoustic properties depending on the background noise level they are exposed to.

Typically, as can be seen in **Figure 2**, anthropogenic noise occupies a spectral range from 1 to 4 kHz, with road traffic noise concentrating the greatest energy mainly at low frequencies (< 2 kHz; [Warren et al. 2006](#); [Nemeth and Brumm 2010](#); [Simmons and Narins 2018](#); [Grenat et al. 2023](#)) and peaks up to 8 kHz (e.g., heavy trucks and trailers), with noise levels of about 85 dB ([Hendriks 1998](#); [Duarte et al. 2019](#)). The calls of *B. pulchella* have average note frequencies ranging between 2.1 and 2.6 kHz ([Baraquet et al. 2007](#); [Ziegler et al. 2011](#); [Baraquet et al. 2013](#); [Ziegler et al. 2018](#); our study). Similar to results reported in birds ([Goodwin and Shriver 2011](#); [Roca et al. 2016](#)), frequency changes appear to be more significant when the typical frequency of anuran completely overlaps with the range of highest noise concentration (e.g., [Cunnington and Parris, 2010](#); [Grenat et al. 2019](#)). However, the variations appear to be slight (< 125 Hz) or non-existent in species with call frequencies greater than approximately 1.5 kHz (e.g., [Lengagne 2008](#); [Parris et](#)

Table 2 Results from the best GLMs fit for the variables analyzed

Factor	CD		N1D		INI		N2D		ICI	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
	AIC = 725.4		AIC = 654.6		AIC = 748.3		AIC = 655.1		AIC = 1,141.8	
Noise condition	19.8	<0.0001	35.3	<0.0001	5.3	<0.01	21.7	<0.0001	1.1	0.3283
Factor	Call rate		DFN1		DFN2		DFN2 – DFN1			
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
	AIC = 860.7		AIC = 1,066.5		AIC = 1,053.9		AIC = 1,015.8			
Noise condition	5.7	< 0.01	7.3	< 0.01	19.9	< 0.0001	8.1	< 0.001		

Models include noise condition as fixed factors and sampling site as random factor. AIC = akaike information criterion. *P*-values significant at the 0.05 level are in bold. CD: call duration; N1D: first note duration; INI: internote interval; N2D: second note duration; ICI: intercall interval; DFN1: dominant frequency of first note; and DFN2: dominant frequency of second note.

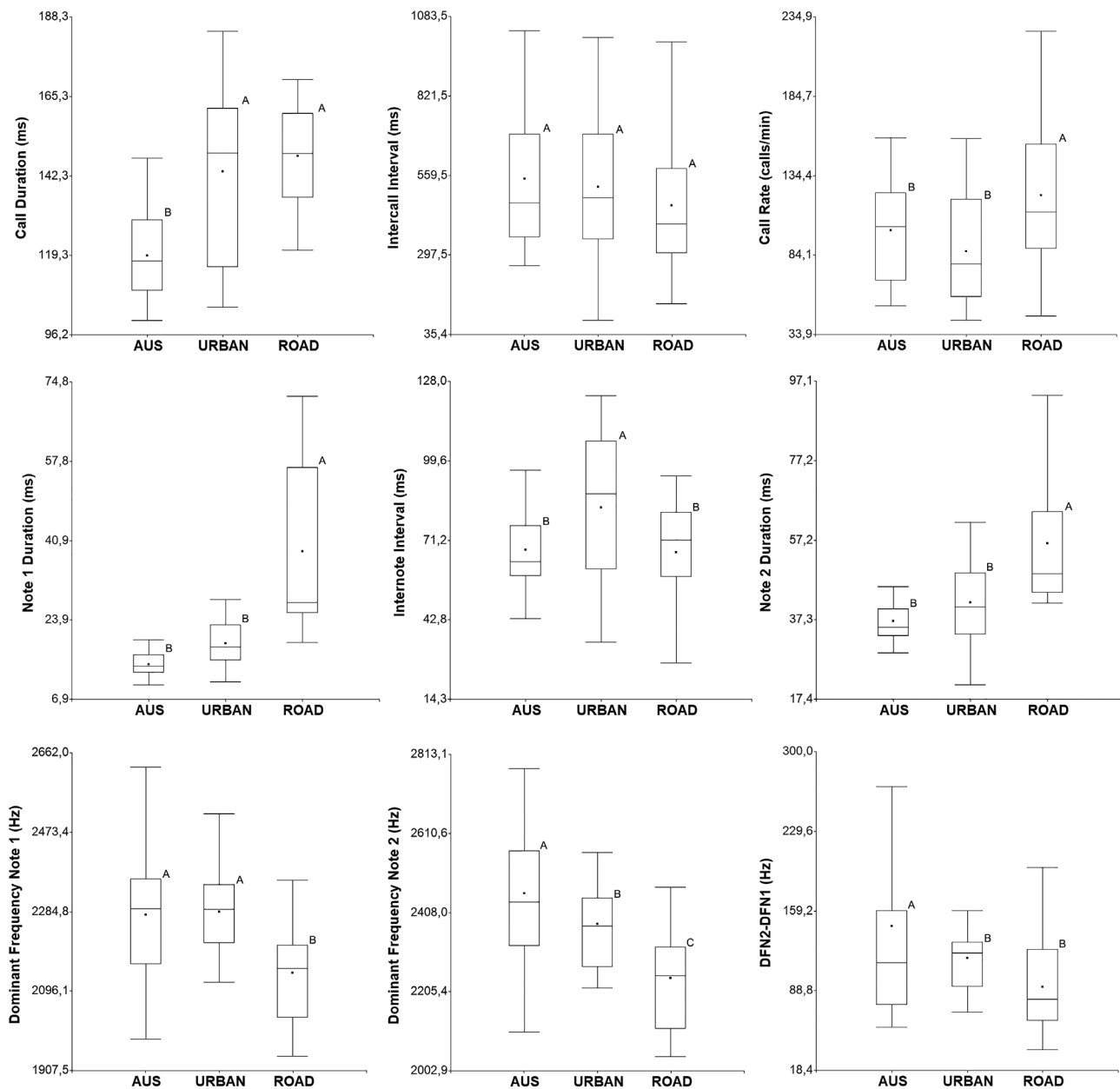


Figure 3 Comparison of mean values of call variables of males calling in all noise conditions: AUS; urban noise (URBAN); and road traffic noise (ROAD). The box plots show the mean, median, 25th and 75th quartiles, and minimum and maximum data. Different letters represent significant differences between means ($P < 0.01$) (Fisher's LSD test). DFN1 and DFN2: dominant frequency of first and second note.

al. 2009; Cunnington et al. 2010; Hanna et al. 2014). Thus, while punctual traffic noise peaks overlap with the vocalizations of *B. pulchella*, the average low frequencies with the highest noise concentration (Figure 2) may not directly mask them.

It is known that individuals living in chronically noisy environments may exhibit calls with higher dominant frequencies than expected to avoid auditory masking (Röhr et al. 2016; Goutte et al. 2018). However, increase the call frequency in response to anthropogenic noise is not a common response in anuran species, which show variable behavior regarding this property (Roca et al. 2016). In fact, we found a significant decrease in the spectral properties of callers from acoustically disturbed environments. Ziegler et al. (2011) reported a similar behavior in *B. pulchella* males for this property in

experiments where the calling environment was physically altered, demonstrating that this may be a common response of individuals to conditions of greater signal attenuation and/or degradation.

In other hylids, such as *Dryophytes cinereus* and *Dryophytes versicolor*, and generally in anurans, the call frequency is considered a static parameter (within-population variation $< 11\%$; Gerhardt, 1991), conserved throughout generations. Therefore, if changes in these traits are immediate or short-term, females could fail to identify conspecifics. Accordingly, males of other hylids such as *Boana leptolineata*, *Hyla arborea*, *D. versicolor*, and *Pseudacris crucifer*, when exposed to high noise levels, do not seem capable of modifying the call frequency to avoid signal masking (Lengagne 2008; Cunnington and Fahrig 2010; Hanna et al. 2014;

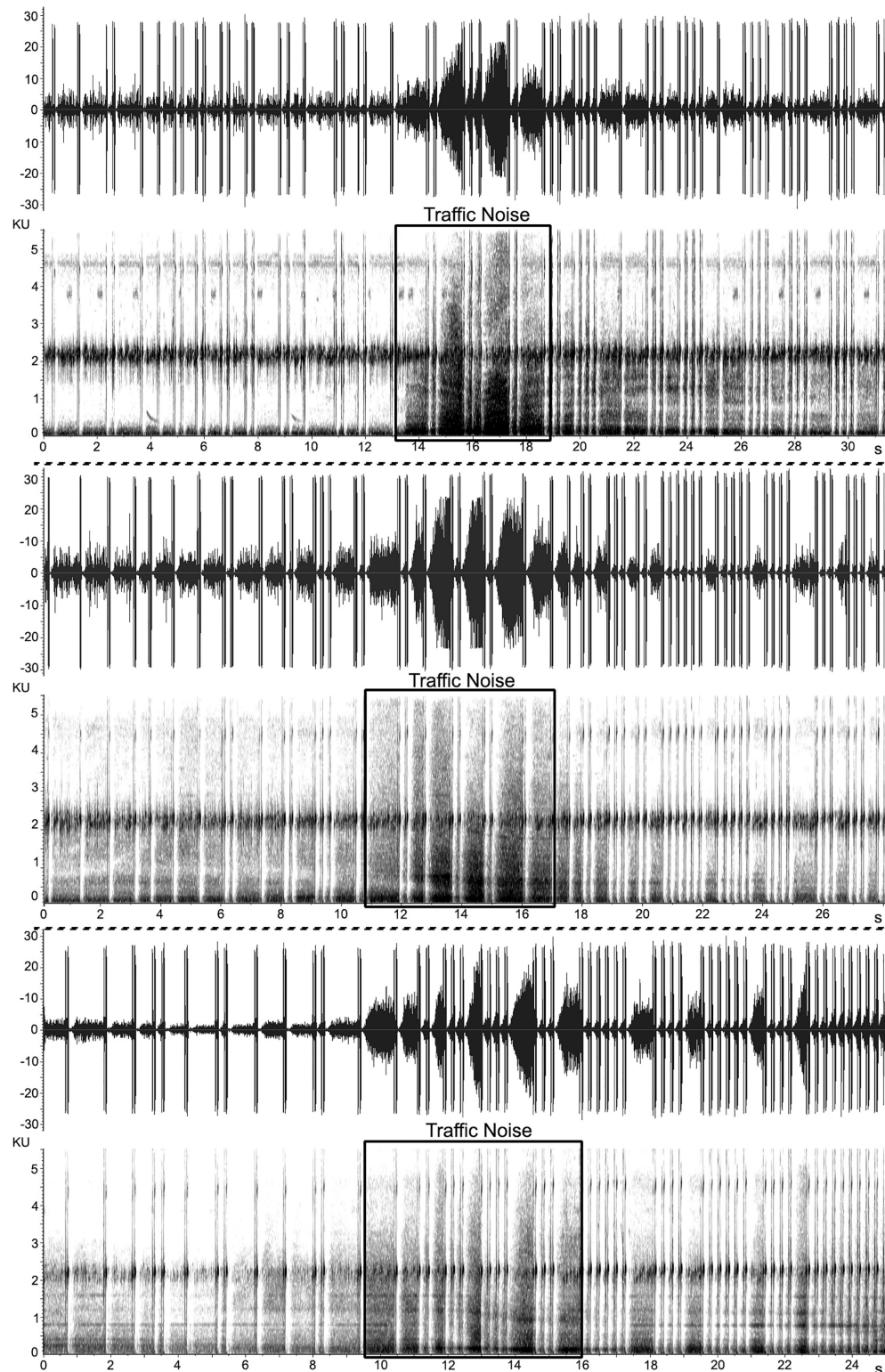


Figure 4 Oscillograms and spectrograms showing immediate responses to vehicular traffic noise in 3 individuals of *B. pulchella* from breeding sites associated with roads, increasing their call rates.

Zaffaroni Caorsi et al. 2017). Based on our results, the lower within-population variations observed in both note frequencies ($CV < 8\%$) suggest that spectral properties would be under stabilizing selection and could be primarily involved in species recognition for *B. pulchella*. However, further studies would be necessary to corroborate the function of the spectral

attributes in the conspecific recognition of this species and, if so, whether these frequency shifts affect mate choice by females in a noisy natural environment.

Males from both ROAD and URBAN conditions exhibited significantly longer calls compared to individuals calling in AUS. The production of longer calls as a short-term

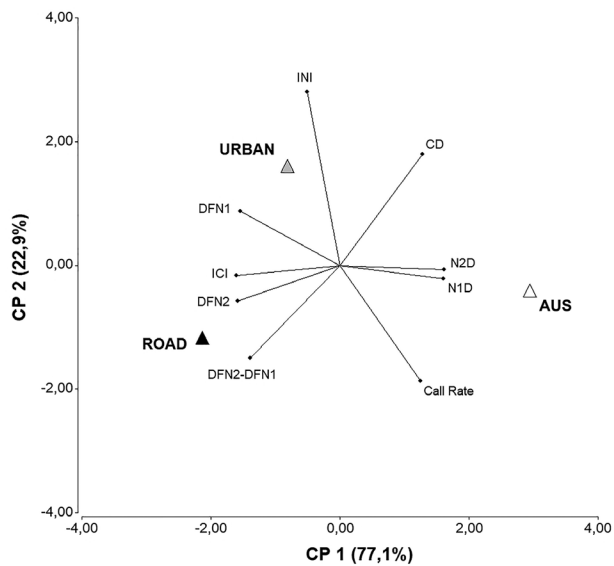


Figure 5 Biplot (PCA) based on acoustical parameters in 3 noisy conditions: AUS; urban noise (URBAN); road traffic noise (ROAD). CD: call duration; ICI: intercall interval; N1D: first note duration; N2D: second note duration; INI: internote interval; and DFN1 and DFN2: dominant frequency of first and second note.

adaptation to temporally increased environmental noise is a common strategy employed by many animals to reduce interference and improve the detectability of brief signals (Brumm et al. 2004). While the CD has the potential to convey communication information (Wells and Schwartz 2007), its function in noisy environments may not only be determined by noise intensity (e.g., Zaffaroni Caorsi et al. 2017), but also by the variation in the notes or elements within the acoustic signal. We observed that while the longer calls in ROAD individuals were primarily due to significantly longer N1D and N2D, URBAN males exhibited note durations similar to AUS callers but significantly longer internote interval. The changes in these temporal call attributes among ROAD callers align with those reported by Ziegler et al. (2011) in studies involving male *B. pulchella* vocalizing in conditions of greater environmental attenuation, demonstrating the short-term flexibility of this species in adjusting their calls in response to the local environment.

Males from ROAD-DS exhibited the shortest intercall intervals, resulting in significantly faster call rates. A similar response was observed in this species under high attenuation conditions (Ziegler et al. 2018). Although several anuran species tend to decrease their vocal activity, likely to avoid degradation or masking of important call traits (e.g., Sun and Narins 2005; Vargas-Salinas et al. 2014), other studies suggest that amphibians increase their call rate as a rapid response to improve call detectability in noisy conditions (Penna et al. 2005; Sun and Narins 2005; Kaiser and Hammers 2009; Kaiser et al. 2011; Kruger and Du Preez, 2016). The calls of many frog and toad species consist of repeated elements (Gerhardt and Huber 2002), likely as an adaptation for communication in chorus conditions (Schwartz and Bee 2013). The calls of *B. pulchella* show a high degree of conservation in terms of the number of notes (Baraquet et al. 2007, 2013; Ziegler et al. 2011). Therefore, increasing the serial redundancy of signals to counteract the communicative constraints of environmental noise could

result in a higher rate of call repetition and enhance the likelihood of females detecting their vocalizations when noise levels are high (Wiley 2006; Schwartz and Bee 2013). This common behavior observed in many species under background noise conditions could be related to the frequency of the neural signal, maintaining the representation of the signal's periodicity in the receiver's auditory system (Simmons and Narins 2018).

Immediate, short-term responses in calling behavior have been documented in several frog species associated with anthropic soundscapes (e.g., Cunnington and Fahrig 2010; Kaiser et al. 2011; Vargas-Salinas and Amézquita, 2013; Zaffaroni Caorsi et al. 2017; Legget et al. 2020; Higham et al. 2021; Jiménez-Vargas and Vargas-Salinas 2021). In our observations, we noted an increase in the call rate in ROAD individuals immediately after peaks of traffic noise, particularly associated with the presence of large vehicles (heavy truck and trailer). The influence of heavy vehicle noise causing immediate, short-term adjustments in call properties, has been reported in other studies (Vargas-Salinas and Amézquita 2013; Higham et al. 2021). Some authors suggest that frogs may discriminate between increasing and decreasing noise levels or detect the vibrations produced by approaching vehicles (Dimmitt and Ruibal 1980; Vargas-Salinas and Amézquita 2013). By taking advantage of lower ambient noise intensity, frogs can increase the signal-to-noise ratio of their calls, thereby enhancing the probability of attracting females. Sun and Narins (2005) observed males of *R. taipehensis* increasing their calling rate when they detected a drop in sound level during a reduction in chorusing activity in response to high levels of anthropogenic noise. Thus, the acoustic activity of neighboring callers may be a more significant factor in modulating calling time than the noise generated by anthropogenic sources (Vargas-Salinas et al. 2014), although this should be further analyzed in future studies.

In conclusion, our study demonstrates that males are capable of short-term adjustments in their calls in different noisy situations, particularly in environments with higher noise levels. Therefore, it is crucial for future studies on this species to determine whether a significant reduction in breeding success occurs or whether call adjustments are effective enough to prevent or mitigate potential negative effects of anthropogenic noise on mate attraction.

Moreover, we believe that behavioral studies can provide valuable insights not only for anurans but also for other vocal animal groups inhabiting similar environments. However, it is important to note that extrapolating results to other species should be done with caution because, as evident from our study and numerous others, different anuran species exhibit diverse and variable responses to different types and levels of anthropogenic noise, even within similar environments.

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Author Contributions

PG, MF, NS, and AM conceived the ideas and study. PG, MF, MB, FP, MO, and SZ collected the data; PG, MF, and MB performed the acoustic analyzes. PG and MF analyzed the data; NS and AM directed the working group. All authors contributed critically to the draft. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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