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VALENTINA ZAFFARONI CAORSI

EFEITO DO RUÍDO ANTROPOGÊNICO NO COMPORTAMENTO ANIMAL

PORTO ALEGRE
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Aprovada em ____ de ____________ de _____.

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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).

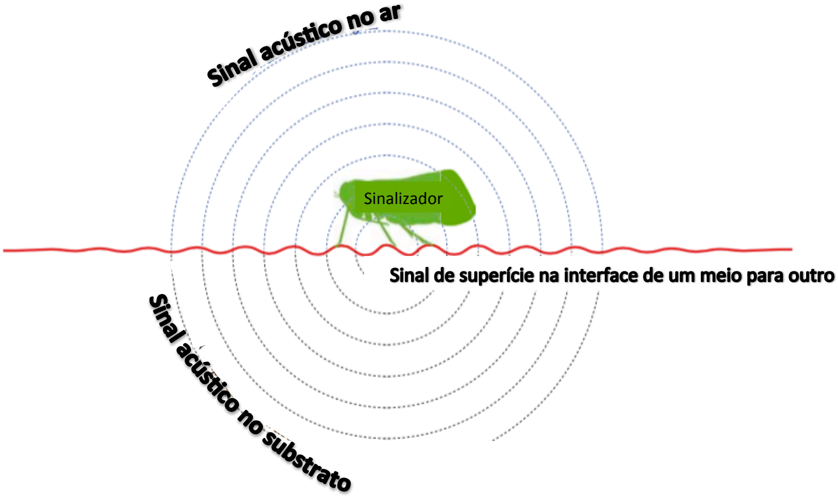

Figura 1. Exemplo de ondas mecânicas emitidas por um animal em diferentes meios e seus limites. Adaptado de Hill and Wessel (2008).

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.

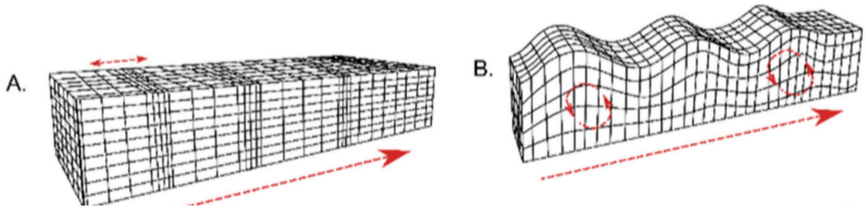

Figura 2. Diagrama de blocos de tipos de ondas em meio 3D com direção de propagação marcada com setas: longitudinal (A), Rayleigh (B). Adaptado de Hill (2008) e Roberts et al. (2016).

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).

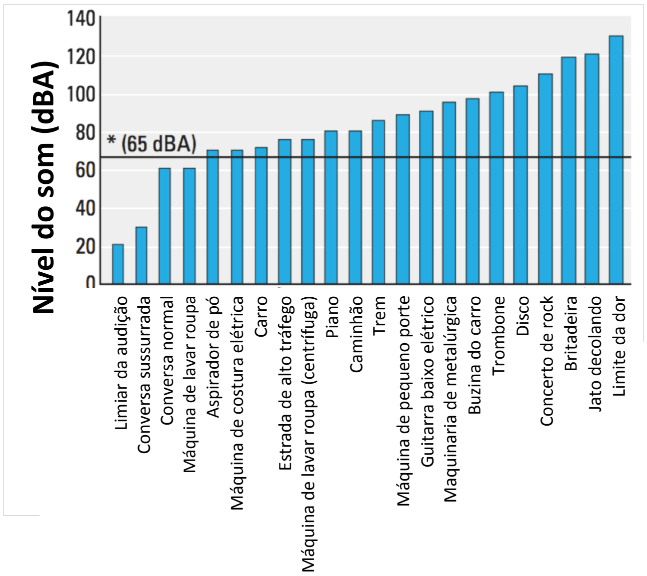

Figura 3. Níveis de ruído audível de diferentes fontes antropogênicas. *Limite de segurança proposto pela Organização Mundial da Saúde (Berglund et al. 2000). Adaptado de Frenzilli et al. (2004).

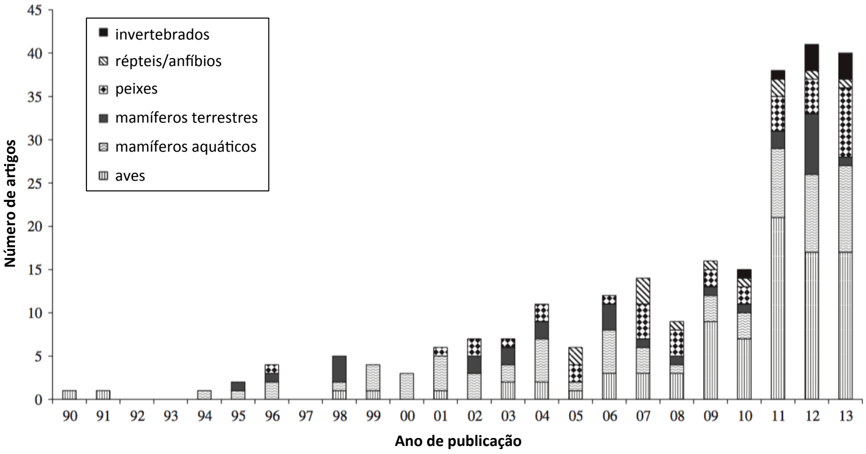

Figura 4. Número de publicações relatando os efeitos do ruído antropogênico em animais entre 1990-2013. Adaptado de Shannon et al. (2016).

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.

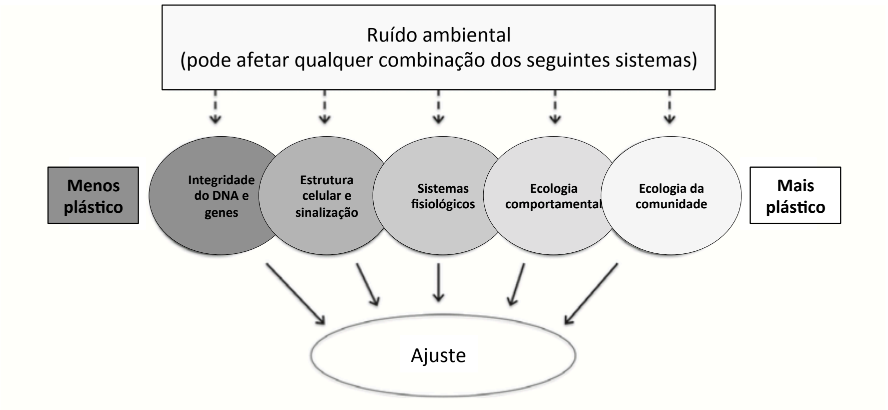

Figura 5. Estrutura conceitual de como o ruído ambiental pode afetar os sistemas biológicos. Adaptado de Kight and Swaddle (2011).

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).

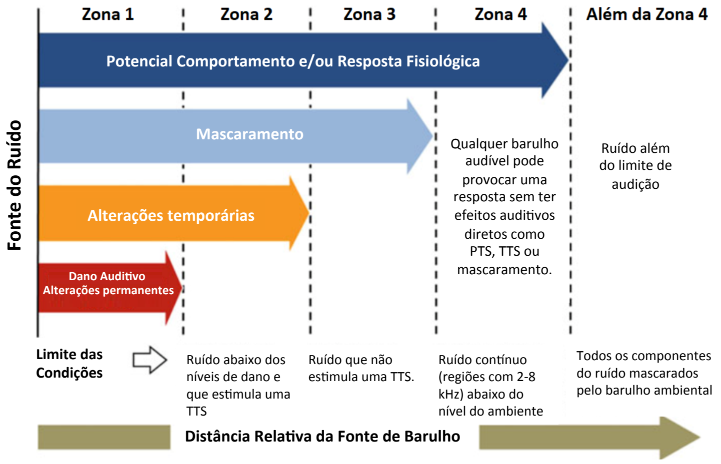

Figura 4. Efeitos dos ruídos antropogênicos em animais. Adaptado de Dooling and Blumenrath (2013).

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.

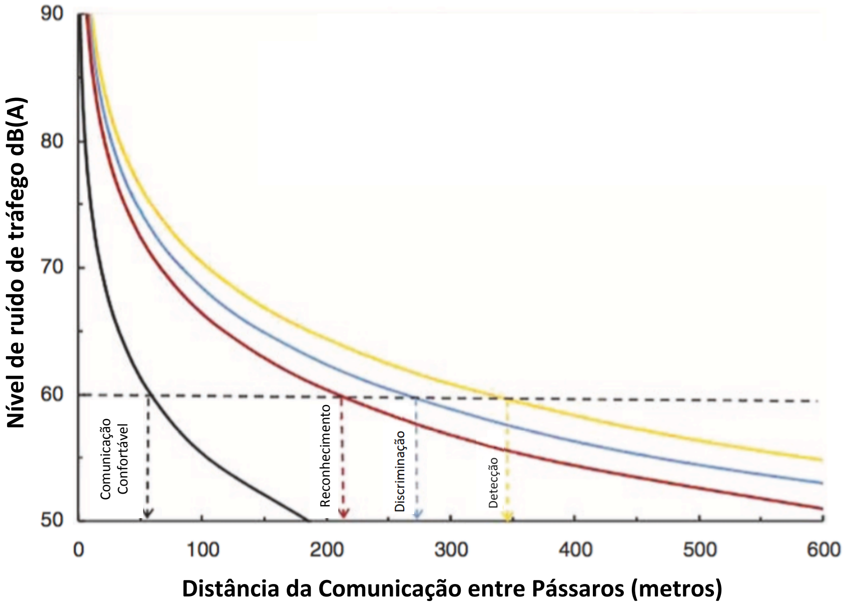

Figure 5. Relação entre o nível geral de ruído e as distâncias de comunicação que permitem detecção, discriminação, reconhecimento e comunicação confortável entre as aves. Adaptado de Dooling and Blumenrath (2013).

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.

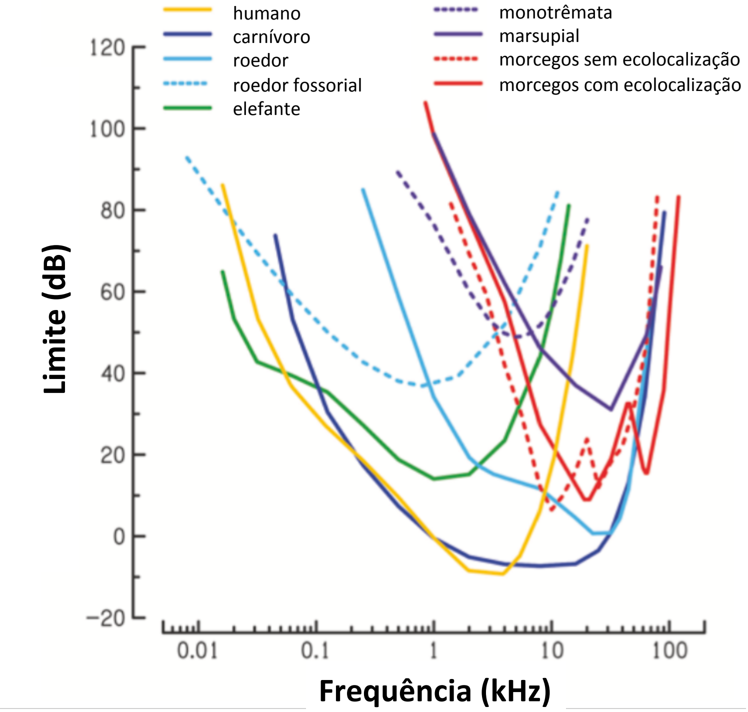

Figura 6. Representação dos limiares auditivos em relação a diferentes frequências em alguns mamíferos terrestres. Adaptado de Slabbekoorn et al. (2018b).

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

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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

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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.

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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

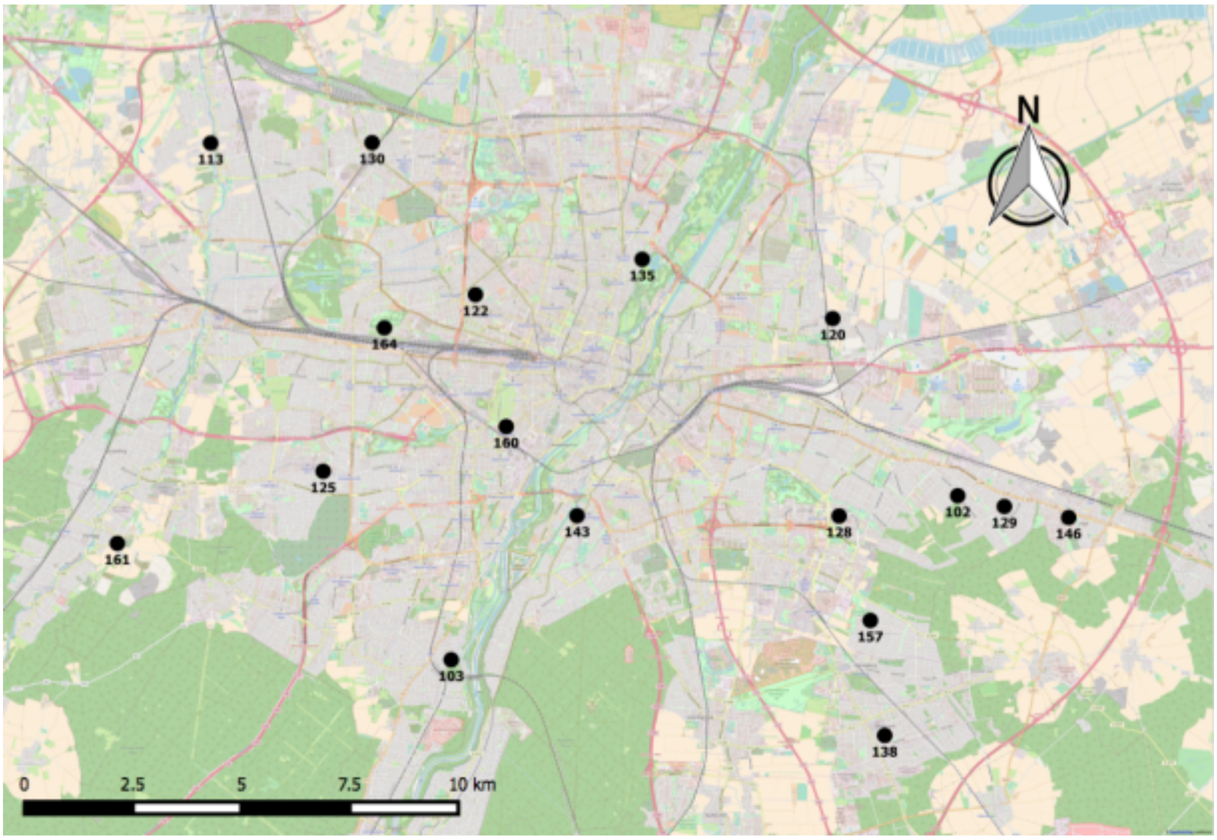

Fig. 1 Distribution of the nest boxes (black dots) in the city of Munich, Germany.

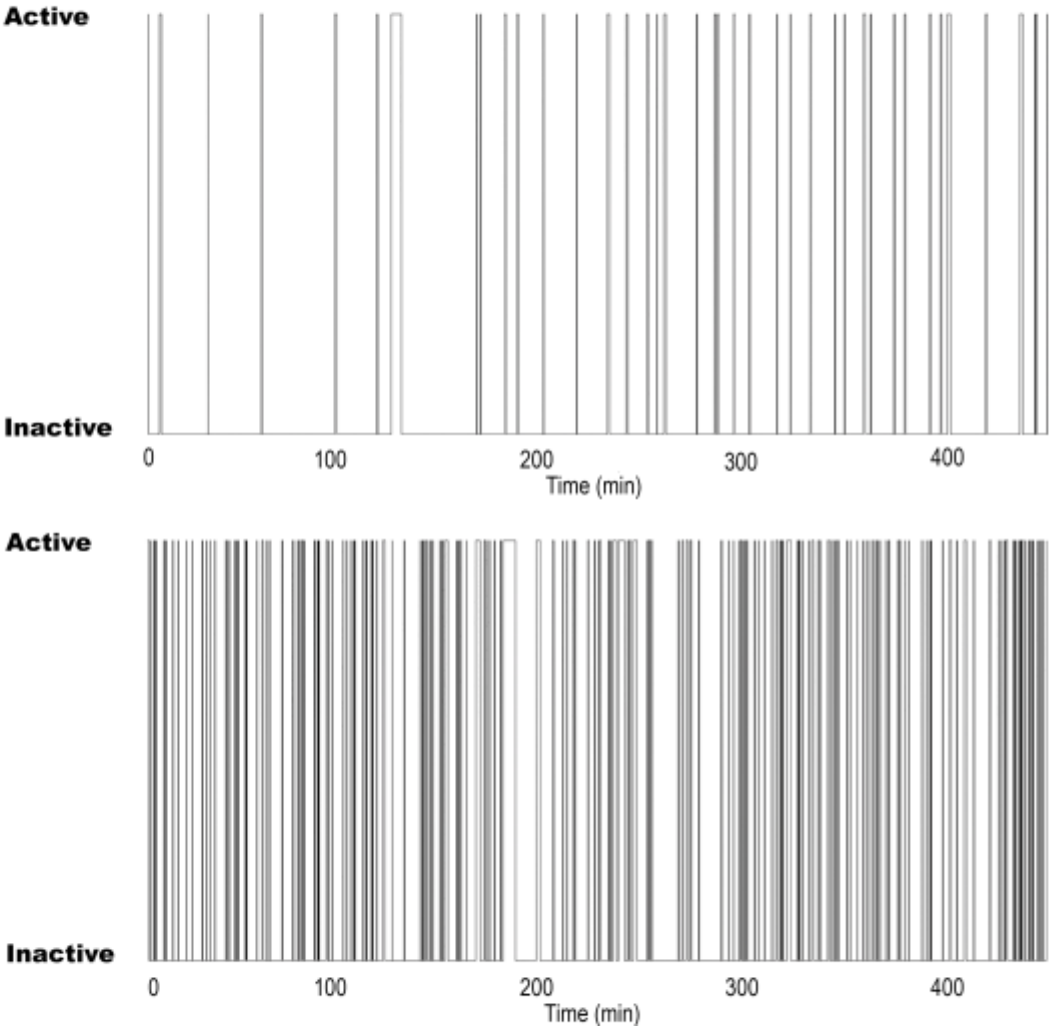

Fig. 2 Hypnogram showing the number and duration of active and inactive states of the least active bird (top) and the most active bird (bottom).

Electronic Supplementary Material

Online Resource 1

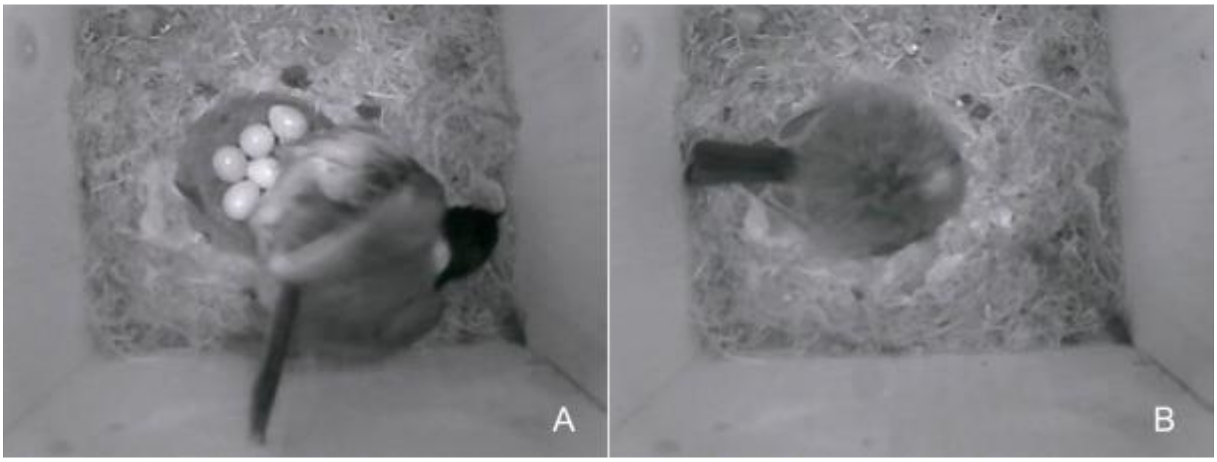

Figure S1 Female great tit (nest box 102) in (A) active and (B) inactive posture during night.

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

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RESEARCH ARTICLE

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

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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.

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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

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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 compostled 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*.

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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.

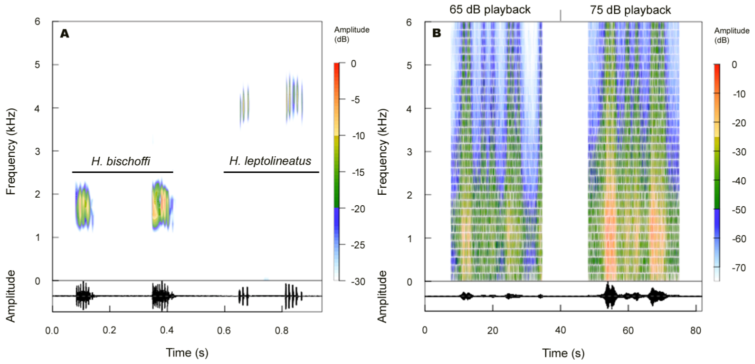

Fig 2. (A) Study species advertisement calls and (B) intensities (dB) of traffic noise used on playbacks. Spectrograms (above) and oscillograms (below) of *Boana bischoffi* and *B. leptolineata*.

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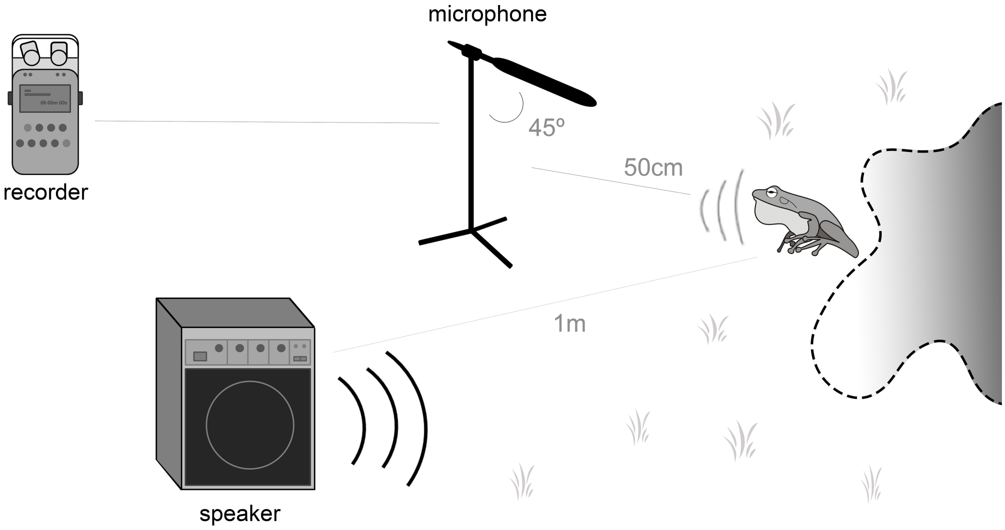

Fig 3. Design of experiments during the field trip to collect data on calling males.

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)

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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

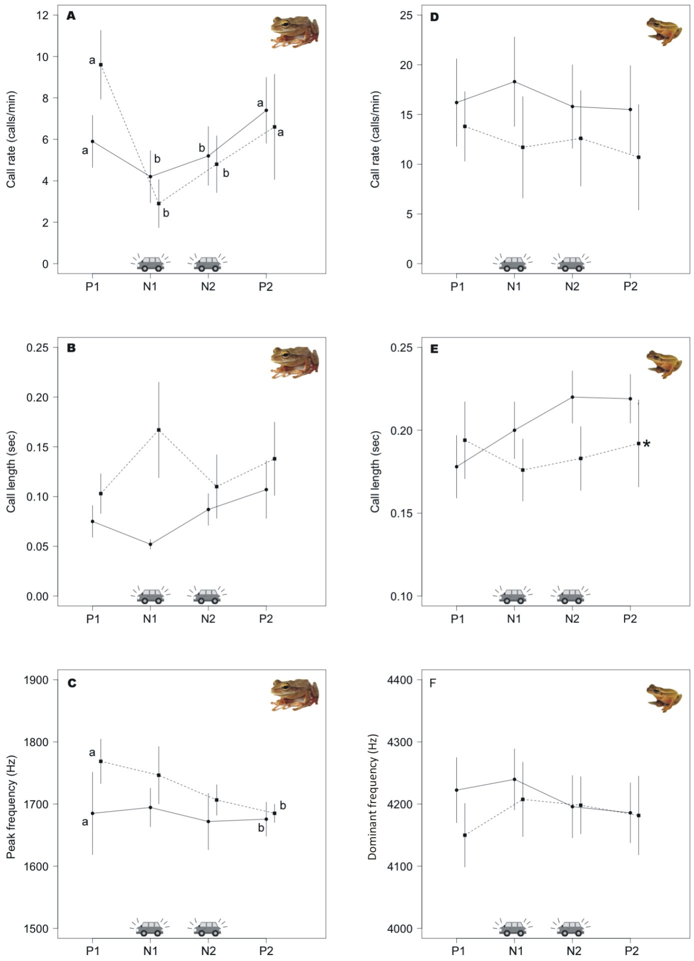

Fig 4. Effects of traffic noise on call parameters of the two hylids. Graphs show call parameter means (±SD) at the four periods of time inside a playback, P1 (pre–stimuli, silence), N1 (noise1), N2 (noise2), P2 (post–stimuli, silence). Dashed line represents the playback order N1 (75dB) followed by 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).

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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)

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7.3. CAPÍTULO III

**Formato nas normas da revista Scientific Reports*

"Bad vibes": Anthropogenic vibrations affect anuran calling activity

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“Bad vibes”: Anthropogenic vibrations affect anuran calling activity

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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.

Key words: anthropogenic activity, noise pollution, substrate-borne vibrations, amphibian, calling activity

Introduction

Environmental pollution has been singled out as one of the major causes of the global biodiversity crisis[1]. With the urban expansion, noise pollution is increasingly becoming a potential threat for biological communities worldwide [2]. This human disturbance has already been shown to negatively affect acoustic communication in many animal groups, such as insects[3], fish[4], birds[5], reptiles[6], amphibians[7] and mammals[8,9]. Yet impacts caused by noise pollution can be larger than previously expected, influencing on species persistence and conservation [2].

Animals can use multiple senses to process their surroundings[10,11]. Over the past 50 years, scientists have described and examined substrate-borne signaling in a variety of taxa, which plays a key role in sexual selection, territory defense, predator-prey interactions or navigation [12–14]. However, below the surface, little is known about how animals perceive substrate vibrations produced by human activities and how they can be affected by these singular environmental cues. Substrate-borne communication and perception by animals have long been neglected and this lack of knowledge impairs our capacity to predict the consequences of anthropogenic vibrations on animal ecology and behavior. In the marine environment, for instance, one study has shown that anthropogenic substrate-borne vibrations induced by human activity affect a hermit crab[17]. Given that studies on acoustics and civil construction reported significant seismic vibrations induced by traffic road or wind turbine[15,16], these anthropogenic vibrations could be a disturbance for animals that scientist have not been taken into account until now.

Among the terrestrial vertebrates, amphibians are likely the most sensitive to vibrations[12], and, therefore, they are a suitable model for assessing potential impacts of human-induced substrate vibrations. The capacity to detect seismic cues is linked to their inner ear, which comprises three organs: *papilla basilaris*, *papilla amphibiorum*, and *sacculus,* the last two presumably capable of detecting low frequency airborne sounds and substrate-borne vibrations[18–21]. Despite their likely seismic sensitivity, vibrational perception and signaling has only been reported for a few species and in limited contexts[13], e.g. intra-specific signaling[22,23], prey detection[24], predator avoidance[25,26], and detection of environmental cues[27,28].

Variations in acoustic parameters of the advertisement call, the most commonly emitted call by anuran males, are being related to sexual selection and female choices, which directly affects male reproductive success[11]. For example, experiments have shown that females prefer advertisement calls with high repetition rates (more acoustic stimulation)[29,30], longer duration[31], and lower frequencies[31,32]. Hence, changes in calling activity can significantly impact individual fitness and species conservation.

Therefore, understanding how anthropogenic substrate-borne vibrations may affect animal communication, which directly mediates species reproduction, could help to embrace future conservation plans, considering a source of disruption that has not been regarded until now. In order to answer the question about the potential effect of anthropogenic substrate-borne vibration disturbance on animals, we performed a series of playback tests using two common human activities (traffic road and wind turbine) to examine behavioral changes in the calling activity of the midwife toad, *Alytes obstetricans*. We expect a negative effect of human-derived substrate-borne vibrations on the toads calling activity, altering its parameters.

Results

A total of 26 males of *Alytes obstetricans* were exposed to the vibratory playback stimuli during calling activity. During the playback tests, eight toads showed avoidance behavior between the first and fourth treatment, ceasing calling activity and abandoning the calling site. These animals were excluded from the rest of the analysis.

Call parameters

The calling activity of the common midwife toad was affected by anthropogenic seismic vibrations (Table 1; Fig.4A). When focal males were exposed to vibratory stimuli, their call rate significantly decreased, as shown by the full-null model comparison (n=18, likelihood-ratio test: x^2=24.5, df=4, p<0.005; Table 2). Call rate was particularly influenced by the recorded traffic and wind turbine stimuli, which caused a mean reduction of 15 and 17.5 calls per min, respectively, while call rate decreased on average in 8 calls per min in response to synthetic stimuli (Table 1; Fig.4A). Furthermore, during original recordings, most frogs completely stopped their calling activity (83% and 92.6%) or reduced call activity to half or a third during the vibratory stimuli. During synthetic stimuli, frogs completely ceased calling activity in 63,6% of the cases for traffic, and 57.1% for wind turbine. The rest of the animals either reduced call to a half or to a third of the baseline. During the playback stimuli, many frogs re-started calling after approximately one min, but all of them with a reduction of call rate. Overall, during no-stimulus period, calling activity reached maximum rates, up to twice as high as during original anthropogenic stimuli (Table 1).

In contrast, call duration and dominant frequency remained unaltered between the exposition to no-stimulus period, synthetic and anthropogenic stimuli (n=17, likelihood-ratio test: x^2=0.28, df=4, p=0.99; x^2=2.29, df=4, p=0.68, respectively; Fig. 4B-C; Supplementary Table 1 and 2). Moreover, baseline acoustic behavior from pre- and post-stimuli periods of the whole experiment showed no differences in any acoustic parameter (likelihood-ratio test: for call rate, x^2=0.02, df=1, p=0.88; for call duration: x^2=0.27, df=1, p=0.60, for dominant frequency: x^2=2.11, df=1, p=0.15). According to the first GLMM, air temperature did not influence call rate during the playback tests (likelihood-ratio test: x^2 = 1.93, df =1, p=0.16, in all cases).

Energy threshold

The amount of sound energy measured at the time when males showed a behavioral change in call rate was around 17.6 dB for traffic and 13.8 dB for wind turbine, but with a wide range of individual variation (Fig.5A). However, toads seem to have a higher tolerance to the synthetic wind turbine stimulus, with a peak tone at 100Hz (24.8 dB) than to that of traffic vibration with a peak tone of 10Hz (12.7 dB). After initiating playback stimuli, the animals kept calling for longer time in wind turbine stimulus and therefore the threshold was higher for this vibrational source.

Discussion

Studies have shown that anthropogenic airborne noise affects acoustic communication [33,34], but to our knowledge this is the first study to demonstrate the effect of ground-borne vibrations induced by human activity on anuran call activity and furthermore on a terrestrial vertebrate. Playback experiments revealed that anthropogenic vibratory stimuli caused a strong reduction on the calling activity in focal males that decreased their mean call rate up to the half. Both traffic and wind turbine vibrations showed similar impact on calling activity. By comparing recorded and synthetic stimuli we found that such response was triggered by the entire spectral component of the vibrations, rather than their peak frequency. Additionally, the sound energy threshold for animals to change their baseline calling activity was lower with recorded stimuli than with synthetic stimuli.

Studies assessing effects of anthropogenic noise on frogs have shown that species coping with this noise use a variety of mechanisms, including short-term adjustment, as signal amplitude, timing or duration or even frequencies[35,36]. However, all these studies focused in airborne transmission of signals. In this study we have found that toads did not change some parameters of the call, as duration and frequency, in response to ground-borne vibration induced by anthropogenic activity, maybe because their airborne signal was not being masked in this channel. Meanwhile, call rate was affected by induced vibrations decreasing the number of calls per minute. Calling activity reached its maximum during no-stimulus period and then decreased during synthetic emission and even more during actual recordings of traffic and wind turbine vibrations.

It is unknown if the studied species, *Alytes obstetricans*, emit any vibrational signal together with airborne sound as has been described for *Leptodactylus albilabris* [21] for instance. However, if that happens, toads could be facing a masking signal underground. A few studies have found differences in perception of seismic cues by anurans between original noise stimuli (such as rain and wind vibrations), which suggested that frogs might possess a sensory system that allows them to discriminate between different types of vibrational stimuli[28]. Furthermore, studies with other animals, which have been proven to use seismic signals to communicate, showed strategies against vibrational interference such as increasing of signal effort, avoidance of temporal overlap, and adjustment of the duration of the signal [28,38]. Although some species with seismic communication appear to be affected by this source of disturbance, we do not know if the midwife toads use this channel for communication and, therefore, this question remains open.

Another important topic to be addressed is that noise studies mainly focus in the signal emitted and background noise, and animals do not use their acoustic system only for sending signal, but also to perceive them and the environment around them. The sensory system detects a wide range of stimuli and are typically optimized to process relevant cues against a background of irrelevant stimuli, often referred to as noise[28,39,40]. Additionally, frogs may use neurological structures in their ears that are sensitive to substrate-borne vibrations[21,22]. Besides the complexity of the auditory system and the variation among species, it is known that two of the three organs of the anuran hearing system are involved in detecting exquisitely low-level substrate-borne vibrations (*sensu*[19,41–44]), the ‘amphibian papilla’ and the ‘sacculus’, the latest one supposable related to detect environmental cues[24,27,45]. This means, these two organs do probably not mainly perceive airborne sound in the midwife toad, but ground-borne vibrations instead.

Our results showed that *Alytes obstetricans* is sensitive to the lower frequencies of traffic and wind turbine recording vibrations, which contains energy in a range of frequencies instead of only one as the synthetic stimuli. Moreover, when looking at a more detailed response of each of the synthetic stimuli, toads appear to be more sensitive to the tone at 10Hz (traffic vibration), given that a lower threshold of noise induced toads to ceased calling activity while with the 100Hz tone (wind turbine), animals called normally until higher intensities were reached. As the amphibian papilla is the organ apparently responsible for perceiving frequencies above 100Hz, we would then suppose that these variations in call rate responses and in threshold between the synthetic stimuli is related to how the animals perceive the vibration noise in the environment. However, this remains to be tested.

Measurements of the neural tuning curves, which represents the frequencies perceived by auditory organs in a scale of sensitivity, have shown to differ between species[46]. Fore instance, some species appear to have a correspondence between vocalization and auditory tuning[47]. This is the case of *Alytes obstetricans*, which showed a high frequency sensitivity center (1571Hz) above the dominant frequency of the call of the species (around 1200Hz). The same study tested the sensitivity of the *torus semicircularis* auditory midbrain of *Alytes obstetricans* to frequencies from 100-5000Hz. The results showed regions of high sensitivity at a low-frequency range, between approximately 100–500 Hz and, at a high-frequency range, between approximately 1200– 2400 Hz. The best threshold of the lower frequency reached values of approximately 40 dB SPL, occurring at the lowest frequency tested (100 Hz), whereas minima in the high-frequency range were between 40 and 50 dB SPL[48,49]. This means the focal species of this study is able to perceive very low frequencies with a high sensitivity, which could explain why the toads decreased calling activity even when their call does not overlap with the noise signal emitted.

Several hypotheses could be raised to explain the results found on this study. For instance, previous studies with anthropogenic noise in airborne sound, showed many species decrease call rate when the interference was too high, for instance high levels of noise [50,51]. We found that *Alytes obstetricans* reduced its call rate during playback of seismic noise, however during the last no-stimulus period of the playback experiment, the animals returned to base line calling activity with a similar rate as in the beginning. This suggests animals could be adjusting signaling during calmer periods, which is consistent with noise-avoidance behavior found in airborne sound studies with anurans[50,51] and other taxa[52,53].

Another possibility could be that the animals are interpreting the anthropogenic unknown vibration as an environmental cue and then making a decision. Previous study mentioned here, reported that frogs apparently discern between wind and rain seismic cues in the water [28]. Furthermore, a study showed that two Iberian toad species were able to respond to rainfall-induced vibrations in the soil emerging to the surface, suggesting that detection of abiotic seismic events might, indeed, be biologically relevant for this group[27]. In this sense, the midwife toads could be interpreting the unknown vibration cue as a predator approach and therefore, reducing calling activity could reduce the risk. It is known that call activity does not only attracts mates, but also predators, so anuran species have strategies as to call in chorus or reduce call to prevent risk[20,54–58]. From a prey perspective, vibration can indicate imminent danger of predation before physical contact occurs, and offers certain advantages over other information channels[25]. Rather than indicating general, ongoing levels of risk or predator presence in the environment as chemical cues often do, vibration indicates the current activity of an individual predator. For instance, a study found that vibrational cues alone could elicit substantial levels of early hatching in *Agalychnis callidryas* anticipating a predator attack[25]. The response of the midwife toad to reduce signaling during playback emissions could be related to the association of this interference to a predator. Noise sources that are a novel or unpredictable as well as similar to a biologically relevant sound are predicted to elicit responses similar to those associated with predation risk[59]. Hence, by reducing call rate and even ceasing calling, it could reduce the chances of being located by the predator.

Despite the reason why animals reduced calling rate, this behavior may have consequences. Several authors have argued that, in addition to other impacts of roads, elevated airborne noise levels also impair the ability of animals to effectively communicate during breeding, thereby impacting reproductive success[60–62]. Reproduction usually depends on a female frog ability to respond correctly to the advertisement signals of a conspecific male[63]. Therefore, sound localization has obvious fitness consequences for anurans (reviewed in [64–66]). Female anurans exhibit phonotaxis towards male choruses[66], and noise may impair an individual ability to detect and respond to biologically critical information[67], affecting mate attraction[62]. We do not know if males of *Alytes obstetricans* emit seismic signals, or if female choice would be affected by these, but we do know that the females hearing system is sensitive at least up to low frequencies around 100Hz[49]. Hence, it should be taken into account, as it is a source of extra information females are receiving from the environment into their acoustic system. Moreover, it is well known for anurans, that calling effort is significantly related to female choice where they prefer males with higher call rates[30,68]. This is not different for *Alytes obstetricans* species[29], which means a decrease on this parameter could affect female choice for different individuals and lower male mating success. The effect of anthropogenic vibration on female choice remains unclear and further studies should be performed in order to answer the true effect of it on mate choice and furthermore on reproduction.

This study shows the first results of the effect of anthropogenic vibrations on terrestrial animal communication. Despite of our study using anurans as a model, any other group might be affected by this underestimated source of disturbance, especially the ones using this channel for communication. Noise produced by human activities is projected to significantly increase in the next decades, accompanying urban expansion[34] and therefore we should not underestimate the effect this source of disturbance might cause, through a complex series of factors in airborne and ground-borne, influencing multiple biological systems both directly and indirectly.

Methods

Theoretical background

The use of the terms sound and vibration is many times not clear[12,70]. Extending the definitions of sound to nonhuman animals occasionally has been difficult and controversial[12]. Sound and substrate-borne vibration are generally understood to be distinct when used in popular connotation, even though this distinction may not be so easy to clearly define. Sound is defined in terms of the hearing sense; however, sound is a vibration transmitted via airborne or water-borne or substrate-borne compressional acoustic waves, which are detected by an animal with pressure receivers, or pressure difference receivers. Thus, substrate-borne vibration, which is the basis of the study of biotremology, is only another type of vibration of a subset of all physical vibrations, which also include sound[14]. In this work we will follow definitions by[14], in which mechanical waves (that transport energy through a medium by oscillation or vibration of the particles of matter) are divided into *acoustic waves* (purely longitudinal waves in a homogeneous medium, as air, liquid or solid, where particle motion travels in the same direction as energy) and *surface-borne waves* (that happen at boundaries between different media, where energy is always transferred from one medium to the other, and vice versa, and where particles oscillate perpendicular to the energy). The focus of this study, vibrations caused by anthropogenic sources, are one kind of surface-borne vibrations, the rayleigh type. This is a combination of longitudinal and transverse waves with the particles moving in an elliptical pathway, and most energy in the vertical direction, perpendicular to the direction of motion[12,71,72]. Surface-borne waves can be carried by any source, for instance a plant; therefore in this study we also use the term ground-borne vibration or seismic vibration, which refer to the waves carried in soil or sand[12].

Study area and species model

Playback tests were conducted in Lago de la Cueva (43º3'N, 6º6'W, 1550m a.s.l.), in the Natural Park of Somiedo, Asturias, Spain. This area has quite minimal interference, allowing accuracy in the simulation of traffic and wind turbine vibration to individuals not previously exposed to it. The focal animals were localized around a mountain lake, above the timberline in alpine grasslands with abundant rocks. Experiments took place from 12 to 25th of June of 2017, when the breeding season of the study species occurs, during clear sky nights and with temperature between 13.6 - 25.6 ºC.

The midwife toad, *Alytes obstetricans*, has terrestrial calling activity and mating (Supplementary Fig.1A). Most males call on top of exposed substrate, but calling males below rocks or inside shallow holes also can be found[73,74]. Their advertisement call (Supplementary Fig.1B) consists of a short tonal note (100-160 ms long), repeated at long intervals (0.5-10 s) and lacking frequency and amplitude modulation. The dominant frequency of the call is around 1200 Hz[75,76].

Vibratory stimuli

In total, we built a set of 5 playback vibratory stimuli (Fig.1): (i) traffic road, (ii) wind turbine, (iii) a synthetic copy of traffic road (iv) and a wind turbine, and (v) no-stimulus. Three different copies of the original stimuli of traffic road and wind turbine were prepared to provide replicates of the playback stimuli. Synthetic copies of original vibrations were used to control the acoustic properties of the stimuli, so that we were able to test behavioral responses to particular spectral components of anthropogenic vibrations. The no-stimulus period was used as control to account for absence of anthropogenic vibrations. Each experiment had, therefore, a total of 9 stimuli.

Stimuli of traffic road and wind turbine vibrations were recorded on the 7th of June of 2017 at Fuencarral-El Pardo road (40º30'16.01 N, 3º45'06.62W, 663m a.s.l., Madrid) and on the 9th of June of 2017 at Canredondo, (40º48'11 N, 2º32'22 W, 1210m a.s.l., Castilla-La Mancha), respectively. The road had two lanes and a maximum allow speed of 60km/h and during recordings the car flow was about 5car/min. The wind shovels had 83m of diameter and turbines are of 2000 kW power (Gamesa G83/2000) and during recordings was operating at 10-12km/h. For the recordings, we used a geophone (OYO – One, Oyo-Geospace), placed 4m from the source (the roadside in the case of the road and wind turbine in the case of the turbine), connected to a custom-built amplifier, which fed a digital recorder (Sound Devices, model 744T). Sounds were recorded using a sampling rate of 48000 Hz and a resolution of 16 bit, and saved in an uncompressed .wav format.

Recordings of the two vibrational noises were input to a MacBook Pro. Intel Core i7 computer and edited using Audacity 2.1.1 audio edition. Segments of 5-10 second judged to have spectra representative of each noise type were selected and pasted to create noise durations up to 120 s. During this process, researcher was very careful to avoid discontinuities of the waveform at the points where the segments were added. To create the synthetic copy of the original vibrations, we first obtained the spectral components of the traffic and wind turbine vibration recordings (Fig.1A-B), using a FFTs (1,024 points, sampling rate 48 kHz, 61.9 Hz bandwidth) in Audacity 2.0.2 and Raven Pro 2.5 software. The traffic and wind turbine stimuli had frequencies ranging from close to zero up to 500 Hz, with peak frequency in 10 Hz and 100 Hz, respectively, in agreement with previous literature[16]. Thus, we constructed two synthetic stimuli by generating a single tone at the dominant frequency of each studied source, i.e. at 10 Hz for traffic and 100 Hz for wind turbine (Fig.1C-D). Thereby, the synthetic stimuli mimicked the main spectral component of the original vibrations but lacking other secondary elements observed in the field recordings.

Playback tests were prepared following the A-B-A protocol[77] and further playback specifications and concerns according to previous studies[78,79]. Each of the 9 playback tracks, containing a single treatment or stimulus, lasted 2 min and was preceded and followed by 2 min interval of no-stimulus period to allow the animal to return to the base line behavior. Thus, the total duration of the test was 38 min for each animal. To standardize the stimuli, all were peak normalized and each stimulus track was subsequently modified by applying a linear 'fade in' amplitude filter from 0 to 100% with duration of 2 min each (Fig.2). This particular amplitude profile was used to expose the focal individuals to an increasing vibration through the playback tests.

Experimental procedures

Playback experiments were carried out during 13 days from sunset until dawn, when male toads were intensively calling for mate attraction. We actively searched for calling males around the study area and set up the equipment close to a focal individual, taking special care to avoid disturbance. Thus, we implemented the following procedure (Fig.3): i) a geophone and a microphone were placed at a distance of 20-30 cm from the focal animal; ii) the tactile transducer was buried 5–10 cm below ground level in front of the individual and, due to the difference in density of rocks, its distance from each focal male was adjusted between 4 and 6 m in order to control the vibration intensity received by all animals; iii) all lights were turned off and observers were positioned seated more than 6 m away from the focal animal, where they waited motionless until the individual starts to call regularly; and iv) playback tests were conducted.

Playback vibrations were generated with a MacBook Air computer using Audacity 2.0.2

software. The audio output of the computer was amplified with a Kenwood KAC-5205 automobile amplifier (frequency range 5 Hz–50 kHz), connected to a tactile sound transducer (Clark Synthesis, Platinum model, frequency range 5 Hz– 17 kHz). The output signal was calibrated by setting the audio output of the computer to a fixed level (-12 dB) and using the amplifier fixed level and bridged output. The vibrations generated by the tactile sound transducer using these settings were monitored with a geophone (Oyo One, Oyo Geospace) placed next to the frog (20-30 cm). The output of the geophone was amplified at 60 dB and connected to the line input of the digital recorder (Sound Devices, model 744T). In order to standardize the amount of energy the animals were receiving, at the beginning of the experiment set, we would emit a one second test using the wind turbine stimulus and control the received energy in the geophone next to the focal animal through the recorded.

During the tests, the order of stimuli was randomized for each animal and air temperature and relative humidity were monitored every 15 min, using an environmental outdoor data logger (HOBO 64K Pendant®Temperature/Alarm and HOBO Pro-V2, respectively). Since previous studies showed fidelity of *Alytes obstetricans* males for calling sites[74], we marked the location of each focal male and moved each night to different areas along the shore of the lake to prevent recording the same animal more than once.

All the equipment was tested on the field using pure tones from 10-300 Hz (generated with Audacity program) and the frequencies used for the synthetic stimuli were further analyzed to check for differences in amplitude due to equipment properties. We did not find differences between the amplitude of the signal sent by the equipment.

Ethical and legal permits

Access permits were obtained from Parque Natural de Somiedo, Asturias, Spain, and study permits were granted by Consejería de Medio Ambiente, Principado de Asturias.

Acoustic analyses

Primary acoustic parameters of the advertisement calls of each focal male were measured using Raven Pro v. 1.4 software: call rate ([number of calls – 1]/min); call duration (sec); and dominant frequency (Hz). Temporal parameters were analyzed on oscillograms, while the spectral parameters were measured on spectrograms created by Hann window type and with a window length of 516 points and 50% of overlap. For these measurements, we previously selected randomly ten notes for each treatment for each toad.

To obtain a proxy of the amount of vibratory stimuli required to trigger a behavioral response to the stimulus, we calculated a fourth parameter termed energy threshold in this study. This was measured as the wave energy (dB) captured by the geophone at the time the animals showed a change in their call rate in comparison to the pre-stimulus period. We did this by subtracting the energy at the moment the animal changed its behavior, from the background noise (using the pre-stimulus). Additionally, we created another categorical variable by classifying the level of behavioral response of the focal animals into four groups according to how their call rates changed with the linear increase of each stimulus: (i) call rate did not change; (ii) call rate reduced to one third; (iii) call rate reduced to half; and (iv) completely ceased calling.

Statistical analyses

General linear mixed-effects models (GLMM; [80]) were used to test the effect of traffic and wind turbine vibrations on the calling activity of the focal individuals. First, a GLMM for each acoustic parameter (call rate, call duration and dominant frequency) was set using Gaussian error structure and identity link function to search for the relationship between these parameters and the vibratory playback stimuli. In these models, the *type of stimuli* was included as a fixed factor, *air temperature* as a covariate, and *recording day, individual* and *track* as random factors. Second, to test whether call parameters varied between the silent periods before and after the exposition to stimuli, similar GLMMs were set using one minute of no-stimulus period previous and after the whole experiment. In these models, *period* (*pre- and post-test*) was included as fixed factors, *air temperature* as a covariate and *recording day* and *individual* as random factors. Additionally, the required random slopes (all except that of type of treatment within track, n = 5) were included in the models in order to keep type I error at the nominal level of 5%[81,82]. Correlation parameters between random intercepts and random slope terms were also added when model convergence was not compromised. To reduce model complexity, interaction terms between fixed factors were excluded.

Model inference and the effect of individual predictors were established by full-null model comparisons as described in[27]. Visual inspection of qqplots and residuals plotted against fitted values revealed no obvious deviation from the canonical assumptions of normally distributed and homogenous model residuals. Collinearity issues were absent from the models according to the Variance Inflation Factor (VIF <1.64, in all cases), estimated with the function vif of the R-package car[83] on a standard linear model excluding the random effects. Besides, model stability was checked by estimating model coefficients in an iterative process that excluded subjects one at a time from the data, which indicated no influential subjects. Confidence intervals of model coefficients were computed through 1000 bootstrap iterations using the function bootMer of the R-package lme4[84]. GLMM were fitted in R[85] using the functions lmer of the package lme4[84].

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Additional information

Figure legends

Figure 1. Power Spectrum (above) and waveform (below) of the (A) traffic and (B) wind turbine seismic vibrations recorded and the (C-D) two synthetic tones constructed.

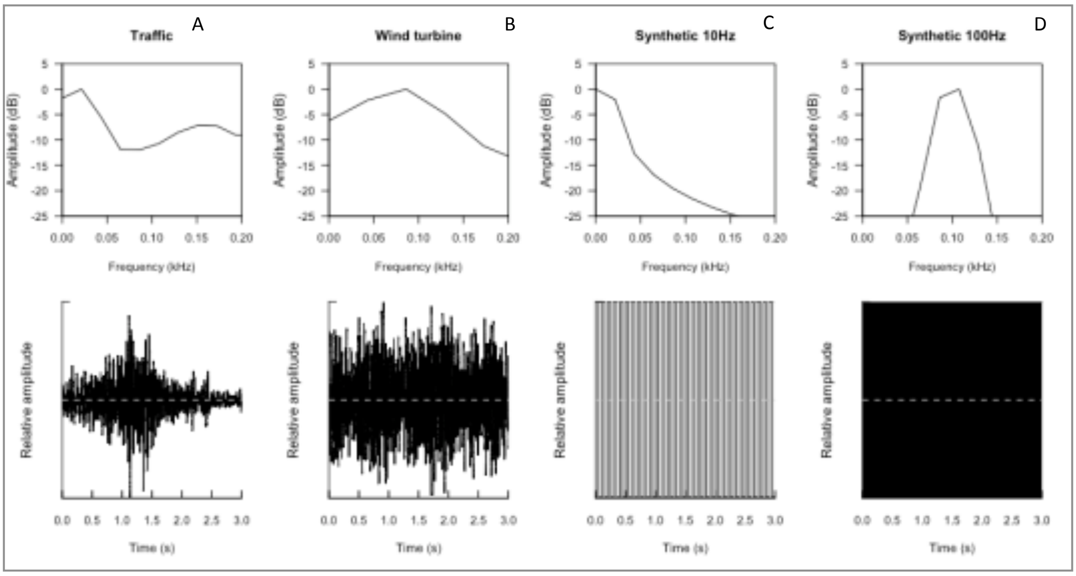
Figure 2. Scheme showing the 38min playback presented to each animal, containing 9 fragments of 5 different stimuli. Blue triangles indicate the increase of amplitude from zero a 100% of the vibration emission within the 2 min of treatment.

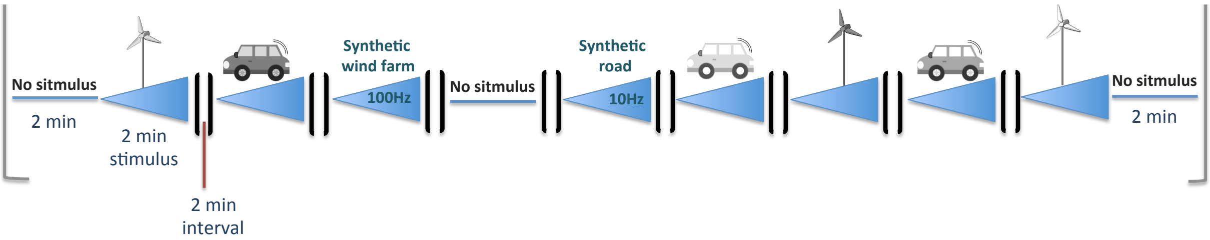
Figure 3. Experimental design *in situ* of playback seismic vibration emissions.

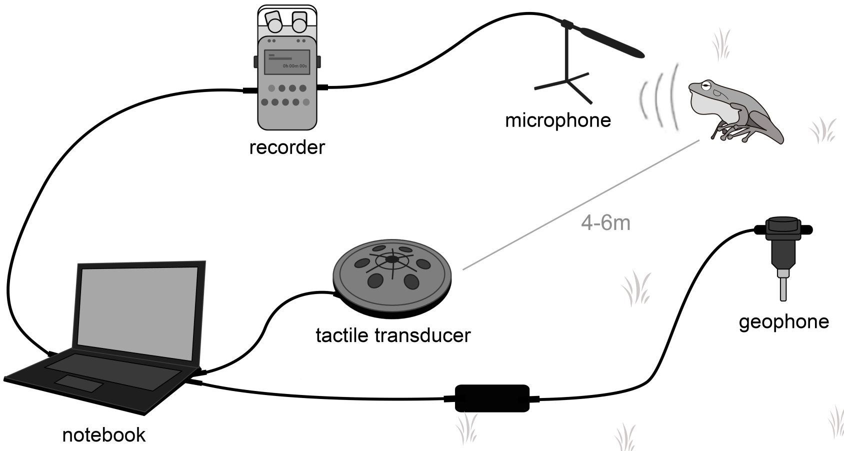
Figure 4. Boxplot showing variation in (A) call rate, (B) call duration and (C) dominant frequency, during each treatment. Box plot displays the median with a center line, a variation of 1st and 3rd quartiles represented by the box, a full range of variation (from min to max) represented by "whiskers" above and below and outliers represented by small circles.

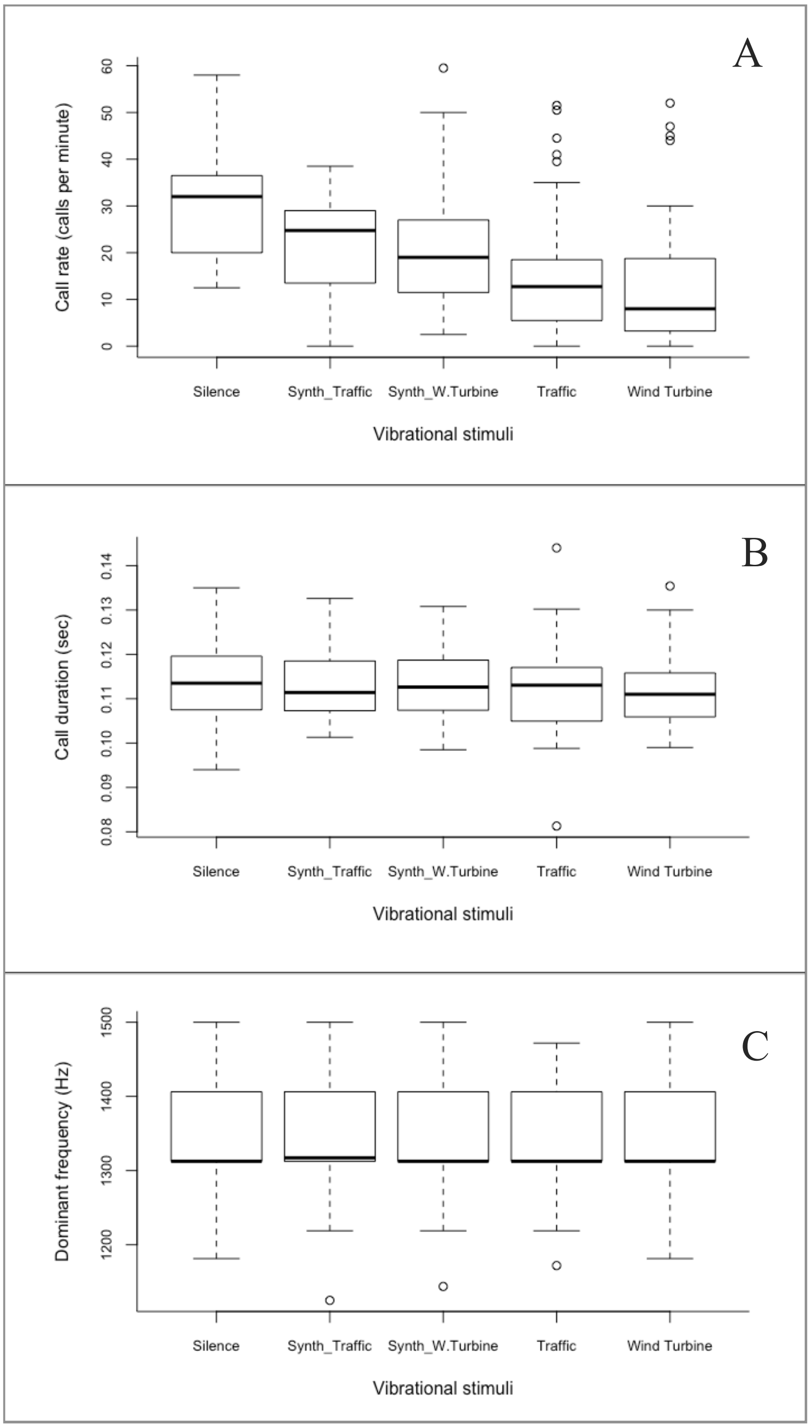
Figure 5. Boxplot showing threshold in dB when the frogs changed their calling activity when exposed to stimuli. (A) Recorded vibrations and (B) Synthetic vibration. Box plot display the median

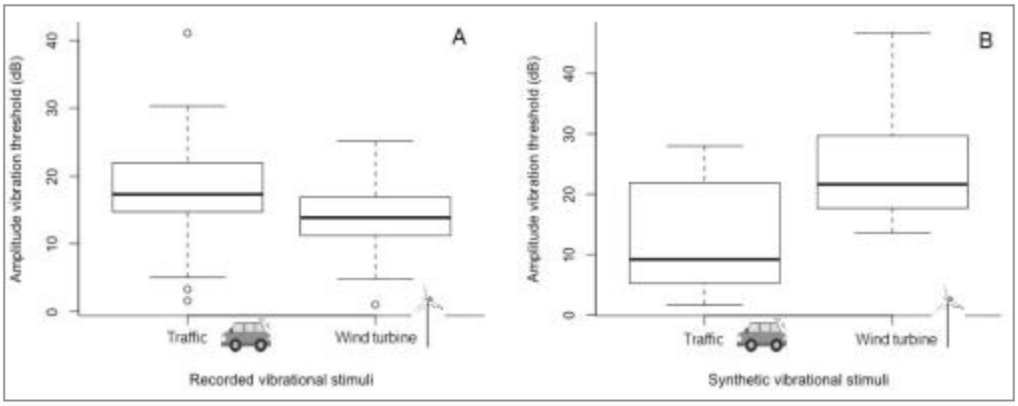
with a center line, a variation of 1st and 3rd quartiles represented by the box, a full range of variation with (from min to max) represented by "whiskers" above and below and outliers are represented by small circles.








A
Call rate (calls per minute)
0
10
20
30
40
50
60
Silence
Synth_Traffic
Synth_W.Turbine
Traffic
Wind Turbine
Vibrational stimuli
B
Call duration (sec)
0.08
0.09
0.10
0.11
0.12
0.13
0.14
Silence
Synth_Traffic
Synth_W.Turbine
Traffic
Wind Turbine
Vibrational stimuli
C
Dominant frequency (Hz)
1200
1300
1400
1500
Silence
Synth_Traffic
Synth_W.Turbine
Traffic
Wind Turbine
Vibrational stimuli


A
Amplitude vibration threshold (dB)
0
10
20
30
40
Traffic
Wind turbine
Recorded vibrational stimuli
B
Amplitude vibration threshold (dB)
0
10
20
30
40
Traffic
Wind turbine
Synthetic vibrational stimuli

Tables

	Call rate (call/min)	Call duration (sec)	Dominant frequency (Hz)	Threshold (dB)
No-stimulus	30.4 ± 11.2 (12.5-58)	0.114±0.01 (0.094-0.135)	1341±83 (1171-1471)	_
Synthetic Traffic	21.9±10.4 (0-38.5)	0.112±0.008 (0.101-0.132)	1339±96 (1125-1500)	12.7±9.5 (1.7-28)
Synthetic Wind turbine	22.2±15.5 (2.5-59.5)	0.113±0.008 (0.094-0.130)	1342±82 (1181-1500)	24.8±9.0 (13.6-46.7)
Traffic	15.05±12.8 (0-51.5)	0.112±0.01 (0.081-0.144)	1343±77 (1143-1500)	17.6±7.6 (1.6-41.1)
Wind turbine	12.9±18.8 (0-52)	0.112±0.008 (0.099-0.135)	1340±74 (1181-1500)	13.8±5.0 (1.0-25.1)

Table 1. Call parameter variations of the advertisement call emitted by males of Midwife toad submitted to traffic, wind turbine and synthetic vibrations stimuli. Data is given by Mean ± Standard deviation (Range).

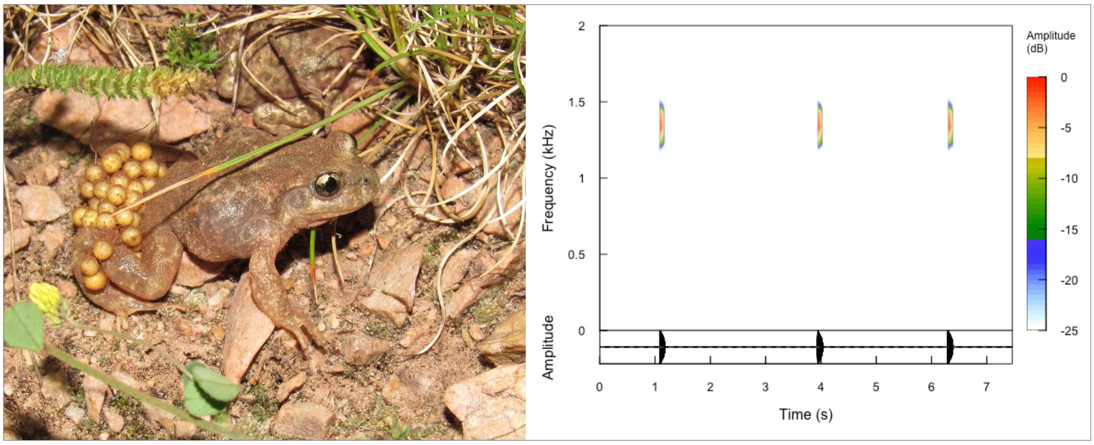
	Coefficient	Std. Error
(Intercept)	33.12	4.75
Synthetic Traffic	-6.37	2.61
Synthetic Wind turbine	-6.38	4.11
Traffic	-15.45	2.90
Wind turbine	-18.93	2.68
Temperature	5.5	3.44

Table 2. Estimated regression coefficients, standard errors, and confidence intervals for GLMM of call rate in response to vibratory playback stimuli. No-stimulus period was the reference category and air temperature was z-transformed.

Supplementary information

Figure legend

Figure 1. Male of *Alytes obstetricans* during calling activity (left) and advertisement call of male (right), both from Lago de la Cueva, Somiedo, Asturias, Spain.



7.4. CAPÍTULO IV

**Formato nas normas da revista Behavioral Ecology*

Effects of anthropogenic noise on anurans: few decades of Science

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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.

Keywords: Anthropogenic noise, Disturbance, Anura, Physiology, Behavior, Conservation

INTRODUCTION

Anthropogenic disturbance has been pointed as the major cause of the world´s biodiversity crisis (Brumm 2013). Some human activities, however, have received less attention from researchers and conservationists because their effects are more difficult to measure, especially when affecting species at a sublethal level, as often occurs with noise (McGregor et al. 2013). Noise disturbance is expanding in scope and intensity commensurate with human population growth and urban development (Shannon et al. 2016). Although there are many non-human sources of noise, including wind, water and other animals, anthropogenic noises are often louder, more frequent and more widespread than other sources of acoustic stimuli (Patricelli & Blickley 2006; Popper & Hastings 2009). This pollutant also knows no boundaries, and aquatic and terrestrial environments are subject to substantial and largely uncontrolled degradation of opportunities to emit and perceive sounds (Barber et al. 2010).

A recently published review on the effects of acoustic disturbance on animals shows how immediate effects on individuals have an impact, risking species conservation (Slabbekoorn, Dooling, et al. 2018). Anthropogenic acoustic disturbance is affecting a wide range of animal groups, including insects (Römer 2013), fishes (Popper & Hastings 2009), birds (Gil & Brumm 2014), reptiles (Brumm

& Zollinger 2017), amphibians (Simmons & Narins 2018), and terrestrial and marine mammals (Finneran & Branstetter 2013; Slabbekoorn, McGee, et al. 2018). Noise exposure poses a significant threat to the integrity of ecosystems, once it can inhibit perception of sounds, an effect called masking (Wiley 2013). However, compromised hearing affects more than acoustical communication (Barber et al. 2010). Noise can influence from DNA integrity and genes, cell structure and signaling, to physiological systems, behavioral ecology and community ecology. Therefore, it is likely to have both diverse and complex impacts on wildlife, as it can influence multiple biological systems both directly and indirectly (Kight & Swaddle 2011).

Between the vertebrates, amphibians are the most endangered class, with 41% of the extant species classified among one of the three IUCN categories of high extinction risk (IUCN, 2018). Sound plays a fundamental role in individual fitness in most anurans through acoustic breeding displays, mate attraction, territory defense and predator detection (Ryan 2001; Gerhardt & Huber 2002). However, noise-masking effect could impair signal emission, perception and communication (Wiley 2013). Anurans produce sounds usually between 100-6000Hz (Capranica 1965; Capranica 1976; Wells 2008), but there are species known to emit ultrasound (Feng et al. 2006) and seismic vibrations (Narins 1990). Besides that, anuran hearing system has three important organs with different sensitiveness: the basilar papilla (frequencies usually above 1000Hz, with the maximum recorded to a frog on 8kH - Geisler et al. 1964; Loftus ‐ Hills & Johnstone 1970); the amphibian papilla, an exclusive organ of the group (frequencies from 50-1000Hz - Feng et al. 1975); and the sacculus, (low frequencies between 20-300Hz). The last two organs are supposable capable of sensing substrate-borne vibrations (Capranica 1976; Lewis et al. 2001; Márquez et al. 2016). Human activities usually produce noises in a range of frequencies about 50-7000Hz (Simmons & Narins 2018). Therefore, given the anuran sensibility of hearing, together with the importance of acoustic (Narins 1995) and seismic (Narins 1990) signals to their communication, it is expected that anthropogenic noises could have a major impact upon this group.

Studies accessing the effects of anthropogenic noise on anurans are very recent, mostly from the last 15 years, and they have accessed mainly the effects of noise on males’ vocal activity. These studies, including some reviews about the topic (Schwartz & Bee 2013; Vélez et al. 2013; Simmons & Narins 2018) provided a useful starting point to understand which life history characteristics, and under what conditions, they might be influenced by noise, but again they were all focused on communication. Nonetheless, nearly 2/3 of all articles on the subject were published in the last five years (Fig.1), emphasizing the perception that the extent of noise effects and impacts remain poorly understood. To better understand the extent of the noise pollution on amphibians it is necessary to take into account all possible effects of noise together.

Here we provide both i) a review of existing literature on the effect of anthropogenic noise on anurans and ii) a compilation of possible mitigation strategies. We hope that this review triggers interdisciplinary collaboration, allowing us to better understand the effects of anthropogenic noise on anurans and suggest possible mitigations to reduce its effect on anurans.

We searched the Web of Sciences database (Thompson Reuters) and Goggle scholar on 2018, without restrictions on publication year and using the following combination of keywords and wildcards: (noise) AND (anuran*) AND (anthropogenic* OR traffic* OR road* OR urban* OR human*) AND (call* OR vocal* OR signal* OR song*). Furthermore, in order to approach this problem to its extent, we included any other study cited by previous one or found outside this search. We have considered studies with amphibian anurans linked to any kind of noise produced by a human activity, including: cities, transportation (traffic, airplanes) and energy production (wind turbine). The preliminary literature search resulted in 27 studies (see Table 1).

We divided the effects of anthropogenic noise into airborne noise (acoustic waves) and substrate-borne noise (surface-borne waves) (Hill & Wessel 2016). We have subdivided each of this two group into two categories of responses according with studies found: (i) Individual level: behavioral, physiological and morphological responses plus reproductive success and fitness; (ii) population and community level: distribution pattern, abundance and species richness. Bellow, we introduce each topic and then bring and discuss the results from the literature for each one.

OVERVIEW OF STUDIES FOCUSING ON ANTHROPOGENIC NOISE AND ANURANS

We present here the result of 27 published studies, which evaluated 57 species from 13 families of anurans. This represents 0,81% of the 6973 anuran species, and 25% of the 52 recognized families (Frost 2018). Despite the worldwide distribution of anthropogenic noise, most studies on anurans were carried out in Europe and US (Fig.2), the same pattern found for terrestrial wildlife (Blickley & Patricelli 2010). This geographic bias in research may limit the knowledge application to certain regions, since the impacts may differ among habitats and species (Blickley & Patricelli 2010). Studies accessed the responses of anurans to anthropogenic noise levels within a range of 40-98.2dB, and mostly produced by transportation activities (Table 1). Most studies were conducted on the field, either comparing areas of high against low noise levels or using a playback (McClure et al., 2013; Ware et al., 2015), in which pre-recorded traffic noise was broadcasted in an otherwise roadless area; a few studies were performed in captivity, exposing animals to noise treatments. From the 27 studies, 22 evaluated male responses and 5 female responses.

AIRBORNE SOUND

1. Individual level

1.1. Behavioral responses

1.1.1. Acoustic communication

Acoustic signals can transmit over long distances through varied habitats and are used throughout much of the animal kingdom to attract mates and to defend resources (Bradbury & Vehrencamp 1998; Tyack 1998; Marler & Slabbekoorn 2004). Sound source perception refers to the auditory system's ability to parse incoming sensory information into coherent representations of distinct sound sources in the environment (Wiley 2013). Such abilities are no doubt key to successful communication in many taxa, but we know little about their function in animal communication systems. Sound plays a fundamental role in most anuran social interactions, including mate attraction, territory defense and predator detection (Gerhardt & Huber 2002; Wells 2008); therefore, changes in the acoustic environment due to anthropogenic noise could limit or inhibit their communication and have a significant negative effect on mating success (Goutte et al. 2013; McGregor et al. 2013). These signal masking occurs when the perception of a sound is affected by the presence of background noise, decreasing the perception of a sound (Wiley 2013). In order to reduce these effects, animals are expected to adjust acoustic structure of their signals to increase the signal-to-noise ratio (Endler 1992). There are long- and short-term mechanisms by which senders can reduce masking of auditory signals: evolutionary changes in call traits and reversible behavioral adjustments, respectively (Brumm & Slabbekoorn 2005). Long-term changes are evolutionary adaptations during which animals are expected to adjust the acoustic structure of their vocalizations to reduce masking effect of noise (Brumm & Zollinger 2013). These evolutionary changes are related with the acoustic adaptation hypothesis (Ey & Fischer 2009), which states that the environment should favor vocalization characteristics that minimize attenuation and distortion (signal degradation) with distance (Brown & Handford 2000). The AAH predicts that individuals should adjust time and place of calling, alter temporal or spectral parameters of the call in order to reduces masking of their signal by a noise in the environment (e.g. (Rabin et al. 2003; Slabbekoorn & Peet 2003). Short-term changes relate to signal plasticity, based on individual signal adjustments (Brumm & Zollinger 2013) and have been reported in species coping with biotic (intra and interspecific) and abiotic (wind, streams) noise. Senders may reduce the negative effects of noise by modifying signal amplitude (Penna & Hamilton-West 2007), timing, duration (Penna et al. 2005), as well as shifting frequencies, so that senders broadcast most energy at frequency where noise intensity is relatively low (Slabbekoorn & Smith 2002; Caldart et al. 2016). Whatever the adjustment is, it is important to highlight that male frog advertisement call is one of the most energetically expensive activities that has been recorded in ectothermic vertebrates, up to 10 to 25 times those at rest (Pough 1992; Ryan 2001). Therefore, energy conservation appear to be a major factor shaping the call strategies of individual frogs (Schwartz et al. 1995; Grafe 1997).

Even though, anurans adjustment and adaptations seem to work with biotic and abiotic noise from the environment, it still to be understood whether this behavioral changes also operate to alleviate the impact of anthropogenic noise (Simmons & Narins 2018). In the following sections we will approach acoustic responses of the advertisement call of males from anuran species exposed to anthropogenic noise.

1.1.1.a. Temporal parameters

Temporal variables such as call/note/pulse rate, call/note/pulse duration, are influenced by environment (e.g. temperature, humidity, wind, vegetation) (Richards & Wiley 1980) and physiology, and therefore, are usually more variable than spectral parameters (frequency), which are more related to the morphology of individuals, although also influenced by physiology (Ryan 2001; Wells 2008). Furthermore, temporal variables can vary among individuals even when environmental variables are held constant (Gerhardt 1991). Therefore, temporal parameters are possibly very variable towards changes in the acoustic environment.

Call rate: According to the studies evaluated here, most anuran species facing anthropogenic noise, responded changing the call rate (number of calls/minute). The strategies varied between decreasing call rate (exposure to 65-88dB), increasing (exposure to 70-98.2dB) or not changing call rate at all (exposure to 52.27-90dB). Decreasing call rate when exposed to noise was observed in several species of Dendrobatidae, Hylidae, Microhylidae and Ranidae to different sources of noise, such as airplane and traffic (Sun & Narins 2005; Cunnington & Fahrig 2010; Vargas-Salinas & Amézquita 2013; Caorsi et al. 2017). 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 and thereby improving communication, increasing the probability of attracting females or repelling other males (Sun & Narins 2005; Vargas-Salinas & Amézquita 2013; Vargas-Salinas et al. 2014). By calling at times when the level of background noise is low, the so-called "gap calling behavior", anurans may reduce the detrimental effects of masking the auditory signals of abiotic noise, either non-human as wind or rain (Douglas Iii & Conner 1999) or anthropogenic (Sun & Narins 2005; Vargas-Salinas & Amézquita 2013). However, decreasing call rate, specially if there are no such gaps of low intensity noise, would not seem as a valid long term strategy, once it is well-established that reproductive success is proportional to calling effort (Whitney & Krebs 1975; Arak 1983; Klump & Gerhardt 1987; Márquez et al. 2008), and that could reduce mate attraction and impact total fitness for the species. Frogs increasing call rate when exposed to noise were observed in Hylidae, Pelodryadidae, Phyllomedusidae e Ranidae species, exposed to playback traffic noise and airplane noise, supposable at intensities high enough to mask their calls (Kaiser & Hammers 2009; Engbrecht et al. 2015; Kruger & Du Preez 2016) or when they were calling next to roads (Hoskin & Goosem 2010) compared to distant sites (up to 100m away). An increase in call rate and/or call intensity is energetically very costly and may reduce the future fitness of signalers (Parris 2002). On the other hand, repeating call notes increases the probability of their detection in noisy environments, compensating for calls masked by noise (Narins 1982; Wiley 1982), so it could be an alternative to increase the temporal window of the sound and reduce masking. A few studies that evaluated background noise, related the anuran response to the total noise of the environment (noise of a chorus assemblage combined with anthropogenic noise) (Sun & Narins 2005). For instance, during an airplane and motorcycle playback stimuli, two species decreased calling activity, causing a dramatic reduction on the intensity of the background noise at the pond, while another species, using the time where the dB at the pond was lower (due to ceasing activity of a very loud one), to increase its calling rate. For last, many species did not adjust call rate in response to noise, as is the case of *Hyla arborea* when exposed either to short (hours) or long (10 days) periods of noise, however most species adjusted at least one other temporal or spectral parameter of the call instead. Remarkably, of the 57 species studied, there was only one species that did not change any of the parameters studied, which was *Anaxyrus americanos* (Cunnington & Fahrig 2010; Vargas-Salinas & Amézquita 2014).

Call length: Only a few studies have tested the effect of anthropogenic noise on parameters other than call rate. Species exposed to noise either maintained the call length constant (exposed to 52.27-90dB), decreased (exposed to 72-88dB) or increased it (exposed to 65-90dB). Observed modifications 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, they would increase call duration (Caorsi et al. 2017; Grace & Noss 2018). As call duration was evaluated only for a few species, it is difficult to make generalizations, however the species that decreased (Lengagne 2008; Caorsi et al. 2017) or maintained call duration constant (Lengagne 2008; Hoskin & Goosem 2010; Kaiser et al. 2011; Troïanowski et al. 2015; Caorsi et al. 2017; Nelson et al. 2017) mostly decreased or not changed the call rate..

Males of some species, when facing an intense noise, may choose to not increase their call effort (more calls or/and longer calls), as energy expenditure might not compensate. Lengagne (2008) found that noise impact differed with noise intensity. The higher the intensity of the background noise broadcasted, the higher decrease of calling effort (29% decrease with a 72 dB stimuli, 50% decrease with a 88 dB stimuli). This modification was mainly due to a decrease in bout duration. The same was observed in *Boana lepetolineata* when the species was first presented to the high noise level, it decreased call effort, also by decreasing call length and not altering call rate (Caorsi et al. 2017).

1.1.1.b. Spectral parameters

Animal signals can be characterized by their frequency content and usually are confined to a particular spectral region or channel (Forrest 1994). Dominant frequency is known as a relevant acoustic parameter, from which information is perceived, for instance the size of the animal, which is frequently associated with reproductive performance and survival. (Ey & Fischer 2009). Environmental noise, frequency-dependent absorption by the medium, and filtering caused by the environment all influence the spectral characteristics of the transmitted signal and consequently communication (Forrest 1994). Following previous studies one would expect that species with higher overlap between signal and noise would be more affected (Cunnington & Fahrig 2010).

Dominant frequency: The effect of noise on anuran call spectral parameters seems to be variable. A recent meta-analysis comparing frequency shift responses of nine anurans species exposed to anthropogenic noises, found a wide range of responses among studies and experiments (Rocca et al 2016). Among species reported in literature, two decreased dominant frequency when facing noise (exposed to 65-80dB), eight increased (43.3-98.2dB) and five did not change it (65-88dB). The two species that decreased frequency, *Boana bischoffi* and *Pelophylax ridibundus*, did not increase other parameter to increase signal-to-noise ratio, actually, the first one decreased call rate and the second decreased amplitude (Lukanov et al. 2014; Caorsi et al. 2017). These species calls at around 1-2kHz, so authors suggested that it might be not feasible to increase frequency beyond the greatest energy frequencies of the noise. Besides the energy expenditure to increase frequency, alternatively, it could be more efficient to reduce it, ensuring longer distance dispersion of the signal, once low frequency waves travel farther (Marten & Marler 1977; Forrest 1994). Species increasing frequency have mostly changed other call parameter as well, as increasing call rate (Kruger & Du Preez 2016), although mostly decreased call rate (Cunnington & Fahrig 2010).

An increase in the dominant frequency of a vocalization comes at an increase of energetic cost (Wells 2001); however, this cost is considerably less than that of increasing the mean amplitude or calling rate of the vocalization (Parris et al. 2009). Animals exposed to playback experiments were reported increasing frequency (Parris et al. 2009; Cunnington & Fahrig 2010; Caorsi et al. 2017). Meanwhile, animals compared between sites (low to high noise), usually showed greater changes (Cunnington & Fahrig 2010; Lukanov et al. 2014; Kruger & Du Preez 2016). On the one hand, once females often prefer low frequency calls to higher frequencies, as frequency is generally correlated with body size, this preference favors larger males (Gerhardt 1994). If elevated levels of traffic noise cause individuals to produce calls with a higher dominant frequency, it is possible that the largest males in the population could be at a disadvantage in attracting females (Cunnington & Fahrig 2010). Some of this studies, which recorded higher frequency calls at noisy places, interestingly, also recorded a significant smaller size of males at this sites (Parris et al. 2009; Hoskin & Goosem 2010). It is known that, in anurans, call frequency is a morphological size dependent variable, with small individuals/species calling at a higher pitch and the same works for the opposite (Giacoma & Castellano 2001). It could be that these frequency increase found in noisy sites is related to smaller males, rather than an actual increase of frequency. Although some anurans may have indeed evolved high frequency calls as an adaptation to abiotic noise, for instance, streams (Feng et al. 2006; Arch et al. 2008), many other species may have been constrained by the effects of morphology and selection acting in contexts such as female choice, species recognition, competition, and reduction of predation risk (Ryan 1988; Gerhardt & Huber 2002). On a study controlling for the effects of body size and phylogenetic signal, Vargas-Salinas & Amézquita (2014) found that although call frequency was significantly higher in anuran species breeding alongside streams, this effect entirely disappeared once the effect of body size on call frequency was statistically removed. This suggests that habitat filtering based on body size rather than convergent acoustic adaptations to noisy habitats explain high call frequency of stream breeders. The authors highlight that selection on body size or on call frequency are not mutually exclusive processes and hence, selection for small body size and selection for high frequency may operate in parallel.

In anthropogenic noise context, it would be possible that high dominant frequencies are easily located by females, due to its lower masking, and then smaller males be easily located and chosen instead of the ones females would normally choose, i.e., the larger males with lower call frequencies. Whether smaller males are calling or animals are actually increasing frequency, mate choice could be affected and shift in mating towards smaller males could result in an increase in genetic variation among populations. These hypotheses have yet to be tested (Cunnington & Fahrig 2010). Finally, species that did not showed shifts in call frequencies, mostly changed some other parameter, either call rate or call length (Lengagne 2008; Hoskin & Goosem 2010; Cunnington & Fahrig 2010; Caorsi et al. 2017).

1.1.1.c. Call amplitude

Another strategy to cope with noise involves increase amplitude in response to noise level. This phenomenon is known as the Lombard effect (Lombard 1911) and has been described in several vertebrates (Brumm & Zollinger 2011), but only recently in anurans (Caldart et al. 2016; W. Halfwerk et al. 2016; Shen & Xu 2016). The Lombard effect allows real-time signal-to-noise ratio adjustment, thereby providing optimal signal detection, localization, and discrimination (Lohr et al. 2003).

Amplitude shifts: Only a few studies have controlled for amplitude variation of the call of males exposed to noise. Of the six species studied, shifts in amplitude were observed only in three, and surprisingly, the animals decreased amplitude, whilst increasing frequency (Cunnington & Fahrig

2010). Authors suggested a high pitch adjustment together with reduction in calling rate and power could result in a vocalization with no net increase in energetic expenditure (Cunnington & Fahrig 2010). Although, there are no studies with anthropogenic noise that observed the Lombard effect yet, studies that tested other abiotic noises as water stream and white noise (Caldart et al. 2016; W. Halfwerk et al. 2016; Shen & Xu 2016), found frogs to increase the amplitude of their calls in relation to background-noise. Although frogs might be able to sing louder, serving as an immediate strategy, this activity has high energetic costs and, therefore, it has been discarded as a long-term adaptation (Wells 2001; Parris et al. 2009; Love & Bee 2010). Hence, it might not serve to cope with noise coming from human activities, once they are mainly constant, very loud and involving a wide range of frequencies (Simmons & Narins 2018). Furthermore, until more studies evaluate this trait, we can only speculate about its effects.

1.1.1.d. Female responses and mate choice

Mating behavior in anurans is by far and large a female choice system (Simmons & Narins 2018). Female anurans exhibit phonotaxis towards male choruses (Gerhardt & Huber 2002; Bee & Swanson 2007) and reproduction usually depends on a female's ability to respond correctly to the advertisement signals of a conspecific male (Gerhardt & Bee 2007). Therefore, sound localization has obvious fitness consequences for anurans (Gerhardt & Huber 2002). This means, an increase in the detection threshold of a signal due to noise may impair or impede an individual's ability to detect and respond to biologically critical information (Barber et al. 2010). Studies focusing on biotic noise found what was expected: female anurans became less discriminating under conditions of moderate-to-high levels of background chorus noise (Gerhardt & Schwartz 2001). These means, anthropogenic noise could also difficult the detection of signals emitted from males to females, which could affect mate attraction and female's choice (Bee & Swanson 2007). Besides the possible masking effect of the signal, we have previously presented here many studies showing males altering mating calls in response to anthropogenic noise. However, these alterations do not ensure positive mate choices or reproduction, once females must receive the signal and encode the message in order to locate a mate and make a choice (Wiley 2013). Here we present the few studies that have tested whether traffic noise (up to 87dB) masks the signal reception and whether alteration in call parameter compensate for the effect of noise in mate attraction.

The first study of this kind was performed by Bee & Swanson (2007) testing the hypothesis that traffic noise could mask female`s perception of males signal in *Hyla chrysoscelis* female frogs. Using a laboratory playback experiment, they found a decrease in orientation and an increase in latency response towards the target signal (artificial calling male) directly related with an increase in the intensity of traffic noise (from 37 e 85dB). Following works, with *Lithobates sylvaticus* and *Rana pirica,* also found a decrease in orientation for both species when traffic noise was presented (Tennessen et al. 2014; Senzaki et al. 2018), whilst latency responses differed between species, with an increase in *R. pirica* and no changes for *L. sylvatius* or *H. arborea* (Tennessen et al. 2014; Troïanowski et al. 2014; Senzaki et al. 2018). Therefore, traffic noise not only leads to adjustment in males call, which might increase energy expenditure and expose them to predators, but potentially results in a lower call detection efficiency by females. Cunnington & Fahrig (2013) tested whether alteration in call parameters compensate for the effect of traffic noise in mate attraction, exposing species previously studied (see Cunnington & Fahrig 2010). For the three species that males adjusted call, the adjustments attracted more females at sites with noise than non-adjusted calls. As well, this adjusted calls in noisy environment attracted as many females as the original call at a quiet site. Therefore, for these species, traffic noise did not affect the number of females attracted. Meanwhile, the species that did not adjusted the call, *Anaxyrus americanus*, was equally able to attract females in the absence or presence of traffic noise, indicating that traffic noise did not negatively affect mate attraction. Although this last study showed no negative effect in number of mate attraction, the other studies showed an increase in latency and increase in disorientation. As well, the same number of females approaching males does not ensure the quality of mates. In species that exhibit plasticity as we previously saw for many anuran calls, males may face a trade-off between transmission efficiency and attractiveness (Patricelli & Blickley 2006).

Accordingly to our previous discussion in 1.1.1.b section, if males emitted higher-pitched calls to avoid the masking effect of background noise, they may increase the number of females who detect their vocalization, but decrease their attractiveness to those females, which in a long time could select smaller males in a population. As well, females preferring males that call at higher rate, shows that increasing call rate might compensate for the masking signal, however the energetic cost of this adjustment might not allow the individual to spend the same temporal effort on the reproductive event as they would usually. For instance, explosive breeders that depend on rain might reproduce very often, but their participation on the breeding event depends on the energy left (Wells 2008). As well, prolonged reproductive species, where males fertilize more than one female over one breeding season, might leave reproductive site earlier. The effect of anthropogenic noise on females' choice remains unclear and further studies should be performed in order to answer the true effect of it on mate choice and on reproduction in general.

1.1.1.e. Locomotion

Any type of movement carries potential costs for amphibians, including spent energy or risk of predation, hence, amphibians move only when necessary (Wells 2008). Jumping performance has been suggested to increases survival (Heinen & Hammond 1997), and therefore it is an essential behavior. A study testing the locomotion of the Marsh frog, *Pelophylax ridibundus,* showed a negative effect of traffic noise, with frogs moving less when exposed to either lower (50dB) or higher (70dB) noise. Nash et al. (1970) found that Leopard frogs (*Lithobates pipiens*) exposed to loud noise (120 dB of signal horn) remained immobile almost eight times longer than did the control group. It could be that sound increased the level of fear, which consequently affected the animal mobility. A study by Brattstrom & Bondello (1983) showed that, during aestivation, Spadefoot toads (*Scaphiopus couchii*) leave their burrows in response to motorcycle noise (up to 95 dB). It is not clear which is the threshold of noise that affects species and which is the exact mechanism that drives them to change their behavior. However, species locomotion is of extreme importance, not only for predation risk but also for displacement and foraging as for migration and, therefore, deserves attention in future studies.

1.1.2. Physiological and Morphological responses

Although most studies have focused on the effect of noise pollution on acoustic transmission and communication, the sublethal effects of noise, including physiological stress and impaired reproduction, remain poorly understood (Kight & Swaddle 2011). It is known that other sources of pollution and habitat fragmentation result in the release of corticosterone, which is the primary glucocorticoid hormone in amphibians and forms part of the highly conserved vertebrate physiological stress response (Ramenofsky & Richardson 1998; Romero 2004; Crespi & Warne 2013). Short-duration elevations of plasma corticosterone concentrations help an organism to respond adaptively to stressors by facilitating the mobilization of energy stores, suppressing unnecessary activities and priming the response to future stressors (Sapolsky et al. 2000; Romero 2004). Chronically elevated corticosterone concentrations, however, can have deleterious effects on survival, reproduction, growth and immune function, due largely to a reallocation of energy away from non-critical functions (Sapolsky et al. 2000). In addition to stress response and immune function, corticosterone is also involved in the regulation of colored signal expression (Eraud et al. 2007). A high level of corticosterone can impact carotenoid-based coloration, as stressed animals have been shown to become paler (Eraud et al. 2007). Carotenoids are pigments responsible for orange-red ornamentations but they have multiple other functions such as immune stimulation or antioxidant effects (Blount et al. 2003). Vertebrates cannot synthetize carotenoids and must acquire them through their food. Hence, in a carotenoid-limited environment, trade-offs between colored ornamentation and immunity can exist. During an immune challenge in animals, carotenoids may preferentially be used for immune function improvement rather than for coloration (Blount et al. 2003; Faivre et al. 2003). Once coloration is known to play an important role in mate selection in many anurans species, changes in this secondary sexual character could have an effect on sexual selection (Hödl & Amézquita 2001). Because traffic noise is widespread in space and time, animal populations might experience a chronic stress level and also face immunosuppression. Bellow we will approach the studies, which have reported physiological and morphological responses due to anthropogenic noise.

1.1.2.a. Stress response

Only a few studies have evaluated the stress response of anthropogenic noise in frogs. Two of them, one with White's treefrog, *Ranoidea caerulea* (Kaiser et al. 2015) and one with the European treefrogs, *Hyla arborea* (Troïanowski et al. 2017), found a significant increase in corticosterone levels when males were exposed to traffic noise. A test on female Wood frogs, *Lithobates sylvaticus,* revealed that corticosterone of individuals exposed to traffic noise were five times greater than control (Tennessen et al. 2014). It thus seems probable that noise affects the well-being of animals independently of its effect on acoustic communication (Wright et al. 2007). Similar elevations in corticosterone concentrations have been documented in anuran responses to habitat loss (Janin et al. 2012) and pollution (Relyea & Mills 2001), which could be also related here as a physiological response to loss of acoustic habitat due to traffic noise (Tennessen et al. 2014). It is important to highlight that the physiological costs of noise-induced stress depends on the dose scale, so for instance, in a short term, glucocorticoids usually help animals respond adaptively to stressors (Sapolsky et al. 2000). However, chronically elevated glucocorticoid levels can have deleterious consequences for animals, including suppressed immune function, and reduce survival and reproduction output (e.g. Romero & Wikelski 2001; Ouyang et al. 2011).

1.1.2.b. Immune system

Increased corticosterone levels triggered by chronic stress has many known trade-off for animals, for instance on immunity (Butler et al. 2009). A recent study found that traffic noise impacts immune response of the European treefrog, *Hyla arborea*, through an immunosuppressive effect of corticosterone (Troïanowski et al. 2017). Furthermore, as immunosuppression may be involved in amphibian decline (Carey et al. 1999), road traffic noise, via its immunosuppressive effect, might be an important threat for amphibian conservation (Troïanowski et al. 2017).

1.1.2.c. Sperm

One study evaluated the sperm of males of *Ranoidea caerulea* exposed to anthropogenic noise (Kaiser et al. 2015). The authors found that frogs exposed to traffic noise stressor showed significant depressions in both sperm count and sperm viability. In other vertebrates, chronic was linked to decreases in gamete count and quality (Campbell et al. 1992; Schreck et al. 2001).

1.1.2.d. Coloration

Visual signaling may represent an alternative or complementary form of communication in anurans (Hödl & Amézquita 2001). Visual signaling in amphibian anurans is diverse and widespread among taxa and has evolved independently in several anuran families, under several ecological conditions, for instance, in noisy environments (waterfalls and fast-flowing streams) and in diurnal aposematic coloration species (Hödl & Amézquita 2001). Among many different visual cues and signals described for anurans (Hödl & Amézquita 2001), color changes on the body can affect the course of interindividual encounters, leading to either agonistic or courtship interactions (Wells 1980). Many anuran species communicate using multimodal signals, as acoustic and visual for instance (Lewis & Narins 1985; Lewis et al. 2001; Grafe & Wanger 2007; Preininger et al. 2013; Caldart et al. 2014; Starnberger et al. 2014). In multimodal signaling, some animals can switch behavior from one sensory channel to another (“multimodal shift”, Partan et al. 2010) depending on the context (Brumm & Slabbekoorn 2005; Partan 2013), which might improve communication efficiency by improving detectability and facilitating discrimination (Preininger et al. 2013; Uetz et al. 2013). When noise compromises the acoustic channel, individuals (either sender or receiver) could shift among channels in order to improve signaling. Hence, a possible solution to cope with anthropogenic noise could be multimodal shift from one sensory modality to another. A study demonstrated, for the first time, that chronic exposure to traffic noise alters visual signals (Troïanowski et al. 2015). After 10 days of exposure, the vocal sac of the European tree frog males (*Hyla arborea*) became paler (high brightness value). In contrast, chroma and hue did not change. Another study with the same species showed that not only traffic noise exposure, but also independent application of corticosterone negatively impact the European treefrog vocal sac coloration. In this experiment, not only the vocal sac became paler, as it became less chromatic (Troïanowski et al. 2017). In treefrogs, losing color is bound to impact sexual selection processes as it has been clearly shown that females' choice towards males exhibiting dark red vocal sacs is achieved by comparing the coloration of all males present (Gomez et al. 2010). Previous studies conducted with the European treefrog have demonstrated that male quality assessment is based on several acoustic parameters, with a female preference for males emitting fast, powerful and low frequency calls (Richardson & Lengagne 2010). On visual parameters the preference is for males with colorful (low brightness, high chroma) orange vocal sacs (Gomez et al. 2010). Therefore, while acoustic signals are embedded in noise, it could be an option for males and females to shift to visual channel to increase the efficiency of their communication. Troïanowski et al. (2014) has investigated whether females of European treefrog shift between multimodal signals during male quality assessment under anthropogenic noise. Surprisingly, when exposed to traffic noise, females chose an attractive acoustic signal associated to poor quality visual signals. For that species, females' reliance on acoustic signal embedded in noise pollution did not decrease in favor of visual signals, therefore showing that females do not necessarily shift between modalities in response to traffic noise (Troïanowski et al. 2014). Although they did not find evidence of multimodal shift, previous work in noisy environment like torrential streams have shown that animals solved the problem of continuous broadband low-frequency noise by both modifying its advertisement call and by using numerous visual signals (Grafe et al. 2012). Preininger et al. (2013) suggested that abiotic noise of the stream did not constrain signal detection in the Small torrent frog (*Micrixalus saxicola*). Actually, the species faced masking caused by conspecific chorus noise, which sound pressure level and frequencies were higher than the noisy ambient. Furthermore, multimodal stimuli from conspecific signals (acoustic and visual) elicited greater response from males and triggered significantly more visual signal responses than unimodal stimuli. Although these results refer to an environment noise context, it is expected that an anthropogenic context would represent a similar challenge to communication. Thus, not only the frequency of the noise masking will be important, but also the intensity of it, i.e. relative importance in the soundscape, to elicit species to shift between multimodal signals.

2. Population and Community level

Urban areas are expanding worldwide and together the average and peak intensity of noise is increasing (Fuller et al. 2007; Barber et al. 2010). Our knowledge of the influence of traffic noise is mostly limited to individual species. However, the cumulative impacts of noise on individuals can manifest at the population level in various ways that can potentially range from population declines up to change entire communities (Blickley & Patricelli 2010). The ambient sound pressure level, with contributions from both biotic and abiotic factors, was shown to be a strong predictor of microhabitat selection for many species (Goutte et al. 2013). This relationship implies that changes in ambient-noise levels, such as increased anthropogenic noise, have the capacity to radically alter calling site selection and communication behavior in chorusing species (Simmons & Narins 2018). One can expect that anthropogenic noise could reduce the richness and/or the abundance of species, changing community composition and structure.

Previous studies have focused on a single species or a selection of species. A few accessed the response at a higher level, as populations or community by approaching two different strategies: (i) compare areas with low or high noise and (ii) playback experiments. Herrera-Montes & Aide (2011) compared paired forest sites near (100 m, dB>60) and far (>300 m, dB<60) from highways, with similar vegetation structure, but different levels of noise. They found that anuran species richness was similar between near and far sites. Authors attributed this results possible to the fact that at the study site, anurans mainly call at night, when traffic activity was low. Another study accessed the abundance of three species in a distance from zero to 100m from the road (Hoskin & Goosem 2010). They found that frog abundance of two species, *Ranoidea rheocola* and *Austrochaperina pluvialis*, increased significantly perpendicularly from the road along two transects. Meanwhile, the abundance of another species of *Ranoidea* was not affected (Hoskin & Goosem 2010). Authors considered the possibility of the decrease of abundance to be related to road kill, once mortality is suspected to cause frog density depression near roads, however both species affected are either completely absent or very rare in road mortality statistics (Fahrig et al. 1995; Fahrig & Rytwinski 2009). Another study comparing different distances of road (15 to 300m) showed that the abundance of *Andinobates bombetes* was correlated with the number of bromeliads, but only marginally affected by traffic noise level (Vargas-Salinas & Amézquita 2013). Authors suggested that the frog population studied deals with the potential masking effect of traffic noise by using the gap calling behavior, and do not modify their spatial distribution. An innovative for this level of effect, was the phantom road, using playback stimuli to test whether traffic noise affected species richness and abundance, by broadcasting noise in a quiet site, whilst having a control site with no noise. They found that traffic noise significantly reduced the total number of anurans observed compared to the control treatment, however species richness remained the same (Grace & Noss 2018), i.e. community structure was modified.

Overall, it is still difficult to access the effect of traffic noise upon entire communities, once species seem to respond in different ways, some tolerating noise more than other, or showing different strategies to cope with it. However, maintenance of species richness does not ensure reproductive success. Kaiser et al. (2011) tested the response of a chorus of *Dendropsophus microcephalus* to anthropogenic noise. The results showed that male frogs exposed to anthropogenic noise (1h per night for approximately 40 days) decreased both the number of days present at the chorus and the nightly chorus duration relative to controls. Frogs at the experimental pond called on average one hour earlier and ended calling activity up to 2 hours before than control ponds. Besides the masking problem of the signal by noise, which was already shown to reduce phonotaxis and latency for many species (Bee & Swanson 2007), these results also indicate that the reproductive period of species might be shortened by noise, which could also reduce chances of finding a mate.

SUSBTRATE BORNE VIBRATION

Previously, we reviewed the impact of anthropogenic noise in the context of foraging and communication in airborne sound (Brumm 2013), as the majority of the studies did. Nevertheless, beyond the surface, a series of studies focusing specially on civil construction have reported ground borne vibration induced by human activities, which generate seismic vibrations (Meunier 2013; Aliyu et al. 2016). In fact, these could be an important source of communication disturbance scientists haven't taken into account until now. Among the terrestrial vertebrates, amphibians are the most sensitive to vibrations (Hill 2008). This capacity for detection is linked especially to their inner ear, which comprises three organs (papilla basilaris, *papilla amphibiorum* and the sacculus), the last two presumably capable of detecting low frequency airborne sounds and substrate-borne vibrations (Capranica 1976; Narins 1990; Lewis et al. 2001; Márquez et al. 2016). Despite the sensitivity of the group, the use of vibrational cues and signals has been reported in only few species and in limited contexts (Hill 2009; Cocroft et al. 2016), e.g. intra specific signaling (Lewis & Narins 1985; Caldwell et al. 2010); prey detection (Solano & Warkentin 2016), predators avoidance (Warkentin 2005; Warkentin et al. 2017), and cues for the environment (Halfwerk et al. 2016; Márquez et al. 2016). Given previous knowledge of the effect of anthropogenic airborne noise on anurans and considering the sensibility of the group to ground-borne vibrations, it is important to understand whether this specific noise source could also have an effect on them and then, if present, to think about how to reduce the impact. The study results present in this review guide us to expect that anthropogenically derived substrate-borne vibrations will also affect anuran calling activity, altering at least some of its parameters, or having other behavioral and physiological consequences.

2.1. Individual level

2.1.1. Behavioral responses

2.1.1.a. Male acoustic activities

Currently, only one study has focused on substrate-borne vibration derived from anthropogenic activity effects upon anurans. Using playback tests, Caorsi et al (unpublished data) assessed how vibrations produced by two sources of anthropogenic activity (road traffic and wind turbine) affect the calling activity of the Midwife toad, *Alytes obstetricans*. Results showed a negative effect of both anthropogenic vibration kinds on the call rate of the species, while call duration and frequency remained stable. Furthermore, call rate was more affected by original traffic and wind turbine (broad band of frequencies) recording than by the pure tone synthetic stimuli (10 and 100Hz) emitted to the toads. As previously discussed, anurans use calls to defend territories and attract reproductive partners, therefore, reducing calling rate, can reduce attractiveness of males, reduce phonotaxis and latency and mate choice and, therefore reduce individual reproductive success (Whitney & Krebs 1975; Arak 1983; Klump & Gerhardt 1987; Ryan & Wilczynski 1991). These are only the first insights of how anthropogenically derived substrate-borne vibrations can affect anurans and certainly these channel of noise deserves more attention.

ANTHROPOGENIC NOISE EFFECTS, A FEW CONCLUSIONS

Besides rare exceptions (Narins 1990; Feng et al. 2006; Caldwell et al. 2010; Shen & Xu 2016), the acoustic system of most anurans is known for producing and perceiving sounds within the frequency range of 50-6000Hz (Fay & Simmons 1999). Anthropogenic noise is a very variable source, with peak energy in the lowest frequencies around 50-7000Hz, but concentrated at frequencies bellow 2000Hz, therefore expected to highly overlap with many species (Cunnington & Fahrig 2010; Simmons & Narins 2018). In this study, we observed that species vary widely in their ability to tolerate introduced anthropogenic noise and exhibited different responses to altered acoustic environments (Fig. 3). This variability makes generalizations about noise impacts among species and among noise sources difficult (Blickley & Patricelli 2010). Studies performed until now showed that anuran species call in a wide range of frequencies, and therefore it is very difficult to separate ones that indeed do not have masking effect of their signal and which will be the threshold of amplitude where the signal wont affect them. Besides that, depending on the intensity of the noise, sound perception might still be impaired for species that lie outside the major spectral energy band. Low-frequency sounds are effective in masking higher frequency sounds, and so masking grows nonlinearly on the high-frequency side (Simmons & Narins 2018). Another important topic to be addressed is the reminder that, even though, noise studies mainly focus on the signal emitted and the background noise, animals do not use their acoustic system only for sending signal, but also to perceive them and the environment. The sensory system detects a wide range of stimuli and is typically optimized to process relevant cues against a background of irrelevant stimuli, often referred to as noise (Brumm & Slabbekoorn 2005; Klein et al. 2013). It has been shown that anuran males appear to recognize when their signal is more likely to be transmitted and detected, avoiding periods of maximal interference, even when their frequency do not highly overlap with noise (Sun & Narins 2005; Vargas-Salinas & Amézquita 2013; Vargas-Salinas et al. 2014). This means, frequency is not the only factor to take into account when evaluating the effects of anthropogenic noise on anurans. The extent of the effect depends on the intensities of the masking sound, the frequency separation between the masker and the stimulus, and the sharpness of the frog's internal auditory filters (Simmons and Narins 2018). On the top of that, calling is probably the most energetically expensive activity a male frog undertakes during his lifetime (Wells 2001), therefore individuals should use vocalizations that conserve energy while maximizing the effective transmission distance (Wells & Schwartz 2007). The findings from this study suggest that most of anuran species males employ a plastic vocalization response when facing anthropogenic noise, depending on many factors, which are still to be understood. These plasticity allows individuals to have a broader tolerance to environmental conditions and therefore higher fitness in multiple or highly variable environments (Wells & Schwartz 2007). Besides the effect of noise on acoustical communication, this review also showed that noise can influence physiological and morphological aspects of anurans, as increase stress levels, reduce immune system, alter coloration pattern and locomotion. Furthermore, these effects not only apply to males, but also to females and mate choice, which can therefore impact reproduction and individual fitness. Findings from this review agree with Kight & Swaddle (2011) that anthropogenic noise is likely to influence multiple biological systems both directly and indirectly.

LEGAL INSTRUMENTS AND MITIGATION MEASURES

The World Health Organization has recognized noise as an important environmental problem for humans. Reviews have shown the impact of anthropogenic noise on human health and a series of legal instruments and guidelines have been proposed in order to deal with this issue (WHO 2007; WHO 2009). However, documents and legal instruments that regulate noise levels are focused on the human well-being. The protection that exists for non-human animals generally relies on regulations that protect habitat and prevent disturbance, rather than regulations that protect against noise *per se* (McGregor et al. 2013). Recently, some studies have suggested and discussed possible actions to reduce noise level and its impact on the environment with a focus on non-human terrestrial animals (Barber et al. 2010; Blickley & Patricelli 2010; McGregor et al. 2013; Nelson et al. 2017). However, the effect of noise seems to vary between species and our knowledge on the subject is still not enough to come up with a standard list of actions to mitigate noise impact (McGregor et al. 2013). The options to deal with this source of disturbance are either to eliminate or to reduce noise levels on target areas. Here, we bring some measurements, based on literature, in order to contribute with future multidisciplinary studies on conservation plans for anuran species.

One possible alternative would be to focus on machine engineering to reduce noise of car engines, wind farm engines, tires, etc. Advancements in environmentally friendly vehicle technology, for instance, have resulted in quieter cars (Komada & Yoshioka, 2014). Besides that, it is possible to choose the pavement to build the roads. It has been proven that low-noise pavements are considered for a cost-effective option to reduce traffic noise. This alternative not only reduces airborne noise, but also ground borne vibration induced by vehicles (Czech 2016). These study shows the difference of induced vibrations of a series of pavements, as concrete, cobble stones and gravel and compares between vehicles types, so it is possible to chose the best pave according to the vehicles mostly using the road. Another possibility to reduce noise on a road is the limit of speed. For instance, an increase in car speed from 30 to 70 km/h induces a 7dB increase of noise level (Favre & Lamure 1987). Besides that, there are also physical ways to reduce noise from roads, cities and so on by implementing a barrier. The installation of sound barriers alongside particularly busy stretches of highway can buffer a considerable amount of noise (McGregor et al. 2013). They can be constructed of artificial building materials, including absorbent sound ones (Sanchez-Perez et al. 2002), or vegetation to create a semipermeable barrier (Van Renterghem et al. 2012), which also provides additional habitat for many animals (Slabbekoorn & Ripmeester 2008). These barriers are also an alternative to reduce ground borne vibration from anthropogenic activities like roads or wind turbines. In this case the barrier would be in-ground (Aliyu et al. 2016). These technique has been proposed to protect buildings from anthropogenic vibrations and the author proposed that a trench or ditch constructed between the site and adjacent buildings can minimize the transmission of vibrations through the ground provided that the ditch is enough deep. All this alternatives are still to be tested for anurans, including other possible side effects of these strategies, which can help reduce risks for species or create new problems.. However, work still needs to be done to implement protection laws for non-human animals. Acknowledging the problem is the first step, but we still need further actions that transform the findings of researches into accessible documents within each country to be implemented in legislation and policies. Most countries only have regulation for humans, and some even lack this kind of documents. For non-human animals, this problem mostly falls into general regulations that protect habitat and prevent disturbance, but not focusing on the noise problem on terrestrial wildlife (McGregor et al. 2013).

CONCLUSIONS

The potential for anthropogenic noise to adversely impact conservation is amply demonstrated (McGregor et al 2013), including in anurans as shown in this review. A decrease in human expansion is unlikely to occur in the near future, making it increasingly important to understand the implications of anthropogenic stressors, such as noise, on wildlife (Kight & Swaddle 2011). Most of the knowledge that exists for the effect of anthropogenic noise on anurans relies on the short-term response behavior of individual males to it (Fig.3). On an individual level, we still lack information on female's response and individual fitness. Besides that, long-term studies are necessary, which answer if this animals are able to cope with this noise and also how it would affect populations, species distribution and communities. It is also extremely important to start testing the efficacy of the mitigation measures. For last, we should work together with governmental institutions in order to create guidelines and legal instruments to be implemented during urban expansion projects. 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.

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Table legends

Table 1. Review of the effects of anthropogenic noise on anuran males.

Table 2. Review of the effects of anthropogenic noise on anuran females.

Figure legends

Figure 1. Cumulative papers published studying the effect of anthropogenic noise on anurans.

Figure 2. Map of anthropogenic biomes of Ellis (2008) and the distribution of the studies focusing on anthropogenic noise and anurans.

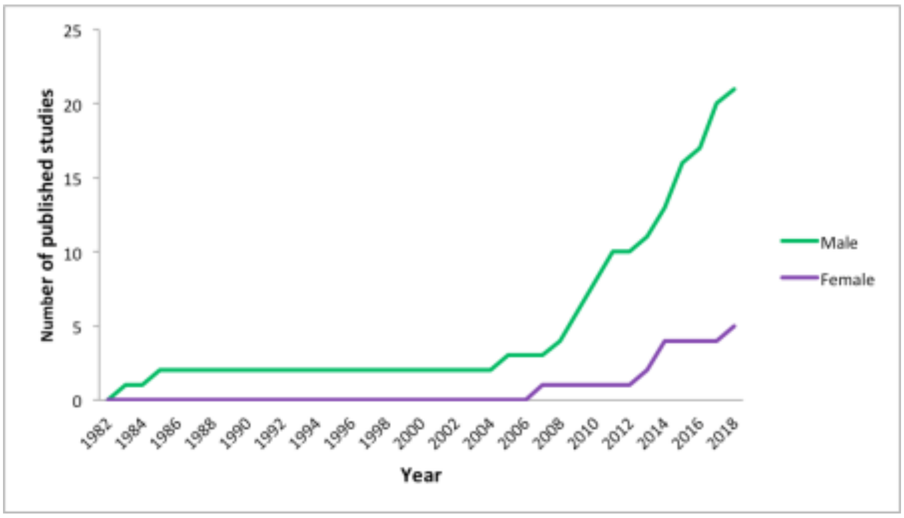
Figure 3. Summary of the effect of antropogenic noise on anurans found on literature.

Family	Species	Source of noise	dB	Analyzed	Call rate	Call length	Amplitude	Dominant Frequency	References
Alytidae	*Alytes obstetricans*	traffic vibration	_	Call	decreased	no changes	_	no changes	Caorsi et al. unpub. data
Alytidae	*Alytes obstetricans*	wind turbine vibration	_	Call	decreased	no changes	_	no changes	Caorsi et al. unpub. data
Bufonidae	*Anaxyrus americanus*	traffic	76	Call	no changes	_	no changes	no changes	Cunnington & Fahrig 2010
Bufonidae	*Anaxyrus americanus*	traffic	73.2	Call	no changes	_	no changes	no changes	Cunnington & Fahrig 2010
Bufonidae	*Anaxyrus americanus*	traffic	73	Call	no changes	_	_	_	Vargas-Salinas et al. 2014
Bufonidae	*Anaxyrus quercicus*	traffic	65-70	Population/species/Call	no changes	increased			Grace & Noss, 2018
Bufonidae	*Anaxyrus woodhousii*	traffic	_	Population/species/Call	_	_		increased	Barras 1985
Bufonidae	*Incilius valliceps*	traffic	90	Call	no changes	increased	_	_	Kaiser et al. 2011
Dendrobatidae	*Andinobates bombetes*	traffic	77.1	Population/species/Call	decreased	_	_	_	Vargas-Salinas & Amézquita 2013
Eleutherodactylidae	*Eleutherodactylus antillensis*	traffic	>60	Population/species	_	_	_	_	Herrera-Montes & Aide 2011
Eleutherodactylidae	*Eleutherodactylus brittoni*	traffic	>63	Population/species	_	_	_	_	Herrera-Montes & Aide 2011
Eleutherodactylidae	*Eleutherodactylus cochranae*	traffic	>62	Population/species	_	_	_	_	Herrera-Montes & Aide 2011
Eleutherodactylidae	*Eleutherodactylus coqui*	traffic	>61	Population/species	_	_	_	_	Herrera-Montes & Aide 2011
Eleutherodactylidae	*Eleutherodactylus planirostris*	traffic	65-70	Population/species					Grace & Noss 2018
Hylidae	*Acris gryllus*	traffic	65-70	Population/species					Grace & Noss 2018
Hylidae	*Boana bischoffi*	traffic	65	Call	decreased	no changes	_	decreased	Caorsi et al. 2017
Hylidae	*Boana bischoffi*	traffic	75	Call	decreased	no changes	_	decreased	Caorsi et al. 2017
Hylidae	*Boana leptolineata*	traffic	65	Call	no changes	no changes	_	no changes	Caorsi et al. 2017
Hylidae	*Boana leptolineata*	traffic	75	Call	no changes	no changes	_	no changes	Caorsi et al. 2017
Hylidae	*Dendropsophus ebraccatus*	traffic	90	Call	no changes	_	_	_	Kaiser et al. 2011
Hylidae	*Dendropsophus microcephalus*	traffic	70 and 90	Call	increased	_	_	_	Kaiser et al. 2011
Hylidae	*Dendropsophus triangulum*	traffic	75	Call	increased	_	_	_	Kaiser & Hammers 2009
Hylidae	*Dendropsophus triangulum*	traffic	75	Call	increased	_	_	_	Kaiser & Hammers 2009
Hylidae	*Hyla arborea*	traffic	72	Call	no changes	decreased	_	no changes	Lengagne 2008
Hylidae	*Hyla arborea*	traffic	88	Call	decreased	decreased	_	no changes	Lengagne 2008
Hylidae	*Hyla arborea*	traffic	76	Physiology	-	-	-	_	Troïanowski et al. 2017

Family	Species	Source of noise	dB	Analyzed	Call rate	Call length	Amplitude	Dominant Frequency	References
Hylidae	*Hyla arborea*	traffic	76	Physiology/Call	no changes	no changes	-	no changes	Troïanowski et al. 2015
Hylidae	*Hyla arborea*	traffic	76	Physiology/Call	no changes	no changes	-	no changes	Troïanowski et al. 2015
Hylidae	*Hyla cinerea*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Hyla cinerea*	_	_	Population/species/Call	_	_	_	increased	Barras 1985
Hylidae	*Hyla femoralis*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Hyla gratiosa*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Hyla squirella*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Hyla versicolor*	traffic	76	Call	decreased	_	no changes	no changes	Cunnington & Fahrig 2010
Hylidae	*Hyla versicolor*	traffic	73.2	Call	decreased	_	no changes	no changes	Cunnington & Fahrig 2010
Hylidae	*Hyla versicolor*	traffic	73	Call	no changes	_	_	_	Vargas-Salinas et al. 2014
Hylidae	*Litoria ewingii*	traffic	47.6-77	Call	_	_	_	increased	Paris et al. 2009
Hylidae	*Osteopilus septentrionalis*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Pseudacris crucifer*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Pseudacris nigrita verrucosa*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Pseudacris ocularis*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Hylidae	*Pseudacris regilla*	traffic	52	Call	no changes	no changes	no changes	increased	Nelson et al. 2017
Hylidae	*Tlalocohyla loquax*	traffic	90	Call	increased	_	_	_	Kaiser et al. 2011
Hylidae	*Tlalocohyla picta*	traffic	90	Call	no changes	no changes	_	_	Kaiser et al. 2011
Hyperoliidae	*Hyperolius pickersgilli*	airplane	85.6-98.2	Call	increased			increased	Kruger & Preez 2016
Leptodactylidae	*Leptodactylus albilabris*	traffic	>60	Population/species	_	_	_	_	Herrera-Montes and Aide 2011
Microhylidae	*Austrochaperina pluvialis*	traffic		Population/species	_	_	_	_	Hoskin & Goosem 2010
Microhylidae	*Gastrophryne carolinensis*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Microhylidae	*Kaloula pulchra*	traffic	_	Call	decreased	_	_	_	Sun& Narins 2005
Microhylidae	*Microhyla butleri*	air plane	_	Call	decreased	_	_	_	Sun & Narins 2005
Microhylidae	*Microhyla butleri*	traffic	_	Call	decreased	_	_	_	Sun & Narins 2005
Microhylidae	*Sylvirana nigrovittata*	air plane	_	Call	decreased	_	_	_	Sun & Narins 2005
Microhylidae	*Sylvirana nigrovittata*	traffic	_	Call	decreased	_	_	_	Sun & Narins 2005
Myobatrachidae	*Crinia signifera*	traffic	43.3-79.3	Call	_	_	_	increased	Paris et al. 2009

Family	Species	Source of noise	dB	Analyzed	Call rate	Call length	Amplitude	Dominant Frequency	References
Pelodryadidae	*Ranoidea caerulea*	traffic	70	Physiology	_	_	_	_	Kaiser et al. 2015
Pelodryadidae	*Ranoidea rheocola*	traffic	_	Population/species	increased	no changes	_	no changes	Hoskin & Goosem 2010
Pelodryadidae	*Ranoidea rheocola*	traffic	_	Population/species	_	_	_	_	Hoskin & Goosem 2010
Pelodryadidae	*Ranoidea serrata*	traffic	_	Call	_	_	_	_	Hoskin & Goosem 2010
Phyllomedusidae	*Agalychnis callidryas*	traffic	90	Call	increased	_	_	_	Kaiser et al. 2011
Phyllomedusidae	*Agalychnis moreletti*	traffic	70	Call	no changes	_	_	_	Kaiser et al. 2011
Ranidae	*Humerana miopus*	traffic	_	Call	decreased	_	_	_	Sun & Narins, 2005
Ranidae	*Hylarana taipehensis*	air plane	_	Call	increased	_	_	_	Sun & Narins 2005
Ranidae	*Hylarana taipehensis*	traffic	_	Call	increased	_	_	_	Sun & Narins 2005
Ranidae	*Lithobates areolatus*	airplane noise	_	Call	increased	_	_	_	Engbrecht et al. 2015
Ranidae	*Lithobates catesbeianus*	traffic	73	Call	decreased	_	_	_	Vargas-Salinas et al. 2014
Ranidae	*Lithobates clamitans*	traffic	76	Call	decreased	_	decreased	increased	Cunnington & Fahrig, 2010
Ranidae	*Lithobates clamitans*	traffic	73.2	Call	decreased	_	decreased	increased	Cunnington & Fahrig, 2010
Ranidae	*Lithobates clamitans*	traffic	73	Call	decreased	_	_	_	Vargas-Salinas et al. 2014
Ranidae	*Lithobates grylio*	traffic	>60	Population/species	_	_	_	_	HerreraMontes & Aide 2011
Ranidae	*Lithobates pipiens*	traffic	76	Call	decreased	_	decreased	increased	Cunnington & Fahrig 2010
Ranidae	*Lithobates pipiens*	traffic	73.2	Call	decreased	_	no changes	increased	Cunnington & Fahrig 2010
Ranidae	*Pelophylax ridibundus*	traffic	50	Physiology	_	_	_	_	Lukanov et al. 2014
Ranidae	*Pelophylax ridibundus*	traffic	70	Physiology	_	_	_	_	Lukanov et al. 2014
Ranidae	*Pelophylax ridibundus*	traffic	80	Population/species	_	_	decreased	decreased	Lukanov et al. 2014
Ranidae	*Rana catesbeianus*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Ranidae	*Rana grylio*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Ranidae	*Rana sphenocephala*	traffic	65-70	Population/species	_	_	_	_	Grace & Noss 2018
Scaphiopodidae	*Scaphiopus couchi*	traffic	95	Physiology	_	_	_	_	Brattstrom & Bondello 1983

Family	Species	Source of noise	dB	Analyzed	Phonotaxis	Latency	References
Bufonidae	*Anaxyrus americanus*	traffic	76	Response to normal male acoustic signal	no changes	no changes	Cunnington & Fahrig 2013
Bufonidae	*Anaxyrus americanus*	traffic	76	Response to altered male acoustic signal	no changes	no changes	Cunnington & Fahrig 2013
Hylidae	*Hyla arborea*	traffic	72-75	Response to male acoustic/visual signal	_	no changes	Troïanowski et al. 2017
Hylidae	*Hyla chrysoscelis*	traffic	37-85	Response to normal male acoustic signal	decreased	increased	Bee & Swanson 2007
Hylidae	*Hyla versicolor*	traffic	76	Response to normal male acoustic signal	decreased	increased	Cunnington & Fahrig 2013
Hylidae	*Hyla versicolor*	traffic	76	Response to altered male acoustic signal	no changes	no changes	Cunnington & Fahrig 2013
Ranidae	*Lithobabtes sylvaticus*	traffic	87	Physiology/ Response to male acoustic signal	decreased	no changes	Tennessen 2014
Ranidae	*Lithobates clamitans*	traffic	76	Response to normal male acoustic signal	decreased	increased	Cunnington & Fahrig 2013
Ranidae	*Lithobates clamitans*	traffic	76	Response to altered male acoustic signal	no changes	no changes	Cunnington & Fahrig 2013
Ranidae	*Rana pirica*	traffic	65	Response to normal male acoustic signal	decreased	increased	Senzaki et al. 2018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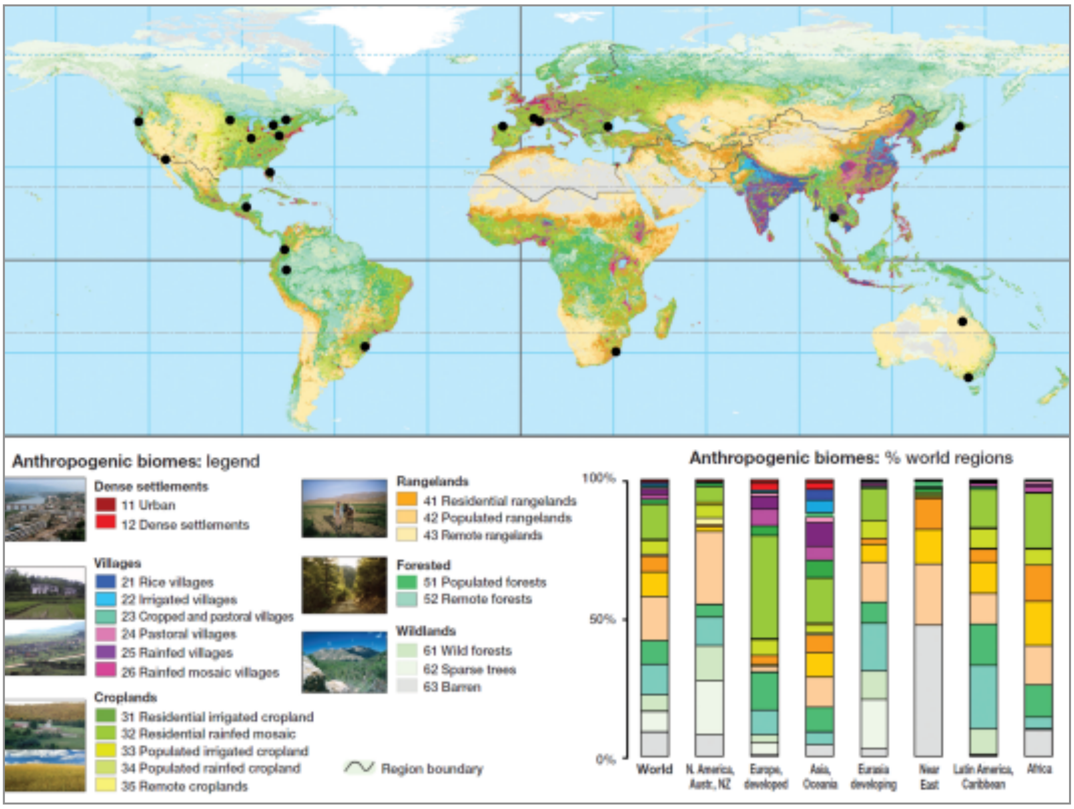
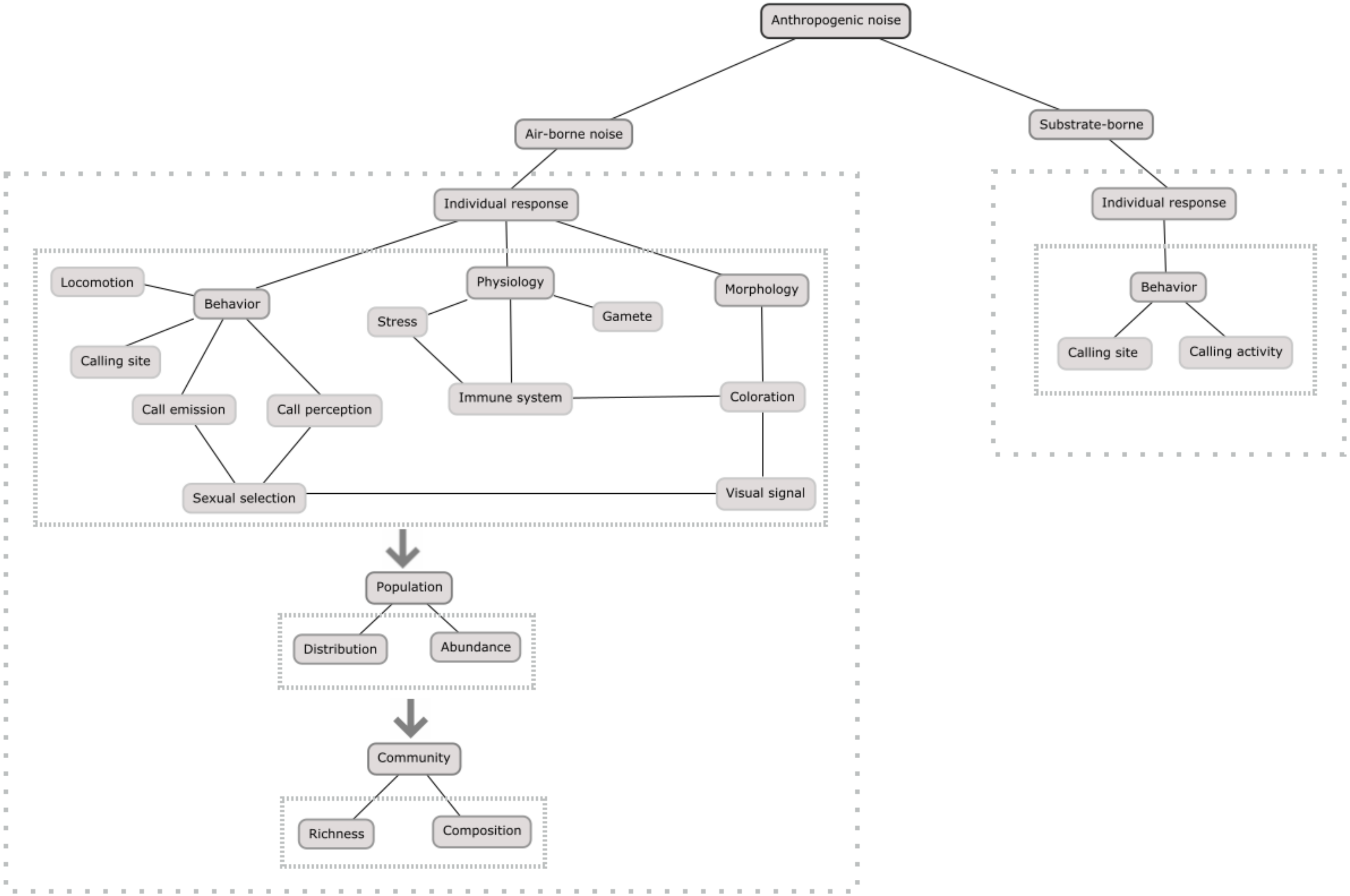

Anthropogenic biomes: legend
Dense settlements
11 Urban
12 Dense settlements
Villages
21 Rice villages
22 Irrigated villages
23 Cropped and pastoral villages
24 Pastoral villages
25 Rainfed villages
26 Rainfed mosaic villages
Croplands
31 Residential irrigated cropland
32 Residential rainfed mosaic
33 Populated irrigated cropland
34 Populated rainfed cropland
35 Remote croplands
Rangelands
41 Residential rangelands
42 Populated rangelands
43 Remote rangelands
Forested
51 Populated forests
52 Remote forests
Wildlands
61 Wild forests
62 Sparse trees
63 Barren
Region boundary
Anthropogenic biomes: % world regions
100%
50%
0%
World
N. America, Austr., NZ
Europe, developed
Asia, Oceania
Eurasia developing
Near East
Latin America, Caribbean
Africa


Anthropogenic noise
Air-borne noise
Substrate-borne
Individual response
Locomotion
Behavior
Calling site
Call emission
Call perception
Sexual selection
Physiology
Stress
Gamete
Immune system
Morphology
Coloration
Visual signal
Population
Distribution
Abundance
Community
Richness
Composition
Individual response
Behavior
Calling site
Calling activity

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.