UFRGS
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PPGBAN
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

VALENTINA ZAFFARONI CAORSI

EFEITO DO RUÍDO ANTROPOGÊNICO NO COMPORTAMENTO ANIMAL

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

Área de concentração: BIOLOGIA E COMPORTAMENTO ANIMAL

Orientador: Prof. Dr. MÁRCIO BORGES MARTINS

Coorientadora: Dra. CAMILA BOTH

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Aprovada em ____ de ____________ de _____.

BANCA EXAMINADORA

Dra. Maria João Ramos Pereira

Dr. Andreas Kindel

Dr. Vinícius M. Caldart

AGRADECIMENTOS

Em poucas palavras, mas verdadeiras, gostaria de agradecer a todos que, de alguma forma, contribuíram para o acontecimento deste trabalho e para meu crescimento.

Desde já agradeço a banca examinadora, doutores Maria João Ramos Pereira, Andreas Kindel e Vinícius M. Caldart, por aceitar o convite e por suas sugestões e contribuições no nosso trabalho.

Ao meu orientador, Márcio Borges Martins, ao qual admiro muito, tanto como pesquisador e como pessoa e sem quem eu não teria realizado este trabalho. Agradecer a ele também por me encorajar em todas as ideias que tive e me mostrar que sempre existe um caminho alternativo. A colega, coorientadora, e além de tudo amiga, Camila Both, que abraçou o projeto desde o princípio e foi uma mentora incrível nestes quadro anos, tanto no lado profissional quanto no pessoal, me dando os melhores conselhos em qualquer hora e para qualquer situação.

À todo o laboratório de herpetologia da UFRGS que me acolheu todos esses anos com apoio e grandes amizades.

À todos aqueles que me ajudaram nas saídas de campo e mantiveram o sorriso e o entusiasmo mesmo as 4am com 9 graus, Deivid, Tiago, Jonathan, Lívia, Debora, Rógger e Camila.

Aos professores e pesquisadores, Sonia, Marco, Maurício, Xande, Santiago, Patrick, Gonçalo, Luke, entre muitos outros, por conversas enriquecedoras e amizade.

Ao time das vibrações sísmicas, em especial ao Rafael, Raíssa, Vinícius, Diego e Peter pela amizade, companheirismo, diversão, saídas de campo e ensinamentos sobre a comunicação em anfíbios de uma maneira encantadora!

Ao time das aves, MPIO, em especial ao Henrik, Sue Anne, Julia, English Sam, Sam, Adri e Teresa pelo acolhimento, parceria, amizade e aprendizado. Sem dúvida, pessoal que levo para a vida.

Aos gestores das UCs, em especial, a Edenice da FLONA SFP, ao pessoal do PRO-MATA e do Parque Natural de Somiedo, por tornar possível e agradável meu trabalho na área.

Ao NuCAR/Curicaca por me mostrar novas alternativas e pela motivação para conservação de anfíbios e répteis.

Ao apoio institucional e financeiro do PPG-BAN, UFRGS, CAPES, CNPq, ICMBio, UCLA, Explorers Club, UFSM, MPIO e FonoZoo MNCN.

Aos amigos da biologia, especialmente, ao Lucas, Mi, Debs, Bru, Bibis, Fe, Di, Rafa, Moreno, Dangi, Lu, Mari, Rodi, Paulinho, Natyzinha, Ceará, Adri, Iri, Mila, Thya, Jessie, Mel e muitos outros, que tornaram todos os momento desse percurso únicos, mas, principalmente, por todos esses anos de amizade.

Aos glows, Michels e Ka, pessoas que foram muito importantes nesta trajetória...cada um da sua maneira especial.

Aos amigos Phil, Matt e a família Johnson, por me acolher em sua família.

Ao Lui e a Raque pela grande amizade, que apesar de ter um oceano no meio, é presente diariamente na minha vida. Pessoas que admiro muito, que me entendem e que me fortalecem.

Aos eternos amigos Fernando, Laura e Thaís que me acham estranha até hoje por trabalhar com sapos, mas que me acompanham a muito tempo e, mesmo assim, sempre me incentivaram.

À minha família, particularmente a Alejandra, Daniel, Facundo e Elena, que sempre acreditam em mim e a quem dedico, especialmente, este trabalho.

SUMÁRIO

1. RESUMO

Distúrbios antropogênicos têm sido apontados como a principal causa da perda da biodiversidade mundial. Dentre eles, a poluição sonora é uma potencial, porém subestimada, ameaça, prevista para aumentar nos próximos anos, juntamente com a expansão urbana. Ruídos antropogênicos podem ter efeitos negativos, especialmente em espécies que dependem da comunicação acústica. Entretanto, a poluição sonora pode afetar não só a comunicação, mas as funções auditivas de maneira geral. Desta forma, ruído pode agir como um estressor geral, influenciando processos vitais, desde a integridade do DNA, a processos fisiológicos ou comportamentais ou até se estender a populações e comunidades. O objetivo desta tese é contribuir com o conhecimento sobre o efeito do ruído antropogênico em animais, estudando sistemas não auditivos e auditivos afetados por ele. O primeiro objetivo teve foco no distúrbio do ruído gerado em cidades no comportamento de sono em aves (i). Os outros três objetivos da tese estão relacionados com a comunicação e o sistema auditivo de anfíbios anuros. Mais especificamente, testamos (ii) o efeito do ruído antropogênico audível (tráfego) e (iii) sísmico (tráfego e eólicos) no canto de algumas espécies; e por último, (iv) apresentamos uma revisão da literatura existente sobre a extensão dos feitos do ruído antropogênico em anuros. Os resultados deste trabalho mostraram que tanto o ruído audível, como o sísmico proveniente de diferentes fontes de atividade humana tem um efeito sobre o comportamento dos animais e que diferentes espécies respondem de distintas maneiras frente a este estressor. Fica claro que a poluição sonora é uma fonte importante de distúrbio em animais, com efeitos adversos e que deve, então, ser levada em conta como possível fator de impacto para as espécies e incluída em futuros estudos e legislações, a fim de controlar seus efeitos na biodiversidade.

PALAVRAS-CHAVE: Ruído antropogênico, Biodiversidade, Fisiologia, Comportamento, Comunicação acústica, Vibrações sísmicas, Conservação.

2. ABSTRACT

Anthropogenic disturbance has been pointed as the major cause of the world´s biodiversity crisis. Among them, noise pollution is a potential underestimated threat, projected to increase in the next decades accompanying urban expansion. Rising levels of noise pollution may result in negative impacts on species, specially the ones depending on acoustic communication. However, compromise hearing affects more than acoustical communication. It has been shown to influence from DNA integrity and genes, to physiological systems, behavioral ecology and community ecology. The aim of this thesis is to contribute with knowledge about the effect of anthropogenic noise in animals, studying non-auditory and auditory systems affected by it. The first goal focused on the effect of urban noise in sleep behavior in birds (i). The other three goals were related to the communication and auditory system of anuran amphibians. More specifically, we tested (ii) the effect of audible (traffic) and (iii) seismic (traffic and wind) anthropogenic noise in the calling behavior of some species; finally, (iv) we reviewed the existing literature on the extent of anthropogenic noise in anurans. The results of this work showed that both audible and seismic anthropogenic noise have an effect on the behavior of the animals, but with species responding in different ways to this stressor. It is clear that noise pollution is an important source of disturbance in animals, with adverse effects and, then, it must be taken into account as a possible impact factor for species and be included in future studies and legislation in order to control it effects on biodiversity.

KEYWORDS: Anthropogenic noise, Biodiversity, Physiology, Behavior, Acoustic communication, Seismic vibrations, Conservation.

3. INTRODUCAO GERAL

Distúrbios antropogênicos têm sido apontados como a principal causa da crise mundial da biodiversidade (Brumm 2010a). Dentre elas, estão atividades como fragmentação e destruição do habitat, introdução de espécies exóticas e poluição de ambientes com contaminantes químicos (Marzluff et al. 2008; Grimm et al. 2008). No entanto, alguns distúrbios recebem menos atenção de pesquisadores e conservacionistas porque seus efeitos são mais difíceis de medir, especialmente quando afetam espécies em um nível subletal, como é o caso da poluição sonora (McGregor 2013).

O ruído pode ser definido como qualquer som indesejado e, mais especificamente no contexto das interações sociais, ele é considerado um fator de interferência na detecção de um sinal e na transmissão de sua informação (Forrest 1994). Ruídos podem ter origem biótica como, por exemplo, os sons emitidos por outros animais, ou origem abiótica, como o vento ou a chuva. Ruídos antropogênicos considerados nessa tese, referem-se àqueles ruídos de origem abiótica resultante de atividades e estruturas sociais humanas como operação de máquinas, transportes, etc.

O ruído antropogênico é derivado de diferentes fontes, por exemplo carros em estradas, e se perpetua como ondas mecânicas em diferentes meios, como no ar ou no solo. Para entender melhor esta divisão de ruídos em diferentes meios vamos usar a definição de Hill and Wessel (2016). Primeiramente, vamos classificar as ondas mecânicas como energia transferida em um meio pela oscilação de matéria (movimento de partículas/vibrações). Essas ondas são divididas em ondas acústicas (ondas puramente longitudinais em um meio homogêneo, como ar, líquido ou sólido) e ondas de superfície (que acontecem em fronteiras entre diferentes meios, onde a energia é sempre transferida de um meio para outro, e vice-versa, e onde as partículas oscilam perpendicularmente à energia) (Fig.1).

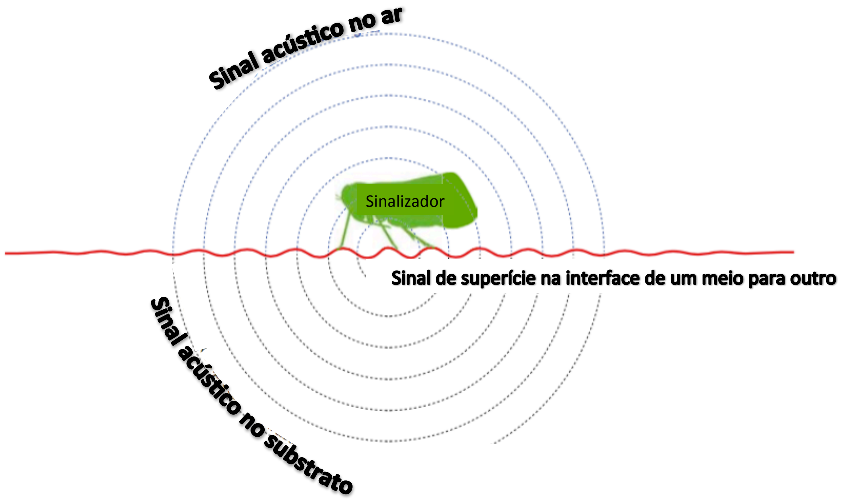

Figura 1. Exemplo de ondas mecânicas emitidas por um animal em diferentes meios e seus limites. Adaptado de Hill and Wessel (2008).

Dentro das ondas acústicas, a vibração mais popularmente conhecida, é o som (onda mecânica longitudinal, onde as partículas oscilam na mesma direção da energia - Fig. 2A). O som é definido em termos de sensibilidade auditiva humana, indo de 20Hz a 20kHz. Tudo abaixo disso é chamado de infrassom (<20Hz), e acima disso é chamado de ultrassom (>20kHz). Já as ondas de superfície, como denominadas por Hill and Wessel (2016), ocorrem na fronteira de um meio para outro e as partículas oscilam perpendicularmente à energia (Markl 1983; Hill and Wessel 2016). Estas ondas podem ocorrer, por exemplo, no solo, derivadas de atividades humanas, como estradas (Aliyu et al. 2016), Estas ondas, conhecidas como Rayleigh, são uma combinação de ondas longitudinais e transversais com partículas se movendo em uma trajetória elíptica, mas com maior intensidade no eixo vertical. Apesar de várias ondas fazerem parte do complexo de vibrações, estas últimas acabam recebendo o termo geral de vibração, embora, teoricamente, o som também seja um tipo de vibração (Hill and Wessel 2016). Isto significa que os ruídos antropogênicos se propagam por mais de um meio e que seus efeitos nos animais podem ser resultantes da percepção em mais de um meio. Neste trabalho iremos usar a denominação de "ruído antropogênico" para denominar o ruído se propaga pelo ar, representando um exemplo de onda em um único meio, e "vibração antropogênica" para o ruído derivado de ondas na superfície entre dois meios, neste caso a interface solo/ar.

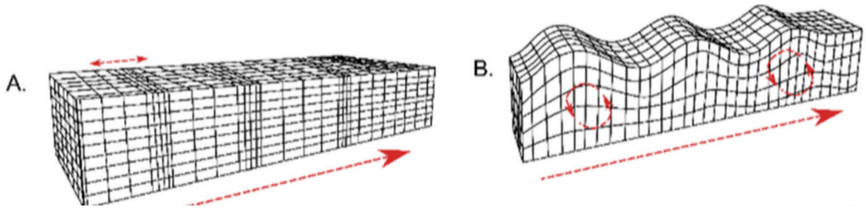

Figura 2. Diagrama de blocos de tipos de ondas em meio 3D com direção de propagação marcada com setas: longitudinal (A), Rayleigh (B). Adaptado de Hill (2008) e Roberts et al. (2016).

A poluição sonora aumentou drasticamente nas últimas décadas como resultado do crescimento populacional, urbanização e globalização das redes de transporte e a estimativa é de que ela seguirá aumentando (Shannon et al 2016). No Brasil, por exemplo, temos o quarto maior sistema rodoviário do mundo (cerca de 1,7 milhão de quilômetros) (Secco et al. 2018). Além disso, novos projetos de construção e expansão estão sendo planejados e executados no momento (PNLT 2011). Embora existam muitas fontes naturais de ruído, incluindo vento, água e outros animais, os ruídos antropogênicos são, geralmente, mais altos (i.e. maior volume), mais frequentes e mais difundidos do que os estímulos acústicos não humanos (Patricelli and Blickley 2006; Popper and Hastings 2009). Em geral o ruído derivado de fontes humanas abrange um amplo espectro de frequência entre 50Hz a 7000Hz (Simmons & Narins 2018). Como o ruído não conhece fronteiras definidas, como as margens das rodovias, os animais estão sujeitos a uma substancial e descontrolada degradação da percepção de sons importantes para sua reprodução e sobrevivência (Barber et al. 2010).

A poluição sonora proveniente de atividades humanas (Fig.3) é uma forma severa de poluição que pode ter impactos maciços na saúde humana e também em outros animais (Brumm and Slabbekoorn 2005). O assunto tem sido foco de pesquisa e regulação em humanos (Murphy and King 2014), com resultados preocupantes para a saúde, incluindo aumento do risco de doença cardiovasculares (Babisch et al. 2005; Hansell et al. 2013), privação do sono (Fyhri and Aasvang 2010) e comprometimento cognitivo (Szalma and Hancock 2011). A Organização Mundial de Saúde, há alguns anos, publicou um relatório sobre o tema, estimando que só na União Europeia mais de 200.000 pessoas morrem todos os anos devido a doenças induzidas pelo ruído (OMS, 2009).

Nas últimas décadas, houve um interesse crescente em questões relativas aos efeitos de sons produzidos pelo homem em animais (Popper and Hawkins 2016). Já se sabe que muitos dos efeitos potenciais do ruído audível antropogênico em humanos (Miedema and Vos 2003; Basner et al. 2014) se aplicam igualmente aos animais (Francis and Barber 2013; Shannon et al. 2016), e cada vez mais trabalhos têm abordado este tema (Slabbekoorn et al. 2018, Fig.4). Entretanto, quantificar a extensão dos efeitos do ruído antropogênico na vida selvagem é uma tarefa desafiadora (Shannon et al. 2016). Uma das dificuldades é o fato de que a sensibilidade ao ruído varia amplamente entre os taxa (Brumm and Slabbekoorn 2005; Brumm 2010, 2013) e também pode variar dependendo do contexto, sexo e história de vida dos organismos (Francis and Barber 2013).

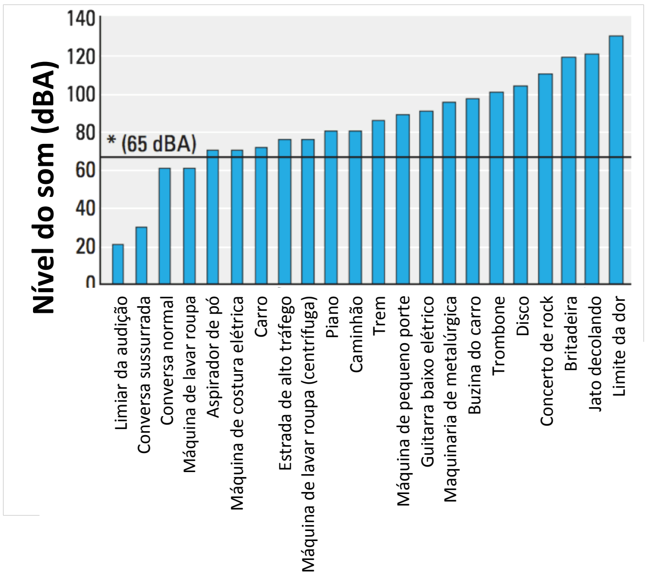

Figura 3. Níveis de ruído audível de diferentes fontes antropogênicas. *Limite de segurança proposto pela Organização Mundial da Saúde (Berglund et al. 2000). Adaptado de Frenzilli et al. (2004).

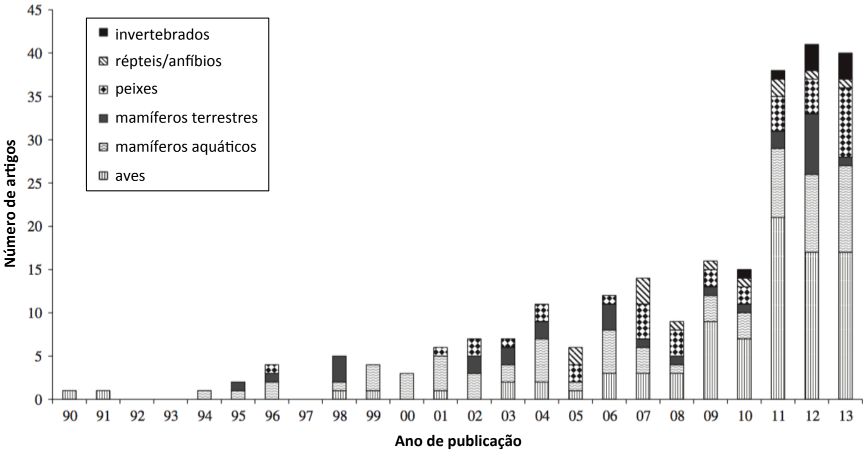

Figura 4. Número de publicações relatando os efeitos do ruído antropogênico em animais entre 1990-2013. Adaptado de Shannon et al. (2016).

A poluição sonora pode ser um problema para as funções auditivas (Barber et al. 2010; Simmons and Narins 2018). As espécies comumente ouvem uma gama mais ampla de sons do que são capazes de produzir e, além disso, a audição continua a funcionar mesmo quando os animais não produzem sons, isso inclui atividade de sono ou hibernação, por exemplo. Isso significa que elas estão expostas intermitentemente aos efeitos do ruído ao seu redor (Barber et al. 2010). Dessa forma, a poluição sonora pode agir, por exemplo, como um estressor geral (Naguib 2013), influenciando vários processos vitais, desde regulação gênica (Cui et al. 2009) a processos fisiológicos como pressão arterial (Evans et al. 2001), resposta imune (Van Raaij et al. 1996; Cheng et al. 2011), medo (Campo et al. 2005) ou atenção e cognição (Cui et al. 2009). Entretanto, os efeitos dos ruídos mais estudados ainda são aqueles relacionados à interferência sobre a produção de som e a comunicação animal (Brumm 2013).

As interações sociais entre indivíduos são baseadas na troca de informações, ou seja, através da comunicação, por exemplo, acústica. Muitas espécies emitem sinais acústicos para comunicar informações, e as mensagens transmitidas podem funcionar, por exemplo, para encontrar um parceiro sexual, competir por recursos e reconhecer filhotes. Portanto, a perturbação da transmissão do sinal por um ruído pode afetar todas estas interações (Forrest 1994; Wiley 2013). Além disso, o ruído pode afetar a escolha do habitat, o espaçamento individual e a densidade populacional (Francis et al., 2009). As respostas biológicas são variadas, em parte porque as respostas dependem da percepção do ruído (Francis & Barber, 2013). Estes mascaramentos de sinais ocorrem quando a percepção de um sinal é afetada pela presença de ruído de fundo, diminuindo a percepção dele (Forrest 1994; Wiley 2013). A fim de reduzir estes efeitos, espera-se que os indivíduos ajustem a estrutura acústica de seus sinais para melhorar a relação sinal-ruído (Endler 1992).

Evidências indicam que as características do sinal acústico (por exemplo, frequência, duração e intensidade) e a biologia da espécie em questão (alcance auditivo, estado comportamental e habitat) são importantes para prever como o ruído pode afetar um organismo em particular (Francis and Barber 2013; Parris and McCarthy 2013). Já que os efeitos da poluição sonora se estendem nos mais diversos níveis, desde o DNA até comunidades (Kight and Swaddle 2011; Fig. 5), nesta introdução iremos abordar algumas destas implicações com foco em vertebrados terrestres. Para tornar o entendimento mais claro, os efeitos serão divididos em dois grupos: efeitos ruído antropogênico (i) aos sistemas não auditivos e (ii) ao sistemas auditivos.

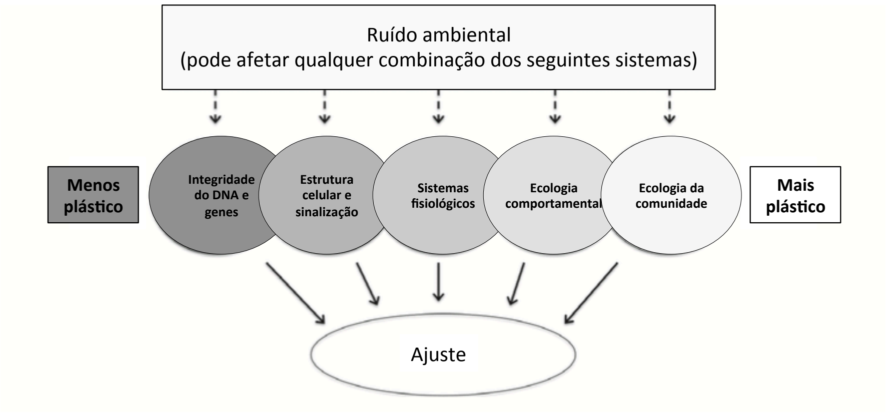

Figura 5. Estrutura conceitual de como o ruído ambiental pode afetar os sistemas biológicos. Adaptado de Kight and Swaddle (2011).

Efeitos não auditivos do ruído antropogênico

Integridade do DNA

Os estressores acústicos podem afetar os genes de duas maneiras principais: desencadeando cascatas químicas que podem levar a danos no DNA e/ou alterações na expressão gênica (Kight and Swaddle 2011). A atividade neural necessária para processar o ruído ambiental leva a um aumento no número de radicais livres, que são conhecidos por causar mutações carcinogênicas (Samson et al. 2005). Níveis de espécies reativas de oxigênio coclear (ROS) também podem aumentar em animais estressados por ruído. Como os radicais livres, os ROS causam danos ao DNA, assim como às proteínas e lipídios. Observou-se que níveis de ROS cocleares foram quadruplicados em camundongos que haviam sido expostos ao ruído e não diminuíram com o tempo (Ohlemiller et al. 1999). Além disso, danos induzidos por ROS foram observados nas glândulas suprarrenais (Frenzilli et al. 2004) e corações (Lenzi et al. 2003) de ratos estressados por ruído. Também foi encontrado um efeito da poluição sonora no comprimento dos telômeros, sequências não codificadoras do DNA, localizadas na extremidade dos cromossomos, que aumentam a estabilidade do genoma (Blackburn 1995). Aves expostas à poluição sonora tiveram uma redução significativa no comprimento dos telômeros em relação aos grupos controle (Meillère et al. 2015; Dorado-Correa et al. 2018). Estes resultados revelam um importante efeito do ruído, uma vez que o maior comprimento dos telômeros tem sido associado positivamente à longevidade (Heidinger et al. 2012) e sobrevivência em vertebrados (Habib et al. 2006; Grimm et al. 2008; Gil and Brumm 2014).

Estresse

Os ruídos antropogênicos podem influenciar, também, os níveis de estresse em várias espécies. Dentre os mamíferos estudados, os humanos (Evans et al. 2001), os cães (Gue et al. 1987) e os pandas (Owen et al. 2004), mostraram aumentos nos níveis de cortisol. Em ratos de laboratório, a corticosterona elevada, produzida em resposta ao estresse, foi associada à redução do consumo de alimentos e diminuição do ganho de peso, mostrando que podem existir efeitos a longo prazo, com consequências para a sobrevivência dos indivíduos (Alario et al. 1987). Da mesma forma, níveis elevados de corticosterona, associados ao estresse, foram observados em galinhas expostas aos ruídos (Chloupek et al. 2009). Altos níveis de corticosterona em aves, foram associados negativamente com respostas imunes (Saino et al. 2003), sobrevivência e recrutamento (Blas et al. 2007). Este conjunto de observações mostrou que, além do ruído antropogênico provocar respostas de estresse imediatas, elas também poderiam ser prejudiciais a longo prazo. Ainda, vale ressaltar que algumas espécies podem ser pouco ou não venham a ser afetadas. Um estudo com corujas-da-califórnia (*Strix occidentalis occidentalis*) expondo os indivíduos ao ruído de uma motosserra, não encontrou alterações nos níveis de corticosterona (Tempel & Gutierrez2003).

Sistema cardiovascular

Os estudos sobre os efeitos dos ruídos no sistema cardiovascular se concentram nos mamíferos. Entre os seres humanos, a exposição a ruídos (tanto temporários quanto de longo prazo) está associada a aumentos nas pressões arteriais tanto em adultos (Andrén et al. 1983), como em crianças (Evans et al. 2001). Pesquisas morfológicas detalhadas em ratos descobriram uma variedade de danos físicos que foram observados no coração quando o sistema foi exposto ao ruído. Dentre elas, o dano mitocondrial nas células miocárdicas (Gesi et al. 2002), que ocorreu tanto nos átrios quanto nos ventrículos (Soldani et al. 1998; Lenzi et al. 2003).

Sistema imunológico

A exposição ao ruído antropogênico também mostrou efeitos sobre o sistema imunológico dos animais. Por exemplo, ratos expostos a ruído mostraram diminuições significativas nas suas respostas imunes humorais, incluindo aumento nos níveis de imunoglobulina, diminuição do número de células T e diminuição na atividade fagocitária (Van Raaij et al. 1996). Além disso, o sistema imune também foi afetado em filhotes durante a gestação. Camundongos com mães expostas ao ruído durante a gravidez tiveram um comprometimento da resposta imune secundaria, apresentando menor peso de timo e menores níveis de ImunoglobulinaG (Sobrian et al. 1997).

Cognição e sono

O ruído antropogênico também mostrou ter efeitos no sistema cognitivo (Stansfeld et al. 2000). A exposição crônica ao ruído em trabalhadores industriais e pessoas que vivem próximas a vias movimentadas de tráfego foi associada à depressão e a sentimentos de agressão (Stansfeld et al. 2000; Ising and Kruppa 2004). Além disso, o aumento dos níveis de ruído foi associado a reduções na memória em crianças (Lercher et al. 2002) e em ratos (Rabat 2007). Além disso, crianças estressadas por ruído tiveram déficits na fala e na capacidade de leitura (Hygge et al. 2002). Um outro estudo comparando crianças de escolas primárias exposta a altos níveis de ruído ferroviário mostrou diferenças significativas nos escores de leitura, com um retraso de idade média de leitura de 3-4 meses (Bronzaft and McCarthy 1975). Por último, estudos realizados ao redor do aeroporto de Heathrow, em Londres, compararam o desempenho cognitivo e as respostas ao estresse de crianças de 9 a 10 anos de idade causados pelo ruído. Os resultados apontaram que, no início, as crianças expostas ao ruído tinham compreensão de leitura e atenção prejudicadas (Haines et al. 2001).

Além dos efeitos de exposição em locais de atividade diurna, o ruído antropogênico pode afetar, por exemplo, o sono (Rabat 2007). Isto pode levar a uma série de efeitos deletérios graves sobre a saúde e a cognição (Stansfeld and Matheson 2003; Muzet 2007; Hume et al. 2012). As recomendações para humanos, em geral, exigem um nível de ruído interior de 30 dB (A) e máximo de 45 dB (A), durante as 8 horas noturnas (OMS 2009). Estima-se que é provável que ocorram distúrbios significativos do sono se houver mais de 50 eventos de ruído por noite com nível igual ou superior a 50 dB (A) (Stansfeld and Matheson 2003). Em humanos, distúrbios crônicos do sono têm sido relacionados a problemas nos sistemas fisiológicos, incluindo cardiovasculares, imunológicos, gastrointestinais ou reprodutivos, bem como uma ampla gama de transtornos mentais e do humor (Knutson et al. 2007; Depner et al. 2014). A perda de sono também está ligada ao envelhecimento celular, incluindo a redução de telômeros (Prather et al. 2015; Tempaku et al. 2015).

O ruído antropogênico, como o tráfego, por exemplo, é conhecido por ser uma causa de interrupção do sono e da sua qualidade em humanos (Lewy et al. 1980; Begemann et al. 1997; Griefahn 2002; Michaud et al. 2008). O impacto dos distúrbios antropogênicos no sono tem recebido maior atenção em humanos, enquanto poucos estudos investigaram esse tópico em animais não humanos. Por exemplo, em ratos, a privação do sono induziu danos ao DNA em células cerebrais e sanguíneas (Andersen et al. 2009), podendo também levar à morte (Naitoh et al. 1990; Rechtschaffen and Bergmann 2002). Fica claro que a perturbação do sono pode ter consequências importantes para os organismos, porém, pouco se conhece sobre os efeitos do ruído antropogênico em animais não humanos ainda.

Efeitos auditivos do ruído antropogênico

O ruído antropogênico pode causar uma variedade de efeitos auditivos adversos nos animais, incluindo lesões ao sistema auditivo pela superexposição acústica até o mascaramento dos sinais acústicos na comunicação e outras pistas importantes do ambiente. As lesões auditivas são geralmente derivadas de traumas causados por exposição a níveis extremos de ruído (por exemplo, além do limiar da dor) ou pela exposição crônica a níveis perigosos (Dooling and Popper 2007). Em casos mais extremos, ele pode causar desde deficiência auditiva até surdez. Além disso, ele pode levar a alterações comportamentais resultando na perturbação de atividades ou até no abandono do local (Kight and Swaddle 2011; Popper and Hawkins 2016).

Geralmente, são considerados quatro tipos de efeitos auditivos dos ruídos antropogênicos em animais (Fig.4). Estes incluem (i) alterações permanente do limiar auditivo e danos no sistema de audição, (ii) alterações temporárias do limiar com dano potencial ao sistema auditivo, (iii) mascaramento e (iv) outros efeitos fisiológicos e comportamentais (Dooling and Blumenrath 2013; Dooling and Popper 2016).

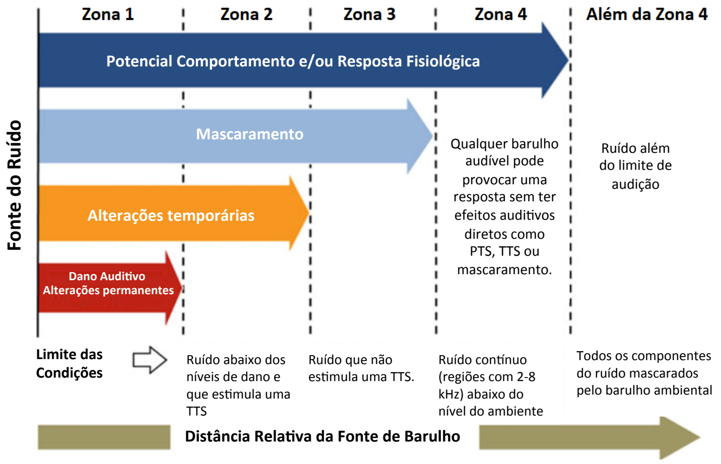

Figura 4. Efeitos dos ruídos antropogênicos em animais. Adaptado de Dooling and Blumenrath (2013).

Os ruídos antropogênicos são possíveis limitadores ou inibidores da comunicação, podendo ter um efeito negativo significativo no sucesso de acasalamento ou outros comportamentos associados a ela. Existem mecanismos de longo e curto prazo utilizados pelos animais para tentar reduzir o mascaramento do sinal acústico: as mudanças evolutivas nos parâmetros do canto e os ajustes reversíveis a curto prazo (Brumm and Slabbekoorn 2005). As mudanças a longo prazo são adaptações evolutivas nas quais se espera que os animais ajustem a estrutura acústica do canto a fim de reduzir o efeito de mascaramento do ruído (Brumm and Zollinger 2013). Essas mudanças estão relacionadas à hipótese de adaptação acústica (Ely e Fisher 2009), que afirma que o ambiente no qual a comunicação acústica ocorre deve favorecer características de vocalização que minimizem atenuação e distorção (degradação do sinal). Isso inclui ajustes no tempo e no local da atividade acústica, assim como alterações nos parâmetros temporais ou espectrais do canto com fins de reduzir o mascaramento de seu sinal por um ruído no ambiente (Rabin et al. 2003; Slabbekoorn and Peet 2003). Já as mudanças de curto prazo estão ligadas à plasticidade do sinal, com base em ajustes individuais (Brumm and Zollinger 2013). Estes ajustes temporários foram relatados em espécies que lidam tanto com ruído biótico (intra e heteroespecífico) como abiótico (vento, córregos). Em geral, os emissores reduzem os efeitos negativos de ruído modificando características do canto como amplitude (Penna and Hamilton-West 2007), duração (Penna et al. 2005) e frequências (Slabbekoorn and Smith 2002). Estas alterações nos parâmetros acústicos do canto foram relatadas para diversos grupos de animais quando os indivíduos enfrentam o ruído antropogênico (Brumm 2013; Slabbekoorn et al. 2018a). A seguir, daremos alguns exemplos do efeito do ruído antropogênico em vertebrados terrestres e como estes animais parecem lidar com o problema.

Anfíbios

A sinalização acústica desempenha um papel fundamental nos anfíbios anuros, tanto na reprodução, com o chamado dos machos para atrair parceiras, quanto na defesa de territórios e detecção de predadores (Gerhardt and Huber 2002). Anuros produzem sons geralmente entre 100-6000Hz (Capranica 1976; Capranica 1965), mas existem espécies que emitem ultrassons (Feng et al. 2006) e vibrações sísmicas (Narins 1990). Além disso, o sistema auditivo do grupo permite a percepção de uma ampla gama de frequências em geral entre 20-10.000Hz, com algumas poucas espécies capazes de perceber vibrações sísmicas e ultrassons (Feng et al. 1975, 2006; Lewis et al. 1982; Narins and Feng 2006; Dijk et al. 2011). A sensibilidade dos anuros aos sons, juntamente com a importância dos sinais acústicos para sua comunicação (Narins 1995; Wells 2008), os tornam suscetíveis a um efeito potencialmente negativo do ruído antropogênico (Schwartz and Bee 2013; Vélez et al. 2013).

Estudos avaliando os efeitos do ruído antropogênico em anuros mostraram que as espécies respondem usando estratégias distintas (Simmons and Narins 2018), incluindo alterações nos parâmetros do canto (temporais e espectrais) (Vélez et al. 2013) ou até evitando a fonte de ruído (Herrera-Montes and Aide 2011; Vargas-Salinas et al. 2014). Para reduzir o efeito de mascaramento, algumas espécies ajustaram a duração do canto ou apenas de algumas notas (Lengagne 2008; Kaiser et al. 2011), alteraram a amplitude dele (Cunnington and Fahrig 2010) ou até mesmo a frequência (Parris et al. 2009; Hoskin and Goosem 2010; Roca et al. 2016). Além dos efeitos dos ruídos nos machos, foi observado em testes de fonotaxia que fêmeas, na presença do ruído antropogênico, diminuíam a orientação em direção ao sinal-alvo (macho) e aumentavam o tempo para alcançá-lo. Isso sugere que o ruído pode atrapalhar a comunicação dos sapos de duas maneiras: alterando ou até suprimindo a atividade de vocalização dos machos e, ao mesmo tempo, diminuindo a capacidade das fêmeas de avaliar e localizar os parceiros (Bee and Swanson 2007). Tais efeitos podem ter consequências diretas na reprodução dos indivíduos.

Répteis

Embora a maioria dos répteis possua capacidade auditiva (Dooling et al. 2000), apenas alguns grupos utilizam o som para comunicação. Apenas duas espécies foram testadas para avaliar o efeito do ruído antropogênico no seu canto. Um exemplo é a lagartixa-tokay (*Gekko gecko*), espécie sensível a faixas de frequência entre 200 a 5000 Hz (Brittan-Powell et al. 2010). Estes animais, quando expostos ao ruído de cidades, aumentam a duração das notas do canto que contém maior amplitude, um comportamento que facilita a detecção de sinais pelos receptores (Brumm and Zollinger 2017). Outro trabalho analisou o lagarto-de-língua-azul (*Tiliqua scincoides*) sobre o efeito do ruído de maquinário de mineração (escavadeira, caminhão de carvão e perfuratriz). Os lagartos expostos ao ruídos passaram mais tempo imóveis. Os autores interpretaram essas reações como indicativas de medo ou estresse (Mancera et al. 2017).

Apesar de existirem apenas estes dois trabalhos com lagartos, sabe-se que jacarés e crocodilos empregam repertórios vocais para comunicação. Os crocodilianos juvenis produzem uma variedade de sons harmonicamente estruturados com energia que se estende até 5000Hz. Os adultos produzem sons nos contextos de corte e acasalamento, com a maior parte da energia concentrada abaixo de 250 Hz

(Vergne et al. 2009). A audição também foi avaliada em várias espécies de tartarugas (testudines). Ferrara et al. (2014) relataram que a tartaruga-da-amazônia (*Podocnemis expansa*) produz uma série de vocalizações distintas com frequências na faixa de aproximadamente 95-460Hz. Os autores especularam que esses animais usam sons em contextos sociais e que o som desempenha um papel importante na sincronização das atividades do grupo durante a época de nidificação. Esses resultados deixam em aberto estudos para avaliar os potenciais efeitos deletérios do ruído antropogênico nestes grupos (Simmons and Narins 2018).

Aves

As aves usam sinais acústicos para uma série de interações sociais cruciais, como defesa de território, atração de parceiros e fuga de predadores. Em geral, as aves produzem sons entre 1.000 Hz e 8.000 Hz (CornelLab 2009) e escutam mais ou menos na mesma faixa de frequências que os humanos (20Hz-20kHz), dependendo do grupo, com melhor sensibilidade entre 2 e 4 kHz (Halfwerk et al. 2018). O efeito do ruído antropogênico na comunicação de aves tem recebido bastante atenção se comparado a outros grupos de vertebrados terrestres. Estudos mostraram que as aves são capazes de aumentar a amplitude e a frequência de seu canto em resposta a altos níveis de ruído (Brumm and Slabbekoorn 2005; Gil and Brumm 2014). Estes resultados sugerem que as aves são capazes, pelo menos em parte, de mitigar o mascaramento de seu sinal ajustando a estrutura do canto. Entretanto, este ajuste é limitado e a efetividade da comunicação cairá quando o limite da capacidade de ajustes for ultrapassada (Brumm and Zollinger 2013). Desta forma, efeitos na eficiência da sinalização podem ter maiores consequências para os indivíduos dependendo da fonte do ruído externo, da amplitude dele e da distancia entre o emissor e o receptor, por exemplo (Fig.5). O ruído pode interferir tanto na detecção como na discriminação e no reconhecimento do sinal e uma série de comportamentos podem ser afetadas devido a este problema (Wiley 2013). O ruído em áreas industriais, por exemplo, mostrou afetar o sucesso de pareamento de casais de mariquita-de-coroa-ruiva (*Seiurus aurocapilla*). Além disso, alterações nos parâmetros do canto também podem afetar preferências sexuais das fêmeas ou até reconhecimento de espécies. Estudos observaram, por exemplo, que na presença de ruído de estradas, as aves conseguiam detectar sinais do canto, mas a discriminação da informação contida nesses sinais fica reduzida (Lohr et al. 2003). Por conseguinte, o ruído antropogênico pode afetar diferentes momentos do processo de comunicação e também aspectos além dele.

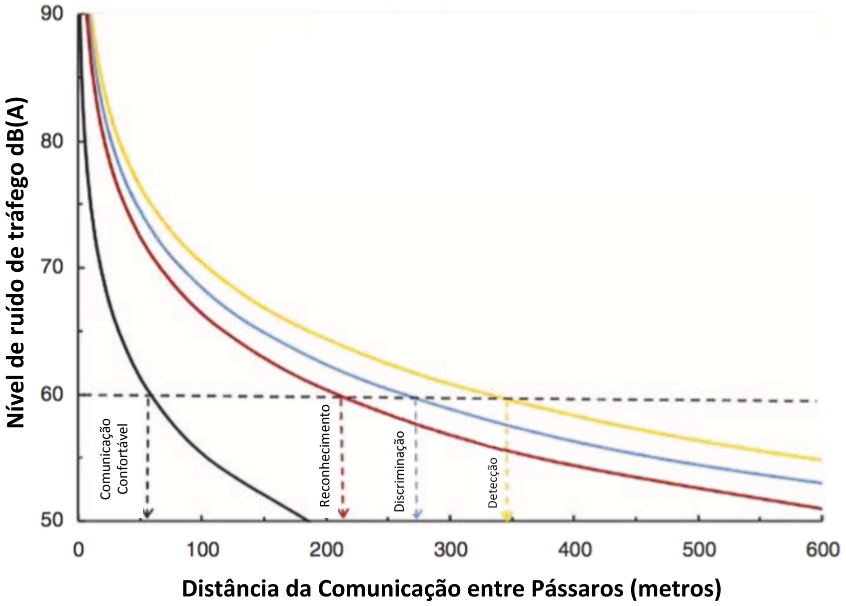

Figure 5. Relação entre o nível geral de ruído e as distâncias de comunicação que permitem detecção, discriminação, reconhecimento e comunicação confortável entre as aves. Adaptado de Dooling and Blumenrath (2013).

Mamíferos

As vocalizações claramente desempenham um papel crítico na comunicação ao longo da vida dos mamíferos. Este grupo habita uma diversidade de ambientes e varia em tamanho corporal, desde pequenas espécies pesando 2g (musaranho-pigmeu) até 6.000kg (elefante). Essa diversidade de formas e tamanhos também se reflete na ampla variedade de performances auditivas (Fig.6). Em relação à capacidade auditiva humana (20-20.000Hz), outros animais têm sensibilidades acima deste limiar (ultrassons), incluindo outros primatas e morcegos, outros têm sensibilidade abaixo deste limiar (infrassons), como os elefantes (Slabbekoorn et al. 2018b)

Determinar a direção e a distância de uma fonte sonora é uma capacidade crítica necessária para localizar parceiros sexuais, presas, detectar predadores e para orientação e navegação no espaço. Foram registradas diversas estratégias em mamíferos terrestres a fim de minimizar a influência do mascaramento do sinal acústico pelo ruído (Slabbekoorn et al. 2018b). Uma estratégia observada é a aproximação da fonte de sinal. Além disso, foi observado o aumento da amplitude da emissão vocal, relatado para a fala humana, além de outros primatas (Sinnott et al. 1975), gatos (Nonaka et al. 1997) e morcegos (Hage et al. 2013). Além de aumentar o nível do som, foi observado aumento na duração dos chamados de espécies de macacos (*Callithrix jacchus* e *Saguinus oedipus*) (Brumm 2004; Roian Egnor and Hauser 2006). A ampla diversidade de limiares auditivos e gamas espectrais de som detectadas por mamíferos terrestres acrescenta uma dimensão de complexidade no esforço para compreender o impacto do ruído produzido pelo homem nos animais.

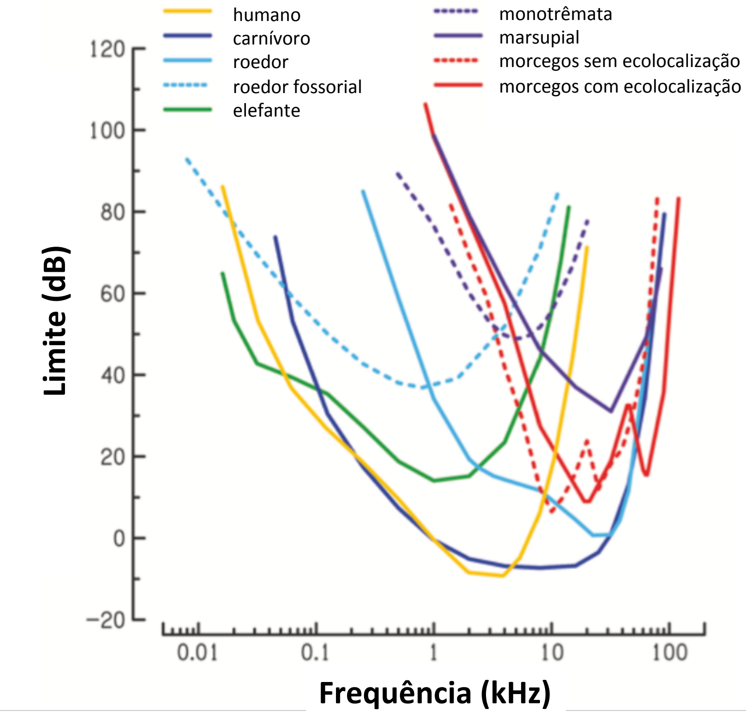

Figura 6. Representação dos limiares auditivos em relação a diferentes frequências em alguns mamíferos terrestres. Adaptado de Slabbekoorn et al. (2018b).

Extensão do problema

Diversos estudos destacaram o potencial efeito negativo dos ruídos antropogênicos sobre a biodiversidade (Brumm 2013). Alguns estudos focaram nos mecanismos por trás desse padrão e testaram a extensão desses efeitos negativos devido ao efeito de mascaramento dos sinais pelo ruído.

Um estudo que compilou pesquisas sobre os efeitos do ruído antropogênico na fauna silvestre observou que, apesar da heterogeneidade das respostas dos animais ao ruído, a faixa de níveis documentada que induzem uma resposta biológica em humanos e em animais silvestres é similar (40 - 100 dB SPL re 20 μPa) (Shannon et al. 2016). As estratégias utilizadas pelos diversos grupos de animais abordados nesta introdução para lidar com o mascaramento do sinal pode trazer diversas consequências. Parâmetros espectrais e temporais são muito importantes na seleção e localização do parceiro (Forrest 1994; Gerhardt and Huber 2002) e o fato de que muitas espécies desenvolveram mecanismos para reduzir os efeitos de mascaramento do sinal não garante seu sucesso no acasalamento. Além das fêmeas mostrarem que sua orientação é reduzida pelo ruído e o tempo para localizar o alvo (macho) aumentado (Bee and Swanson 2007), os parâmetros acústicos são importantes na seleção sexual (Gerhardt 1991; Márquez et al. 2008), podendo alterar a escolha ou inclusive selecionar indivíduos, que incialmente não seriam escolhidos.

No contexto dos efeitos do ruído derivado de atividade antrópica, o efeito das vibrações antropogênicas na biodiversidade terrestre ainda permanece desconhecido. Dentre os vertebrados terrestres, os anfíbios são conhecidos como o grupo mais sensíveis às vibrações (Hill 2008). Apesar de alguns trabalhos mostrando a emissão e percepção deste tipo de onda pelo grupo (Narins 1990; Warkentin 2005; Márquez et al 2016), o efeito desta provável fonte estressora permanece desconhecido.

Além disso, ruído antropogênico não é apenas uma alteração nas características do meio de transmissão e da comunicação; na verdade, é também uma ameaça à saúde que pode diminuir a sobrevivência dos animais (Troïanowski et al. 2017). Do ponto de vista individual, as mudanças na atividade do canto podem ter consequências negativas, como aumento da exposição a predadores e altos custos energéticos (Ryan 1988; Wells 2001). Portanto, a mudança nas características do canto pode afetar não apenas a atividade de vocalização, mas indiretamente a função de vida dos animais e as taxas vitais (McGregor et al. 2013; Francis and Barber 2013; Kaiser et al. 2015). Além de consequências individuais, áreas urbanizadas ou próximas a estradas podem ter um efeito negativo na densidade e na presença de indivíduos (Pellet et al. 2004; Hamer and Parris 2011). No geral, as evidências atuais indicam que a abundância de algumas espécies é afetada negativamente pelo ruído produzido pelo homem e - entre outras coisas - o comprometimento da comunicação acústica é uma das razões para o declínio .

Após uma breve revisão do efeito do ruído antropogênico em animais terrestres, mostrando a extensão do problema, que vai desde o efeitos na integridade do DNA até funções fisiológicas e comportamentais, esta tese busca contribuir com conhecimento para este problema.

4. OBJETIVOS

4.1. OBJETIVO GERAL

O objetivo desta tese é compreender o efeito de ruídos antropogênicos em animais, abordando aspectos não auditivos e auditivos, incluindo a comunicação, em aves e anfíbios anuros.

4.2. OBJETIVOS ESPECÍFICOS

1. Investigar se fatores ambientais urbanos, tanto antropogênicos (ruído e luz artificial), quanto outros fatores abióticos como temperatura e humidade, predizem padrões de atividade de sono em uma ave urbana (*Parus major*). Com base na literatura, espera-se que o que frequência de interrupção do sono seja maior em níveis crescentes de ruído antropogênico, luz artificial e temperatura.

2. Testar o efeito do ruído antropogênico audível do tráfego de veículos na atividade de vocalização de duas espécies de anuros hylídeos (*Boana bischoffi* e *B. leptolineata*). Baseado na literatura, espera-se que o ruído do tráfego altere parâmetros acústicos do canto, especialmente na espécie em que a frequência do canto tem maior sobreposição com a frequência do ruído.

3. Testar o efeito do ruído antropogênico sísmico (tráfego de veículos e turbinas aerogeradoras) na atividade de vocalização do sapo-parteiro-comum (*Alytes obstetricans*). Espera-se um efeito negativo das vibrações sísmicas proveniente de atividades humanas sobre a atividade de vocalização dos machos, alterando seus parâmetros de canto.

4. Revisar a literatura sobre os efeitos auditivos e não auditivos do ruído antropogênico em anuros.

5. Propor medidas de mitigação e redução do impacto da poluição sonora em anuros.

5. ESTRUTURA DA TESE

1. Efeitos não auditivos do ruído antropogênico
    1.1. Ruído de cidades e o sono em aves (CAPITULO I)
2. Efeitos auditivos do ruído antropogênico
    2.1. Ruído antropogênico sonoro de estradas e a atividade acústica em anuros (CAPITULO II)
    2.2. Ruído antropogênico sísmico de estradas e eólicos e a atividade acústica em anuros (CAPITULO III)
    2.3. Revisão do efeito do ruído antropogênico em anuros (CAPITULO IV)

6. REFERÊNCIAS

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7.1. CAPÍTULO I

**Submetido na Behavioral Ecology and Sociobiology*

Nocturnal activity and resting behaviour in urban great tits and its relation to anthropogenic disturbance and microclimate

Valentina Caorsi [a,b], Philipp Sprau[c], Sue Anne Zollinger[b], Henrik Brumm[b]

[a] Programa de Pós–Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

[b] Max Planck Institute for Ornithology, Communication and Social Behaviour Group, Seewiesen, Germany

[c] Behavioral Ecology, Department of Biology, Ludwig Maximilians University of Munich, Germany

Normas conforme o Periódico Behavioral Ecology and Sociobiology

https://www.springer.com/life+sciences/behavioural/journal/265?detailsPage=pltci_109021

Behavioral Ecology and Sociobiology

Nocturnal activity and resting behaviour in urban great tits and its relation to anthropogenic disturbance and microclimate

--Manuscript Draft--

Manuscript Number:	BEAS-D-18-00209R1	
Full Title:	Nocturnal activity and resting behaviour in urban great tits and its relation to anthropogenic disturbance and microclimate	
Article Type:	Original Article	
Corresponding Author:	Henrik Brumm Max-Planck-Institut fr Ornithologie Seewiesen, GERMANY	
Order of Authors:	Valentina Caorsi	
	Philipp Sprau	
	Sue Anne Zollinger	
	Henrik Brumm	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Max-Planck-Institut fr Ornithologie	
Corresponding Author's Secondary Institution:		
First Author:	Valentina Caorsi	
First Author Secondary Information:		
Order of Authors Secondary Information:		
Funding Information:	Deutsche Forschungsgemeinschaft (BR2309/8-2)	Dr. Henrik Brumm
	Deutsche Forschungsgemeinschaft (Br2309/9-1)	Dr. Henrik Brumm
	Deutsche Forschungsgemeinschaft (SP 1450/3-1)	Dr. Philipp Sprau
	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES No. 88881.135522/2016-01)	Ms Valentina Caorsi
Abstract:	The ecological novelty of urbanisation poses many challenges to animals. We investigated whether anthropogenic disturbance (artificial light at night and noise) and abiotic factors in cities (temperature and humidity) predict nocturnal activity and rest in free-living urban great tits (Parus major). Our study is the first to relate nocturnal rest in wild birds to levels of noise pollution during the night, an issue that has been shown to be particularly damaging to human health. Unlike previous work on nocturnal behaviour of urban birds, we considered the combined effect of anthropogenic disturbance and urban microclimate to acknowledge that the umwelt of an animal is comprised of multiple environmental variables. Using infrared cameras, we observed the nocturnal resting behaviour as a proxy for sleep in seventeen birds in nest boxes deployed across the city of Munich, Germany. Although we found marked differences in resting behaviour between individuals, this variation was not related to the measured environmental factors. This finding contrasts earlier studies that reported nocturnal resting behaviour of birds to vary with temperature and light exposure. Although we did not find evidence that urban environmental factors disrupt resting behaviour in great tits, their sleep might still be impaired by the anthropogenic disturbances. To elucidate this issue, further studies are necessary that, for instance, measure brain activity.	
Response to Reviewers:	RESPONSES TO REVIEWER COMMENTS - BEAS-D-18-00209	

Nocturnal activity and resting behaviour in urban great tits and its relation to anthropogenic disturbance and microclimate

Valentina Caorsi[a,b], Philipp Sprau[c], Sue Anne Zollinger[b], Henrik Brumm

Abstract

The ecological novelty of urbanisation poses many challenges to animals. We investigated whether anthropogenic disturbance (artificial light at night and noise) and abiotic factors in cities (temperature and humidity) predict nocturnal activity and rest in free-living urban great tits (*Parus major*). Our study is the first to relate nocturnal rest in wild birds to levels of noise pollution during the night, an issue that has been shown to be particularly damaging to human health. Unlike previous work on nocturnal behaviour of urban birds, we considered the combined effect of anthropogenic disturbance and urban microclimate to acknowledge that the umwelt of an animal is comprised of multiple environmental variables. Using infrared cameras, we observed the nocturnal resting behaviour as a proxy for sleep in seventeen birds in nest boxes deployed across the city of Munich, Germany. Although we found marked differences in resting behaviour between individuals, this variation was not related to the measured environmental factors. This finding contrasts earlier studies that reported nocturnal resting behaviour of birds to vary with temperature and light exposure. Although we did not find evidence that urban environmental factors disrupt resting behaviour in great tits, their sleep might still be impaired by the anthropogenic disturbances. To elucidate this issue, further studies are necessary that, for instance, measure brain activity.

Keywords: anthropogenic disturbance, artificial light at night, noise, *Parus major*, sleep, urbanisation

Significance Statement

Urbanisation is a subject of growing concern among scientists, conservationists and policy makers alike. Yet surprisingly little is known about the impact of urbanisation on wildlife. We investigated whether anthropogenic disturbance (artificial light at night and noise) and microclimate (temperature and humidity) predict patterns of nocturnal activity and resting behaviour in urban great tits (*Parus major*). Although patterns of resting behaviour differed markedly between individuals, this variation was not related to any of the four measured environmental factors. Our findings are in contrast to previous studies on the effects of urban microclimate and light pollution. At the same time they suggest that opposing effects of different urban ecological factors may level each other out and thus should be considered in combination.

Acknowledgments

We thank students and field assistants of the Behavioural Ecology group of the Ludwig-Maximilians University Munich, as well as all citizen scientists for help with collecting the data. Walter Schimmel and Dietfried Molter are acknowledged for technical support. Also, we thank Niels Rattenborg, Constantino Macías Garcia, and two anonymous reviewers for their comments on the manuscript and Sam Hardman, Samuel Kaiser, Julia Schäfer, Adriana Dorado, and Luke Eberhart-Phillips for discussions. We further thank the citizen scientists for hosting the nest boxes and providing access to the cameras. Funding was provided by grants by the Deutsche Forschungsgemeinschaft to P.S. (award SP 1450/3-1) and H.B. (awards BR 2309/8-2 and BR 2309/9-1) and a research fellowship to V.C. awarded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES No. 88881.135522/2016-01).

Introduction

Urbanisation is among the human activities causing the most drastic and permanent habitat transformations (McKinney 2002). These transformations include a loss of natural resources, habitat fragmentation, changes in environmental factors such as temperature and precipitation, and an increase of anthropogenic disturbances, e.g. chemical, noise, and light pollution (Marzluff et al. 2008; Grimm et al. 2008; Shanahan et al. 2014). Ultimately, the ecological changes associated with urbanisation cause exceptional environmental variation (Sprau et al. 2016), which may pose a threat to biodiversity (Kappelle et al. 1999).

Increases in temperature, which are typically observed in cities ("urban heat islands"), have been suggested to affect a whole suite of physiological, behavioural and ecological traits in animals, such as body growth, breeding phenology, reproductive success, predator-prey relationships, and community composition (Avondet et al. 2003; Visser et al. 2006; Peach et al. 2008; Murphy et al. 2016; Brans et al. 2017; Schäfer et al. 2017).

Another characteristic of urban areas is the presence of artificial light. An increase in light intensity during the night may have fundamental ecological and evolutionary implications for animal populations, which may in time reshape entire ecosystems (Hölker et al. 2010). On an individual level, artificial lighting at night can alter behaviour, with often drastic effects on biological rhythms, activity budgets, and reproduction (Kempenaers et al. 2010; Dominoni et al. 2013, 2014; Raap et al. 2015).

In addition to artificial light and temperature changes, a wide range of species, from terrestrial to aquatic animals, are also affected by noise pollution (Brumm 2010; McGregor et al. 2013). Over the past decades, many studies have shown that anthropogenic noise may negatively affect animals on different systemic levels. An obvious effect of anthropogenic noise is on animal communication since noise can impair the detection of acoustic signals, which may disrupt, for instance, anti-predator or reproductive behaviours (Brumm 2013; Templeton et al. 2016). However, anthropogenic noise also has subtler, but nevertheless equally profound, impacts beyond signal masking. On a proximate level, chronic noise exposure may affect animal physiology, neural function, cellular ageing, and gene expression (Kight and Swaddle 2011, Kleist et al. 2018, Dorado-Correa et al. 2018). In terms of behaviour and ecology, there is a growing body of evidence that anthropogenic noise can impair foraging, reduce reproductive success, and change animal density and community structure (Barber et al. 2010).

Behavioural responses to the urban environment are usually studied during the active period of animals, however, disruptions during the inactive period (e.g. during the night for diurnal animals) are also crucial because sleep disturbance may have severe consequences. Sleep is a widespread and important behaviour in animals (Siegel 2008; Cirelli and Tononi 2008; Rattenborg et al. 2017) and many studies have shown that sleep deprivation can result in a wide range of negative health effects (Shaw et al. 2002; Stephenson et al. 2007; Andersen et al. 2009). The impact of anthropogenic disturbances on sleep has received the most attention in humans (Lewy et al. 1980; Begemann et al. 1997; Griefahn 2002; Michaud et al. 2008), while only few studies have investigated this topic in non-human animals. Urban birds are known to advance their activity to early morning and night hours, depending on the level of ambient light and noise pollution (Fuller et al. 2007; Dominoni et al. 2014). Moreover, experimental studies, applying artificial illumination inside nest boxes or cages, show that light exposure during the night disrupts resting behaviour in birds (Raap et al. 2015; de Jong et al. 2016a; Sun et al. 2017). Similarly, artificial light from lamp posts was reported to reduce nocturnal rest in birds roosting outside nest boxes (Ouyang et al. 2017, but see Raap et al. 2017).

A crucial gap in our knowledge is whether current levels of noise pollution disrupt nocturnal resting periods in urban birds. Moreover, to our knowledge, no previous studies have considered the combined effects of urban factors, i.e. the synergistic impact of artificial light intensities, noise levels, temperature and humidity on nocturnal resting periods in urban birds. As birds have become a common and useful model system in the study of urban ecology (Marzluff 2001; Gil and Brumm 2013), approaching these questions in an urban avian species may be particularly relevant.

In this study, we investigated whether urban environmental factors, both anthropogenic and abiotic, predict patterns of nocturnal activity and rest in free-living great tits (*Parus major*). Great tits are one of the commonest birds in Eurasian cities and previous studies on this species showed that nocturnal activity may vary with temperature (Stuber et al. 2015, 2017) and light intensity (Raap et al. 2015; de Jong et al. 2016b). However, it is not known whether these factors actually disrupt resting behaviour in urban habitats. Based on the previous literature, we predicted great tit resting behaviour during the night to (1) be more disrupted with increasing levels of artificial light and anthropogenic noise and to (2) be more disrupted at higher temperatures. Unlike previous works, this study considers the combined effect of anthropogenic disturbance and urban microclimate, which may help to elucidate the influence of urbanisation on animal behaviour (Sprau and Dingemanse 2017).

Material and Methods

Study site and data collection

Between 2 April and 3 May 2016 we observed nocturnal resting behaviour of great tits in the city of Munich, Germany (48° 8' 6.45" N 11° 34' 55.132" E). Great tits are secondary hole nesters, utilizing natural holes in trees and artificial nest boxes (Perrins 1965). They are primarily active during the day and are considered nocturnal sleepers (Amlaner and Ball 1983; Stuber et al. 2015). During egg incubation and after hatching of the young, the female spends the night on the nest, whereas the male sleeps outside the nest cavity. This study was performed within the framework of a larger previous study (Sprau et al. 2016). From a total number of 157 great tit territories in the study population, we selected a subset of 23 territories. These 23 nest boxes were distributed across the entire city, thus covering a range of human disturbances on a gradient from highly disturbed habitats in the city centre to relatively undisturbed habitats in suburban areas (Fig. 1). Territorial pairs bred in nest boxes deployed in the gardens of private homes of collaborating citizen scientists. For the analysis, we used only 17 of these nest boxes, the rest were excluded due to technical problems or to the excessive presence of ectoparasites in one nest, which is known to affect the nocturnal activity of infected birds (Christe et al. 1996). In order to investigate whether urban induced environmental variation influences resting activity of female great tits during incubation, we chose locations of the nest boxes that allow environmental variation (Fig. 1). At each site we measured four environmental factors: temperature (ºC), atmospheric humidity (%), artificial light intensity (lux) and nocturnal noise levels (dB(A) re. 20µPa). Measurements were taken at each nest box every minute during the time of observation using custom-made environmental loggers (MSR Electronic GmbH, Switzerland) installed outside the nest boxes approx. 10 cm above the entrance hole. The minimum noise level detectable by the loggers was 39 dB(A), which corresponds to the minimum noise level measured at night in urban bird habitats in previous studies (Fuller et al. 2007; Dominoni et al. 2016). Light levels were measured in lux, which is the luminous flux per square meter. A value of about 100.000 lux correspond to direct sunlight, 100 lux to a dark overcast day, and 0.05–0.3 lux to a full moon on a clear night (Ryer 1997; Kyba et al. 2017). Inside each nest box, an infrared digital internet protocol camera (INSTAR GmbH, Germany) was installed to remotely monitor the birds' resting behaviour. In order to minimize disturbance induced by the cameras, LEDs with a wavelength of 940 nm were used, a colour that birds cannot see. We recorded a still photograph every one second between 19:00 and 03:00 every night in each nest box for three consecutive nights during the incubation period to measure the activity of the females. However, only data for two nights could be used for three birds because of technical failure, resulting in a mean number of 2.8 analysed nights per bird. Pictures taken by the cameras were automatically stored on a server at the Ludwig Maximilians University. For technical reasons, the system was limited to a recording time of 8 hours (i.e. 28,800 pictures) per night and nest box.

Data analysis

A single observer visually analysed all pictures using a MacBook pro. To exclude (unconscious) observer-expectancy biases (Traniello and Bakker 2015; Brumm et al. 2017), scoring of the images was done blindly, i.e. the person analysing the images was not informed about the environmental data of the nest boxes. For each night, we analysed all photographs by scoring the behavioural state depicted in each, and tallying the number of images that depicted each of two behavioural categories: (a) active, when the head was up, bill was out, facing forwards or the bird was actively moving inside the nest box, then considered awake (Online Resource 1a), or (b) inactive (resting), when the bird was in a "sleep posture", with the bill pointed backwards, tucked under the scapulars (Online Resource 1b) (Amlaner and Ball 1983). Since it is not possible to determine if a bird was physiologically asleep without recording brain activity, we used the "sleep posture" as a behavioural proxy for sleep, which we describe here as an “inactive” state. From the photographic counts, we then calculated (i) *the number of nocturnal movements* (number of times that the animal changed from an inactive position to an active one) and (ii) *the proportion of night spent in active and inactive states*.

We calculated an average of the environmental factors for each nest box, using only the data taken during the picture recording times (Online Resource 2). Following Sprau et al. (2016), we excluded spurious noise events above 90 dB(A) (likely elicited by wind). As intended, the average environmental parameters varied markedly between sites. The mean temperature was 5.4ºC (SD=4.0°C, range: 0.2-17.0ºC). The mean ambient humidity was 77.1% (SD=15.1%, range: 44.4-95.9%). The mean artificial light level during the night was 16.8 lux (SD=19.4, range: 0-92.9 lux). Noise levels had a mean amplitude of 56.9 dB(A) SPL (SD = 20.4, range: 39.0-76.2 dB(A)).

Statistical analysis

We performed a principal component analysis (PCA) with varimax rotation to investigate whether our environmental factors (temperature, humidity, light and noise) could be summarized into a single axis (principal component). The PCA resulted in two components (PC1 and PC2) with eigenvalues higher than one, describing two orthogonal axes of environmental factors (Table 1). We fitted univariate mixed-effect models to estimate sources of variation in resting behaviour. We investigated sources of variation in each of the two focal behaviours (number of nocturnal movements and proportion of night spent in each state) separately. Random effects included in the models were nest box and date. The two components resulting from the PCA and a variable separating the night into two halves (factor: early vs. late night) were fitted as fixed effects. The factor (early night: 1900–2300 hours, late night: 2301-0300 hours) was included because previous work found that nocturnal behaviour of great tits can vary with the period of the night (Stuber et al. 2015, 2017). We assumed a Gaussian error distribution for number of nocturnal movements and proportion of the night spent in inactivity, which was confirmed by visual inspection of model residuals. All covariates were further centred on their mean value (Kreft et al. 1995). For each specified relationship, we calculated the parameter estimate with its associated 95% credible interval. Credible intervals that do not cross zero indicate statistical significance (i.e., $p<0.05$) in the frequentist's sense. All statistical analysis were performed in R environment (version 3.4.1) using the packages "stats" (version 3.1.27) (R Core Team 2016), "lme4" (version 1.1–7) (Bates et al. 2015), "ggplot2" (version 2.2.1) (Wickham 2009) and "dplyr" (version 0.7.4) (Wickham et al. 2017). The software QGIS (version 2.4.0) (QGIS 2017) was used to plot the map in Fig.1.

Results

Patterns of resting behaviour during the night varied notably between females (Fig. 2, Online Resource 2), with some animals moving more than six times as often as others ($\bar{x}$=84.5, SD=25.7, range= 29-180). On average, the birds spent 93.3% of the night in resting position, with 97.1% resting time in the most inactive night and 82.1% in the least inactive night.

However, this variation was not related to the composite measures of environmental factors (Table 2). Neither PC1 (temperature, humidity and noise) nor PC2 (artificial light) had an effect on the proportion of the night spent at rest or on the continuity of rest (number of movements). When analysing artificial light and noise levels separately, models also did not show an effect of these factors on nocturnal movements [light: β=0.05; 0.16-0.25 (95% CI) and noise β=0.05 0.05; -0.12-0.20 (95% CI)] or on the proportion of night resting [(light β= -0.05; -0.28-0.20 (95% CI) and noise β=0.01; -018-0.18 (95%CI)].

Discussion

Our study found that variation in nocturnal resting behaviour between female great tits was not related to the environmental factors ambient light, noise, temperature and humidity. In particular, we did not find an effect of environmental factors on the number of nocturnal movements or on the proportion of time spent at rest during the night, both when considering composite measures of environmental factors within the same model and when looking at the effects of artificial light and noise separately.

These findings contrast earlier studies that reported nocturnal resting behaviour of birds varied with temperature and light exposure. For instance, free-ranging great tits were found to exhibit more nocturnal bouts of activity and to spend a greater proportion of the night active when temperatures were higher (Steinmeyer et al. 2010; Mueller et al. 2012; Stuber et al. 2015, 2017). Likewise, increased light intensities were found to reduce nocturnal rest in great tits, both in a correlative study (Stuber et al. 2015) as well as in response to experimental internal illumination of nest boxes (Raap et al. 2015, 2016; Stuber et al. 2017). When it comes to anthropogenic noise, we know of no previous study that has investigated whether noise affects bird resting behaviour or sleep. However, laboratory studies with other vertebrates have addressed the relation between environmental noise and sleep disturbances, and these studies found that chronic exposure to noise can permanently reduce and fragment sleep (Rabat 2007). Additionally, sleep deficits have been linked to compromises in the immune system (Majde and Krueger 2005), and animals chronically exposed to noise may even develop pathologies linked to poor sleep (McEwen and Wingfield 2003). However, in this study we did not find an effect of noise levels, or any of our tested urban environmental factors on resting behaviour in great tits, either in the number of nocturnal activity bouts, or on the proportion of time spent resting at night.

Although all our nest boxes were located in urban and suburban areas, the data loggers registered a large variation of the environmental factors between recording sites (see Online Resource 2) and, thus, the lack of environmental correlates of disruptions of rest time cannot be explained by lesser variation in environmental factors in our study. Indeed, we deliberately chose the nest box locations to cover a wide range of noise and light levels. As a result, our variation in artificial light levels ranged between 0 and 92.2 lux, which is much greater than the variation of light levels that have been previously related to reduced nocturnal rest in great tits (Raap et al. 2016; Sun et al. 2017), although these studies used artificial illumination inside nest boxes, whereas we measured the natural variation of ambient urban light levels outside the nest boxes. In addition, our average nocturnal noise levels varied substantially between nest boxes and were well within the range of noise levels that induces behavioural changes in urban birds, including great tits (Brumm 2004; Dominoni et al. 2016; Zollinger et al. 2017). Although not explicitly planned during the design of the study, the average temperatures also varied markedly between our nest box sites, namely by 13°C, which is similar to the temperature difference of 15°C, and bigger than the 5°C rise, that triggered a modification of great tit nocturnal rest in two previous studies (Lehmann et al. 2012; Stuber et al. 2017).

The discord between this study and previous work on temperature and light effects on resting behaviour in birds may be accounted for by our novel integrative approach that considers environmental variation as a unit. Ecological studies of urbanisation often focus largely on simple urban versus rural comparisons (Marzluff 2001; Marzluff and Rodewald 2008). Only recently have researchers started to integrate quantitative environmental measures and their variation in studies on the impacts of urbanisation on life histories (Sprau et al. 2016). In this study we tested multiple environmental factors within the same model, which acknowledges the complexity of urban habitats and, therefore could yield different results than studies including only one of these factors. The statistical difference between the two approaches is that in our case variance is partitioned whereas in previous studies variation is composed only of one factor. This partitioning may result in different effects levelling each other out in our study. However, as the umwelt of an animal is comprised of multiple environmental variables, it is important to consider the combined effects of external influences. Thus, we feel an integrative approach is better suited to the investigation of responses of animals to the combination of various environmental variables. However, even when considering light pollution levels in isolation, we did not find an effect on nocturnal resting behaviour. These contrasts with previous studies may be, at least partly, accounted for by methodological differences. Previous studies used artificial illumination inside nest boxes or cages (Raap et al. 2015; de Jong et al. 2016a; Sun et al. 2017), whereas we measured the natural variation of urban light levels outside the nest boxes. Obviously, nest boxes and natural nesting cavities can shield birds, to some extent, from light pollution. Hence, our results might also be taken as a hint that the effects of light pollution may be reduced for birds sleeping in cavities. In line with this notion, Ouyang et al. (2017) found that great tits in artificially illuminated areas were less active during the night when roosting inside nest boxes compared to birds roosting outside nest boxes (but see Raap et al. (2017) for a critique).

Moreover, anthropogenic disturbances may also affect other biotic factors that could, in turn, influence nocturnal resting behaviour of birds. For example, if increased levels of noise or light pollution decrease predation risk during the night, great tits might be more likely to be active in less noisy/illuminated urban areas, which would mask an effect of these anthropogenic disturbances in our data. To further investigate this issue, one would need to assess whether light or noise pollution affects the density or the behaviour of nocturnal nest box predators. If it does, then artificial illumination inside the nest box, as used in previous experimental studies, may not be the most ideal method in terms of ecological relevance.

Another explanation for our findings could be that individuals that are better adapted to anthropogenic disturbances settle in areas with high levels of light and noise pollution. Phenotype-matching habitat choice has been suggested as an explanation for settlement patterns in relation to human disturbance in dunnocks, *Prunella modularis*, (Holtmann et al. 2017). Moreover, it has been found that urban great tits differ in consistent behavioural traits from their rural conspecifics (Hardman and Dalesman 2018). It might well be that non-random distributions of great tit phenotypes also occur at smaller spatial scales within cities, such that individuals that are less sensitive to nocturnal disturbance are more likely to settle in disturbed areas.

Alternatively, it is also possible that city great tits have habituated to the environment and therefore no correlation between the environment and resting behaviour could be found. So far, little is known about whether and how birds habituate to anthropogenic disturbance. A study on human-induced flight behaviour indicates that urban birds habituate faster than their rural conspecifics (Vincze et al. 2016). In addition, several reports suggest that animals may respond less to loud noise events after repeated exposure (Boudreau 1968; Weisenberger et al. 1996; Krausman et al. 1998). While American black ducks (*Anas rubripes*) habituated to experimental aircraft noise exposure and reduced their vigilance and flight behaviours over the course of several days, wood ducks (*Aix sponsa*) did not habituate (Conomy et al. 1998). Laboratory experiments on rodents demonstrated that habituation to noise often occurs only on the behavioural level, but animals can still be affected physiologically (Rabat 2007). Thus, even if urban birds are able to restore their resting behaviour in chronic noise, nocturnal noise exposure may still be harmful to them because covert micro-arousals and decreased sleep intensity might go unnoticed in the behavioural observations.

It is important to bear in mind that this study, like previous ones on environmental effects on “sleep” in wild birds (Steinmeyer et al. 2010; Stuber et al. 2015, 2017, Raap et al. 2015, 2016; Ouyang et al. 2017, Sun et al. 2017), did not measure sleep but used nocturnal resting behaviour as a proxy for it. While it is obvious that the scope for sleep is decreased when a bird is more active, a caveat of this behavioural proxy is that inactivity does not necessarily mean that a bird is indeed sleeping. Even without detectable differences in total sleep duration, environmental influences may still affect the quality of sleep (Aulsebrook et al. 2016). For instance, humans may subjectively habituate to nocturnal noise, in that they are not awakened by it, but noise events still cause responses of the autonomic nervous system, such as elevated heart rate and vasoconstriction of sleeping individuals (Muzet 2007). Moreover, it has been found that noise exposure can result in a suppression of sleep intensity in humans without affecting sleep duration, which could over the long-term have adverse effects on health (Tasali et al. 2008). This means that although we did not find evidence that urban environmental factors disrupt resting behaviour in great tits, their sleep might still be impaired by anthropogenic disturbance. To elucidate this issue, it is therefore necessary to advance from behavioural sleep correlates to measuring brain activity. Laboratory set-ups to record EEG patterns in birds (Rattenborg et al. 2004; Lesku et al. 2011) could be used to investigate whether sleep is affected by light and noise pollution. Moreover, as the progress in technology now enables researchers to measure EEG-defined sleep also in wild animals (Rattenborg et al. 2017), it may be feasible in the near future to measure brain activity in free-ranging city birds. For this purpose, the integrative ecological approach that we used in this study may be particularly fruitful for future research on animal sleep in urban environments.

Compliance with Ethical Standards

The authors declare that they have no conflict of interest. Permits were obtained from the Bavarian government and the Bavarian regional office for forestry LWF (permit numbers 55.2-1-54-2532-140-11; 55.2-1-54-2532-59-12).

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Tables

Table 1 Results of the PCA using the four environmental factors.

	PC1	PC2	PC3	PC4
Standard deviation	1.37	1.09	0.88	0.41
Proportion of variance	0.47	0.3	0.2	0.04
Cumulative	0.47	0.76	0.96	1
Noise [dB(A)]	0.47	-0.22	0.81	-0.27
Light (lux)	-0.41	0.7	0.26	-0.52
Temperature (ºC)	-0.47	-0.66	-0.1	-0.57
Humidity (%)	0.62	0.13	-0.52	-0.57
Eigenvalues	1.87	1.18	0.78	0.17

Table 2 Sources of variation in the number of active bouts and total duration of activity/inactivity in relation to temperature, humidity, and noise (PC1) and light (PC2, see Table 1). All models control for variation induced by time of night (early and late night) and included random intercepts for nest box and day. We present fixed parameters (β) and random parameters (σ) with their 95% credible intervals (CrIs); effects with credible intervals that do not include zero are considered to be likely important.

	Number of active bouts	**Proportion of night inactive**
Fixed effects	β [CrIs]	β [CrIs]
Intercept	0.28 [-0.34–0.79]	0.53 [-0.10–1.14]
PC1	0.03 [-0.19–0.25]	-0.06 [-0.31–0.19]
PC2	0.00 [-0.17–0.18]	0.02 [-0.18–0.21]
Time of night	0.14 [-0.35–0.08]	-0.36 [-0.70–-0.04]
Random effects	σ [CrIs]	σ [CrIs]
Nest box	0.88 [0.58–2.15]	0.44 [0.29–1.09]
Day	0.03 [0.01–0.04]	0.00 [0.00–0.00]
Residual	0.25 [0.20–0.35]	0.66 [0.49–0.90]

Figures and Captions

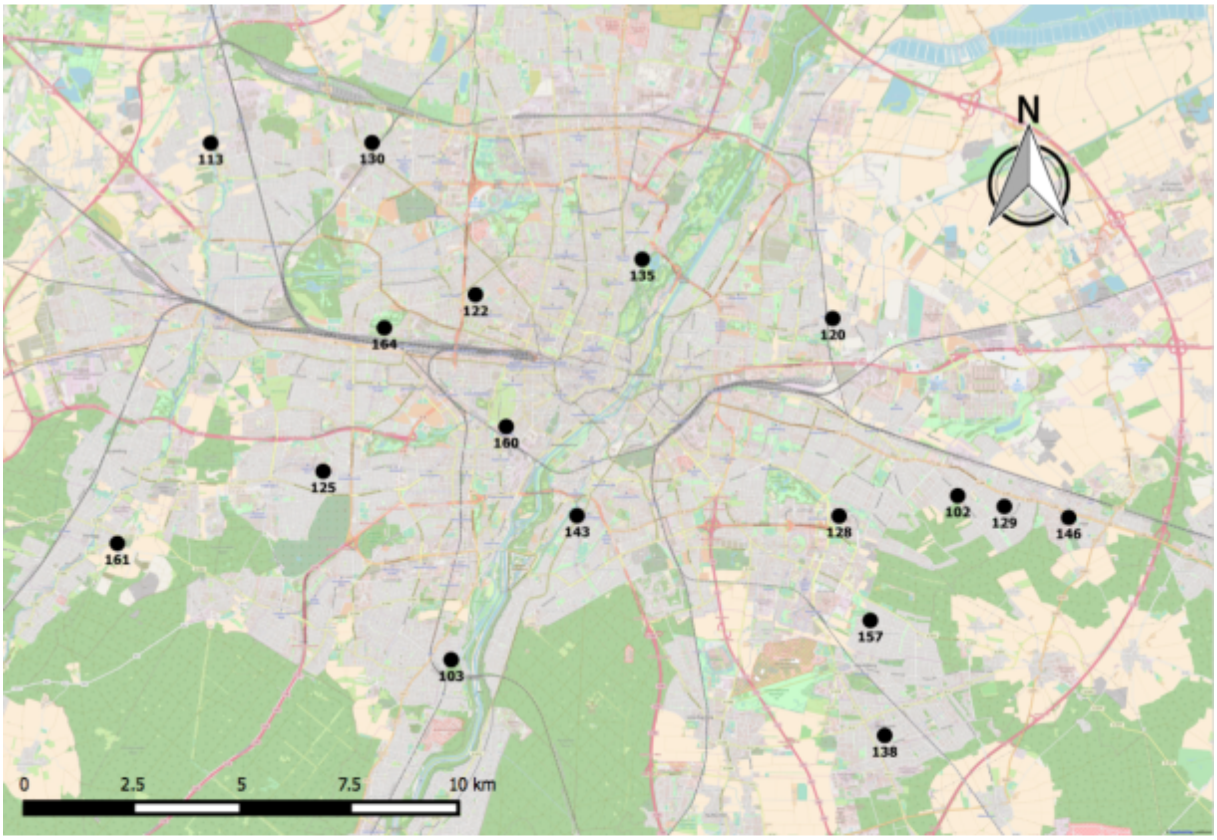

Fig. 1 Distribution of the nest boxes (black dots) in the city of Munich, Germany.

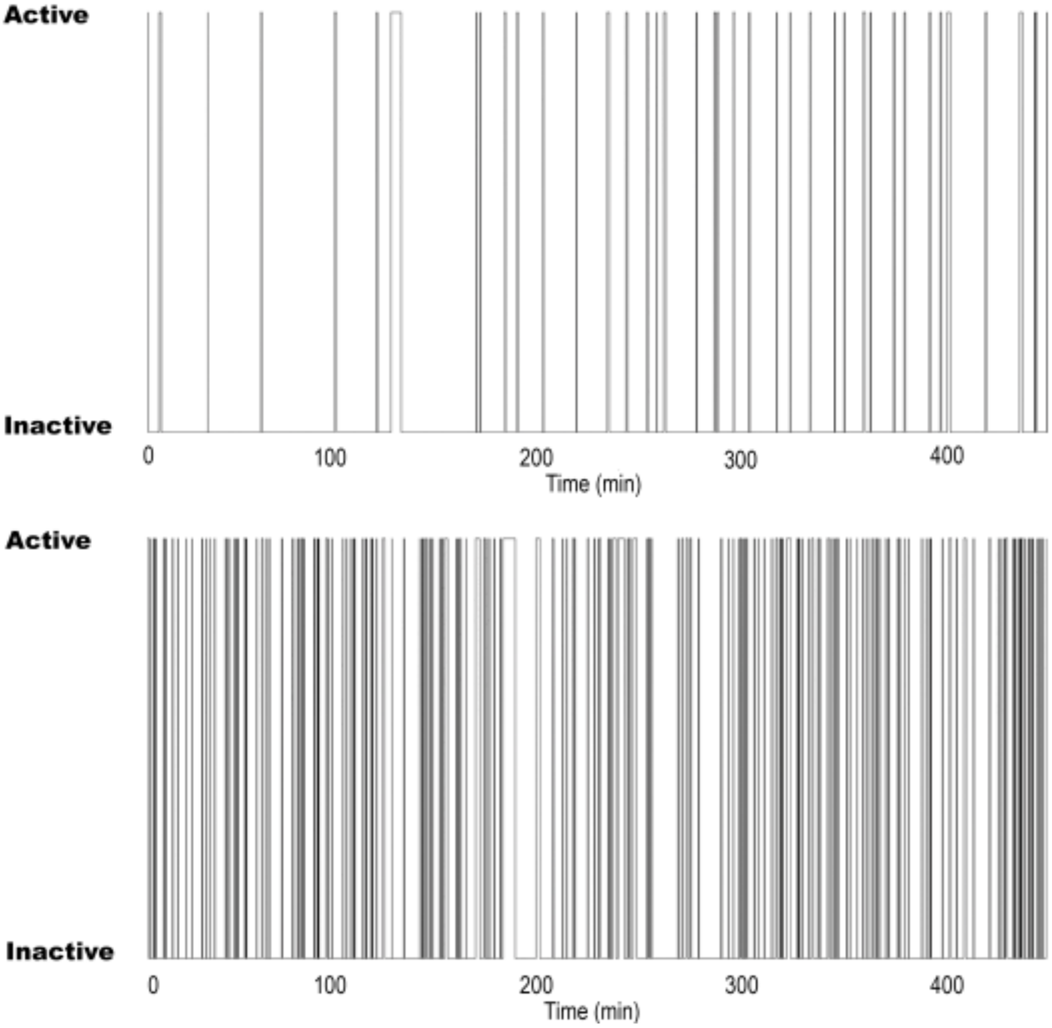

Fig. 2 Hypnogram showing the number and duration of active and inactive states of the least active bird (top) and the most active bird (bottom).

Electronic Supplementary Material

Online Resource 1

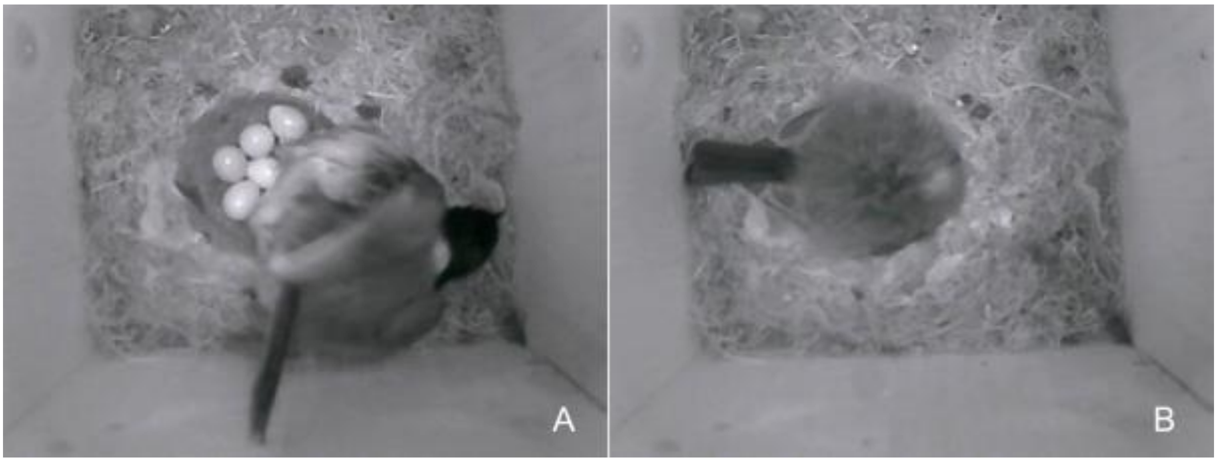

Figure S1 Female great tit (nest box 102) in (A) active and (B) inactive posture during night.

Online Resource 2

Table S1 Summary of nocturnal behaviour and environmental variables (mean ± standard deviation).

Nest box	Number of nocturnal movements	Percentage of night inactive	Noise (dB SPL)	Light (lux)	Temperature (ºC)	Humidity (%)
102	120 ± 15.6	95 ± 0.8	56.5 ± 9.3	9.7 ± 5.5	3.4 ± 1.5	86 ± 2.6
103	63.5 ± 9.2	95.5 ± 0.6	67.6 ± 1.5	15.5 ± 8.9	3 ± 1.2	83.6 ± 5.5
113	83 ± 2	91.8 ± 2	47 ± 28.5	2.3 ± 1.0	7.9 ± 0.6	82.8 ± 9.6
120	69 ± 8.2	95.2 ± 1	49.4 ± 28.6	65.3 ± 18.3	2.4 ± 0.9	58.4 ± 25.7
122	55 ± 10	93.9 ± 1.7	51.3 ± 21.3	20.1 ± 9.3	4.0 ± 0.7	83.9 ± 5.9
125	107 ± 4.2	95.5 ± 0.6	43.3 ± 10.7	15.9 ± 11.2	4 ± 1.1	85 ± 3.2
128	38 ± 11.5	90.1 ± 3.4	62.5 ± 4.7	17.6 ± 10.0	10.1 ± 0.8	69.1± 12.3
129	87 ± 6	96.3 ± 0.6	63.4 ± 10.7	34.5 ± 7.6	1.5 ± 1	81.9 ± 5.5
130	146 ± 48.1	88 ± 8.2	39.0 ± 35.2	29.2 ± 14.0	15.0 ± 2.5	50 ± 7.3
135	91.7 ± 1.5	91.1 ± 4.1	70 ± 1.2	0.0 ± 0.0	5.1 ± 0.8	91.1 ± 2.8
138	79 ± 28.6	94.8 ± 0.7	73.3 ± 2.5	0.0 ± 0.0	2 ± 0.6	80 ± 8.3
143	74.7 ± 9.6	93.6 ± 0.5	65.5 ± 6.7	5.5 ± 3.3	9.9 ± 0.6	66.3 ± 9
146	80.3 ± 6.3	93.3 ± 0.8	52.1 ± 9.6	29.5 ± 7.1	3.6 ± 1.5	78.6 ± 10.9
157	95.7 ± 15.3	94.5 ± 2	68 ± 1	14.2 ± 10.5	3.4 ± 1.2	81.6 ± 6.5
160	98 ± 12.2	94.9 ± 0.7	69.7 ± 1	2.8 ± 0.8	3.4 ± 1.4	77 ± 9.1
161	88.3 ± 3	91.7 ± 6.9	39.0 ± 14.6	36.2 ± 25.0	2.9 ± 1.1	86.1 ± 6.1
164	100.3 ± 10.3	90.8 ± 1.6	65.3 ± 3.7	0.1 ± 2.7	10 ± 2.3	68.0 ± 20.8

7.2. CAPÍTULO II

**Publicado na PLoS ONE*

Effects of traffic noise on the calling behavior of two Neotropical hylid frogs

Valentina Zaffaroni Caorsi1, Camila Both2, Sonia Cechin2, Rógger Antunes1, Márcio Borges-Martins1

1 Programa de Pós–Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil,

2 Programa de Pós- Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

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RESEARCH ARTICLE

Effects of traffic noise on the calling behavior of two Neotropical hylid frogs

Valentina Zaffaroni Caorsi[1☯]*, **Camila Both**[2☯], **Sonia Cechin**[2‡], **Rógger Antunes**[1‡], **Márcio Borges-Martins**[1‡]

1 Programa de Pós–Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, **2** Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

☯ These authors contributed equally to this work.
‡ These authors also contributed equal to this work.
* valenzc@gmail.com

Citation: Caorsi VZ, Both C, Cechin S, Antunes R, Borges-Martins M (2017) Effects of traffic noise on the calling behavior of two Neotropical hylid frogs. PLoS ONE 12(8): e0183342. https://doi.org/10.1371/journal.pone.0183342

Editor: Stefan Lötters, Universitat Trier, GERMANY

Received: March 21, 2017

Accepted: August 2, 2017

Published: August 30, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by research fellowships from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (www.capes.gov.br / CAPES No.3471301), Conselho Nacional de Desenvolvimento Científico e Tecnológico (www.cnpq.br / CNPq 401076/2014-8; 312968/2013-2), and finally the Universidade Federal do Rio Grande do Sul Postgraduate program in Animal Biology (www.ufrgs.br/ppgban) and The Explorers Club (explorers.org / New York,

Abstract

Anthropogenic disturbance has been pointed to as one of the major causes of the world´s biodiversity crisis. Among them, noise pollution is a potential underestimated threat, projected to increase in the next decades accompanying urban expansion. Rising levels of noise pollution may result in negative impacts on species highly dependent on acoustic communication. Amphibians have long served as model organisms for investigating animal acoustic communication because their reproduction depends on transmitting and receiving acoustic signals. A few studies have investigated the effects of anthropogenic noise on anurans, but there is still limited knowledge on how it affects them. In this study, we test the effect of two intensities of traffic noise on calling males of two Neotropical treefrogs species. We expect to record more changes in call parameters, to avoid masking effect, at higher intensity noise treatments, and in the species with higher call/noise frequency overlap. We performed a set of field playback experiments exposing male frogs to road noise at two different intensities (65dB and 75dB). Focal species are *Boana bischoffi* (high call/noise frequency overlap) and *B. leptolineata* (low call/noise frequency overlap). Both species changed acoustic parameters during or after the exposure to traffic noise. Advertisement call rate of *B. bischoffi* decreased during road noise, and dominant frequency decreased over time. Call length of *B. leptolineata* increased or decreased, depending on the order of noise intensity. We also observed spatial displacement in both species, which moved away from the noise source. Our results provide evidence that traffic noise affects anuran calling behavior, and noise intensity is an important factor affecting how species respond.

Introduction

Habitat fragmentation, introduction of exotic species and overexploitation are among the major causes of the world´s biodiversity crisis [1]. Nevertheless, many other anthropic activities play an important role in the process of biodiversity loss. Some, however, are

USA) for financial support of the equipment and field activities.

Competing interests: The authors have declared that no competing interests exist.

underestimated because their effects are more difficult to measure, especially when affecting species at a sub lethal level. Such is the case of noise pollution. Noise produced by human activities is projected to significantly increase in the next decades, accompanying urban expansion and its necessary connections, roads [2]. Rising levels of noise disturbance become a potential threat for many species, especially those depending on transmission and detection of acoustic signals [3], because background noise may limit the distance over which animals are able to communicate [4].

A recently published review of the effects of acoustic disturbance on animals shows how immediate effects on individuals have an impact, risking species conservation [5]. Anthropogenic acoustic disturbance is affecting a wide range of animal groups, including insects [6], fishes [7,8], birds, [9–11], amphibians [12–14], and terrestrial and marine mammals [15–17]. Several species, when facing spectral overlap from background noise, display a variety of mechanisms in order to reduce masking effects, like change duration, intensities or even frequencies of their calls, even though these strategies are not always sufficient to ensure transmission and detection of signal, or subsequent mating success [5,13,18].

Amphibians are the most endangered class of vertebrates, with 42% of the extant species classified among one of the three IUCN categories of high extinction risk [19]. As anuran reproduction depends directly on emitting and perceiving sounds, if background noise interferes, limits or inhibits their communication, it may have a significant negative effect on mating success [4,5]. Anurans present a variety of communication–related adaptations, and their morphology and physiology allows them to perceive and emit sounds within a high range of frequencies, including ultrasound and seismic vibrations [20–23]. For these reasons, frogs have long served as model organisms for investigating the mechanisms, function and evolution of animal acoustic communication [24]. Studies assessing effects of anthropogenic noise on frogs have shown that species respond using distinct strategies [13,24], including changes in both temporal and spectral parameters of their calls [24] and/or the avoidance of the noise source [25,26]. To reduce the masking effect of noise, some frogs may adjust the timing of whole calls or just some notes [27,28], change call amplitude [14] or call frequency [18,29,30].

One should expect a close relationship between the degree of frequency overlap between calls and noise and the type or intensity of call modification. Indeed, species calling at frequencies within the noise spectral range will tend to be more affected [31], and therefore are more likely to have to adjust their calls towards a reduction in temporal and spectral overlap. Changes in call pattern may also be directly related to the intensity of the noise [14,32,33], as background noise can limit the distance over which an individual can perceive acoustic signals [3]. If the intensity of the noise is related to the distance from the source to the receiver [34], we would expect that anthropogenic noise emitted at lower distances (i.e. at higher intensity) would have a higher effect on anuran communication. This variation in the efficiency of signaling is proved to have major fitness consequences for other animal groups [35].

In this sense, it is imperative to determine whether the traffic road noise affects anuran males calling behavior and how animals attempt to reduce the masking effect between their signal and the noise. Furthermore, it is poorly understood how different noise intensities affect the anuran calling behavior. We hypothesize that traffic noise influences the anuran calls, depending on the extent of frequency overlap and the intensity of the noise emitted. To test this hypothesis we performed a set of field experiments intending to measure the effects of traffic noise of different intensities on the call of two anuran species in the Atlantic forest in southern Brazil. We selected one species with call frequencies highly overlapping noise frequencies and one little overlapping. We expect to record more changes in call parameters, to avoid masking effect, at higher intensity noise treatments, and in the species with higher call/noise frequency overlap.

Material and methods

Study area

To observe how species react to traffic noise we choose a study site with quite minimal road traffic, a research reserve, 50 km way (off-road conditions) from the closest highway. Therefore, we could simulate the effects of traffic noise upon calls of anurans not exposed to it. Experiments were conducted at the Centro de Pesquisas e Conservação da Natureza Pró–Mata, São Francisco de Paula, Rio Grande do Sul, Brazil (29˚35'S, 050˚15'W), from October to December 2015 (Austral Spring).

Focal species

We chose two anurans with distinct vocal profiles. The first species, *Boana bischoffi* (Fig 1A), is a medium size hylid (Snout–to–vent–length–SVL between 38–43mm), found mainly in permanent ponds close or within to forestall fragments, with two main types of call. The advertisement call is composed of one or two multipulsed notes, with duration between 0.05–0.1 seconds (Fig 2A). The call rate ranges from 3–24 notes per minute and the dominant frequency between 1.4–2.1 kHz [36,37]. The other call emitted by the species, probably territorial, is composed by one note with a series of pulses, which lasts in average 1.26s and presents dominant frequency between 1.7–2 kHz [37]. The second focal species was *Boana leptolineata* (Fig 1B), a small hylid (males SVL between 30–36mm) found mainly in open grassland on streams and ponds with clear water. It presents two main call types: i) the advertisement call of the species is composted by 3 to 4 multipulsed notes, and last from 0.04–0.1s (Fig 2A); ii) the aggressive call is longer than the advertisement call, with 11–21 pulses and lasting between 0.004–0.015s. Both calls have dominant frequency between 3.5–5.2Hz [36].

Traffic noise

We recorded the traffic noise for the playback experiments at a major highway located in the South of Brazil (BR 389). Recordings were taken 10m from the edge of the paved road, at July

Fig 1. Calling activity of (A) *Boana bischoffi* and (B) *B. leptolineata*.

https://doi.org/10.1371/journal.pone.0183342.g001

14th of 2015, beginning at 18h during winter season, for 30 minutes (S1 File). We chose this day and time for its similarity to the vehicle fluxes during the summer breeding season of the anurans in the area recorded. We used a portable sound level meter (SLM–Instrutemp ITDEC 4000, 0.1dB precision, C-weighting) to measure the mean amplitude (dB) of the traffic noise. We also measured the amplitude of the traffic noise at distances of 50m and 100m from the edge of the road. All sounds recorded in this study were obtained using a portable SONY PCM–D50 recorder, and a uni-directional microphone Sennheiser ME 67 equipped with a windscreen and a dynamic stereo headphone to monitor recordings.

Sound editing

We used Audacity 2.1.1 software to observe and edit traffic sounds (.wav) for the playback tests. The playbacks were constructed using traffic noise and intensities previously recorded and measured on the field, as described above. The recordings used for the stimuli presented a range of frequencies from close to zero Hz up to approximately 15 kHz, with higher intensity on the lower frequencies (up to 3 kHz) and dominant frequency on 1125 Hz (dB) (Fig 2B). Sound edition included the selection of 3min traffic noise, intensity calibration (dB) for each treatment chosen and the inclusion of a silent period at the beginning and ending of each playback sound. Noise stimuli were divided into two different intensities of traffic noise: 65dB (treatment N1) and 75dB (treatment N2), which represents the mean intensity of the noise measured at 100m and 50m from the edge of the traffic road, respectively. These distances are representative to the real distances of water bodies found near roads in Rio Grande do Sul.

Playback experiments

Playbacks followed the P1–N1–N2–P2 protocol [38] and were programed to play: three minutes of pre–stimulus (P1–silence), three of traffic noise of treatment (N1), three minutes of the treatment (N2) and for last, three minutes of post–stimulus (P2–silence), totalizing 12 minutes of playback experiment. We constructed two different playbacks ordering the treatments of traffic noise on the two possible alternative ways: Silence–65dB–75dB–Silence and Silence–75dB–65dB–Silence. Individuals were assigned to one playback type only. The first individual received the 65dB–75dB treatment and, following we alternated playback types for all others.

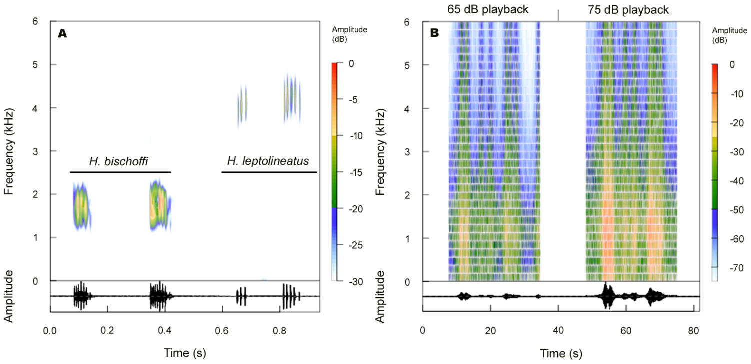

Fig 2. (A) Study species advertisement calls and (B) intensities (dB) of traffic noise used on playbacks. Spectrograms (above) and oscillograms (below) of *Boana bischoffi* and *B. leptolineata*.

https://doi.org/10.1371/journal.pone.0183342.g002

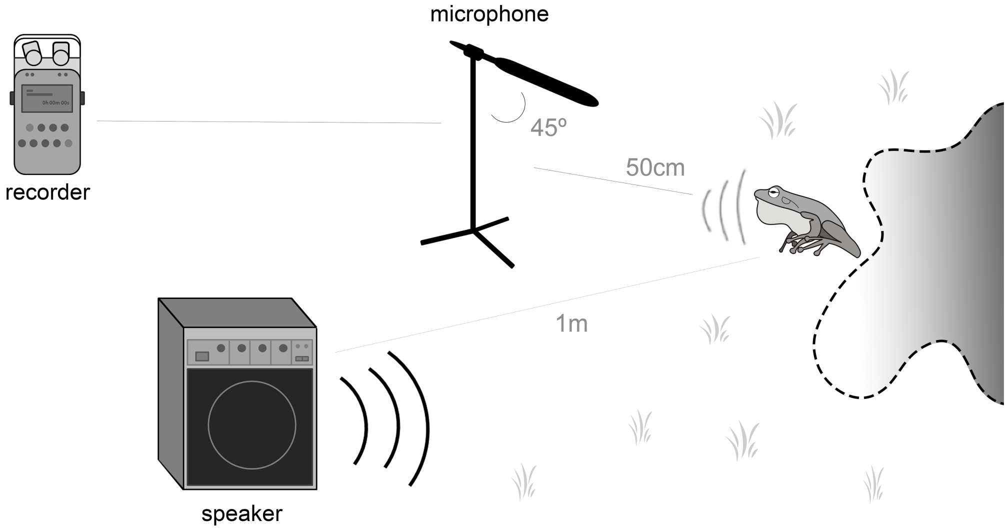

Fig 3. Design of experiments during the field trip to collect data on calling males.

https://doi.org/10.1371/journal.pone.0183342.g003

Experiments were carried out during 18 days at dark hours, beginning one hour after the sunset until the cessation of most animals' activity. During the study period, the air temperatures on the ponds ranged from 14.1–23.7˚C, and relative humidity from 70.8–91.5%. We actively searched for calling males of the two focal species. For each individual found we implemented the following procedure: i) we actively searched for conspecific males within 5 m of the focal male and removed all those detected, to avoid any recording responses by any other individuals other than the focal male for that single experiment; ii) loudspeaker was placed at a distance of 1–4m from the animal, and the microphone within 50cm of the calling male with an inclination of 45˚ (Fig 3); iii) observer would get away from the focal male and waited from five to twenty minutes until the individual re-started its vocalizations; iv) playback levels were adjusted in the field using the sound level meter to reproduce the intensity observed and measured in the original road, also taking into account the distance between the focal male and the speaker; v) playbacks were performed.

The placement of the loudspeaker at different distances was necessary because its size/weight (522mm x 427mm x 267mm / 14.3Kg), which requires it to be at a stable ground. The speaker used for these experiments was carefully chosen by its characteristics to emit signals in the spectrum of frequencies of the traffic noise and do not distort low frequencies. The loudspeaker used, Oneal 360–12v, answers to frequencies from 10Hz to 70kHz and the battery lasted up to 24h on the field, so it did not need an external energy supply. After every recording, environmental sound was measured 1 m from the water body with the sound level meter.

Specimens handling procedures, and ethical and legal permits

Once a recording was concluded, we measured male body temperature at the calling spot (using a infrared thermometer GM300, 0.1˚C resolution) and hand captured it to measure body mass and SVL, using a scale to the nearest 0.1 g and a caliper (Starrett–798) to the nearest

0.1 mm respectively. Captive individuals were kept in containers for up to 5 days with vegetation and wet cotton at ambient temperatures to avoid pseudoreplication. At the end of each species experiment, the recorded individuals were released at the same water body where they were collected. All experimental procedures were approved by the applicable Brazilian biodiversity agency and local institutional committee on research and ethics: *Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios–Instituto Chico Mendes de Conversação da Biodiversidade* (RAN–ICMBIO–Permit No. 52021–1), by *Comissão de Pós-Graduação* (Project n° 28872—PPGBAN/UFRGS), *Comissão de Pesquisa* (COMPESQ/IB/UFRGS) and *Comissão de Ética no Uso de Animais* (CEUA/UFRGS).

Acoustic analyses

Using Audacity 2.1.1 software, we divided each record into 3 min files, corresponding to a pre–stimulus, two stimuli and a post–stimulus time periods. Afterwards, all acoustic analyses were carried out on software Raven Pro v. 1.4 for Mac [39].

Call rate (calls– 1)/min was calculated by counting the number of calls per individual at each 3 minute interval during the playback experiment. For this parameter, we analyzed advertisement and aggressive calls separately, by counting all the signals emitted during that time period. Further, we also measured one spectral and three temporal parameters on the advertisement calls: dominant frequency (call frequency containing most energy); call length (time from the beginning to the end of one call); note length (time from the beginning to the end of one note); and interval between notes (distance between two consecutive notes) except for *Boana bischoffi* as most of the calls present a single note. These call parameters were measured by randomly selecting ten notes in *B bischoffi* and 15 notes in *B. leptolineata* for each 3 minute period the playback. Selection was made in Excel software (rand function; Microsoft Excel 2010. available from: https://products.office.com/pt–BR/excel). In a few cases, males emitted equal or less notes than stipulated for each species. In these cases, we used all observed notes emitted in the period to measure acoustic parameters and calculate the respective means.

Statistical analyses

To test if noise significantly affected any of the call parameters in the two species we used a Permutational Multivariate Analysis of Variance Using Distance Matrices and post–hoc pairwise comparisons to asses which group significantly differed [40]. Stimuli type and time period (P1–N1–N2–P2) were considered as fixed factors and the individuals were considered as blocks. We also considered the order of exposure– 65/75dB or 75/65dB–as a factor. All analyses and figures were carried out in R environment [41] using Vegan: Community Ecology Package [42]; oscillograms and spectrograms were done using the Seewave package [43].

Results

Boana bischoffi

We recorded 19 males, and four of them showed avoidance behavior when exposed to the noise stimuli. Three individuals changed their initial position and moved away from the source of traffic noise, but remained calling. One male ceased the calling activity and apparently left the area, as we were not able to track it again. Call rates were calculated for all recorded males. Other call parameters were measured from 14 males only, due to the low quality from the recordings from a few males (moving males plus one).

Seventeen animals emitted both advertisement and aggressive calls in at least one period of the playback. Advertisement call rate was affected by traffic noise (F = 7.13; p = 0.001; Table 1),

Table 1. Effects of traffic noise playback stimuli on call parameters of the focal species. Measurements of each parameter are given by means and (standard error); Dominant frequency is given in Hz. Letters "a" and "b" and numbers in bold indicate significant differences between groups.

	Time	Treatment	Aggressive call	Advertisement call				
			Call rate (call/ min)	Call rate (call/ min)	Call length (seconds)	Note length (seconds)	Interval (seconds)	Dominant frequency
Boana bischoffi	1	Silence	0.9 (0.4)	**5.9 (1.3)**^{a}	0.07 (0.02)	0.05 (0.006)	_	**1685 (66)**^{a}
	2	65 dB	0.5 (0.2)	**4.2 (1.3)**^{b}	0.05 (0.005)	0.05 (0.006)	_	1694 (31)
	3	75 dB	0.9 (0.3)	**5.2 (1.4)**^{b}	0.09 (0.02)	0.05 (0.007)	_	1672 (45)
	4	Silence	1.2 (0.4)	**7.4 (1.6)**^{a}	0.11 (0.03)	0.05 (0.007)	_	**1676 (27)**^{b}
	1	Silence	1.0 (0.4)	**9.6 (1.7)**^{a}	0.10 (0.02)	0.06 (0.004)	_	**1769 (36)**^{a}
	2	75 dB	0.5 (0.3)	**2.9 (1.6)**^{b}	0.16 (0.05)	0.06 (0.005)	_	1746 (47)
	3	65 dB	0.7 (0.4)	**4.8 (1.4)**^{b}	0.11 (0.03)	0.06 (0.005)	_	1706 (25)
	4	Silence	1.5 (0.8)	**6.6 (2.6)**^{a}	0.14 (0.04)	0.06 (0.004)	_	**1685 (15)**^{b}
Boana leptolineata	1	Silence	0.9 (0.3)	16.2 (4.4)	0.18 (0.02)	0.06 (0.005)	0.08 (0.005)	4222 (54)
	2	65 dB	1.2 (0.3)	18.3 (4.5)	0.20 (0.02)	0.06 (0.004)	0.08 (0.005)	4240 (49)
	3	75 dB	1.4 (0.4)	15.8 (4.2)	0.22 (0.02)	0.06 (0.004)	0.08 (0.005)	4196 (50)
	4	Silence	1.5 (0.5)	15.5 (4.4)	**0.22 (0.01)**^{*a}	0.06 (0.005)	0.09 (0.005)	4186 (48)
	1	Silence	2.4 (0.7)	13.8 (3.5)	0.19 (0.02)	0.07 (0.007)	0.09 (0.02)	4150 (51)
	2	75 dB	1.8 (0.6)	11.7 (5.1)	0.18 (0.02)	0.07 (0.007)	0.08 (0.009)	4207 (60)
	3	65 dB	1.4 (0.7)	12.6 (4.8)	0.18 (0.02)	0.08 (0.1)	0.08 (0.01)	4198 (46)
	4	Silence	1.1 (0.7)	10.7 (5.3)	**0.19 (0.03)**^{*b}	0.07 (0.004)	0.09 (0.01)	4182 (63)

https://doi.org/10.1371/journal.pone.0183342.t001

but not by time periods. The order of treatments was not significant ($p > 0.05$). Male calling rates significantly decreased from an average of 7.5 call/min during silence periods to an average of 4.6 and 4.3 call/min during treatments of 65 dB ($F = 3.99$, $p = 0.012$) and 75 dB ($F = 3.99$, $p = 0.011$) respectively (Fig 4A). Aggressive call rate showed no differences between stimuli types, periods or the ordination of noise ($p > 0.05$). Males also tended to increase the duration of their advertisement calls in response to traffic noise (Fig 4B). Advertisement calls lasted in average 0.009 sec longer in response to 75 dB traffic noise stimulus than during silence or the 65 dB stimulus although these differences were marginally non-significant ($F = 1.1$, $p = 0.09$). The order of the treatments was also marginally non-significant ($F = 3.73$; $p = 0.06$). Males first exposed to 75 dB traffic noise showed even longer calls. Males tended to change their calls to both noise intensities first presented, returning close to their original call lengths during the second noise stimuli presented. Note duration was not affected by stimulus, time period or ordination of noise ($p > 0.05$ for all cases). The dominant frequency differed significantly across time periods ($F = 2.39$; $p = 0.04$), decreasing from time 1 to time 4 ($F = 2.07$; $p = 0.047$) (Fig 4C). The frequency did not change in response to stimuli type, and the ordination of noise was also non-significant ($p>0.05$).

Boana leptolineata

We recorded 23 males. Three individuals changed their initial position to farther away of the source of noise. Nevertheless, even moving, they all continued the calling activity during playbacks. Twenty animals emitted both advertisement and aggressive call in at least one period of the noise playback. Call rates were calculated for all individuals, and other parameters for 20 males, (moving males were not used). Males did not increase advertisement call rate during the noise stimuli (Fig 4D; Table 1). Statistical analyses also did not show any significant differences between period or ordination of noise ($p>0.05$). Aggressive calls followed the same pattern and were not affected by stimuli type or period ($p>0.05$). Nevertheless, we found

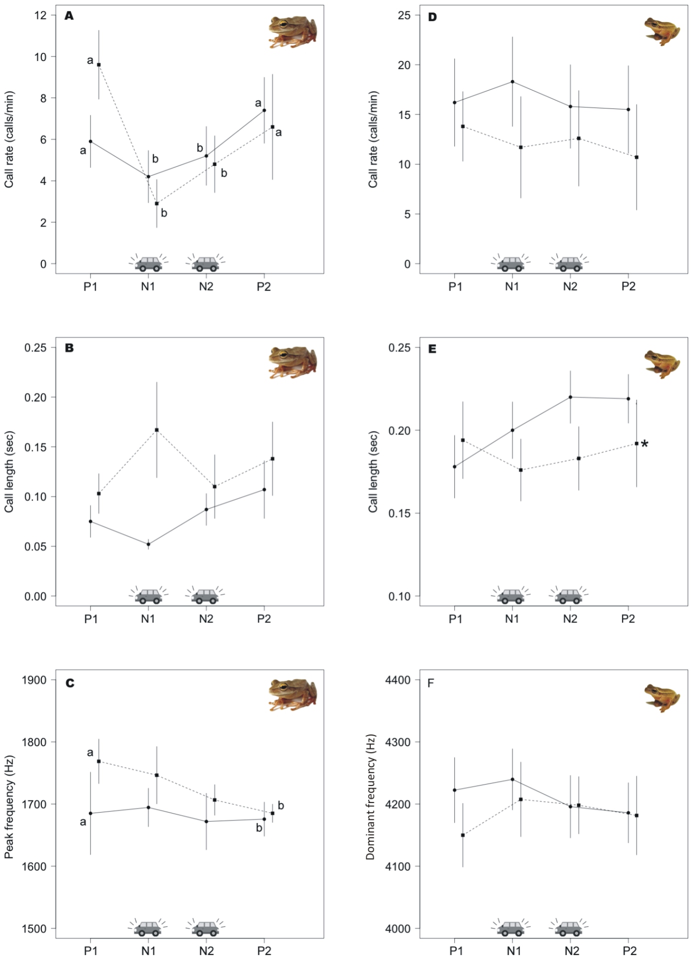

Fig 4. Effects of traffic noise on call parameters of the two hylids. Graphs show call parameter means (±SD) at the four periods of time inside a playback, P1 (pre–stimuli, silence), N1 (noise1), N2 (noise2), P2 (post–stimuli, silence). Dashed line represents the playback order N1 (75dB) followed by N2 (65dB) and solid line the other way around N1 (65dB) and N2 (75dB). During road noise treatments, *Boana bischoffi* decreased call rate (A). Peak frequency was significantly different for *B. bischoffi*, decreasing from period P1 to P2 (C). Call duration showed changes in *B. leptolineata* depending on the order of the treatment (E). Letters "a" and "–b" indicate statistically different values due to treatments (intensity) or playback periods, and "*" indicate differences due to playback type/order (65 or 75dB first).

https://doi.org/10.1371/journal.pone.0183342.g004

significant differences in advertisement call length depending on the order of noise intensity (F = 2.85, p = 0.04). Males showed progressively longer calls in response to the noise (Fig 4E), when first exposed to 65dB, and slightly shorter calls when first exposed to 75dB. Note length and dominant frequency (Fig 4F) did not change significantly in response to the period, intensity level or order ($p > 0.05$ for all cases).

Discussion

In this study we found evidence that traffic noise leads to changes in anuran calls, supporting the idea that anthropogenic noise has the potential to adversely impact biodiversity [4]. Temporal parameters of the calls changed significantly during road noise treatments, affecting call rate of *Boana bischoffi* and call length *of B. leptolineata*. The species with low frequency call altered its dominant frequency in the last time period, after been exposed to both noises intensities for six minutes in total. Besides, we also reported a few cases of spatial displacement of males from both species, which moved away from the experimental traffic noise. Our results point out important effects of traffic noise upon frogs calling activity and shows that noise intensity is an important factor affecting species calls. Following, we discuss in detail the implications of our findings.

Impact of traffic noise on call temporal parameters

Acoustic communication in anurans depends on the transmission and detection of signals, therefore, anthropogenic noise can have many different effects on species, especially when the interference of background noise has a masking effect on the species signaling [13]. According to this, we would expect that species whose call frequencies are within the same range of frequencies as the ones of the noise they are subject to, to present more evident changes in their acoustic behavior, potentially affecting the efficiency of their communication. Our results supported these expectations for the calling rate behavior of both species. They showed significant changes in the calling pattern of *B. bischoffi* during traffic noise stimuli, the species with high call/traffic noise frequency overlap. *Boana letptolineata*, with low spectral overlap, kept similar calling rates during stimuli.

Both intensities of traffic noise stimuli affected *B. bischoffi* call rate. It decreased more than 60% in average at both noise intensities, 65dB and 75dB, when compared to pre and post–stimulus periods (silence). These intensities represent traffic noise at 100m and 50m from the edge of the road, respectively, showing that for this species the traffic noise has a strong effect on its calling activity even at these distances. In a study with *Hyla chrysoscelis* female frogs, Bee and Swanson (2007) reported increases in latency response and decreases in orientation towards the target signal (artificial calling male) directly related with an increase in the intensity of traffic noise (37 e 85dB). Therefore, traffic noise not only leads to a decrease in call rate emission by males, but potentially results in a lower call detection efficiency by females. This may have a significant synergistic deleterious effect in mate selection, which is yet to be better investigated [34,44]. Anuran species decreasing signal rate when exposed to noise, like *B. bischoffi*, were observed in several species of Hylidae, Microhylidae and Ranidae to different sources of noise, either natural or anthropic, such as air plane, motorcycle engine and traffic noise [12,14,26]. Males of different species appear to recognize when their signal is more likely to be transmitted and detected, avoiding periods of maximal interference based on the total background noise (native + artificial/anthropic stimuli) of the pond.

On the other hand, we did not detect changes on call rate for *B. leptolineata*. This result corroborates our initial hypothesis that the species with high frequency call and little spectral overlap would be less affected by traffic noise. Such absence of response would be related to the little overlap between the signal and the background noise, as seen in other species calling in higher frequencies [14]. Still, *B. leptolineata* is known to change call rate in response to the calls of invasive frogs, even when their calls present little spectral overlap, as well as in response to continuous white noise [31]. In sum, these results point out that adjustments in call rate are likely to be stimulus-specific, and that the triggers for that adjustment are yet to be fully explored.

Only a few studies have tested the effect of anthropogenic noise on parameters other than call rate [28,30,45]. Our results showed that call length of *B. bischoffi* also tended to be affected, specially when males were exposed first to the most intense stimulus. Males slightly increased call length in this condition, although were results were only marginally significant. However, when analyzing temporal parameters of the call of *B. leptolineata*, we found that when exposed to traffic noise, males seems to modify the length of their calls and their strategy depend on which intensity of noise is first presented. Males showed progressively longer calls in response to the noise when first exposed to 65dB traffic stimulus. The modification observed could be an alternative adopted to increase the temporal window of the sound produced by the animal in the environment Instead of increasing call rate as other species attempted [12,27,30,45–47], they increased call duration. Contrastingly, calling males emitted slightly shorter calls after being first exposed to 75dB noise. Therefore, the noise intensity might be determinant to the call modification strategy to be adopted. In front of an intense noise, males may choose to not increase their call effort (more calls or longer calls), as *B. leptolineata* males actually did in response to the playback stimuli beginning with the less intense.

Impact of traffic noise on call spectral parameters

The impact of noise on anuran call spectral parameters seems to be variable. Previous studies detected an increase in the dominant frequency of species whose calls overlap noise frequency range [14,29,48], however others reported a decrease [29,49] or no changes at all [28]. A recent meta-analysis comparing frequency shift responses of birds and anurans exposed to anthropogenic noises, found that while birds are prone to increase the frequency in response to noise, anurans would less commonly display such strategy [18]. Because anurans share acoustic environments among themselves, and other species for that matter, they have evolved towards emitting signals within high temporal and spatial ranges [21,23]. Nevertheless, it is plausible to expect them to adjust their tones and timing to workaround the masking effect problem.

Boana bischoffi males decreased call's dominant frequency in response to traffic noise. This species calls at 1.7kHz, so it is not feasible an increase beyond 3kHz (frequency at which the energy of the traffic noise decreases), once frequency changes usually not exceed 300Hz in anurans [14,45]. Alternatively, it could be more efficient to reduce the frequency, ensuring longer distance dispersion of the signal [44,50]. For the high pitch call species studied, *B. leptolineata*, we did not detect any changes in call dominant frequency in response to period or intensity ordination, a result consistent with previous reports for other anurans with frequencies above those of noise stimuli [14,18].

Potential effects of traffic noise on frog's reproductive behavior

Several studies alerted for the potential of anthropogenic originated sounds to adversely impact biodiversity, however only a few studies focused on the mechanisms behind such pattern, and tested to what extension such negative effects are due to the masking effect from the noises such as traffic. For instance, urbanized surfaces and the proximity to roads may have negative impact on the density and the presence of calling males [51,52]. We reported in this study that some individuals of *B. bischoffi* and *B. leptolineata* attempted to displace away from the source of noise, and even ceased calling. This behavior was also reported for *Hyla arborea* during manipulative experiments [28]. Our study was not designed to understand if noise might directly affect habitat selection for these species; nevertheless, it indicates a promising line of investigation. Since some anuran species have restricted distribution ranges and low dispersal capacity, their ability to move to quitter sites if background noise disrupts acoustic communication is low, therefore this topic certainly deserves the scientists' attention [53].

All these spectral and temporal parameters are very important in mate selection and localization [44,54] and the fact that many species have developed mechanism to reduce masking effects of signal does not ensure their success on mating. In this study we observed that call modifications in response to noise might be directly related to the degree of frequency overlapping between the species call and the noise. Our study is based on a short-term exposure to traffic noise, and based on individuals not previously exposed to it. Therefore, we only accessed very immediate effects caused by noise and cannot exclude the possibility of additional changes in call parameters, which might occur in a long-term exposure. Besides, we only tested males, i.e. the emitters of acoustic signals, and exogenous acoustic noise generally decreases the ability of a receiver to decode a message [55]. It is known that female frogs exposed to traffic noise might increase the time to find and decreased orientation towards males' calls [13]. Therefore, it is yet to be understood whether changes on call parameters helps on the transmission and detection of signals emitted and if it really increases chances of mating in anthropogenic noise environments. Alternatively, it is possible that habitats such as those close to roads might work as an environmental filter for low pitch species. In this scenario, given time, we should expect a spatial effect on community composition (filtered by species call frequency) in a disturbance gradient from high to low traffic noise caused by roads.

Traffic noise is not only an alteration of transmission channel characteristics; actually, it is also a health threat that could decrease animal survival [56]. From an individual perspective, changes on calling behavior to achieve communication may have individual negative consequences, as increased exposure to predator and high energy costs [23,57]. The energetic cost of calling in frogs is well recognized [57] and so the consequences of increased vocal output in response to noise, which could lead to a use of more energy reserves [27]. Therefore, although its yet to be more explored, changing call parameter can affect not only calling activity, but indirectly the animals life function and vital rates [5,34,58].

Supporting information

S1 File. Traffic noise sample. Sample (24 seconds) of traffic noise recorded for the playback experiments at a major highway (BR 389) located in southern Brazil, Xangrilá municipality, Rio Grande do Sul. 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.
(MP3)

S1 Table. *Boana bischoffi* call rate. Table containing original data used for the analysis of the Call rate of *Boana bischoffi*.
(TXT)

S2 Table. *Boana leptolineata* call rate. Table containing original data used for the analysis of the Call rate of *Boana leptolineata*.
(TXT)

S3 Table. *Boana bischoffi* advertisement call. Table containing original data used for the analysis of the temporal and spectral parameters of *Boana bischoffi*.
(TXT)

S4 Table. *Boana leptolineata* advertisement call. Table containing original data used for the analysis of the temporal and spectral parameters of *Boana leptolineata*.
(TXT)

Acknowledgments

We are grateful to Camila Ineu Medeiros, Debora Bordignon, Jonathan Della Flora, Lívia Roese Miron, Thiago Theis and Alejandra Caorsi for their help in field and/or laboratory activities; to Andreas Kindel, Maria João Ramos Pereira, Patrick Colombo, Rafael Márquez and Vinícius M. Caldart for the encouragement on this work and revision suggestions. We also thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for authorization to conduct this research (No. 42411–1).

Author Contributions

Conceptualization: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Márcio Borges-Martins.

Formal analysis: Valentina Zaffaroni Caorsi, Camila Both, Rógger Antunes.

Funding acquisition: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Márcio Borges-Martins.

Investigation: Valentina Zaffaroni Caorsi, Camila Both, Rógger Antunes.

Methodology: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Márcio Borges-Martins.

Project administration: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Márcio Borges-Martins.

Resources: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Márcio Borges-Martins.

Supervision: Sonia Cechin, Márcio Borges-Martins.

Visualization: Valentina Zaffaroni Caorsi, Camila Both, Rógger Antunes.

Writing – original draft: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Rógger Antunes, Márcio Borges-Martins.

Writing – review & editing: Valentina Zaffaroni Caorsi, Camila Both, Sonia Cechin, Rógger Antunes, Márcio Borges-Martins.

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7.3. CAPÍTULO III

**Formato nas normas da revista Scientific Reports*

“Bad vibes”: Anthropogenic vibrations affect anuran calling activity

[1]Valentina Z. Caorsi, [2]Vinicius Guerra, [3]Raíssa Furtado, [4]Diego Llusia, [5]Lívia Rose Mirón, [1]Márcio Borges-Martins, [6]Camila Both, [7]Peter M. Narins, [8]Rafael Márquez

1 - Programa de Pós–Graduação em Biologia Animal, Dep. de Zoologia, Inst. de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

2 - Laboratório de Herpetologia e Comportamento Animal, Departamento de Ecologia, Inst. de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brazil.

3 – Programa de Pós-Graduação em Ecologia, Dep. de Ecologia, Inst. de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

4 – Terrestrial Ecology Group (TEG), Departamento de Ecología, Universidad Autónoma de Madrid, Madrid, Spain.

5 - Departamento de Ecologia e Evolução, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

6 – Programa de Pós-Graduação em Biodiversidade Animal, Dep. de Ecologia e Evolução, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

7 - Departments of Integrative Biology & Physiology, and Ecology & Evolutionary Biology, University of California Los Angeles, Los Angeles, CA, USA

8 - Fonoteca Zoológica. Dept. de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain.

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“Bad vibes”: Anthropogenic vibrations affect anuran calling activity

[1]Valentina Z. Caorsi, [2]Vinicius Guerra, [3]Raíssa Furtado, [4]Diego Llusia, [5]Lívia Rose Mirón, [1]Márcio Borges-Martins, [6]Camila Both, [7]Peter M. Narins, [8]Rafael Márquez

Anthropogenic disturbance is one of the major causes of the biodiversity crisis. In particular, the threat of substrate vibrations caused by anthropogenic activities in animal behavior has been, so far, underestimated. Amphibians are the terrestrial vertebrates most sensitive to vibrations, and since communication is crucial to their survival and reproduction, they are a suitable model for investigating this pioneer subject. Using playback tests, we assessed how vibrations produced by two sources of anthropogenic activity (road traffic and wind turbine) affect the calling activity of a native population of terrestrial toads (never exposed to these disturbances before). Traffic and wind turbine vibrations were recorded *ex situ* and synthetic copies generated digitally. In their natural habitat, we used a tactile sound transducer below ground to simulate the seismic sources: (a) traffic, (b) wind turbine, (c) synthetic traffic, (d) synthetic wind turbine, and (e) a no-stimulus period as a control. We analyzed the toads’ acoustic response by measuring some of the most important parameters for reproductive success: call rate, call duration and dominant frequency. Our results showed a negative effect of both seismic sources on the call rate of *Alytes obstetricans*, while call duration and frequency remained stable. Furthermore, call rate was more affected by original traffic and wind turbine recordings than by synthetic stimuli. Since anurans use calls to attract reproductive partners and other activities, as defend territories, this study suggests that anthropogenically derived substrate-borne vibrations could reduce individual reproductive success. Our study demonstrates the effect of anthropogenic vibrations in anuran communication and claims for further investigations about the impact of anthropogenic disturbances that humans may not easily perceive.

7.4. CAPÍTULO IV

**Formato nas normas da revista Behavioral Ecology*

Effects of anthropogenic noise on anurans: few decades of Science

Valentina Caorsi[1], Camila Both[2], Marina Debon[3], Rafael Márquez[4], Márcio Borges-Martins[1].

1 - Programa de Pós–Graduação em Biologia Animal, Dep. de Zoologia, Inst. de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

2 – Programa de Pós-Graduação em Biodiversidade Animal, Dep. de Ecologia e Evolução, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

3 - Dep. de Zoologia, Inst. de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

4 - Fonoteca Zoológica. Dept. de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain.

Normas conforme o Periódico Behavioral Ecology

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Effects of anthropogenic noise on anurans: few decades of Science

Valentina Caorsi[1], Camila Both[2], Marina Debon[3], Rafael Márquez[4], Márcio Borges-Martins[1].

Abstract

Anthropogenic noise is widely spread in the environment and has been shown to have an array of negative effects on wildlife. Noise exposure poses a significant threat to the integrity of terrestrial ecosystems, once it can inhibit perception of sounds. However, compromised hearing affects more than acoustical communication. Noise has been shown to affect from DNA integrity and genes to physiological systems and behavioral ecology. Amphibians are a group in which sounds plays a fundamental role, with species relying on acoustic communication for social behavior and, therefore, noise can have a negative effect on its activities. Studies accessing the effects of anthropogenic noise on anurans are very recent and they have accessed mainly the effects of noise on males' vocal activity. Knowing the extent of effects of human noise on wildlife, we believe it is important to look at all possible consequences of anthropogenic noise together in order to better understand the extent of this source of pollution in amphibian anurans. In light of that, we provide in this study a review of existing literature on the effect of anthropogenic noise on anurans. We gather here 27 studies, including 57 species from 13 families, that showed the effect of noises derived from cities, transportation (traffic, airplanes) and energy production (wind turbine). Studies showed that airborne and seismic anthropogenic noise affected a wide range of systems including behavioral, for instance acoustic signals and mate selection and physiological, as stress, immunity and coloration. Anthropogenic noise also negatively affected species abundance and, furthermore, the individual's persistence over the

reproductive season. Furthermore, these effects not only applied to males, but also to females and mate choice, which can therefore impact reproduction and individual fitness. Finding suggest that anthropogenic noise is likely to influence multiple biological systems both directly and indirectly, however most of the knowledge that exists relies on the short-term response behavior of individual males. We still lack information on female's response and individual fitness. Besides that, long-term studies are necessary showing effects of noise from an individual level to populations, species distribution and communities. Further, researchers should work together with governmental institutions in order to create guidelines and legal instruments to be implemented during urban expansion projects to reduce the effects of this pollutant on wildlife. The key challenges and future challenge on this topic is to work with multidisciplinary teams in order to test effects, access the impacts, propose mitigation and conservation actions and actually implement them.

8.CONCLUSÃO

O aumento global dos níveis de ruído antropogênico, tanto em habitats humanos quanto em habitats naturais, são um desafio para a conservação, especialmente quando considerados em conjunto com outras ameaças aos ecossistemas. De forma geral, esta tese fornece resultados e uma revisão com evidencias consideráveis de que o ruído antropogênico é prejudicial à vida selvagem. Os estudos mostram a extensão do problema do ruído que pode afetar desde funções fisiológicas até comportamentais, desde o nível de indivíduos até populações e comunidades. Além disso, os estudos mostram uma dificuldade em padronizar e quantificar estes efeitos para compreender os impactos associados ao ruído e à diversidade de taxa em diferentes contextos biológicos. A maioria dos trabalhos testa o efeito a curto prazo, mas ainda é necessário compreender melhor como isto afeta os animais a longo prazo. Além disso, é preciso investigar a eficácia das medidas de mitigação propostas pelos trabalhos para reduzir o impacto do ruído nos animais. Com isso, ficará mais acessível fornecer orientações para avaliação do impacto do ruído e para as politicas de conservação.