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**TRAFFIC NOISE AND DANCING FROGS: EFFECTS OF NOISE ON THE
ADVERTISEMENT CALL AND VISUAL DISPLAYS IN A NATURALLY NOISY
ENVIRONMENT**

PORTO ALEGRE

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Dissertação/Tese apresentada ao Programa de Pós- Graduação em Biologia Animal, Instituto de Biotecnologia da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre/Doutor em Biologia Animal.

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Aprovada em _____ de _____ de _____.

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RESUMO

Diversos fatores relacionados às atividades humanas têm causado impactos negativos sobre a biodiversidade. A urbanização crescente do planeta tem intensificado muitos destes fatores, incluindo o aumento da poluição sonora, que afeta diretamente e indiretamente diversas espécies de vertebrados e invertebrados. Ruídos antrópicos, como os produzidos por veículos automotores podem afetar a comunicação sonora em anfíbios anuros. Contudo, a intensidade e extensão desse problema ainda são pouco compreendidos. Nesse estudo testamos o efeito do ruído de veículos em estradas através de experimento de “playback” em uma espécie com comunicação acústica e visual que habita ambientes naturalmente ruidosos. A espécie deste estudo (*Hylodes meridionalis*) habita córregos e cascatas com água corrente, possui frequências de canto sobrepostas às frequências do ruído e sete sinalizações visuais descritas. Os resultados deste estudo mostraram que emissões do ruído de tráfego causam alterações nas emissões de sinais acústicos e visuais dos machos. A taxa média de canto de *H. meridionalis* diminuiu 11,8% em comparação com o pré-estímulo. A duração do canto teve um aumento médio de 8,98% durante o tratamento em comparação com o pré-estímulo. Além disso, houve um intervalo menor entre as notas do canto em comparação com o pré-estímulo. Foi observado aumento no número de sinais visuais durante o tratamento em comparação ao pré-estímulo: na soma de todos os sinais visuais, *arm lifting*, *toe flagging* e *leg lifting*. Estes resultados destacam a importância de realizar estudos comportamentais *in situ*, considerando os efeitos do ruído do tráfego em anuros com diferentes modos de comunicação, bem como na sinalização visual.

Palavras-chave: *Hylodes meridionalis*; Parâmetros acústicos; Ruído antropogênico; Sinais visuais.

ABSTRACT

Several factors related to human activities has negative impacts on biodiversity. The growing urbanization of the planet has intensified many of these factors, including increased noise pollution, which directly and indirectly affects various vertebrate and invertebrate species. Anthropogenic noises, such as those made by motor vehicles, can affect sound communication in anuran amphibians. However, the intensity and extent of this problem is still poorly understood. In this study, we tested the effect of vehicle noise on the roads through the playback experiment in a species with acoustic and visual communication that inhabits naturally noisy environments. The species of this study (*Hylodes meridionalis*) inhabit streams and waterfalls with running water, have call frequencies that overlap with noise frequencies and seven visual signals described. The results of this study showed that traffic noise emissions cause changes in male acoustic and visual signal emissions. The average call rate of *H. meridionalis* decreased by 11.8% compared to the pre-stimulus. Call length had an average increase of 8.98% during treatment compared to pre-stimulus. There was an increase in the number of visual signals during treatment compared to pre-stimulus: in the sum of all visual signals, arm lifting, toe flagging and leg lifting. Our results highlight the importance of conducting behavioral studies in situ, considering the effects of traffic noise on frogs with different modes of communication, as well as on visual signaling.

Keywords: Anthropogenic noise; Call parameters; *Hylodes meridionalis*; Visual signals.

INTRODUÇÃO

Durante o século XX, a população humana mais que triplicou e atualmente existem por volta de 7,7 bilhões de pessoas no mundo (Schwart & Bee 2013; ONU 2019). Enquanto isso, as populações de animais vertebrados do planeta tiveram, entre os anos 1970 e 2005, declínio de aproximadamente 30% e aumento nas taxas de extinção (Hails *et al.* 2008; Vié *et al.* 2008). Os declínios são atribuídos a diversos fatores, especialmente a interferência humana, como: alteração de habitat, sobre-exploração, introdução de espécies invasoras, mudanças climáticas, doenças e poluição (química, luminosa e sonora). (Marzluff *et al.* 2008; Dood Jr 2009; Stuart *et al.* 2008; Brumm 2010; Shanahan *et al.* 2014; Ceballos *et al.* 2015).

A crescente urbanização do nosso planeta tem aumentado a quantidade de tráfego (local e global), afetando direta e indiretamente diversos grupos de animais, vertebrados e invertebrados (Brumm 2010). Estudos identificaram muitas perturbações ecológicas pelos efeitos de estradas, como perda e fragmentação de habitats, poluição, mortalidade por atropelamento e interferência acústica, o que torna cada vez mais preocupante a crescente proporção de estradas que cobrem nosso planeta (Forman *et al.* 2003; Warren *et al.* 2006; Parris *et al.* 2009). O ruído do tráfego de veículos automotores pode se estender mais de quatro quilômetros da margem da estrada dependendo do volume e da velocidade do trânsito, e das condições climáticas (Parris *et al.* 2009; Department of Transport 1988).

Estudos recentes têm indicado que o ruído do tráfego tem efeitos, como estresse e o mascaramento do sinal acústico, sobre diversos táxons, como insetos, peixes, aves, anfíbios e mamíferos (Brumm 2010; Bruum 2013; Herrera-Montes & Aide 2011). Em diversas espécies a comunicação acústica está diretamente ligada à seleção sexual e a reprodução (Wells 1977; Bradbury & Vehrencamp 1998; Gerhardt & Huber 2002; Rheindt 2003; Narins *et al.* 2006), podendo também ser utilizada para outras funções, como a defesa de território, alerta de predadores, socialização, localização de presas ou orientação espacial (Hollén & Radford 2009; Nogueira *et al.* 2012; Reby *et al.* 1999; Rheindt 2003). A interferência na comunicação de invertebrados e vertebrados causada por ruídos pode afetar diretamente a fisiologia reprodutiva, por exemplo, pela perda de oportunidades de acasalamento, perda das chances de obtenção de alimentos, interações agressivas desnecessárias ou outros comportamentos atípicos, resultando em aumento do gasto energético (Bruum 2013; Edge & Marcum 1985; Krausman *et al.* 1986; Bradbury & Vehrencamp 1998). Ações que podem causar alterações da distribuição territorial e/ou declínios populacionais (Fahrig *et al.* 1995; Brumm 2010; Vos & Chardon 1998).

Os anuros são considerados organismos modelo para estudar os mecanismos, a função e a evolução da comunicação sonora animal, uma vez que respondem rapidamente a variações sonoras no meio. Eles possuem especializações morfológicas e fisiológicas que permite emitir e detectar sinais de diversas frequências, incluindo ultrassons e vibrações sísmicas (Vélez *et al.* 2013; Feng *et al.* 2006; Narins 1990; Wells

2007). Os machos desse grupo cantam para atrair as fêmeas, essa vocalização é um critério utilizado pelas fêmeas na seleção do parceiro (Wells 2007). Também, foram descritas para anuros várias exibições visuais em diferentes contextos sociais (Hödl & Amézquita 2001; Biju *et al.* 2014; Furtado *et al.* 2017). Por exemplo, em Hylodidae, os movimentos dos membros e a elevação da postura são as exibições visuais mais relatadas durante interações intraespecíficas agressivas e / ou reprodutivas (Caldart *et al.* 2014; Forti & Castanho 2012; de Sá *et al.*, 2016).

Sabe-se que o efeito do ruído de tráfego de automóveis altera o comportamento de canto de algumas espécies de anuros (Bee & Swanson 2007; Cunnington & Fahrig 2010; Hoskin & Goosem 2010; Kaiser *et al.* 2011; Lengagne 2008; Parris *et al.* 2009; Vargas-salinas *et al.* 2014; Caorsi *et al.* 2017). Estudos sobre o efeito do ruído antropogênico apontam que anuros podem utilizar estratégias alternativas para tentar contornar o mascaramento de seu sinal acústico (Bee & Swanson 2007; Vélez *et al.* 2013). Foram observadas espécies que quando expostas a ruídos antrópicos mudaram seus parâmetros temporais e espectrais de canto (Vélez *et al.* 2013; Caorsi *et al.* 2017), podendo alterar a duração do canto ou das notas, mudar a amplitude do canto ou variar a frequência dele (Kaiser *et al.* 2011; Lengagne 2008; Cunnington & Fahrig 2010; Halfwerk *et al.* 2015; Shen & Xu 2016; Hoskin & Goosem 2010; Parris *et al.* 2009). Entretanto, essas alterações podem ter consequências negativas para os indivíduos, como o aumento do custo energético e a maior exposição a predadores, além de diminuir a efetividade da transmissão e recepção do sinal, assim como o sucesso reprodutivo (Wells 2007; Bee & Swanson 2007; Mcgregor *et al.* 2013). Outras espécies adotam a estratégia de evitar a fonte do ruído, por exemplo, cantando em intervalos menos ruidosos (Vargas-salinas *et al.* 2014). Algumas estratégias estão relacionadas diretamente à intensidade do ruído, de modo que os anuros aumentam as mudanças nos parâmetros do canto quando enfrentam perturbações de alta energia (Cunnington & Fahrig 2010; Halfwerk *et al.* 2015; Shen & Xu 2016). Estudos observaram espécimes que se locomoveram para longe das fontes do ruído de tráfego em estradas, possivelmente como forma de evitar o mascaramento de seu canto (Caorsi *et al.* 2017; Lengagne 2008; Herrera-montes & Aide 2011).

O efeito do ruído do tráfego altera o comportamento de canto em alguns anuros de ambientes lênticos (Bee & Swanson 2007; Cunnington & Fahrig 2010; Hoskin & Goosem 2010; Kaiser *et al.* 2011; Lengagne 2008; Parris *et al.* 2009; Vargas-salinas *et al.* 2014; Caorsi *et al.* 2017). Entretanto espécies que vocalizam em ambientes lóticos, como riachos, carecem de informações. Shen & Xu (2016) observaram que *Odorrana tormota* (Ranidae), espécie que vocaliza em cursos d'água ruidosos, ao ser exposta a ruídos de playback altera seus padrões de canto – aumentando a amplitude e a frequência - para evitar a nova fonte de mascaramento. Em Hylodidae, foi visto que *Crossodactylus schmidti* ajusta as características de canto de acordo com o ruído de fundo, variando a frequência e amplitude do canto (Caldart *et al.* 2016). Além disso, nenhum estudo com ruído de tráfego foi realizado em espécies que possuem sinalização visual. Portanto, é importante avaliar se o ruído de tráfego afeta o comportamento de canto dos anuros de ambientes lóticos aparentemente ruidosos em espécies que possuem também o registro

de sinalização visual. A partir disso, foram realizados experimentos *in locu* com a intenção de medir os efeitos do ruído de tráfego sobre o comportamento acústico e visual da rã-das-corredeiras (*Hylodes meridionalis* - Hylodidae), espécie que habita córregos e cascatas com água corrente limpa e que possui frequências de canto sobrepostas às frequências do ruído (Garcia & Segalla 2010; Lingnau *et al* 2013). Durante o período reprodutivo (outubro-fevereiro), os machos cantam durante o dia e nas primeiras horas da noite (Kwet *et al.* 2010). Os machos são territoriais e defendem seus locais de chamada usando sinais acústicos (Lingnau *et al.* 2013). Esta espécie também é caracterizada por sinais visuais complexos para comunicação. Furtado *et al.* (2019) descreveram sete tipos de exhibições visuais para *H. meridionalis*: *toe flagging* (movimentos lentos para cima e para baixo de um ou mais dedos), *arm lifting* (movimentos rápidos para cima e para baixo de um braço), *leg lifting* (rápido movimentos para cima e para baixo de uma perna), *arm waving* (levantando um braço e agitando-o em um arco), *both legs kicking* (esticando rapidamente os dois membros posteriores em direção à parte traseira), *foot flagging* (levantando lentamente um membro posterior em uma semi movimento da arca) e *throat display* (pulsação de um ou ambos os sacos vocais laterais pareados sem produção sonora). A sobreposição espectral do canto da espécie com o ruído de tráfego pode induzir maiores mudanças comportamental (como menor taxa de canto, alteração de parâmetros do canto e/ou mais sinais visuais), consequência do efeito de mascaramento. Nossa hipótese é que, o ambiente mais ruidoso (devido o ruído de tráfego de automóveis) reduziria o número de chamadas e aumentaria o número de respostas visuais. Além disso, esperávamos que durante o estímulo houvesse alteração da duração de canto e/ou frequência de canto.

Este estudo foi realizado na Floresta Nacional de São Francisco de Paula (FLONA-SFP), sul do Brasil (29 ° 25'22,4"S; 50 ° 23'11,2"W). A FLONA-SFP possui uma área de 1607 ha, 56% coberta pela Floresta Ombrófila Mista nativa (Narvaes *et al.*, 2005; Sonogo *et al.* 2007). O clima da região é temperado super-úmido, com temperatura média anual de 14,5°C e precipitação anual superior a 2000 mm (Moreno, 1961; Backes *et al.* 2005). As experiências foram realizadas durante 11 dias não consecutivos em fevereiro de 2019.

Buscas ativas foram realizadas atrás de machos vocalizando durante a luz do dia, entre 13 e 18h. Cada indivíduo foi submetido a um modelo artificial de resina para simular a presença de um intruso no território do residente e estimular a emissão de sinais visuais. Posteriormente, era posicionado um alto-falante para emissão do tratamento (ruído do tráfego de veículos automotores) e também eram posicionados: um gravador portátil e uma câmera de vídeo para registrar o experimento.

Foram gravados 14 indivíduos e 210 minutos de áudio e vídeo. Ao todo, 593 sinais foram contabilizados, sendo 419 visuais e 174 acústicos. Dentre os sinais visuais, foram registrados seis tipos: *Leg lifting* (177), *Arm lifting* (80), *Arm waving* (57), *Toe flagging* (29), *Foot flagging* (5) e *Both legs kicking* (1). Além disso, foi registrada a mudança de posição ou local dos machos no total de 56 vezes.

Nos testes acústicos, o ruído de tráfego foi associado negativamente à taxa de canto ($r = -0,451$; $p < 0,0001$) e à duração do canto ($r = -0,256$; $p < 0,0001$). O comprimento do canto também foi correlacionado com a etapa do experimento ($p < 0,0001$), negativamente entre as etapas 1 e 2 ($r = -0,384$), negativamente entre as etapas 1 e 3 ($r = -0,384$) e positivamente entre as etapas 2 e 3 ($r = 0,500$).

Nos testes de sinais visuais, o ruído de tráfego foi associado negativamente à soma de todos os sinais visuais ($r = -0,184$; $p < 0,0001$) e *Leg lifting* ($r = -0,263$; $p < 0,0001$). A etapa foi associada à soma de todos os sinais visuais ($p < 0,0001$), negativamente entre as etapas 1 e 2 ($r = -0,307$), negativamente entre as etapas 1 e 3 ($r = -0,296$) e positivamente entre as etapas 2 e 3 ($r = 0,537$). A etapa também foi associada à sinalização *Toe flagging* ($p = 0,00593$), negativamente entre as etapas 1 e 2 ($r = -0,445$), negativamente entre as etapas 1 e 3 ($r = -0,441$) e positivamente entre as etapas 2 e 3 ($r = 0,757$). O deslocamento dos indivíduos foi associado negativamente à umidade ($r = -0,999$; $p = 0,00199$).

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CAPÍTULO 1

TRAFFIC NOISE AND DANCING FROGS: EFFECTS OF NOISE ON THE ADVERTISEMENT CALL AND VISUAL DISPLAYS IN A NATURALLY NOISY ENVIRONMENT

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TRAFFIC NOISE AND DANCING FROGS: EFFECTS OF NOISE ON THE ADVERTISEMENT CALL AND VISUAL DISPLAYS IN A NATURALLY NOISY ENVIRONMENT

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Abstract

Several factors related to human activities has negative impacts on biodiversity. The growing urbanization of the planet has intensified many of these factors, including increased noise pollution, which directly and indirectly affects various vertebrate and invertebrate species. Anthropic noises, such as those made by motor vehicles, can affect acoustic communication in anuran amphibians. However, the intensity and extent of this problem is still poorly understood. In this study, we tested the effect of vehicle noise on the roads through the playback experiment in a species with acoustic and visual communication that inhabits naturally noisy environments. The species of this study (*Hylodes meridionalis*) inhabit streams and waterfalls with running water, have call frequencies that overlap with noise frequencies and seven visual signals described. Our results showed that traffic noise emissions cause changes in male acoustic and visual signal emissions. The average call rate of *H. meridionalis* decreased by 11.8% compared to the pre-stimulus. Call length had an average increase of 8.98% during treatment compared to pre-stimulus. There was an increase in the number of visual signals during treatment compared to pre-stimulus: in the sum of all visual signals, toe flagging and leg lifting. Our results highlight the importance of conducting behavioral studies in situ, considering the effects of traffic noise on frogs with different modes of communication, as well as on visual signaling.

Keywords: Anthropogenic noise; Call parameters; *Hylodes meridionalis*; Visual signals.

Introduction

During the 20th century, the human population has more than tripled, and currently there are around 7.7 billion people in the world (Schwart & Bee 2013, UN 2019). Meanwhile, vertebrate animal populations have declined, by approximately 30%, in 35 years along with an increase in extinction rates (Hails *et al.*, 2008, Vié *et al.* 2008). These

declines are attributed to several factors, especially to human interference, such as habitat alteration, overexploitation, introduction of invasive species, climate change, diseases and pollution (e.g. chemical, light and noise) (Marzluff *et al.* 2008; Stuart *et al.* 2008; Dood Jr 2009; Brumm 2010; Shanahan *et al.* 2014; Ceballos *et al.* 2015). The growing urbanization of our planet has increased the amount of local and global traffic, directly and indirectly affecting several animals groups, in vertebrates and invertebrates (Brumm 2010; Slabbekoorn *et al.* 2018). Many ecological disturbances have been identified due to the effects of roads, such as habitat loss and fragmentation, pollution, road crash mortality and acoustic interference, which makes the increasing proportion of roads that cover our planet increasingly worrisome (Forman *et al.* 2003; Warren *et al.* 2006; Parris *et al.* 2009; Gonçalves *et al.* 2018).

The noise of motor vehicle traffic can reach more than four kilometers from the roadside, depending on the volume and speed of traffic along with weather conditions and topography (Department of Transport 1988; Parris *et al.* 2009). Research indicated that this noise stress and mask the acoustic signal of several taxa, such as insects, fish, birds, amphibians and mammals (Brumm 2010; Herrera-Montes & Aide 2011; Bruum 2013). Interference in the communication of invertebrates and vertebrates caused by noise can directly affect reproductive physiology, for example, loss of mating opportunities, loss of chances of obtaining food, unnecessary aggressive interactions or other atypical behaviors, resulting in increased energy expenditure, actions that may result in changes in territorial distribution or even in population declines (Edge & Marcum 1985; Krausman *et al.* 1986; Fahrig *et al.* 1995; Bradbury & Vehrencamp 1998; Vos & Chardon 1998; Brumm 2010; Brumm 2013).

In several species, acoustic communication is directly linked to sexual selection and reproduction. Furthermore, it is also used for defense of territory, predator alert, socialization, prey location or spatial orientation (Wells 1977, Bradbury & Vehrencamp 1998; Reby *et al.* 1999; Gerhardt & Huber 2002; Rheindt 2003; Narins *et al.* 2006; Hollén & Radford 2009; Nogueira *et al.* 2012). Communication depends on the transmission, detection and understanding of the signals, in anurans noise can have various types of effects, especially when interference from background noise has a masking effect on species signaling. Thus, it is expected that noises may have a greater effect on the communication of species that call in the same frequency spectrum (Schwart & Bee 2013; Vargas-Salinas *et al.*, 2014).

Anurans are considered model organisms for studying the mechanism, function and evolution of animal sound communication. They respond rapidly to sound variations in the environment, because they have morphological and physiological specializations that allow them to emit and detect signals of different frequencies, including ultrasound and seismic vibrations (Narins 1990; Narins 1995; Feng *et al.* 2006; Wells 2007). Males call to attract females, and the attributes of this call is used by females in the selection of the partner (Wells 2007). Therefore, acoustic interference can affect sexual behavior and reproductive success, since background noise may limit the distances at which animals can detect the signals. Over time, these effects could also lead to population declines

(Brumm 2010; Goutte *et al.* 2013). Although anurans communicate mainly through acoustic signals, visual signals is also important during social interactions in some species. (Ryan 2001; Preininger *et al.* 2013; Grafe & Tony 2017). Visual displays have been described for anurans in different social contexts (Hödl & Amézquita 2001; Biju *et al.* 2014; Furtado *et al.* 2017). In Hylodidae, for example, limb movements and posture raising are the most common visual displays during aggressive and reproductive interactions (Caldart *et al.* 2014; Forti & Castanho 2012; de Sá *et al.* 2016).

Several studies have demonstrated that some anuran species when exposed to traffic noise changed the call parameters (Bee & Swanson 2007; Lengagne 2008; Parris *et al.* 2009; Cunnington & Fahrig 2010; Hoskin & Goosem 2010; Kaiser *et al.* 2011; Vargas-salinas *et al.* 2014; Caorsi *et al.* 2017). Effect of anthropogenic noise indicate that anurans may exhibit different strategies to try avoiding masking their acoustic signal (Bee & Swanson 2007; Vélez *et al.* 2013). Most species exposed to noise changed parameters of their calls (Simons & Narins 2018), but in some extreme cases, animals even abandoned the calling sites (Caorsi *et al.* 2017). Species may change the rate, duration, amplitude or frequencies of their calls (Lengagne 2008; Parris *et al.* 2009; Cunnington & Fahrig 2010; Hoskin & Goosem 2010; Kaiser *et al.* 2011; Halfwerk *et al.* 2015; Shen & Xu 2016). However, little is known about species that vocalize in noisy water bodies, such as streams. It has been observed that *Odorrana tormota* (Ranidae), a species that calls in noisy watercourses, when exposed to playback noises increases the amplitude and frequency of the call to avoid the new source of masking (Shen & Xu 2016). In Hylodidae, it was seen that *Crossodactylus schmidti* adjusts the call characteristics according to background noise, changing considerably the spectral structure of call (Caldart *et al.* 2016). Besides that, no study with traffic noise has been performed on species that have visual signaling. Therefore, it is important to evaluate whether traffic noise affects the anuran calls, that have visual signaling record, in natural noisy environments. What strategies would these species adopt to reduce the masking effect of their signals? From this, field experiments were carried out with the intention of measuring the effects of traffic noise on the acoustic and visual communication of the torrent frog (*Hylodes meridionalis* - Hylodidae). This species inhabits streams and cascades with clean running water and has call frequencies overlapping with traffic noise frequencies (Garcia & Segalla 2010; Lingnau *et al.* 2013). The spectral overlap of the call was expected to be an important indicative for noise effect, as greater overlap may induce behavioral changes (e.g. lower call rate, alteration of parameters or more visual signals) as a response to avoid the masking effect. Our hypothesis is that the car traffic noise would reduce the number of calls and increase the number of visual responses as an attempt to compensate for the reduction in the number of calls. In addition, we expected that during the stimulus there will be change in call duration and / or call frequency.

Material and methods

Study area

This study was conducted in the Floresta Nacional de São Francisco de Paula (FLONA-SFP), southern of Brazil (29°25'22.4"S; 50°23'11.2"W). FLONA-SFP has an area of 1607 ha, 56% covered by the native Mixed Ombrophilous Forest (Narvaes *et al.*, 2005; Sonogo *et al.* 2007). The region climate is Temperate Superhumid, with average annual temperature of 14.5°C and annual precipitation exceeding 2000 mm (Moreno 1961; Backes *et al* 2005). The experiments were performed during non-consecutive 11 days on February of 2019.

Focal species

The torrent frog (*Hylodes meridionalis*) (Fig. 1A) is small-sized specie (snout–vent length of 35-43mm for males and 45-50mm for females) endemic to the southern Atlantic rain forest. It occurs in streams and cascades with clear running water of the northeastern region of Rio Grande do Sul and in the extreme South of Santa Catarina states, in altitudes at 400-1000 m. (Kwet *et al.* 2010; Garcia & Segalla 2010). During the reproductive period (October-February) males call during the day and in the first hours of the night (Kwet *et al.* 2010). Males are territorial and defend their calling sites using acoustic signals (Lingnau *et al.* 2013). The advertisement call is composed by long, high-pitched, whistled trills with a harmonic structure, consisting of 32 to 82 notes. Call duration is between 2.71s to 5.69s and the dominant frequency ranges from 3.9 to 4.9 kHz (Lingnau *et al.* 2013). *Hylodes meridionalis* advertisement call has the frequency spectrum within the frequency of noise emitted by vehicles on the roads. This species also is characterized by complexes visual signals for communication. Furtado *et al.* (2019) described seven types of visual displays for *H. meridionalis*: toe flagging (slow up-and-down movements of one or more toes), arm lifting (rapid up-and-down movements of one arm), leg lifting (rapid up-and-down movements of one leg), arm waving (lifting an arm and waving it in an arc), both legs kicking (rapidly stretching both hind limbs towards the back), foot flagging (slowly raising one hind limb in a semi-arch movement) and throat display (pulsation of one or both paired lateral vocal sacs without sound production).

Traffic noise

For the playback experiment we use recording of vehicles traffic noise on the ERS-389 highway (Estrada do Mar, Rio Grande do Sul, Brazil) of Caorsi *et al.* 2017. Recordings were taken 10m from the edge of the paved road, at July 14th of 2015, beginning at 18h during winter season, for 30 minutes. This day and time was chose for its similarity to the vehicle fluxes during the summer breeding season of the anurans. Was used a sound level meter (SLM-Instrutemp ITDEC 4000, 0.1dB precision, C-weighting) to measurement the mean intensity (dB) produced by the traffic at distance of 50 meters of the road. The noise was recorded using a portable recorder (SONY PCM-D50) coupled to a unidirectional

microphone (Sennheiser ME 67) equipped with a windscreen and a dynamic stereo headphone to monitor recordings.

Sound editing

We used Audacity 2.1.3 software to observe and edit the traffic sound (.wave format) for the experiment. The playback was constructed with the traffic noise and intensity recorded and measured on ERS-389. The recordings used for the stimuli had a frequency between zero kHz and 15 kHz, with dominant frequency on 1.125 kHz. . The playback followed the protocol A-B-A with a total of 15 minute proposed by McGregor *et al.* (1992). It was constructed using five minutes of traffic noise at intensity 75dB (treatment), which represents the mean intensity of noise measured at 50m from the edge of a road. Before and after treatment (traffic noise), we included two silent periods of the same duration, one at the beginning and one at the end of each playback. The distance used correspond to the reality of the bodies of water found near roads in Rio Grande do Sul.

Artificial model

For the visual stimuli we constructed an artificial model (Fig. 1B) using a male specimen deposited at the Zoological Collection from the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). We created a plaster mold, and then filled with a white plastic polymer (manufacturer Redelease). Later, we painted the artificial models with oil-based paint, following the color and pattern of the species.

Playback experiments

We actively searched for calling males at daylight, between 13 and 18h. Each individual was submitted to the artificial model as a visual sign to simulate the presence of an intruder male in the resident's territory, at approximate 30-50 cm of distance for the male to emit visual signals, this distance that proved to be adequate in tests performed before this study, since less than 30 cm the male withdrew from the place to fight with the model and when more than 50 cm the male was indifferent to the model. Later, we placed a speaker (Oneal 360-12v, which issues frequencies 0.01 kHz a 70 kHz) at a distance of 1-3 m of the specimen, a portable SONY PCM-D50 recorder at 1-3 m and the microphone Sennheiser ME 67 (same used to record the stimulus) at 1-4 m. A video camera (Panasonic HC-W850) was used to record the experiment (Fig. 2) also to a distance of 1-4 m. Finally, when the calling male returns to baseline behavior we started to record.

After recording each individual, we measured the noise level at the place where the male called, the temperature and the humidity. In this study, individuals were not handled. The experiment was previously approved by Ethics Committee on the Use of Animals (CEUA/UFRGS) and by the Instituto Chico Mendes de Conservação da Biodiversidade—ICMBio, Brazil (permits no. 51201-2).

Acoustical and Visual analyzes

We divided each recording made in the field into five minutes files, corresponding to pre-stimulus, stimulus and post-stimulus. Each file was separately analyzed using Raven Pro 64 v1.5 for Windows (Bioacoustics Research Program 2017), at 16 bit resolution, 44 kHz sampling rate and Fast Fourier Transformation 512 (FFT). For each file analyzed four different parameters of the call of the males were recorded: (a) call rate (number of calls per minute); (b) call length (time between the beginning and the end of each call); (c) interval between notes (interval between two consecutive notes within the same call) and (d) dominant frequency (frequency of the call with the highest sound energy). Parameters are according to Kohler *et al.* 2017.

For the visual communication, we analyzed the video recordings to observe the visual responses according to the displays described to *H. meridionalis* (Furtado *et al.* 2019). Each record of one movement was categorized and further we counted the number of visuals signals in their respective time and stimuli.

Statistical analyses

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) was performed to test differences in call parameters and visuals signals, with specimens as a random factor. To visual signals we used poisson distribution, except sum of all visual signals that we use a gaussian distribution, well as to acoustical signals. We test all possible interactions between the predictors: treatment (traffic noise), period, temperature, humidity and background noise. The best model was selected using the Akaike information criterion (AIC) (Burnham & Anderson 2002). The statistical analyses were performed in R software version 3.3.1 (R Core Team 2015), using “lme4” package (Bates *et al.* 2019).

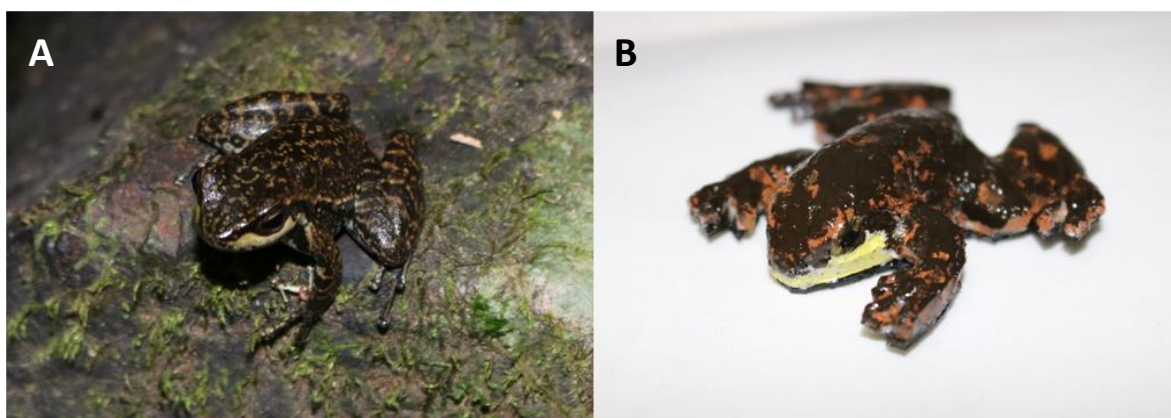


Figure 1. Focal species of this study: *Hylodes meridionalis*. (A) Male in situ; (B) Artificial model based in focal species.

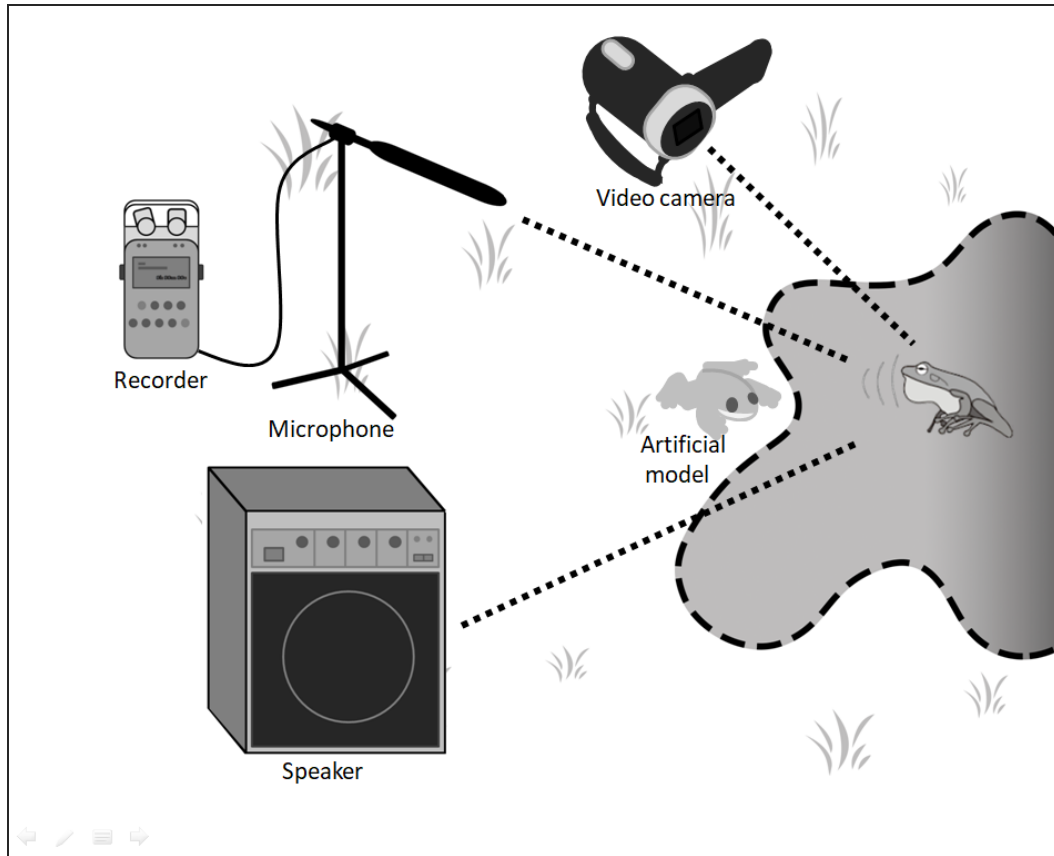


Figure 2. Playback experiment conducted in the field to expose the torrent frog to traffic noise stimuli.

Results

We recorded 14 individuals (total of 210 minutes of audio e video recordings). We counted 593 signals, being 419 visuals and 174 acoustical signals. For the acoustic analyzes call rate was analyzed for all males ($n=14$), but to call length, interval between notes and dominant frequency were measured for only 11 males due to the lack of calls during some of the periods. We observed six categories of visuals signals: Leg lifting (177), Arm lifting (80), Arm waving (57), Toe flagging (29), Foot flagging (5) and Both legs kicking (1). Foot flagging and both legs kicking was excluded from the individual analysis due to the low quantity but were used in the total visual signal analysis (sum of all signs visual). Besides the visual signals, the males changed their position a total of 56 times.

To acoustical tests, traffic noise was negatively associated with call rate ($r = -0.451$; $p < 0.0001$) and call length ($r = -0.256$; $p < 0.0001$). Call length also was correlated with step ($p < 0.0001$), negatively among step 1 and 2 ($r = -0.384$), negatively among step 1 and 3 (r

= -0.384) and positively among step 2 and 3 ($r = 0.500$). Table 1 shows means for call parameters and visuals signals of individuals in pre-stimulus, stimulus (traffic noise) and pos-stimulus conditions. Table 2 shows results from the bests GLMM fit for the variables analyzed to call parameters, include traffic noise, step and humidity, also only random factor (Null).

In visual signals tests, traffic noise was negatively associated with sum of all visuals ($r=-0.184$; $p<0.0001$) and leg lifting ($r=-0.263$; $p<0.0001$). Step was associated with sum of all visuals ($p<0.0001$), negatively among step 1 and 2 ($r = -0.307$), negatively among step 1 and 3 ($r = -0.296$) and positively among step 2 and 3 ($r = 0.537$). Step also was associated with toe flagging ($p=0.00593$), negatively among step 1 and 2 ($r = -0.445$), negatively among step 1 and 3 ($r = -0.441$) and positively among step 2 and 3 ($r = 0.757$). Displacement was negatively associated with humidity ($r=-0.999$; $p=0.00199$). Table 3 shows results from the bests GLMM fit for the variables analyzed to visuals signals include traffic noise, step and humidity, also only random factor (Null).

Table 1. Mean of call parameters and visuals signals measurements taken on *Hylodes meridionalis* from experiment conducted in the field to expose: pre-stimulus (silence), stimulus (traffic noise) and pos-stimulus (silence).

		pre-stimulus	stimulus	pos-stimulus
Call parameters	Call rate	1.171	0.917	0.904
	Call length	4.013	4.370	4.297
	Interval between notes	0.082	0.046	0.046
	Dominant frequency	4743.879	4816.147	4809.926
Visuals signals	Toe flagging	0.286	0.929	0.857
	Arm lifting	1.286	2.000	1.286
	Leg lifting	3.857	4.500	3.786
	Arm waving	1.500	1.357	1.214
	Both legs kicking	0.000	0.000	0.071
	Foot flagging	0.071	0.000	0.286
	Advertisement call	4.929	3.643	3.500
	Displacement	1.214	1.000	1.643

Table 2. Results from the bests GLMM fit for the variables analyzed to call parameters. Models include Traffic noise (TN), only random factor (Null), stage of experiment duration (Step) and humidity as fixed factors and specimens as random factor. AICc = Akaike Information Criterion. Delta AICc = difference between ranked models. AICcWt = Akaike weights. K = number of parameters. P-values significant at the 0.05 level are in bold.

Call parameters	Factor	Null	TN	Step	Humidity
		K			
Call rate	<i>p</i>	<0.0001	<0.0001	<0.0001	0.759
	<i>AICc</i>	53.37	57.24	59.42	61.03
	<i>Delta AICc</i>	0.00	3.87	6.05	7.66
	<i>AICcWt</i>	0.76	0.11	0.04	0.02
Call length	<i>p</i>	<0.0001	<0.0001	<0.0001	0.5235
	<i>AICc</i>	55.25	57.4	58.34	61.61
	<i>Delta AICc</i>	0.00	2.15	3.09	6.36
	<i>AICcWt</i>	0.53	0.18	0.11	0.02
Interval between notes	<i>p</i>	<0.0001	0.00025	0.00062	0.06104
	<i>AICc</i>	-67.67	-60.11	-53.55	-59.09
	<i>Delta AICc</i>	0.00	7.56	14.12	8.58
	<i>AICcWt</i>	0.96	0.02	0.00	0.01
Dominant frequency	<i>p</i>	<0.0001	<0.0001	<0.0001	0.03982
	<i>AICc</i>	432.65	424.99	416.51	426.08
	<i>Delta AICc</i>	34.49	26.83	18.35	47.92
	<i>AICcWt</i>	0.00	0.00	0.00	0.00

Table 3. Results from the bests GLMM fit for the variables analyzed to visuals signals. Models include Traffic noise (TN), stage of experiment duration (Step), only random factor (Null) and humidity as fixed factors and specimens as random factor. AICc = Akaike Information Criterion. Delta AICc = difference between ranked models. AICcWt = Akaike weights. K = number of parameters. P-values significant at the 0.05 level are in bold.

	<i>Factor</i>	Null	TN	Step	Humidity	
		<i>K</i>				
Visuals signals	Sum of all visuals	<i>K</i>	2	3	4	3
		<i>p</i>	<0.0001	<0.0001	<0.0001	0.514
		<i>AICc</i>	280.89	280.44	282.64	282.8
		<i>Delta AICc</i>	0.46	0.00	2.21	2.36
		<i>AICcWt</i>	0.28	0.24	0.12	0.07
		<i>p</i>	0.0355	0.0227	0.00593	0.279
	Toe flagging	<i>AICc</i>	91.78	92.45	90.72	93.16
		<i>Delta AICc</i>	1.06	1.74	0.00	2.44
		<i>AICcWt</i>	0.23	0.08	0.39	0.11
		<i>p</i>	0.602	0.0795	0.144	0.117
	Arm lifting	<i>AICc</i>	149.24	148.57	151.02	149.33
		<i>Delta AICc</i>	0.67	0.00	2.45	0.75
		<i>AICcWt</i>	0.14	0.2	0.06	0.14
		<i>p</i>	<0.0001	<0.0001	<0.0001	0.81
	Leg lifting	<i>AICc</i>	206.39	207.67	210.11	208.66
		<i>Delta AICc</i>	0.00	1.28	3.72	2.27
		<i>AICcWt</i>	0.35	0.19	0.04	0.08
		<i>p</i>	0.481	0.492	0.517	0.503
	Arm waving	<i>AICc</i>	135.02	137.35	139.37	136.89
		<i>Delta AICc</i>	0.00	2.32	4.35	1.87
<i>AICcWt</i>		0.34	0.11	0.03	0.13	
<i>p</i>		0.531	0.251	0.345	0.00199	
Displacement	<i>AICc</i>	137.53	138.46	140	132.58	
	<i>Delta AICc</i>	4.94	5.87	7.42	0.00	
	<i>AICcWt</i>	0.04	0.02	0.01	0.43	

Discussion

Our experiment demonstrated that vehicle traffic noise appears to have an effect on torrent frog behavior, as there are changes in the call parameters (call rate and call length) and visual signals (toe flagging, leg lifting and sum of all visual signals) during traffic noise playback (treatment). These results highlight the importance of conducting behavioral studies *in situ*, considering the effects of traffic noise on frogs with different modes of communication, as well as on visual signaling.

Call rate reduction when exposed to traffic noise has been observed in several species of Dendrobatidae, Hylidae and Ranidae (Cunnington & Fahrig 2010; Vargas-Salinas & Amézquita 2013; Caorsi *et al.* 2017). Decreased signal rate may be associated with the choice of the best time for sound emission by the animal, when their signal is more prone to transmission and detection avoiding singing when ambient noise is unfavorable (Sun & Narins 2005; Vargas-Salinas & Amézquita 2013; Vargas-Salinas *et al.* 2014).

Dominant frequency had no differences in *H. meridionalis*. Other studies, however, have reported changes in dominant frequency in species that also have call frequency overlapping with traffic noise: some increasing (Cunnington & Fahrig 2010; Parris *et al.* 2009; Barrass 1985) and others decreasing (Parris *et al.* 2009; Lukanov *et al.* 2014). Frequency is a parameter influenced by the environment, due to absorption, filtering and background noise (Forrest 1994). Anurans share acoustic environments and have acquired strategies such as the emission of calls at different frequencies and the synchronized emission with time intervals of other transmitting sources, avoiding the problem of masking effect (Narins 1995; Wells 2007). Studies in which species did not showed shifts in call frequencies, changed some other parameter, either call rate or call length (Lengagne 2008; Hoskin & Goosem 2010; Cunnington & Fahrig 2010; Caorsi *et al.* 2017). The fact that the dominant frequency remains unchanged probably reflects the biology of this species that calls in naturally noisy environments.

Call length had an average increase during noise treatment compared to pre-stimulus. Increased call length has also been observed in other studies, and could be an alternative adopted to increase the temporal window in response to anthropogenic noise, thus reducing the masking effect (Kaiser *et al.* 2011; Caorsi *et al.* 2017; Grace & Noss 2018).

During the noise of traffic the increase in the averages of the sum of all visual signals, toe flagging and leg lifting shows the importance of this method of communication for dancing frogs, which are from normally noisy environments. *H. meridionalis* possibly increased the visual signals in response to the stimulus, because there was an increase in ambient noise (in this case, of anthropogenic origin). In addition, Furtado *et al.* 2019 observed that toe flagging and leg lifting were performed when the individual enters into

visual contact with another male, showing the effectiveness of the artificial model used in this study.

Toe flagging and leg lifting also had their higher averages after the stimulus than the initial silence, suggesting a short-term behavioral change caused by contact with a stimulus that had never been heard by the local population.

Although this study deals with traffic noise, a relationship was found between the repositioning of individuals and the humidity of the environment. Other studies have reported cases of movement during traffic noise in *Boana bischoffi*, *Boana leptolineatus* and *Hyla arborea*, however, these individuals attempted to move away from the noise source and even cease calls (Caorsi et al. 2017; Lengagne 2008). In our study, individuals who switched places did not stop producing acoustic and visual signals. Thus, the noises did not influence the position of the individuals in the reproductive place, but the humidity of the place.

Apparently, even species adapted to noisy environments that have secondary communication behaviors are affected by traffic noise. Our study is based on short-term exposure to traffic noise to individuals never previously exposed to traffic noise. Therefore, we record only the immediate effects and it is not possible to exclude the possibility of further behavioral changes in long-term exposure. Despite this, after playback, the averages of visual signals were still higher than before the stimulus, which may be an indication that populations exposed in the long term to traffic noise have an average of visual signals greater than those never exposed.

Although the species changes its behavior, it is not known whether the changes help in the transmission and detection of signals (acoustic and visual), increasing the chances of mating species in environments with anthropogenic noise. The reduction in the call rate and call length may cause the population reduction of the species in the future, since it is known that the call has functions beyond communication. In species that males vocalize for long periods of time with females in the same habitat, the calls appear to stimulate hormone production in females, maintaining their reproductive condition (Wells 2007). Lea & Halliday (2001) observed that *Alytes muletensis* pregnant females only when stimulated with co-specific call continued to maintain and mature their eggs, while that the females kept silent or the stimulated with calls heterospecific reabsorbed their ovules. In addition, the increase in visual signals can increase the wear of the individual who in turn will have less time to reproduce. The data obtained in this study showed behavioral changes, both visual and call, caused by the traffic noise in a species that normally inhabits noisy environments, inferring that populations that live near the roads may behave differently from those in protected areas.

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CONCLUSÕES GERAIS

Este trabalho demonstrou que o ruído do tráfego de veículos afeta o comportamento da rã-das-corredeiras. Foram observadas alterações na quantidade de sinais visuais emitidos pelos machos em atividade reprodutiva, assim como na taxa de canto, comprimento do canto e intervalo entre notas durante o estímulo de ruído. A taxa média de canto de *H. meridionalis* diminuiu 11,8% durante o ruído em comparação com o pré-estímulo (silêncio). A duração do canto teve um aumento médio de 8,98% durante o tratamento em comparação com o pré-estímulo. Além disso, foi observado um aumento no número de sinais visuais durante o tratamento em comparação ao pré-estímulo: na soma de todos os sinais visuais (+ 25,51%), *toe flagging* (+ 224,825%) e *leg lifting* (+ 16,67 %). Embora este estudo lide com o ruído do tráfego, foi encontrada uma relação entre o reposicionamento dos indivíduos e a umidade do ambiente, indicando aumento no número de reposicionamento de indivíduos quando a umidade relativa era maior. Nosso estudo é baseado na exposição de curto prazo ao ruído do tráfego a indivíduos nunca expostos a esta perturbação anteriormente. Portanto, analisamos apenas os efeitos imediatos causados, e, portanto, não é possível excluir a possibilidade de outras mudanças comportamentais ou se acostumar em exposições de longo prazo. Este e outros estudos geram informações importantes que podem ser utilizadas nos planos de manejo e contribuem significativamente para a conservação das espécies, beneficiando anuros e outros grupos.