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EFECTOS DE LA CONTAMINACIÓN ANTROPOGÉNICA SOBRE LA COMUNICACIÓN ACÚSTICA DE ANFIBIOS EN AMBIENTES URBANOS

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar por el grado de Maestría Académica en Biología

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Dedicatoria

A Dios y a mi familia, quienes siempre han estado a mi lado y son mi más grande apoyo.

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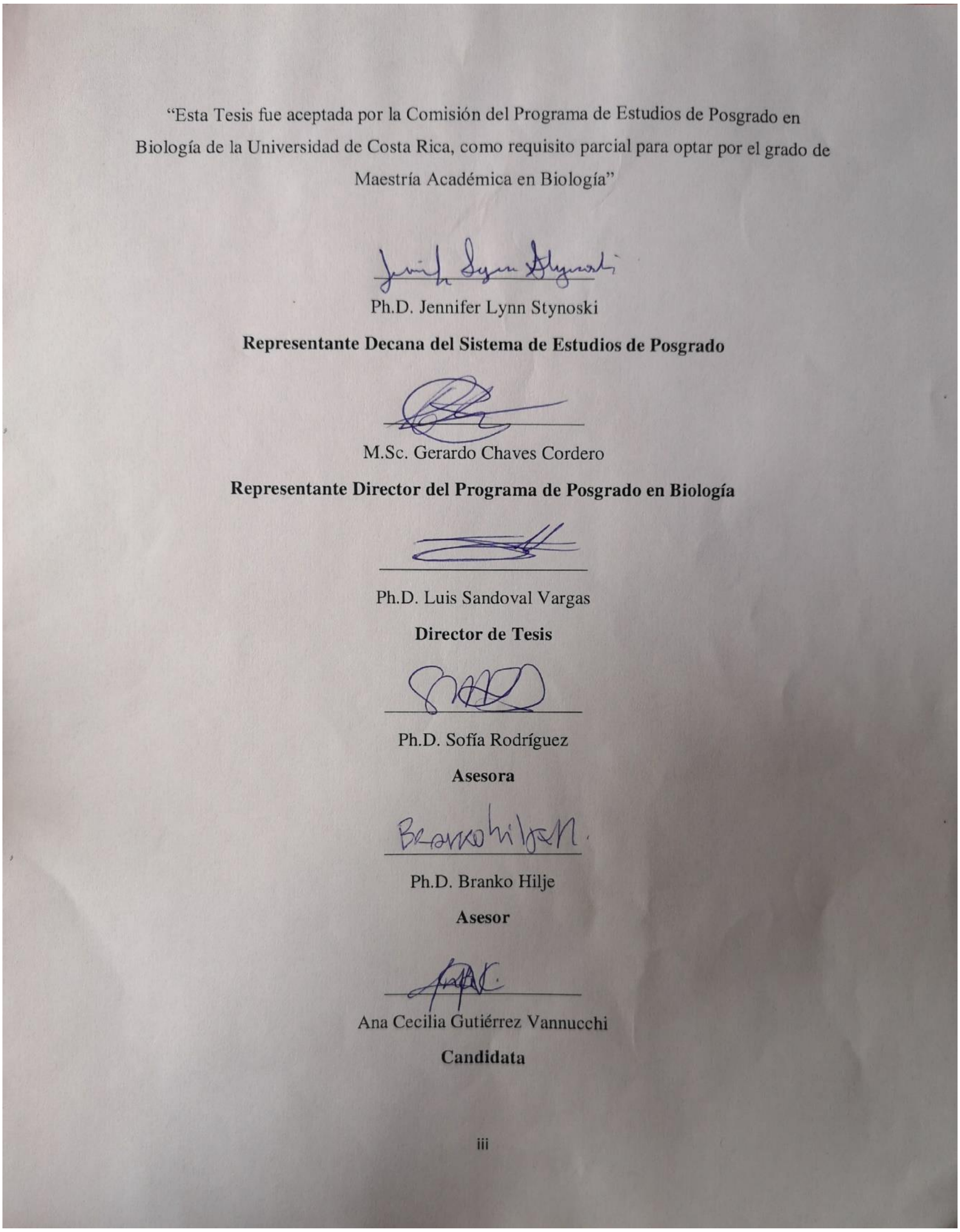

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Resumen

El proceso de urbanización que se da de manera creciente a nivel mundial, conlleva cambios en el ambiente, entre los cuales se encuentra la aparición de contaminantes de origen antropogénico, en zonas donde antes no existían. Entre estos contaminantes se encuentran el ruido antropogénico y la contaminación lumínica, provocada por luces de origen artificial. Estos contaminantes pueden afectar distintos procesos en diferentes grupos de animales, entre los que se encuentra la comunicación acústica.

La comunicación acústica es el principal método de comunicación en anuros, sin embargo, los efectos de la urbanización y más específicamente de la contaminación lumínica y el ruido antropogénico han sido menos estudiados en este grupo, que en otros grupos de animales como aves y mamíferos. Debido a esto, el conocimiento que tenemos sobre cómo los contaminantes antropogénicos pueden estar afectando la superviviencia de anuros en zonas urbanas, especialmente a largo plazo, es aún limitado.

Nuestro objetivo con esta investigación fue determinar los efectos de la contaminación antropogénica en la comunicación acústica de anfibios presentes en ambientes urbanos. Más específicamente, queríamos determinar los efectos del ruido antropogénico y la contaminación lumínica sobre las características acústicas de las vocalizaciones de la rana de vidrio *Hyalinobatrachium fleischmanni* y sobre su actividad vocal. Esta especie de rana vive y se reproduce en quebradas pequeñas en ambientes urbanos, donde vocaliza desde las hojas, y está presente en sitios con distintos niveles de desarrollo urbano, con distintos niveles de intensidad lumínica y ruido. Además, el rango de frecuencia del canto de esta especie, traslapa en su parte baja con las frecuencias de ruido antropogénico, lo cual podría dificultar su comunicación acústica en ambientes con altos niveles de ruido.

Realizamos una revisión bibliográfica de literatura científica a nivel global, para determinar el estado de conocimiento sobre anfibios en ambientes urbanos. Y obtuvimos la locación geográfica de los estudios, clasificamos las especies como “N/A” (cuando no teníamos suficiente información para clasificarlas),“explotadoras”, “sobrevivientes” y “evitadoras”, y los efectos de la urbanizaicón como “positivos”, “neutros” y “negativos”. Encontramos que la mayor cantidad de estudios pertenecieron al norte global, y fueron en su mayoría realizados en Estados Unidos. El mayor porcentaje de especies fue categorizada como N/A, seguido por especies evitadoras. Además, el mayor porcentaje de estudios indicó efectos negativos, lo cuales fueron reportados principalmente para las

especies evitadoras. El efecto negativo más reportado fue baja abundancia y/o ocurrencia de especies, y el efecto positivo más reportado fue alta abundancia y/o ocurrencia.

Para investigar los efectos del ruido antropogénico en las características acústicas del canto de *H. fleischmanni*, trabajamos en dos sitios con distinto grado de desarrollo urbano. Grabamos 56 machos y medimos 7 caracterísiticas acústicas para el canto de cada macho y las relacionamos con el nivel de ruido antropogénico en ambos sitios. Encontramos que la duración del canto y el tiempo entre cantos fueron mayores en el sitio urbano, el cual tenía un nivel de ruido crónico mayor, y que la duración de la llamada y el tiempo entre llamadas disminuyó conforme aumentó el nivel de ruido crónico. La frecuencia mínima y la frecuencia de máxima amplitud también fueron mayores en el sitio con mayor nivel de ruido crónico. En cuanto al ruido instantaneousáneo, encontramos que el tiempo entre llamadas disminuyó con el aumento del ruido instantaneousáneo, pero el resto de las características acústicas no variaron. Es decir, el ruido crónico e instantaneousáneo, afectan de manera distinta las características acústicas del canto de *H. fleischmanni*.

Para determinar los efectos de la contaminación lumínica y el ruido en la actividad vocal de *H. fleischmanni,* trabajamos en dos sitios con diferente grado de urbanización, ruido y abundancia de luces artíficiales. Medimos la intensidad lumínica y el ruido de fondo en cada punto de muestreo dentro de cada sitio de estudio, y dividimos los puntos de muestreo entre “iluminados” y “oscuros”, dependiendo de la cantidad de luz artificial en cada uno. Grabamos la actividad vocal de *H. fleischmanni* de las 16:00 a las 6:00 h en cada sitio de muestreo y obtuvimos una proporción de cantos por hora. El pico de actividad de la especie de estudio fue entre las 19:00 h y las 22:00 h. Al relacionar la proporción de cantos con los niveles de contaminación lumínica y ruido de fondo, encontramos que el ruido de fondo afectó los patrones de actividad vocal de manera distinta dependiendo de la hora, sin embargo, no encontramos un efecto de la contaminación lumínica sobre los patrones de actividad vocal de *H. fleischmanni*.

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INTRODUCCIÓN

El proceso de urbanización es un fenómeno creciente a nivel mundial que conlleva la expansión de zonas urbanas hacia zonas rurales, y la de estas hacia zonas naturales (Joyce, 2006). Este proceso se asocia a un acelerado crecimiento poblacional en escala global, durante las últimas tres décadas (Stein et al., 2000; McKinney, 2002; Estado de la Nación, 2018). En Costa Rica el crecimiento urbano ha sido incentivado por las políticas económicas y sociales, causando una rápida expansión horizontal del Gran Área Metropolitana (GAM; Limitada al este por Ochomogo, al oeste por San Ramón y Atenas, al norte por la Cordillera Volcánica Central y al sur por los Cerros de Escazú) (Fig.1) (Estado de la Nación, 2018). Esto ha provocado la eliminación de plantaciones (de café, caña o pasto), zonas de crecimiento secundario, o remanentes de bosques, para la construcción de viviendas y comercios (Joyce, 2006; Pauchard et al., 2006; Estado de la Nación, 2018).

El aumento de la urbanización produce un incremento en la contaminación sónica, lumínica, del aire y los suelos, aumenta la temperatura, la compactación de suelos y acelera la pérdida de los parches de vegetación natural (Medley et al., 1995; Pickett et al., 2001; McKinney, 2002). Estos cambios en la estructura del ambiente causan una disminución o desplazamiento de la mayoría de las especies nativas de la zona, debido al cambio de las condiciones ambientales necesarias para su supervivencia (Marzluff, 2001; McKinney, 2002; Biamonte et al., 2011). Este desplazamiento de especies nativas genera un aumento en un número pequeño de especies colonizadoras que contribuye a la homogenización de la diversidad en sitios urbanos (Blair, 2001).

Con los cambios en el ambiente producto de la urbanización aparecen tres clasificaciones para los animales, que dependen de su respuesta a estos cambios. Existen 1) las especies "evitadoras", que se alejan del centro urbano y se mantienen en el interior de los bosques, 2) las especies "adaptadoras" que usualmente tienen cierto grado de tolerancia a los cambios por urbanización, y que normalmente se encuentran en las periferias de los centros urbanos y por último 3) las especies "explotadoras", las cuales suelen ser comensales y toman provecho de los recursos que la urbanización les presenta, como fuentes estables de alimento y agua de origen antropogénico; este último grupo de especies ha sido capaz de adaptarse y sobrevivir bajo estas condiciones. (McKinney, 2002). Algunos ejemplos de especies "evitadoras" son las aves *Crypturellus soui* y *Myadestes melanops* (Biamonte et al., 2011), lagartijas como *Uta stansburiana* y *Phrynosoma solare* (Germaine & Wakeling, 2001) y algunos mamíferos como el zorro rojo (*Vulpes vulpes)* (Randa & Yunger, 2006). En cuanto a ejemplos de especies "adaptadoras" se encuentran algunos anfibios como el sapo *Alytes obstetricans* y las ranas *Pelophylax ridibundus* y aves como la lechuza *Strix aluco* y la garza *Ardea cinerea* (Laurian, 2012). Por último existen varios ejemplos comunes de especies "explotadoras" como las palomas comunes (*Columba livia)* y los zanates (*Quiscalus mexicanus),* los mapaches (*Procyon lotor)* y ratas (*Rattus rattus)* y anfibios como la rana coquí (*Eleutherodactylus coqui*), que alcanzan densidades mucho mayores en zonas urbanizadas que en zonas naturales (Woolbright et al., 2006).

Los estudios de especies que han logrado adaptarse a zonas urbanas son limitados y la mayoría están representados por reportes de aves (Blair, 2001; Marzluff, 2001; Biamonte et al., 2011) y de riqueza de especies en ambientes urbanos versus ambientes rurales para mamíferos, lagartijas y algunos insectos (Mackin-Roglaska et al., 1988; Germaine & Wakeling, 2000; McIntyre, 2000; Pawlikowski & Polorniecka, 1990; Nuhn & Wright, 1979). Para anfibios, existe aún menos información sobre las especies que han logrado adaptarse a estos cambios en el ambiente y los efectos que la urbanización ha tenido sobre el grupo en general (compilado en Mitchell et al., 2008). Dado el acelerado crecimiento del proceso de urbanización, es de vital importancia tener conocimiento sobre la ecología de diversos grupos, tanto en ambientes naturales como urbanos, para comprender las adaptaciones a nuevas fuentes de contaminación. Por ejemplo, la contaminación acústica por ruido antropogénico (motores, música) y la contaminación lumínica por la aparición de fuentes de luz artificial durante la noche, que pueden afectar la ecología de diferentes especies de animales.

Contaminación acústica

La comunicación acústica representa una de las formas de comunicación principal en muchos grupos de animales como aves, mamíferos, anfibios y reptiles (Ryan, 2001; Bradbury &Vehrencamp, 1998). Ya que es utilizada en las interacciones sociales como cortejo, copula, defensa de territorio, o encuentros agonísticos (McGregor, 2005; Brumm, 2013; Vitt & Caldwell, 2013). Los sonidos utilizados en la comunicación acústica están determinados por múltiples parámetros o características espectro-temporales como lo son la energía del sonido en decibeles, la frecuencia en hertzio, la duración del sonido y la temporalidad (momento del día en que se realiza) (Bradbury & Vehrencamp, 1998).

Las características del ambiente (como ruido por viento y fuentes de agua, densidad de la vegetación y temperatura), influyen en la forma en que se comunican acústicamente los animales, como lo propone la Hipótesis de Adaptación Acústica (Morton, 1975; Hansen, 1979). Una nueva característica del ambiente que puede afectar la comunicación acústica es el ruido antropogénico producto de los motores de los vehículos (ej. automóviles, trenes, aviones), motores de fábricas, el paso de personas, la música, y cualquier otra fuente de sonido producido por los humanos. El ruido antropogénico, contrario al ruido de origen natural (producido por ríos, viento, cantos de otras especies), es más constante en el tiempo, es de origen muy reciente (desde mediados del siglo XVIII con la revolución industrial), y ocurre en sitios que antes no poseían altos niveles de ruido (Kunc & Schmidt, 2019). Lo anterior dificulta que muchas especies de animales puedan comunicarse por medio de vocalizaciones (señales acústicas) dentro de zonas urbanas, ya que las frecuencias de sus vocalizaciones traslapan con las frecuencias del ruido antropogénico (enmascaramiento de las señales acústicas) que ocurre por debajo de los 5kHz (Wood &Yezerinac, 2006; Hanna et al., 2014).

Se ha encontrado que algunos grupos de animales tienen la capacidad de modificar las características espectro-temporales de sus vocalizaciones para evitar el enmascaramiento por ruido antropogénico (Slabbekoorn & Peet, 2003). Dentro de las estrategias que utilizan distintos grupos de animales para evitar el enmascaramiento acústico, está aumentar las frecuencias de su canto para reducir o eliminar el traslape con el ruido, como sucede para varias especies de aves como *Parus major* (Slabbekoorn & Peet, 2003) y algunos anfibios como la rana *Litoria ewingii* (Parris et al., 2009). También pueden modificar los periodos de vocalización para evitar las horas con mayores niveles de ruido como ocurre en algunos mamíferos como el mono *Callicebus nigrifrons* (Duarte et al., 2018) o modificar su tasa de canto como el caso de las ranas *Microhyla butleri* y *Rana taipehensis* (Sun & Narins, 2005).

Esta variación de las características espectro-temporales del canto o periodos de vocalización, se conoce como plasticidad acústica y esta a su vez puede variar entre poblaciones o entre individuos de una misma población (Cunnington & Farig, 2010). La plasticidad acústica permite a las especies minimizar los efectos de enmascaramiento por ruido antropogénico. Sin embargo, se sabe que modificar la intensidad y frecuencia del canto, vocalizar una mayor cantidad de horas, o cantar a horas distintas de las horas usuales de actividad implica un gasto de energía mayor para algunos individuos (Brumm, 2013). El efecto del ruido en las vocalizaciones de anuros ha sido poco estudiado en comparación con otros grupos de animales y los estudios han tenido resultados diversos en cuanto a modificación de las vocalizaciones en este grupo (Sun & Narins, 2005; Bee & Swanson, 2007; Lengagne, 2008; Herrera-Montes & Aide, 2011). De modo que las estrategias que utilizan los anfibios, para evitar el enmascaramiento acústico por ruido antropogénico y las implicaciones que esto tiene en la plasticidad de sus vocalizaciones aún son poco conocidas.

Contaminación lumínica

La urbanización aumenta la cantidad de fuentes de luz no naturales durante las noches. Este tipo de iluminación está constituida por cualquier fuente de luz de origen antropogénico -las luces de las calles, estadios, casas, carros, anuncios- (Perry et al., 2008), tiende a ser mucho más brillante que la luz natural y afectan todas las áreas urbanas (Cinzano et al., 2001; Bennie et al., 2015). Esto genera en dichas áreas contaminación lumínica, definida como un exceso de luz proveniente de fuentes antropogénicas. Adicionalmente, el brillo de estas luces se refleja en las nubes causando una iluminación nocturna mayor a la natural, especialmente en las áreas adyacentes a los centros urbanos (Cinzano et al., 2001). En consecuencia los efectos de la contaminación lumínica se expanden más allá de los sitios donde están presentes las fuentes de luz artificial.

A pesar del gran alcance que puede llegar a tener la contaminación lumínica, sus efectos sobre la fauna y el ambiente en general han sido mucho menos estudiados que los de otros tipos de contaminación de origen antropogénico como la contaminación por sólidos, la contaminación acústica, o la química (Longcore & Rich, 2004; Perry et al., 2008). La mayoría de los estudios sobre contaminación lumínica, ha estudiado su efecto en aves, siendo el resultado principal el cambio en los patrones temporales de actividad acústica en aves diurnas (Miller, 2006; Da Silva et al., 2015). Al haber un exceso de luz durante la noche, las aves extiendan sus horas de actividad aumentando las horas de vocalizaciones en el día, y por ende reduciendo el tiempo de descanso (Byrkjedal et al., 2012; Raap et al., 2015). En peces como salmones se ha encontrado que la luz artificial causa una detección mayor de presas y altera el tiempo de migración nocturna (Metcalfe et al., 1997; Riley et al., 2012). En mamíferos, como algunos roedores nocturnos, se han encontrado cambios en su ciclo circadiano y una actividad nocturna reducida (Sharma et al., 1997; Kramer & Birney, 2001). Los estudios para medir el efecto de esta contaminación en reptiles son escasos (Perry et al., 2008) y se centran básicamente en el análisis de la actividad de forrajeo y reportes de ocurrencias de especies cerca de centros con luz artificial (Case et al., 1994; Meshaka et al., 2004; Powell et al., 2005; Perry & Fisher, 2006). La excepción en este grupo son los estudios que han analizado los efectos adversos de la iluminación artificial en el comportamiento de desove de las tortugas marinas (Witherington & Martin, 2000; Tuxbury & Salmon, 2005; Bourgeois et al., 2009; Kamrowski et al., 2012), por ser importante para la conservación de las playas de anidación.

Los anfibios tampoco son la excepción a la falta de información del efecto de la contaminación lumínica, pese a que son un grupo principalmente nocturno (Savage, 2002; Perry et al., 2008). La comunicación acústica en anuros (ranas y sapos) es de vital importancia para todas sus interacciones sociales, y por ende la temporalidad de su actividad acústica se vuelve también un factor determinante para la comunicación efectiva entre individuos (Schwartz & Bee, 2013; Vitt & Caldwell, 2013; Bevier, 2016; Colafrancesco & Gridi-Papp, 2016). Un aumento en el nivel de iluminación podría significar un cambio en las horas de actividad y en el comportamiento de varias especies (Meshaka et al., 2014, Henderson & Powell, 2001; Perry & Fisher, 2006). Los efectos que esto pueda tener sobre las relaciones entre individuos (ej.: atracción de pareja, defensa de territorio, o detección de depredadores) tanto a nivel intra- como inter-específico son aún desconocidos. Por lo tanto, estudiar el efecto de la contaminación lumínica sobre las especies de anuros que habitan áreas urbanas es necesario para generar medidas correctivas y de manejo que faciliten la conservación de estas especies.

Especie de estudio

A pesar de los efectos de la contaminación acústica y lumínica sobre la comunicación acústica, se conoce que hay especies de anfibios que sobreviven y se reproducen en ambientes urbanos como los anuros (Perry *et al*., 2008). La rana de vidrio *Hyalinobatrachium fleischmanni* vive y se reproduce dentro de pequeños ríos en ambientes urbanos, desde donde cantan sobre las hojas (Kubicki, 2007). El canto de esta especie presenta un rango de frecuencias entre 3.8 y los 5.3 kHz (Savage, 2002; Kubicki, 2007), traslapando así con las frecuencias del ruido antropogénico. Al ser una especie común en ambientes urbanos, puede ver afectada su comunicación acústica por los altos niveles de ruido o la iluminación intensa y constante presente en estos ambientes. Esta situación puede provocar el enmascaramiento de sus señales acústicas, evitando que el mensaje sea transmitido al receptor de forma efectiva. Debido al traslape entre el canto y el ruido, y la presencia de iluminación artificial en ambientes urbanos, espero que *H. fleischmanni* modifique las características espectro-temporales de su canto y las horas de actividad acústica, para mejorar la eficiencia de su comunicación.

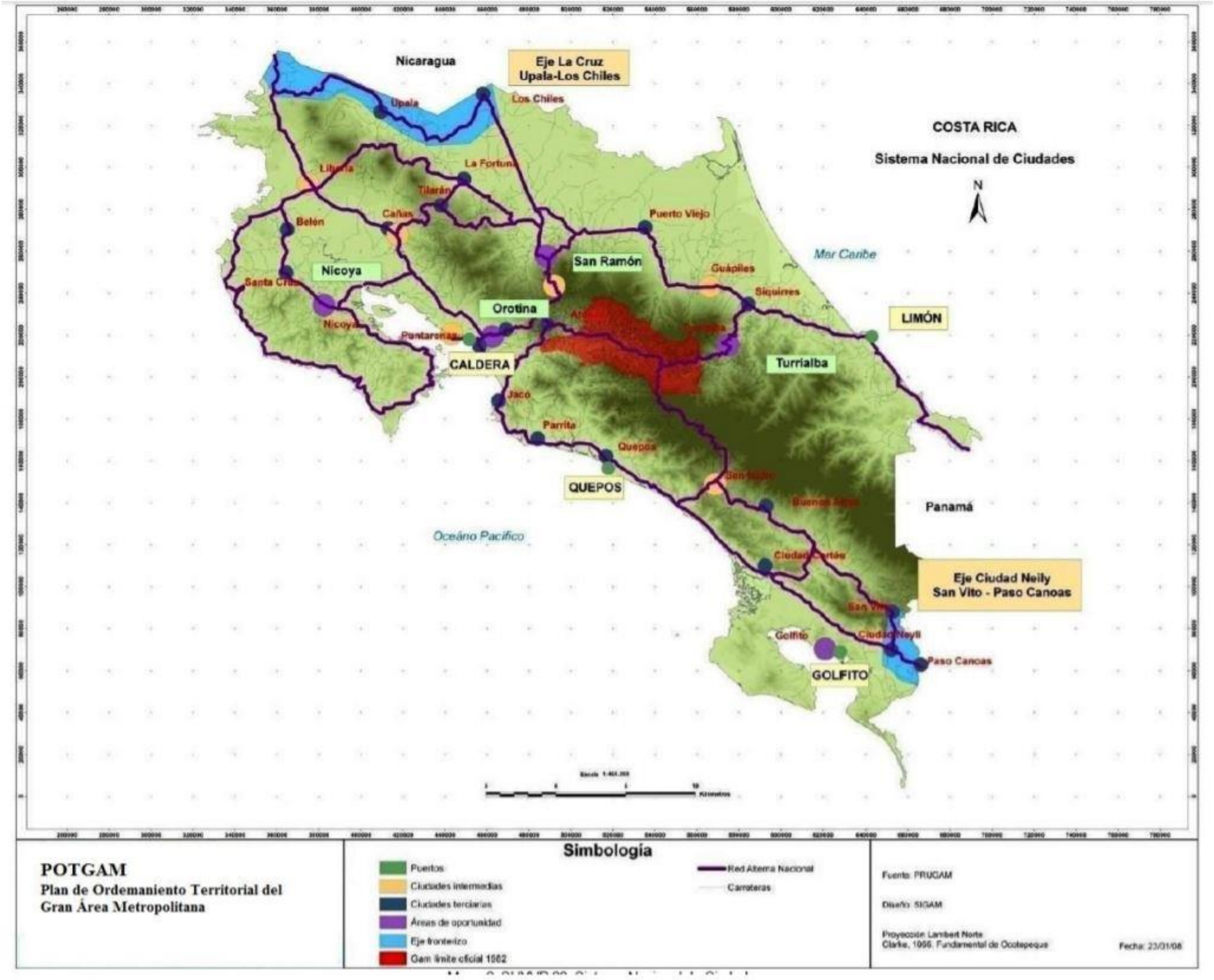

Figura 1**.** Mapa de Costa Rica con el Gran Área Metropolitana señalada en rojo. Tomado del Plan de Ordenamiento Territorial de la Gran Área Metropolitana 2011-2030 (Rosales, 2012).

Capítulo 1. Occurrences and effects of urbanization on amphibians: a review

Abstract. Urbanization causes changes in natural landscapes. It causes a decrease or displacement of native species from urban areas, contributing to homogenization of diversity. Based on the response of a species to changes in the environment, they can be classified into urban “exploiters”, “survivors”, and “avoiders”. Because amphibians in urban areas have been less studied than other animal groups, our knowledge of the effects of urbanization on amphibians is limited. Our goal was to conduct a literature review about the occurrence of amphibians in urban areas, and the effects of urbanization on amphibians at a global scale, to identify information gaps and propose future research directions. We conducted a search of peer-reviewed scientific literature and from 104 selected studies; we extracted the geographical location, the effects of urbanization reported (positive, negative, neutral) and the species abundance/occurrence to classify them as exploiters, survivors or avoiders. The United States had the largest number of studies, and the majority of studies belonged to the Global North. The highest percentage of species was categorized as N/A followed by avoiders. The highest percentage of studies indicated negative effects, and those negative effects were mostly reported for avoider species. The most commonly reported negative effect was low abundance and/or occurrence, and the most commonly reported positive effect was high abundance and/or occurrence. Only a small group of species (exploiters) obtained benefits from urban sites, and the large majority of studies come from the Global North, where species richness is lower. This, along with a lack of long-term studies, can compromise our understanding of the effects of urbanization on amphibians. We recommend increasing the number of long-term studies in the Global South, making direct comparisons between urban and natural environments, and continuing to make species classifications regarding their response to urbanization, to better standardize future revisions.

Key words: urban ecology, city, amphibians

The process of urbanization is a growing phenomenon worldwide, which entails the expansion of urban areas towards rural areas, and the expansion of rural areas to natural areas (Joyce, 2006). It is associated with a rapid population growth worldwide, especially during the last three decades (Stein et al., 2000; McKinney, 2002; Estado de la Nación, 2018). Currently, urban areas constitute approximately 4% of the earth’s surface and support more than 50% of the human population (Rosen, 2000; United Nations Department of Economic and Social Affairs, 2019). Urbanization encompasses the physical, anthropological, and economic processes that change natural landscapes to those dominated by human activities (Mitchell & Brown, 2008). The increase in urbanization produces an increase in noise, light, air and soil pollution, temperature, soil compaction, and accelerates the loss of natural vegetation patches (Medley et al., 1995; Pickett et al., 2001; McKinney, 2002) producing a decrease or displacement of native species of fauna from urban areas (Marzluff, 2001; McKinney, 2002; Biamonte et al., 2011). This displacement of native species generates an increase of colonizing species that contribute to the homogenization of diversity in urban areas (Blair, 2001).

Based on how species respond to changes in the environment that are produced by urbanization, species are classified in three groups (McKinney, 2002). "Avoider" species move away from the urban center and stay inside the forests. "Survivor" species usually have a certain degree of tolerance to changes caused by urbanization, and are normally found on the outskirts of urban centers. “Exploiter” species are usually commensals and take advantage of the resources offered by urbanization, such as stable sources of food and water of anthropogenic origin, and are able to adapt and survive under these urbanized new conditions, and might become dependent on urban resources (McKinney, 2002). Studies of species that adapt to urban areas are limited and are most represented by reports of birds (Blair, 2001; Marzluff, 2001; Biamonte et al., 2011), as well as studies of species richness in urban environments versus rural environments for mammals, lizards, and some insects (Mackin-Roglaska et al., 1988; Germaine & Wakeling, 2000; McIntyre, 2000; Pawlikowski & Polorniecka, 1990; Nuhn & Wright, 1979). Therefore, to understand which species are part of each group (“avoiders”, “survivors”, and “exploiters”), and how they respond to environmental changes, it is key to analyze the impact of urbanization on multiple animal groups and species.

Amphibians have been less studied in urban areas relative to birds (*e.g.*, Erz, 1966; Marzluff, 2001; Seress & Liker, 2015; Isaksson, 2018), plants (*e.g.*, Pysek, 1989; King & Buckney, 2000; Chocholouskova & Pysek, 2003, Witting, 2004), and arthropods (*e.g.*, McIntyre, 2000; Raupp et al., 2010; Bang & Faeth, 2011; Fenoglio et al., 2020). However, reports of amphibians in urban areas appeared as early as 1902, from the city of Washington, with a list of the batrachia and reptilia of the District of Columbia and vicinity (Hay, 1902), and in 1905, with a report of the batrachians in the vicinity of New York City (Ditmars, 1905; Mitchell & Brown, 2008). More recently, studies about the effect of urbanization on amphibians have focused on determining which species are present and can survive in urban areas (Entiauspe-Neto et al., 2016; Hill et al., 2017; Melo et al., 2018;

Ingle et al., 2019; Konowalik et al., 2020) and on the causes of population declines in cities (Martinez-Solano & García-Paris, 2001; Mollov, 2005; Price et al., 2006; Mitchell & Brown, 2008). But, information about other ecological processes (e.g., diet changes, mortality rate, or reproductive success), evolutionary responses (e.g., behavioral adaptation, or genetic and morphology changes), and changes in communication (e.g., changes in duration and frequency of calls) of amphibians in urban areas is still limited (Mitchell & Brown, 2008). Additionally, the majority of studies about amphibians in urban areas are from temperate regions (Mitchell, Brown & Barholomew, 2008). Given the rapid growth of urbanization, and very limited knowledge of its effect on amphibians, it is timely to generate a literature review to better understand how species are responding to urbanization. Therefore, our main goal was to conduct an exhaustive literature review about the ocurrence of amphibians in urban areas and the main effects of urbanization on this group. We also want to identify information gaps and propose future research directions to improve the knowledge of amphibian urban ecology and conservation.

Methods

Literature search

We conducted a search of peer-reviewed scientific literature in November 2020 using Google Scholar (https://scholar.google.com/) and Web of Science databases (http://webofknowledge.com). Both databases were used to search for the following terms and their combinations: “Urban herpetofauna”, “Urban amphibians”, “cities” AND “herpetofauna”, “cities” AND “amphibians”, “cities” AND “frogs”, “cities” AND “salamanders”, “cities” AND “newts”, “cities” AND “caecilians”.

For each search, we checked only the first 50 references that were generated, because after 50, the references started to repeat between searches, or the results were not very relevant to the term searched. In addition, we reviewed the bibliography of all selected peer-reviewed manuscripts in order to include other sources of information in the review. Also, we decided to exclude meta-analyses and previous reviews that included amphibians (n =43), because we preferred not to use previously analyzed data, and rather use the original source of information. Therefore, we considered the literature used in these reviews and meta-analyses, and included the references that met our choice criteria (see below), if they were not found previously under the search terms in both databases.

Data collection and analysis

We obtained 245 studies from the database search and 41 from reviewing the literature of the studies selected from the database, meta-analyses, and other reviews. To be included in the analysis, the studies needed to report the occurrence of amphibians in urban environments (presence, abundance, or comparison between urban or other habitats) or report the effects of urbanization on amphibian morphology, behavior, survival, diet, or evolution. We excluded studies that analyzed the effect of anthropogenic pollutants in laboratories or experimentally inside cities. We also excluded reports of amphibian deaths on roads, if roads were outside of cities (*i.e.*, a road outside of a natural park or in the middle of a desert), or did not compare city roads versus rural, or natural roads or a gradient of road density as a measure for urbanization. After all these selection criteria, 104 studies met the inclusion criteria and were used in this study (Appendix 1).

From all selected studies, we extracted the geographical location of the reports, the effects of urbanization on amphibians when compared to natural or rural environments or reported by the authors (*i.e.*, positive, negative, neutral), and its relative or quantitative abundance. We used the species abundance or occurrence in each study (when include in the study) to classify the species into three groups: exploiters, survivors, or avoiders. Species were classified as exploiters if there was a reported increase in population size or colonizing events when compared between rural or natural environments, when the population size or abundance was greater at urban sites compared to rural or natural sites, if the species were classified as “abundant”, “very common” or “common” by the authors, or if they had an occurrence percentage of 50% or more in the urban study sites. Species were classified as urban survivors if the population size was not larger in either rural or natural areas but species were still present in urbanized areas, if the population size was constant over time as long as it was not a species classified as “abundant”, “very common” or “common”, or if the occurrence percentage was between 15% and 49% in the study sites. Finally, species were classified as avoiders if the population size was very small or the species were not found in the more urbanized areas, if the species were classified as “uncommon” or “rare”, or if they had fewer than 15% of occurrence in the study sites. The criteria we used for the classification of amphibians and the effects of urbanization in this review were based on and then adapted from the definitions of McKinney (2002). When the studies did not give enough information according to our criteria to put the species in one of the three aforementioned categories, we classified them as N/A.

For the effect of urbanization on amphibian biology we classified it as positive when there was an increase in population size, a higher number of colonization events, the species was more common or abundant in urban areas than in natural areas or there was a more specific positive effect reported by the authors (increase in the individual size or lower mortality). We classified the effect as negative when the population size decreased due to urbanization or the species was only found in non-urban areas, or when there was a more specific negative effect reported by the authors (low genetic variability, mortality due to road collisions, tissue damage). When the effect could not be clearly classified as positive or negative, or when there was not enough information in the study to classify it in one of the aforementioned categories according to our criteria, we classified it as neutral.

We calculated the percentage of each species category (exploiter, survivor, avoider, and N/A) per study, and then estimated the average for all studies. We also calculated the average number of species per category, in the studies that reported each category (average number of exploiter species in the studies that reported exploiter species). We calculated the percentage of studies for each effect category of urbanization on amphibians (positive, negative, or neutral), and the percentage of each effect category per amphibian category (exploiter, survivor, avoider, or N/A). We also calculated the percentage of studies per geographical location.

Finally, we classified the negative and positive effects of urbanization into categories, to count which were the most common negative and positive effects reported in the literature. We classified the negative effects into: 1) Reduction or small population size, 2) Reduction or low genetic variability, 3) Low abundance and/or occurrence in urban spaces, 4) Lower species richness, 5) Local extinction of one or more species in a study site due to urbanization, 6) Mortality reports due to urbanization, 7) Negative reproductive effects, and 8) Physiological damage (tissue damage or negative hormonal effects). We classified the positive effects into: 1) Increase in or large population size, 2) High abundance and or/occurrence, 3) Higher species richness, 4) Colonization events, 5) Fewer diseases, and 6) Positive effects on survival. Then, we counted the number of studies that reported positive or negative effects on each category, per species category (avoiders, survivors, exploiters, and N/A). We did this in order to identify where the majority of information is generated (and therefore, where there is a lack of information) geographically, how the majority of amphibians are responding to urbanization (exploiters with positive effects or avoiders with negative effects), and what are the most commonly reported effects (whether positive or negative) for amphibians in urban environments.

Results

We found 104 studies that met inclusion criteria, which were conducted in 29 different countries or regions. The United States had the largest number of studies, representing 37.5%, followed by Australia with 14.4%, and Brazil with 6.7%. The other 26 countries each accounted for less than 5% of the studies and 41.3% combined (Fig. 1). When we divided the studies into eight geographic regions (according to the Department of Homeland Security of the United States, https://www.dhs.gov/geographic-regions), North America accounted for the highest percentage of studies with 42.3%, followed by Europe with 25.0%, and Oceania with 14.4%. The other geographic regions accounted for less than 10% of the studies (Fig. 2).

On average, the highest percentage of species in the studies were categorized as N/A (40.6% ± 47.2%), followed by avoiders (23.9% ± 32.5%), survivors (18.7% ± 30.1%), and the lowest percentage was classified as exploiters (16.8% ± 27.8%). For the studies in which species were classified as N/A, the average number of N/A species studied was 5.6 ± 6.5. For the studies that reported survivors, the average number of survivor species studied was 5.4 ± 6.8. For the studies that reported avoiders, the average number of avoider species studied was 4.5 ± 3.6; and for the studies that reported exploiters, the average number of exploiter species studied was 3.2 ± 1.6.

Most studies indicated negative effects of urbanization on amphibians, followed by neutral effects, and then positive effects (Fig. 3). When we combined the species classifications and the effects classifications we found that for avoider species, the highest number of studies indicated negative effects. For the survivor species, the highest number of studies indicated neutral effects, followed by negative effects. For the exploiter species, the highest number of studies indicated positive effects, and for the N/A species, the highest number of studies indicated neutral and negative effects (Fig. 4).

The most commonly reported negative effect of urbanization for amphibians was low abundance and occurrence (n = 54), and it was mostly reported for avoider species (n = 35), followed by lower richness (n = 13), and reduction of population size (n = 12), also mostly reported for avoider species (n = 5 and n = 4, respectively; Fig. 5). The most commonly reported positive effects of urbanization for amphibians were high abundance and/or occurrence (n = 22) mostly reported for exploiter species (n = 20), and colonization events (n = 3), and higher population size (n = 2), also mostly reported for exploiter species (n = 3 and n = 2, respectively; Fig. 6).

Discussion

Regional differences in study publications

We found that most of the studies included in this review were conducted in North America, more specifically in the United States, followed by the European region and Australia, which means most of the studies were conducted and produced by the Global North. This might be because these are regions with high levels of urban development (United Nations Department of Economic and Social Affairs, 2019), as well as greater economic resources (as developed countries) compared to other areas of the world like Central America, South America, and Africa, where developing countries are located (United Nations Department for Economic and Social Affairs, 2020). This agrees with the findings of Karlsson et al. (2007), who found that most of the papers included in their review were published in the Global North or temperate/cold regions (around 80%), while the least number of studies were published from and about the Global South or sub-tropical/tropical regions (around 13%). Several authors have also demonstrated that the number of scientific contributions are related to the economic resource of countries (Rodriguez-Navarro & Brito, 2022; Allik et al., 2020; Cimini et al., 2014), which is also supported by our results.

Species classification and urban effects

Many studies did not provide enough information, according to our criteria, to classify the species into avoiders, survivors or exploiters, but when we were able to classify the species into these categories, the majority of reports were of avoider species, and the minority of exploiter species. The studies that reported exploiter species had, on average, a lower number of species per study than the studies that reported avoider and survivor species. This indicates that, according to our criteria, most of the amphibian species included in urbanization studies were avoiders, and the least were exploiters. Our results support Blair´s (2001) idea about diversity homogenization in cities, because there is evidence for a lower number of species that are able to adapt to the urbanization process, live, and reproduce successfully in urban centers (urban exploiters). Therefore, it is likely that the species commonly found in urban areas and major cities of a region are the same. This colonization phenomenon by urban exploiters may be increased, since the effects of urbanization present a threat and cause displacement of native species in urban areas (Marzluff, 2001; McKinney, 2002; Biamonte et al., 2011).

The majority of the studies we included in this review reported negative effects of urbanization on amphibians, and the minority reported positive effects. This suggests that according to our results, the negative effects of urbanization on amphibians overcome the positive effects. The type of species with higher positive reports was the exploiters, and there were no positive effects reported for avoider species, so the positive effects are only benefiting a restricted group of species. We found the same for the survivor and N/A species, which did have some positive effects reported, but the majority were either neutral or negative.

The most common negative effect reported was a lower abundance and/or occurrence of amphibian species in urban sites. For example, for the salamander species *Eurycea cirrigera*, larval abundance decreased with an increase in the gray area (e.g. buildings, houses and streets) around studied streams, due to a low basal flux of the stream, and changes in the water chemistry in urban and sub-urban sites of North Carolina, USA (Miller et al., 2007). The same was found in Atlanta, Georgia, USA with the salamander *Desmognathus fuscus fuscus*, where urbanization produced physical instability of riparian habitats because of increased runoff and erosion at most disturbed sites, and for this reason population density was inversely proportional to the urbanization degree (Orser & Shure, 1972). For the species *Hyla arborea* in Switzerland, urbanization had a negative effect on the occurrence in studied ponds, even within a 1 km buffer (Pellet et al., 2004), and a different study found that this species was not present, or that it was rare in the most urbanized areas of the city of Plovdiv, Bulgaria, because of a lack of suitable habitat (Mollov, 2005). The species *Eurycea tonkawae* had a significantly lower average density in developed areas of Texas, USA (with more than 10% of impervious surface) associated with urbanization, compared to undeveloped areas (less than 10% impervious surface; Bowles et al., 2006). A study in the central Amazonia, in Brazil, reported 13 different amphibian species with a lower abundance in urban sites compared to rural sites, and five other species had a higher abundance in urban sites (Menin et al., 2019).

The second most commonly reported negative effect for amphibians was lower species richness in urban sites, compared to less urbanized or natural sites. In a study of the larval amphibian community in wetlands along an urbanization gradient in central Pennsylvania, USA, it was found that there was a lower richness of amphibian larvae in wetlands with a higher level of urbanization, compared to rural wetlands, as well as a decrease in the occurrence of adults of the frog *Rana sylvatica*, and the salamanders *Ambystoma maculatum* and *Ambistoma jeffersonianum* (Rubbo & Kiesecker 2005). In central Amazonia, Brazil, researchers determined the composition and abundance of anurans in urban and rural sites, and reported a lower richness of species in the urban areas, with 10 anuran species only present in the rural site and not in the urban site (Menin et al. 2019). A similar pattern was found in western Georgia, USA, when other researchers studied multiple amphibian species in watersheds distributed along different categories of urbanization, with a lower species richness reported for the site with a higher urbanization level (Barret & Guyer, 2008).

Other common negative effects reported were a reduction in population size, lower genetic diversity, and mortality events. For example, in North Carolina, USA, the populations of *Eurycea cirrigera* and *Desmognathus fuscus* frogs decreased 12% and 9% respectively, from 1972 to 2000, which was associated with the conversion of natural areas into urban areas (Price et al. 2006), In Texas, USA, researchers also found a population decline of the salamander *Eurycea tonkawae*, which was strongly correlated with an increase in urban area (Bendik et al. 2014).

In Quebec and Montreal (Canada), New York (USA), and Melbourne (Australia), four different studies have found a low genetic diversity, including lower allelic richness, in amphibian species like *Litoria raniformis*, *Plethodon cinereus,* and *Desmognathus fuscus*, mostly because of habitat fragmentation caused by urbanization, which tends to isolate amphibian populations (Nöel et al., 2007; Nöel & Lapointe, 2010; Munshi-South et al., 2013; Keely et al., 2015). Finally, several authors have reported mortality events, mostly related to car and bicycle collisions in urban areas. For example, in Indiana, USA, authors conducted surveys of multi-species road-kills in order to identify habitat characteristics associated with road-kills, and found that 95% of the individuals in the study were herpetofauna, with at least eight amphibian species found as road-kill (Glista et al., 2008). In Falconbridge, Australia, researchers surveyed 1.4 km of suburban streets and found that six frog species had high mortality due to collisions in roads, representing 33% of the local frog fauna (Wotherspoon & Burgin, 2011). Similarly, Laurijssens & Stark (2013) found that five amphibian species suffered mortality by collisions with cyclists, in a bikeway in the vicinity of a university area in Leeuwarden, Netherlands.

On the other hand, the most common positive effect reported was a high abundance and/or occurrence of amphibians in urban sites. For example, *Rana temporaria* was found reproducing in most of the Sustainable Drainage Systems (SuDS) surveyed in Inverness, UK, over a period of 3 years, and the authors highlight the ability of SuDS inside the city to support amphibian breeding in these areas, given that they can be suitable habitats for reproduction (O´Brien 2015). In Australia, researchers studied amphibian assemblage structure in five natural and five urban sites with similar characteristics, and found that five out of the six amphibian species studied were only present in the urban site (Lane & Burgin, 2008) which might indicate that these species found benefits from the urban setting. Lane & Burgin (2008) hypothesize that chemicals present in urban contaminated waters might protect those five frog species from diseases such as chitridiomycosis, which can reduce mortality compared to natural sites. Menin et al. (2019) found five amphibian species within their study that were most common in urban areas, as compared to rural areas, in central Amazonia, Brazil, where authors determined the composition and abundance of anurans in urban and rural sites.

The second and third most reported positive effects were bigger population size and colonization events, respectively. Mollov (2005) found that *Bufo viridis* had a larger population in urbanized zones compared to non-urbanized zones, showing a possible benefit from urbanization for this specific frog species. Konowalik et al. (2020) found that five amphibian species had a greater number of colonization events than extinction events during the study period inside urban areas. According to Konowalik et al. (2020), the permanency of ponds, the occurrence of those ponds near river valleys, and a high ratio of green area around ponds are positively correlated with amphibian species richness in the city. Therefore, these characteristics might be helping amphibian species colonize ponds within the urban limits of the city. Many studies also described positive, neutral, and negative effects of urbanization for different amphibian species within the same study (How & Dell, 2000; Ghiurcă & Gherghel, 2008; Pulev & Sakelarieva, 2013; Entiauspe-Neto et al., 2016; Ingle et al., 2019; Konowalik et al., 2020). For example, Ghiurcă & Gherghel (2008) studied the composition and distribution of the herpetofauna in urban and peri-urban areas in Romania, and found that four amphibian species were very common in highly urbanized sites and peri-urban areas, with many individuals present both in artificial and natural ponds, whereas four other amphibian species in the same study were rare. There were few individuals found only in the peri urban sites and not in the areas with a higher concentration of buildings and houses. Entiauspe-Neto et al. (2016) did a fauna inventory of amphibians and reptiles in an urban area with different levels of anthropogenic alteration in Rio Grande do Sul, Brazil, and found that out of 16 amphibian species, eight were present either in sites with low anthropogenic alteration or in sites with high anthropogenic alteration but in low numbers (uncommon in these areas). Other six amphibian species in the same study were only found in sites with no anthropogenic alteration, and two were abundant in sites with higher anthropogenic alteration within the urban site studied. This shows that different amphibian species can respond differently to urbanization, which is why it is important to pay attention to the general trends as well as to species-specific responses.

In conclusion, our results showed that the majority of studied amphibian species (avoiders and survivors) inside urban areas suffered negative effects, mostly pertaining to low abundance, richness and smaller population size (Pellet et al., 2004; Price et al., 2006; Miller et al., 2007; Barret & Guyer, 2008; Menin et al., 2019). Conversely, a small group of species (exploiters) obtained benefits from urban sites and became the most common species inside urban areas (Mollov, 2005; Lane & Burguin, 2008; Menin et al., 2019). Notably, the large majority of urban amphibian studies came from the Global North (i.e., rich countries) where the amphibian diversity is lower (Duellman, 1999), and for that reason, our understanding of urban effects in this taxonomic group is biased to that small representation of species. The lack of studies in the Global South, where the majority of amphibian species occur, makes direct comparisons between urban and natural sites difficult in this area. Also the lack of long-term studies in general, which can describe the effects of urbanization over time, compromises our understanding of the general effects of urbanization on amphibians as urbanization progresses. Therefore, our recommendation would be to focus on this type of studies in future research on amphibian urban ecology. In comparison with other taxonomic groups such as birds (Blair, 2001; Marzluff, 2001; Seress & Liker, 2015; Isaksson, 2018) and arthropods (Pawlikowski & Polorniecka, 1990; McIntyre, 2000; Raupp et al., 2010; Bang & Faeth, 2011; Fenoglio et al., 2020), our knowledge about the effect of urban development on amphibians is very limited, which reduces the potential reach of the information to impact informed decisions on their conservation within urban areas and to help decision makers improve urban planning in the future.

Future directions for urban amphibian studies

Our recommendation is to increase the number of studies of urban amphibian ecology in the Global South. More specifically, we recommend focusing on studies that make direct comparisons between urban and natural environments, so that the effects of urbanization on this group can become more directly evident. Also, it is important to focus on long-term studies in order to better describe the effects and responses of amphibians to urbanization over time. In such long-term studies, in order to make future revision on this subject easier and more standardized, it would be important to describe the study sites thoroughly, place them in an urbanization category (urban, suburban, peri-urban, rural, etc), classify the study species into exploiters, survivors, or avoiders as well as include the reason why the species was classified in a certain category. Also, we recommend clarifying if the matter that is being studied affects the amphibian species in a positive or negative way, to more clearly understand the effects of urbanization on anuran and other amphibian species.

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Tables and figures

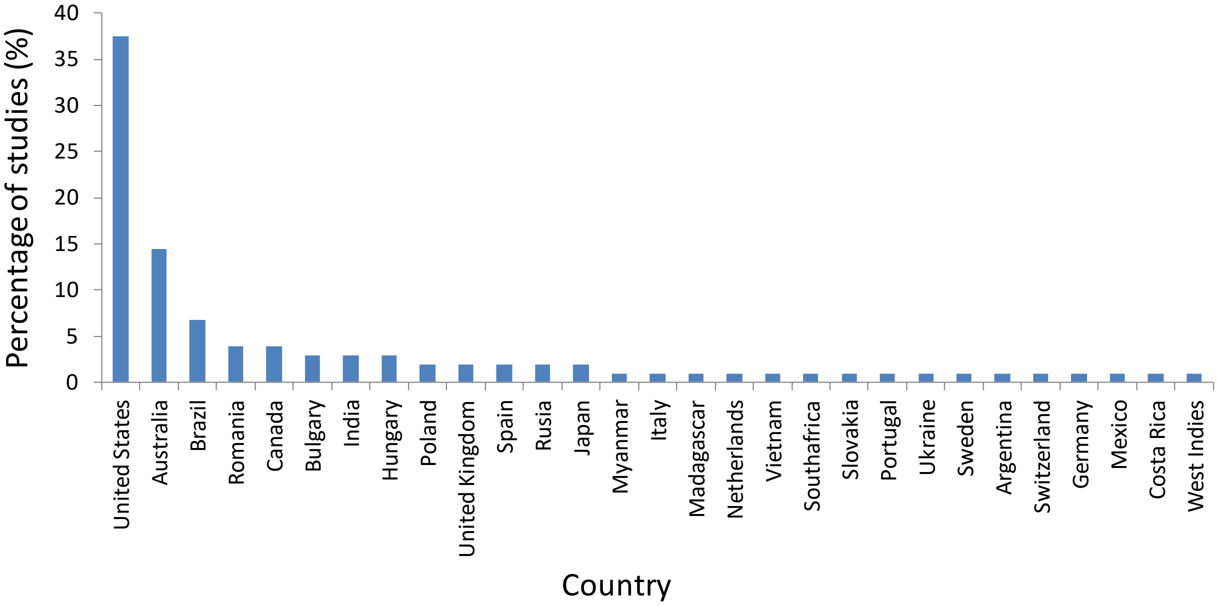

Figure 1. Percentage of urban effect studies on amphibians per country or region prior to November 2020 (n =104).

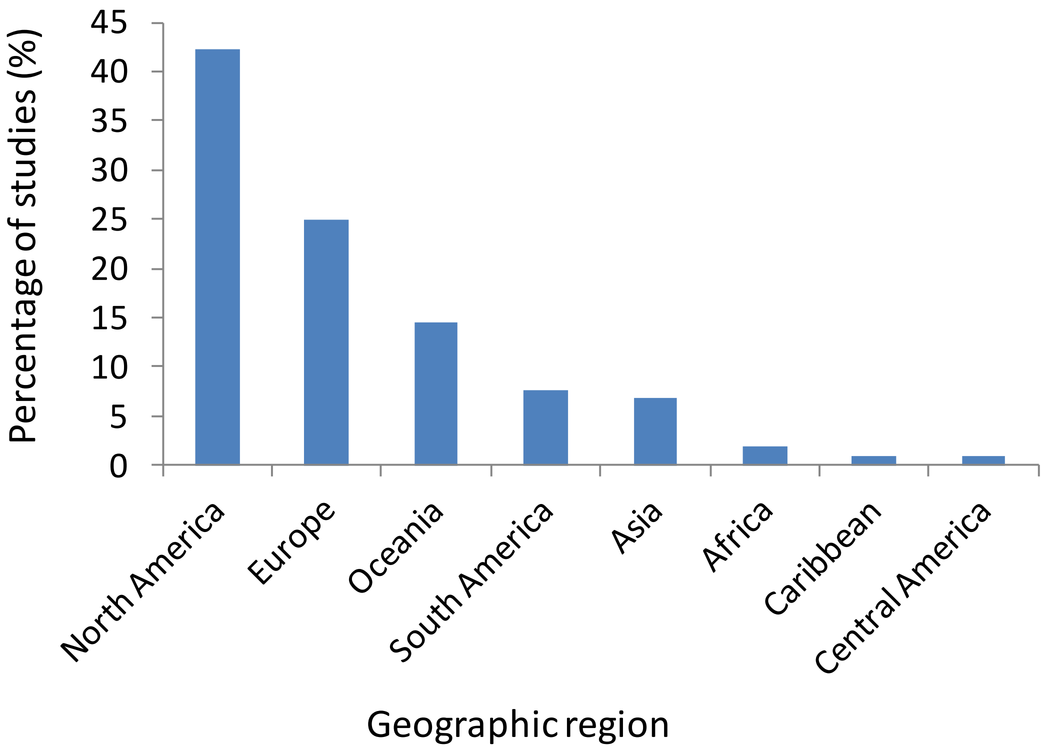

Figure 2. Percentage of urban effect studies per geographic region prior to November 2020 (n = 104).

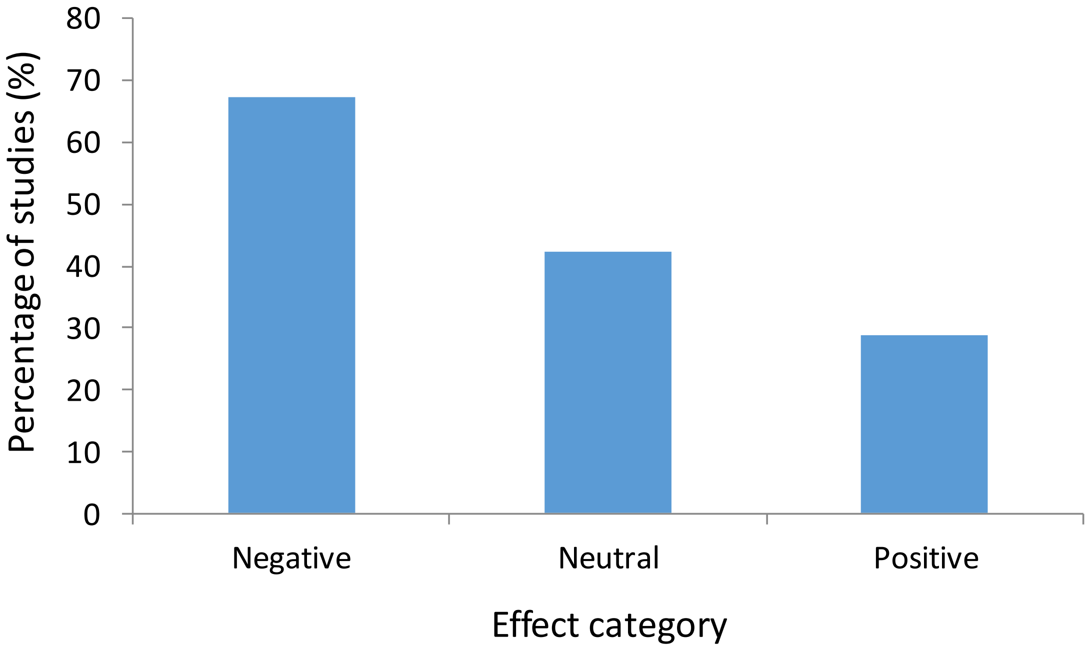

Figure 3. Percentage of studies including amphibians in urban areas, separated by effect category of urbanization on amphibian species. Percentages add up to more than 100% because some studies reported more than one effect in the same publication, for example, a positive effect for one species but a negative effect for another species.

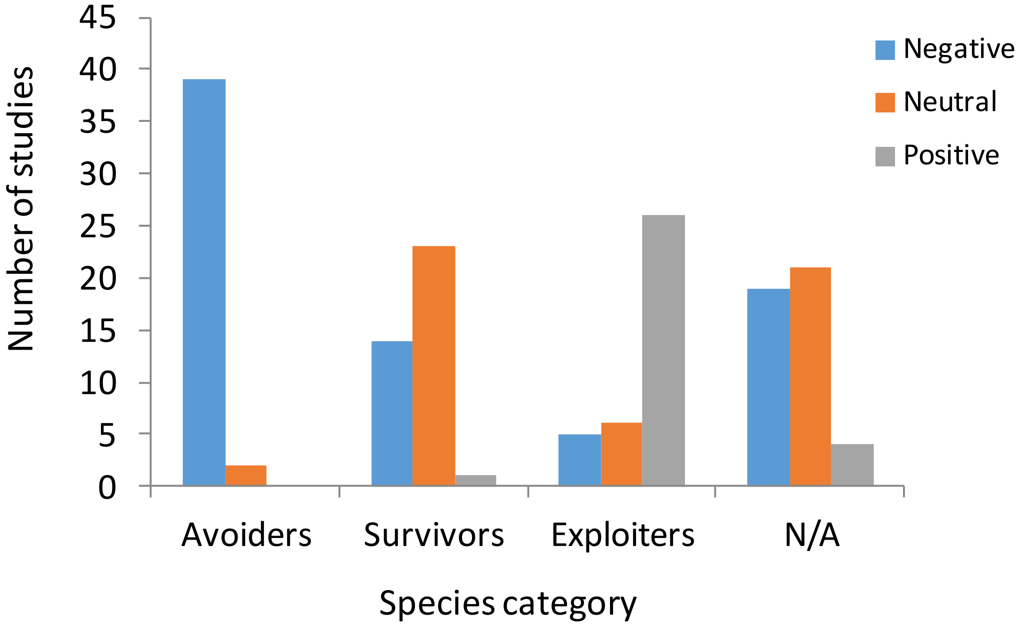

Figure 4. Number of studies with negative, neutral and positive effects for each species category.

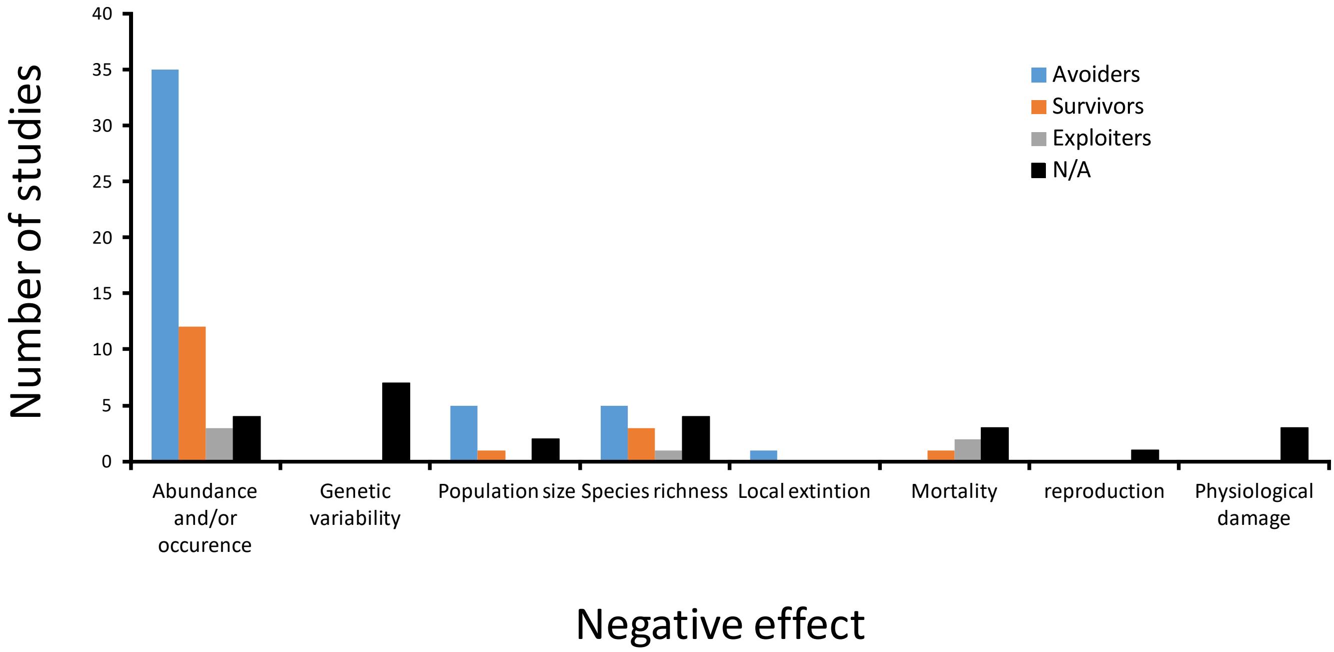

Figure 5. Number of studies reporting each type of negative effect category, separated by species category (avoiders, survivors, exploiters, and N/A).

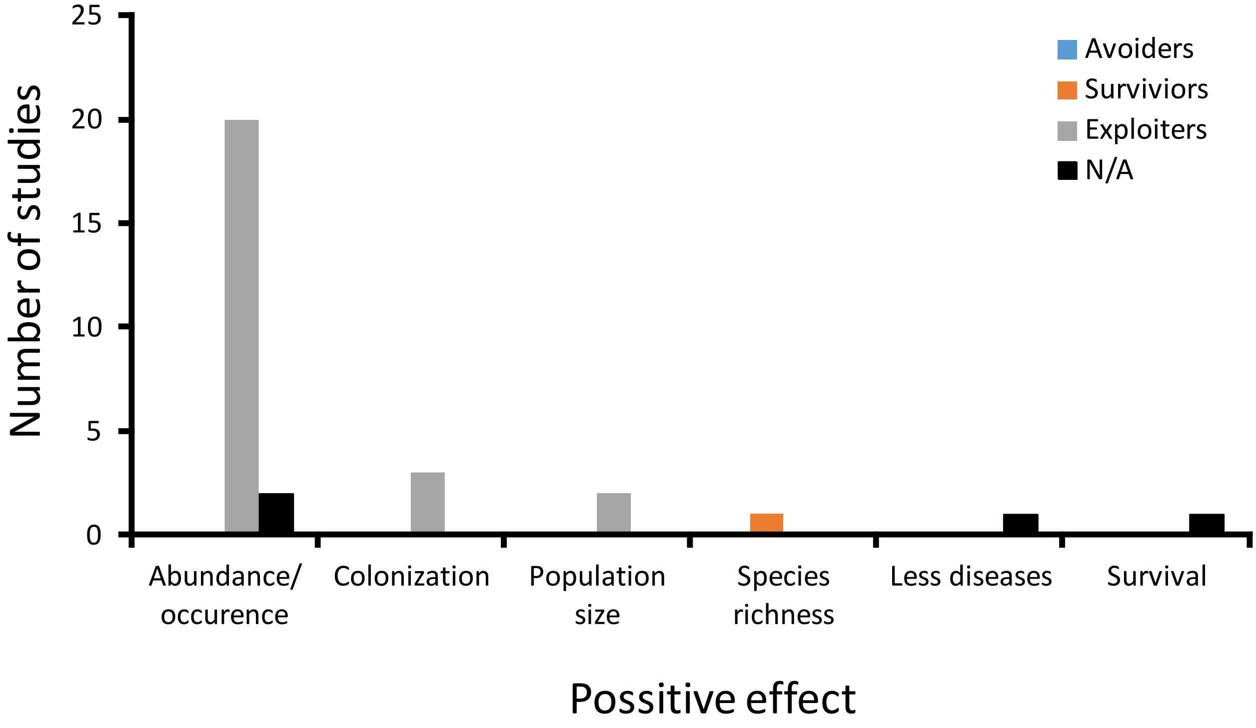

Figure 6. Number of studies reporting each type of possitive effect category, separated by species category (i.e. avoiders, survivors, exploiters, and N/A).

Appendix 1

List of studies included in the present review, found by searching the terms: “Urban herpetofauna”, “Urban amphibians”, “cities” AND “herpetofauna”, “cities” AND “amphibians”, “cities” AND “frogs”, “cities” AND “salamanders”, “cities” AND “newts”, “cities” AND “caecilians”. The studies included in the analysis needed to report the occurrence of amphibians in urban environments (presence, abundance, or compare between urban or another habitats) or report the effects of urbanization on amphibian morphology, behavior, survival, diet, or evolution. Studies that analyze the effect of anthropogenic pollutants in laboratories or experimentally inside cities were excluded, as well as reports of amphibians’ deaths in roads, if roads were outside of cities or did not compare city roads versus rural, or natural roads or a gradient of road density as a measure for urbanization.

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Capítulo 2. Effects of anthropogenic noise on the acoustic characteristics of a glass frog call in urban conditions

Abstract. Many animal groups use acoustic communication as their main form of communication for social interactions. Characteristics of the environment influence the way animals communicate acoustically. Anthropogenic noise is a new feature of the environment within urban areas that can make acoustic communication more difficult because it can overlap with animal vocalizations, producing acoustic masking. Multiple strategies have been used by different animals groups to avoid acoustic masking, but our understanding of acoustic plasticity in amphibians is limited compared to other animal groups. Therefore, our objective was to describe how the Fleischmann's Glass Frog *Hyalinobatrachium fleischmanni* males avoid the effects of anthropogenic noise on the spectral characteristics of its advertisement calls. We selected two sites with different degrees of urban development. We recorded 56 calling males in total, measured 7 acoustic characteristics for each male, and related them to anthropogenic chronic and instantaneousaneous noise levels at each site. The urban site had a higher chronic noise level than the rural site. When related to chronic noise, both call duration and time between calls were longer in the urban site and the call duration and time between calls decreased as the chronic noise increased. Minimum frequency and frequency of maximum amplitude were higher at the urban site. Regarding instantaneousaneous noise, time between calls decreased when instantaneousaneous noise increased but the rest of the acoustic characteristics did not vary. We found that chronic and instantaneousaneous noise affect the acoustic characteristics of Fleischmann's Glass Frog calls differently, and apparently for this species the chronic noise levels have more of an effect on the structure of calls.

Key words: urbanization, anuran, amphibian, *Hyalinobatrachium fleischmanni*, bioacustic, vocalization

Acoustic communication represents one of the main forms of communication in many groups of animals such as birds, mammals, amphibians, and reptiles (Ryan, 2001; Bradbury & Vehrencamp, 1998), and it is used in social interactions such as courtship, copulation, territory defense, or agonistic encounters (McGregor, 2005; Brumm, 2013; Vitt & Caldwell, 2013). The sounds used in acoustic communication have multiple parameters or spectral characteristics such as sound energy (dB), frequency (Hz), duration of the sound, and diel patterns (time of day when it is produced) that allow communication of different messages (Bradbury & Vehrencamp, 1998).

The characteristics of the environment, such as noise from wind and water sources as well as density of vegetation and temperature, influence the way in which animals communicate acoustically, as proposed by the Acoustic Adaptation Hypothesis (Morton, 1975; Hansen, 1979). A new feature of the environment that may affect acoustic communication is the anthropogenic noise produced by vehicle engines (e.g., cars, trains, or planes), factory engines, people, music, and any other source of sound produced by humans (Slabbekoorn, 2013). Anthropogenic noise, contrary to noise of natural origin (produced by rivers, wind, songs of animals), is more constant over time, is of very recent origin (since the mid-eighteenth century with the industrial revolution), and occurs in sites that previously did not have high noise levels (Kunc & Schmidt, 2019). This makes it difficult for many species of animals to communicate using sounds (acoustic signals) within urban areas, given that the frequencies of their vocalizations overlap with the frequencies of anthropogenic noise, masking acoustic signals that occur below the 5 kHz where most of the noise frequencies occur (Wood & Yezerinac, 2006; Hanna et al., 2014).

It has been found that some groups of animals have the ability to modify the spectral characteristics of their sounds to avoid masking by anthropogenic noise (Slabbekoorn & Peet, 2003). Among the strategies used by different groups of animals to avoid acoustic masking, one of the most studied is an increase of the lowest frequencies of their sounds to reduce or eliminate the overlap with noise (Brumm & Slabbekoorn, 2005). This behavior has been reported in several species of birds such as Great Tits *Parus major* (Slabbekoorn & Peet, 2003), Common Blackbird *Turdus merula* (Slabbekoorn & den Boer-Visser, 2006), Rufous-collared Sparrow *Zonotrichia capensis* (Laiolo, 2011), and Rainbow Lorikeet *Trichoglossus haematodus* (Hu & Cardoso, 2010). It has also been found in some amphibians such as the southern brown treefrog *Litoria ewingii* (Parris et al., 2009), the concave-eared torrent frog *Odorrana tormota* (Zhang et al., 2015), the green frog *Rana clamitans*, and the northern leopard frog *Rana pipiens* (Cunnington & Fahrig, 2010). Animals also modify their diel patterns to avoid the hours with higher noise levels. For example, the black-fronted titi *Callicebus nigrifrons* (Duarte et al., 2018) and the cotton-top tamarin *Saguinus oedipus* (Egnor et al., 2007) 2007) produce more vocalizations at times when anthropogenic noise is lower, and some species like the diurnal European Robin *Erithacus rubecula* even shift to sing at night (Fuller et al., 2007). Finally, other species increase their calling rate when anthropogenic noise level is higher because this increases the probability of the receiver getting the message. This behavior has been found in frogs like the Taipei grass frog *Rana taipehensis* (Sun & Narins, 2005) and the triangle tree frog *Dendropsophus triangulum* (Kaiser & Hammers, 2009). Such variation of the acoustic characteristics of sounds or vocalization periods is known as acoustic plasticity (Brumm & Slabbekoorn, 2005).

Acoustic plasticity varies between populations or between individuals of the same population (Cunnington & Farig, 2010; Mendez et al., 2021; Juárez et al., 2021), and it allows species to minimize the masking effects of anthropogenic noise. The effect of anthropogenic noise on the vocalization periods and acoustic characteristics of anuran vocalizations has produced mixed results. For example, the painted chorus frog *Microhyla butleri*, the black-striped frog *Rana nigrovittata,* and the banded bullfrog *Kaloula pulchra* were found to decrease their calling rate due to exposure to anthropogenic noise, whereas the Taipei frog *Rana taipehensis* increased the calling rate with the same stimulus (Sun & Narins, 2005). The males of the common tree frog *Hyla arborea* decreased their calling activity after exposure to traffic noise (Lengagne, 2008), whereas spring peepers *Pseudacris crucifer* modified their call duration and frequencies, but not their call rate after anthropogenic noise exposure (Hanna et al., 2014). Consequently, our understanding of the acoustic plasticity in amphibians to avoid acoustic masking is still limited. For this reason, our main objective is to describe how Fleischmann's Glass Frog *Hyalinobatrachium fleischmanni* males avoid the effects of anthropogenic noise on the spectral characteristics of its advertisement calls. More specifically, we analyze the changes in duration, frequency, call rate, and energy entropy in advertisement calls under different anthropogenic noise levels between individuals and populations. We selected this glass frog as our focal species because it inhabits and breeds on vegetation over small rivers and creeks inside urban environments (Kubicki, 2007; Gutiérrez-Vannucchi et al. 2019). The call of this species has a frequency range between 3.8 and 5.3 kHz (Savage, 2002; Kubicki, 2007), thus overlapping with the frequencies of anthropogenic noise, between 0 and 5 kHz (Wood & Yezerinac, 2006; Hanna et al., 2014). Therefore, acoustic communication in this species may be masked by anthropogenic noise levels, disrupting the effective transmission of the message to the receiver in noisy areas. Due to the overlap between call and noise, we expect Fleischmann's Glass Frog to modify the spectro-temporal characteristics of its calls, to improve the efficiency of its communication. Consequently, we predict that males calling in sites with higher anthropogenic noise levels will have calls with longer call durations (s), higher calling rate (calls/s) (therefore, shorter time between calls), higher lowest, maximum and maximum amplitude frequencies (kHz) and higher average entropy. Entropy describes the amount of disorder for a sound within a selection, by analyzing the distribution of energy in that sound. Higher entropy values mean a great disorder in the sound (Charif et al., 2010). At the population level, we predict that populations with higher anthropogenic noise levels will have males with higher calling frequencies (kHz), higher calling rates (calls/min), longer call duration (s) and higher average energy entropy.

Methods

Study sites

We selected two sites with different urban development to measure the effects of anthropogenic noise on the acoustic characteristics of Fleischmann's Glass Frog call: 1) Rodrigo Facio Campus (hereafter urban site), San Jose province (09°56′ N & 84°05′ W, 1 200 m.s.n.m), which has a mix of secondary forest patches, one to five floor buildings, gardens and isolated trees, surrounded by small secondary roads with low levels of traffic and bigger primary roads with a high level of traffic, and a small creek that runs through the main campus area (Juarez et al., 2021). 2) Calle Ciénega (hereafter rural site), Heredia province, Costa Rica (10°56′ N & 84°04′ W, 1 500 m.s.n.m), that has a mix of secondary forest, coffee plantations, pastures, family houses with gardens, small creeks, and small streets with low levels of traffic. We selected these sites because they are the most contrasting habitats for the Fleischmann's Glass Frog within the Gran Área Metropolitana (the area with more people inhabiting and urban development in Costa Rica; Joyce, 2006). (Fig.1).

Recording and noise level measurement

We recorded a total of 56 calling males from September to October 2021, between 19:00–22:00 h. We recorded 27 males in the rural site and 29 males in the urban site. To avoid recording the same male, we made sure they were at least 5 m apart from each other, and since we recorded them all in the same night, it is very unlikely that the same male moved a long distance into another territory during our recording time. We used a Marantz PMD661 solid state recorder (with WAV recording format, 44.1 kHz sampling rate and 16 bits precision) and a unidirectional Senheiser K6/ME66 microphone to record each male. Each male was recorded continuously for 5 min between 1 – 5 m distance. Additionally, during the recording time of each male we took three measurements of anthropogenic noise level (during 0 – 1 min, 2 – 3 min, and 4 – 5 min) using a SperScientific 840014 mini soundmeter (measurement range 32-130 dB) at fast response and with type A weight. We oriented the soundmeter in the opposite direction of the calling male and we covered with our bodies the sound source produced by calling males to avoid including that sound energy in our anthropogenic noise measurements. We took the maximum noise (hereafter instantaneousaneous noise) and minimum noise (hereafter chronic noise) level in each measurement at 1 m height. Instantaneousaneous noise is a type of noise that is non-stationary and that arises and disappears suddenly in the environment (Lin & Goubran, 2005). Conversely, chronic noise would be the environmental noise that is more constant and stationary over time (Barber et al., 2010; Hohmann et al., 2013). Finally, we obtained temperature and humidity data from World Weather Online (worldweatheronline.com) during the recording days and hours, because anuran activity is known to be highly affected by temperature and humidity conditions (Steelman & Dorcas, 2010; Ospina et al., 2013).

Recording analysis

To analyze the effect of anthropogenic noise on male call characteristics, we selected 5 calls/min randomly, without using the first and the last call recorded to avoid losing one of our measurements (see below). We used calls not overlapping with other sounds or vocalizations and with a high signal-to-noise ratio. We measured the following seven acoustic characteristics in each male call (Table 1): 1) duration (s), 2) minimum frequency (kHz), 3) higher frequency (kHz), 4) frequency of maximum amplitude (kHz), 5) frequency range (kHz), 6) average energy entropy (bits), and 7) average time between calls (s) that was estimated averaging the duration between the previous and posterior call with respect to the call where we measured all other acoustic characteristics (s). We used Raven Pro 1.6 software (Cornell Lab of Ornithology, Ithaca, New York, USA) to obtain each measurement. We used the spectrogram window (Hann window with 50 % overlap and 256 Hz transform size, temporal resolution of 5.8 ms, and frequency resolution of 188 Hz) to select the calls for measurement. We used a threshold of -30 dB related to vocalization´s peak amplitude to obtain the frequency measurements in the power spectrum window (Podos, 1997; Mendez et al. 2021). We used the wave form window to obtain the three duration measurements.

Statistical analysis

We compared the chronic and instantaneousaneous noise levels between the urban and rural populations using two one-way analyses of variance. In both analyses, we used the study site (urban and rural) as the independent variable, and for the first one we used the chronic noise as our dependent variables, and for the second one we used the instantaneousaneous noise as our dependent variable. We performed several Linear Mixed-effect Models (LMM) to test the effects of chronic and instantaneousaneous anthropogenic noise on each of the seven acoustic characteristics of Fleischmann's Glass Frog's calls. In all LMMs, call duration, average time between calls, minimum frequency, higher frequency, frequency range, frequency of maximum amplitude, and the average entropy were used as dependent variables, and the male ID, temperature, and humidity measurements as random effects. In the first seven LMMs, we used the study site (urban or rural), the average chronic noise level per male recorded (continuous variable), and the interaction of study site and chronic noise level as independent variables. For the second seven LMMs, because there was no difference in the instantaneousaneous noise between study sites (see results), we used only the instantaneousantaneous noise level (continuous variable) as our independent variable, but we added study site as another random variable in the model. All analyses were conducted in JMP 7.0 (SAS Institute, Cary, NC, U.S.A.). We reported all values as mean ± SD.

Results

We found differences in the chronic noise level between the urban and rural sites ($t = -5.73$, $df = 36$, $p < 0.001$) where we studied Fleischmann's Glass Frog. The urban site had a higher chronic anthropogenic noise level (53.74 ± 0.92 dB) than the rural site (46.17 ± 0.95 dB). But, there was no difference in the instantaneousaneous noise level between the urban site (59.60 ± 1.39) and the rural site (60.65 ± 1.45; $t = 0.52$, $df = 30$, $p = 0.60$).

Effects of chronic anthropogenic noise on male advertisement calls

The Fleischmann's Glass Frog male call duration was longer in the urban site compared to the rural site ($F_{1.45} = 4.54$, $p = 0.039$; Table 1), and the call duration decreased as the chronic noise increased ($F_{1.45} = 4.84$, $p = 0.033$; Fig. 2). The time between calls was longer in the urban site compared to the rural site ($F_{1.45} = 21.84$, $p < 0.00.1$; Table 1), and the time between calls also decreased as the chronic noise increased ($F_{1.45} = 6.60$, $p = 0.01$; Fig. 2). The minimum frequency ($F_{1.46} = 10.27$, $p = 0.003$; Table 1) and frequency of maximum amplitude were higher at the urban site ($F_{1.46} = 23.35$, $p < 0.00.1$; Table 1). But, males did not change their minimum frequency ($F_{1.46} = 0.36$, $p = 0.55$; Fig. 2) or frequency of maximum amplitude when the chronic noise increased ($F_{1.46} = 0.08$, $p = 0.77$; Fig. 2). The higher frequency ($F_{1.46}= 3.60$, $p=0.064$), the average energy entropy ($F_{1.47} = 1.49$, $p = 0.23$), and the frequency range ($F_{1.46} = 1.86$, $p = 0.18$) were similar between both study sites (Table 1). We also found that the higher frequency ($F_{1.46} = 2.48$, $p = 0.121$), the average energy entropy ($F_{1.47} = 0.01$, $p = 0.91$; Fig. 2), and frequency range ($F_{1.46} = 0.85$, $p = 0.36$) did not change when the chronic noise increased (Fig. 2). None of the seven acoustic characteristics measurements varied according to the interaction between study site and chronic noise ($p > 0.07$ for all interactions).

Effects of instantaneous anthropogenic noise

We found that the time between calls of Fleischmann's Glass Frog males decreased when the instantaneous noise increased ($F_{1.46} = 5.75$, $p = 0.021$; Fig. 3). However, the call duration ($F_{1.47} = 3.57$, $p = 0.07$), the minimum frequency ($F_{1.47} = 0.031$, $p = 0.086$), the higher frequency ($F_{1.47} = 0.50$, $p = 0.48$), the frequency range ($F_{1.48} = 0.81$, $p = 0.37$), the frequency of maximum amplitude ($F_{1.47} = 0.0075$, $p = 0.93$), and the entropy ($F_{1.48} = 0.93$, $p = 0.34$) did not change when the instantaneous noise increased.

Discussion

As expected, the chronic anthropogenic noise was higher in the urban site compared to the rural site, because urbanization means an increase in the density of people living in one site, and along with this, an increase in the traffic, industries, and overall human activities that cause anthropogenic noise pollution, which tends to be most constant over time (Kunc & Schmidt, 2019). We found no difference in the instantaneous noise between sites, probably because even though the study sites have different levels of urbanization, the instantaneous noise sources such as passing vehicles are very similar in both sites. The urban site is surrounded by larger roads than the rural site, but the closest road to the recording areas in the urban population is smaller and, therefore, similar to the one in the rural site in terms of the type of vehicles passing by.

The higher minimum frequency and higher frequency of maximum amplitude at the urban site may be a strategy used for the Fleischmann's Glass Frog males to decrease the acoustic masking produced by higher chronic anthropogenic noise (Slabbekoorn & Peet, 2003; Brumm & Slabbekoorn, 2005), increasing their chances of being heard by both conspecifics and heterospecifics (Brumm et al., 2009; Brumm, 2013). Increasing the minimum frequency and putting more energy on a higher frequency may reduce the overlap with anthropogenic noise, which tends to have more energy at lower frequencies (Brumm & Slabbekoorn, 2005). This strategy has been reported in other anuran species such as the brown tree frog (*Litoria ewingii*) (Parris et al., 2009; Higham et al., 2021), leopard frog (Cunnington & Fahrig, 2010), and the concave-eared torrent frog (*Odorrana tormota*) (Zhang et al., 2015), and several species of birds (Slabbekoorn & Peet, 2003, Slabbekoorn & den Boer-Visser, 2006; Laiolo, 2011), which vocalize at higher frequencies at noisier sites.

The lack of relationship between the chronic anthropogenic noise variation with the four frequency measurements and average energy entropy of the glass frog call was probably caused because the variation in chronic noise between each male during the recording period was not large enough to elicit a differential response. This idea is supported by our results, because we found differences in chronic noise between the two studied sites, so this means that the variation in chronic noise measured during each male recording within sites was similar within the studied site. Additionally, the lack of a relationship between changes in noise levels within each individual territory or singing perch with frequency characteristics of vocalizations has been reported in both white-eared ground-sparrow *Melozone leucotis* (Mendez et al. 2021) and tree swallows *Tachycineta bicolor* (McIntyre et al., 2014). In these species, the authors mentioned as a possible cause that the species may adjust the amplitude of the call instead of the frequency (McIntyre et al., 2014), which may also be the case for our study. The authors also mentioned that the differences in the call characteristics were related to the learning process from other individuals as well as calling time, rather than the noise (Mendez et al., 2021), which would not explain what we found in this study because anuran vocalizations are innate and not learned.

We found that Fleischmann’s Glass Frog males decreased the time between calls when chronic and instantaneous noise increased (they call at higher call rates). This increase in the call rate has also been found in the neotropical tree frog *Dendropsophus triangulum* (Kaiser & Hamers, 2009), and in the Taipei grass frog *Rana taipehensis* (Sun & Narins, 2005), where both species increased their call rates when exposed to higher levels of anthropogenic noise. A similar pattern has been found for bird species like domestic chickens *Gallus gallus domesticus* (Brumm et al., 2009) and Japanese Quail *Coturnix japonica* (Potash, 1972), which increased their call rate when noise increased. This strategy most likely increases the probability of being heard by potential receivers during short quiet moments that might take place during noisier moments (Brumm & Zollinger, 2013).

We also found that the call duration was longer at the urban site compared with the rural site, which is a pattern similar to that observed in gray tree frogs *Hyla versicolor* (Shwartz et al., 2013), common marmosets *Callithrix jacchus* (Brum et al., 2004), and killer whales *Orcinus orca* (Foot et al. 2004). All of these species increased the duration of their calls with increasing background noise level, which agrees with the Call Detection Hypothesis (Schwartz et al., 2013). This hypothesis states that males may increase call duration even if they lower their call rate with increasing background noise because they can be more easily detected, and therefore may increase the detection of their calls by females (Schwartz et al., 2008, Schwartz et al., 2013)

In conclusion, we found that the two most common anthropogenic noise levels (chronic and instantaneous) have distinct effects on the acoustic characteristics of Fleischmann’s Glass Frog calls. Our results show that apparently for Fleischmann´s glass frog males, the levels of chronic noise are more critical and have more of an effect on the structure of their call, as compared to the instantaneous noise. It is important to take into account the variation in the noise between sites and within sites when anthropogenic noise levels are measured, as this can help better explain the patterns found. Finally, it is also important to start conducting more studies that compare the responses to chronic and instantaneous noise, to better understand how animals adapt to this pollutant.

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Tables and figures

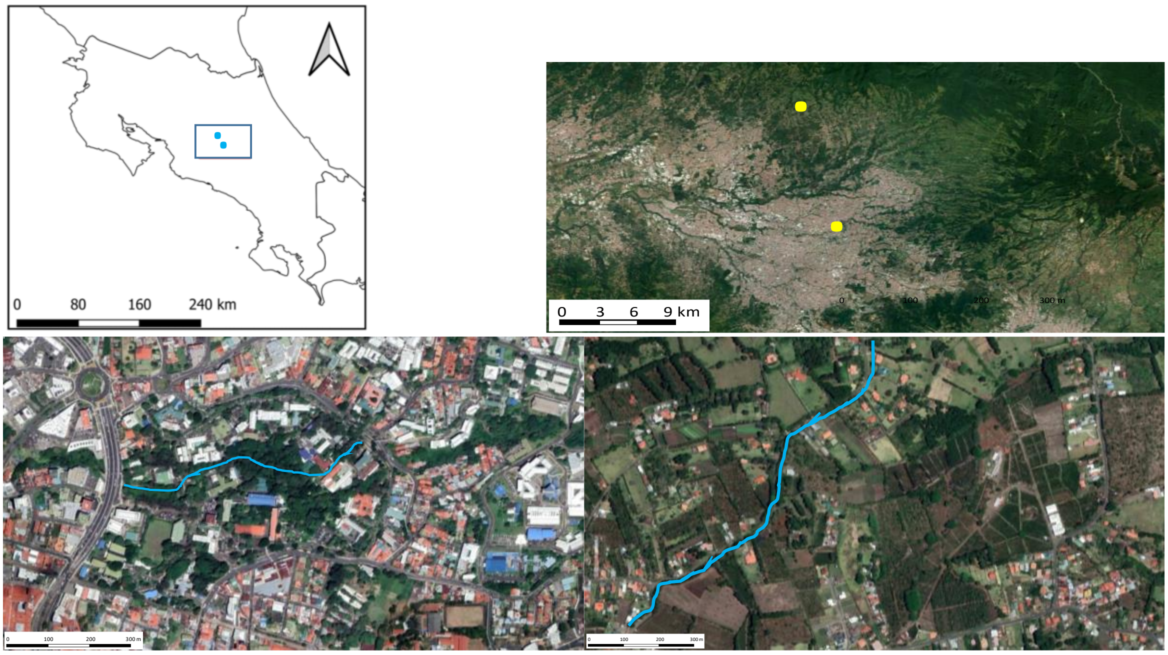

Figure 1. A. Map of Costa Rica with the two study sites marked with blue dots, and the square marking the Gran Área Metropolitana, where the study sites are located. B. Yellow dots mark the two study sites, the yellow dot on the bottom marks the urban study site, and the yellow dot on the top marks the rural study site. C. Urban study site with a creek marked in blue. The course of this creek was followed to place the automated recorders. D. Rural site with the creek followed to place the automated recorders marked in blue.

Table 1. Acoustic characteristics of Fleischmann's Glass Frog calls, in two sites with different levels of urbanization. Values are average ± SD.

Acoustic characteristics	**Urban (N = 29)**	**Rural (N = 27)**
Call duration (s)	0.20 ± 0.05	0.18 ± 0.04
Time between calls (s)	9.73 ± 4.1	6.76 ± 3.2
Minimum frequency (dB)	3645.1 ± 170.3	3471.2 ± 172.9
Higher frequency (dB)	4764.05 ± 176.7	4574 ± 169.9
Frequency range (dB)	1119.0 ± 178.32	1103.8 ± 184.0
Frequency of maximum amplitude (dB)	4462.04 ± 177.8	4181.3 ± 188.01
Entropy	2.03 ± 0.20	1.95 ± 0.43

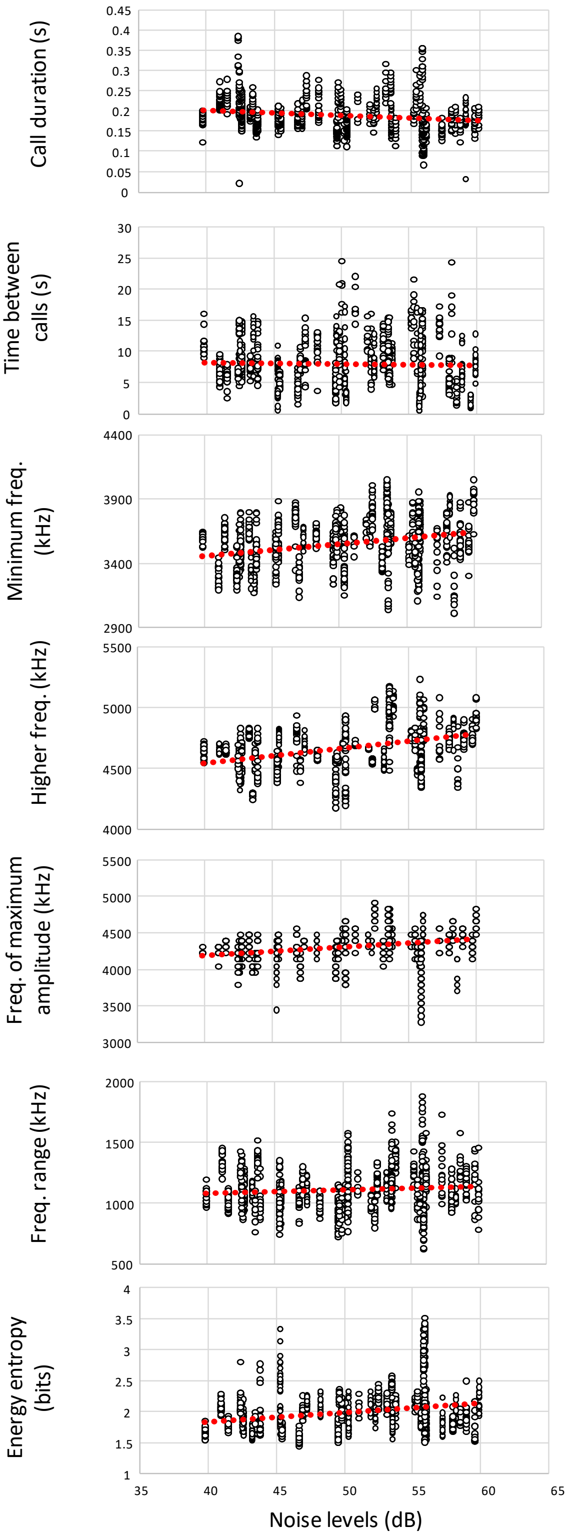

Figure 2. Relationship between seven acoustic characteristics of Fleischmann's Glass Frog male calls, and the chronic anthropogenic noise level (dB).

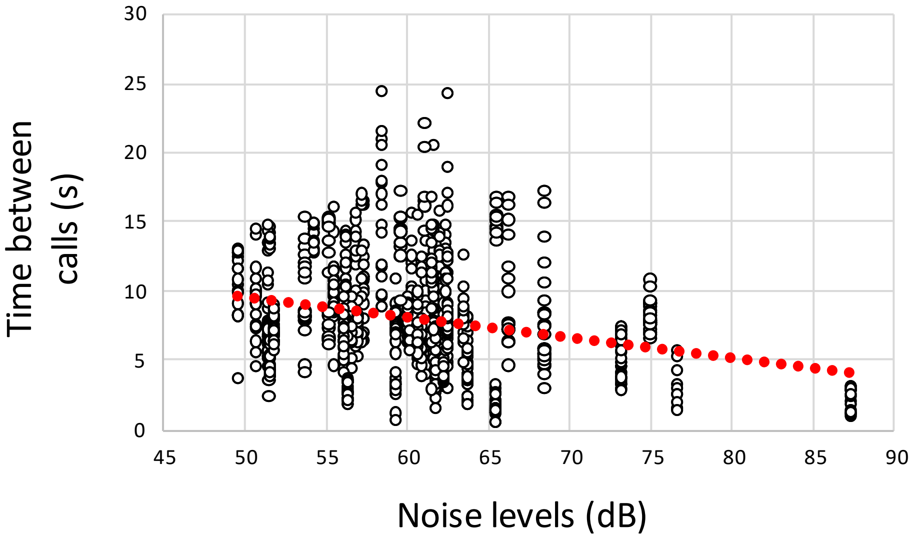

Figure 3. Relationship between the time between calls in Fleischmann's Glass Frog male calls and the instantaneous anthropogenic noise level (dB).

Capítulo 3. Effects of light pollution and noise on the vocal activity of a glass frog in urban conditions

Abstract. Urbanization causes light pollution which alters natural patterns of light and dark. Light pollution may have a great impact on multiple organisms, but its effects on animals have been less studied than the effects of other pollutants. Another common pollutant present in urban areas is noise, which, as well as light pollution, can affect important processes for animals including communication. Acoustic communication in anurans is important for their social interactions, and variation in the acoustic activity can become a determining factor for their effective communication. Our main goal was to determine the possible effects of light pollution and noise on the acoustic activity of the Fleischmann’s Glass Frog. We worked at two sites with different levels of urbanization, noise, and abundance of artificial lights, and measured the light intensity and background noise at each sampling point within each site. We divided sampling points into “light” and “dark” within each study site and recorded vocal activity from 16:00 h to 6:00 at each sampling point. We calculated a proportion of calls per hour at each sampling point and related it to the background noise and light intensity levels. There was a difference in the call proportion at different hours of the night, with the peak calling activity from 19:00 to 22:00 h. Background noise affected calling activity patterns differentially throughout the night, which might be related to mating status and female availability. There was no effect of light intensity on the calling activity of Fleischmann’s Glass Frog. Light pollution might be more critical to anuran species living in more open areas where there is less vegetation that can cover them from artificial lights, and for species that need almost complete darkness to call at night.

Key words: urbanization, amphibian, anuran, *Hyalinobatrachium fleischmanni*, diel pattern

Urbanization increases the amount of artificial light sources at night (Longcore & Rich, 2004; Hopkins et al., 2018). This type of light includes any light source of anthropogenic origin - street lights, stadiums, houses, cars, advertisements - (Perry et al., 2008), which tends to be much brighter than natural light and affects all urban areas worldwide (Cinzano et al., 2001; Bennie et al., 2015). This causes light pollution in urban and natural areas, which is defined as an excess of light from artificial sources that alters the natural patterns of light and dark (Cizano et al., 2001; Longcore & Rich, 2004). Additionally, the brightness of the artificial lights is reflected in the clouds during nights, causing a greater illumination in areas adjacent to urban centers (Cinzano et al., 2001), expanding light pollution beyond the sites where artificial light sources are present.

Despite the great impact that light pollution may have on animals, plants, and ecosystems, its effects have been much less studied than those of other types of pollution of anthropogenic origin such as solid pollution, noise pollution, or chemical pollution (Longcore & Rich, 2004; Perry et al., 2008, Corrales-Moya et al., 2021). However, studies in light pollution include mostly birds, fish, insects, mammals, and reptiles (Metcalfe et al., 1997; Sharma et al., 1997; Witherington & Martin, 2000; Kramer & Birney, 2001; Tuxbury& Salmon, 2005; Miller, 2006; Bourgeois et al., 2009; Byrkjedal et al., 2012; Riley et al., 2012; Kamrowski et al., 2012; Raap et al., 2015). In diurnal bird species, such as American Robin *Turdus migratorius,* European Robin *Erithacus rubecula,* Common Blackbird *Turdus merula,* Eurasian Wren *Troglodytes troglodytes,* and Great Tit *Parus major* changes were reported in their singing patterns, such that birds vocalized earlier in the mornings and later at night, reducing their resting time (Miller, 2006; Byrkjedal et al., 2012;;Raap et al., 2015). In fish such as the Atlantic Salmon *Salmo salar*, artificial light increased prey detection, allowing them to feed longer, and altered nocturnal migration timing (Metcalfe et al., 1997; Riley et al., 2012). Mammals, such as some nocturnal rodents (e.g., Patagoninan Leaf-eared Mice *Phyllotis xanthopygus* and Field Mice *Mus booduga*), changed their circadian cycle and reduced nocturnal activity (Sharma et al., 1997; Kramer & Birney, 2001). Studies to measure the effect of this contamination on reptiles have focused mainly on the study of the adverse effects of artificial lighting on sea turtle nesting behavior (Witherington & Martin, 2000; Tuxbury & Salmon, 2005; Bourgeois et al., 2009; Kamrowski et al., 2012), being important for the conservation of nesting beaches. Finally, amphibians are not the exception to the lack of information on the effect of light pollution, despite being a mainly nocturnal group (Savage, 2002; Perry et al., 2008).

Another important pollutant present in urban areas is noise, especially from anthropogenic sources (Slabbekoorn, 2013). This type of noise can alter behavioral and physiological processes, including communication (Cronin et al., 2022). In general, noise may mask animals' acoustic signals if it overlaps with their frequency range (Hannah et al., 2014), because it interferes with the propagation of sound waves, making acoustic communication more difficult at sites where noise is higher (Fuller et al., 2007). One of the strategies animals can use to avoid being masked by background noise is by calling at times where background noise is lower (Shwartz & Bee, 2013). For example, the European Robin *Erithacus rubecula* has been found to call at night, when the background noise level is lower, despite the fact that it is typically a diurnal bird (Fuller et al., 2007). Mammals like the Black-Fronted Titi *Calicebus nigrifrons* have been found to call more when noise levels are lower (Duarte et al., 2018). Another strategy used by some animals to overcome acoustic interference by background noise is by modifying their calling rate. For example, *Rana taipehensis* and *Dendropsophus triangulum* (Sun & Narins, 2005; Kaiser & Hammers, 2009) call at higher rates when noise levels are higher, presumably to increase the chances of being heard.

Acoustic communication in frogs and toads is very important for all their social interactions (Ryan, 2001). Therefore, the diel variation of their acoustic activity also becomes a determining factor for effective communication between individuals (Schwartz & Bee, 2013; Vitt& Caldwell, 2013; Bevier, 2016; Colafrancesco & Gridi-Papp, 2016). An increase in illumination and noise at night may change the hours of activity, by reducing the vocal activity or delaying the start of vocalization time (Meshaka et al., 2014, Henderson & Powell, 2001; Perry & Fisher, 2006). How these changes in vocal activity may affect mate attraction, territory defense, or predator detection is still greatly understudied. This lack of information about light pollution and noise effects on frogs and toads is more critical in urban areas, because the levels of light pollution and noise are higher and more constant, potentially generating negative effects for the species that live in those habitats. Therefore, studying the effect of light pollution and noise on anuran species that inhabit urban areas is necessary to generate corrective and management measures that facilitate the conservation of these species.

Consequently, our main objective is to determine the possible effects of light pollution and noise on the acoustic activity of an urban frog species, the Fleishman’s Glass Frog *Hyalinobatrachium fleischmanni*. We chose the Fleishman’s Glass Frog as our model species, because is very commonly found in urban areas of Costa Rica, where it uses creeks and small rivers to call and reproduce (Kubicki, 2007). Inside those habitats, it is common to find it calling in very illuminated areas as well as in very dark sites. Given that this is a nocturnal species that is common in urban areas, its acoustic communication (e.g., temporal pattern or amount of vocalizations) may be affected by the intense and constant artificial lights and both constant and sudden noise present in these environments. We predict calling activity patterns of Fleishman’s Glass Frog will be affected by both light pollution and noise. We expect the species to have different diel patterns between sites with higher and lower levels of light pollution and to call proportionally more at times with lower levels of noise, to avoid acoustic masking.

Methods

We worked at two sites with different levels of urbanization, noise, and abundance of artificial lights. 1) A more urbanized site that was at the Rodrigo Facio Campus, Montes de Oca, San Jose province, Costa Rica (09°56′ N & 84°05′ W, 1 200 m.s.n.m.). This was the site with more artificial lights from street posts, more buildings, and a very high internal traffic level. Also, this site is surrounded by two major highways that add extra noise and light pollution from the cars that move around the study area. 2) A less urbanized site that was at Calle Hernández , San Rafael, Heredia province, Costa Rica (10°56′ N & 84°04′ W, 1 500 m.s.n.m.). This site includes a mix of houses and coffee plantations, with a single street with a very low traffic level, and therefore it tends to have lower levels of anthropogenic noise. There is a street post approximately every 100 m. We chose these sites because they have different levels of light pollution and noise within the Gran Área Metropolitana, because we could easily find areas with higher and lower levels of light pollution within each site, and because the glass frog species is abundant at both sites.

Light measurements and recordings

In order to analyze the effect of light pollution on the acoustic activity of Fleishman's Glass Frog during the night in sites with different levels of light pollution, we selected sampling points with high levels (light point) and low levels (dark point) of light intensity. The minimum distance between points of the same or different light type was 30 m and the maximum 200 m. At each point, we took five maximum and minimum light intensity measurements (in lux) at 1.5 m from the ground, using a digital luxometer EXTECH Instruments LightMeter LT 300. One measurement was made at the center of point where the autonomous recorder was placed (see below), and the other four measurements at 2 m away from the center, 90 degrees from each other (Fig. 1). We took the light intensity measurements on new moon nights, to avoid the effect of moon light intensity in measurements. This sampling approach allowed us to compare between light and dark sampling points inside each and between the two study sites that vary in average light pollution levels.

We recorded frog vocal activity from 16:00 to 6:00 h (14 h of recording per sampling point) at seven light and seven dark points at the site with higher level of urbanization; and five light and three dark points at the lower site with lower level of urbanization. At each sampling point we recorded 59 minutes/hour, using song Meter SM2+ and SM3 autonomous recorders (Wildlife Acoustics) set to 32kHz sampling rate and 16-bit precision in WAV format. The recordings were made between the months of September and November 2021, which are within the rainy season in Costa Rica and when Fleishman's Glass Frog has its breeding peak (Savage, 2002; Kubicki, 2007). Finally, we measured the temperature each hour using the internal thermometer of the Song Meter SM2+, because anuran activity is known to be affected by environmental conditions (Savage, 2002).

Recording analysis

We analyzed 10 minutes per hour (from minute 20 to 30), for one day (from 16:00 h to 6:00 h) on each dark and light sampling point. We selected only 10 minutes because after analyzing the whole hour for 14 hours, we found the same patterns for both measurements (Appendix 1). We counted the total number of Fleshman's Glass Frog calls in the selected 10 minutes per hour using the automatic Band Limited Energy Detector for the spectrogram in Raven Pro 1.6 (Cornell Lab of Ornithology, Itaca, New York, USA) sound analysis software using the following configuration: 3500 kHz minimum frequency, 4600 kHz maximum frequency, 0.13 seconds minimum duration, 0.30 seconds maximum duration, 0.01 seconds minimum separation, 50% minimum occupancy and 5dB SNR threshold. With this configuration we obtained the least amount of false positives and negatives. After the automatic detection we conducted a manual review to correct for false positives or negatives.

We used Raven Pro 1.6 (Cornell Lab of Ornithology, Itaca, New York, USA) to extract noise values manually from the 10 minutes analyzed in each hour recorded. We measured sections of 0.5 s in the spectrogram window, at times that did not overlap with frog vocalizations at the beginning (before the first 5 analyzed minutes) and at the end (after the first 5 analyzed minutes). From each 0.5 section, we obtained the relative values of amplitude (average amplitude in FSdB, Raven Pro user manual) for eight 1/3-frequency octave bands from 44.7 to 7079 kHz. We used the first 1/3 of each octave band to define the low and high frequency limits of each 0.5 s section (Sanchez et al., 2022). With these measurements we calculated the average relative ambient noise levels for every 10 minutes analyzed. The microphones used were not calibrated to obtain absolute measurements of amplitude, and therefore the noise levels obtained are relative amplitude values in which noisy sites have values closer to 0 and less noisy sites have more negative values (Sanchez et al., 2022).

Statistical analysis

As the total number of calls every 10 minutes per hour changes according to the total number of individuals in each sampling site, a comparison of the raw data between sites will produce inaccurate results. Therefore, we estimated the proportion of calls counted during each 10 minutes according to the total calls counted from 16:00 to 6:00 h per sampling site. This call activity value was not affected by the difference in the number of males between sampling sites, and made all of the sites comparable. We transformed the proportion value for every 10 minutes using the arc sine square root transformation, and we used this transform value as our response variable in all our analysis.

We estimated the average of minimum and maximum light intensity for the light and dark sampling points, and we used these two values as our measurements of light pollution in the analysis. We compared if light and background noise levels changed between both treatments (light and dark sites) and between study sites (more and less urban site) using a linear mixed model that included the site where we placed the recordings as our random variable. We conducted a generalized linear mixed model with zero-inflated and Gaussian distribution to test if the proportion of calls per hour changed according to recording hour (14 levels), light treatments (light and dark sites), and background noise levels (continuous) and its second order interactions. In this model we used the study site, the site where we placed the recordings, and the average temperature per hour as random variables. This analysis was conducted with R package NBZIMM (https://github.com/nyiuab/NBZIMM).

Results

We analyzed a total of 196 h in the more urbanized site (Light site: 98 h. Dark site: 98 h), and 112 h in the less urbanized site (Light site: 70 h. Dark site: 42 h). Light intensity level was higher at the less urbanized site ($F_{1,272} = 854.9$, $p < 0.001$; Fig. 2), contrary to what we expected, and it was higher in the “light” sites compared to the “dark” sites ($F_{1,272} =$

2792.6, $p < 0.001$; Fig. 2). “Light” sites had a higher light intensity level at the less urbanized sites, but “dark” sites were similar in both study sites ($F_{1,272} = 1295.5$, $p < 0.001$; Fig. 2). The background noise level was higher at the site with more urbanized site compared to the site with the less urbanized site ($F_{1,274} = 17.4$, $p < 0.001$; Fig. 2), and background noise was also higher in the “dark” sites compared to the “light” sites ($F_{1,272} = 15.4$, $p < 0.001$; Fig. 2), but did not show an interaction effect ($F_{1,272} = 0.03$, $p = 0.86$; Fig. 2).

We found a difference in the call proportion across the hours of the night (Table 1, Fig. 3). The call proportion had a rapid increase at the beginning of the night with an activity peak from 19:00 to 22:00 h, followed by a progressive decrease in the calling activity after 22:00 h (Fig. 3). The lowest activity was at 16:00 and 05:00 h (Fig. 3). We found that proportion of calling activity changed differently depending on the hours and background noise (Table 1, Fig. 4). At the beginning of the night (16:00-19:00 h), the proportion of calls was lower when background noise was higher (Fig. 4). At the peak activity hours (20:00-23:00 h) the proportion of calls remained constant at different levels of background noise (Fig. 4). After the peak activity to the end of the night (00:00-05:00 h) the proportion of calls were higher at higher background noise levels (Fig. 4). Finally, we found no effects of the treatment (dark and light sites), noise, the interaction between treatment and noise, or the interaction between hour and treatment in the calling activity of Fleishman’s Glass Frog (Table 1).

Discussion

We found differences in artificial light intensity between sites, with the less urbanized site having a higher level of artificial light intensity, contrary to our expectations. Although there were fewer artificial light sources in the less urbanized site (pers. obs.), the intensity of each one of these lights was higher (street lights with LED bulbs) than the intensity of the lights used at the more urbanized site (i.e., incandescent light bulbs). This showed the importance of not only taking into account the amount of artificial light sources at the different study sites, but also the different types of lights used. We found lower levels of background noise at the less urbanized site, agreeing with our expectations, because more urbanized areas have higher number of anthropogenic noise sources (Parris, 2013; McMullen et al., 2014; Tennessen et al., 2016). In our studies sites, this was likely due to the more urbanized site being surrounded by larger roads and being located inside of a university campus, where more cars arrive each day.

We found that the peak calling activity of Fleishman's Glass Frog males occurred between 19:00-22:00 h. This diel pattern was similar to the pattern reported for *Dendropsophus, Boana,* and *Leptodactylus* species at Goiás, Brazil, where the peak calling activity was between the first and fourth hour after sunset (Guerra et al., 2020). This diel pattern has been related to the amount of energy stored by males of prolonged breeder species, like the Fleishman's Glass Frog (Kubicki, 2007), which need to save energy to keeping calling on consecutive nights (Bevier, 1997; Castellano & Gamba, 2011). Therefore, cessation of calling after midnight will save energy for the next night, given that calling is a very expensive activity in frogs due to the high level of oxygen consumption compared to basal metabolic rate (Wells & Taigen, 1989; Wells, 2007).

Frogs in our study presented a differential response to background noise levels depending on the hour of the night. It is possible that at the beginning of the night it is less critical to be heard by females, because there are still many hours in the night to attract a possible mate and copulate, and several males are calling (Fig. 3). Additionally, females probably are more available and searching males more actively also during the first hours of the night, increasing the chance of finding a mate without calling if it is noisier (Woolbright, 1985; Lopez & Narins, 1991). However, as the night progresses, it becomes more critical for males that have not yet found a mate to be heard by females, because their chances of finding a female and copulate decrease. This could be a reason that males are maintaining, and in some cases increasing, the proportion of calls later in the night at high levels of background noise.

Another possibility to explain this differential response across the hours of the night is that this species might be responding differently to different sources of background noise that can appear at different times of the night. Such a pattern has been found previously for Blue Whales *Balaenoptera musculus* that did not have a diel pattern in response to noise, but did have a differential response depending on the noise source (Melcon et al., 2020). Blue whales were less likely to call when mid-frequency active sonar was present, and even more at closer distances, but the calls increased in response to ship noise when they were close (Melcon et al., 2020). A similar result was also found for the white-throated sparrow *Zonotrichia albicolis,* in which this species changed its singing behavior in response to different types of noise. White-throated Sparrows were less likely to sing in the presence of vocalization from other birds and car noise, but instead changed the duration and frequency of their songs in response to choruses of spring peeper *Pseudacris crucifer* (Lenske & La, 2014). Thus, later at night in both study sites the dominant background noise came from insects (i.e., crickets or katydids) that produce sounds at higher frequencies than anthropogenic noise (Gerhardt & Huber, 2002), having less of an impact on frog communication.

We did not find an effect of light pollution (amount of light intensity) on the call proportion of Fleishman's Glass Frog throughout the night. This result means that there was no difference in the calling activity patterns between our light and dark sites. This may be because Fleishman's Glass Frog males are very small (SVL= 22-27 mm) and vocalize while perched on leaf vegetation (Kubicki, 2007), which means they may have the ability to shelter from light under the layer of vegetation. For this reason, light pollution at night may not influence vocal activity as has been reported in others anuran species that call in more open areas like pastures or street puddles (Baker & Richardson, 2006; Hall, 2016; McMahon et al., 2017; Dias et al. 2019). Additionally, the lack of differences in calls between light and dark sites may be result of acclimatization to light after being continuously exposed, which can make species behave in the same way under different light pollution conditions (Dias et al., 2019). To test this acclimatization effect in Fleishman's Glass Frog calling activity, it would be necessary to compare populations that have never experienced light pollution with populations that have been exposed, such as in our two studied populations.

Another possible explanation for our results is that the intensity of light at our study sites is not intense enough to elicit a differential response in Fleishman's Glass Frog males, especially if individuals are hiding in the vegetation to call (Kubicki, 2007). The illumination reported for a partly sunny day is 50 000 lux. In comparison a lighted parking lot at night has an illumination level of 10 lux (Rich & Longcore, 2013), similar to the average light intensity that we measured in the light sites of the less urbanized study site (Fig. 2). Frogs that are active at night could have a disruption of their nocturnal activity and photoperiodic behavior if artificial light at night is bright enough to be similar to diurnal dim light or twilight (Buchanan, 2006), but presumably that would be much higher than the levels we measured. Light pollution might be more critical for species that have almost never been exposed to light (e.g., interior forest species), but this is not the case for our study species, which we observed calling several times after 15:00 h if it was a rainy day and the temperature was under 20° C (pers. obs.). Low levels of light pollution or a small increase in nocturnal light levels might affect the calling activity of species that are more recently exposed to urbanization and its pollutants, or that never call during daylight hours as Fleishman's Glass Frog does.

In conclusion, we found no significant effect of light pollution on the vocal behavior of Fleishman's Glass Frog, possibly due to a combination of factors that include: low intensity levels of light pollution, the habitat where this species lives in urban centers, and a long period of exposure that might be producing acclimatization of the species, especially because this glass frog does not need complete darkness to call. Light pollution might be more critical to anuran species living in less dense vegetation or open areas, and especially for species that have only recently been exposed to urbanization and an increase in light levels at night, and that need extremely low levels of light to call. We found that background noise, including anthropogenic noise, affects the calling activity patterns of Fleishman's Glass Frog differentially throughout the night, and may be associated with mating status and female availability during the night. Both ideas need to be tested in the field or under laboratory conditions.

We recommend focusing future studies of light pollution and noise to amphibians in tropical regions, which would help to increase our understanding of the effects of urbanization pollutants on amphibians and thus the decisions and changes we need to implement in our cities to ensure the survival of this group in urbanized areas. We also recommend taking into consideration other aspects of light pollution aside from intensity, such as the sources, amounts, and spectral compositions of artificial lights. Doing so would contribute to filling the knowledge gaps in the effects of light pollution on anuran species.

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Tables and figures

Table 1. Summary of the results for generalized linear mixed models with zero-inflated and Gaussian distribution, used to test if the proportion of calls changed according to different variables and their interactions.

	F	df	p
Hour	15.11	1,263	< 0.001
Treatment	0.37	1,263	0.54
Noise	2.31	1,263	0.12
Hour x Treatment	0.52	1,263	0.47
Hour x Noise	16.96	1,263	<0.001
Treatment x Noise	0.39	1,263	0.53

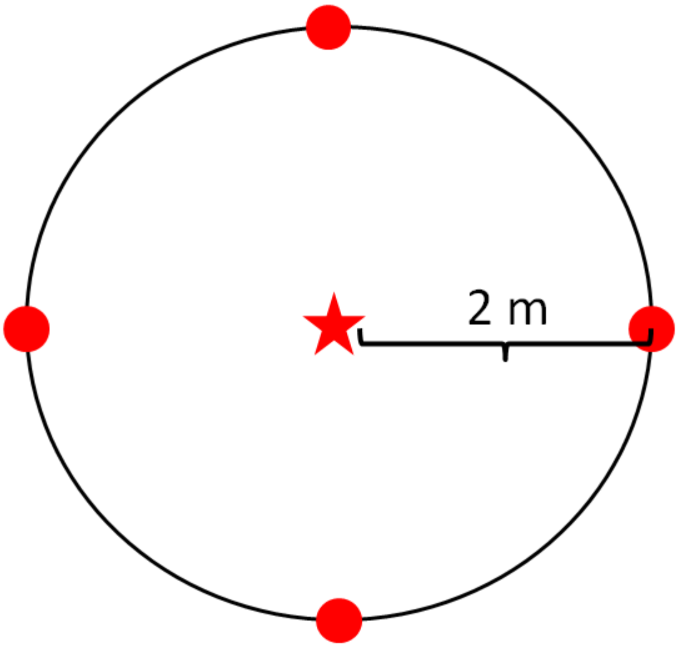


Figure 1. Light intensity measurement points within each light and dark sampling point. The star at the center marks the location of the recorder and one light intensity measurement point. The red dots mark four light intensity measurement points taken 2 m apart from the recorder and at 90° from each other.

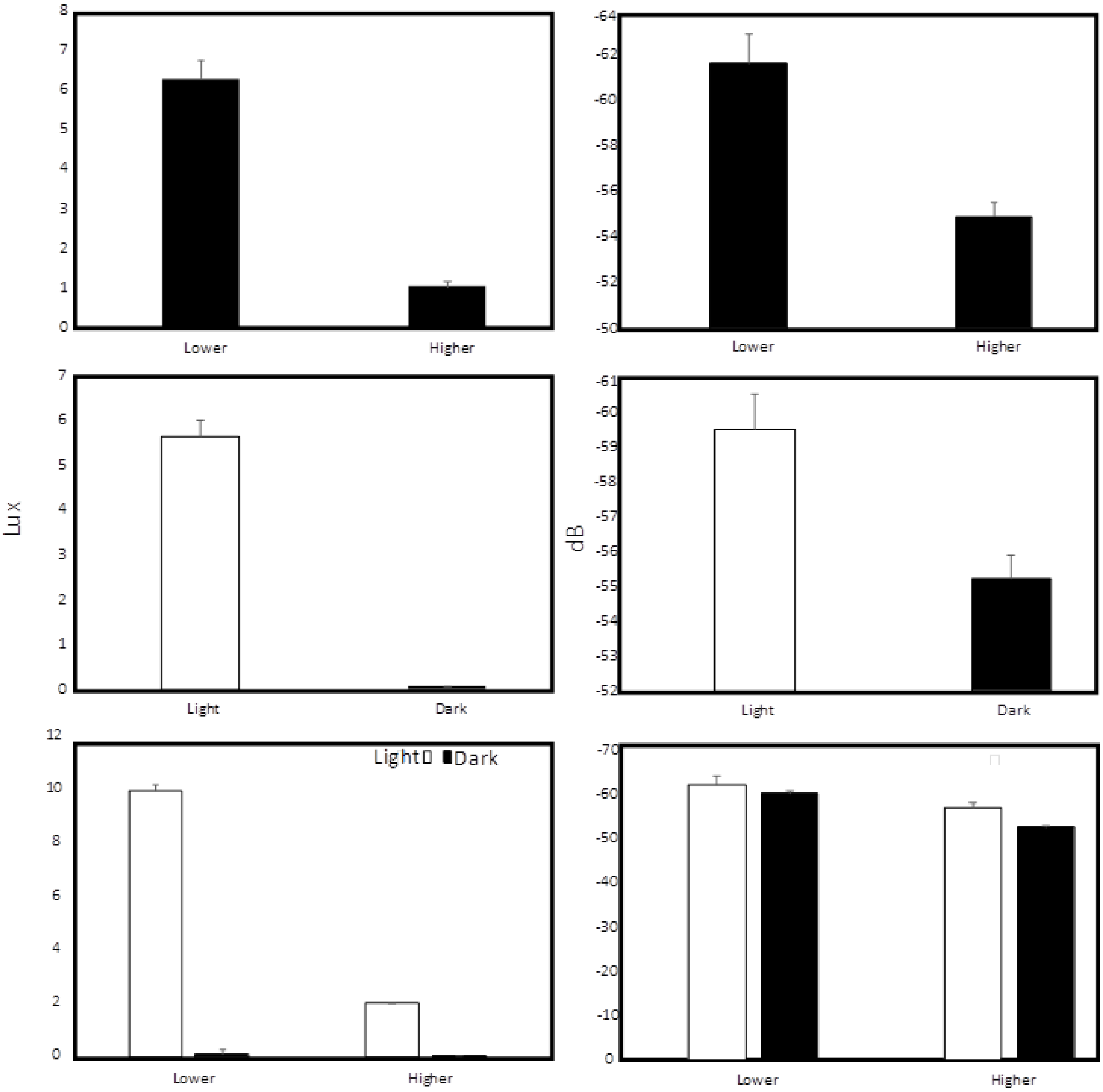


Figure 2. Figure shows average levels of light intensity (lux) and background noise level (dB -1) between sites with lower and higher levels of urbanization in our study (first line), between light and dark sites in our study (second line), and for the interaction of light intensity and study site (third line on the left), and the interaction of background noise and study site (third line on the right).

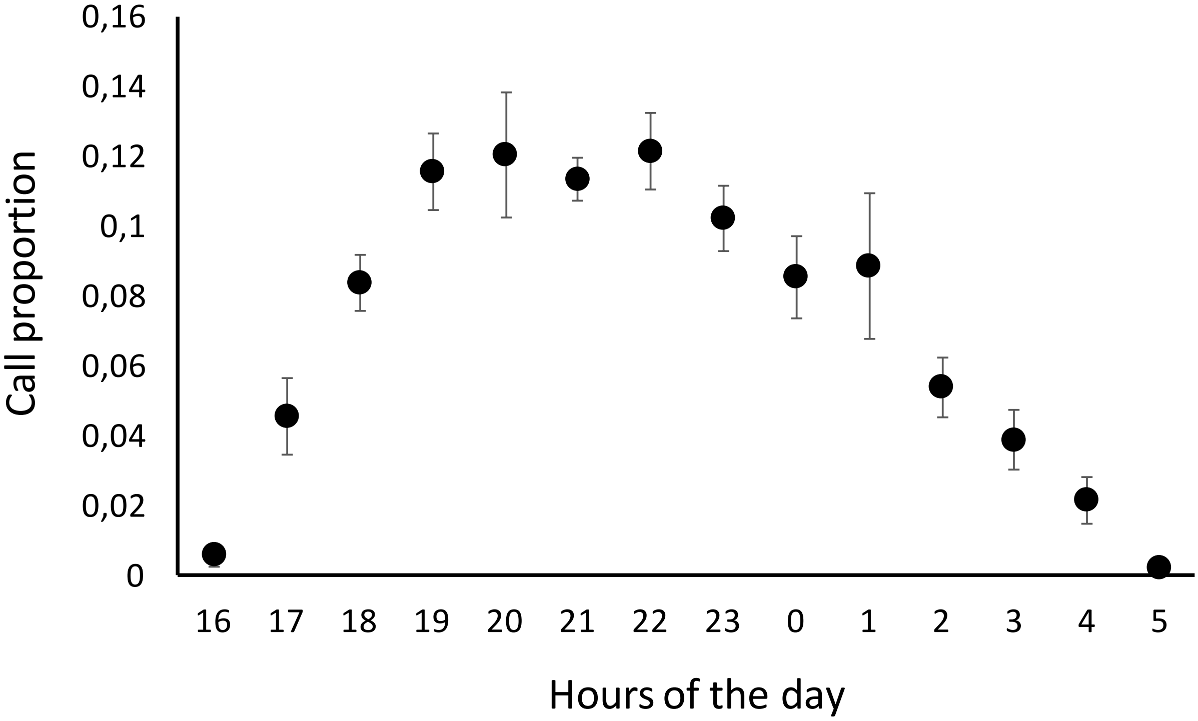


Figure 3. Proportion of Fleischmann’s Glass Frog calls at different hours of the day. The dots represent the mean call proportion per each recorded hour including all sampling points in the study, with their standard deviation represented by the error bars.

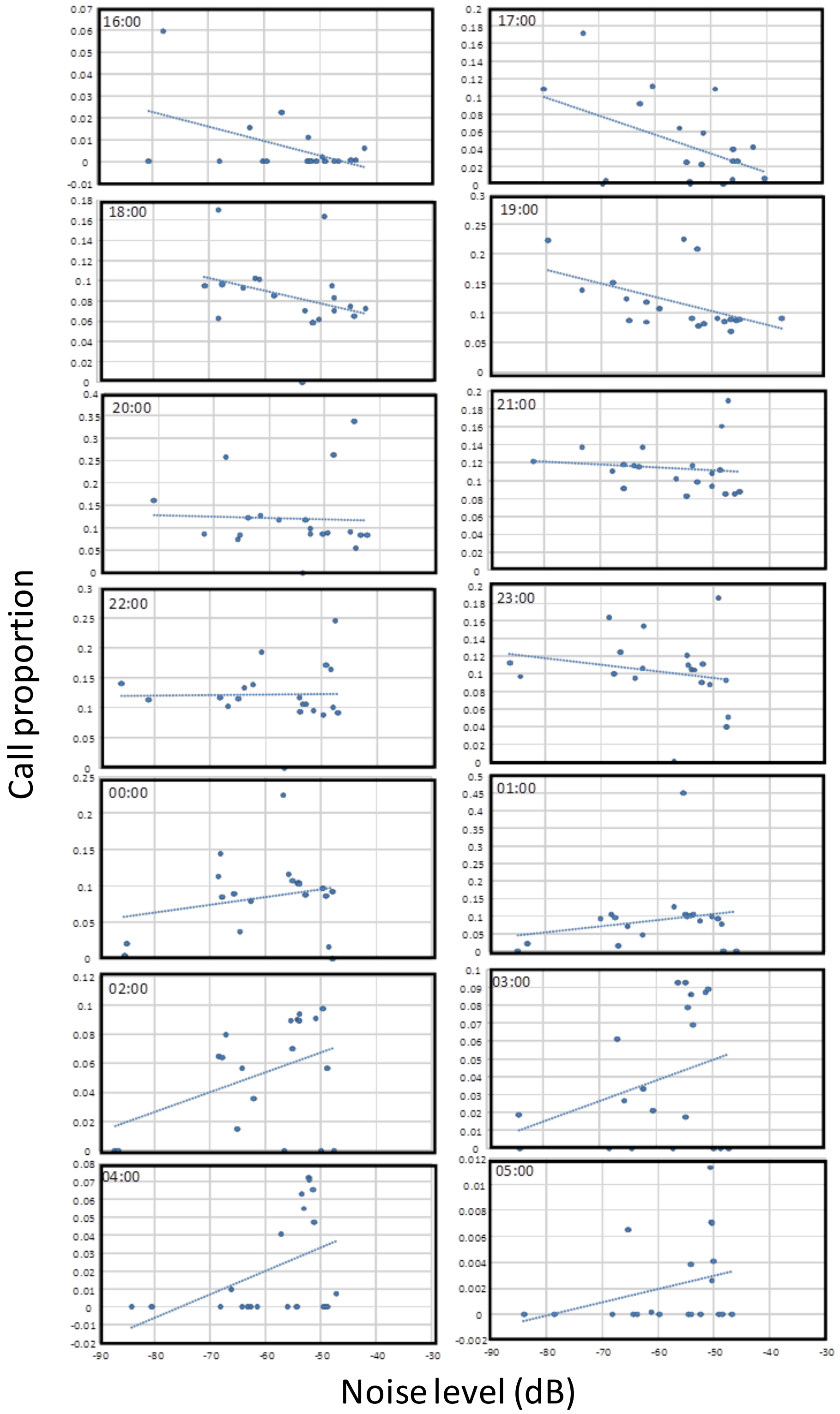
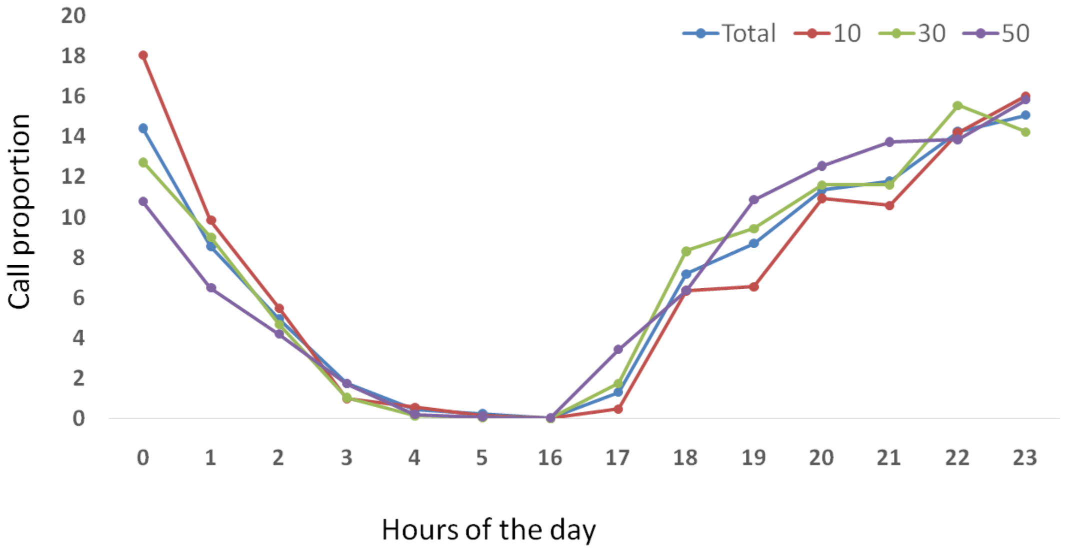


Figure 4. Proportion of calls in relation to the background noise level (dB) for each recorded hour from 16:00 h to 6:00 h. Each square shows the call proportion distribution according to the background noise level (dB) for each of the 14 recorded hours separately.

Appendix 1



Fleischmann’s Glass Frog calling activity throughout the night. Blue line indicates distribution of calls when a full hour was analyzed, red line shows distribution when analyzing minutes from 10.1 to 20, green line shows distribution when analyzing minutes from 20.1 to 30 and purple line shows distribution analyzing minutes from 50.1 to 59 to Fig 1 shows the distribution of call proportion throughout the night when we analyzed the full hour and 3 different options of 10 minute intervals. We performed a Kolmogorov-Smirnov test and found that there are no differences between the call distribution of a full analyzed hour and any of the 10 minute interval options (D=0.14, P=0.99). For this reason, we selected the interval between 20.1 to 30 minutes to analyze, because it is in the middle of the recording period and there is less chance of any mistake that can be made in the minutes between one hour and the next.

CONCLUSIONES

Los resultados de nuestro análisis de la literatura muestran que la urbanización representa un efecto negativo para la mayoría de especies de anfibios estudiadas en zonas urbanas. Estos efectos negativos estuvieron asociados principalmente a baja abundancia y riqueza de especies y tamaños de poblaciones más pequeños que en zonas naturales o con menos grado de urbanización. Y que, por el contrario, un grupo de especies reducido obtuvo beneficios por parte de los sitios urbanos y se convirtieron en las especies más comunes dentro de estas áreas. Otra importante conclusion, es que existe una disparidad en la cantidad de estudios provenientes del norte-global en comparación con el sur-global, viviendo la mayoría de estudios del norte-global donde la riqueza de anfibios es menor. Esta disparidad hace que nuestro entendimiento de los efectos de la urbanización en anfibios este sesgada a un grupo pequeño de especies. Además, la falta de estudios sobre el tema en el sur-global hace difícil la comparación directa entre sitios urbanos y naturales en esta área del mundo.

Por otra parte, hay una falta de estudios a largo plazo tanto para los efectos generales de la urbanización, como para los efectos de contaminantes más específicos como el ruido antropogénico y la contaminación lumínica. Esta falta de información de largo plazo reduce el potencial impacto que puede tener la información generada en diversos estudios a la hora de tomar decisiones informadas en cuanto a la conservación de anfibios en áreas urbanas, y para el mejoramiento de la planeación urbana a futuro.

Otra importante concusión en nuestro estudio es que el ruido antropogénico, tanto crónico como instantaneousáneo, es capaz de afectar las características acústicas de las vocalizaciones de *Hyalinobatrachium fleischmanni*, sin embargo, se necesitan más estudios para determinar el efecto que esos cambios en estructura. Es por ende, altamente posible que el ruido antropogénico sea capaz de alterar también las vocalizaciones de otras especies de anuros presentes en ambientes urbanos, o en sitios con un alto nivel de ruido, los cuales son cada vez más comunes debido al rápido desarrollo urbano en la actualidad. Para *H. fleischmanni* particularmente, el ruido crónico afecta de mayor manera la estructura de sus vocalizaciones, comparado con el ruido instantaneousáneo, por lo que es importante prestar especial atención a este tipo de ruido.

Además, el ruido de fondo, incluyendo el ruido antropogénico, afecta la actividad vocal de *H. fleischmanni* de manera distinta a lo largo de la noche. Es decir, la especie responde de manera diferente a altos niveles de ruido, dependiendo de la hora de la noche en la que se encuentre vocalizando. Esto podría estar asociado con el estado de apareamiento o disponibilidad de hembras a lo largo de la noche, sin embargo, estas ideas deben probarse en campo o laboratorio.

No encontramos efectos significativos de la contaminación lumínica sobre la actividad vocal de *H. fleischmanni*, posiblemente debido a una combinación de factores que incluyen: baja intensidad en los niveles de contaminación lumínica, el hábitat donde se encuentra la especie dentro de las zonas urbanas y tiempos prolongados de exposición a la luz artificial que podrían estar causando aclimatación en esta especie. Es posible que la contaminación lumínica sea más crítica para especies de anuros que viven en sitios con vegetación menos densa, donde les sea más difícil esconderse de la luz artificial, y especialmente para especies que hayan sido expuestas a contaminación lumínica de manera reciente y que necesiten de oscuridad casi total para vocalizar.

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