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Effects of traffic noise on calling activity of *Aplastodiscus leucopygius* (Anura, Hylidae)

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Abstract. Advertisement calls are the main communication form of anurans, and other individuals can use it to evaluate several aspects of the calling individual. In this context, environmental disturbances, such as traffic noise, can potentially affect this recognition. Therefore, this study aims to evaluate the response of *Aplastodiscus leucopygius* to traffic noise in a fragment of Atlantic Forest within the city of São Paulo. The experimentation consisted of recording the calling individual previously, during and after an exposure to urban noise. After that, individuals were measured to evaluate the Scaled Mass Index (SMI), and individual and environmental temperatures were taken. Also, considering that individuals of this species present harmonic shifting, we tried to evaluate which factors (individual, acoustic, or environmental) are associated with this phenomenon. We observed that the individuals showed an increase in call activity after exposure to traffic noise, but none of the evaluated aspects here could explain the harmonic shifting in their calls. Considering that this increasing on call activity also means an increasing of individual's expense of energy, traffic noise is potentially harmful to the communication of *A. leucopygius* and, consequently, to its permanence in the site.

Keywords. Amphibian, Hylinae, anthropogenic noise, advertisement call, Atlantic Forest.

INTRODUCTION

Males of anurans use advertisement calls to attract females and segregate territories (Toledo et al., 2015). While these calls are emitted, other individuals use hearing to evaluate several aspects of the calling individual through call characteristics. These characteristics can be divided into two groups: spectral, such as dominant frequency, frequency bandwidth, and harmonics, and temporal, such as call rate, call duration, and interval between calls (Köhler et al., 2017). Spectral variables are less sensitive to environmental characteristics, such as temperature and precipitation, and are more related to intrinsic aspects of calling individual (Tonini et al., 2020; Maria et al., 2023), unlike temporal variables, which can be influenced by several aspects of environment surrounding the calling individual (Lingnau and Bastos, 2007; Both and Grant, 2012; Caorsi et al., 2017).

Since most anuran communication is performed through sound, some sound-related aspects must influ-

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ence this process. One of them is environmental noise, which can modify the call of individuals or even impair communication since it interferes with the auditory information transmitted to the receiver (Feng and Schul, 2007). Among these environmental noises, it is possible to distinguish two groups: natural noises, which are a consequence of the natural environment where each individual is inserted, such as rivers or wind (Lingnau and Bastos, 2007), and anthropogenic noises, which are human-produced and can promote an impact on natural populations. Among the effects of anthropogenic noises, such as traffic noise, it is possible to observe an increase in recognition time of males by females in the reproductive display (Bee and Swanson, 2007), decreased activity, which reduces the reproductive success (Kaiser et al., 2011), and the increase of the amplitude of call, which potentially results in waste of energy that could be used for reproduction (Gerhardt and Klump, 1988; Lima et al., 2022).

One of the characteristics of calls on several species is the presence of harmonics. They consist of frequencies that are separated in bands multiple of the lowest resulting from periodic patterns of oscillation (Köhler et al., 2017). Several anuran species present their calls consisting of observable harmonics, such as *Boana albomarginata* (Giasson and Haddad, 2006; Rebouças et al., 2020; Rebouças, 2021), *Eleutherodactylus iberia* (Estrada and Hedges, 1996) and those of *Aplastodiscus* genus (Zina and Haddad, 2006a,b). In this way, it was already observed that some species present the dominant frequency of their calls shifting between harmonics, such as *Boana albomarginata* (Rebouças et al., 2020) and *Aplastodiscus leucopygius* (Zina and Haddad, 2006b), but the possible causes of this phenomenon remain understudied.

Although anthropogenic noise can have harmful effects on anuran populations, its specific effects are highly variable (Zaffaroni-Caorsi et al., 2023). Some species modify their call activity, increasing the call rate and duration in noisy environments (Lima et al., 2022), while others present no effect on call activity (Cunnington and Fahrig, 2010), or even some species are reported to shift their call frequency (Parris et al., 2009). Thus, evaluating anthropogenic noise effects on anuran calling activity is necessary to predict consequences of communication disturbance in population or species level. In this study, we aimed to experimentally assess the impact of anthropogenic noise on the call activity of an isolated population of *Aplastodiscus leucopygius* in an urban forest fragment within the municipality of São Paulo, Brazil. Considering that this species commonly occurs in habitats far from anthropogenic noise sources, we evaluated if this noise could represent a factor that could impair this occupancy. Also, we evaluated which factors are able to predict the shifting of dominant frequency in the harmonics of calls. Here, we tested the hypothesis that individuals modify their calling activity structure as a consequence of anthropogenic noise. Specifically, we evaluated if the magnitude of this modification is related to (i) intrinsic aspects of individual calling, such as body condition, or (ii) temperature of the environment where each individual is inserted. Also, we evaluated if (iii) harmonic shifting is more related to extrinsic than intrinsic aspects, as proposed by Zina and Haddad (2006b).

MATERIALS AND METHODS

Sampling site and species

Individuals of *Aplastodiscus leucopygius* were captured in the Parque Estadual das Fontes do Ipiranga (PEFI), an urban Atlantic Forest fragment in the municipality of São Paulo, Brazil. The specific collecting site was between two boulevards, Avenida do Cursino and Avenida Miguel Stefano (23º38’21.55”S, 46º37’7.25”W), at a distance of 514 m and 891 m, respectively. We selected this specific place to minimise the influence of other anthropogenic noise in our experiment (Fig. 1).

Aplastodiscus leucopygius is a species of the Hylidae family, with occurrence in the Atlantic Forest in the states of Rio de Janeiro and São Paulo, Brazil (Frost, 2023). It breeds in small streams or ponds, calls in marginal vegetation above the water body, and lays eggs in subterranean nests constructed by males (Zina and Haddad, 2006a). Males of this species present three call types: territorial, multi-note call, and advertisement call, which is the most

Fig. 1. Sampling site of *Aplastodiscus leucopygius* in the Estadual das Fontes do Ipiranga, municipality of São Paulo (photo by Victor Fávaro).

common (Haddad and Sawaya, 2000; Zina and Haddad, 2006b). Advertisement calls are described as composed by four visible harmonics, with the dominant frequency in the first or third harmonic.

Experiment

We conducted this study from November 2020 to April 2021, between 19:00 and 22:30, in the natural calling site of individuals. Our experiment consisted of recording the call of 20 males of *A. leucopygius* during three uninterrupted minutes while they were exposed to three consecutive trials of one minute each: (i) pre-playback, when each individual was recorded with no influence of noise (control trial); (ii) playback, when each individual was recorded during the emission of traffic noise by a speaker; and (iii) post-playback, when each individual was recorded after speaker turned off. Thus, each individual was exposed sequentially to a pre-playback, playback and post-playback trial. Both male's calls and traffic noise recordings were made with a YOGA 9600 unidirectional microphone and a Tascam DR-40 digital recorder. The traffic noise sound for the playback trial was recorded in the Avenida Miguel Stefano during the rush hour, for one minute. In all playback trials, individuals were exposed to the same traffic recording (Supplementary Materials, Fig. S1). During the recording of calls, the microphone was placed at a distance of 1 m from the focused individual, and during the playback trial, the speaker was placed at the same distance in a parallel position to the microphone, in a position of ~45º of the individual, to reduce the interference of sound emission into the recording (Fig. 2). The recordings were made at a sampling rate of 44.1 kHz and with 16 bits of resolution. For the playback trial, we used a JBL Extreme speaker because of its relatively good frequency response (Fig. S2) and Bluetooth connection, which allowed us to perform the experiment in the natural environment of individuals. The noise was emitted through its connection to a cell phone. We kept the traffic noise emission as it was recorded, which implies some variation of levels, which ranged from -55 until -85.4 dBFS (scale C), measured with a digital decibel meter Instrutherm DEC-500 during all recording periods. Thus, we used a decibel meter to calibrate the sound pressure of the speaker to the same levels at 1 m distance (Fig. 3). Although inserted between two avenues, the noise generated by them does not reach the collecting site (see Lima et al., 2022). We avoided performing the experiments on rainy or windy days to reduce the further influence of other noises that were not the playback, and to record individuals close to each other to ensure that individuals would be exposed to only the specified time of noise.

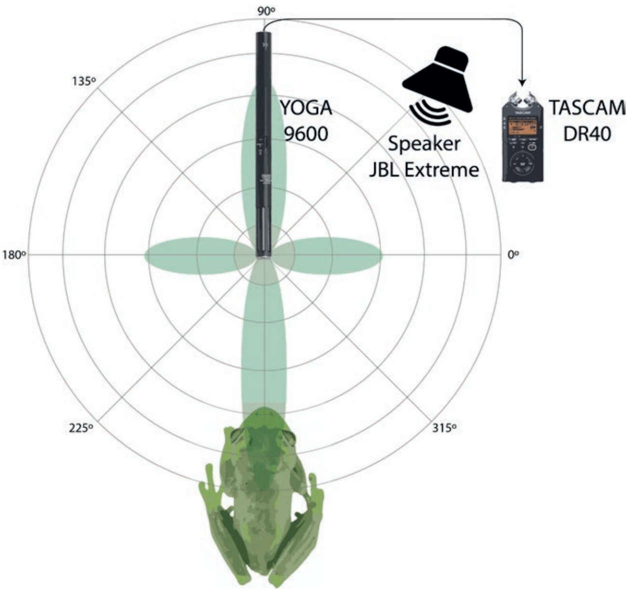

Fig. 2. Experimental design, with the location of the speaker according to the recording range of the microphone, with the aim to reduce the influence of traffic noise exposed to the recorded individual on posterior analysis (individual not in scale).

After the experiment, we captured the individuals and measured their snout-vent length (SVL) with a digital calliper (to the nearest 0.01 mm), and their weight with a digital scale (to the nearest 0.1 g). These measurements were used to calculate the Scaled Mass Index (SMI) of individuals (Peig and Green, 2009). This is a measurement based on the population parameters used as an indicator of energy reserves of an animal (Peig and Green, 2009). At the collecting site, we evaluated the air temperature with a mercury thermometer (to the nearest 0.1 ºC) and the body temperature of the focal individual, with an infrared thermometer (to the nearest 0.1 ºC). To avoid performing the experiment twice with the same individual and consequently avoiding pseudo replications, each individual was marked with Visible Implant Elastomer, applied subcutaneously in the ventral part of the thigh (Nauwelaerts et al., 2000), and recordings of recaptured individuals were discarded.

Call Analysis

We analysed all calls in Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics, 2019) with the following settings: Hann window type with size of 512 samples, 3 dB filter bandwidth of 2.7 kHz, time grip with an overlap of 50%, hop size of 256 samples, DFT size of 512 samples and spectral resolution of 1.88 kHz. Spectrograms were visualised with contrast of 75% and bright of 60%. Recordings in all trials were deposited according to previous recommendations (Dena et al., 2018, 2020) in Fonoteca Neotropical Jaques Veilliard (FNJV 58961 - 59020).

We used four spectral and three temporal variables in call analysis. As spectral, we used the dominant frequency through the function 'peak frequency'; minimum and maximum frequency, obtained through the function 'frequency at 5%' and 'frequency at 95%', respectively; and the bandwidth, which was the difference between the minimum and maximum frequencies. We used this latter function to avoid the inclusion of frequency measurements that were not related to individual calls (see Köhler et al., 2017). As temporal variables, we used the interval between calls, the number of calls in the recorded minute, and the duration of the call.

Statistical Analyses

To verify multicollinearity between variables in all models, we performed an initial model and used the variation inflation factor (VIF) through the "vif" function of the "*car*" package (Fox and Weisberg, 2019). We checked the performance of each model with the package "*performance*" (Lüdecke et al., 2020) (Fig. S3-S9). We considered an indicator of multicollinearity when the variables reached a VIF higher than 10 (Quinn and Keough, 2002). We used a Generalised Linear Mixed Models analysis (GLMM) to evaluate if the trial (pre-playback, playback, and post-playback) influenced each of the measured variables of calls. We excluded the minimum frequency and frequency bandwidth of analysis during the playback trial since traffic noise overlapped these measurements. Considering that we have several measurements of the same individual in each trial, we used "individual" as a random factor and Gaussian family with identity link, for analysis with dominant, minimum, and maximum frequencies, frequency bandwidth, call duration and the interval between calls as response variables. To evaluate the influence of trial on the number of calls, we used a GLMM with a Poisson family and logit link. Additionally, we ran a GLM, with Gaussian distribution and "identity" link, using the residuals of those models, which showed the influence of trial on a specific call variable as a response, and SMI, individual temperature and air temperature as predictors to evaluate which factor influenced in the response of individuals to traffic noise. Also, to evaluate which factor is better predicting the harmonic shifting in calls (Zina and Haddad, 2006b), we also used a GLMM with harmonic of dominant frequency (coded as 0 for the first harmonic and 1 for the third) as the response variable and, as the predictor, the trial (only used pre- and post-playback trials, since playback could give a false estimative of first harmonic due to experimental noise), temporal variables (call duration, interval between calls), minimum and maximum frequencies, individual variables (SMI and body temperature), and habitat variables (air temperature and number of surrounding individuals calling). We used a binomial distribution with logit link and individual as random factor.

To determine the effect of each factor on the response variable, we used the analysis of variance with the type II Wald chi-square test through the "Anova" function of the "*car*" package (Fox and Weisberg, 2019). All analyses were performed in R 4.2.1 (R Core Team, 2022) with a confidence interval of 95 %, parameters of all models are available in supplementary material, and information in tables were provided according the best practices to allow transparency and reproducibility with the package "*report*" (Makowski et al., 2023).

RESULTS

Calls of *Aplastodiscus leucopygius* consisted of a single-pulsed note with most energy concentrated in three harmonics (Fig. 3). We observed that among spectral parameters of call, individuals of *A. leucopygius* showed a reduction in the dominant frequency during playback trial which was not observed for maximum frequency. However, in relation to temporal parameters, during playback trial calls were less frequent and more spaced (Table 1, Fig. 4).

None of our variables presented a VIF higher than 10, so we considered all in our analysis (minimum frequency: 1.83; maximum frequency: 7.67; dominant frequency: 1.37; bandwidth: 8.81; interval between calls: 2.21; number of calls: 2.64; call duration: 1.26). Our analyses showed that the complete trial (pre-playback, playback and post-playback) presented a significant influence on dominant frequency ($\chi^2 = 10.28$, $P = 0.006$), call duration ($\chi^2 = 7.17$, $P = 0.03$), interval between calls ($\chi^2 = 43.47$, $P < 0.001$) and number of calls ($\chi^2 = 494.87$, $P < 0.001$), but presented no influence on maximum frequency ($\chi^2 = 1.36$, $P = 0.51$), minimum frequency ($\chi^2 = 0.19$, $P = 0.66$), and bandwidth ($\chi^2 = 0.44$, $P = 0.51$) (Table 2, Figs. S3-S9). Specifically, during the playback trial, individuals showed a reduction in call duration, call rate, and dominant frequency and an increase in the interval between calls. Additionally, during the post-playback trial, the call rate increased compared to the two previous trials (Table 2).

Individuals presented a weight of 4.09 ± 0.47 g (3.1-4.9 g), SVL of 38.56 ± 2.03 mm (32.9-42 mm) and SMI of 4.11 ± 0.52 (3.37-5.21). Body temperature had an average of 20.77 ± 1.18 °C (18.4-22.6 ºC), and air temperature had an average of 22.68 ± 0.98 °C (20-24 ºC). None of these variables were excluded based on their VIF (SMI: 1.22, body temperature: 1.36, air temperature: 1.46). None of these variables showed any influence on the response of individuals to traffic noise (Table 3).

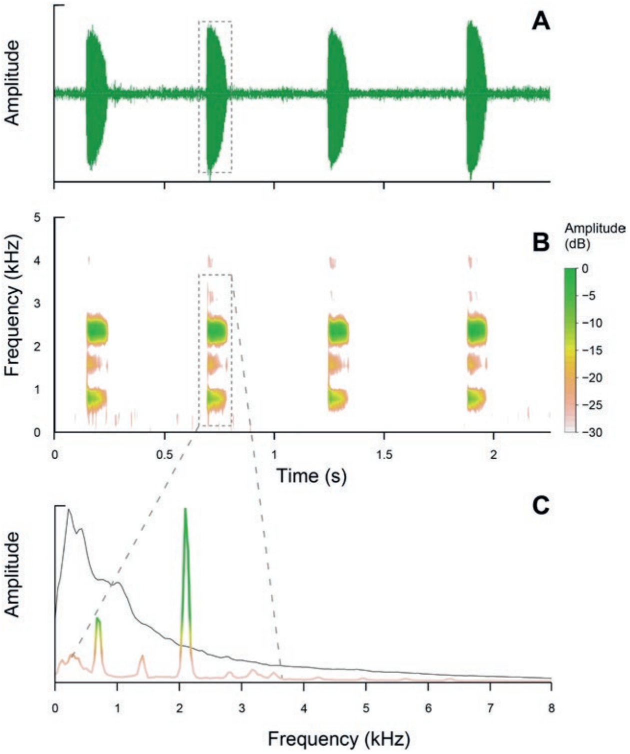

Fig. 3. Call of *Aplastodiscus leucopygius*: Oscillogram (A), Spectrogram (B) and frequency spectrum of the call in relation to the noise of boulevard (grey) (C).

Table 1. Summary statistics of *Aplastodiscus leucopygius* call during pre-playback, playback and pos-playback trials.

	Pre-playback	Playback	Post-playback
Minimum Frequency (Hz)	740.1 ± 95.83 (598.7 - 1097.8)	-	736.4 ± 85.57 (580.9 - 1015.9)
Maximum Frequency (Hz)	2551 ± 265.37 (2212 - 3201)	2535 ± 213.1 (2248 - 3159)	2558 ± 292.4 (2205 - 3328)
Dominant Frequency (Hz)	1830.5 ± 686.56 (750 - 2449.2)	1629.5 ± 632.7 (703.1 - 2374.2)	1832.3 ± 704.74 (750 - 2437.5)
Bandwidth (Hz)	1811 ± 281.67 (1254 - 2451)	-	1821 ± 300.09 (1333 - 2578)
Call duration (s)	0.098 ± 0.008 (0.081 - 0.109)	0.093 ± 0.11 (0.067 - 0.107)	0.097 ± 0.008 (0.082 - 0.109)
Interval between calls (s)	0.709 ± 0.28 (0.4 - 1.67)	2086 ± 1.53 (0.64 - 7.16)	0.604 ± 0.183 (0.384 - 1.079)
Number of calls	79 ± 2.39 (33 - 121)	33.25 ± 18.14 (3 - 80)	89.35 ± 21.73 (51 - 126)

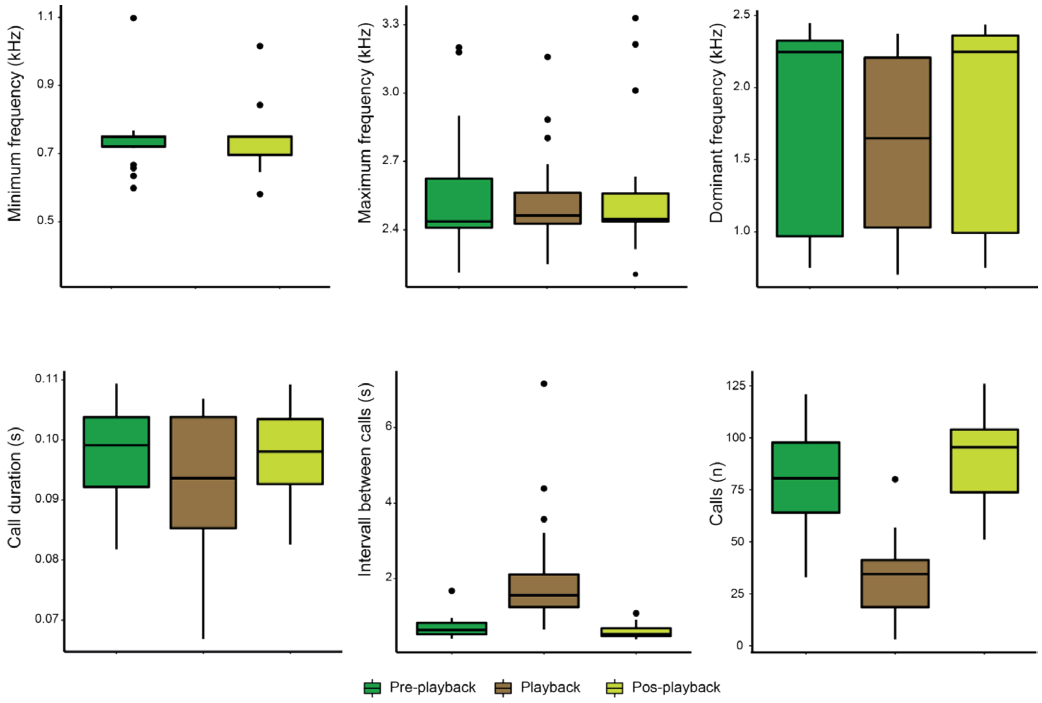

Fig. 4. Spectral and temporal variables of the call of *Aplastodiscus leucopygius* in the three trials: pre-playback (green), playback (brown) and post-playback (yellow).

Table 2. Coefficients of the Generalised Linear Mixed Effects model considering the influence of each trial (pre-playback, playback, and post-playback) on each call parameter as response variables (t or z values are corresponding to Gaussian and Poisson families, respectively).

Response	Parameter	Coefficient	95% CI	t/z	P	Effects	Group	Std. Coef.	Fit
Call Duration	Intercept	0.1	0.09, 0.1	44.39	< 0.001	fixed		-0.32	
	exp [POS]	4.29E-03	0, 0.01	2.22	0.031	fixed		0.45	
	exp [PRE]	4.66E-03	0, 0.01	2.41	0.019	fixed		0.49	
		7.10E-03				random	individual		
		6.12E-03				random	residual		
	AICc								-368.44
	R2 (conditional)								0.59
	R2 (marginal)								0.05
	Sigma								0.006
Minimum frequency	Intercept	740.12	698.92, 781.32	36.43	< 0.001	fixed		0.02	
	exp [POS]	3.71	-21.10, 13.67	-0.43	0.67	fixed		-0.04	
		86.71				random	individual		
		27.11				random	residual		
	AICc								432.02
	R2 (conditional)								0.91
	R2 (marginal)								4.28e-04
	Sigma								27.11
Maximum frequency	Intercept	2534.98	2418.89, 2651.07	43.76	< 0.001	fixed		-0.05	
	exp [POS]	22.83	-17.44, 63.1	1.14	0.261	fixed		0.09	
	exp [PRE]	15.97	-24.3, 56.24	0.79	0.43	fixed		0.06	
		251.15				random	individual		
		63.54				random	residual		
	AICc								728.65
	R2 (conditional)								0.94
	R2 (marginal)								0.001
	Sigma								63.54
Bandwidth	Intercept	1810.83	1678.85, 1942.81	27.83	< 0.001	fixed		-0.02	
	exp [POS]	10.58	-21.78, 42.93	0.66	0.51	fixed		0.04	
		286.62				random	individual		
		50.45				random	residual		
	AICc								500.45
	R2 (conditional)								0.97
	R2 (marginal)								3.39e-04
	Sigma								50.45
Dominant frequency	Intercept	1629.53	1326.89, 1932.17	10.79	< 0.001	fixed		-0.2	
	exp [POS]	202.81	57.1, 348.53	2.79	0.007	fixed		0.3	
	exp [PRE]	200.97	55.25, 346.69	2.76	0.008	fixed		0.3	
		635.01				random	individual		
		229.93				random	residual		
	AICc								862.05
	R2 (conditional)								0.89
	R2 (marginal)								0.02
	Sigma								229.93

(Continued)

Table 2. (Continued).

Response	Parameter	Coefficient	95% CI	t/z	P	Effects	Group	Std. Coef.	Fit
Interval between calls	Intercept	2.09	1.68, 2.49	10.31	< 0.001	fixed		0.85	
	exp [POS]	-1.48	-1.98, -0.98	-5.91	< 0.001	fixed		-1.32	
	exp [PRE]	-1.38	-1.88, -0.87	-5.49	< 0.001	fixed		-1.23	
		0.44				random	individual		
		0.79				random	residual		
	AICc								167.67
	R2 (conditional)								0.51
	R2 (marginal)								0.36
	Sigma								0.79
Number of calls	Intercept	3.46	3.3, 3.62	43.19	< 0.001	fixed		3.46	
	exp [POS]	0.99	0.9, 1.08	21.79	< 0.001	fixed		0.99	
	exp [PRE]	0.87	0.77, 0.96	18.74	< 0.001	fixed		0.87	
		0.31				random	individual		
	AICc								532.75
	R2 (conditional)								0.95
	R2 (marginal)								0.63
	Sigma								1

Finally, our GLMM analysis showed that neither call, individual aspects nor environmental variables explained the harmonic shift between the first and the third harmonic (Table 4).

DISCUSSION

In our analyses, we observed that urban traffic noise had a significant influence on several aspects of *Aplastodiscus leucopygius* calls, even when it was not present anymore. Also, we observed that neither the body nor environmental aspects measured are related to these responses, which probably means that all individuals are subjected to this modification, independently of their body condition or temperature. We observed, during the playback trial, an influence of noise on almost all aspects of the call, except for the maximum frequency. All temporal variables showed a significant influence of playback trial, with calls becoming shorter, less frequent, and with a larger interval between them. It is consistent with most anuran species, since a recent study showed that 49% of anuran species decrease their call rate during exposure to a noise (Zaffaroni-Caorsi et al., 2022). These changes in the call pattern can directly imply communication with females. Similar results were also observed for *Scinax nasicus* (Leon et al., 2019), *Hyla arborea* (Lukanov and Naumov, 2019), *Rana clamitans*, *R. pipiens*, *H. versicolor* (Cunnington and Fahrig, 2010), and *Pseudacris crucifer* (Hanna et al., 2014), with calls presenting less duration in noisy environments than in silent ones. In *Bokermannohyla hylax*, a species from the same subfamily of *A. leucopygius*, when in noisy environments, males present longer, more frequent and less spaced calls (Lima et al., 2022), similar to the one observed for *Dendropsophus triangulum* (Kaiser and Hammers, 2009). It reveals that the effect of anthropogenic noise on anuran call is not the same for all species (Zaffaroni-Caorsi et al., 2022), but that they probably tend to modify temporal aspects of the call, with only some exceptions (e.g., Parris et al., 2009; Grenat et al., 2019).

In the post-playback trial, individuals presented a significant increase in the number of calls when compared to the playback trial, but the estimate of our models showed slight increase in the number of calls also in relation to the pre-playback trial. Consequently, in the post-playback trial, individuals emitted calls at shorter intervals. It probably means that traffic noise stimulates individuals to increase the call rate, i.e., spend more energy on calling activity, even when the noise stimulus is no longer present. Similar results were observed for *Hyperolius pickersgilli,* a native species from South Africa, which presents an increase of 18% in call rate after anthropogenic noise stimulus, in this case, aeroplane noise (Kruger and Du Preez, 2016). Calling is one of the most energetic spending activities of anurans (Ryan, 1988; Grafe and Thein, 2001; Wells and Schwartz, 2007), with metabolic rates rising up to tenfold over the resting metabolism (Wells and Schwartz, 2007). Consequently, the increasing calling activity after anthropogenic noise

Table 3. Coefficients of Generalised Linear Models between residuals of models which showed a significant influence of traffic noise, as response, and Scaled Mass Index (SMI), body temperature and air temperature as predictive variables.

Model	Parameter	Coefficient	95% CI	t/z	*P*	Fit
Call Duration	Intercept	0.01	-0.02, 0.05	0.71	0.48	
	SMI	-1.77E-04	0,0	-0.14	0.89	
	Air temperature	-6.35E-04	0,0	-0.68	0.5	
	Body temperature	7.91E-05	0,0	0.11	0.91	
	AICc					-453.67
	R2 (conditional)					-452.56
	R2 (marginal)					-443.2
	Sigma					5.26E-03
Dominant frequency	Intercept	-174.72	-1531.99, 1182.56	-0.25	0.8	
	SMI	7.03	-99.98, 85.92	-0.15	0.88	
	Air temperature	4.51	-62.05, 71.08	0.13	0.89	
	Body temperature	4.89	-47.13, 56.91	0.18	0.85	
	AICc					806.49
	R2 (conditional)					807.6
	R2 (marginal)					816.96
	Sigma					191.14
Interval between calls	Intercept	1.18	-4.01, 6.38	0.45	0.65	
	SMI	-0.06	-0.42, 0.3	-0.33	0.74	
	Air temperature	-0.02	-0.27, 0.24	-0.14	0.89	
	Body temperature	-0.03	-0.22, 0.17	-0.25	0.8	
	AICc					138.72
	R2 (conditional)					139.83
	R2 (marginal)					149.19
	Sigma					0.73
Number of calls	Intercept	-0.84	-9.93, 8.26	-0.18	0.86	
	SMI	-0.18	-0.8, 0.45	-0.55	0.58	
	Air temperature	4.77E-03	-0.44, 0.45	0.02	0.98	
	Body temperature	0.07	-0.28, 0.41	0.37	0.71	
	AICc					205.85
	R2 (conditional)					206.96
	R2 (marginal)					216.32
	Sigma					1.28

stimulus can induce individuals to spend more energy, and consequently impair some other activities which also demand great amounts of energy, such as reproduction. In an experiment with *Hyla chrysoscelis,* evaluating the time response of females to mating calls, it was observed that in silent environments, females tend to respond faster to the call of males than in noisy environments, which means that anthropogenic noise masks the mating call emitted by males in a chorus (Bee and Swanson, 2007).

Furthermore, a study in Belize showed that anthropogenic noise promoted a decrease in the number of males present in choruses and the duration of the chorus during the night, and considering that females join the reproduction site lately than males, which could substantially reduce reproductive success in these species (Kaiser et al., 2011). Unlike *B. hylax* (Lima et al., 2022), *A. leucopygius* only breeds at sites far from the boulevard in the PEFI (Lisboa et al., 2021). Therefore, individuals are probably not used to the levels of anthropogenic noise of

Table 4. Coefficients of Generalised Linear Mixed Effects Models analysis (GLMM) using harmonic (first or third) as response variable and trial, temporal variables (call duration and interval between calls), spectral variables (minimum and maximum frequencies), individual variables (SMI and body temperature) and habitat variables (air temperature and number of individuals calling) as predictors.

Parameter	Coefficient	95% CI	z	*P*	Effects	Group	Std. Coef.	Fit
Intercept	232.33	-5.62E+06, 5.62E+06	8.11E-05	> 0.99	fixed		235.29	
exp [POS]	-207.19	-6.62E+06, 6.61E+06	-6.14E-05	> 0.99	fixed		-211.76	
exp [PRE]	-106.09	-6.68E+06, 6.68E+06	-3.11E-05	> 0.99	fixed		-105.02	
Call duration	-0.55	-2.32E+06, 2.32E+06	-4.64E-07	> 0.99	fixed		-0.03	
Interval between calls	2.76	-2.19E+06, 2.19E+06	2.47E-06	> 0.99	fixed		0.26	
Minimum frequency	-4.93	-2.40E+06, 2.40E+06	-4.02E-06	> 0.99	fixed		-2.95	
Maximum frequency	2.38	-2.46E+06, 2.46E+06	1.90E-06	> 0.99	fixed		4.49	
SMI	10.75	-2.92E+06, 2.92E+06	7.23E-06	> 0.99	fixed		10.69	
Body temperature	-15.03	-2.71E+06, 2.71E+06	-1.09E-05	> 0.99	fixed		-15.4	
Dominant frequency	266.95	-2.27E+06, 2.27E+06	2.30E-04	> 0.99	fixed		269.6	
Air temperature	8.6	-2.83E+06, 2.83E+06	5.94E-06	> 0.99	fixed		10.35	
n individuals	-52.25	-2.51E+06, 2.51E+06	-4.08E-05	> 0.99	fixed		-58.75	
	0.01				random	individual		
AICc								26.09
R2 (conditional)								1
R2 (marginal)								1
Sigma								1
Log loss								2.22E-16

the playback trial. However, it highlights that, as previously observed in other species (Bee and Swanson, 2007; Leon et al., 2019; Lukanov and Naumov, 2019), this type of noise can be harmful to individuals of *A. leucopygius* and consequently could be a factor that explains the non-occurrence of this species close to anthropogenic noise sources. Finally, we did not test for other noise sources, such as white noise or waterfall noise, to verify if the results observed here are specifically related to anthropogenic noise (e.g., white noise or traffic noise) or to any sort of noise those individuals are not used to (e.g., waterfall noise). However, considering that individuals of *A. leucopygius* typically occur in very silent habitats (Zina and Haddad, 2006a), probably both noise sources (anthropogenic and natural) could present an influence on their call parameters, and further studies are still necessary to evaluate this aspect.

We observed that individuals of *A. leucopygius* have the dominant frequency in the third of the three visible call harmonics. However, it also presented the dominant frequency in the first harmonic in several calls. It was consistent with observation for other species of the same genus, such as *A. albosignatus* (Moser et al., 2022), for other species of a different genus but in the same family, such as *Boana albomarginata* (Rebouças et al., 2020) and *B. punctata* (Brunetti et al., 2015), and for other species from a different family, such as *Thoropa lutzi* (Nunes-de-Almeida et al., 2016). However, we observed that none of the examined variables were able to explain this phenomenon. Although Zina and Haddad (2006b) reported that individuals of *A. leucopygius* present dominant in the first harmonic when calling in antiphony and dominant frequency in the third harmonic when calling alone, we did not evaluate the number of individuals calling in the habitat in this study. This aspect requires further studies explicitly designed to observe this harmonic shifting, especially in an experimental approach.

This study demonstrated that individuals of *A. leucopygius* present calling activity influenced by anthropogenic noise, with a reduction of calling activity during the exposure to noise and a significant increase after that. Also, we observed that the harmonic shifting observed in this species is not related to traffic noise, nor to individual and environmental aspects. These results reinforce that further studies are still needed and that anthropogenic noise, generated by human activities in the city surrounding the habitat of this species (Lisboa et al., 2021), represents a potentially harmful influence on this population.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 15334.

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