



Traffic Noise Affects Brazilian Mundo Novo Treefrog Calling Behavior

Rógger L. T. Antunes¹ · Márcio Borges-Martins¹ · Giorgia Guagliumi² · Valentina Zaffaroni-Caorsi²

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Abstract

Anthropogenic disturbance, including noise, is a major cause of biodiversity decline worldwide. Especially in anurans, the effect of noise pollution is known to have major consequences on their reproduction since acoustic communication is an essential part of this process. In this study, we tested the effect of three levels of traffic noise (55 dB, 65 dB, and 75 dB) corresponding to three different distances from the road (200, 100, and 50 m, respectively) on the Brazilian Mundo Novo treefrog (*Boana marginata*). The results of the playback experiments showed an effect on call type B. More specifically, there was a decrease in the advertisement call rate, a reduction in the number of pulses, and a lengthening of the interval between pulses, particularly at 65 dB. These findings suggest that at distances of up to 100 m, the masking effect significantly influences acoustic communication on the species. However, a 55 dB stimulus—equal to 200 m from the road edge—did not change the call in this species, suggesting a minimal distance to implement noise-inflicting infrastructures. We recommend that new studies adopt sampling methods from this distance to refine the threshold of the traffic noise effect.

Keywords Anthropogenic disturbance · Noise · Acoustic communication · Animal behavior

1 Introduction

One of the foremost contemporary environmental challenges is the alarming decline in biodiversity [1]. Hails et al. [2] reported a staggering 30% decrease in the number of vertebrate species between 1975 and 2010, with a concurrent increase in extinction rates, as noted by [3]. This decline can be attributed to many factors, especially anthropogenic disturbances such as habitat fragmentation, the introduction of exotic species, overexploitation, pollution, climate change, and disease [4, 5].

The rapid urbanization of our planet has led to a substantial surge in both local and global traffic, consequently exacerbating noise pollution [6]. This pervasive issue directly and indirectly impacts a diverse array of animal groups, encompassing both invertebrates and vertebrates [7]. Acoustic communication plays a pivotal role in several species

and is intricately linked with processes such as sexual selection and reproduction [8–11]. Additionally, it serves multiple other functions, such as territory defense, predator alarm, socialization, prey location, and spatial orientation [12–15].

The disruption of acoustic communication can significantly impact reproductive fitness by leading to missed mating opportunities, lost feeding chances, unnecessary aggressive interactions, or other atypical behaviors, resulting in increased energy expenditure [9]. These behavioral changes may consequently culminate in alterations in territorial distribution or population decline [6]. This is the case of most amphibian anurans, whose reproduction depends directly on acoustic communication [16].

Research into the impact of anthropogenic noise on anurans has revealed a variety of impacts caused by it and the mechanisms employed to mitigate its masking effects [17]. For instance, some anuran species exhibit noise avoidance behavior, where individuals adjust their calling patterns to synchronize with periods of reduced noise [18]. Alternatively, the strategy involves alterations in the temporal and/or spectral parameters of their calls [19]. However, these changes may have adverse consequences, including increased energy costs and exposure to predators [16]. Furthermore, such modifications may diminish the efficiency of signal transmission and reception, ultimately impacting reproductive success [20, 21].

✉ Valentina Zaffaroni-Caorsi
valentina.zaffaronicaorsi@unimib.it

¹ Programa de Pós-Graduação em Biologia Animal, Department of Zoology, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

² Department of Earth and Environmental Sciences, University of Milan Bicocca, Milan, Italy

These strategies can be directly related to noise intensity, prompting anurans to augment their alterations in call parameters when confronted with high-energy disturbances, as observed in studies by [22–24]. In an open field area, the amplitude of the noise is contingent on the proximity of the source to the receiver. Therefore, it would be reasonable to anticipate that anthropogenic noise sources in closer proximity would exert a more pronounced impact on anuran communication, while more distant sources would induce comparatively less interference, as seen by [25–27].

In this study, we tested three different amplitudes of traffic noise on the call parameters of the Brazilian Mundo Novo treefrog (*Boana marginata*; Hylidae), a species that vocalizes in streams and has a call frequency that overlaps with the traffic noise [28]. We expect that higher levels of traffic noise will induce more alterations in the spectral and temporal call parameters of the species. Considering the naturally noisy lotic environment that this species inhabits, we expect that the effect of traffic noise can be less pronounced than that recorded for other lentic species.

2 Material and Methods

2.1 Study Area

We conducted this study at the Floresta Nacional de São Francisco de Paula (FLONA-SFP, 29° 25' 22.4" 'S; 50° 23' 11.2" W), which is located in the municipality of São Francisco de Paula, Rio Grande do Sul, Brazil. The FLONA-SFP is inserted in the formations of the top fields of the mountain chains and forest with Araucaria (mixed ombrophylous forest or Atlantic forest—*lato sensu*). The FLONA-SFP is a conservation unit of sustainable use and has an area of 1606 ha with regions with elevations above 900 m. The locality is among the most humid in southern Brazil, with rainfall above 2000 mm per year and an annual average temperature of approximately 14.5 °C (FLONA 2016). The experiments were performed in the early evening, approximately one hour after sunset, from March to October 2016.

2.2 Focal Species

The Brazilian Mundo Novo treefrog (*Boana marginata*) is endemic to southern Brazil and occurs on the slopes of the southern portion of the Atlantic Forest (the states of Rio Grande do Sul and Santa Catarina). It is a medium-sized species (males 46–50 mm and females 50–57 mm in snout-vent length) with dorsal greenish coloration and the presence of dark brown and white lateral lines [29]. The call of the males of the species is composed of two types of multipulsed notes that are emitted in sets of up to 12 (Fig. 1a).

The first note (A) consists of 10 to 18 pulses and has an average duration of 0.49 s (ranging from 0.38 to 0.62 s) and a dominant frequency between 1.5 and 2.3 kHz, with an average of 1.8 kHz. The following notes are of the second type (B) and are composed of 22 to 48 pulses with an average duration of 0.57 s (ranging from 0.36 to 0.71 s) and a dominant frequency between 1.4 and 2.1 kHz, with an average of 1.7 kHz [28]. The call of *B. marginata* is within the frequency spectrum of the noise emitted by vehicles on roads.

2.3 Recording Traffic Noise

To test whether traffic noise from transport vehicles affects the call of *Boana marginata*, a series of vehicles passing were recorded on the RS-389 road (Estrada do Mar, Rio Grande do Sul, Brazil) at a distance of 10 m from the road. In order to understand the differences of noise level (dB) at different distances from the road, a sound-level meter (SLM-Instrutemp ITDEC 4000, C-weighting) was positioned on an open area at distances of 50, 100, and 200 m from the road. All the recordings made in this study were obtained using a SONY PCM-D50 coupled to a unidirectional microphone (Sennheiser ME 67) equipped with windscreens and stereo headphones to monitor the recordings. The playback was constructed using Audacity 2.1.1 software (in the WAVE format) based on the traffic noise recordings. The same file was used for the three different noise levels while the amplitude was defined using Audacity, with each noise file 10 dB higher than the other (55–65–75 dB). The final calibration of the right level of noise to be received by each frog was performed in the field using a sound-level meter to ensure the level.

2.4 Playback Experiment

The stimuli were constructed using traffic noise previously recorded and measured in an open field with no vegetation around, as described above. The recordings used for the stimulus had a frequency range of approximately 0 (zero) Hz to approximately 15 kHz and overlapping with the call of the target species (Fig. 1b). The noise stimuli were divided into three different traffic intensities: 55 dB (treatment B1), 65 dB (treatment B2), and 75 dB (treatment B3), which represent the noise intensities measured at 200 m, 100 m, and 50 m, respectively, of the road. The distances used correspond to the reality of the bodies of water found near roads in Rio Grande do Sul, Brazil.

The sound reproductions followed the protocol A-B-A proposed by [30] and were programmed for the following sequence of tracks: three minutes of pre-stimulation (A-silence), three minutes of treatment (B2), three minutes of treatment (B3), three minutes of treatment (B3), and three minutes of post-stimulus (A-silence), totaling 15 min of the

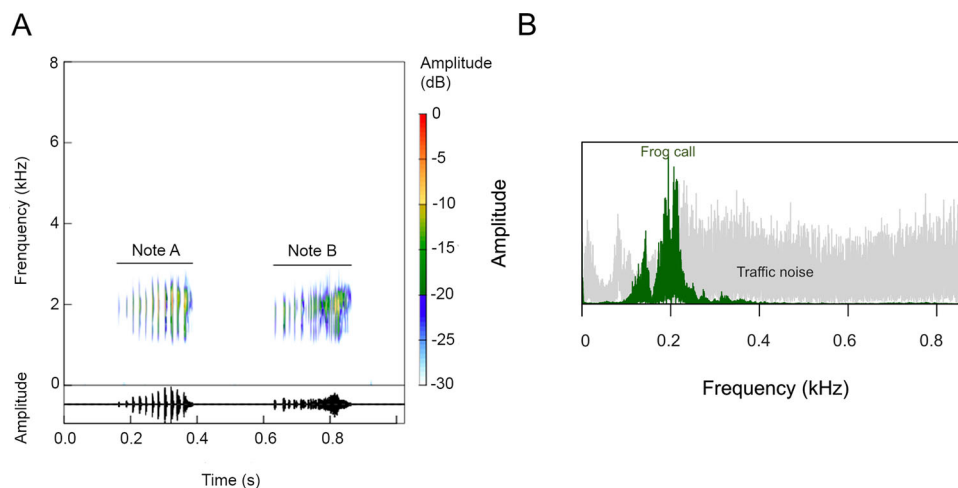


Fig. 1 Advertising call of *Boana marginata* and road traffic noise used as a stimulus. Spectrogram (above) and oscillogram (below) in **A** the two types of notes of *B. marginata* and in **B** a the power spectrogram of both the call of the species (green) and the traffic noise (gray) showing the overlap of frequencies of traffic noise with the species call

playback experiment. The three playbacks at different levels were combined on different orders, totalizing, six different possible tracks (for details see [25]).

Treatments were randomized, and one of the sequences was presented to each recorded specimen. Reproduction levels were field adjusted using the SLM to reproduce the intensity observed and measured on the original road. For each recorded specimen, the following procedure was implemented: a calling male was located; a sound box was positioned at a distance of approximately 1–2 m from the animal; and the microphone was positioned at 50 cm with an inclination of approximately 45° (for more detail, see [25]). Finally, a five- to twenty-minute pause was made for the animal to resume basal calling activity. The speaker used (Oneal 360-12v) emits frequencies from 10 Hz to 70 kHz and has an internal battery lasting up to 24 h.

Following each recording session, the animal's body temperature at the calling spot was assessed using an infrared thermometer (GM300, resolution 0.1 °C), and later, it was hand captured to measure body mass and snout-vent length (SVL) using a scale to the nearest 0.1 g and a caliper (Starrett–798) to the nearest 0.1 mm, respectively. The captured specimens were kept in captivity in containers with vegetation and cotton wool at room temperature for up to four days, avoiding possible pseudoreplicates. At the end of each trial period, the recorded individuals were released into their respective bodies of water. All experimental procedures were approved by the applicable Brazilian biodiversity agency and local institutional committee on research and ethics: Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios–Instituto Chico Mendes de Conservação da Biodiversidade (RAN–ICMBIO–Permit No. 52021–1), Comissão de Pós-Graduação (Project n° 28,872—PPGBAN/UFRGS),

Comissão de Pesquisa (COMPESQ/IB/UFRGS), and Comissão de Ética no Uso de Animais (CEUA/UFRGS).

2.5 Acoustic Analyses

Audacity 2.1.2 software was used to construct the recording files, while Raven Pro 64 v1.5 beta software for Windows (Bioacoustics Research Program 2018) was used to analyze the call parameters. The following features were analyzed for both notes A and B separately: note rate (notes – 1)/min, note length (time between the beginning and the end of each note) in seconds; pulse length (time between the beginning and end of a pulse present on the note) in seconds; interval between pulses only for type note B (interval between two consecutive pulses) in seconds; and dominant frequency (frequency in the note with the highest energy) in kHz. The parameters analyzed followed a description by [31]. The parameters were measured for 10 notes of each type inside each 3-min track. The notes analyzed were randomized in Excel software ("randomize" function, Microsoft Excel 2010. Available at: <https://products.office.com/en/excel>).

2.6 Statistical Analyses

To evaluate the effect of traffic noise on the call parameters of *B. marginata*, a permutational multivariate analysis of variance using distance matrices was performed with a post hoc t-test [32]. The type and period (A-B1-B2-B3-A) of the stimuli were considered fixed factors, and the individuals were considered blocks. The null hypothesis was that any note and/or any signal rate would be emitted by a given individual at any time and during any stimulus. All analyses and figures were made and constructed in R v3.3.1

software using "Vegan: Community Ecology Package" [33]; the oscillograms and spectrograms were constructed using the Seewave package [34].

3 Results

We recorded a total of 18 specimens, of which only nine emitted calls during all periods; the others remained silent during some of the traffic noise stimuli.

Regarding notes A and B, both notes were issued by all specimens in at least some period. The number of notes A (Fig. 2a) was less frequent than that of the B notes, and there was no difference between the periods in any of the analyzed parameters ($p > 0.05$). On the other hand, there were differences in B values between the treatments (Fig. 2b–d). During the stimuli, there was a decrease in the number of notes per minute ($p = 0.048$). During the silence period (pre- and post-treatment), the average rate was 7.8 notes/min, while during the traffic noise period, the average fell to 5.8–6.2 (notes/min) (Table 1). The lowest average was recorded when individuals were exposed to noise at an intensity of 65 dB. In addition, the mean number of pulses in the B notes decreased ($p = 0.019$) during noise stimuli (9.0 to 9.6 pulses/0.2 s) compared to that during silence (10.3 pulses/0.2 s), with an increase in the interval between pulses ($p = 0.003$), highlighted between silence (0.021 s) and stimuli of 65 dB (0.030 s) (t-test, $p = 0.029$), while the duration of the pulses did not change ($p > 0.05$).

4 Discussion

In this study, we found evidence that traffic noise can lead to changes even in anurans living in naturally noisy lotic environments, such as streams. More specifically, *Boana marginata* showed a decrease in the advertisement note rate, a decrease in the number of pulses, and an increase in the interval between pulses, especially at 65 dB, indicating that, at least until 100 m, the masking effect has an impact on its acoustical communication. Moreover, at a 55 dB stimulus—equal to 200 m from the road edge—there were no significant changes in the call of the species.

More into detail, during periods of traffic noise, the call B note rate decreased to 25.6% during the 65 dB treatment, which represents a distance of 100 m from the road. Other studies also showed a reduction in the signal rate in several species of Hylidae, Microhylidae, and Ranidae when exposed to different sources of noise (airplane, motorcycle, and road traffic) [18, 22, 35, 36]. In Brazil, experiments with different levels of traffic stimuli were performed for two other species of Hylidae, one with overlapping spectral noise, *B. bischoffi*, and the other without, *B. leptolineata*. As in our work, the

authors observed a significant decrease in the *B. bischoffi* calling rate by more than 60% under acoustic reproductions of 65 and 75 dB, whereas in *B. leptolineata*, there was no alteration [25]. The decrease in the signal rate can be associated with the choice of the best moment for the emission of sound by the males, that is, the selection of the moment when their signal is more prone to transmission and detection, so as not to call when the environmental noise is unfavorable [18, 36].

To date, in addition to the signal rate, few studies have tested the effect of anthropogenic noise on other temporal parameters. In this study, the analysis of the effect of noise on the call of *B. marginata* also revealed a reduction in the number of pulses of the B note with an increase in the interval between them, with the largest interval between the silence and the stimulus being 65 dB. Furthermore, the lower number of pulses during the stimuli seems to explain how the interval between pulses changed without changing the average duration of the notes.

The response of *B. marginata* males to stimuli, by reducing the note rate and pulses, can have consequences on their chances of reproduction because they are directly related to the selection and location of males by females [37, 38]. Although the Mundo Novo treefrog is a species adapted to live in usually noisy environments due to the sound of current flows, this study shows that it may be susceptible to other types of masking, especially from anthropogenic sources such as traffic noise. Moreover, although many species have developed mechanisms to reduce the masking effects on their calling, there is no guarantee of their reproductive success.

In addition, the calling function in some anurans can also act beyond communication, as in species in which males vocalize for long periods of time with females in the same habitat, calls seem to stimulate the production of hormones in females, maintaining their reproductive condition [16]. In pregnant females of *Alytes muletensis*, females stimulated with a conspecific call continued to maintain and mature their ovules, while females kept silent or stimulated with calls of heterospecifically reabsorbed ovules [39].

Furthermore, during the experiment, two specimens moved to a different calling site; however, no animal left the stream. Cases of movements during noise stimuli were also reported in studies by *Boana bischoffi*, *B. leptolineata* and *Hyla arborea*; however, these individuals attempted to move away from the source of noise and even ceased calling [25–35]. This may indicate that, in some species, noise directly affects habitat selection [40]. However, testing this effect was not an objective of this work.

Our study is based on short-term exposure to traffic noise and individuals not previously exposed to it. Therefore, we only assessed very immediate effects caused by noise and cannot exclude the possibility of additional changes in call parameters, which might occur during long-term exposure.

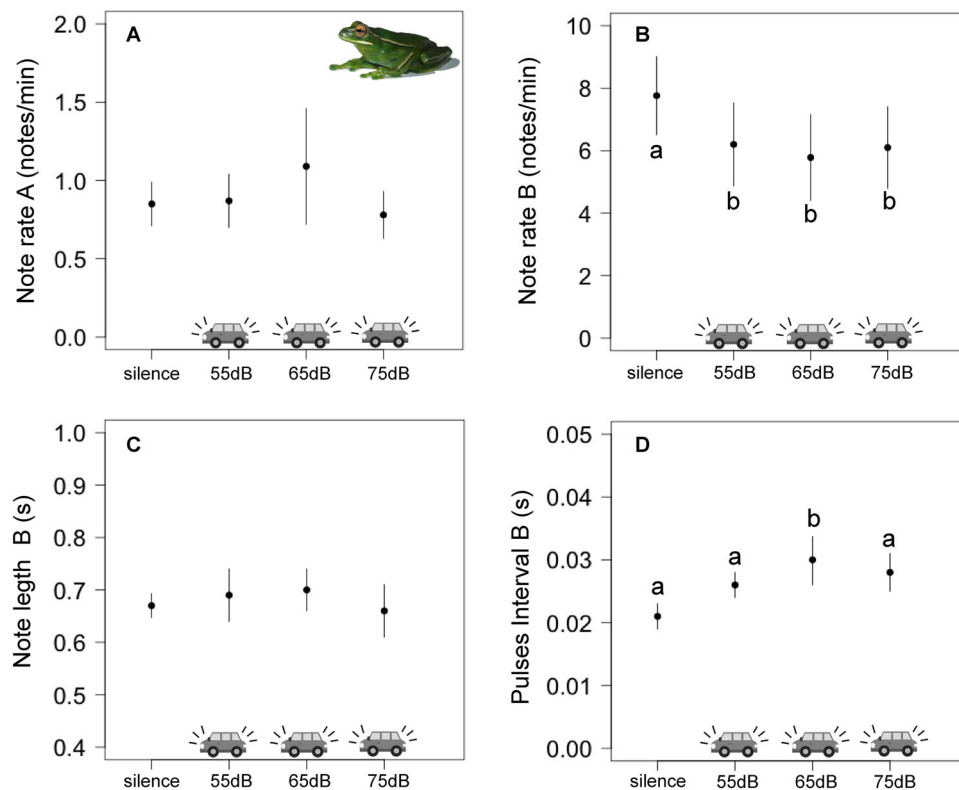


Fig. 2 Main effects (average and standard error) of road traffic at three different amplitudes on the call parameters of the Mundo Novo treefrog (*Boana marginata*). Letters **a**, **b** represent significant differences between treatments for each parameter tested

Table 1 Effect of road traffic noise on temporal and spectral parameters of the advertising call of *Boana marginata* at three different intensities

Treatment		Silence	55 dB	65 dB	75 dB
Note rate A	(note/min)	0.9 (0.1)	0.9 (0.2)	1.1 (0.4)	0.8 (0.1)
Note rate B*	(note/min)	7.8 (1.3)	6.2 (1.3)	5.8 (1.4)	6.1 (1.3)
Note length A	(seconds)	0.6 (0.03)	0.6 (0.03)	0.6 (0.05)	0.6 (0.06)
Note length B	(seconds)	0.7 (0.02)	0.7(0.05)	0.7 (0.04)	0.7 (0.05)
Pulse length A	(seconds)	0.03 (0.002)	0.03 (0.002)	0.03 (0.002)	0.03(0.001)
Pulse length B	(seconds)	0.01 (0.001)	0.01 (0.001)	0.02 (0.001)	0.01 (0.001)
Pulse interval A	(seconds)	0.035 (0.001)	0.036 (0.001)	0.034 (0.001)	0.039 (0.003)
Pulse interval B*	(seconds)	0.021 (0.003)	0.026 (0.002)	0.030 (0.004)	0.028 (0.003)
Pulse rate B*	(pulses/0.2 s)	10.3 (0.9)	9.0 (0.7)	9.3 (0.8)	9.6 (0.6)
Dominant frequency A	(kHz)	1.8 (0.03)	1.8 (0.03)	1.8 (0.03)	1.8 (0.02)
Dominant frequency B	(kHz)	1.6 (0.02)	1.7 (0.02)	1.6 (0.03)	1.6 (0.02)

The mean and standard error of each parameter are shown; * $p < 0.05$ between silence and treatments

In addition, we tested only males, i.e., the emitters of acoustic signals, and exogenous acoustic noise generally decreases the ability of a receiver to decode a message [9]. Therefore, new studies with anurans should be performed to better understand how changes induced by anthropogenic noise affect males and females, be it in physiology, behavior and choice of companions, thus increasing the contribution to the definition of strategies to reduce sound impacts. In this study, the

stimulus that caused most of the changes in the *B. marginata* call was 65 dB (equivalent to 100 m of roadside), while the stimulus of 55 dB (equivalent to 200 m of roadside) did not have any significant effect. This finding might indicate that this method is promising for study and may be safe for the communication of some anuran species.

Studies testing the effect of anthropogenic noise on anurans include not only traffic but also a series of human sources

to which the environment is constantly under pressure, such as airplanes [36, 41], wind farms [42], and even underground vehicle vibrations [43]. Therefore, we suggest that new studies adopt sample designs that can refine this effect limit from different distances to different sources of human-generated noise.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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