



## Acoustic monitoring of anuran communities in road noise disturbed soundscapes

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Vocalisation is the main form of communication in frogs. These signals have different social structures and functions. Road noise has increased over the past few decades to the point where it can mask acoustic signals with impacts for animal communication. Anurans are sensitive to noise masking, but few studies have investigated how noise impacts their vocal behaviour. Here, we compared noise levels and activity, as well as calling activity and richness of aurally-identified species, between two sites; one near and one far from a road. We also assessed the potential of noise masking. Noise was significantly higher at the site near the road and during the day at both sites, while vocalisations were more frequent at the far site and during the night. Species richness and composition was the same at both sites, however, *Boana albopunctata*, *B. cipoensis*, and *Scinax curicica* had greater vocal activity at the far site while *S. squalirostris*, *Leptodactylus jolyi*, and *Dendropsophus minutus* had greater vocal activity at the near site. Traffic noise was found to overlap with the frequencies occupied by vocalisations. Since many natural areas around the world are bordered by roads, we emphasise the importance of establishing regulations for the control and monitoring of road noise.

**Keywords:** Anurans, vocalisation, passive acoustic monitoring, noise pollution

### INTRODUCTION

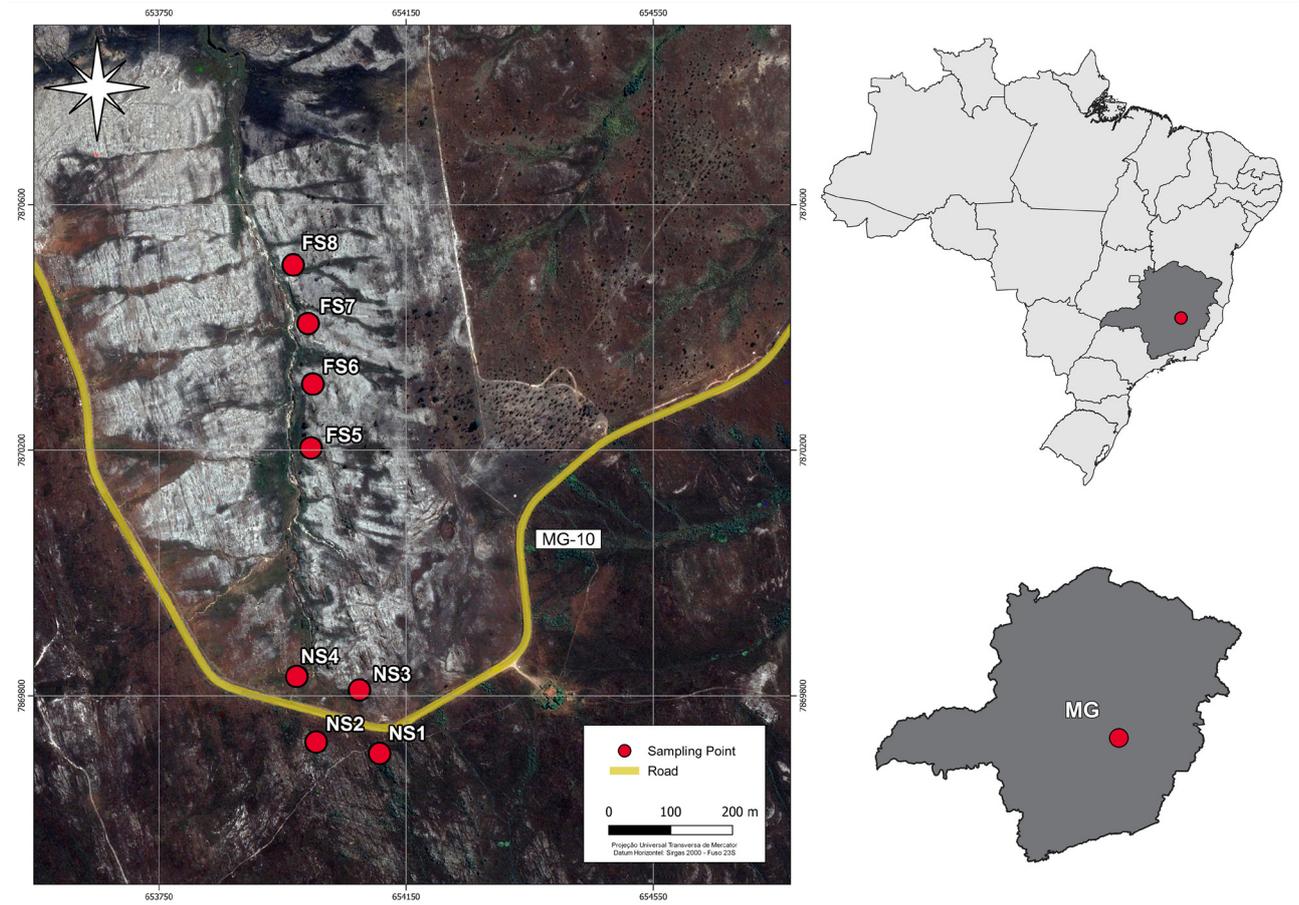
Amphibians are facing a global extinction crisis and represent the most threatened taxon of the world (IUCN, 2021). It has become clear that it is very important to understand the factors contributing to amphibian declines (Houlahan et al., 2000; Stuart et al., 2004). According to Fahrig & Rytwinsky (2009), anurans are among the taxa most threatened by roads, mainly due to roadkill, and studies have found lower species richness and/or abundance in noisier environments (Gibbs & Shriver, 2005; Colino-Rabanal & Lizana, 2012; Grace & Noss, 2018). This finding may be due to animals moving away from noisy places, either temporarily or permanently. On the other hand, some anuran species can persist at noisy areas by adjusting their vocal behaviour to improve communication in the presence of noise (Brumm, 2013). Some such documented vocal adjustments are: avoidance of vocalising during noisy times of the day; increase call amplitude (Lombard effect); and increase or decrease call frequencies, number of notes, syllables and calling rate (Schwartz & Bee, 2013, Leon et al., 2019). However, it is unclear if these adjustments are sufficient to overcome acoustic interference imposed by traffic noise and its negative consequences (Parris

et al., 2009). Furthermore, the production of sounds by anurans involves high energetic costs (Taigen & Wells, 1985), and so to change call characteristics to effectively communicate in noisy areas can be even more costly and bring negative consequences for species fitness (Bucher et al., 1982; Taigen et al., 1985; Prestwich et al., 1989; Wells & Taigen, 1989; Emerson, 2001).

Vocalisation is the main communication mechanism in frogs (Morais et al., 2012; Forti et al., 2015; Heard et al., 2015; Köehler et al., 2017). Many species are capable of producing sounds with different functions that vary depending on the social context (Duellman & Trueb, 1986; Narins et al., 2006; Wells & Schwartz, 2007; Toledo et al., 2015). For frogs, acoustic signals are important for species recognition (Gambale et al., 2014; Guerra et al., 2017), for sexual partner attraction (Haddad & Cardoso, 1992; Lingnau et al., 2004; Costa & Toledo, 2013), for warning about the presence of predators and for the defense and maintenance of breeding sites (Toledo et al., 2015).

Anurans can be severely affected by noise, as it can mask their acoustic signals and prevent individuals from effectively receiving and interpreting the sounds of interest (Barber et al., 2010; Dowling et al., 2012; Kunc & Schmidt, 2019; Leon et al., 2019). One of the characteristics of road

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**Figure 1.** Sampling points located at the near site (NS1-NS4) and the far site (FS5-FS8) from road MG-10, in an area of campo rupestre (rupestrian grassland) at Alto do Palácio, municipality of Morro do Pilar, Minas Gerais, Brazil.

traffic noise is that it has more spectral energy (amplitude) at low frequencies, below 1000 Hz (Bee & Swanson, 2007; Cho & Mun, 2008; Cunnington & Fahrig, 2010). Thus, low frequency signals, such as those produced by many frog species, are expected to experience greater masking than species with higher frequency call types (Legett et al., 2020). The masking effect consists of temporal and spectral overlap (total or partial) between sounds and occurs, for example, when noise is emitted at the same time as the vocalisations of animals and occupies the same frequencies and amplitudes (or higher). Studies with frog species report behavioural changes (Sun & Narins, 2005; Vargas-Salinas et al., 2014; Zhang et al., 2015; Nelson et al., 2017; Medeiros et al., 2017; Higham et al., 2021), physiological (Tennesen et al., 2014; Kaiser et al., 2015) and serious consequences during reproduction (Wollerman & Wiley 2002; Bee & Swanson, 2007; Grace et al., 2017; Simmons & Narins, 2018; Schou et al., 2021).

In the present study we analysed calling activity of two anuran communities in a noise disturbed soundscape at an important site of the Espinhaço Mountain Range in Brazil, that houses endemic species. Anuran species were acoustically monitored at two sites, one near (50 metres) and another far (500 metres) from a paved road, to test the following hypothesis: (1) Areas near and far from the road differ in background noise and anuran calling activity. We predict higher noise levels and lower calling activity at

the site near the road. (2) Anuran species richness differ between the sites. We expect greater species richness at the site far from the road.

## MATERIALS & METHODS

### Study area

Data were collected in an area of rupestrian field, one of the most endangered ecosystems of the world (Pieretti et al., 2015), located in the municipality of Morro do Pilar, southern Espinhaço Mountain Range (EMR), Minas Gerais State, south-east Brazil (19° 15' S, 43° 31' W). The EMR is the second largest mountain range in south America, with a length of 1000 kilometres (Giulietti et al., 1987; Eterovick et al., 2020). The south portion of EMR is considered an area of high richness and endemism of both flora and fauna, including anuran species (Leite et al., 2012). Furthermore, it is located between the Cerrado and Atlantic Forest domains, two global biodiversity hotspots (Myers et al., 2000; Mittermeier et al., 2005). Although recognised as a priority area for conservation in Minas Gerais (Drummond et al., 2005), agriculture and mining activities are increasing in southern EMR, as is tourism, causing accelerated modification of the environment, including the expansion of roads and increased road traffic (Eterovick et al., 2005; Lopes et al., 2012). As tourism and human settlement increase in the vicinities

of Parque Nacional da Serra do Cipó (PNSC), which is occurring in southern EMR, new barriers can appear for the migration of species susceptible to disturbances in the area as a whole. According to the Köppen-Geiger classification, the climate of the study area is 'subtropical highland' (de Sá Jr. et al., 2011) with two well defined seasons - a dry season from April to September and a rainy season from October to March. Higher precipitation rates are concentrated between November and February (de Sá Jr. et al., 2011).

### Data collection

The study was conducted from January to March during the 2018 rainy season. Data were collected using passive acoustic monitoring sensors (Song Metre II, Wildlife Acoustics, Inc., Massachusetts, USA). Four sensors were installed at sampling points at a location within the Morro da Pedreira Environmental Protection Area around the PNSC. The site, henceforth called 'near site', is approximately 50 metres from the MG-10 state highway at km 121, with two sensors installed on the right bank of the internal road and two on the left bank. The MG-10 highway is an access road between the municipalities of Santana do Riacho and Conceição do Mato Dentro, an area known as Alto Palácio, which has intense tourist activity, especially during school holidays (January) and long holidays, such as Carnival (February). For comparison purposes, another four sensors were installed in a quieter area, approximately 500 m away from the road, in a location henceforth called 'far site' (Fig. 1). The far site was chosen because it is easily accessible and has floristic and physical characteristics similar to the near site with the presence of streams and temporary ponds, which tend to be full during the rainy season and used by anurans as breeding sites. The sensors were configured to record soundscapes 24 hours a day, two consecutive days a month, always in the first weekend of the month, totaling 1,152 hours of recordings. At each site the sensors were installed 1.5 metres above the ground and spaced 100 metres apart at each sampling site. The distance between the near and far sites was approximately 500 metres. The sensors were programmed to record at a sampling rate of 44.1 kHz, 16-bit, in stereo channels, using two waterproof omnidirectional microphones with a flat frequency response between 0.020 – 20 kHz, sensitivity of  $-36 \pm 4$  dB and gain of 36 dB.

We conducted noise level measurements for 15 minutes at each recording point using a Z-weighted B&K 2270 sound level metre to verify differences between the studied sites. Noise measurements were done at every sampling point during three periods of the day: (1): 0600–0900 - intense road traffic; (2): 1600–2000 - moderate road traffic, and (3): 2300–0500 low road traffic. These time intervals were chosen based on previous analysis of the soundscape recorded with the same type of sensor, which permitted us to identify periods of more and less intense vehicular traffic. We excluded from the recordings all animal sounds close to the microphone using BZ5503 software. The standard sound pollution

measurement of Equivalent Sound Levels (Leq) was then extracted from the recordings (Rossing, 2007).

### Data analysis

#### I. Anuran calling and noise

Data were subsampled by analysing one minute every ten minutes of the recordings (Pieretti et al., 2015). We used Arbimon software (Sieve Analytics) to automatically detect anuran vocalisations present in every recorded file. The automatic detection was performed after we created a species-specific model of vocalisation for each anuran species detected in the recordings. For these models we selected species with vocalisations that had the possibility of registration in at least 50 Regions of Interest (ROIs). To produce conservative models, we determined an acceptable average of only 5 % false positive detections (Aide et al., 2013). Arbimon detected the presence or absence of each species in every one-minute analysed per recording. All species automatically detected by the Randon Forest approach (RF) were checked and adjusted manually. Contrary to vocalisations, the road traffic noise presented high spectral variation, making it impossible to automatically detect the different sources of noise recorded. Therefore, we manually detected the presence or absence of noise in every one-minute analysed per recording.

#### II. Spectral analysis

Thirty advertisement calls of each anuran species were randomly selected from the recordings to perform spectral characterisation in Raven pro 1.5 software. We measured the following acoustic parameters: minimum, maximum, and peak frequencies, bandwidth and duration. In order to determine the potential for masking by noise we conducted the same analysis for 30 randomly selected noisy events emitted by road traffic.

#### III. Statistical Analysis

We performed the Kruskal-Wallis test to assess differences in noise levels between diurnal and nocturnal periods and vocalisation detections among months. We used Student's t-test to assess differences between noise levels at the near and far sites. The Mann-Whitney test was applied to assess differences between the near and far sites in relation to detections of noise and vocalisations. Analyses were done using R software (R Core Team, 2022). Results were considered significant when  $P < 0.05$ .

## RESULTS

### Characterisation of road noise

The 'near site' had noise levels measuring 1 to 18 dB (Z) higher than the far site ( $t = 3.45$ ,  $df = 22$ ,  $P < 0.001$ , Table 1). Noise levels at both sites were higher during 0600–0900 hours (near:  $H = 7.63$ ,  $df = 2$ ,  $P < 0.05$ ; far:  $H = 7.81$ ,  $df = 2$ ,  $P < 0.05$ ).

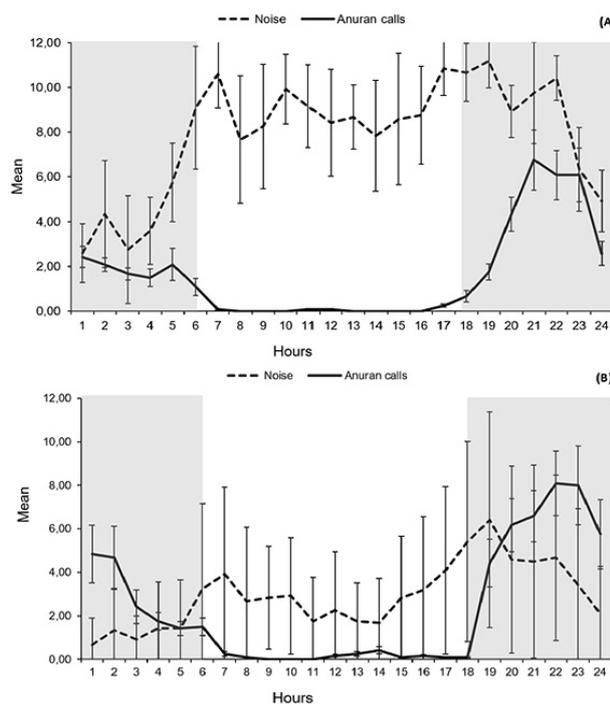
The main sources of road noise identified in this study were car, truck, and motorcycle traffic. Manual detection of these noise sources confirmed the results of the noise level measurements, since vehicle traffic noise was

**Table 1.** Noise levels- Leq dB (Z)- recorded at the sites near and far from the road MG-10, municipality of Morro do Pilar, Alto Palácio, Minas Gerais, Brazil.

Sampling point	0600–0900	1600–2000	2300–0500
Near 1	44	46.9	34.5
Near 2	41.7	46.2	34.2
Near 3	47	39.4	34.5
Near 4	43.5	41.2	33
Far 1	39.6	32.3	31
Far 2	39.5	30.1	29.9
Far 3	40.4	28.7	28.1
Far 4	38.6	34.2	30.9

**Table 2.** Spectral characteristics of the different categories of noise recorded at road MG-10, at Alto do Palácio, municipality of Morro do Pilar, Minas Gerais, Brazil. Values are represented by mean  $\pm$  SD.

Noise source (N=30)	Duration (s)	Peak frequency (Hz)	Maximum frequency (Hz)	Minimum frequency (Hz)	Bandwidth (Hz)
Car	7.8 $\pm$ 4.7	5.7 $\pm$ 21.8	7363.6 $\pm$ 3013.7	0.0 $\pm$ 0.0	7363.6 $\pm$ 3013.7
Truck	27.1 $\pm$ 10.3	14.3 $\pm$ 32.6	12054.6 $\pm$ 3215.2	0.0 $\pm$ 0.0	12054.6 $\pm$ 3215.2
Motorcycle	15.5 $\pm$ 6.7	2.8 $\pm$ 15.7	13172.9 $\pm$ 4173.2	0.0 $\pm$ 0.0	13172.9 $\pm$ 4173.2



**Figure 2.** Means for road noise and anuran calls detected per hour from January to March of 2018 at the site near (A) and the site far (B) from road MG-10, at Alto do Palácio, Municipality of Morro do Pilar, Minas Gerais, Brazil. The white area represents the diurnal period (0600–1759) and grey the nocturnal period (1800–0559).

detected significantly more at the near site ( $U = 12535$ ,  $Z = 14.4$ ,  $P < 0.001$ ). Vehicle traffic at the near site was significantly lower in March ( $H = 18.69$ ,  $df = 2$ ,  $P < 0.001$ ).

There was no significant difference in vehicle traffic among the sampled months for the far site ( $H = 2.70$ ,  $df = 2$ ,  $P = 0.25$ ). Traffic at the near site was higher in the diurnal period (0600–1759) than in the nocturnal period (1800–0559) ( $U = 6093$ ,  $Z = 6.04$ ,  $P < 0.001$ , Fig. 2), while there was no significant difference in vehicle traffic noise between the diurnal and nocturnal periods for the far site ( $U = 9837$ ,  $Z = 0.75$ ,  $P = 0.22$ , Fig. 2).

Noise of the passage of trucks had the longest duration (27.10 s  $\pm$  10.32 s) while the noise produced by motorcycle

traffic had the highest maximum frequencies (13.17 kHz  $\pm$  4.17 kHz). The spectral characteristics of each type of noise are presented in Table 2.

### Anuran calling activity

We identified vocalisations of seven anuran species belonging to three families at both the near and far sites: Hylidae *Boana albopunctata*, *B. cipoensis*, *Dendropsophus minutus*, *Scinax squalirostris*, *S. curicica*, Brachycephalidae *Ischnocnema juipoca*, and Leptodactylidae *Leptodactylus jolyi*. Species composition did not differ between the two sites.

We detected more anuran vocalisations at the far than at the near site ( $U = 11167$ ,  $Z = 3.28$ ,  $P < 0.001$ ,  $N_{\text{far}} = 162$ ,  $N_{\text{near}} = 174$ ). The species *B. albopunctata* ( $t = -7.31$ ,  $P < 0.05$ ), *B. cipoensis* ( $t = -3.96$ ,  $P < 0.05$ ) and *S. curicica* ( $t = -2.86$ ,  $P < 0.05$ ) had greater calling activity at the far site. In contrast, the species *D. minutus* ( $t = 4.24$ ,  $P < 0.05$ ), *S. squalirostris* ( $t = 4.13$ ,  $P < 0.05$ ), and *L. jolyi* ( $t = 3.11$ ,  $P < 0.05$ ) had significantly greater calling activity at the near site. The calling activity of *I. juipoca* did not differ significantly between sites ( $t = 1.02$ ,  $P = 0.15$ ).

The calling activity of species differed among the sampled months. We registered more calling activity in February at both the near and far sites (near:  $H = 10.30$ ,  $df = 2$ ,  $P < 0.05$ ; far:  $H = 11.61$ ,  $df = 2$ ,  $P < 0.05$ ). All species had greater calling activity during the nocturnal period at both sites (near:  $U = 1$ ,  $Z = 3$ ,  $P < 0.001$ ; far:  $U = 2.5$ ,  $Z = 2.81$ ,  $P < 0.001$ ).

We registered more vehicle traffic noise than anuran vocalisations in the nocturnal period ( $U = 4462$ ,  $Z = 8.8$ ,  $P < 0.001$ , Fig. 2) at the near site, while significantly more vocalisations than road noise ( $U = 79.94$ ,  $Z = 4.8$ ,  $P < 0.001$ ) were recorded nocturnally at the far site.

### Potential of noise masking

All of the recorded anuran species emitted vocalisations within the frequency ranges occupied by the noises produced by vehicle traffic (Fig. 2). Vocalisations of *B. albopunctata* had the lowest minimum and peak frequencies among the registered species. Table 3 presents the spectral characterisations of the detected anuran calls.

**Table 3.** Spectral characteristics of anuran vocalisations recorded at the site near and the site far from road MG-10, at Alto do Palácio, municipality of Morro do Pilar, Minas Gerais, Brazil. Values are presented in mean  $\pm$  SD. N = 30 sampled vocalisation for each species.

Species	Duration (s)	Peak freq. (Hz)	Max. Freq. (Hz)	Min. Freq.(Hz)	Bandwidth (Hz)
<i>B. albopunctata</i>	0.55 $\pm$ 0.10	1929.3 $\pm$ 152.7	3175.6 $\pm$ 414.8	849.6 $\pm$ 290.5	2326.0 $\pm$ 675.1
<i>B. cipoensis</i>	0.06 $\pm$ 0.01	4002.2 $\pm$ 167.3	4509.7 $\pm$ 153.7	3414.1 $\pm$ 152.2	1095.5 $\pm$ 142.7
<i>D. minutus</i>	0.04 $\pm$ 0.01	4203.3 $\pm$ 1130.8	5525.4 $\pm$ 125.8	2168.1 $\pm$ 121.3	3357.2 $\pm$ 177
<i>S. curicica</i>	0.78 $\pm$ 0.08	3327.5 $\pm$ 384.8	3989.8 $\pm$ 164.5	1163.7 $\pm$ 100.5	2826 $\pm$ 188
<i>S. squalirostris</i>	0.34 $\pm$ 0.03	4062.6 $\pm$ 121.9	10539.2 $\pm$ 1236.3	2262.9 $\pm$ 349.6	8276.2 $\pm$ 1291.6
<i>I. juipoca</i>	0.53 $\pm$ 0.14	3353.4 $\pm$ 158.2	4106.7 $\pm$ 170.2	2498.7 $\pm$ 206.4	1608 $\pm$ 255
<i>L. jolyi</i>	0.03 $\pm$ 0.01	2156.2 $\pm$ 99.8	2692.5 $\pm$ 78.4	1263.4 $\pm$ 79.8	1429.1 $\pm$ 97.9

## DISCUSSION

We provide the first assessment of the anuran vocal activity using passive acoustic monitoring at the study area, which is recognised for high species richness and endemism and a priority for conservation. It is also the type locality for the treefrog *S. curicica* (Pugliese et al., 2004). Our results showed that road noise was loudest and most frequent at the location close to the road. Because it has the ability to compromise the amount of information that can be extracted from a signal (Bee & Swanson, 2007; Duarte et al., 2019), recorded road noise has the potential to mask anuran vocalisations. Although road traffic noise was greater during the day and anuran vocalisations were concentrated at night, we found more noise than anuran vocalisations in the nocturnal period at the site near the road, while the opposite was observed at the site far from the road. This finding is evidence that noise is competing for acoustic space with anuran vocalisations at the site near the road.

Noise can affect species richness and abundance and change the composition of animal communities, including those of anurans (Francis & Barber, 2013; Duarte et al., 2015, Alvarez-Berríos et al., 2016; Potvin, 2016; Grace & Noss, 2018). As noise can compete with animal vocalisations for acoustic niches, it is expected that species that are more sensitive to noise (such as those that vocalise at lower frequencies, for example) will avoid noisy areas (Duarte et al., 2015). We did not find any differences in species richness and composition between the two sampled sites. Anuran species generally have restricted ranges and poor dispersal capacity in comparison to birds, which limits their ability to move from areas with high noise disturbance (Alvarez-Berríos et al., 2016). Moreover, some anuran species can tolerate a high degree of habitat modification (Herrera-Montes & Aide, 2011). The factors described above can explain the similar species richness and composition between the two sites. However, such similarity also suggests that anuran responses to road noise can be related to other factors, such as a difference in abundance or changes in calling rate and spectral and/or temporal characteristics of vocalisations.

Anuran species respond to anthropogenic noise using distinct strategies (Sun & Narins, 2005; Halfwerk et al., 2016; Caorsi et al., 2017; Lima et al., 2022). For example, they can change both temporal and spectral parameters of their calls, such as calling rate, amplitude, duration, and frequency (Schwartz & Bee, 2013). Overall, we detected more call activity at the location far from the road than at the location close to the road, mainly from the species *B. albopunctata*, *B. cipoensis* and *S. curicica*, the last two endemic to Espinhaço Mountain Range. This result can be interpreted as a response of frogs to road noise, which is penetrating and occupies the same frequency bands as frog vocalisations. Thus, with less acoustic space available at the site close to the road, the species could be vocalising less, compared to the community inhabiting the site far from the road (Vargas-Salinas et al., 2014). Studies have shown that species tend to expend less energy calling where there is interference from traffic noise (Sun & Narins, 2005; Cunnington & Fahrig, 2010; Love & Bee, 2010), which may be the case for *B. albopunctata*, a species very susceptible to masking due to its lower call frequency, and *B. cipoensis* and *S. curicica*. Vocalising is one of the most energetically expensive activities for frogs (Taigen & Wells, 1985; Bradbury & Verenhcamp, 2011), and so males should call more when there is an effective distance of transmission of calls (Wells & Schwartz, 2007). Lengagne (2008) also found that noise triggered a decrease in male calling activity. Grace & Noss (2018) found that traffic noise caused a significant reduction in anuran vocalisations, but the effect was absent when the traffic noise was digitally altered to remove frequencies that overlap with anuran vocalisations. On the other hand, the higher calling activity found at the site far from the road can simply be a result of a greater abundance of individuals at this site, which is difficult to estimate using passive acoustic monitoring (Duarte et al., 2015; 2019).

The species *D. minutus*, *S. squalirostris*, and *L. jolyi* emitted more vocalisations at the site near the road. Narins (2013) reports that responses to noise may vary between species. While some anurans decrease their calling rate with the presence of noise, others can increase their calling rate in response to an identical stimulus. Increasing calling rate in a noisy environment can be

interpreted as an attempt to increase the efficiency of the communication and guarantee that the signal will be received by the receptor. However, the costs associated with this behavioural change can be high since calling activity is energetically demanding (Taigen & Wells, 1985; Lukanov et al., 2014; Yeo & Sheridan, 2019). On the other hand, higher rates of vocalisation at the site near the road may be a result of a greater abundance of individuals, although this seems improbable since many studies have found lower abundances of anurans near roads (Colino-Rabanal & Lizana, 2012; Eigenbrod et al., 2009; Fahrig & Rytwinski, 2009; Witte et al., 2001; Tennesen et al., 2014). In addition, species may be able to tolerate high levels of interference (Herrera-Montes & Aide, 2011) and have their occurrence associated with locations close to sources of anthropic noise (Herrera-Montes & Aide, 2011; Alvarez-Berríos et al., 2016; Deichmann et al., 2017).

Considering that the human transportation network continues to rapidly expand throughout the world (Laurance et al., 2014), our results bring to light a problem that needs an urgent protocol for control (Kunc & Schmidt, 2019). It becomes even more urgent considering the global decline of amphibians (Ford et al., 2020). The highway network in Brazil is among the largest in the world with a total length of 1,720.9 km, of which 12.4 % is paved and 78.5 % is unpaved roads (National Confederation of Transport, 2021). Even with an understanding of the importance of acoustic communication for frogs and the effects of road noise on many species, it is not always politically, economically, and logistically feasible for governments to eliminate or reduce noise. There is no doubt that many policy approaches set noise standards in order to limit noise levels. However, it is important to establish norms that balance economic and environmental factors, since in the development of standards the priority is aimed at human benefit (Blickley & Patricelli, 2010; Kunc & Schmidt, 2021). In Brazil, there are no laws or regulations that require noise control and monitoring in areas uninhabited by humans. Thus, there is no noise control in nature reserves such as national and state parks bordered by roads, even though in some parts of the world this is already happening (e.g. Dumyahn & Pijanowski, 2011; Pijanowski et al., 2011). The results of our study showed that road noise is present in areas of high biodiversity and can force frog species to decrease or increase vocalisation activity. Road noise has the potential to mask and disturb frog calls as it overlaps the frequencies of their vocalisations. Our findings show the importance of establishing norms for noise control and monitoring in natural areas.

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