

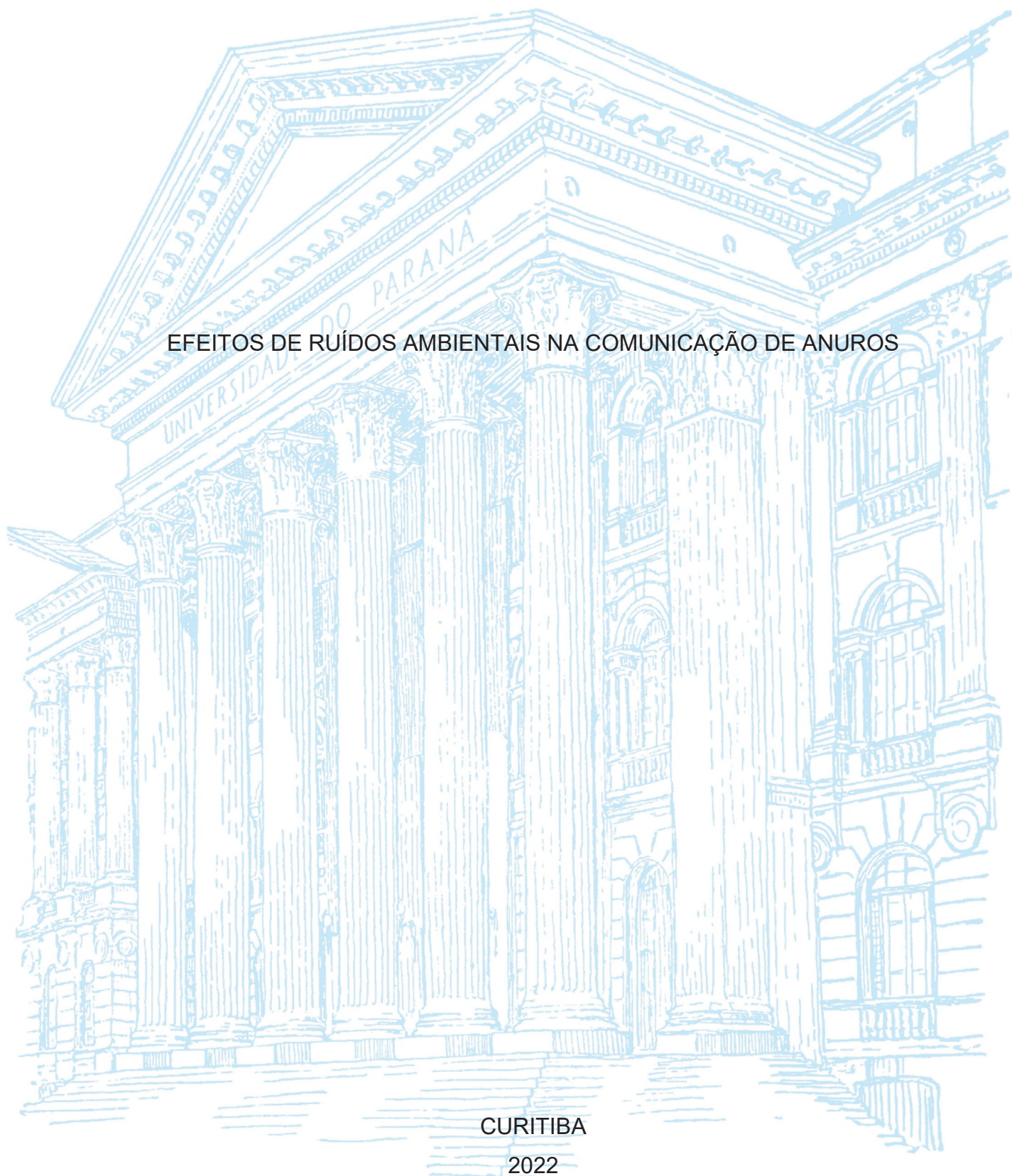
UNIVERSIDADE FEDERAL DO PARANÁ

MICHELLE MICARELLI STRUETT

EFEITOS DE RUÍDOS AMBIENTAIS NA COMUNICAÇÃO DE ANUROS

CURITIBA

2022



MICHELLE MICARELLI STRUETT

EFEITOS DE RUÍDOS AMBIENTAIS NA COMUNICAÇÃO DE ANUROS

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Orientador: Prof. Dr. Maurício O. Moura

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A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

A comunicação é efetiva quando um emissor transmite um sinal e o receptor processa e responde este sinal. No entanto, os ruídos ambientais (natural e antropogênico) podem afetar a transmissão dos sinais durante as interações, principalmente em animais que se comunicam por som, como os anuros. Além de prejudicar a comunicação, os ruídos podem causar alterações fenotípicas, fisiológicas e comportamentais nos animais. Assim, o objetivo da tese foi investigar os efeitos de ruídos ambientais na comunicação dos anuros. Este trabalho está dividido em três capítulos, sendo que no primeiro forneço uma revisão global dos efeitos dos ruídos naturais e antropogênicos no comportamento dos anuros utilizando uma análise cienciométrica. Já o segundo e o terceiro capítulo envolveram experimentos em campo para investigar como o ruído de uma estrada afeta a comunicação acústica e visual das rãs de corredeira (*Hylodes heyeri*) em dois níveis/perspectivas: individual e interações agonísticas macho-macho. Os resultados indicaram que o comportamento acústico é a variável mais investigada em estudos de ruído ambiental. Nos experimentos com *H. heyeri* não houve alterações na emissão de sinais visuais na presença do ruído. No entanto, a nível individual *H. heyeri* vocalizou mais alto, com maior duração, maior frequência central e largura de banda no ruído de maior intensidade. Enquanto que na interação agonística, os machos aumentaram a frequência dominante e as taxas de vocalização, porém diminuíram o intervalo entre cantos. Sendo assim, uma vez que toda a estrutura espacial e dinâmica populacional é dependente da comunicação efetiva e algumas populações vivem próximas às estradas, compreender as respostas comportamentais dos indivíduos quando expostos a diferentes ruídos e contextos sociais tem implicações para a conservação de *H. heyeri* que é uma espécie endêmica da Mata Atlântica.

Palavras-chave: Anfíbios. Interações sociais. Comportamento. Ruídos de fundo. Sinalização.

ABSTRACT

Effective communication occurs when a sender transmits a signal and the receiver processes and responds to this signal. However, environmental noises (natural and anthropogenic) can affect the transmission of signals during interactions, especially in animals that communicate by sound, such as frogs. In addition to impairing communication, noise can cause phenotypic, physiological, and behavioral changes in animals. Thus, the aim of the thesis was to investigate the effects of environmental noise on frog communication. This work is divided into three chapters, the first of which provides a global review of the effects of natural and anthropogenic noise on frog behavior using scientometric analysis. The second and third chapters involved field experiments to investigate how road noise affects the acoustic and visual communication of Torrent Frogs (*Hylodes heyeri*) at two levels/perspectives: individual and male-male agonistic interactions. The results indicated that acoustic behavior is the most investigated variable in studies of environmental noise. In the experiments with *H. heyeri* there were no changes in the emission of visual signals in the presence of noise. However, at the individual level, *H. heyeri* vocalized louder, with longer duration, higher central frequency, and bandwidth in higher intensity noise. While in the agonistic interaction, males increased the dominant frequency and vocalization rates but decreased the interval between calls. Therefore, since the entire spatial structure and population dynamics are dependent on effective communication and some populations live close to roads, understanding the behavioral responses of individuals when exposed to different noises and social interactions have implications for the conservation of *H. heyeri*, which is a species endemic to the Atlantic Forest.

Keywords: Amphibians. Social interactions. Behavior. Background noises. Signaling.

SUMÁRIO

1 INTRODUÇÃO GERAL	16
2 REFERÊNCIAS	21
3 CAPÍTULO 1	24
4 INTRODUCTION	25
5 METHODS	27
5.1 BIBLIOGRAPHIC SEARCH.....	27
6 RESULTS	28
6.1 HISTORICAL TRENDS	28
6.2 GEOGRAPHIC DISTRIBUTION, MAIN FAMILIES AND THREAT STATUS OF SPECIES AFFECTED BY BACKGROUND NOISE	29
6.3 AIM STUDIES, METHODS AND SOURCE NOISE.....	30
7 DISCUSSION	32
8 REFERENCES	34
9 CAPÍTULO 2	43
9.1 INTRODUCTION	45
9.2 METHODS.....	46
9.2.1 Focal species and study area.....	46
9.2.2 Stimulus design and experimental procedure.....	47
9.2.3 Acoustic and data analysis	48
9.3 RESULTS	49
9.4 DISCUSSION	52
9.5 REFERENCES	55
10 SUPPLEMENTARY MATERIAL	59
11 CAPÍTULO 3	66
12 INTRODUCTION	67
13 METHODS	69
13.1 STUDY SPECIES AND SITE	69
13.2 ROBOT FROG AND PLAYBACK TRIALS	71
13.3 EXPERIMENTAL DESIGN	74
13.4 DATA ANALYSIS AND STATISTICS	75

14 RESULTS.....	76
15 DISCUSSION	78
16 ACKNOWLEDGMENT	79
17 REFERENCES.....	80
18 CONSIDERAÇÕES FINAIS	84
19 REFERÊNCIAS.....	85
20 APÊNDICE.....	95

1 INTRODUÇÃO GERAL

Nas últimas décadas, a poluição sonora aumentou como resultado do crescimento da taxa populacional e da globalização (Shannon et al., 2016). Os países em desenvolvimento como Turquia, Índia e China são considerados os países com maior poluição sonora (Mehdi et al., 2011), além de superpopulosos. O ruído ambiental tem sido considerado uma ameaça à fauna, principalmente por ser uma fonte “invisível” de estresse (Ware et al. 2015), ou seja, apenas sentimos seu impacto. Esses ruídos podem ser de origem natural como, por exemplo, riachos, chuva, sons de heteroespecíficos, conspecíficos ou pode ser antropogênico, como ruídos de rodovias, estradas, ferrovia e avião (Larom et al., 1997; Rundus e Hart, 2002; Both e Grant, 2012; Brumm, 2013). Enquanto que os sons de origem antrópica possuem mais impacto para os animais silvestres e urbanos porque podem afetar diversos fatores biológicos direta ou indiretamente, por exemplo, ratos com exposição crônica ao ruído tiveram diminuição na expressão gênica no hipocampo prejudicando a memória e aprendizagem (Cui et al., 2009). Além disso, o barulho urbano também foi capaz de diminuir a riqueza de aves nidificantes, por outro lado aumentou a quantidade de ninhos porque o ruído reduziu a presença de um predador de ovos naquela comunidade (Francis et al., 2009). Desta forma, os ruídos podem impactar desde o nível de genes, fisiológico, comportamental, populacional e até nas comunidades (Kight e Swaddle, 2010).

A comunicação ocorre quando há transmissão de mensagens entre dois indivíduos, onde um emissor emite um sinal e um receptor processa, decodifica e responde a este sinal (Littlejohn, 1977; Scott-Phillips et al., 2012). Esse sistema de comunicação varia entre os táxons e pode ser composto por um ou diversos sinais, sendo eles o sinal acústico, visual, químico, tátil e/ou sísmico (Vitt e Caldwell, 2009). O sinal acústico é o mais comumente estudado em diversos grupos, principalmente em aves e anuros, porque pode fornecer informações relativas à identidade individual e espaço-temporal do organismo através do som (Marler e Slabbekoorn, 2004; Wells e Schwartz, 2007; Zhao et al., 2016). Desta forma, está associado com interações sociais, escolha de parceiros, localização de hospedeiros por parasitas, de defesas e manutenção de território determinando um papel importante em aspectos evolutivos e de história natural (Narins, 2001; Narins, Hödl e Grabul, 2003).

As vocalizações em anuros podem ser reconhecidas em 13 tipos e classificadas em três grupos de acordo com o contexto social: cantos reprodutivos, agressivos e defensivos (Toledo et al., 2014). Também, podem variar conforme o sexo, estágio de vida e da faixa espectral de emissão sonora (audível, infrassom e ultrassom) (Toledo et al., 2014). Foram identificados seis cantos reprodutivos, sendo o mais comum o canto de anúncio que é realizado pelos machos ou fêmeas para atração de parceiros. A vocalização do tipo defensiva é dividida em três cantos para sinalização de alerta no ambiente, como a presença de um predador no local. Por fim, são quatro tipos de cantos agressivos, estes são realizados quando há defesa de território ou durante um combate físico, assim ocorrem a curta distância entre dois ou mais indivíduos (Toledo et al. 2014). Geralmente os cantos agressivos também são emitidos durante a reprodução para afastar possíveis intrusos. Desta forma, há um repertório de vocalização que varia entre as espécies e que dependerá do contexto social e de outros fatores como disponibilidade de recursos, presença de predadores e condições climáticas.

Uma vez que há um crescimento da urbanização, e como resultado do tráfego urbano e rodoviário, em torno de áreas naturais em vários lugares do mundo, é fundamental entender se e como um animal supera os níveis de ruído com que se depara. Nos anuros, o comportamento vocal é o principal canal sensorial através do qual todas as interações sociais entre os indivíduos são construídas (Brumm e Slabbekoorn 2005; Grenat et al., 2019). Assim, a comunicação acústica pode ser afetada negativamente por ruídos de fundo que influenciam na discriminação e detecção do sinal pelo receptor (Wiley e Richards, 1982; Luther e Gentry, 2013).

A exposição dos anuros à poluição sonora antropogênica é comum, sendo que muitas espécies se reproduzem em buracos ou valetas que existem em estradas ou próximos, como as lagoas temporárias ou permanentes (Tennesen et al., 2014). Algumas espécies de anuros, assim como aves e insetos, possuem a capacidade de evitar, a curto prazo, a interferência acústica por três formas: alterar a emissão do sinal, evitar a fonte do barulho e/ou modificar a estrutura da vocalização (Littlejohn, 1959; Lengagne, 2007). No entanto, a modificação na estrutura e emissão do sinal acústico é diversa entre as espécies, podendo alterar de diferentes formas os parâmetros temporais (taxa e duração da vocalização) ou espectrais como a frequência (Caorsi et al., 2022).

No entanto, essa interferência na sinalização acústica pode beneficiar algumas espécies (Stansbury et al., 2016). Por exemplo, os ruídos do motor de barco aumentaram a vulnerabilidade para a predação do caranguejo-eremita, porque as presas se distraíam com o ruído de fundo e assim diminuam o estado de alerta (Chan et al., 2010). Desta forma, o benefício ou prejuízo dependerá do objeto de estudo analisado e de como o ruído antropogênico afeta essa relação social.

Há poucos estudos que investigam os efeitos de ruídos ambientais em anuros que habitam ambientes lóticos. Por exemplo, os machos de *Odorrana tormota* (Wu, 1977) aumentaram sua frequência e amplitude de vocalização de anúncio em respostas a diferentes intensidades do ruído de riacho. Também foi avaliado o potencial auditivo desta espécie diante do ruído e concluíram que não há mascaramento dos neurônios auditivos (*torus semicircularis* na região do mesencéfalo) diante do barulho do riacho, sendo assim, considerados adaptados a este tipo de ambiente (Liu et al., 2015, Shen e Xu, 2016). Também, houve alterações da amplitude e frequência das vocalizações de *Crossodactylus schmidtii* Gallardo, 1961 associado ao ruído do riacho (Caldart et al. 2016). Essas estratégias comportamentais são fundamentais para garantir a comunicação e a sobrevivência dos indivíduos no ambiente ruidoso.

Nos anuros a comunicação é composta, principalmente, por sinais visuais e acústicos. Indivíduos que utilizam comunicação multimodal alternam a emissão de dois ou mais sinais, ou seja, é o uso de mais de uma modalidade sensorial para a transmissão de informações entre os indivíduos, mediando interações sociais como a reprodução e a competição (Hebets e Papaj, 2005; Stange et al., 2016). Essa troca de emissão de sinais pode aumentar a eficiência na comunicação em um ambiente ruidoso, aumentando a probabilidade de detecção e facilitando a discriminação (Troianowski et al., 2014). Como a reprodução e as interações sociais dependem da transmissão efetiva dos sinais, os anuros são um bom modelo para avaliar como o ambiente e os comportamentos estão associados à comunicação.

Uma metodologia que tem sido amplamente utilizada em estudos de comportamento animal é o uso de *playback*. Este envolve a reprodução de sinais previamente gravados (naturais) ou sintetizados para simular uma interação de comunicação para que possa medir a resposta de um animal para os sinais apresentados (McGregor, 1992; Reichert, 2014), além de permitir condições controladas no experimento em campo ou em laboratório. Também, o uso de

estímulos fixos pode ser mais interpretável, pois os efeitos no indivíduo focal podem ser atribuídos ao sinal de *playback* (Reichert, 2014). O mais comum é o *playback* acústico que é reproduzido por uma caixa de som, mas há também como reproduzir os sinais visuais do animal por vídeo (Rosenthal et al., 2004). Recentemente, alguns estudos têm simulado através de um modelo eletromecânico ou modelo robótico um conjunto de características que imitam a morfologia bem como sua comunicação multimodal, através dos sinais sonoros e visuais (Narins et al., 2003; Narins et al., 2005; de Luna et al., 2010, Taylor et al., 2011; Starnberger et al., 2014; Caldart et al., 2020). Estes sinais podem ser emitidos isolados ou combinados permitindo testar a função dos sinais (Caldart et al., 2020), estratégias comportamentais durante uma disputa entre machos (Narins et al., 2003) ou escolha do parceiro reprodutivo pela fêmea (Taylor et al., 2011).

Hylodidae Günther, 1858 é constituída por 48 espécies diurnas que são reofílicas, ou seja, habitam riachos ou corredeiras no interior da Mata Atlântica (Haddad et al. 1996). Esta família possui quatro gêneros com diferentes comunicações complexas: *Crossodactylus* Duméril e Bibron, 1841 (13 espécies), *Hylodes* Fitzinger, 1826 (26 espécies), *Megaelosia* Miranda-Ribeiro, 1923 (1 espécie) e *Phantasmarana* Vittorazzi, Augusto-Alves, Neves-da-Silva, Carvalho-e-Silva, Recco-Pimentel, Toledo, Lourenço, Bruschi, 2021 (8 espécies) (Frost, 2022). Os anuros que habitam os riachos são menores em tamanho do que os anuros que se reproduzem longe dos riachos (restrições morfológicas) e vocalizam com maior frequência dominante (Hz) porque vivem em um ambiente ruidoso (Duellman e Trueb 1986, Vargas-Salinas e Amézquita 2014). *Hylodes* Fitzinger 1826 é o gênero mais numeroso representado por espécies com machos territoriais e com distribuição desde o norte do Espírito Santo até o estado do Rio Grande do Sul (Frost, 2022). Além da comunicação acústica, os machos de *Hylodes heyeri* Haddad, Pombal e Bastos, 1996 utilizam sinalização visual e tátil para defender territórios e atrair as fêmeas. Os sinais visuais consistem em oito tipos: abaixar o corpo (*body lowering*), abrir a boca (*mouth opening*), estiramento da perna (*leg stretching*), elevação do membro (*limp lifting*), exibição da garganta (*throat display*), postura ereta (*upright posture*), saltos (*jump display*) e sinalização com os dedos dos pés (*toes flagging*) (Beltramin, 2014). Os sinais acústicos para esta espécie são os cantos territoriais, de anúncio e de cortejo (Lingnau e Bastos, 2007). Então, *Hylodes*

heyeri possui um repertório de sinalização visual, tátil e acústica sendo assim, uma espécie com comportamento multimodal.

O objetivo geral desta tese é avaliar o efeito de ruídos ambientais na comunicação dos anuros. Adicionalmente, considerando que os ruídos estão presentes em ambientes urbanos e rurais, que existe uma tendência no aumento da poluição acústica pelo aumento do uso de estradas, agricultura mais mecanizada e expansão das cidades em direção as áreas naturais ao redor, espera-se que indivíduos com comunicação multimodal mantenham uma comunicação eficaz mesmo na presença de ruído. Assim, as espécies podem aumentar a detecção e discriminação de informações combinando um ou mais tipos de sinais. Desta forma, utilizamos como modelo de estudo experimental uma espécie multimodal para testar nossa hipótese de que *H. heyeri* altera o comportamento acústico e visual em resposta ao ruído antropogênico em dois níveis/perspectivas biológicas: individual e interações agonísticas macho-macho. Além disso, forneço uma revisão dos efeitos dos ruídos naturais e antropogênicos no comportamento dos anuros do mundo. Assim, uma vez que toda a estrutura espacial e dinâmica populacional é dependente de comunicação efetiva e algumas populações vivem próximas às estradas, compreender as respostas comportamentais dos indivíduos quando expostos a diferentes ruídos tem implicações para a conservação de *H. heyeri*.

Por fim, minha tese é composta por três capítulos que já estão em formatação de artigo conforme a revista que será submetida para publicação:

1. *Review of natural and anthropogenic noise effects on anurans*
2. *When distance matters: Brazilian torrent frogs change their calls according to the distance of road traffic noise*
3. *Facing the enemy: are the agonistic behaviors affected by road noise in Brazilian torrent frog?*

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3 CAPÍTULO 1

REVIEW OF NATURAL AND ANTHROPOGENIC NOISE EFFECTS ON ANURANS

In progress to submission

Review of natural and anthropogenic noise effects on anurans

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Abstract.

In the last decades, urbanization is growing and noise pollution tends to increase. Therefore, animals also face natural noise in their environment. Thus, several studies have reported the background noise effects on anurans at different levels (individual, population and community) which can be influenced and shaped by behaviour. Here, we used scientometric review to provide an overview of natural and anthropogenic noises studies in the frog's behaviours at a global scale. Also, we provide aspects about the frequency of the studies across main families and threat status of species, geographical distribution, historical trends in this area, the most used method to test each noise source and suggestions for future studies. The scientometric analysis was performed in Google Scholar and Web of Science data bases, we not considered studies that did not test the effects of noise, thesis/master studies or review articles, resulting in 74 papers (31 anthropogenic and 39 natural noises). The first studies were carried out in the 1980s, however, from 2005 onwards, studies with noise began to grow. Most studies that have evaluated the effects of both noises have focused on vocal behaviour, followed of mating behaviour in natural noises and multiples categories in anthropogenic noises. Also, the majority experiments were performed in field and with playback methods. We expect this work to provide a basis for studies in the field of background noise in anurans to elucidate new avenues for future research. Finally, we would recommend studies that consider evaluating multiple behaviours and also if the information carried to the receiver is affected by noise.

Keywords: Background noises, Behaviour, Communication, Frogs.

4 INTRODUCTION

The main kinds of background noise in the world are originated by anthropogenic or natural sounds. Natural sounds can have a biotic origin, such as heterospecific and conspecific species emitting sound during the breeding season, or an abiotic origin, such as low frequency

noise of streams, wind and rainfalls (Vargas-Salinas and Amézquita, 2014). On other hand, anthropogenic noises are man-made noise and include roadway traffic, airplane overflights till boat noise (Sordello et al., 2019). As the frequency and intensity of anthropogenic noise have continuously increased in the last decades due to the expansion of human occupation (Frisk, 2012; Gill et al., 2014), also raises concerns about the potential effects of man-made noise on humans health and quality of life and other animals (Castaneda et al., 2020). Usually, anthropogenic noise varies from a high intermittent (train buzzing) impact noise to almost continuous background noise (stream). Then, when the noise has more intensity than the sound animals use to social interactions, it can affect the transmission or comprehension of the social content that these sounds represent among individuals (Simmons and Narins, 2018). This way, the implications of background noise for wildlife includes changes for many aspects of animals' life.

Many studies around the world have investigated different effects of anthropogenic noise in several anuran species (e.g. Brumm, 2013; Shannon et al., 2016; Simmons and Narins, 2018). Animals have behavioural responses and adaptations to have an efficient communication (Schwartz and Bee, 2013; Simmons and Narins, 2018), thus, it is crucial to know if and how different species of frogs respond to anthropogenic noise, as wells as if they have the same capacity and uses the same behavioural responses to abiotic and biotic noise. Because many species have a long-term exposition to noise in natural conditions, it is probable they may be able to responds more quickly to anthropogenic noise. Thus, to fully understand how noise affects communication in anurans it is essential to include studies on the influence of natural background noise on behavioural responses of anurans, such as wind, rain, or heterospecific species.

Most of animals use acoustic signals to communicate in several social interactions such as to finding a mate, avoiding predators, locating food and defend their territories (Gerhardt and Huber, 2002). Because of that, it is important to communicate efficiently during these behavioural interactions, avoiding information loss or failure in the interpretation of the signals by the receivers. There are a number of ways of how sender and receiver can communicate under different background noises to reduce this acoustic disturbance. Senders can alter the amplitude or frequency to avoid spectral overlap (Halfwerk et al. 2016). Indeed, some animals are known to change their call duration to avoid temporal overlap with the noise (Grafe, 1996; Hanna et al. 2014). Another strategy includes choose the caller position which maximizes the propagation and effective area of the calls (Parris, 2002). Moreover, individuals which communicate with multimodal signals (two or more types of

communication employed together) can shift to other sensory channels to cope with the noise (Troianowski et al., 2014). For example, *Staurois parvus* use visual displays linked to acoustic signals as solution to communicate in fast flowing stream (Grafe et al., 2012). Thus, behaviour and short-term acoustic adaptations are some of the strategies to compensate for background noise.

In the last century, there was an increase in industrial and residential development, as well as in the size of human population growth more than triple (Schwartz and Bee, 2013). This contributes to an increase in environmental noise closer to wildlife. Beyond that, the species need to compensate for other natural noises already present in each environment. Because that, different from others reviews about this theme, we include the natural noises in our study. Here, we used a scientometric review framework to provide an overview of the anthropogenic and natural noises research on anurans behaviour in the world. Also, we provide aspects about the frequency of the studies with impact of background noise on anurans across main families and threat status of species, geographical distribution, historical trends in this area, the most used method to test each noise source and suggestions for future studies.

5 METHODS

5.1 BIBLIOGRAPHIC SEARCH

We performed a bibliographic search of articles with the research question is about the effect of natural or anthropogenic noises on anuran behaviour, excluding those who did not test for the noise effects (descriptive papers), thesis/master studies or review papers. We carried out our search on *Web of Science* and *Google Scholar* databases, using the key-words: “*anura*”, “*frogs*” and “*noise*” combined with “*background*”, “*natural*”, “*anthropogenic*”, “*environment*”, “*acoustic interference*”, “*pollution*”, “*ambient*”, “*anthropogenic pollution*”. Also, we searched the references each paper mention. For this survey, we considered studies until December 2021 which resulted in 74 studies, 31 of anthropogenic noises, 39 of natural noises and 4 investigated both background noises.

From each selected study we gather information and built a database with: (1) numbers of publications in each year, (2) species and family name, (3) countries of research, (4) the threatened status of species from the IUCN Red List of Threatened Species, (5) study type (modelling, field, in laboratory or in both), (6) method used (modelling, natural experiment, playback experiment or both approaches), (7) noise sources (natural, anthropogenic and both) and (8) aim studies (investigated behaviour category). The last one,

we followed the categorical classification of biological response by Shannon et al. (2016) to classify the behaviours investigated and how species responds in each study. A total of eight categories of aim studies: physiological, mating behaviour, population, vocal behaviour, visual behaviour, movement behaviour, foraging behaviour, ecosystem or multiple (more than one category).

We generate a map of sampled localities of each study to show which country or continent has most studied the effects of noise. Also, with this information, it is possible to establish correlations with the population size or development stage of the country. For this, we extracted the country of study and for the theoretical studies, we chose to indicate the country of the first author or location of taxon. Finally, we mapped numbers of studies by region using *ggplot2* package and function in the R environment (R Core Team, 2022).

6 RESULTS

6.1 HISTORICAL TRENDS

Based on our scientometric review, 74 articles studied the influence of background noise in anurans in the last 39 years (1982 to 2021) (Figure 1). The first published study meeting our criteria was in the earliest 1980 (Narins, 1982), describing how natural background noise affect anuran communication. Narins (1982) simulated a synthetic note that elicits a response when an intruder male call and also, masked this note with natural noise to evoke and evaluate the ability of males of *Eleutherodactylus coqui* Thomas, 1966 to respond to an intruder in the presence of noise. Also, Tuttle and Ryan (1982) tested whether the presence of waterfall noise would affect the predation success of the bat *Trachops cirrhosus* (Spix, 1823) on the frog *Smilisca sila* Duellman and Trueb, 1966. The results showed that bats respond more for complex and asynchronous frog's vocalizations, they also preyed *S. sila* located further away from the waterfall. After 1980s, there is a decrease in published papers, which rises again from 2002, with the peak of publications between 2014 and 2017 (Figure 1).

Studies that tested the influence of background noise caused by human, also started in earlier 1980. The first study (Brattstrom and Bondello, 1983), showed that *Scaphiopus couchii* Baird, 1854 leaves their burrows in presence of motorcycle sounds (up to 95 dB) during aestivation. As with natural noise, there is a gap of studies of anthropogenic noise impacting frogs until 2005 with an increase in 2014 and the peak of studies in 2017 and 2019 (Figure 1).

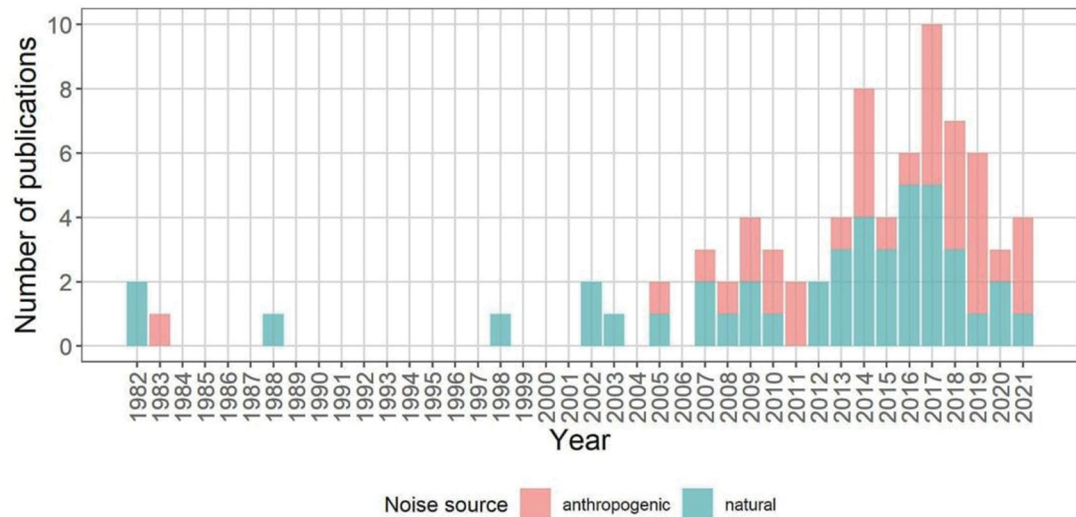


Figure 1. Number of publications of anthropogenic (pink) and natural (blue) noises effects on anurans behaviour published between 1980 and 2021.

6.2 GEOGRAPHIC DISTRIBUTION, MAIN FAMILIES AND THREAT STATUS OF SPECIES AFFECTED BY BACKGROUND NOISE

In total, the majority of studies were conducted in North America ($n = 22$, 30%), followed by Asia ($n = 18$, 25%) and South America ($n = 16$, 22%). Also, the countries with more published papers on noise effects on Anurans were United States of America ($n = 14$, 19%), Japan ($n = 5$, 7%), Brazil ($n = 5$, 7%), and Australia ($n = 5$, 7%; Figure 2).

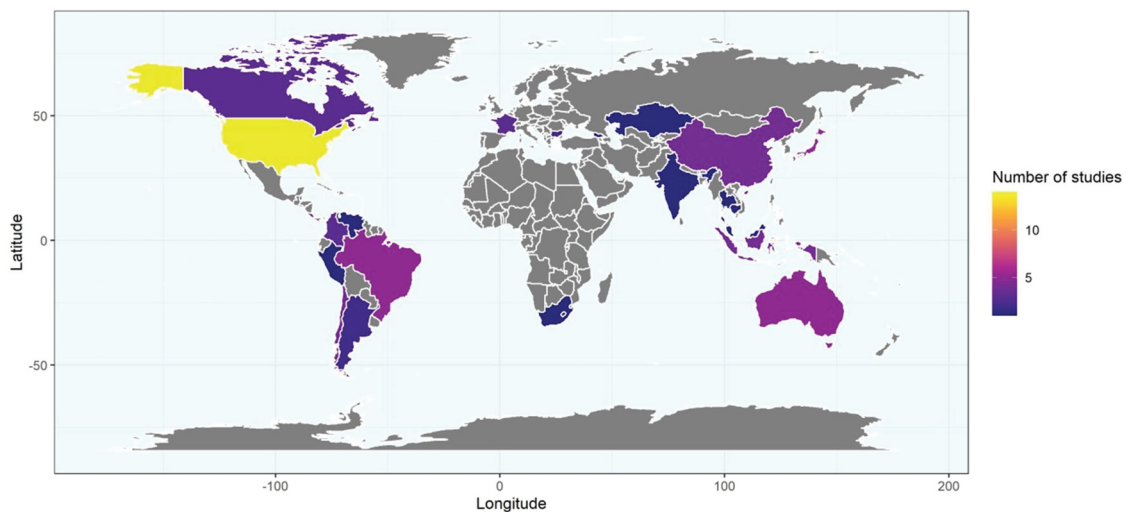


Figure 2. Geographic distribution of background noises studies on anurans behaviour. Coloured shading indicates the number of studies by nation.

The studies included in this review showed that 176 different anuran species have been tested for response to environmental or man-made noise. It represents 75 genera and 23

families around the world. The frequency of species which had been most studied belong to families: Ranidae ($N = 117$; 47%), Hylidae ($N = 42$; 17%), Rhacophoridae ($N = 17$; 7%) and Dendrobatidae ($N = 11$; 5%) (Figure 3). Overall, the vast majority of the species are classified as of Least Concern (LC, $N = 140$, 80%) by IUCN, followed by those classified as Vulnerable (VU, $N = 9$; 5%), Near Threatened ($N = 7$; 4%), Critically Endangered ($N = 7$; 4%) and Data Deficient ($N = 2$; 1%). From the 31 studies of human-caused noise, only two were performed with species that are not classified as LC (VU and EN – Endangered). However, from the 39 studies of natural noises, the most species have LC status, while 4 are classified as Vulnerable, two as Near Threatened (NT), one as Critically Endangered (CR) and one as Data Deficient.

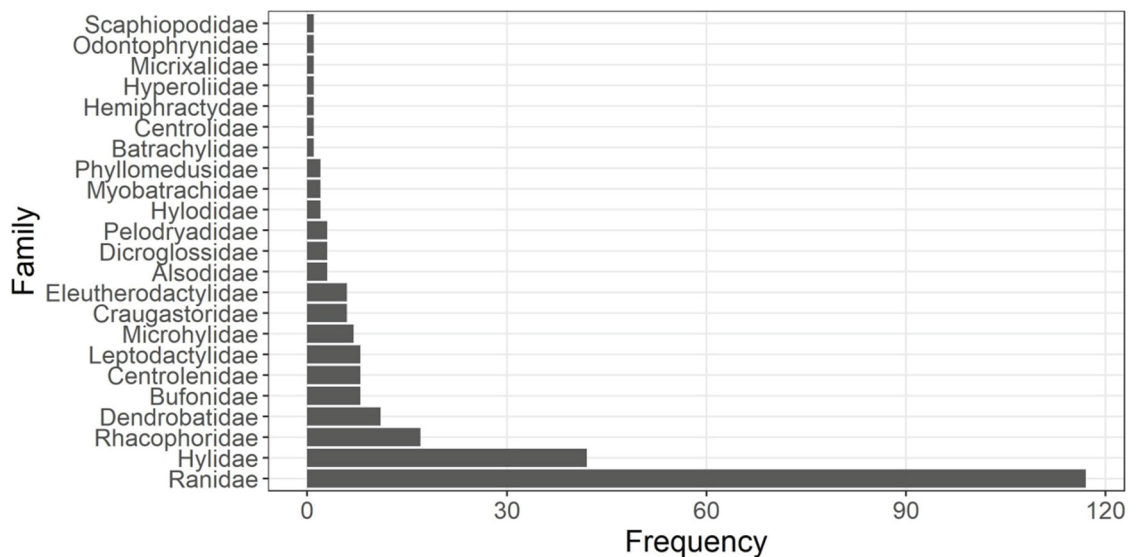


Figure 3. Frequency of studies by anurans family which were test for anthropogenic and environmental noise

6.3 AIM STUDIES, METHODS AND SOURCE NOISE

The principal behaviour categories investigated by the papers in this review (Figure 4) were the background noise effects on vocal behaviour in both noise sources. However, in natural noises studies the second category more investigated was mating behaviour ($N=10$, 26%), while in anthropogenic were multiple parameters ($N=7$, 23%). The multiple categories most of times combines the vocal behaviour with other aspects, such as visual and vocal behaviour (multimodal behaviour), physiological and vocal behaviour, mating and vocal behaviour, and movement associated with vocal behaviour.

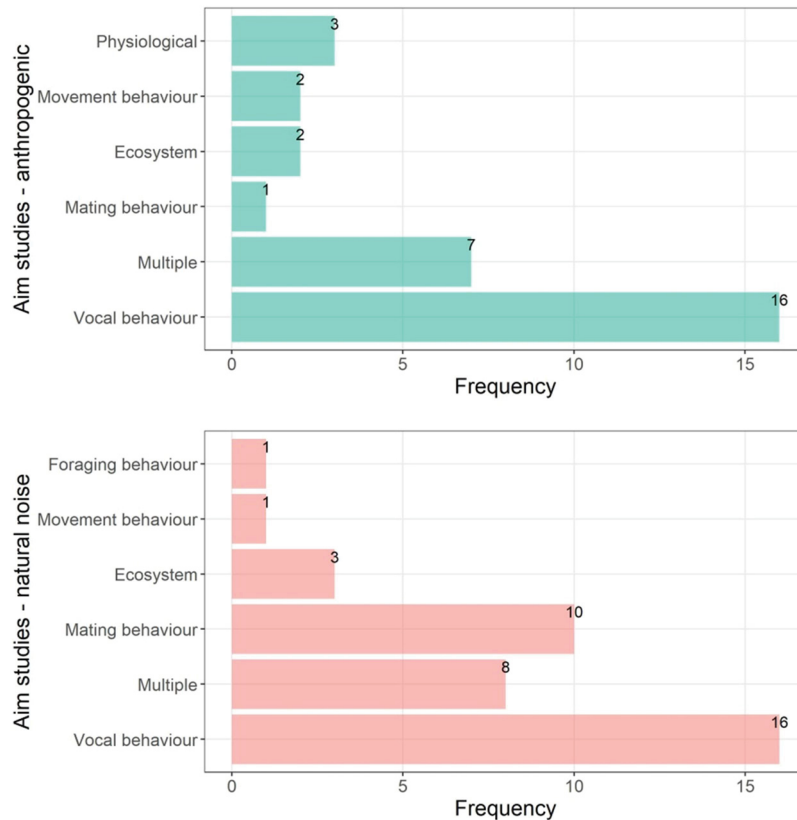


Figure 4. Number of studies that investigated different categories in the anuran behaviour in the overview of the background noises research on anurans in the world.

The majority of studies uses playback methods to test their hypotheses ($N = 42$, 58%), followed by natural experimental conditions ($N = 24$, 33%). Regarding the study type, the authors run the experiment mainly in the field ($N = 50$, 68%). However, in 28% ($N = 20$) of the studies selected run experiments in the laboratory and only 7% use mathematical modelling ($N = 5$) to test their hypotheses.

The most evaluated anthropogenic noise ($N = 31$) in the studies on anuran behaviour was traffic road ($N = 25$, 81%) and airplane noise ($N = 3$, 10%), but we also found noise generated by motorcycle ($N = 2$, 7%) and music ($N = 1$, 5%). Unlike it, in natural noise studies ($N = 39$) the most investigated noise was stream noise ($N = 15$, 40%). Also, others noise sources mentioned were conspecifics ($N = 11$, 30%) and heterospecifics chorus ($N = 3$, 9%), wind and rain ($N = 3$, 9%), synthetic noise (white noise, $N = 2$, 6%) and other unidentified noise sources ($N = 3$, 9%).

7 DISCUSSION

This review is an overview of 74 articles which tested the influence of background noises on frog behaviour. We found 39 studies that evaluated for natural noises, 31 for anthropogenic noises and 4 to both noises, a relatively small difference considering the increasing high urbanization pace in the world. The first formal test was published in 1982, with natural noise, regardless of this, research in the area of background noise has increased since 2005. Moreover, in the last 30 years, the field of bioacoustics has technological advances with the devices and the analytical tools becoming more accessible prompting an increase in behavioural and other bioacoustics studies from the mid-20th century (Mundy, 2009; Penar et al., 2020).

As anuran relies heavily on acoustic communication it is not unexpected that it became a model system to understand acoustic communication, since it uses mainly this channel to modulate social interactions (Gerhardt and Huber, 2002). As most frog species that vocalize are males, they are the main subject in all tests. Only studies with focus on mating behaviour or physiological responses target female choice or female stress level. Tennessen et al (2014) investigated the physiological stress levels (corticosterone levels) generated by anthropogenic noise on the female *Lithobates sylvaticus* (LeConte, 1825) found that traffic noise affected negatively her travel to find the male in the chorus noise thus, affecting mating behaviour.

Although, acoustic signals are important to communication over relatively long distances in several animal species (Boeckle et al. 2009; Brumm and Slabbekoorn, 2005), the environment in which the signal travels can impose constraints to an effective communication (Goutte et al., 2018). So, the optimal solution to this problem will depend on the environment and the species traits. Regarding noise environments, one of these possibilities is to combine one or more signal components to increase the detection of the stimulus by the receiver. This strategy was found mainly in torrent frogs that inhabiting streams as way to avoid interference of natural noise, such as *Staurois parvus*, *Micrixalus saxicola* and *Staurois latopalmatum* (Grafe et al. 2012, Preininger et al. 2013, Preininger et al., 2019). Another possibility is to call at higher frequencies than the noise, as species of Ranidae, Hylodidae, Hylidae and Dendrobatidae do. Usually, authors believe that higher frequency calls (most above 3.5kHz) are shaped by natural selection as a response to environmental noise such as streams (Wilkins et al., 2013, Zhao et al. 2018, Goutte et al. 2018). In addition, urban and natural habitats differ in many more traits than just background noise (Shochat et al. 2006), such as predators and prey abundance, amount of vegetation that can be a sound transmission barrier and other

pollution types. For example, *Lithobates sylvaticus* living in urban habitats already showed some adaptations, their leukocyte and gluco-corticoid production were unaffected by traffic noise, which allow it to overcome human noise impacts (Tennesen et al., 2018). Although some species show behavioural plasticity which allows coping with a noise environment, some species can take years to adapt to a new environmental noise, in contrast to animals that have evolved from noisy places.

The North America was the continent with more publication in studies of effects of background noises on anurans, leaded by USA. This country is a reference in investments for research (Okhovati, 2015). Although most studies are in North America, it does not have a high incidence of noise pollution compared to other countries, such as China and India. Road traffic is the main noise source in urban areas and has been reported to be high in developed nations such as USA and Japan (Mehdi et al., 2011). Beyond that, Turkey, India, and China are considered countries with higher noise pollution, also China and India are known for being the world's most populous country (United Nations, Department of Economic and Social Affairs, Population Division, 2022).which can explains the high incidence of noise. The increasing urbanization expands humans to non-urban areas and thus increases the destruction and fragmentation of habitat closer to protected areas, which can affect animals in several ways.

The experiments on the effect of background noises were performed mainly using playback methods. These methods facilitate the controlled nature of stimuli presented to the animal and are commonly performed in animal communications experiments (Reichert, 2014, McGregor, 1992). Beyond that, the majority of studies carried the experiments in the field, not in laboratory. The last one has the advantage of having controlled environmental conditions, in contrast to field experiments where there are numerous variables occurring at the same time but more closed to the reality in which behavioural acts occur.

The most common species where noise response has been tested belong to widespread anuran families (Figure 3). It can be related to the species richness of each family as well as biological characteristics that make them to occur in habitats close to urban sites or altered landscapes (Toledo et al., 2014). Here, we found the most studied species belong to Ranidae, Hylidae, and Rhacophoridae. In constrast, some anuran families are declining quickly than others animal groups due habitat loss, for example Rheobatrachidae, Leptodactylidae, Bufonidae and Ambystomatidae (Stuart, 2004). Although anurans has a large number of species classified as vulnerable (IUCN, 2022) most of the published papers uses focal species classified as Least Concern in the IUCN - Red List of Threatened Species. This can be

explained because they are said to be common species, that is, with a greater area of occurrence, and usually more habitat generalist than other species (Toledo et al., 2014). These two characteristics habitat generalists and abundance seem to be the two most common characteristics used by researchers to select species for noise experiments, probably because they imply a high possibility of interaction with anthropogenic impacts. Then is uncommon to carry out studies with threatened species due to their lower abundance combined with their threatened status.

Overall, the findings of this review indicate that most studies focus on the effects of background noises in vocal behaviour. Although the acoustic signal is considered the main channel of communication in anurans, it is important to associate this signal with others signals to understand the potential effects on different song parameters or behaviours. In addition, it would be important to consider the receivers of information for future studies, not just analyze the sender because communication occurs between two individuals. Beyond that, few studies have integrated environmental noise when carrying out experiments in urban areas. The discussion in these studies is different when it comes to noise sources, generally anuran species in a noisy natural environment have adaptive behaviour strategies to be there, while in the urban environment species have the ability to short-term responses to noise. Finally, these studies are crucial to understand how different noise sources can affect anuran behaviour.

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9 CAPÍTULO 2

**WHEN DISTANCE MATTERS: BRAZILIAN TORRENT FROGS CHANGE THEIR
CALLS ACCORDING TO THE DISTANCE OF ROAD TRAFFIC NOISE**

In submission - Behavioral Ecology and Sociobiology

When distance matters: Brazilian torrent frogs change their calls according to the distance of road traffic noise

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Abstract

In the last years with increasing urbanization, many species are more exposed to noise, mainly caused by road traffic noise close to natural environments. Thus, the impact of the sound is related to the distance from the noise source, but some anuran species are able to respond with short-term adaptations to communicate. However, it is unclear how noise intensities affect vocal behavior in anurans. Here, we tested the influence of two levels of road traffic noise in the advertisement call of the Brazilian torrent frog (*Hylodes heyeri*). Also, we evaluated whether acoustic parameters are affected by climate and individual traits. We predicted a stronger response in the higher noise treatment than in the lower noise treatment with call parameters directly proportional with body traits. We produced the road traffic noise playback at two distances and played those noise recordings to spontaneous vocalization of male frogs. We found that the sound pressure level of a call was related to male body size and humidity. Also, the call rate increase with temperature. In the more distant noise treatment (less noise intensity), males did not change any acoustic parameters (as compared to controls with no noise). In the near noise treatment, calls were louder, lasted longer, had a higher center frequency and greater bandwidth. Thus, our predictions were supported and the Brazilian Torrent Frog responds differently depending on noise levels. We conclude that short-term adaptations allow species to compensate for different environmental noises.

Significance statement

Anthropogenic noises are an environmental problem that affects any sound-based communication. Using playback experiments, we evaluate whether males of Brazilian Torrent frogs adjust their advertisement calls when exposed to higher and lower intensity road traffic noise. Our results demonstrated that anthropogenic noise affects torrent frog communication. When in a high noise environment *Hylodes heyeri* needs to call in higher frequencies compared to noise, reducing the overlap of frequencies and allowing a clear channel for communication. Also, we found there is a threshold in sound intensity that triggers calling intensity to raise. It implies that frequency parameters, the most used bioacoustics traits, do not convey all the information we need to understand behavioral noise responses.

Key-words Anthropogenic noise. Anura. *Hylodes*. Plasticity. Vocal behavior.

9.1 Introduction

Pollution noise has received much attention in the last years because it is one of the major problems caused by humans that provide many negative outcomes for wildlife. Overall, communicating in a noisy environment is challenging, especially to those animals relying on sound to perform behavioral and ecological interactions. For example, noise can interfere in male advertisement calls which are used to attract females and thereby affect mate choice (Senzaki et al. 2018). Also, can influence foraging behaviors in tadpoles (Castaneda et al. 2020), corticosterone levels (Tennesen et al. 2014), vocal sac coloration (Troïanowski et al. 2017), and abundance (Vargas-Salinas and Amézquita 2013).

Noise is the primary cause of interference mainly in acoustic communication in animals. Since there is a growth in urban and road traffic and urbanization around natural areas at several places in the world it is imperative to understand if and how an animal overcomes the noise levels it is faced with. In anurans, the vocal behavior is the main sensory channel through which all social interactions between individuals are built (Brumm and Slabbekoorn 2005; Grenat et al. 2019). Then, the perception and transmission of sound between individuals can have negative consequences because of the signal masking by noise (Bee and Swanson 2007; Simmons and Narins 2018). However, frogs have vocal plasticity and can adjust their signal characteristics to avoid noise interference through the short or long-term adaptations (Brumm and Slabbekoorn 2005). For example, some anuran species use the regulation of signal amplitude (Lombard effect) to increase the signal-to-noise ratio, signal duration, and/or serial redundancy (Halfwerk et al. 2015; Shen and Xu, 2016; Yi and Sheridan 2019). In other species, males can increase their call rate, change the frequency, or avoid calling during the noise (gap calling) (Kaiser and Hammers 2009; Vargas-Salinas and Amézquita 2013; Luther and Magnotti 2014). Besides that, anuran calling can also be influenced by temperature and body size (Lingnau and Bastos 2007; Moser et al. 2022), microhabitat (Goutte et al. 2013; Camurugi et al. 2015), and other natural features of their environments. So, the acoustic communicating system in frogs is complex and these factors should be considered to evaluate anuran vocal plasticity.

The sound intensity is known to changes depending on the distance from the source. This implies that propagating sound is reduced inversely with the distance between the source and receiver (Larsen and Radford, 2018), then at longer distances from the road it is expected that noise has minor masking interference than at shorter distances. Also, the effect of sound will be different in quiet and natural noise environments (e.g. conspecific chorus, stream, wind, rain). This array of possibilities of sound effects on environments elicits specific responses in anurans, such that each anuran species have different behavioral adjustments to noise (Caorsi et al 2022). Here we experimentally test whether Brazilian Torrent frogs (*Hylodes heyeri*) modify their

advertisement calls when faced with road traffic noise at two levels of intensity. Also, we test how individual traits and climate influence call parameters. The road traffic noise playback here is an additive sound to the natural background noise where this species inhabits. We expect changes mainly in temporal and intensity calls in the higher noise than lower noise because *H. heyeri* already calls at higher frequency due to the low frequency stream noise. It lowers the possibility of another increase in frequency since it is the same frequency as road traffic noise.

9.2 Methods

9.2.1 Focal species and study area

Test subjects were 11 males of the Brazilian Torrent Frog (body mass: 5.3 g, range 4.8–5.8g; snout-vent length: 38.4 mm, range 36.7–41.7mm) located in the field. *Hylodes heyeri* is a diurnal species and a member of the small family Hylodidae, comprising four genera, all found in Atlantic Forest streams (Frost 2022). *H. heyeri* occurs in waterfalls with rushing water that produces a persistent background natural noise, which selects for calling with higher dominant frequency than the rushing water to reduce the masking interference of the running water noise (Goutte et al. 2016, 2017; Zhao et al. 2021). The males of several species of *Hylodes* call above rocks to establish territories (Narvaes and Rodrigues, 2005) and perform a defensive behavior emitting territorial calls when an intruder approaches a resident male. Over the stream, individuals are separated from each other at distances between 2 to 11 m (mean distance 5.08 ± 2.45 m; Lingnau and Bastos 2007). In addition to vocal communication, members of this family also use visual communication for mating and agonistic interactions (Haddad and Giaretta 1999; Hödl and Amézquita 2001). Although *H. heyeri* can occur in sympatry with other torrent-dwelling frogs (e.g. de Carli Monteiro et al. 2014), at our study site, it is the only species of torrent frog.

We conducted experiments in a private farm near of São José dos Pinhais municipality (25°42'8.98" S; 49° 4'19.35" W), state of Paraná, southern Brazil. This site was chosen because it is located at least more than 5 km of routes and urban areas and so, we can test how *H. heyeri* without previous exposition to anthropogenic noise will respond to it. The area has an average altitude of 980 meters and it is an ecotone between dense rain forest and Araucaria Forest (Maack, 1981). The climate is mesothermal humid subtropical, without a dry season, with cool summers and the occurrence of severe and frequent frosts, corresponding to the Cfb climate of the Köppen scale (Maack, 1981). We performed the experiments during the summer, from January to April 2019. This period is considered the peak activity and reproductive period of this diurnal species (09:00– 18:00, Lingnau and Bastos 2007, personal observation).

9.2.2 Stimulus design and experimental procedure

In order to simulate the effects of road traffic noise in calling behavior, we constructed the playback through recordings of a road traffic noise nearest of Universidade Federal do Paraná (25°26'52.99" S; 49°14'7.82" W; BR-116 – Brazilian road with an extension of 496 km, extending from state of São Paulo to state of Paraná). This road has 7 meters wide and has six lanes separated by a lane-specific for bus, where cars, bus, motorcycles and trucks travel. We recorded the road traffic noise, at January 24th of 2019, during 5 minutes at 1 hour intervals from 7:00 a.m to 7:00 p.m (which corresponds to *Hylodes heyeri* activity time) with a uni-directional microphone (Yoga HT-81) and a Zoom H2n Handy Recorder placed at 10 m from the road to better record and avoid vegetation of area and at a 1 m height with default recording settings (44.1 kHz/24 bit sampling rate, and wav file format). Also, we measured the peak of sound pressure level (SPL, dB re. 20 μ Pa) at 50 m and 100 m from road during 5 minutes at 1:00 p.m (the traffic peak time) using a Minipa MSL-1325A sound level meter (C - weighted). From the road recordings, we selected randomly several sections of different time recordings and mixed together and normalized each one to common peak amplitude of 60 and 78 dB, which correspond to the expected amplitude at 100m and 50m respectively (Audacity Team 2015). We created the four treatments for playbacks to experiments in Audacity® 2.1.0 (Audacity Team, 2015): no noise is a silent treatment generated by software (S1 - natural background pre-stimulus and S2 - natural background post-stimulus), and the two noise levels at 100 m (60 dB - low) and 50 m (78 dB - high, Fig. 1). Each playback experiment lasted 12 min and stimuli were presented continuously to the focal males in the field, three min per treatment, with the order of the two noises randomized for each individual sampled.

Focal males were found and checked if it was more than 5 m (average distance between males in the stream, Lingnau and Bastos, 2007) from any other potentially interacting male. During each experimental trial, we recorded only spontaneous advertisement calls because males emit these calls to attract females for reproduction along the day. We did not use playbacks to attract males and avoid inducing males to defend territories using territorial calls. We placed a sound level meter (Minipa MSL-1325A; Peak dB C - weighted, fast response, manufacturing calibration) at 50 cm from the focal male to measure the sound pressure level (SPL) of calls. This is calibrated equipment and it is ideal for measurements of amplitude (Zollinger et al. 2012). To measure SPL, we attached a camera (Canon PowerShot SX520 HS) to a tripod and record the values that appeared on screen of the sound level meter, then we had the SPL noise values (without call) and SPL values of noisy with the call to use in downstream analysis. We recorded spontaneous vocalizations with a microphone attached to a tripod at 50 cm from the focal male. For playback (road traffic noise treatments), we placed the water- proof speaker (JBL

Charge 3, frequency response = 65 Hz–20 kHz) 1 m from the focal male with the dB values of noise playback calibrated previously with the SPL meter. The sound level meter and the microphone were placed in front and at the same height as the focal male, while the speaker was placed at 45° angle from others equipment. All distances were measured with a laser distance meter (Starrett - KLMS-30; 0,05m a 40m) to standardize the distance. After each experimental trial, we captured, measured snout-vent length (SVL), weighed (to the nearest 0.01 g on a digital balance), and uniquely marked individuals with colour-coded “belts” (Narvaes and Rodrigues, 2005) to ensure that each male was tested only once. We measured air temperature and relative humidity (digital thermohygrometer – Incoterm) upon releasing the male.

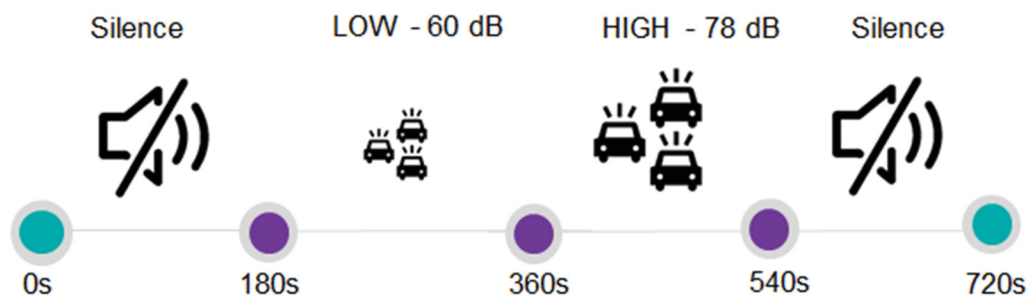


Fig. 1 Schematic representation of the stimulus design. Silence is natural background pre-stimulus noise, low represents quieter noise at 100 m of road, high represents louder noise at 50 m and Silence is a natural background post-stimulus. The order of two noise treatments was randomized for each individual.

9.2.3 Acoustic and data analysis

We measured acoustic parameters of 132 calls (randomly chosen three per treatment) from 11 males using Raven Pro 1.5 (Bioacoustics Research Program, 2011) with a sampling rate of 44.1 kHz, 16-bits, FFT size 1.024, 75% Overlap, threshold-based at -20 dB below peak amplitude and Hamming window. We also measured the following seven parameters: bandwidth (maximum minus minimum frequency, Hz), call rate (calls min $^{-1}$), call duration (in seconds), center frequency (splits the call into two bands with equal amounts of energy, Hz), dominant frequency (Hz), the interval between consecutive calls (in seconds) and SPL call (sound pressure level of call). We measured temporal parameters on the oscillograms while the spectral parameters were measured on the power spectra (Zollinger et al. 2012; Brumm et al. 2017). We determined SPL values with the formula following Brumm and Zollinger (2011). We extracted the SPL noisy and SPL call from the screen of sound pressure level video recorded during experiments.

To test the prediction that *H. heyeri* adjusts their call levels to compensate road traffic noise, we used a linear mixed model (LMM), with acoustic parameters as the response variables and treatments (Silence, LOW

- 100 m, HIGH – 50 m, Silence) as explanatory variables. Individuals were set as random factors to control for the intra-individual variation if present. Our study involved focal animals in the field, then it was not recorded data blind. We fit each random intercept model using *lmer* function in the *lme4* package (Bates et al. 2016). We use the *anova* function (car package) to obtain Wald F tests and the *emmeans* package (Lenth and Lenth 2018) for contrast comparison. All analysis we run in R (R Core Team 2018).

To test whether air temperature, humidity and individual variables influenced the call parameters, we decided to use calls from 14 males emitted during the S1 treatment (no road traffic noise added) because it is a spontaneous call without interference and all explanatory variables should remain unchanged (or with a minimum variation) over the 12 min experiment. Also, as SVL and body mass were not correlated ($r = 0.10$, $P = 0.7233$), probably due to the small body size variation in this population, we used both variables in our models. We used a multiple linear regression analysis in which the response variables were the acoustic parameters (mean values for each male recorded) in S1 treatment (silence) and the additive explanatory variables were the mean values of air temperature, relative humidity, snout-vent length (SVL), and body mass (BM). The individual traits (SVL and BM) were \log_{10} transformed to attain a normal distribution. Also, relative humidity was arcsine transformed. We performed this test using the *lm* function in the stats package in R (R Core Team 2018). Also, the figures 2,3,4 and 5 were performed in R (R Core Team 2018).

9.3 Results

Our study population emitted advertisement calls with a mean dominant frequency of 4577 Hz (range from 4312 – 4875 Hz) and a mean SPL of 75.37 dB (range from 67.33 – 82.23 dB). These calls of *H. heyeri* are high-pitched whistle-trills that have a peak of energy at the third harmonic (Fig. 2). These acoustic parameters have little spectral overlap with the noise treatments (frequency range 0.1 - 4 kHz; Fig. 3). We found that two call parameters were influenced by weather and body traits ($R^2 = 0.82$, $F_{2,8} = 19.03$, $P = <0.001$). There was an inverse relationship between humidity and SPL calls ($\beta = -26.6$, C.I = -44 – -9; Fig. 4a), while SVL was directly related to SPL – that is, larger frogs emitted louder sounds ($\beta = 1.98$, C.I = 0.86 – 3.11; Fig. 4b). Also, call rate was found to increase with air temperature ($F_{4,9} = 5.29$, $P = 0.046$; Fig. 4c).

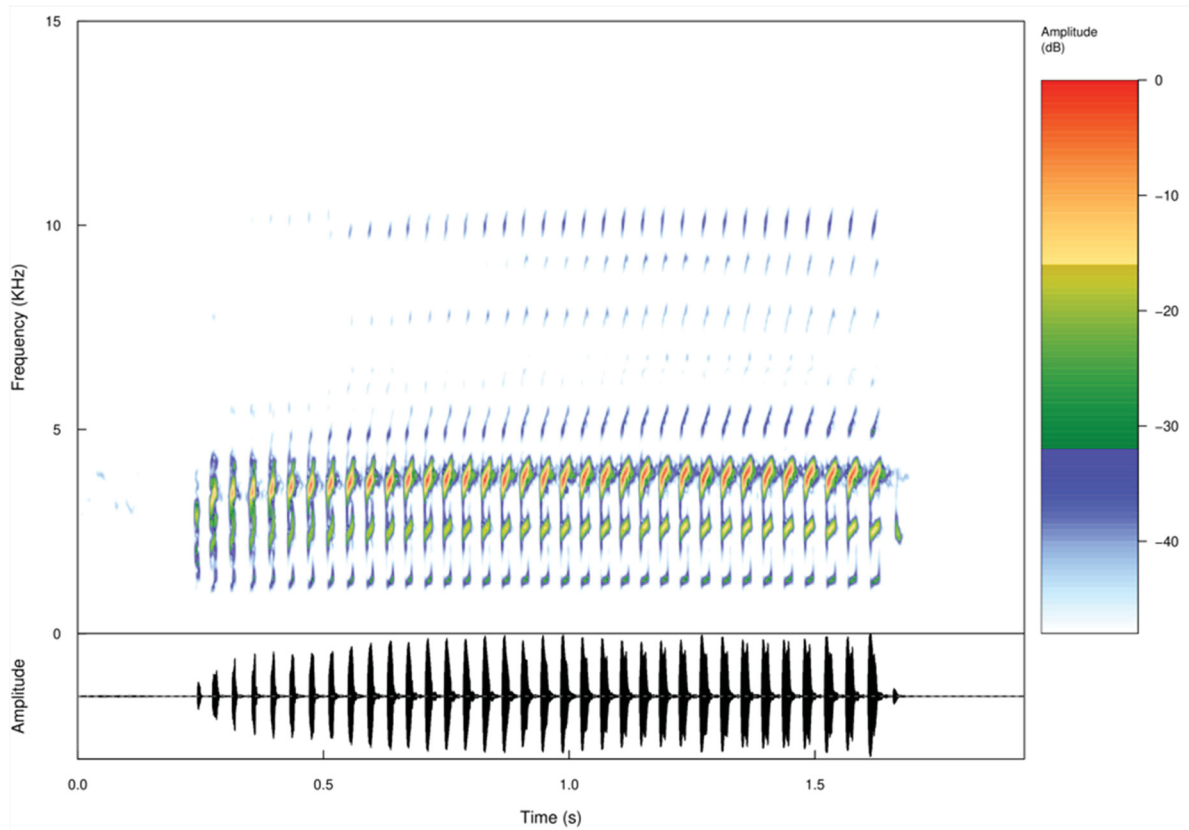


Fig. 2 Spectrogram and waveform of the advertisement call of a *H. heyeri* male recorded in São José dos Pinhais municipality – state of Paraná, Brazil in January of 2019 with 21.5°C temperature.

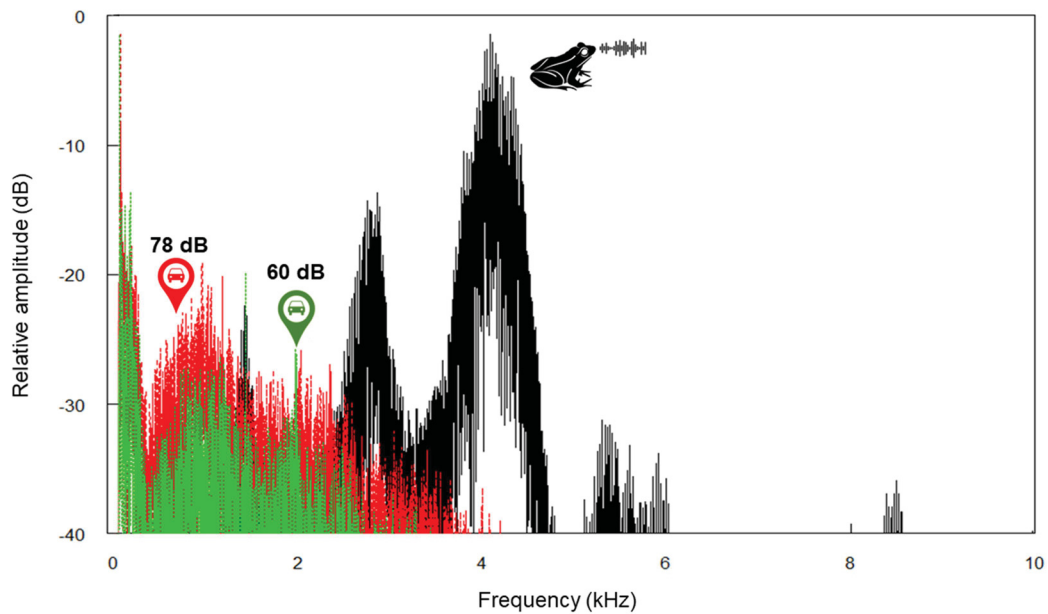


Fig. 3 Power spectra of advertisement call of *H. heyeri* male (84 dB) and the road noise in two intensities (78 dB is high and 60 dB is low treatment) used in this experiment.

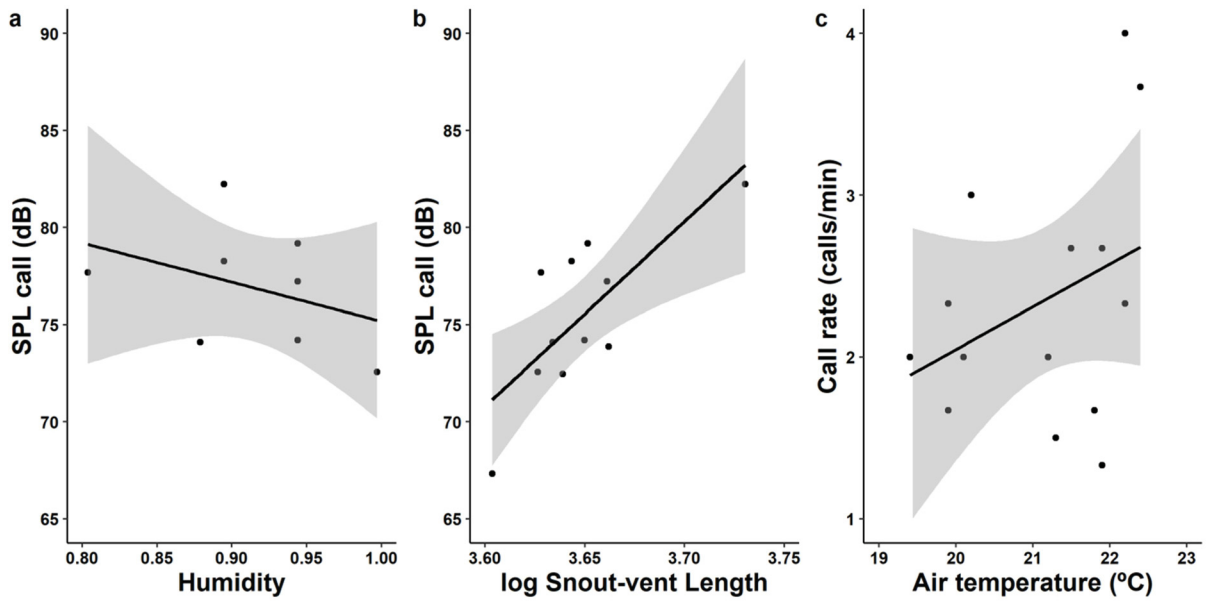


Fig. 4 The relationship of air temperature, humidity and individual variables in the acoustic parameters of *H. heyeri* (N = 14 males) in S1 treatment. **A** SPL call (dB) is inversely related to humidity, **B** SPL call (dB) is directly related to snout-vent length (log), **C** Call rate is directly related to air temperature (°C). Gray shadow represents the 95 % confidence interval

Individuals changed their vocal behavior, especially in noise treatment which simulated the nearest road (50 m). Male SPL calls were louder ($F_{3,128} = 35.89$, $P < 0.0001$, Fig. 5a) in the high treatment (Figure 5). The center frequency of the high treatment was greater than the S2 treatment ($F_{3,128} = 3.05$, $P = 0.003$, Fig. 5b). Call duration was greater in the high treatment than S1 ($F_{3,128} = 4.38$, $P = 0.005$, Fig. 5c). Bandwidth was greater in the high treatment than the S2 treatment ($F_{3,128} = 3.85$, $P = 0.01$, Fig. 5d). All results of post hoc contrast tests of the linear mixed model are provided in the supplementary material S1.

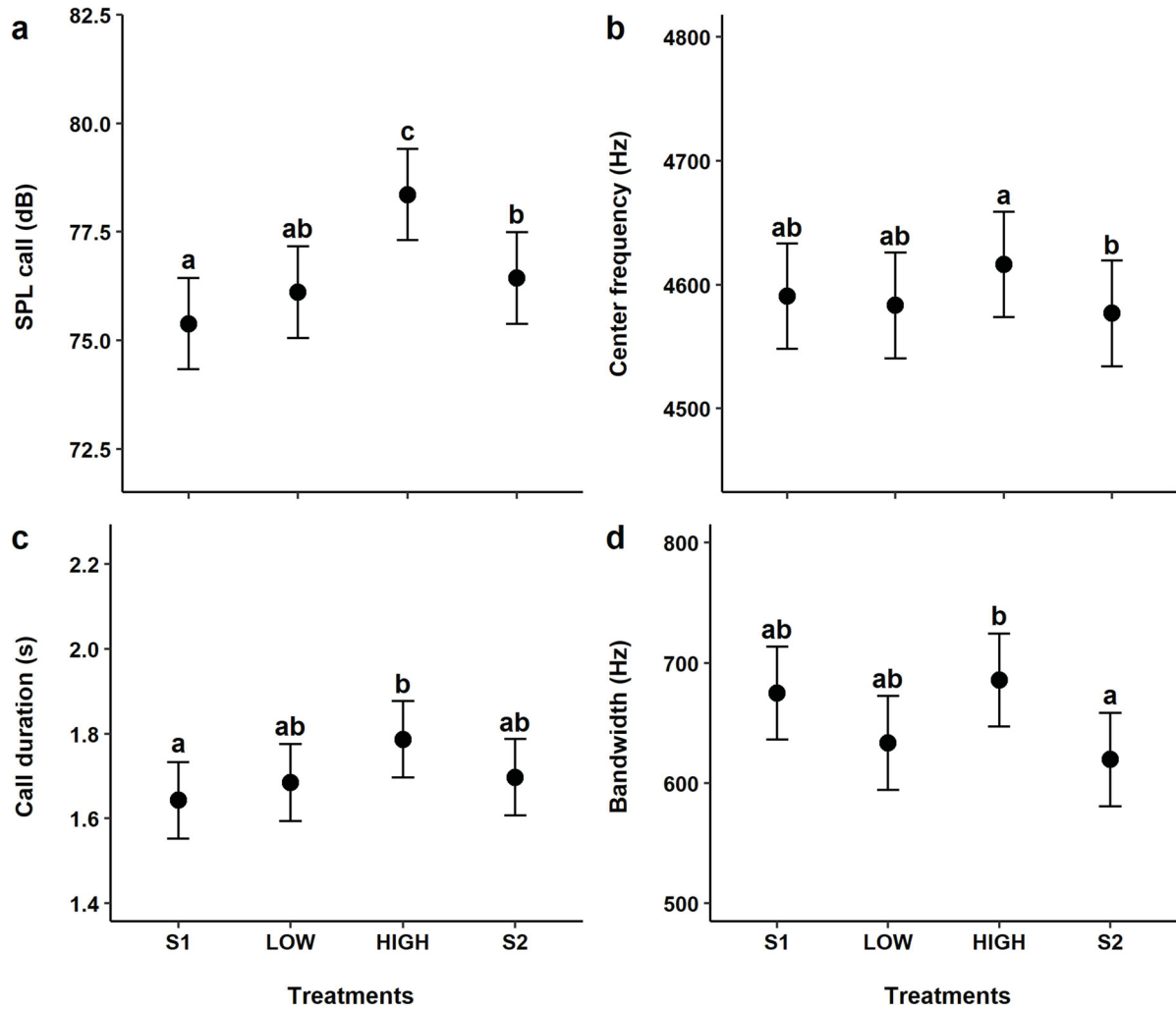


Fig. 5 Main effects (Least-squares model predicted means and standard error) of two distance of road noise playback on acoustic parameters of *Hylodes heyeri* (N = 11). **A** sound pressure level (SPL) call, **B** center frequency, **C** call duration, and **D** bandwidth. The four treatments are **s1** pre-stimulus noise, **low** quieter noise at 100 m of road, **high** louder noise at 50 m, and **s2** post-stimulus. Different letters above indicate statistically significant pairwise differences ($P < 0.05$)

9.4 Discussion

How species respond to traffic noise is fundamental to understand individual and population level consequences of living in anthropogenic or near anthropogenic areas, especially for species relying on acoustic communication, such as Anura. In this study, we tested whether two levels of road traffic noise affected the vocal behavior in *H. heyeri* and whether these acoustic parameters respond to climate variables and the body size traits of males. We show that *H. heyeri* males when faced with noise stimulus call with louder SPL, have longer calls, higher center of frequency and bandwidth, the last two parameters compared to the natural background

post-stimulus. We also found that a spectral trait (dominant frequency and SPL) scales with body size. Similarly, we show that SPL, a spectral parameter, decreases while humidity increases and that call rate increases with temperature.

The Brazilian Torrent Frog changed four call parameters in response to high-intensity noise, which represents the nearest road traffic from the site. Three spectral parameters (SPL, bandwidth, center frequency) and one temporal parameter (call duration) increased in the 50 m (loudest anthropogenic noise) treatment. The increased call duration in high noise treatment found here is similar to the response showed by *Boana leptolineata* (Caorsi et al. 2017) which also increases call length in response to louder road traffic noise. Both species call in high frequencies and have little spectral overlap with noise. Moreover, the response of increasing the center frequency and bandwidth of *H. heyeri* also occurred in *Hypsiboas albomarginatus* (Both and Grant 2012) which calls at higher dominant and center frequencies in the presence of the invasive *Lithobates catesbeianus* calls. Also, *Litoria ewingii* decreases mean dominant frequency and call rate with increasing distance from the road >200 m (Higham et al. 2021). These changes in temporal and spectral parameters (i.e call duration and frequency, respectively) seem to be general responses anurans use to cope with interference noise.

Overall, most anurans show plastic vocal responses employing different adaptive strategies including no responses to anthropogenic noises (Zaffaroni-Caorsi et al., 2022). Short-term adaptations in calls can include a change in sound intensity (Lombard effect), an increase in call rate, and changing frequencies (Kaiser and Hammers 2009; Luther and Magnotti 2014). Our results showed that the increase of 18 dB in traffic road playback resulted in an average of 2 dB louder call intensity (without social interference), this increase is known as Lombard effect. This strategy of increase sound intensity when calling in noise environments was employed by six anurans species: *Leptodactylus albilabris*, *Engystomops pustulosus*, *Odorrana tormota*, *Kurixalus chaseni*, *Dryophytes chrysoceles* and *Bokermannohyla hylax* (Lopez et al. 1988; Love and Bee 2010; Halfwerk et al. 2015; Shen and Xu 2016; Yi and Sheridan 2019; Lima et al. 2022). Overall, these species rise their call intensity between 1 and 3 dB, which is similar to *H. heyeri*, that rose their call intensity by 2dB. Anurans that inhabit streams are smaller than anurans breeding away from streams (morphological constraints) and they call at a higher frequency because the background is noisy (Duellman and Trueb 1986, Vargas-Salinas and Amézquita 2014). Although body size imposes a biological limitation to sound producing, in stream anurans the environmental noise seems to be the key driver of the higher frequency calls (Goutte et al. 2016, 2017; Zhao et al. 2021). Beyond that, biotic factors such as predator, an intruder or female can influence their acoustic parameters.

The positive relationship between spectral traits (dominant frequency and SPL) and body size occurs in the majority of frog species (Gerhardt and Huber 2002; Köhler et al. 2017). Our findings that humidity is negatively related to SPL calls were different from the response of *Physalaemus cuvieri*, which was positively related to relative humidity (Gambale and Bastos, 2014). The humidity of this study ranged from 0.80 to 1.07 (Fig. 4) and *H. heyeri* males usually vocalize above the rocks inside of the stream. As both species occur in different environments, rivers and forests, it could be that the physical structure of the environments elicits different responses because the noise is transmitted differently in each environment (Goutte et al. 2013).

Temperature is a weather factor that modulates amphibian physiology because they are ectothermic animals, so changes in temperature also change metabolic reactions (Köhler et al. 2017). In addition, calling involves muscular contractions which are linked to temporal parameters such as call rate, call duration, interval call, and pulse rate (Gerhardt 1994; Köhler et al. 2017). Then, temperature affects mainly temporal calls. Consistent with this, *H. heyeri* increases their call rate with air temperature. However, other studies that found similar results measured water temperature or the air temperature in each microhabitat (Wong et al. 2004; Camurugi et al. 2015). This shows the importance of considering the interaction of other factors besides the air temperature in the vocal activity of anurans.

In summary, our study showed that the Brazilian Torrent Frog changes the acoustic parameters in presence of anthropogenic noise, also their SPL call is related to humidity and SVL. The evidence of this study implies that *H. heyeri* has the ability to make real-time little adjustments in their calls when exposed mainly to louder noises that accompany roads in their natural noisy environment. Results so far have encouraging more attention with different intensities of noise resulting in different call responses. This behavioral plasticity is important for species to cope with environmental noise.

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Authors’ contribution MMS conceived and performed the experiments. MMS and MOM analyzed the data and wrote the manuscript.

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Data availability The datasets generated are available in the supplementary material (S2 and S3).

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The Ethics Committee for Animal Use from the Biological Sciences Section of the Federal University of Paraná (CEUA/BIO – UFPR) in agreement with the Brazilian Guidelines for Care and Use of Animals for Scientific and Teaching purposes established by the National Council for Control of Animal Experimentation (CONCEA) and with the international guidelines for animal experimentation permit no. 23075.068081.

Conflict of interest The authors declare that they have no conflict of interest.

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10 SUPPLEMENTARY MATERIAL

When distance matters: Brazilian torrent frogs changes their calls according to the distance of road noise

Behavioral Ecology and Sociobiology

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S1 Results of post hoc contrasts tests of the linear mixed model used to test the effect of two noise intensities in call parameters of *Hylodes heyeri*. Males were exposed to four treatments of 3 min each, totalizing 12 min. *P* value with statistical significance contains the stars.

<i>Acoustic parameters</i>	<i>Contrast</i>	<i>Estimate</i>	<i>SE</i>	<i>t ratio</i>	<i>P value</i>
<i>Bandwidth</i>	HIGH – LOW	52.2	23.2	2.251	0.1160
	HIGH – S1	10.8	22.3	0.484	0.9625
	HIGH – S2	66.2	22.9	2.889	0.0237*
	LOW – S1	-41.4	23.0	-1.798	0.2797
	LOW – S2	14.0	23.7	0.591	0.9348
	S1 – S2	55.4	22.9	2.424	0.0785
<i>Call rate</i>	HIGH – LOW	-0.1601	0.173	-0.923	0.7925
	HIGH – S1	-0.0574	0.169	-0.339	0.9865
	HIGH – S2	-0.3469	0.171	-2.024	0.1856
	LOW – S1	0.1027	0.175	0.587	0.9358
	LOW – S2	-0.1868	0.177	-1.055	0.7176
	S1 – S2	-0.2895	0.173	-1.675	0.3421
<i>Call duration</i>	HIGH – LOW	0.1022	0.042	2.410	0.0811
	HIGH – S1	0.1439	0.040	3.532	0.0033**
	HIGH – S2	0.0894	0.041	2.136	0.1483
	LOW – S1	0.0417	0.042	0.991	0.7551
	LOW – S2	-0.0127	0.043	-0.294	0.9911
	S1 – S2	-0.0544	0.041	-1.303	0.5629
	HIGH – LOW	33.1	14.3	2.319	0.1000

<i>Center frequency</i>	HIGH – S1	25.5	13.7	1.859	0.2520
	HIGH – S2	39.5	14.1	2.801	0.0302*
	LOW – S1	-7.6	14.2	-0.536	0.9500
	LOW – S2	6.4	14.6	0.439	0.9716
	S1 – S2	14.0	14.1	0.995	0.7525
<i>Dominant frequency</i>	HIGH – LOW	34.6830	20.3	1.707	0.3249
	HIGH – S1	37.2380	19.5	1.908	0.2309
	HIGH – S2	37.143	20.1	1.851	0.2554
	LOW – S1	2.5550	20.2	0.127	0.9993
	LOW – S2	2.4601	20.7	0.119	0.9994
	S1 – S2	-0.0949	20.0	-0.005	1.0000
<i>Interval call</i>	HIGH – LOW	5.441	2.24	2.429	0.0775
	HIGH – S1	2.739	2.15	1.272	0.5824
	HIGH – S2	3.703	2.21	1.673	0.3426
	LOW – S1	-2.701	2.22	-1.214	0.6191
	LOW – S2	-1.738	2.29	-0.760	0.8720
	S1 – S2	0.963	2.21	0.437	0.9720
<i>SPL call</i>	HIGH – LOW	2.251	0.309	7.286	<.0001**
	HIGH – S1	2.977	0.299	9.945	<.0001**
	HIGH – S2	1.922	0.305	6.299	<.0001**
	LOW – S1	0.726	0.309	2.346	0.0940
	LOW – S2	-0.329	0.315	-1.042	0.7250
	S1 – S2	-1.055	0.307	-3.435	0.0046**

S2 Raw data of acoustic parameters of males of *Hylodes heyeri* (N=11) measured for each treatment. For each treatment we measured three calls randomly from individual.

Individual	Treatment	Center frequency (Hz)	Dominant frequency (Hz)	Bandwidth (Hz)	Call duration (s)	Interval call (s)	Call rate (call/min)	SPL call (dB)
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N17	S1	4547	4500	603	1.549	22.649	2	73.2
N17	S1	4547	4500	891	1.409	33.055	2	71.7
N17	S1	4547	4547	602	1.067	33.048	NA	NA
N17	HIGH	4547	4500	1060	1.688	25.03	2	75.98
N17	HIGH	4547	4500	884	1.425	25.037	2	73.4
N17	HIGH	4594	4500	874	1.562	18.947	2	75.41
N17	LOW	4547	4500	705	1.454	22.162	1	72.6
N17	LOW	4547	4500	592	1.34	34.038	2	71.8
N17	LOW	4547	4500	559	1.28	33.856	2	73
N17	S2	4547	4500	613	1.388	21.906	2	72.2
N17	S2	4547	4500	624	1.412	28.862	2	72.8
N17	S2	4547	4500	639	1.455	25.443	2	72.9
N15	S1	4500	4453	693	1.496	41.25	2	78.1
N15	S1	4453	4453	602	1.311	33.33	1	78.9
N15	S1	4406	4453	490	1.623	21.6	2	77.8
N15	LOW	4453	4453	685	1.772	26.14	2	78.6
N15	LOW	4453	4453	685	1.733	29.52	3	79.2
N15	LOW	4500	4453	792	1.733	22.37	2	79.6
N15	HIGH	4500	4453	789	1.737	23.78	2	79.4
N15	HIGH	4500	4500	780	1.919	34.79	2	81.1
N15	HIGH	4453	4453	650	1.454	43.08	1	79.4
N15	S2	4500	4453	627	1.423	49.55	2	77.8
N15	S2	4453	4453	540	1.616	33.68	2	78.5
N15	S2	4453	4453	684	1.632	19.75	2	79.1
N10	S1	4359	4359	987	1.087	46.757	1	76.2
N10	S1	4359	4359	963	1.756	25.307	2	78.4
N10	S1	4406	4359	960	1.625	42.99	1	78.4
N10	LOW	4500	4594	625	1.731	30.081	2	78.3
N10	LOW	4547	4547	542	1.486	28.083	2	78.4
N10	LOW	4547	4547	591	1.927	22.554	2	78.8
N10	HIGH	4594	4594	530	1.836	24.594	2	81.6
N10	HIGH	4547	4547	572	1.855	26.604	3	80
N10	HIGH	4594	4594	751	1.269	34.144	2	77.8

N10	S2	4547	4594	724	1.772	27.745	2	77.9
N10	S2	4594	4594	746	1.888	21.367	3	78.1
N10	S2	4547	4594	718	1.735	24.812	2	78.4
N3	S1	4594	4594	413	1.92	29.297	2	73.4
N3	S1	4594	4594	444	2.147	30.635	1	74.9
N3	S1	4547	4594	420	2.124	30.635	NA	74.9
N3	HIGH	4547	4547	440	1.917	75.942	1	78.9
N3	HIGH	4594	4594	459	2.12	61.294	1	76.15
N3	HIGH	NA	NA	NA	NA	NA	NA	NA
N3	LOW	4547	4547	452	2.007	23.904	1	73.7
N3	LOW	4547	4547	452	1.921	23.904	1	74.4
N3	LOW	NA	NA	NA	NA	NA	NA	NA
N3	S2	NA	NA	NA	NA	NA	NA	NA
N3	S2	NA	NA	NA	NA	NA	NA	NA
N3	S2	NA	NA	NA	NA	NA	NA	NA
N11	S1	4781	4640	647	1.344	20.134	2	74.1
N11	S1	4875	4922	646	1.385	16.154	2	74.6
N11	S1	4781	4640	659	1.465	17.183	3	73.9
N11	HIGH	4828	4922	651	1.272	22.084	2	74.66
N11	HIGH	4828	4922	650	1.32	18.67	3	78.93
N11	HIGH	4922	4922	604	1.805	20.17	2	76.3
N11	LOW	4875	4922	659	1.353	13.386	4	75.2
N11	LOW	4781	4922	646	1.274	16.051	3	75.7
N11	LOW	4734	4640	635	1.363	14.455	3	74.7
N11	S2	4828	4922	620	1.384	19.81	1	74.9
N11	S2	4828	4922	622	1.378	14.554	4	75
N11	S2	4734	4640	614	1.489	14.561	3	75.9
N13	S1	4359	4312	642	1.701	27.239	2	72.3
N13	S1	4312	4312	712	1.476	36.935	2	72.3
N13	S1	4312	4312	611	1.712	26.722	2	73.1
N13	LOW	4359	4312	615	1.525	28.709	2	72.9
N13	LOW	4359	4312	690	1.869	18.431	2	72.2
N13	LOW	4359	4312	650	1.795	22.028	2	72.9

N13	HIGH	4406	4312	727	2.008	40.972	1	75.63
N13	HIGH	4406	4453	733	2.007	22.783	2	74.77
N13	HIGH	4406	4500	726	1.981	25.122	2	73
N13	S2	4359	4312	654	1.762	24.042	3	72.8
N13	S2	4359	4312	646	1.752	28.339	1	71.8
N13	S2	4359	4312	654	1.693	22.87	2	72.8
N0	S1	4594	4547	745	1.684	15.211	3	76.7
N0	S1	4781	4828	662	1.59	12.651	4	77.5
N0	S1	4781	4828	639	2.126	11.095	5	77.5
N0	LOW	4781	4828	666	1.488	13.583	4	77.9
N0	LOW	4828	4828	617	1.946	12.32	4	77.7
N0	LOW	4687	4734	724	1.432	10.986	5	77.7
N0	HIGH	4781	4828	625	2.23	12.493	3	79.7
N0	HIGH	4781	4828	660	1.834	13.247	5	79.7
N0	HIGH	4687	4781	659	1.919	12.35	4	79.4
N0	S2	4687	4781	656	1.642	12.086	5	78.2
N0	S2	4781	4828	626	2.105	13.364	4	78.4
N0	S2	4734	4781	659	1.635	10.349	5	78.2
N1	S1	4453	4500	824	2.186	51.228	2	82.9
N1	S1	4453	4500	655	2.305	20.902	1	80.9
N1	S1	4500	4500	678	2.123	22.159	3	82.9
N1	LOW	4453	4500	746	2.404	26.977	2	83.2
N1	LOW	4453	4500	690	2.458	24.888	2	83.4
N1	LOW	4406	4500	753	2.163	23.22	1	81.8
N1	HIGH	4500	4500	628	2.193	18.012	3	84.1
N1	HIGH	4453	4500	735	2.384	26.204	1	84.8
N1	HIGH	4500	4500	670	2.079	49.746	1	83.2
N1	S2	4453	4500	682	2.285	30.792	3	82.6
N1	S2	4500	4500	693	2.412	27.249	1	82.4
N1	S2	4500	4500	712	2.239	24.909	1	82.4
N7	S1	4687	4687	625	1.405	21.91	2	78.7
N7	S1	4687	4687	585	1.443	45.206	1	80.9
N7	S1	4640	4687	463	1.171	40.067	2	77.9

N7	HIGH	4640	4687	612	1.518	28.881	1	80.5
N7	HIGH	4687	4687	560	1.424	44.116	2	81.4
N7	HIGH	4687	4687	725	1.518	26.564	2	82.4
N7	LOW	4640	4687	476	1.348	23.042	1	79.4
N7	LOW	4687	4687	543	1.419	30.764	2	79.7
N7	LOW	4640	4687	467	1.187	26.067	2	79.4
N7	S2	4640	4687	516	1.477	37.639	2	81.7
N7	S2	4640	4687	425	1.381	41.539	2	79.7
N7	S2	4640	4640	447	1.047	18.967	3	79.1
N8	S1	4734	4734	429	1.422	14.706	2	65.9
N8	S1	4781	4781	764	1.362	15.246	2	67.4
N8	S1	4781	4828	436	1.506	16.659	3	68.7
N8	LOW	4547	4547	639	1.547	18.833	2	69.5
N8	LOW	4594	4547	522	1.626	29.918	3	69.9
N8	LOW	4594	4687	495	1.392	30.973	2	70.8
N8	HIGH	4594	4547	557	1.608	16.949	3	76.5
N8	HIGH	4640	4687	495	1.687	19.416	3	77
N8	HIGH	4594	4687	589	1.65	17.912	2	77
N8	S2	4547	4687	435	1.416	10.258	4	72.1
N8	S2	4594	4687	550	1.375	25.075	3	72.9
N8	S2	4547	4547	551	1.383	13.162	4	72.1
N9	S1	4828	4969	930	1.889	17.15	4	73.1
N9	S1	4828	4969	959	1.822	14.839	2	73.3
N9	S1	4687	4687	895	1.978	25.686	2	75.2
N9	HIGH	4828	4969	934	1.801	26.961	2	75.8
N9	HIGH	4828	4875	927	1.914	49.523	1	77.9
N9	HIGH	4734	4687	897	1.925	24.805	2	76.9
N9	LOW	NA	NA	NA	NA	NA	NA	NA
N9	LOW	NA	NA	NA	NA	NA	NA	NA
N9	LOW	NA	NA	NA	NA	NA	NA	NA
N9	S2	4640	4687	778	1.925	42.8	1	75.5
N9	S2	4594	4687	868	1.959	28.189	2	75.3
N9	S2	4687	4687	890	1.92	25.836	2	75.3

S3 Raw data of acoustic parameters of males of *Hylodes heyeri* (N=14) measured in S1 treatment (natural background pre-stimulus). Each line represents an individual. RU = relative humidity, SVL = snout-vent length, SPLvoc = sound pressure level vocalization.

SVL (mm)	Mass (g)	SPLvoc (dB)	Temperature (°C)	RU	Call_rate (call/min)	Dominant_frequency (Hz)	Call_duration (s)	Interval_call (s)	Center_frequency (Hz)
41.7	5	82.23	20.1	78	2	4500	2.2	31.42	4468
37.58	5.1	NA	21.5	86	2.67	4359	1.91	18.74	4343
37.86	4.9	74.1	21.3	77	1.5	4594	2.06	30.18	4578
38.82	5.8	NA	20.2	88	3	4640	1.58	36.87	4593
39.06	5.5	NA	22.4	80	3.67	4547	2.71	12.92	4531
38.53	5.2	79.17	21.8	81	1.67	4687	1.33	35.72	4671
36.73	4.8	67.33	19.9	87	2.33	4781	1.43	15.54	4765
38.94	5.4	73.87	21.9	85	2.67	4875	1.9	19.23	4781
37.64	5.4	77.67	21.9	72	1.33	4359	1.48	38.35	4374
38.47	5	74.2	22.2	81	2.33	4734	1.39	17.82	4812
38.91	5.8	77.23	22.2	81	4	4734	1.8	12.98	4718
37.58	5.4	72.57	21.2	84	2	4312	1.62	30.29	4327
38.22	5.2	78.27	19.9	78	1.67	4453	1.47	32.06	4453
38.05	5.8	72.45	19.4	88	2	4515	1.34	29.58	4547

11 CAPÍTULO 3

**Facing the enemy: are the agonistic behaviors affected by road noise in
Brazilian torrent frog?**

In progress – Ethology, Ecology and Evolution

Facing the enemy: are the agonistic behaviors affected by road noise in Brazilian torrent frog?

Michelle M. Struett, Joel Rapp and Maurício O. Moura

Abstract

Noise pollution is known to negatively affect social interactions in some animal species, with consequences for fitness and reproductive success. Many frog species use multimodal signals in social interactions, including competition between males, when they compete for resources such as breeding territories. We experimentally tested in the field the effects of traffic road noise on auditory and visual signals in *Hylodes heyeri* during agonistic context. We expect that resident males increase the frequency of visual signals, change temporal acoustic parameters, and increase the latency of detecting an intruder during traffic road noise. We exposed resident males to a robotic frog that emitted visual and acoustic signals with and without road traffic noises playback. Our results demonstrate that *H. heyeri* modifies only acoustic signals in experimental noise environments. Males increase their call rates and dominant frequency, and decrease interval calls when exposed to road noise. Also, noise did not affect the male's ability to detect an intruder model frog. This is one of the first studies to concurrently examine the effects of road noise in responses of receivers and the multimodal signals during agonistic behavior. These results illustrate that frogs can respond with a short-term acoustic strategy to a different noise in the agonistic context so that their signal quality remains strong.

Key-words: Agonistic interaction, Anthropogenic noise, Behavior, *Hylodes heyeri*, Signals.

12 INTRODUCTION

Animal communication occurs when a sender transmits the message to a receiver via signals (Hödl and Amézquita, 2001). We expect that natural selection will favor signals, and the associated behavior, that could be detected and discriminated from others as well as from the background noise (Endler, 1992). So, the social and environmental conditions during signaling affect how the information (signal content) is transmitted detected and recognised (Endler, 1992, Brumm & Slabbekoorn, 2005; MacGregor et al 2013).

Acoustic signal is the most commonly signal used by animals to communicate during social interactions mostly over long distances due to sound propagation capacity (Brumm and Slabbekoorn, 2005). However, sounds are subjected to several environmental constraints that affect transmission and thus, how the receiver perceives the information. Anthropogenic noises (from automobiles, trains, airplanes, construction, and so on) often occur in association with anthropic habitat fragmentation leading to a modification of the soundscape experienced

by animals (Vos and Chardon, 1998; Wiley, 2009; Hardman and Dalesman, 2018). For example, in anurans, anthropogenic noise can interfere with male vocalization masking the call, and thereby influencing mate choice through impossibility to the female to recognize the emitted signal (Senzaki et al., 2018). The anthropogenic noise effects are broad and can also influence movement patterns (Vargas-Salinas and Amézquita, 2013), foraging behaviors (Tuttle and Ryan, 1982), physiology (Tennessen et al., 2014), vocal sac coloration (Troïanowski et al., 2017), and population density (Vargas-Salinas and Amézquita, 2013).

Overall, anthropogenic noise interferes with signal detection and recognition and then, with how species communicate with each other (Brumm and Slabbekoorn, 2005; Brumm, 2013). To efficiently communicate, animals have some strategies to improve effective sound transmission relative to a background noise, either natural or anthropogenic. For example, increasing call amplitude and the signal-to-noise ratio (Brumm and Zollinger, 2011), timing shifts such as change call durations or interval calls (Halfwerk et al., 2016) or frequency shifts that minimize call overlap with noise (Simmons and Narins, 2018; Jiménez-Vargas and Vargas-Salinas, 2021).

Also, the ability to shift among sensory channels can lead an individual to transmit more information in a noise environment. For example, animals that can change from an acoustic channel to a silent channel (visual signal) continue to emit the same or even more information to the receiver, but without any interference in the signal transmission (Partan et al., 2010). Many species emit more visual signals than acoustic signals when in a noise environment, such as *Staurois parvus* (Anura: Ranidae) which display foot flagging preceding the advertisement call in presence the another male or that *Hyperolius marmoratus* (Anura: Hyperoliidae) females which uses more visual cues in noisy areas (Grafe et al., 2012; Hödl and Amézquita, 2001). Then, the use of more than one signal is associated with an improvement in communication.

Several anuran species defend calling or oviposition sites, displaying several levels of behavioral aggression through visual and acoustic signaling, sometimes ending up in combat (Wells, 2007). As occurs with the detection of the intruder, territory defense in some anurans involves more than one kind of signal, or sensory modality (multimodal signal, Narins et al., 2003). The social context that leads to the emission of acoustic aggressive calls is widely studied in birds, usually to test the ability to detect intruders, performance of territorial songs and territory-defense behaviors in noisy environments (Brumm, 2004, Kleist et al. 2016). More specifically in frogs, most studies examine the influence of acoustic signals on mate

choice (Ryan, 1980; Ryan, 1985) and few describe/test signaling behavior in agonistic interactions (Halfwerk et al., 2014). In addition, we found studies of aggressive calls testing the "dear enemy" hypothesis (Chuang et al., 2017), function of aposematic signals (Crothers and Cummings, 2015), description of acoustic signals in territorial contexts (Brasileiro et al., 2020) and evaluating how sound shapes interspecific aggressive interactions (Reichert and Gerhardt, 2014). However, none of these studies test the effect of any noise during these interactions. Yet, male–male interactions that determine who occupies a favored location to attract females are also important in a reproductive context (Gardner and Graves, 2005). Although these interactions entail costs to males, for example, expending higher energy in their calls and being more exposed to predators, and also some flies that are attracted by the calls can transmit parasites to these frogs (Hamilton and Zuk 1982; Caldart et al., 2016). Also, competition is another costly interaction in which natural/sexual selection can act and influence resource distribution (Milinski and Parker, 1991) and reproductive success between males.

In an agonistic arena, anthropogenic noise can interfere with the sounds (signals) emitted by the territory holder (sender) and, if the sender is aware of that interference, it will also influence its behavior, which in turn can influence responses to those signals by the intruder (receiver, McMullen et al., 2013). The effects of anthropogenic noise in agonistic interaction and how it affects vocal and behavioral interactions in anurans has received less attention than the effects in mate choice. To contribute to fill this gap, we used a robotic frog to test how visual and vocal signals emitted by *Hylodes heyeri* during an agonistic male-male interaction are affected, for instance, by road noise. Also, as far as I know this is the first study to test, in field, the influence of road noise playback during agonistic behavior in a Neotropical species using a multimodal model frog. Following Francis (2015), we predicted that in a noisy setting, males increase the rate of visual signals and calls, and both dominant frequency and call duration increase, while call interval decreases. Also, the latency time to resident male detect intruder is higher during noisy than no noisy treatment.

13 METHODS

13.1 STUDY SPECIES AND SITE

The Brazilian torrent frog *H. heyeri* is a diurnal species member of a small family, Hylodidae, comprising four genera occurring in Atlantic Forest streams (Haddad et al., 2013; Frost, 2022). *Hylodes heyeri* lives in fast-flowing streams which impose noise in

lower frequencies, 0–3kHz (Vargas-Salinas and Amézquita, 2013). Also, this species emits advertisement calls using rocks as calling site located inside of the river (Haddad et al., 2013; Lingnau and Bastos, 2007), which is defended against approaching males using territorial calls and also, where courtship calls to attract females are emitted (Lingnau and Bastos, 2007). The calls can have little variation, our previously study population (N=13 males, chapter 2) have advertisement calls with 4578 Hz of dominant frequency, 1.7 s of call duration, 36 notes per call (Fig. 1) and 38 mm average of snout–vent length of male (range 36.7–41.7mm). The territorial calls (N=7 males) have a dominant frequency of 4259 Hz, call duration of 0.25 s and 4 notes per call.

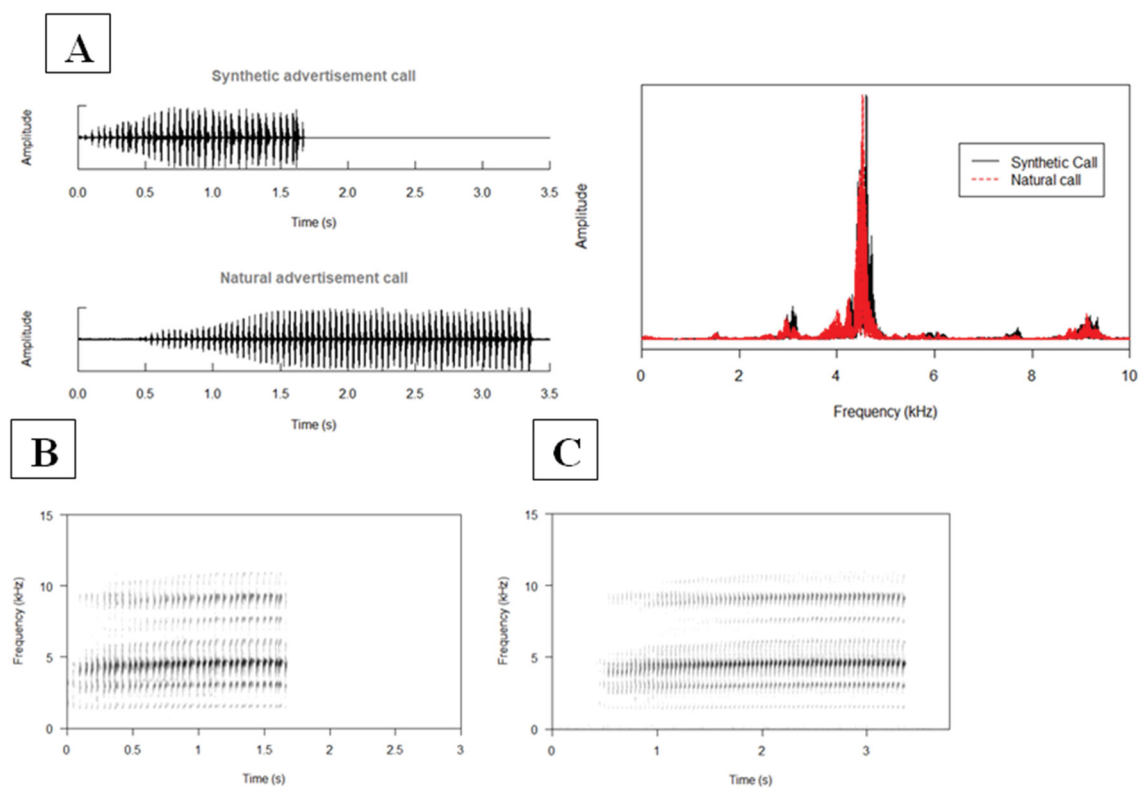


Figure 1. Oscillograms and power spectra (A) of synthetic (mean population) and natural advertisement call of one individual displaying the amplitude of the calls in time and frequency; spectrograms of synthetic (B) and natural (C) advertisement call displaying the frequencies of the calls and harmonics (darker shading corresponds to higher energy).

In addition to vocal communication, members of this family also use visual communication for mating and agonistic interactions (Haddad and Giaretta, 1999; Hödl and Amézquita, 2001). If the territorial calls are not sufficient for the intruder to give up, then the opponent can enter the territory of the resident male and the agonistic behavior could escalate to a corporal fight (Beltramin and Moura, in prep). Previous studies showed that some species

in Hylodidae perform several visual signals, limbs and body movements such as: foot flagging, body jerking, arm lifting, toes flagging, head bobbing, throat display (De Sá et al., 2016; Augusto-Alves, 2018). Because that, this species can be considered a good model for testing our predictions of the effect of anthropogenic noise on visual and acoustic communication. Also, this species not show aggregate behavior neither calling in dense choruses and has prolonged breeding seasons (September to March).

We conducted the experiments in a stream located on a private property near to the municipality of São José dos Pinhais (25°42'9" S; 49° 4'19" W), state of Paraná, southern Brazil. This site was chosen because it is located at least more than 5 km of routes and urban areas and so, we can test how *H. heyeri* without previous exposition to anthropogenic noise will respond to it. The area has an average altitude of 980 meters, it is an ecotone between dense rain forest and mixed rain forest or Araucaria Forest (Maack, 1981). The climate is mesothermal humid subtropical, without a dry season, with cool summers and the occurrence of severe and frequent frosts, corresponding to the Cfb climate of the Köeppen scale (Maack, 1981). Also, *H. heyeri* is the only species we found inhabiting this stream. The part of the stream sampled was approximately 30 m by 1.5 m wide with few rocks causing water fall, so the noise was relatively low 50-60 dB. Experiments were carried out from February to March 2020 and October to December 2020, which is the austral summer and breeding season for this species, between 07:00 and 18:00 hours.

13.2 ROBOT FROG AND PLAYBACK TRIALS

We modeled a frog out of artistic modeling clay based on a 3-D printed model of an individual with a mean snout-vent length of 38.4 mm, the mean size of individuals in other population of *Hylodes heyeri* (Struett, Ropper and Moura, subm). We separately made the arm of silicone so that it could be moved remotely and painted the model to be as similar to the species with a Polyvinyl Acetate matte ink (PVA ink), varnishing it to preserve its color in the presence of humidity. The frog was mounted on a hollow styrofoam base that was painted to resemble rock and in which we placed the mechanisms to manipulate behavior and vocalizations. Both the frog and rock were varnished for waterproofing. These mechanisms were three tiny servo-motors that were controlled by a microcontroller board (Arduíno UNO R3) and powered by a 9v battery, all housed within the artificial rock (Fig. 2). One microcontroller was attached as support to the rotating platform with the model frog above and rotated in time determined by a script according to previously video recordings of individuals. Another two microcontrollers were connected to mechanical rods to move the

model right arm and vocal sacs. Robot frogs perform behaviors that were previously associated with a territorial dispute in *Hylodes* species (Fig 3; De Sá et al., 2016; Augusto-Alves, 2018; Beltramin and Moura, in prep). It was programmed to elevate their right arm, show their dual vocal sacs, and rotate the entire body, which was a platform rotation. Rather than inflation per se, the gular sacs were moved in and out by the motor synchronized with vocalizations. All movements were controlled by a script in Arduino 1.8.10 software (Hughes, 2016).

The speaker (JBL Go 2, frequency response = 180 Hz–20 kHz) with playback calls was attached in styrofoam behind the model frog (Fig. 2). Below the rock base, we constructed a “quadruped” made of PVC tubes and placed it below the rock base to control the height and stability of the prototype on the ground. Also, most of the time this structure allows keeping the robotic male at the same calling-perch height as the resident male.

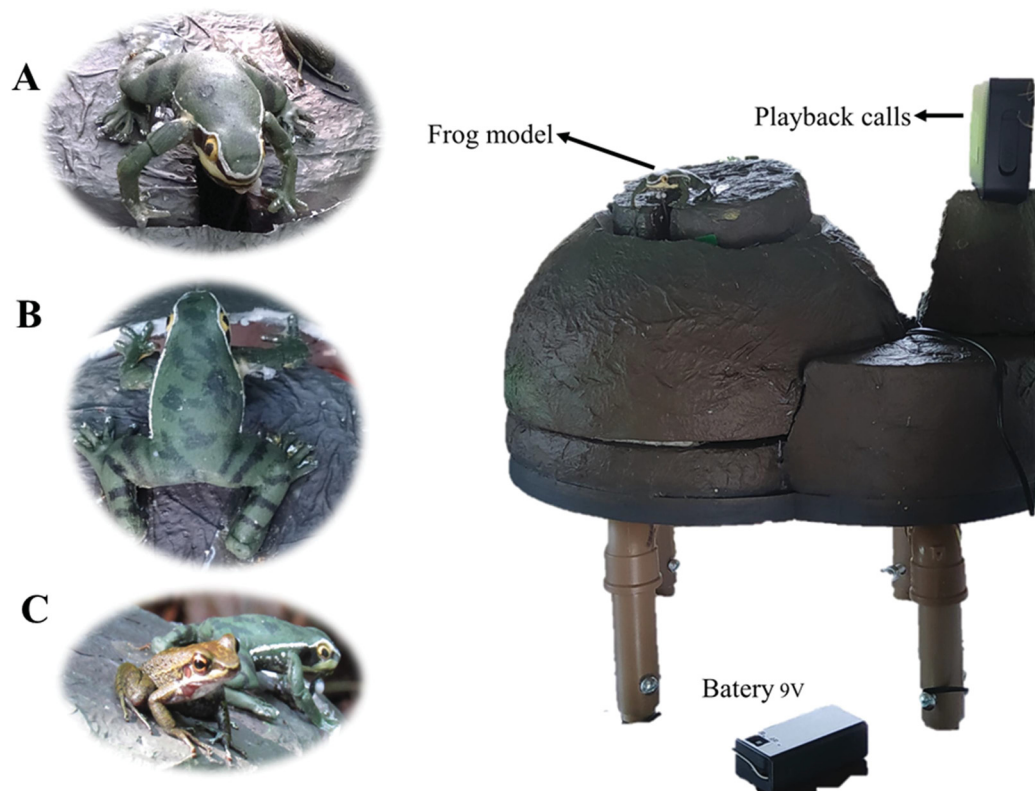


Figure 2. Rock (hollow Styrofoam) with support connected in battery 9V and the robot frog model used to experiment. The servo-motors are inside of artificial rock and the playback

calls were emitted by speaker. A – Zoom in model frog, B - model top view and C – *Hylodes heyeri* male and model frog.

We synthesized male vocalizations from male recordings of the previously year population to produce the acoustic signal used in the experiment (Fig. 1). All vocalizations were averages of advertisement and territorial calls based on 3 calls per male (N=39 calls, 13 males) and 5 calls per male (N=35 calls, 7 males), respectively (Fig. 3). We observed that *H. heyeri* alternates between advertisement and territory calls during agonistic interactions, because this we built the playback with both calls. The synthesized advertisement calls have a dominant frequency of 4578 Hz, 1.7 s duration, 36 notes per call and represent a male with an average of 38 mm snout–vent length (range 36.7–41.7mm). As well the territorial calls have a dominant frequency of 4259 Hz, call duration of 0.25 s and 4 notes per call.

We recorded the road traffic noise, at January 24th of 2019, during 5 minutes at 1 hour intervals from 7:00 a.m to 7:00 p.m (which corresponds to *Hylodes heyeri* activity time). For this, we used a directional microphone (Yoga HT-81) with a Zoom H2n Handy Recorder, at a distance of 10 m from the highway (BR-116 – Brazilian highway, 25°26'52.99" S; 49°14'7.82" W) at a 1 m height with default recording settings (44.1 kHz/24 bit sampling rate, and wav file format). Also, we measured the peak of sound pressure level (SPL, dB re. 20 μ Pa) at 50 m from road during 5 minutes at 1:00 p.m (the traffic peak time) using a Minipa MSL-1325A sound level meter (C - weighted). From the road recordings, we selected randomly several sections of different time recordings and mixed together and normalized each one to common peak amplitude of 78 dB, which correspond to the expected amplitude at 50m with low frequency range 500Hz-2kHz (Audacity Team 2015). This intensity was tested previously and has ability to interfere in acoustic parameters of this species. We created six-minute treatments with the following structure: two minutes of sound followed by two minutes of silence and then another two minutes of sound in Audacity® 2.1.0 (Audacity Team, 2015). The aim of our study was to test how road traffic noise affects the receiver's response during the agonistic behavior, then the first treatment was only a synthesized male vocalization (Call treatment) while the second included synthesized male vocalization and road traffic noises (78 dB, Call + Noise treatment) taken from our recordings at the highway (Fig. 3). The treatments were randomized for each resident male, in this way we expect that males respond with more frequency signals to the model frogs with road traffic noise independently of order. The visual behaviors were emitted in both treatments, so the experiment simulates a multimodal model intruder of *H. heyeri*.

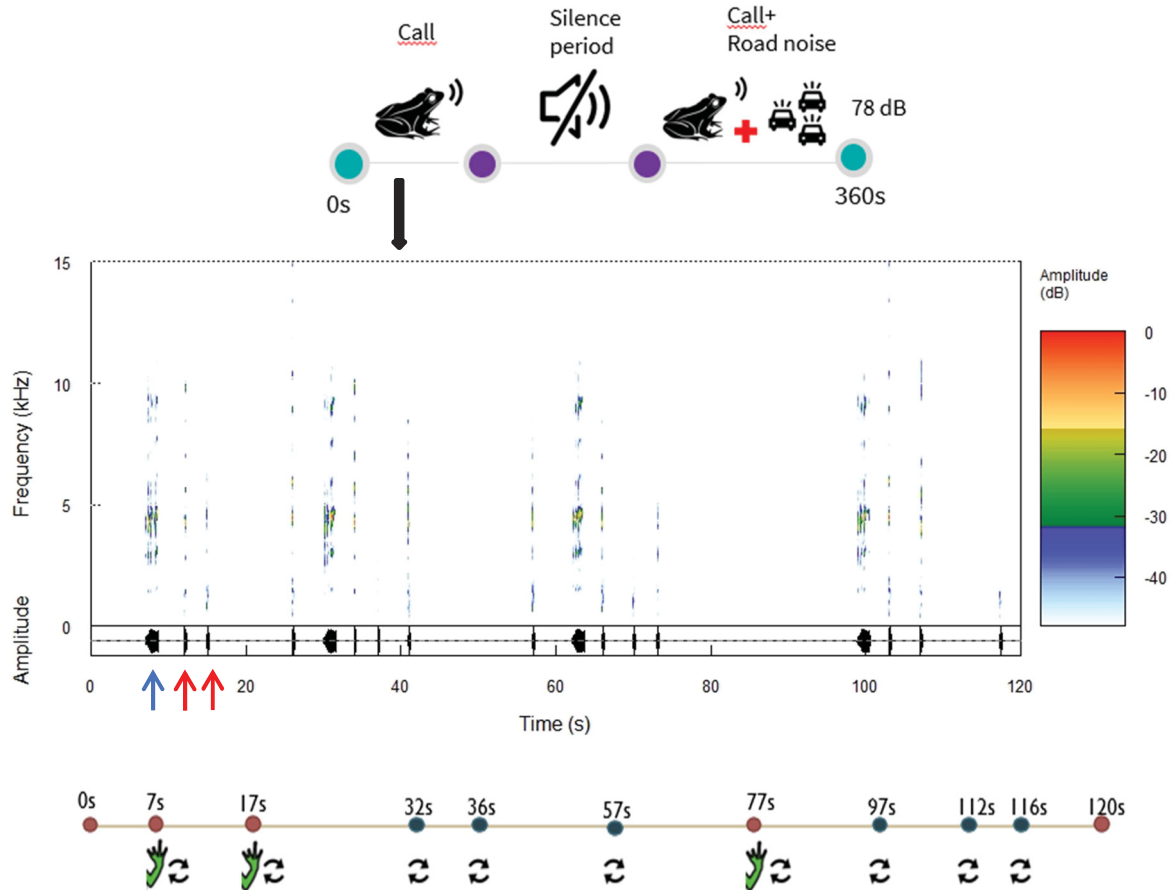


Figure 3. Playback design: auditory (spectrogram and oscillogram) and visual signals emitted by a robot frog model during two minutes of each treatment in each experiment. The larger waveform in oscillogram is advertisement calls (blue arrow) and the short is territorial calls (red arrow). The vocal sacs movement occurred simultaneously with calls playback, the arm elevations are represented by arm illustration and platform rotation by circle arrows in order of timeline. The order of treatments was alternated for each individual subject.

13.3 EXPERIMENTAL DESIGN

Prior to applying any treatment, we first found a continuously vocalizing male at least 5 m from any other individual to avoid male interference. We then placed the frog robot with loudspeaker, camera (Canon PowerShot SX520 HS) and sound recorder in front of the active frog (30-50 cm, Fig. 4). Another speaker (JBL Charge 3, frequency response = 65 Hz–20 kHz) was placed on either side of the frog, at least 1 m (distances were measured with a laser distance meter) from the active frog for road traffic noise playback. We initiated the experiment by simultaneously turning on all equipment, all of which was controlled by Bluetooth from a cell phone, except the servo motor. Road traffic noise amplitude was set at 78 dB for 1 m and the call stimulus was set at 75 dB at 1 m using a sound level meter (Minipa

MSL-1325A). The playback sound of road noise and vocalization were emitted separately in different speakers. The model frog with call speaker and microphone were placed in front and at the same height as the focal male, while the speaker with road traffic noise was placed at 45° angle from others equipment. The treatments were alternated for each individual tested. After each trial, we captured and marked the frogs with color-coded waist bands (Narvaes and Rodrigues, 2005) to avoid pseudoreplication.

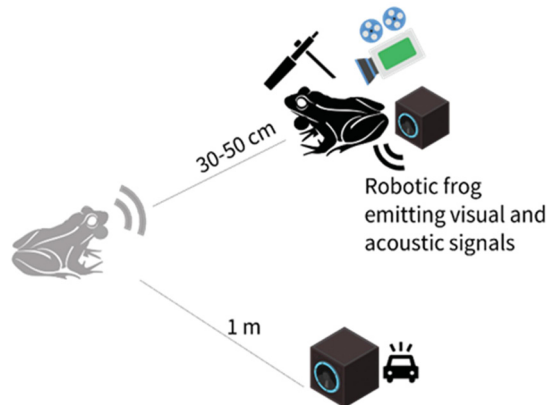


Figure 4. Experimental design of playback trials in the field. The gray frog indicates the test subject resident male, and the black frog indicate the frog robot.

13.4 DATA ANALYSIS AND STATISTICS

We have tested 42 individuals in the field. We excluded from analysis males that did not respond to the stimulus ($N = 1$), records with a presence of a female detected during the experiment ($N=2$) and videos or audios with low quality ($N = 5$) were excluded. A final sample size of 34 responsive males was used to measure 1381 calls in Raven Pro 1.5.0 software (Bioacoustics Research Program, 2011). All acoustic parameters were measured with a sampling rate of 44.1 kHz, 16-bits, FFT size of 1024 and 75% Overlap. Also, we remove the background noise below 1000 Hz with a Band filter. We measured the following four parameters in all calls emitted in each treatment: call rate ($\text{calls}^{-1\text{min}}$), call duration (in seconds), call interval (in seconds) and dominant frequency (Hz). This terms and definitions were based by Köhler et al. 2017. Video recordings were analyzed and the visual signals emitted by the resident male as well as the number of each movement: body, body jerking, arm, fingers and legs during the trial interval were counted by the same person. Latency (perception and response of resident) was measured as the difference between the times we turned on the equipment to the time that the individual first responded, with either visual or vocal signals.

We tested whether a total frequency of visual signals are different between treatments using an analysis of variance (ANOVA). Also, we ran linear mixed models (LMM) with acoustic parameters as response variables and treatments (Call + Noise and Call) as explanatory variables. Individuals were set as random effects added to the main regression (a random intercept model). For each model, the analysis was performed using *lmer* function in the *lme4* package (Bates et al., 2016), the *anova* function to obtain the F statistic of the *car* package and the *emmeans* package for posteriori tests (Lenth and Lenth, 2018). All analysis was performed in R (R Core Team 2018).

14 RESULTS

A total of 34 individuals were tested and no one rise the rate of the visual signal emitted in the road traffic noise treatment (ANOVA: $F_{1,59} = 0.24$, $P = 0.625$). Overall, individuals emitted 10.7 ± 7.85 (mean \pm SD, $N = 28$) visual signals in Call treatment and 11.7 ± 7.72 (mean \pm SD, $N = 33$) in Call + Noise treatment. Similarly, the time to the resident to respond to the intruder model frog (latency) is independent of the first treatment tested because the treatment were randomized ($F_{1,32} = 0.63$, $P = 0.432$), but males took a longer time to respond to the Call treatment (37.8 ± 15.2 s, $N=18$) than did to the Call + Noise treatment (32.6 ± 22.7 s, $N=16$).

All males emitted only territorial calls in response to the robotic frog. Our analyses revealed significant main effects of road traffic noise in acoustic parameters of resident *H. heyeri* during the agonistic interactions (ANOVA: dominant frequency: $F_{1,31} = 4.48$, $P < 0.01$; call duration: $F_{1,31} = 0.014$, $P = 0.9036$; call rate: $F_{1,33} = 8.93$, $P < 0.005$ and interval call: $F_{1,31} = 7.53$, $P = 0.004$). During the road noise treatment, the call duration was the only parameter that not changes with noise. Then, males tend to increase their dominant frequency in on average of 191 Hz (least-squares means contrast), increase 3 calls per minute (call rate) and decrease interval calls on average 5.6 s (Table 1, Fig. 5). Beyond that, the call duration is similar between the treatments.

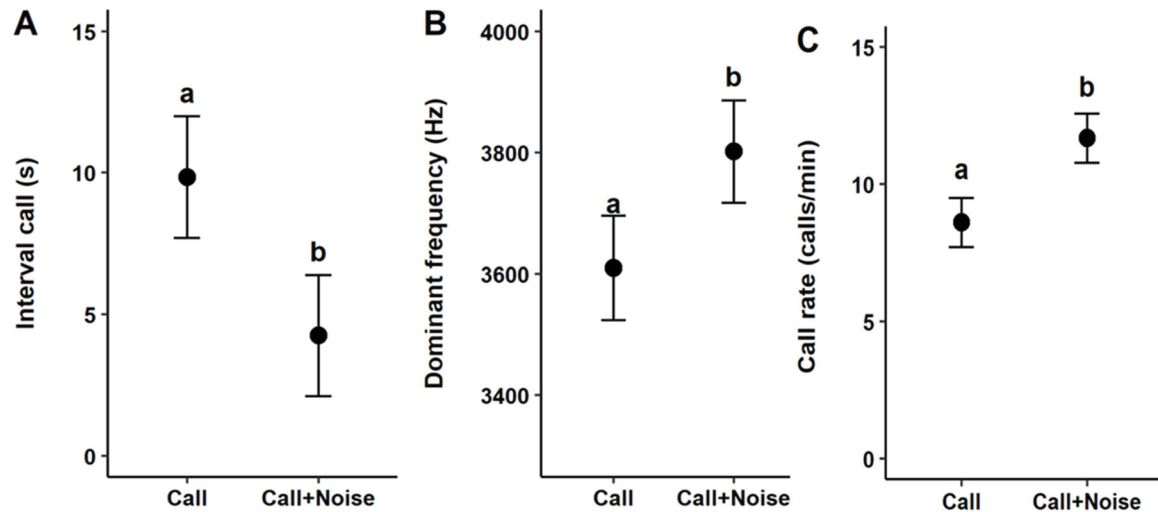


Figure 3. Response of males (Least-squares model predicted means and standard error) to playback of robotic male intruder in each treatment. **A** interval call, **B** dominant frequency and **C** call rate. Different letters above indicate statistically significant pairwise differences ($P < 0.05$).

Table 1. Results of post hoc contrast tests of the linear mixed model used to test the effect of road traffic noise in call parameters of *H. heyeri* during agonistic interactions. Males (N=34) were exposed to two treatments of 2 min each separated by two minutes of silence, totalizing 6 min. P value with statistical significance contains the stars.

Acoustic parameters	Contrast	Estimate	SE	t ratio	P value
Call rate	Call – Call + Noise	-3.07	1.03	-2.989	0.0052*
Call duration	Call – Call + Noise	0.001	0.011	-0.122	0.9036
Dominant frequency	Call – Call + Noise	-191	90.4	-2.118	0.0422*
Interval call	Call – Call + Noise	5.6	1.8	3.108	0.0040*

15 DISCUSSION

Our study shows that acoustic and visual signals are not functionally linked in the *Hylodes heyeri* mainly during agonistic interactions and noise presence. As well, this study is the first to consider responses of receivers in presence of anthropogenic noise and the multimodal signals. A personal observation in our experiments was that without presence of another individual closer of resident, the male not emit visual signals, probably because these signals are associated with response at short distances, for individuals to see and receive this signal. Surprisingly, the resident males did not show significant differences in latency to detect an intruder in presence or absence of road traffic noise. Then, the synthetic call was sufficient to evoke an agonistic response by males and the signal was transmitted independently of road noise playback. Beyond that, our results suggest that road traffic noise impaired territorial communication in *Hylodes heyeri*. However, it influences only the acoustic signal, not affecting visual communication of the Brazilian torrent frog during agonistic interactions.

The torrent frog uses two common strategies to cope with the masking effect of noise (anthropogenic or natural), an increase in the dominant frequency and an increase in the repetition of the signal. Both strategies suggest that road noise increases the threshold of acoustic signal detection. For example, *Batrachyla taeniata* (Batrachylidae) increases its advertisement call rate when exposed to four abiotic noises: wind, creek, rain, and band-pass noise (Penna and Zúñiga, 2014). Also, *Andinobates bombetes* (Dendrobatidae) raise their advertisement call dominant frequency on average of 130.76 Hz in the presence of a roadway noise (Jiménez-Vargas and Vargas-Salinas, 2021). All these results are evidence of background noise effects on courtship and territorial behavior in anurans. Specifically for *Hylodes heyeri* territorial behavior maintains the spatial structure in river populations and so breaking this kind of communication can have profound effects on population structure. Also, *H. heyeri* lives in noisy streams, their dominant frequencies are higher than the other frogs that do not live in streams (Carvajal-Castro and Vargas-Salinas, 2016). These findings indicate that anthropogenic noise associated with stream noise does not mask the responses during agonistic behavior.

Another way to enhance communication in noisy environments is to rely in multimodal communication, that is to combine acoustic signals with visual signals or others signals to improve the signal to noise ratio (Endler, 1992; Hödl and Amézquita, 2001; Narvaes and Rodrigues, 2005). Although *H. heyeri* has an extensive visual repertoire

(Beltramin, 2014) as well as other species of *Hylodes* (Haddad and Giaretta, 1999; De Sá et al., 2016), visual signals were not an additional strategy used by *H. heyeri* to compensate noise because the frequency of these signals, although small, did not change significantly between treatments. Considering the multimodal communication there are three groups of hypothesis associated with the presence of these signals: content-based, efficacy-based and inter-signal interaction hypothesis (Hebets and Papaj, 2005). The inter-signal interaction hypotheses states that the initial signal alerts the receptor to subsequent signals, for example, in *Staurois parvus* (Ranidae), another stream species, the advertisement call is an alert signal that elicits subsequent foot flagging behavior (Grafe et al., 2012). Another possibility to explain why visual signals did not change in the road noise treatment is a decision strategy, where resident males observe each movement of the opponent to acquire some information about their fighting abilities and if decided to challenge to a conflict (Enquist and Leimar, 1983; Owen and Gordon, 2005). Also, this decision is influenced by resource dynamics and intruder pressure (Hinsch and Komdeur, 2017). Overall, it is necessary to acquire more detailed information about natural history to set up experiments to test the signal function of *H. heyeri*.

In conclusion, our experimental study presents the visual and acoustic behaviors of males' residents of *H. heyeri* in presence of road traffic noise playback. Our procedure could be applied to others species and with different social contexts. The findings of this study indicate the importance of short-terms acoustic strategy for defense and maintenance of territory and calling site. Moreover, these findings may help to elucidate gaps in the understanding of the behavior and ecology of Neotropical anurans affected by anthropogenic noises.

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18 CONSIDERAÇÕES FINAIS

Os impactos ambientais, como a fragmentação do habitat, queimadas e poluições, tem se intensificado nas últimas décadas devido ao crescimento urbano e avanço tecnológico. A poluição sonora pode ser considerada um risco invisível, porque possui efeitos em sua maioria negativos que não são visuais e, que atuam no âmbito fisiológico, biológico que se escalonam em diferentes níveis desde populacionais até de comunidade. Considerando que os ruídos antropogênicos e naturais impactam os animais, os efeitos mais investigados destes ruídos são no contexto de: como os anuros alteram o comportamento vocal, seguido do comportamento reprodutivo (escolha de parceiros) ou de sua combinação.

Os experimentos realizados com a rã-de-corredeira *Hylodes heyeri* para avaliar os efeitos do ruído de estrada em dois contextos sociais mostraram que os machos possuem plasticidade comportamental na vocalização. No contexto reprodutivo, os machos de *H. heyeri* alteram as vocalizações de anúncio conforme a distância da estrada. Enquanto que no contexto territorial, o ruído foi capaz de alterar o comportamento acústico, mas não alterou o comportamento de sinal visual da espécie. Estes resultados indicam que mesmo a espécie sendo adaptada a viver em ambiente naturalmente ruidoso (riachos), os machos modificam principalmente o sinal acústico mesmo a curta distância de outro indivíduo. Sendo assim, a presença do ruído antropogênico causa uma perturbação no comportamento dos indivíduos que pode acarretar em um aumento no custo energético e até de risco de predação. Espero que este trabalho forneça uma base e ajude a elucidar futuros estudos na área de ruído de fundo e na comunicação de anuros.

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20 APÉNDICE

Study	Species	Family	Country	Noise sources	Noise type	Aim study	IUCN status	Study type	Method
Narins (1982)	<i>Eleutherodactylus coqui</i>	Eleutherodactylidae	Puerto Rico	Natural	Background	vocal behaviour	LC	Field	Playback
Tuttle and Ryan (1982)	<i>Smilisca sila</i>	Hylidae	Panama	Natural	Waterfall	foraging behaviour	LC	Field	Playback
Brattstrom and Bondello (1983)	<i>Scaphiopus couchi</i>	Scaphiopodidae	USA	Anthropogenic	Traffic	movement behavior	LC	Lab	Playback
Gerhardt and Klump (1988)	<i>Hyla cinerea</i>	Hylidae	Georgia	Natural	Chorus noise conspecific	mating behaviour	LC	Lab	Playback
Wollerman (1998)	<i>Dendropsophus ebraccatus</i>	Hylidae	Costa Rica	Natural	Chorus noise conspecific	mating behaviour	LC	Lab	Playback
Parris (2002)	<i>Pseudacris crucifer</i>	Hylidae	USA	Natural	Chorus noise conspecific	vocal behaviour, movement behaviour	LC	Modeling	Modeling
Wollerman and Wiley (2002)	<i>Dendropsophus ebraccatus</i>	Hylidae	Costa Rica	Natural	Chorus noise conspecific	mating behaviour	LC	Lab	Playback
Castellano et al (2003)	<i>Bufo viridis</i>	Bufo	Kazakhstan	Natural	Abiotic and biotic	vocal behaviour	LC	Field	Playback
Sun and Narins (2005)	<i>Rana taipehensis</i>	Ranidae	Thailand	Anthropogenic	Airplane and motorcycle	vocal behaviour	LC	Field	Playback
Pennell et al (2005)	<i>Eupsophus calcaratus</i>	Alsodidae	Chile	Natural	Wind, rain, creeks, surf noise with conspecific advertisement call	vocal behaviour	LC	Field	Playback
Bee and Swanson	<i>Hyla chrysosceli</i>	Hylidae	USA	Natural and	Road and	mating behaviour	LC	Lab	Playback

(2007)	s			Anthropogenic	chorus noise conspecific				
Penna and Hamilton-West (2007)	<i>Eupsophus emiliopugini</i>	Alsodidae	Chile	Natural	Wind, rain, creeks, surf noise with conspecific advertisement call	vocal behaviour	LC	Field	Playback
Lengagne (2008)	<i>Hyla arborea</i>	Hylidae	France	Anthropogenic	Road	vocal behaviour	LC	Lab	Playback
Feng and Narins (2008)	<i>Odorrana tormota</i>	Ranidae	China	Natural	Stream	physiological and mating behaviour	VU	Field	Natural experiment
Kaiser and Hammers (2009)	<i>Dendropsophus triangulum</i>	Hylidae	Peru	Anthropogenic	Motorcycle engine and music	vocal behaviour	LC	Field	Playback
Parris et al (2009)	2 spp	Hylidae , Myobatrachidae	Australia	Anthropogenic	Road	vocal behaviour	LC	Field	Modeling
Preininger et al (2009)	<i>Staurois latopalmatius</i>	Ranidae	Malaysia	Natural	Stream	visual behaviour, vocal behaviour	LC	Field	Natural experiment
Boeckle et al (2009)	<i>Staurois Latopalmatius</i>	Ranidae	Borneo	Natural	Stream	vocal behaviour	LC	Field	Natural experiment
Love and Bee (2010)	<i>Hyla chrysoscelis</i>	Hylidae	USA	Natural	Chorus noise conspecific	vocal behaviour	LC	Lab	Playback
Cunnington and Fahrig (2010)	4 spp	Ranidae, Hylidae , Bufonidae	Canada	Anthropogenic	Traffic	vocal behaviour	NA	Field	Playback

Hoskin and Goosem (2010)	2 spp	Microhylidae, Pelodyadidae	Australia	Anthropogenic	Traffic	vocal behaviour; population			
Kaiser et al (2011)	7 spp	Bufoidea, Phyllomedusidae, Hylidae	Belize	Anthropogenic	Traffic	ecosystem	LC	Field	Playback
Herrera-Montes and Aide (2011)	6 spp	Eleutherodactylidae, Leptodactylidae, Ranidae	Puerto Rico	Anthropogenic	Traffic	ecosystem	LC	Field	Natural experiment
Both and Grant (2012)	<i>Hypsiboas albomarginatus</i>	Hylidae	Brazil	Natural	Chorus noise heterospecific (<i>Lithobates catesbeianus</i>)	vocal behaviour	S	Field	Playback
Grafe et al (2012)	<i>Staurois parvus</i>	Ranidae	Borneo	Natural	Stream	vocal behaviour, visual behaviour	VU	Field	Playback
Vargas-Salinas and Amézquita (2013)	<i>Andinobates bombetes</i>	Dendrobatidae	Colombia	Anthropogenic	Road, agriculture field	vocal behaviour, movement behaviour, population	VU	Field	Natural experiment
Preininger et al (2013)	<i>Micrixalus saxicola</i>	Micrixalidae	India	Natural	Stream	vocal behaviour, visual behaviour	VU	Field	Playback
Moreno-Gomes (2013)	<i>Eupsophus roseus</i>	Alsodidae	Chile	Natural	Abiotic and biotic noise	mating behaviour	NT	Lab	Playback
Vargas-Salina and Amezquita (2013)	2 spp	Dendrobatidae	Colombia	Natural	Stream	vocal behaviour	CR	Field	Natural experiment
Vargas-Salinas et al	5 spp	Bufoidea,	Canada	Anthropogenic	Road	vocal behaviour	LC	Field	Natural experiment

(2014)		Hylidae , Ranidae		c					nt
Troianowski et al (2014)	<i>Hyla arborea</i>	Hylidae	France	Anthropogenic	Road	mating behaviour, vocal behaviour, visual behaviour	LC	Lab	Playback
Lukanov et al (2014)	<i>Pelophylax ridibundus</i>	Ranidae	Bulgaria	Anthropogenic	Anthropogenic	vocal behaviour, movement behavior	LC	Lab and Field	Playback and Natural experiment
Caldwell and Bee (2014)	<i>Hyla chrysoscelis</i>	Hylidae	USA	Natural	White noise	mating behaviour	LC	Lab	Playback
Penna and Zúñiga (2014)	<i>Batrachyla taeniata</i>	Batrachylidae	Chile	Natural	Wind, rain, creeks, band-pass noise	vocal behaviour	LC	Field	Playback
Vargas-Salina et al (2014)	<i>Andinobates bombetes</i>	Dendrobatidae	Colombia	Natural	Stream	vocal behaviour, population	VU	Field	Natural experiment
Hanna et al (2014)	<i>Pseudacris crucifer</i>	Hylidae	Canada	Natural	White noise	vocal behaviour	LC	Field	Playback
Tennessee et al (2014)	<i>Lithobates sylvaticus</i>	Ranidae	USA	Anthropogenic	Traffic	physiological	LC	Lab	Playback
Bleach et al (2015)	2 spp	Hylidae , Myobatrachidae	Australia	Natural	Chorus noise heterospecific (<i>Rhinella marina</i>)	vocal behaviour	LC	Field	Playback
Höbel (2015)	<i>Hyla cinerea</i>	Hylidae	USA	Natural	Chorus noise heterospecific	mating behaviour	LC	Lab	Playback
Engbrecht et al (2015)	<i>Lithobates areolatus</i>	Ranidae	USA	Natural and Anthropogenic	Chorus noise and Airplane	vocal behaviour	NT	Field	Playback

Kaiser et al (2015)	<i>Litoria_cerulea</i>	Pelodyadidae	USA	Anthropogenic	Traffic	physiological	LC	Lab	Playback
Halfwerk et al (2016)	<i>Physalaemus pustulosus</i>	Leptodactylidae	Panama	Natural	Chorus noise conspecific	vocal behaviour	LC	Lab	Playback
Caldart et al (2016)	<i>Crossodactylus schmidti</i>	Hylodidae	Brazil	Natural	Stream	vocal behaviour	NT	Field	Natural experiment
Goutte et al (2016)	37 spp	Ranidae	Southeast Asia	Natural	Stream	ecosystem		Modeling	Modeling
Tarano and Carballo (2016)	<i>Eleutherodactylus johnstonei</i>	Eleutherodactylidae	Venezuela	Natural	Chorus noise conspecific	mating behaviour	LC	Field	Natural experiment
Krueger and Preez (2016)	<i>Hyperolius pickersgilli</i>	Hyperoliidae	South Africa	Anthropogenic	Airplane	vocal behaviour	EN	Field	Natural experiment
Carvajal-Castro and Vargas-Salinas (2016)	38 spp	Bufonidae, Dendrobatidae, , Centrolenidae, Hylidae, , Hemiphractyidae, Craugastoridae, Leptodactylidae, Ranidae	Panama	Natural	Stream	ecosystem	NA	Modeling	Modeling
Troianowski et al (2017)	<i>Hyla arborea</i>	Hylidae	France	Anthropogenic	Road	physiological, visual behaviour	LC	Lab	Natural experiment
Caorsi et al (2017)	2 spp	Hylidae	Brazil	Anthropogenic	Road	vocal behaviour	LC	Field	Playback
Grace and Noss (2017)	<i>Anaxyrus quercicus</i>	Bufonidae	USA	Anthropogenic	Road	vocal behaviour, population	LC	Field	Playback

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Vélez and Gordon et al (2017)	2 spp	Hylidae	USA	Natural	Road	vocal behaviour	LC	Field	Natural experiment
Kollarits et al (2017)	<i>Allobates talamancae</i>	Dendrobatidae	Costa Rica	Natural	Natural noise (reconhecimento de conspecíficos devido chorus noise heterospecific - Silverstoneia flotator)	mating behaviour	LC	Field	Playback
Zhao et al (2017)	<i>Amolops torrentis</i>	Ranidae	China	Natural	Stream	mating behaviour	VU	Lab	Playback
Lee et al (2017)	<i>Hyla chrysoscelis</i>	Hylidae	USA	Natural	Chorus noise	physiological, mating behaviour	LC	Lab	Natural experiment
Nelson et al (2017)	<i>Pseudacris regilla</i>	Hylidae	USA	Anthropogenic	Anthropogenic	vocal behaviour	LC	Field	Modeling (Spatiotemporal communication model)
McMahon et al (2017)	<i>Engystomops pustulosus</i>	Leptodactylidae	Panama	Natural and Anthropogenic	Natural and Anthropogenic noise	population	LC	Field	Natural experiment
Senzaki et al (2018)	<i>Rana pirica</i>	Ranidae	Japan	Anthropogenic	Road	mating behaviour vocal behaviour	LC	Lab (arena)	Playback
Goutte et al (2018)	79 species	Ranidae, Rhacophoridae, Dicoglossidae	Asia	Natural	Stream	ecosystem		Modeling	Modeling

		Microhylidae							
Augusto-Alves et al (2018)	<i>Megaelosia apuana</i>	Hylodidae	Brazil	Natural	Stream	visual behaviour, mating behaviour	DD	Field	Natural experiment
Tennessee et al (2018)	<i>Lithobates sylvaticus</i>	Ranidae	USA	Anthropogenic	Traffic	physiological	LC	Lab	Playback
Zhao et al (2018)	<i>Amolops torrentis</i>	Ranidae	China	Natural	Stream	vocal behaviour	VU	Field	Playback and Natural experiment
Nakano et al (2018)	<i>Hyla japonica</i>	Hylidae	Japan	Anthropogenic	Traffic	movement behavior	LC	Field	Playback
Grenat et al. (2019)	<i>Odontophrynus americanus</i>	Odontophrynidae	Argentina	Natural and Anthropogenic	Low noise, biological noise, atmospheric noise	vocal behaviour	LC	Field	Natural experiment
Leon et al (2019)	<i>Scinax nasicus</i>	Hylidae	Argentina	Anthropogenic	Traffic	vocal behaviour	LC	Field	Natural experiment
Yi and Sheridan (2019)	<i>Kurixalus appendiculatus</i>	Rhacophoridae	Borneo	Anthropogenic	Traffic	vocal behaviour	LC	Field	Natural experiment
Lukanov and Naumov (2019)	<i>Hyla arborea</i>	Hylidae	Bulgaria	Anthropogenic	Traffic	vocal behaviour	LC	Field	Natural experiment
Gutiérrez-Vannucchi et al (2019)	<i>Hyalinobatrachium fleischmanni</i>	Centrolidae	Costa Rica	Anthropogenic	Traffic	vocal behaviour	LC	Field	Natural experiment
Legett et al (2020)	<i>Buergeria japonica</i>	Rhacophoridae	Japan	Anthropogenic	Traffic	vocal behaviour	LC	Field	Playback
Legett et al (2020)	<i>Buergeria japonica</i>	Rhacophoridae	Japan	Natural	Chorus noise conspecific	vocal behaviour	LC	Field	Playback
Estrela et al (2020)	<i>Scinax nebulosus</i>	Hylidae	Brazil	Natural	Chorus noise	movement behavior	LC		Playback

					consp cific				
Higham et al (2021)	<i>Litoria ewingii</i>	Pelodry adidae	Australia	Anthro pogeni c	Road	vocal behaviour	LC	Field	Natural experime nt
Legett et al (2021)	<i>Buergeria japonica</i>	Rhacop horidae	Japan	Natural	Chorus noise consp cific	mating behaviour	LC	Field	Playback
Schou et al (2021)	<i>Litoria fallax</i>	Pelodry adidae	Australia	Anthro pogeni c	Traffic	mating behaviour	LC	Lab	Playback
Zhao et al (2021)	4 spp	Dicrogl ossidae , Microh ylidae, Ranida e, Rhacop horidae	China	Anthro pogeni c	Airplan e	vocal behaviour	LC	Field	Natural experime nt