

DOES TRAFFIC NOISE AFFECT THE ADVERTISEMENT CALL OF *DENDROPSOPHUS NANUS* (ANURA, HYLIDAE)?

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Abstract.—Generally, the effect of noise pollution on wildlife is not considered in environmental assessments, although noise scares animals away and, in anuran amphibians that communicate by vocalizations, it can significantly interfere with their reproduction. The alteration of soundscapes is an aspect scarcely explored in the field of conservation. Here, our aim was to assess the impact of noise pollution from vehicular traffic on the advertisement call of the Dwarf Treefrog (*Dendropsophus nanus*) in northwestern Corrientes Province, Argentina. Specifically, we analyzed whether there were alterations to the acoustic parameters of the advertisement call in *D. nanus* in environments exposed to traffic noise. We recorded calls using a digital recorder coupled to a directional microphone targeted on individuals vocalizing in environments without traffic noise (control) and others in places close to roads with high vehicular traffic. Permutational Multivariate Analysis of Variance showed significant differences between noisy sites and without-noise sites, and post-hoc analysis indicated significant differences in two temporal parameters (duration of the complex call and note rate) and three spectral parameters (dominant frequency of B note and upper limit of frequency band in A and B type notes). Our study shows that traffic noise affects roadside vocalizing populations of *D. nanus*. Understanding how anthropogenic noise pollution can affect amphibian populations is a first step in better understanding the effects of expanding urban development on wildlife.

Key Words.—acoustic communication; amphibia; noise pollution; South America

INTRODUCTION

Vocalizations in anurans represent a crucial resource for communication and the development of social behaviors. Several call types are known in anurans (Köhler et al. 2017), including advertisement calls. These species-specific calls help females locate conspecific males (Gerhardt and Huber 2002) and are responsible for initiating courtship and reproduction. On the basis of certain call characteristics, females can recognize conspecific males and choose the most suitable males for reproduction (Wells 2007; Toledo et al. 2015; Köhler et al. 2017). Any interference or interruption in the transmission of the male advertisement call could negatively affect reproduction, which in turn can negatively affect population size and persistence.

Several studies have shown the influence of anthropogenic noise on vertebrate communication, especially in amphibians and birds (Brumm 2004; Sun and Narins 2005). In addition to interference in communication, anthropogenic noise can cause

physiological stress (i.e., high corticosterone levels) and alterations in migratory behavior associated with reproduction (Witte et al. 2001; Halfwerk et al. 2011; Tennesen et al. 2014, 2018; Troïanowski et al. 2017). For anurans, anthropogenic noise can interfere with reproductive and social behaviors, as these activities are highly dependent on acoustic communication (Bee and Swanson 2007; Cunningham and Fahrig 2013; Brumm and Slabbekoorn 2005; Simmons and Narins 2018; Grenat et al. 2019). Anurans often modify their acoustic parameters to avoid overlapping with environmental noise (sound masking; Sun and Narins 2005; Amézquita et al. 2006; Hanna et al. 2014; Roca et al. 2016; Zaffaroni-Caorsi et al. 2023), and several studies have documented alterations in anuran vocalizations at sites with noise pollution, such as traffic noise (Cunnington and Fahrig 2010; Simmons and Narins 2018; Yi and Sheridan 2019; Gomes et al. 2022; Zaffaroni-Caorsi et al. 2023). Studies document changes in temporal parameters (Kaiser and Hammers 2009), spectral parameters (Parris et al. 2009; Roca et al. 2016; Kunberger et

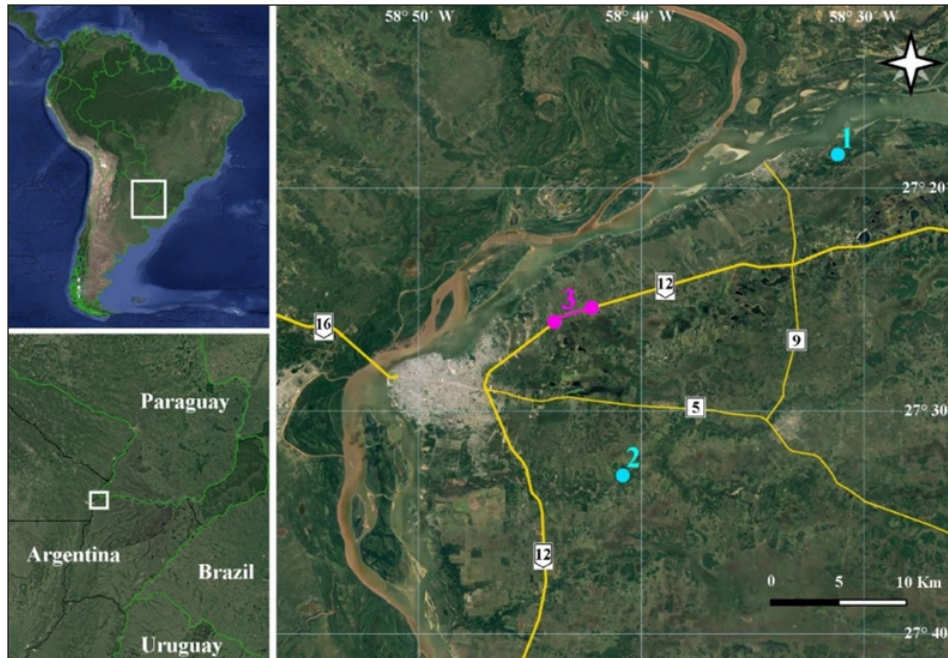


FIGURE 1. Study area in Corrientes province, Argentina, where we recorded calls of the Dwarf Treefrog (*Dendropsophus nanus*). Without-noise sites (blue) were (1) Estancia San Pedro, San Cosme Department and (2) Laguna Yacaré, Parque Provincial San Cayetano, Capital Department. Noisy sites (purple; 3) were a portion of National Route N° 12 between Km 1038 and Km 1041, Capital Department. Yellow lines represent national routes. (Images from Google Earth Pro).

al. 2024), or both (Leon et al. 2019; Cunningham and Fahrig 2010; Higham et al. 2021), alteration of the amplitude (Yi and Sheridan 2019; Gomes et al. 2022), or avoidance of source noise (Vargas-Salinas and Amezcua 2013).

In South America, and particularly in Argentina, there are few studies that analyze the effect of traffic noise on anuran species (Leon et al. 2019; Grenat et al. 2019, 2024). While the above examples provide clues as to what we might expect, the breadth of responses to traffic noises observed in anurans elsewhere highlights the need for regional- and species-specific data to make informed assessments of anthropogenic noise effects in any given region. For this reason, we assessed whether traffic noise alters the advertisement call of the Dwarf Treefrog (*Dendropsophus nanus*). We chose this species because it has a wide distribution in South America and is abundant in environments close to roads. In addition, *D. nanus* exhibits prolonged reproductive activity (Prado et al. 2005), with males calling during both wet and dry periods. *Dendropsophus nanus* vocalizations have a simple structure that allowed us to easily compare vocalizations close to and away from road noise. We expected that traffic noise pollution alters both temporal and spectral parameters of the vocalizations of *D. nanus*.

MATERIALS AND METHODS

Study area.—We recorded the advertisement call of *D. nanus* males in northwestern Corrientes Province (Argentina) in March, April, and November 2022, between sunset and midnight (Fig. 1). The area is located in the Chacoan Phytogeographic Province, Oriental Chaco District (Cabrera 1976). We used two conditions: without-noise sites, considered a control group ($n = 33$ males), and noisy sites, the test group ($n = 34$ males). Without-noise sites included a semi-permanent water body (depth 50 cm, area 3.6 ha), located in Estancia San Pedro (San Cosme Department; $27^{\circ}18'19''\text{S}$, $58^{\circ}31'05''\text{W}$) and a permanent water body, Laguna Yacaré (depth 50 cm, area 0.35 ha; Fig. 2), located inside Parque Provincial San Cayetano (Capital Department; $27^{\circ}32'56''\text{S}$, $58^{\circ}40'39''\text{W}$). Both sites were 7–8 km from National Route N° 12. Noisy sites were located next to National Route N° 12 between Km 1038 and Km 1041 ($27^{\circ}25'55''\text{S}$, $58^{\circ}43'46''\text{W}$ to $27^{\circ}25'24''\text{S}$, $58^{\circ}42'18.29''\text{W}$; Fig. 2). Noisy sites correspond to three temporary water bodies where individuals were vocalizing 10–60 m from the road. Each body of water was approximately 450–1,600 m from each other, with a depth of approximately 40 cm and a



Figure 2. Examples of a without-noise site (A) and a noisy site (B) in northeastern Corrientes Province, Argentina. (Photographed by Víctor Zaracho).

width of 3 m.

During sampling we observed a low activity of anuran calling, probably related to a dry period in the region in the preceding 3 y. *Dendropsophus nanus* was often the only species calling, but occasionally we recorded other species. In both site conditions we recorded Dwarf Frog (*Physalaemus cristinae*), Sanborn's Treefrog (*D. sanborni*), Chaco Treefrog (*Boana raniceps*), and Mato Grosso Snouted Treefrog (*Scinax acuminatus*). In noisy sites we also recorded Lesser Snouted Treefrog (*Scinax nasicus*), Oven Frog (*Leptodactylus latinasus*), Pointed Belly Frog (*L. podicipinus*), and Helvetia Dwarf Frog (*P. santafecinus*), and in without-noise sites we recorded Paraguayan Swimming Frog (*Pseudis platensis*) and Uruguay Harlequin Frog (*Lysapsus limellum*).

Study species.—*Dendropsophus nanus* is a small treefrog (approximately 24 mm snout-vent length) commonly found in emergent vegetation, and it reproduces during the rainy season (spring-summer) with males vocalizing from dusk to dawn (Zaracho et al. 2012). It has a wide distribution that ranges from the state of Mato Grosso do Sul in Brazil to the Rio de la Plata Basin in Argentina (<https://amphibiansoftheworld.amnh.org/index.php>). In northeastern Argentina, *D. nanus* inhabits most bodies of water, even in urban areas (Zaracho et al. 2012).

Recording details.—We measured the noise amplitude at each site to the nearest 1.5 dB in a range of 30–130 dB using a MASTECH® MS 6701 decibel meter (MASTECH, Guandong, China). At noisy sites, we took measurements at 1 m height and 20 m from the road. Vialidad Nacional (http://transito.vialidad.gob.ar:8080/SelCE_WEB/index.html)

reported a mean daily vehicular traffic of 6,000 vehicles (cars, trucks, and buses) per day for the studied road section. At without-noise sites, we measured the noise amplitude at 1 m height in the center of the semi-permanent water body; there were no trees in the surrounding area. The maximum sound level ranged from 38–42 dB at the without-noise sites and from 66–78 dB at the noisy sites. Several studies report that traffic noise occupies low-frequency spectral ranges of around 2 kHz, sometimes reaching 8 kHz (Warren et al. 2006; Nemeth and Brumm 2010; Grenat et al. 2024). A spectrogram of one of our recordings shows the low-frequency spectral range occupied by a truck and the frequency of the call of the study species (Fig. 3). The highest energy of traffic noise is concentrated around 1 kHz; there is a range of frequencies above this value that overlaps slightly the frequency of the call of *D. nanus*.

We recorded vocalizations with an M-Audio MicroTracK II (M-AUDIO®, Irwindale, California, USA) recorder attached to a directional microphone (Sennheiser ME66 K6; Sennheiser electronic SE & Co. KG, Wedemark-Wennebostel, Germany) using the same volume and sound input level settings for each recording. We made recordings of approximately 1 min for each individual. We used a sampling rate of 48 kHz and 16-bit resolution. We made each recording at a distance of 45–55 cm from the individual. Once we located a frog, we waited 5 min before recording to allow the frog to continue natural calling behavior.

During each call, we recorded the air temperature with a digital thermohygrometer. After call recording, we captured the males and measured the snout-vent length (SVL) and body mass (BM), then released them at their original capture location (Supplemental Information Table S1). We measured

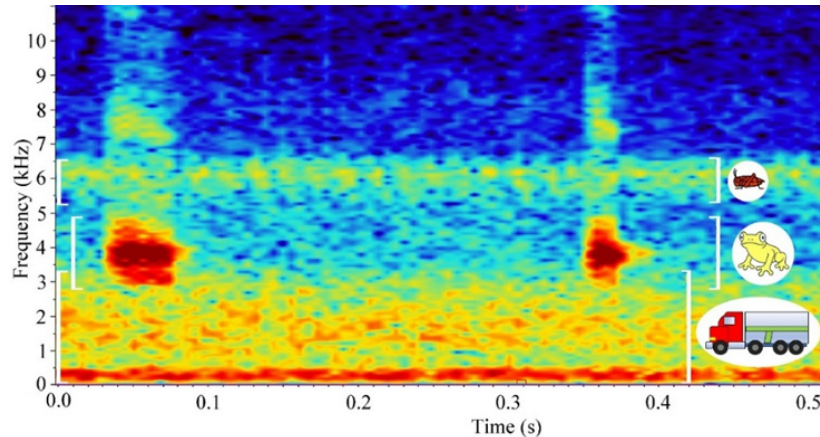


FIGURE 3. A portion of a recording from a noisy site (National Route N°12 between Km 1038 and Km 1041) in northeastern Corrientes Province, Argentina, showing the frequency band occupied by traffic noise (in this case, a truck), the dominant frequency of Dwarf Treefrog (*Dendropsophus nanus*) call, and an insect sound. Colors in the spectrogram are proportional to the power expressed in decibels (relative to an arbitrary reference power), with warmer colors (e.g., red and orange) indicating higher intensities and cooler colors (e.g., blue) indicating lower intensities.

SVL to the nearest 0.01 mm with a digital caliper and mass to the nearest 0.01 g with a Traveler TA302 balance (OHAUS Latinoamerica, Ciudad de Mexico, Mexico). To avoid recording a male more than once,

we sampled each area a single time only. To avoid variations due to habitat selection, we made recordings at the same distance from the ground or water surface as these factors can cause frequency enhancement or attenuation (Catchpole et al. 1995; Zollinger and Brumm 2015). We cataloged the recordings with the Fonoteca Zoológica of the Universidad Nacional del Nordeste (FZ-UNNE), Corrientes, Argentina.

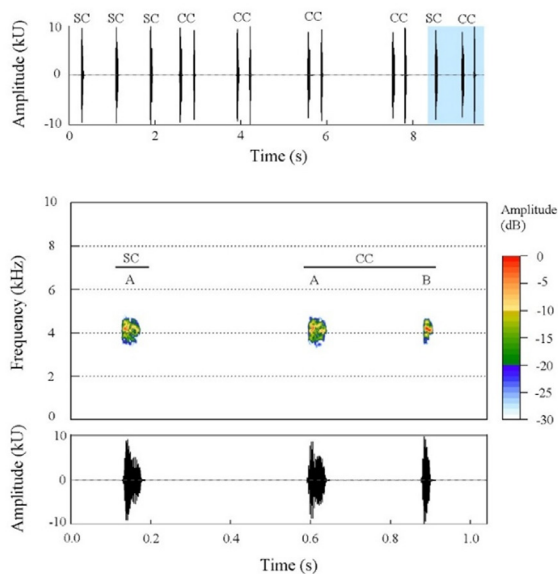


FIGURE 4. A recording showing simple calls (SC) composed of a single A note and complex calls (CC) composed of an A note followed by a B note, in the advertisement call of the Dwarf Treefrog (*Dendropsophus nanus*) in northeastern Corrientes Province, Argentina. Upper panel (oscillogram) shows amplitude for several calls. The signal amplitude is presented in kilo-units (kU), an uncalibrated relative amplitude values based on the digital waveform of Raven Pro. The section highlighted in blue indicates the section represented in the middle and lower panels of the figure. Middle panel (spectrogram) shows the frequency of a simple call followed by a complex call. Color represents the relative amplitude of the signal, expressed in decibels (dB) on a logarithmic scale; values are shown relative to the maximum power (0 dB). The signal amplitude is presented in kilo-units (kU) as described for upper panel.

Analysis of vocalizations.—The advertisement call of *D. nanus* is composed of two types of notes: a long or introductory note (A) and a short or secondary note (B; Martins and Jim 2003; Fig. 4). Two types of calls can be recognized: simple calls composed of a single A note and complex calls composed of an A note followed by a series of B notes. In the same recording, an individual can emit both simple and complex calls (Fig. 4). We use the term simple call and complex call following the terminology of Köhler et al. (2017).

We measured acoustic variables in Raven Pro 1.4 software (Cornell Lab of Ornithology, Ithaca, New York, USA), following the terminology of Köhler et al. (2017). The analyzed temporal variables were: (1) note duration; (2) number of pulses per note; (3) complex call duration; (4) number of notes in complex call; (5) notes/min (note rate; we only calculate the note rate for complex calls); (6) call rate (number of simple or complex calls emitted in a defined period of time); (7) inter-call interval (interval between two consecutive simple or complex calls); and (8) inter-note interval (measured in complex calls). The spectral variables analyzed were: (1) dominant frequency; (2) upper limit of frequency

band; and (3) lower limit of frequency band. We included as variables the upper and lower limit of the frequency band in which the call was concentrated because these measures provide relevant biological information (Köhler et al. 2017) and the dominant frequency appears to be a conserved pattern within the species, so modifications in this type of variable could be more complex (Gomes et al. 2022). To know whether the frequency band can shift, even slightly, in response to the soundscape could provide information about the acoustic space occupied by each species and how each species uses this space in relation to environmental disturbances. We developed spectral components with a 512-point fast Fourier transform (FFT) and Hanning window function, with brightness and contrast at 50%. For the analysis we considered 10 type A notes (five emitted as simple calls and five emitted in complex calls) and 10 type B notes (as part of complex calls) for each individual. Because there were no differences between the A notes of simple or complex calls, they were considered as a single note type (A). To calculate the rate of repetition of notes in the complex call, we analyzed five randomly selected complex calls per individual. We measured the duration of each complex call and divided it by the number of notes of each call. To determine the call rate, we considered 1 min of recording and identified all simple and complex calls present per individual, dividing the number of each call type by the selected recording minute.

Statistical analysis.—We evaluated the normality of the data using the Shapiro–Wilk Test and homogeneity of variances using Levene’s Test. We performed Linear Regressions between call temporal properties and air temperature to assess the effect of this environmental variable on acoustic traits. We corrected the acoustic properties significantly correlated with temperature by adjusting their values to 20° C following Heyer and Reid (2003). We also analyzed the relationship between size and spectral call variables and corrected spectral properties significantly correlated with size (SVL) by adjusting their values to 20 mm. To test if noise significantly affected any call parameters in the two conditions, we analyzed the call data adjusted for temperature and SVL using a Permutational Multivariate Analysis of Variance Using Distance Matrices using the similarity index Euclidean. Because some of the variables analyzed did not have a normal distribution, we used a nonparametric test for the multivariate analysis (Permutational Multivariate Analysis of

TABLE 1. Results of simple Linear Regressions between spectral call parameters and body size (snout-vent length; first six rows), and between temporal call parameters and environmental temperature (last six rows), for the Dwarf Treefrog (*Dendropsophus nanus*) in northeastern Corrientes Province, Argentina. Only significant relationships ($P < 0.05$) are shown. The direction of the relationship (positive or negative) is indicated in the r column. Abbreviations are DF = dominant frequency; FB = frequency band.

	r	r^2	$F_{1,65}$	P
DF A note (Hz)	-0.547	0.298	27.71	< 0.001
DF B note (Hz)	-0.578	0.334	32.61	< 0.001
Upper limit of FB A note (Hz)	-0.469	0.219	18.29	< 0.001
Lower limit of FB A note (Hz)	-0.399	0.159	12.29	< 0.001
Upper limit of FB B note (Hz)	-0.467	0.219	18.29	< 0.001
Lower limit of FB B note (Hz)	-0.444	0.196	15.95	< 0.001
A note duration (ms)	-0.407	0.165	12.93	< 0.001
B note duration (ms)	-0.283	0.079	5.64	0.021
Complex call duration (ms)	-0.401	0.160	12.42	< 0.001
Inter-call interval (ms)	-0.596	0.355	55.79	< 0.001
Calls/min (call rate)	0.599	0.359	36.52	< 0.001
Notes/min (note rate)	0.736	0.542	77.06	< 0.001

Variance). Bonferroni correction was performed to get the appropriate level of significance and *post-hoc* pairwise comparisons assessed which variables differed significantly. According to the normality of the data, we used Mann-Whitney U-test and Student’s t-test. We conducted statistical analyses using Past 4.09 (Hammer et al. 2001). For all tests $\alpha = 0.05$

RESULTS

We analyzed advertisement calls from 67 individuals (33 from without-noise sites and 34 from noisy sites) with 1,340 notes (670 A notes and 670 B notes). Most calling individuals were on emergent vegetation 10–30 cm above the water surface. We found a significant negative relationship between six spectral parameters and SVL (dominant frequency A note, dominant frequency B note, upper limit of frequency band A note, lower limit of frequency band A note, upper limit of frequency band B note,

TABLE 2. Comparison of parameters of the advertisement call of the Dwarf Treefrog (*Dendropsophus nanus*) between without-noise sites and noisy sites in northeastern Corrientes Province, Argentina. Values are mean $\pm$ standard deviation, followed by the range of values in parentheses. Statistical tests were Student's *t*-tests (*t* and *P* values) and Mann-Whitney tests (*U* and *P* values). Abbreviations are DF = dominant frequency and FB = frequency band. Superscript 1 = values adjusted to 20 mm snout-vent length and superscript 2 = values adjusted to 20° C.

	Without-noise sites (n = 33)	Noisy sites (n = 34)	Statistical tests df = 65
A note duration (ms) ²	39.8 $\pm$ 7.8 (25.7–54.3)	41.05 $\pm$ 6.3 (22.8–52.5)	<i>t</i> = 0.747 <i>P</i> = 0.457
B note duration (ms) ²	21.5 $\pm$ 4.1 (14.7–32.8)	22.5 $\pm$ 2.7 (17.8–27.7)	<i>t</i> = 1.307 <i>P</i> = 0.195
A Notes pulse number	9.8 $\pm$ 1.8 (6.4–13.2)	9.8 $\pm$ 1.7 (5.1–12.3)	<i>t</i> = 0.079 <i>P</i> = 0.938
B Notes pulse number	4.7 $\pm$ 1.1 (2.3–6.8)	4.8 $\pm$ 1.1 (2.7–7.8)	<i>t</i> = 0.383 <i>P</i> = 0.702
Complex call duration (ms) ²	424.5 $\pm$ 124.9 (288.1–822.6)	477.9 $\pm$ 136.3 (329.9–1,111.05)	<i>U</i> = 365 <i>P</i> = 0.014
Number of notes of complex call	2.4 $\pm$ 0.4 (2–3.6)	2.4 $\pm$ 0.5 (2–4.6)	<i>U</i> = 493.5 <i>P</i> = 0.390
Calls/min (call rate) ²	36.8 $\pm$ 11.3 (21.3–67.7)	36.2 $\pm$ 12.0 (18.6–66.7)	<i>U</i> = 539 <i>P</i> = 0.782
Notes/min (note rate) ²	207.9 $\pm$ 25.83 (159.7–281.7)	184.8 $\pm$ 21.8 (157.3–268.6)	<i>U</i> = 229 <i>P</i> < 0.000
Inter-note interval (ms)	243.7 $\pm$ 51.9 (157.9–363.7)	247.1 $\pm$ 43 (147.8–343.8)	<i>t</i> = 0.291 <i>P</i> = 0.771
Inter-call interval (ms) ²	1,145.9 $\pm$ 223.04 (791.3–1,541.7)	1,204.8 $\pm$ 219.3 (849.9– 1,653.5)	<i>t</i> = 1.090 <i>P</i> = 0.279
DF A note (Hz) ¹	4,303.5 $\pm$ 170.2 (3,882.2–4,625.2)	4,376.8 $\pm$ 220.2 (3,902.9–4,788.4)	<i>t</i> = 1.522 <i>P</i> = 0.132
DF B note (Hz) ¹	4,279.4 $\pm$ 152.6 (3,914.3–4,629.4)	4,373.8 $\pm$ 202.7 (3,789.8–4,909.5)	<i>t</i> = 2.149 <i>P</i> = 0.035
Upper limit of FB A note (Hz) ¹	5,001.7 $\pm$ 223.2 (4,555.7–5,439.6)	5,148.7 $\pm$ 286.2 (4,280.3– 5,568.2)	<i>t</i> = 2.340 <i>P</i> = 0.022
Lower limit of FB A note (Hz) ¹	3,376.7 $\pm$ 265.3 (2,983.9–4,101.7)	3,471.4 $\pm$ 213 (3,138.3– 3,871.9)	<i>t</i> = 1.614 <i>P</i> = 0.111
Upper limit of FB B note (Hz) ¹	4,941.5 $\pm$ 212.6 (4,492.2–5,400.1)	5,076.6 $\pm$ 359.8 (4,288.2–5,516.7)	<i>t</i> = 2.324 <i>P</i> = 0.022
Lower limit of FB B note (Hz) ¹	3,460.9 $\pm$ 239.9 (3,100.2–4,107.5)	3,541.3 $\pm$ 194.6 (3,210.7– 4,063.5)	<i>t</i> = 1.507 <i>P</i> = 0.136

and lower limit of frequency band B note; Table 1). We also found a negative relationship between temperature and A note duration, B note duration, inter-call interval, and complex call duration, and a positive relationship between temperature and both call rate and note rate.

We found significant differences between noisy and without-noise sites ($F_{1,65} = 3.158$, $P = 0.019$). Duration of complex call was significantly longer in noisy sites than in without-noise sites and note rate was significantly greater in without-noise sites than in noisy sites Table 2; Fig. 5). Upper limit of

frequency band of A notes, dominant frequency of B notes, and upper limit of frequency band of B notes were all significantly higher at noisy sites than at without-noise sites (Table 2; Fig. 5). For the rest of the variables analyzed, we found no significant differences (Table 2).

DISCUSSION

It has been reported that one of the ways that anurans can respond to changes in the soundscape is by modifying their acoustic parameters (Cunnington

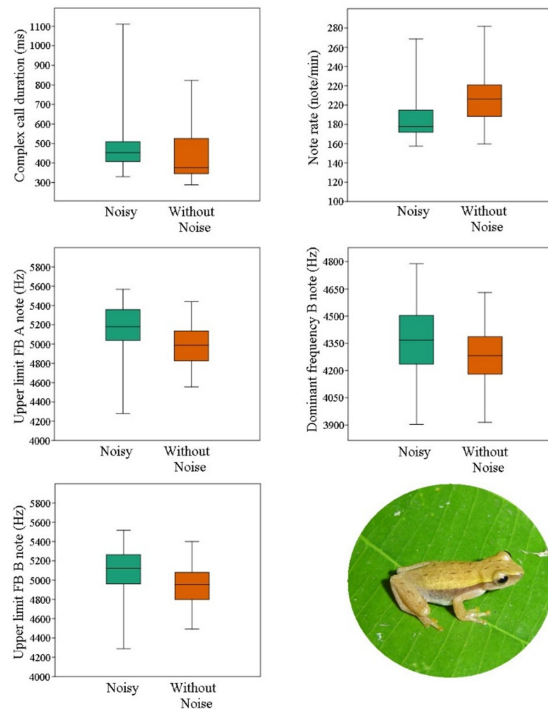


FIGURE 5. Call parameters of Dwarf Treefrog (*Dendropsophus nanus*) males calling in noisy (green) and without-noise (red) sites recorded in northeastern Corrientes province, Argentina. The abbreviation FB = frequency band. Horizontal lines are medians, boxes represent 25 percentiles, and vertical lines represent ranges. (Photographed by Victor Zaracho).

and Fahrig 2010; Roca et al. 2016; Leon et al. 2019; Higham et al. 2021; Zaffaroni-Caorsi et al. 2023). We found that *Dendropsophus nanus* vocalizing in noisy sites had higher upper limits of the frequency band than those vocalizing in without-noise sites, and the dominant frequency of B notes was higher in noisy sites than in without-noise sites. Leon et al. (2019) found similar results in *Scinax nasicus* where the upper and lower limits of the frequency band and the dominant frequency were higher in populations inhabiting noisy sites than in noiseless sites. Because the dominant frequency appears to be a conserved pattern within the species, changes in the upper and lower limits of the frequency bands may be first responses to anthropogenic noise (Köhler et al. 2017; Gomes et al. 2022). These variables, however, are not commonly analyzed. A shift in the entire frequency band occupied by the call of a species may be an adaptive response to traffic noise.

A higher dominant frequency was also reported in Green Frogs (*Rana clamitans*) and Leopard Frogs (*Rana pipiens*) in sites with high traffic noise compared to sites with low traffic noise (Cunnington and Fahrig 2010). In *D. nanus*, however, it is important to note

that we only observed a higher dominant frequency in one of the notes (B note), which comprises part of the complex call. Future studies are needed to understand this differential effect on the A and B notes that constitute the complex call, which could be related to the function of each note.

We also found that the duration of the complex call was longer and note rate was lower at noisy sites compared to without-noise sites. The effects of traffic noise on temporal parameters can vary among anurans. For example, Butler's Narrowmouth Frog (*Microhyla butleri*), Black-striped Wood Frog (*Hylarana nigrovittata*), and Painted Bullfrog (*Kaloula pulchra*) significantly decreased their call rate under airplane fly-by noise and motorcycle sounds (Sun and Narins 2005). In contrast, Taipeh Slender Frog (*Hylarana taipehensis*), Triangle Treefrog (*Dendropsophus triangulum*), and *Scinax nasicus* exhibited increases in temporal variables in noisy environments (Sun and Narins 2005; Kaiser and Hammers 2009; Leon et al. 2019). In *D. triangulum*, the note rate was nearly double in individuals exposed to anthropogenic noises (Kaiser and Hammers 2009). Given our observation that *D. nanus* lowered their call rate, it is notable that congeneric species respond differently to variations in the soundscape. Thus, we caution against generalizing about responses from one species to others, even if the species are closely related. Complementary studies are needed to further understanding of the effects of traffic noise on different species.

Temporal adjustments of calls in the presence of noise pollution could improve the transmission of information for intraspecific communication, allowing its persistence in disturbed areas. Longer call durations in noisy environments may help females find males more easily (Zaffaroni-Caorsi et al. 2023). For *D. nanus* in noisy sites, we observed that each call lasted longer, but the males called less frequently, which could indicate that males are making a trade-off between the two variables. While these modifications do not facilitate the transmission of information *per se*, they could help males improve their performance when vocalizing in traffic noise. Further studies are needed to analyze or compare the effectiveness of males in transmitting their message to females.

Several studies show that modifications in spectral parameters occur in species with high acoustic overlap with traffic noise (Cunnington and Fahrig 2010; Caorsi et al. 2017; Higham et al. 2021; Zaffaroni-Caorsi et al. 2023; Kunberger et al. 2024). Our work represents

one of the few examples of spectral modification for a species with little acoustic overlap with traffic or other anthropogenic noise (Kruger and Du Preez 2019). While no explanation has yet been proposed for this phenomenon, previous studies have suggested that it may be the amplitude (loudness) rather than frequency that is influential. This may explain our observations, given that traffic noise at our study sites had high amplitude. Additionally, traffic and airplane noise are often accompanied by vibrations that may directly disturb nearby amphibians, which could lead them to modify certain parameters of their vocalizations. Experiments analyzing vocal responses of amphibians to vibrations related to high intensity noise even when there is no direct spectral overlap between the call and the low frequency sound would help clarify how anthropogenic noise amplitude and vibrations affect anuran calls.

In some species females choose males for their spectral characteristics, and a 200 Hz increase or decrease in call frequency could affect the choice of females (Gerhardt 1991). For this reason, if males modify the spectral characteristics of their call even slightly, they may appear less attractive to females and consequently their reproductive success would likely be reduced. In the case of *D. nanus*, we observed a higher upper limit of the frequency band of A notes and B notes, and a higher dominant frequency of B notes, for frogs in noisy sites. Modifications of this type may affect mate choice, because females of some frog species prefer the low frequencies of conspecific calls (usually associated with larger males; Gerhardt 1994; Márquez 1995; Lode and Le Jacques 2003; Zaffaroni-Caorsi et al. 2023). Consequently, if males of *D. nanus* modify certain spectral parameters to avoid sound masking, this may have negative consequences for mate choice and reproduction. Future studies on mate choice in this species, however, are needed to determine the preferences of females.

It has also been suggested that changes in temporal or spectral call variables could be a short-term adaptation to noisy sites, in contrast with changes in call structure or in the communication channel itself, which may represent long-term evolutionary shifts (Brumm and Slabbekoorn 2005). We did not observe changes in the structure of *D. nanus* calls, but rather in temporal and spectral variables, so our observations likely represent short-term adaptations. To confirm this, future studies could examine whether the parameters of the call are genetically encoded

and whether there are permanent genetic changes associated with them. Our study shows that traffic noise affects vocalizations of roadside populations of *D. nanus*. Understanding how anthropogenic noise pollution can affect amphibian populations is a first step in better understanding the effects of expanding urban development on wildlife.

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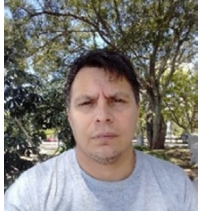
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