

TERRESTRIAL AMPHIBIANS INHABITING AN URBAN XERIC ECOSYSTEM: AN ASSESSMENT OF FROGS AND SALAMANDERS IN PROTECTED AREAS FROM MEXICO CITY

ANFIBIOS TERRESTRES QUE HABITAN EN UN ECOSISTEMA XÉRICO URBANO: UNA EVALUACIÓN DE RANAS Y SALAMANDRAS EN ÁREAS PROTEGIDAS DE LA CIUDAD DE MÉXICO

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Received: 2021-07-12. Accepted: 2022-02-21. Published: 2022-03-17.

Editor: Irene Goyenechea Mayer Goyenechea, México.

Resumen.— Los anfibios son particularmente sensibles a las perturbaciones causadas por la urbanización. Los disturbios urbanos impactan a los anfibios en diferentes escalas ecológicas que incluyen una disminución de la biodiversidad, aumento del estrés y cambios en el comportamiento vocal. Sin embargo, se sabe poco sobre los efectos urbanos sobre los anfibios terrestres. En este estudio, cuantificamos la abundancia (por tasas de encuentro) y la riqueza de especies de anfibios, y las características de sus hábitats en el Pedregal del Xitle, un ecosistema xerofítico en la Ciudad de México. Además, evaluamos la actividad vocal de las ranas del Pedregal bajo diferentes exposiciones al ruido dentro de las tres áreas protegidas. Durante 2015 y 2016, con el apoyo de voluntarios, se realizaron varios registros nocturnos directos estratificados en tres áreas urbanas protegidas. Además, utilizando una clasificación no supervisada de imágenes de satélite, analizamos la cobertura de los diferentes tipos de vegetación y calculamos la equitatividad de los anfibios. Con un esfuerzo de 238 horas-persona, localizamos 73 individuos de anfibios, entre ellos tres salamandras pletodóntidas (*Aquiloerycea cephalica*, *Chiropterotriton orculus* y *Pseudoeurycea leprosa*), y la rana del pedregal, *Eleutherodactylus grandis*. En conjunto, las capturas de *C. orculus* y *E. grandis* representaron el 90% de los anfibios encontrados. Encontramos que el 75% de los anfibios ocupaban el microhábitat de suelo rocoso. La tasa de cantos de *E. grandis* fue consistentemente más baja en los sitios con mayor exposición al ruido. Entre las tres áreas protegidas urbanas, Ecoguardas sobresale por ser la que tiene mayor abundancia y diversidad de anfibios y mayor proporción de superficies boscosas. Nuestro estudio destaca el valor ecológico del ecosistema del Pedregal para los anfibios terrestres, ya que son las especies más amenazadas (la rana *E. grandis* y la salamandra *C. orculus*) aquellas que tienen mayor presencia en los sitios estudiados.

Palabras clave.— anfibios urbanos, aplicaciones de percepción remota, ecología urbana, especies en peligro de extinción, perturbación del ruido.

Abstract.— Amphibians are particularly sensitive to disturbances caused by urbanization. Urban disturbances impact amphibians at different ecological scales that include a decrease in biodiversity, increment of stress, and changes in vocal behavior. However, little is known about urban effects on terrestrial amphibians. In this study, we quantified abundance (by encounter rates) and species richness of amphibians, and the characteristics of their habitats in Pedregal del Xitle, a xerophytic ecosystem in Mexico City. In addition, we evaluated the vocal activity of Pedregal frogs under different noise exposures within the three protected areas. During 2015 and 2016 with the support of volunteers, several stratified direct night searches were conducted in three urban protected areas. Additionally, using a non-supervised classification of satellite images, we analyzed the coverage of the different vegetation types, and calculated the evenness of amphibians. With an effort of 238 person-hours, we located 73 individuals of amphibians, including three plethodontid salamanders (*Aquiloerycea cephalica*, *Chiropterotriton orculus* and *Pseudoeurycea leprosa*), and the Pedregal frog, *Eleutherodactylus grandis*. Altogether, the captures of *C. orculus* and *E. grandis* represented 90% of the amphibians found. We found that 75% of the amphibians occupied the rocky ground microhabitat. The call rate of *E. grandis* was consistently lower at sites with the highest noise exposure. Among the three urban protected areas, Ecoguardas stands out as the one with the greatest abundance and diversity of amphibians and the largest proportion of forested area. Our study revealed the ecological value of the Pedregal ecosystem for terrestrial amphibians, since the most threatened species (the frog *E. grandis* and the salamander *C. orculus*) are those with the greatest presence in the urban protected areas studied.

Keywords.— endangered species, noise disturbance, remote sensing applications, urban amphibians, urban ecology.

INTRODUCTION

The presence of protected and relict areas embedded in megalopolis has a positive effect on local biodiversity (Gaston et al., 2006; Goddard et al., 2010; Pimm et al., 2014). However, these areas also have the presence of threats associated with biodiversity, such as species displacement, local extinctions (McDonald et al., 2008) and changes on phenotypic traits, for example behavioral changes of feeding, migration and singing (Alberti, 2015). The establishment of big cities contributes to the fragmentation of natural areas, therefore, monitoring efforts to evaluate biodiversity in fragmented urban landscapes are a priority for the conservation of local fauna. Current monitoring efforts are mainly focused on estimating effects on species replacement along disturbance gradients (McDonnell & Hahs, 2008; Beninde et al., 2015). Unfortunately, these studies are taxonomically biased by birds and insects, probably because these groups exhibit greater diversity, and there is a more complete knowledge about their biology compared to other taxa (Beninde et al., 2015). For this reason, the population status of local taxa, such as amphibians, in urban landscapes is probably underestimated (Hamer & McDonnell, 2008; MacGregor-Fors et al., 2015).

Amphibians are a group of animals particularly sensitive to changes in environmental conditions produced by urbanization (Smallbone et al., 2011), mainly due to humidity levels and water

quality required for their survival and reproduction (Sodhi et al., 2008). Several urban conditions, such as noise, luminosity, and isolation, severely impact the diversity, distribution (Smallbone et al., 2011; MacGregor-Fors et al., 2013; Oda et al., 2017), stress levels (Tennessee et al., 2014; Troianowski et al., 2017), and larval development of amphibians (Grace et al., 2020). Today, an increasing number of studies are investigating the effects of urbanization on amphibians, they mainly focus on evaluating the consequences of anthropogenic noise on the vocalization of anurans (Warren et al., 2006; Roca et al., 2016; Simmons & Narins, 2018). To evaluate the effects of urban pollution on acoustic communication, many studies use remote sensing tools, such as automatized recording units. The remote sensing tools accomplish a double function by informing the presence of species that produce sounds, and assessing their response to urban disturbances (Dorcas, 2010; Teixeira et al., 2019). Until now, there is no single response pattern in the anuran communication signals with urban interference. Some responses include the decrease in the number of calls and the call rate, others the increase in the dominant frequency or the duration of the calls, and in many species, there is an absence of an effect (Sun & Narins, 2005; Cunnington & Fahrig, 2010; Kaiser et al., 2011; Simmons & Narins, 2018).

In the case of non-vocalizing amphibians, as salamanders and caecilians, studies of the effects of urbanization on their communities are rather scarce (Murphy et al., 2016). Indeed, the

study of amphibian populations within cities has been centered on aquatic (pond) breeding species, whose assemblages are strongly altered by water pollution, and are dependent on broad forested land areas (Rubbo & Kiesecker, 2005; Parris, 2006; Hamer & McDonnell, 2008; Smallbone et al., 2011; Scheffers & Paszkowski 2012; Barrett & Price, 2014; Oda et al., 2017). Nevertheless, the life cycle of amphibians is diverse, several species of anurans, as salamanders and caecilians, are direct-developing terrestrial breeders, lacking an aquatic phase, some include viviparity (Crump, 2015). Although terrestrial amphibians are widely distributed and considered as climatic

generalists (Wake & Hanken, 1996; Bolochio et al., 2020), the effects of urbanization on this particular amphibian functional group are not well documented. This is unfortunate considering that, in terms of evolution, the ecological transition from aquatic to terrestrial breeders has occurred multiple times and in a wide variety of environments (Gomez-Mestre et al., 2012; Liedtke et al., 2017).

An example of a terrestrial amphibian with a restricted distribution, and whose habitat has been highly degraded in Mexico City, is the endangered *Eleutherodactylus grandis*. This is

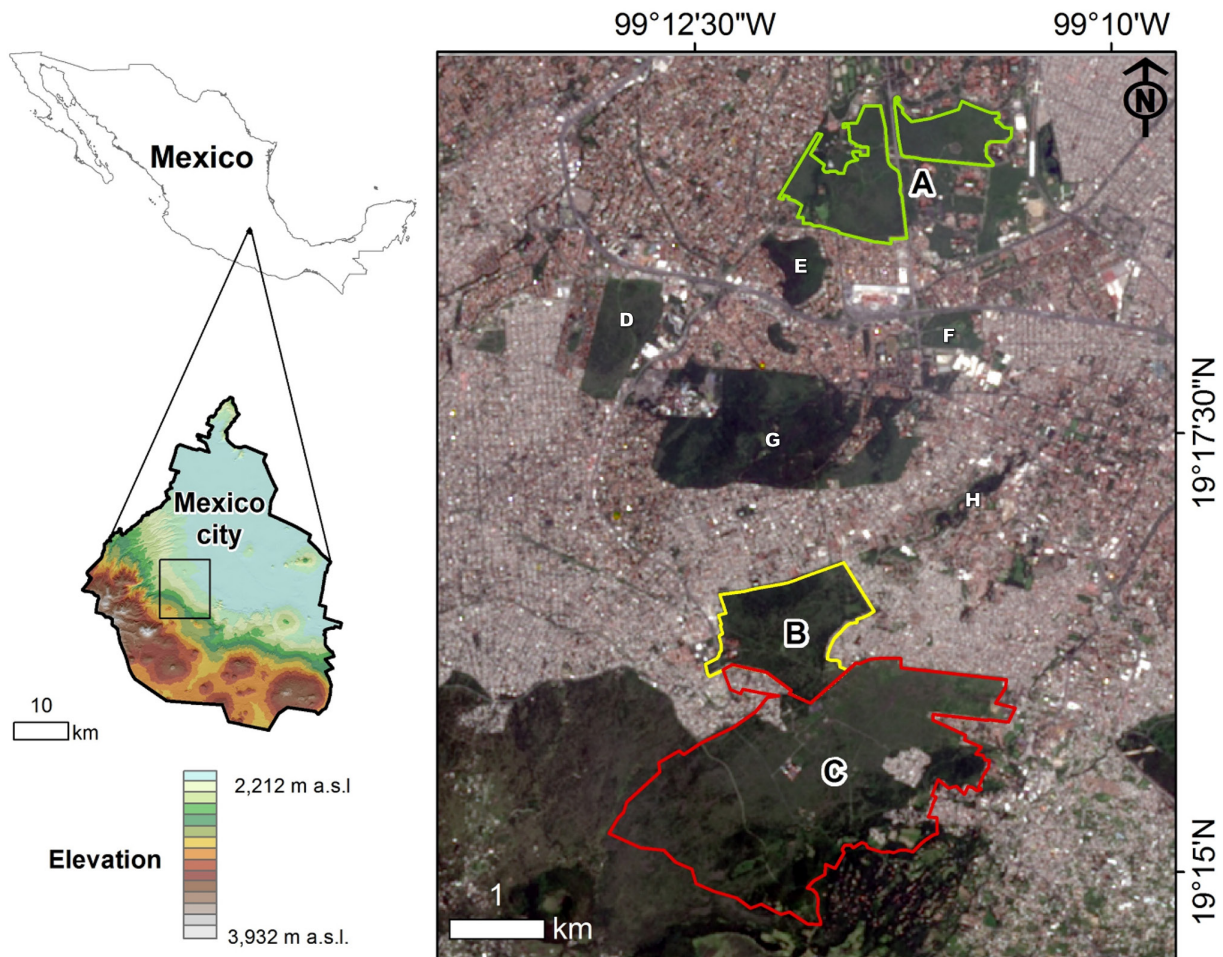


Figura 1. El ecosistema del Pedregal del Xitle está inmerso en un gradiente altitudinal y de urbanización que son paralelos de norte a sur en la Ciudad de México (izquierda). Las tres reservas urbanas estudiadas se indican en la imagen de la derecha: A. Reserva del Pedregal de San Ángel (REPSA), B. Ecoguardas y C. Parque Ecológico de la Ciudad de México (PECM). Otros fragmentos de Pedregal relevantes pero no incluidos en este estudio son: D. Predio Los Encinos, E. Cerro Zacatepetl, F. Cuicuilco, G. Bosque de Tlalpan, y H. Parque Nacional Fuentes Brotantes de Tlalpan.

Figure 1. Pedregal del Xitle ecosystem is immersed in an altitudinal and urbanization gradient which are parallel from north to south in Mexico City (left). The three urban reserves studied are indicated in the right image: A. Reserva del Pedregal de San Ángel (REPSA), B. Ecoguardas and C. Parque Ecológico de la Ciudad de México (PECM). Other fragments of Pedregal relevant but not included in this study are: D. Predio Los Encinos, E. Cerro Zacatepetl, F. Cuicuilco, G. Bosque de Tlalpan, H. Parque Nacional Fuentes Brotantes de Tlalpan.

a microendemic amphibian restricted to the Pedregal del Xitle ecosystem (IUCN SSC Amphibian Specialist Group, 2020a), a unique xerophytic formation in a lava field supporting around 1500 native species, including animals, plants, and fungi at the south of Mexico City (Lot & Cano-Santana, 2009). In addition to *E. grandis*, other endemic amphibians have been collected in the Pedregal del Xitle ecosystem, of these, four are terrestrial and three have mixed aquatic and terrestrial habits (Díaz de la Vega-Pérez et al., 2016). Despite the importance of this ecosystem, and others surrounding Mexico City, the effects of urbanization on populations of amphibians have rarely been studied in Mexican cities (reviewed by Domínguez-Vega et al., 2019). The aim of this study was to characterize the presence of amphibians in Pedregal del Xitle, pursuing the following objectives 1) to quantify the abundance (by encounter rate) and richness of amphibians in urban protected areas with different conditions due to the level of surrounding urbanization, 2) characterize the habitat of each protected area using remote sensing tools and 3) measure the microhabitat in situ for each species, and 4) evaluate the vocal activity of the Pedregal frog under different exposures to noise within the protected areas. To do this, we integrated field data, obtained from a direct search by experts and volunteers, passive acoustic monitoring, and land-cover composition analysis from remote sensory tools.

MATERIALS AND METHODS

Study area. The Xitle volcano is located within the South of the Valley of Mexico, a Key Biodiversity Area considered as a priority site in the Alliance for Zero Extinction (AZE, 2018). Pedregal del Xitle is a fragmented xeric ecosystem surrounded by the urban area of Mexico City. Among the coordinates 19.327222° N, 99.235736° W and 19.231389° N, 99.153889° W and an altitudinal range of 2200–3100 m a.s.l. (Fig. 1), this highly fragmented ecosystem (Suárez et al., 2011) is located on a lava spill of vegetation dominated by xeric shrubs (Cordova et al., 1994; Cano-Santana et al., 2006). This study was conducted in three of the largest urban protected areas of Pedregal del Xitle: Parque Ecológico de la Ciudad de México (PECM), Ecoguardas, and Reserva del Pedregal de San Ángel (REPSA). These three urban protected areas are located within an altitude and urbanization gradient, ranging from 2300 to 3100 m a.s.l. and from 250 to 25 inhabitants per ha, respectively and in parallel (Suárez et al., 2011). In this gradient, REPSA corresponds to the lower and most urbanized area (2280–2320 m a.s.l.), and Ecoguardas (2440–2762 m a.s.l.) and PECM (2422–2540 m a.s.l.) correspond to the higher and semi-urbanized areas (Fig. 1), in the lowlands, and a mixture of xeric shrub with oak forests in the highlands (Castillo-Agüero et al., 2004; Mendoza-Hernández et al., 2013).

Amphibian sampling. Three urban protected areas were visited during June and August of 2015, and in March of 2016. Transects of 100 m long and 5 to 8 m wide (depending on how rugged the terrain was), separated by at least 250 m, were outlined within each protected area. Transects were oriented towards the four cardinal points (N, S, E, W) from the center of each protected area. Thus, each cardinal point had one or two transects with a similar orientation but located on a different surface to avoid resampling. Each transect was visited once by at least four persons, for three to five hours at night-time (20:00 and 03:00 h). To sample terrestrial amphibians, each transects was exhaustively searched visually, inspecting with the help of volunteers the available microhabitats in the Pedregal ecosystem: between soil cavities, under rocks, over standing and dead vegetation, and litter (Vonesh et al., 2010).

The specimens found were captured by hand using new disposable nitrile gloves, to minimize pathogen exposure (Phillott et al., 2010). After species identification, the individuals were released at the exact site of their capture. We measured the temperature and relative humidity of the exact collection point with a mini environmental quality meter (Sper Scientific 850070). The microhabitat where each amphibian was found was categorized as rocky ground, leaf litter, or plants. To describe the vegetation of the microhabitat around the collection point each amphibian was found, two observers counted the number of plant species and individuals of the same plant species using a 50 cm ruler to delimit a 1 m² circle. In addition, the area covered by moss and leaf litter was visually determined within this circle.

Passive acoustic monitoring and noise exposure. Calling activity of the microendemic frog, *E. grandis*, was recorded using automatic sound recording systems SM3 (Wildlife Acoustics) from June 20 to August 30, 2015. Four recording systems were installed on trees at an average height of 1 m above the ground and located in areas within the urbanization and elevation gradient having two conditions of exposure to urban noise: 1) high noise exposure, at 150 m from the edge of the urban protected area with high traffic roads, and 2) low noise exposure, at a distance of >500 m from the nearest road. Because Ecoguardas and PECM are adjacent urban protected areas and the first is no longer than 1 km in length, the high noise exposure recording system was placed in Ecoguardas, and the low exposure recording system was placed in PECM. Thus, recording systems were in four conditions: urbanized lowlands with high and low noise exposure (Eastern and Western REPSA, respectively), and semi-urbanized highlands with high and low noise exposure (Ecoguardas and PECM, respectively; further down in Fig. 4 the location of recorder systems is sketched).

Sounds were recorded in stereo files for five minutes every half hour, at a sample rate of 24 kHz, without filters, and in WAV format. Recordings were analyzed in the software Audacity 2.1.0 (Audacity Team, 2015). The amphibian call detection was made under bias visual inspection by a single person, from temporal and spectral characteristics of *E. grandis*, reported by Serrano (2016). Calling activity was recorded as call rate, i.e., the number of total calls/min recorded by visual and auditory counts following Dorcas et al. (2010). To investigate the physical environment influence on calling activity, daily mean environmental conditions of temperature, relative humidity, and precipitation were obtained from three automatic meteorological stations of the National Meteorological System (Sistema Meteorológico Nacional; <https://smn.conagua.gob.mx/es/observando-el-tiempo/estaciones-meteorologicas-automaticas-ema-s>) near or within each protected area. Environmental data analyzed were obtained during equivalent acoustic monitoring dates.

Land-cover composition. We estimated land use and vegetation coverage of PECM, Ecoguardas, and REPSA from a satellite image Landsat 8 Oli (geographic extent: 19° 19' 38.69" N, 99°14' 8.65" W and 19°13' 53.48" N, 99° 9' 14.28" W), available by US Geological Survey throughout Global Visualization Viewer (<http://glovis.usgs.gov>). The image was taken on July 13, 2015, a date that coincided with the sampling period and presented the lowest percentage of clouds for a better processing. The image had a pixel resolution of 30x30 cm with a 12% cloud coverage. Landsat image was processed employing the "Layer Stack" tool of the software Arcmap 10 (ESRI, 2011), and was converted in a multi-spectral image with a pixel resolution of 15 x1 5 m. We performed a non-supervised classification of 15 coverage classes. Subsequently, considering the similarity in structure and reflectance of the original Landsat image, and with the help of Google Earth, validation and unification of coverage classification were finally obtained in the following six classes: shrub, forest, mixed shrub forest, urban constructions, urban trees, and lawn. Finally, employing ArcMap 10 (ESRI, 2011), we calculated the coverage areas for each class and urban protected area.

Data analysis. The total number of organisms and amphibian species found, and the searching effort was recorded per transect and per visit to each protected area. Amphibian observation data was used to calculate the Simpson's diversity index for each protected area. Simpson's index was used due to its sensibility to detect variations in diversity by one or a few components within communities (Nagendra, 2002). We calculated the encounter rate (ER) as the total of amphibians recorded during person-hours of searching added for each area. The ER permits to

compare the amphibian abundance between sites even without identical searching efforts (Rovito et al., 2009; Sandoval-Comte et al., 2012; Aguilar-López et al., 2017).

Micro-environmental conditions among different amphibian species and frogs call rate among different sites were compared using ANOVA and pairwise comparisons using Wilcoxon rank sum test. Relationships of call rate with environmental conditions were analyzed using Pearson correlations. When required, normality criteria were verified for all the variables using the graphical method of quantile-quantile plot (Crawley, 2013). All analyzes were realized using the software R v.3.2.3 (R Core Team, 2016), and the packages car (Fox et al., 2013) and pgirmess (Giraudoux et al., 2018).

RESULTS

Amphibian surveys. A total of 73 individuals belonging to four amphibian species (*Chiropterotriton orculus*, *Pseudoeurycea leprosa*, *Aquiloerycea cephalica*, and *Eleutherodactylus grandis*) were observed in the three urban protected areas (Table 1; Fig. 2). The salamander *C. orculus* was the most common species, found only in Ecoguardas and PECM and representing 52% of the total captures, followed by the frog *E. grandis* found in all protected areas, with 38% of the captures. The least common salamanders (*A. cephalica* and *P. leprosa*) were found only in Ecoguardas and REPSA (Table 1). The amphibian counts were highly variable between visits, and the number of amphibians found in the

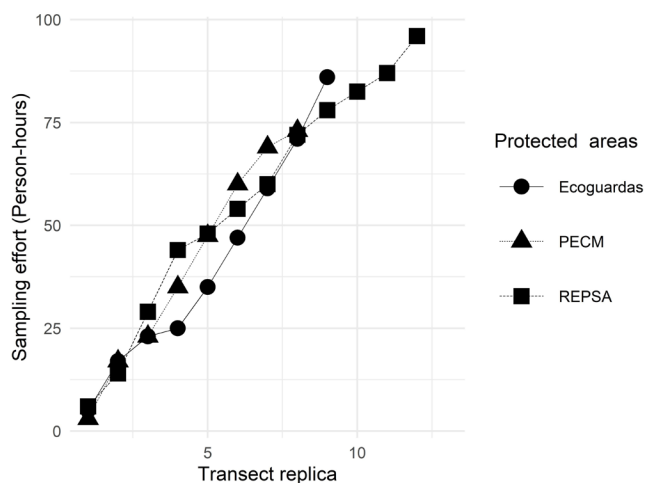


Figure 2. Esfuerzo de muestreo acumulado para los transectos dentro de cada área urbana protegida. Cada punto representa una réplica de un transecto dentro del área urbana protegida.

Figure 2. Cumulative sampling effort for the transects within each urban protected area. Each point represents a transect replica within the urban protected area.

Tabla 1. Especies de anfibios observadas y esfuerzo de muestreo en las tres áreas protegidas urbanas del Pedregal del Xitle. Abreviaturas: PECM = Parque Ecológico de la Ciudad de México; REPSA = Reserva Ecológica del Pedregal de San Ángel; ER = Tasa de encuentro (anfibios / persona-horas); *A. cephalica* = *Aquiloerycea cephalica*; *C. orculus* = *Chiropterotriton orculus*; *E. grandis* = *Eleutherodactylus grandis*; *P. leprosa* = *Pseudoeurycea leprosa*.

Table 1. Amphibian species observed and sampling effort in the three urban protected areas of Pedregal del Xitle. Abbreviations: PECM= Parque Ecológico de la Ciudad de México; REPSA= Reserva Ecológica del Pedregal de San Ángel; ER= Encounter Rate (amphibians/person-hours); *A. cephalica*= *Aquiloerycea cephalica*; *C. orculus*= *Chiropterotriton orculus*; *E. grandis*= *Eleutherodactylus grandis*; *P. leprosa*= *Pseudoeurycea leprosa*.

Total of individuals observed (ER per species)									
Urban protected area	Transects	Visits	<i>A. cephalica</i>	<i>C. orculus</i>	<i>P. leprosa</i>	<i>E. grandis</i>	Total individuals	Sampling effort (person-hours)	ER per site (amphibian/person-hours)
REPSA	4	8	1	0	2	15	18	81	0.22
Ecoguardas	4	7	3	24	1	9	37	84	0.44
PECM	4	7	0	14	0	4	18	73	0.24
Total	12	22	4 (0.02)	38 (0.16)	3 (0.01)	28 (0.12)	73	238	0.31

three protected areas had a relationship with the person-hours effort ($F= 8.377, p= 0.008$; Fig. 3). Regarding amphibian diversity, Ecoguardas is the protected urban area with the highest value of the Simpson index. In contrast, REPSA and PECM had a similar number of amphibians counts and Simpson index values (see Table 4).

The total effort searching in the three protected areas was 238 person-hours. So, our results suggest an effort of 3.2 person-hours required to find one amphibian in Pedregal del Xitle. In particular, the ER in the three reserves studied was 0.31 amphibians/person-hours. Ecoguardas had the highest number of amphibians found with 37 individuals and an ER= 0.44, followed by PECM and REPSA with 18 individuals for each site and 0.24 and 0.22 amphibians/person-hours, respectively. Per species, the vulnerable salamander *C. orculus* had the highest with ER= 0.16, followed by the endangered frog *E. grandis* with= 0.12. In contrast, the salamanders *A. cephalica* and *P. leprosa* showed a lower ER with 0.01 and 0.02, respectively (Table 1). Thus, the effort needed to find the most common salamander, *C. orculus* (N= 38 individuals), was around 6.3 person-hours; but to find the least common, *P. leprosa* (N= 3 individuals), it was required 100 person-hours.

During fieldwork, 75% of the amphibians were found over rocky ground, 18% over leaf litter, and only 7% over plants. *Eleutherodactylus grandis* and *P. leprosa* were exclusively found on rocky ground, and *A. cephalica* was exclusively found on leaf litter. *Chiropterotriton orculus* showed the highest diversity of microhabitats, it was found on rocks, leaf litter, oak trees, and Agave plants. A single microhabitat feature associated to amphibian captures differed between protected areas, the

moss and leaf litter percentage was lower in REPSA ($F= 11.280, p< 0.001$) compared to Ecoguardas ($p= 0.020$) and PECM ($p= 0.009$), respectively (see Table 2).

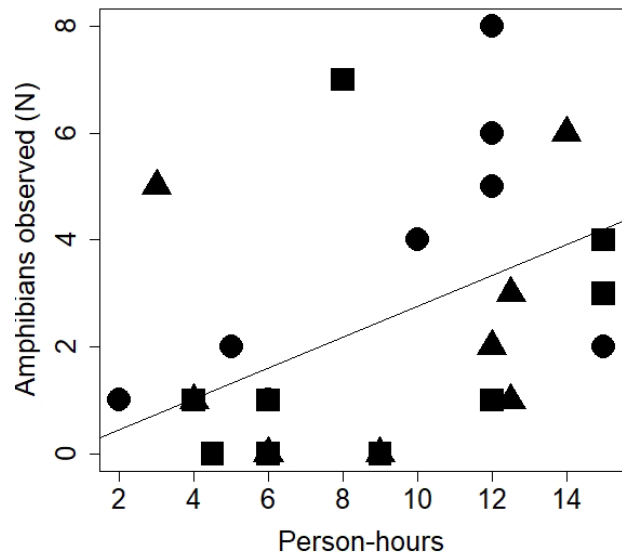


Figura 3. Recuento de anfibios y relación horas-persona. Cada punto representa una réplica de un transecto entre el área urbana protegida representada por símbolos (Ecoguardas = círculos; PECM = triángulos; REPSA = cuadrados). La línea de tendencia única sugiere que las tres áreas protegidas urbanas tuvieron un esfuerzo de muestreo similar.

Figure 3. Amphibian counts and person-hours relationship. Each point represents a transect replica between the urban protected area represented by symbols (Ecoguardas= circles; PECM= triangles; REPSA= squares). The single trend line suggest that the three urban protected areas had similar sampling effort.



Tabla 2. Características medias de los microhábitats utilizados por los anfibios terrestres dentro de las áreas urbanas protegidas del Pedregal del Xitle. Abreviaturas: PECM = Parque Ecológico de la Ciudad de México; REPSA = Reserva Ecológica del Pedregal de San Ángel; R= suelo rocoso, L= hojarasca, P = plantas; a y b indican diferencias post hoc entre áreas urbanas protegidas.

Table 2. Microhabitat mean features used by terrestrial amphibians within urban protected areas in Pedregal del Xitle. Abbreviations: PECM= Parque Ecológico de la Ciudad de México; REPSA= Reserva Ecológica del Pedregal de San Ángel; R= rocky ground, L= leaf litter, P= plants; a and b indicate post hoc differences between reserves.

Urban protected area	% Moss & lichen	# Plant individuals	# Plant species	Microhabitats used	Temperature (°C)	% Relative humidity
REPSA	6.4a	11.3	6.1	R	19.1	76.0
Ecoguardas	73.1b	13.9	4.4	R, L, P	19.7	67.1
PECM	55.0b	20.6	7.8	R, L, P	16.2	72.1
Total	45.7	14.9	5.5	R, L, P	17.9	72.4

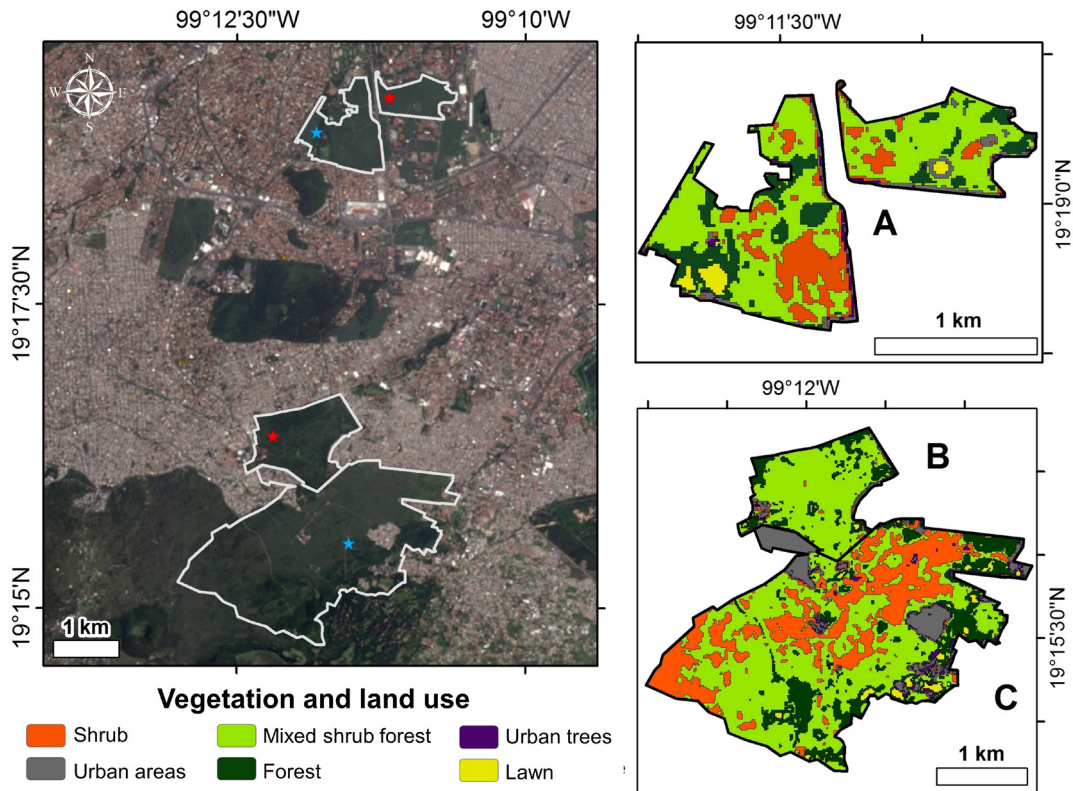


Figura 4. Uso del suelo y cobertura vegetal obtenida a partir de imágenes de percepción remota. La exposición al ruido detectada por el sistema de grabación acústica está indicada para una alta exposición en rojo y una baja exposición en puntos azules en el mapa sin procesar, respectivamente, dentro de las condiciones de urbanización: urbana en REPSA occidental y oriental (A) y semiurbana en Ecoguardas (B) y PECM (C). Abreviaturas: PECM = Parque Ecológico de la Ciudad de México; REPSA = Reserva Ecológica del Pedregal de San Ángel.

Figure 4. Land use and vegetation coverage obtained from remote perception images. Noise exposition sensed by acoustic recording system are indicated for high exposition in red and low exposition in blue points on the raw map, respectively, within the urbanization conditions: urban in Western and Eastern REPSA (A) and semi-urban in Ecoguardas (B) and PECM (C). Abbreviations: PECM= Parque Ecológico de la Ciudad de México; REPSA= Reserva Ecológica del Pedregal de San Ángel.

Tabla 3. Se muestran la exposición al ruido dentro de las áreas urbanas protegidas, los rangos de llamadas por minuto y las características ambientales. Los coeficientes de correlación de Pearson y las significaciones se indican de la siguiente manera: * p <0.05; ** p <0.01; *** p <0.001. Abreviaturas: PECM = Parque Ecológico de la Ciudad de México; REPSA = Reserva Ecológica del Pedregal de San Ángel; NR = No registrado.

Table 3. Noise exposure within urban protected areas, ranges of calls per minute and environmental features are shown. Coefficients of Pearson correlation and significances are indicated as follows: *p < 0.05; ** p < 0.01; *** p < 0.001. Abbreviations: PECM= Parque Ecológico de la Ciudad de México; REPSA= Reserva Ecológica del Pedregal de San Ángel; NR= Non-registered.

Urbanization / Noise exposure	Urban protected area	Calls / min	Temperature (°C)	r	Relative humidity (%)	r	Precipitation (mm ³)
Urban / High	Eastern REPSA	0 - 38.8	14.5 - 21.9	-0.68***	51 - 88	0.63***	0 - 32.8
Urban / Low	Western REPSA	0 - 56.8	14.5 - 21.9	-0.72***	51 - 88	0.65***	0 - 32.8
Semi-Urban / High	Ecoguardas	0 - 32.4	9.7 - 14.3	-0.40	68 - 96	0.70*	NR
Semi-Urban / Low	PECM	0 - 57.4	15.7 - 19.6	-0.94**	52 - 83	0.93**	NR

Calling activity and noise exposure. The four sound recording systems recorded a total of 460 hours. Calling activity differed among urban and semi-urban protected areas, having contrasting noise exposure ($F=37.040, p<0.001$). Lower call rates

were observed in the sites with high noise exposure (Eastern REPSA and Ecoguardas), and higher call rates were observed in sites with low noise exposure (Western REPSA and PECM) (Table 3; Fig. 4). However, call rate differed between the two sites with

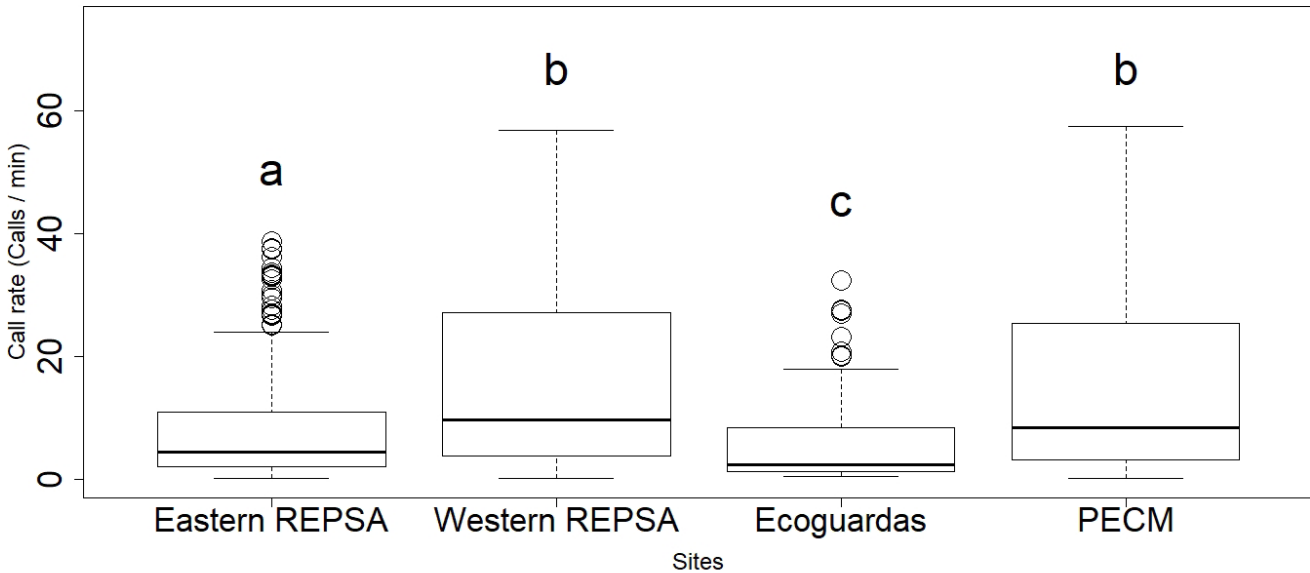


Figura 5. Tasa de llamados registrada entre sitios expuestos a condiciones contrastantes de urbanización (urbano y semiurbano) y exposición al ruido (alto y bajo) de la siguiente manera: REPSA oriental = urbano / alto; REPSA occidental = urbano / bajo; Ecoguardas = semiurbano / alto; PECM = semiurbano / bajo. Las letras a y b indican diferencias post hoc entre sitios de áreas protegidas urbanas.

Figure 5. Call rate registered among sites exposed to contrasting conditions of urbanization (urban and semi-urban) and noise exposition (high and low) as follows: Eastern REPSA= urban/high; Western REPSA= urban/low; Ecoguardas= semi-urban/high; PECM= semi-urban/low. Letters a and b indicate post hoc differences among sites of urban protected areas.



high noise exposure but did not differ between the two sites with low noise exposure, after post hoc analysis (Fig. 5). The calling activity of *E. grandis* was mainly nocturnal, starting at 18 hours and ending at 6 hours (Fig. 6). The call rate was positively related to the relative humidity, and negatively related to temperature in all sites, except in Ecoguardas, where the temperature was not related to the call rate (Table 3). Precipitation was only available for analysis in REPSA, so we did not analyze the relationship of call rates with this variable (Table 3).

largest area, with more than 6 km², while REPSA and Ecoguardas have an area of approximately 1.6 and 1.4 km², respectively. As a result of the vegetation classification, we found six categories of vegetation and land use, all of which were present in the three sites (Table 4). In general, the study areas of the three sites are constituted by mixed shrub forest (53%), followed by shrub (21%), and forests (15%), and approximately 11% coverage associated with urbanization (such as 8% buildings, 2% urban trees or 1% lawns) within these three protected areas.

Characterizing the land-coverage of the study area. The three urban protected areas together sum up 9.4 km²; PECM represents the

Two protected areas of Pedregal del Xitle, REPSA and PECM, showed a similar composition in terms of the percentage of

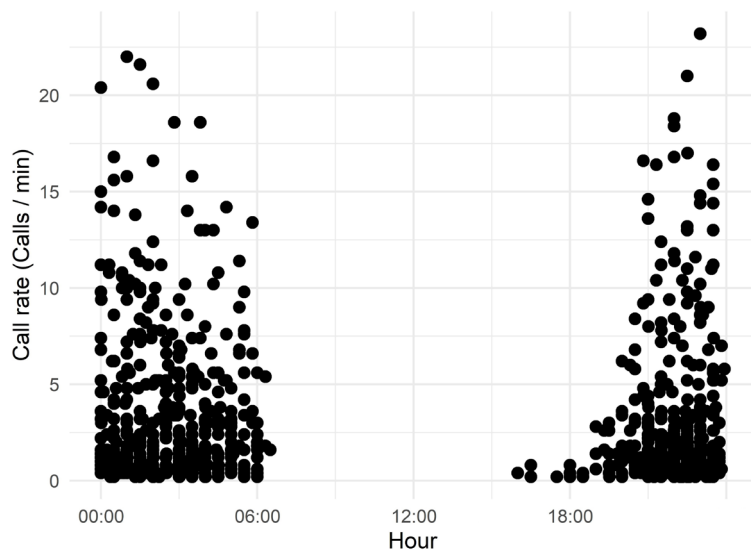


Figura 6. Tasa de llamados de *Eleutherodactylus grandis* a lo largo del día, registrada en las tres áreas protegidas urbanas del Pedregal del Xitle.

Figure 6. Call rate of *Eleutherodactylus grandis* throughout the day, registered in the three urban protected areas of Pedregal del Xitle.

Tabla 4. Uso del suelo y cobertura de vegetación (indicado como porcentaje) e índice de diversidad de anfibios de Simpson en las tres áreas urbanas protegidas. Abreviaturas: PECM = Parque Ecológico de la Ciudad de México; REPSA = Reserva Ecológica del Pedregal de San Ángel.

Table 4. Land use and vegetation coverage (indicated as percentage) and Simpson's diversity index of amphibians in the three urban protected areas. Abbreviations: PECM= Parque Ecológico de la Ciudad de México; REPSA= Reserva Ecológica del Pedregal de San Ángel.

Urban protected areas	Natural vegetation (%)			Urban land use and vegetation (%)			Simpson's Index	Area (km ²)
	Mixed shrub forest	Forest	Shrub	Lawn	Urban trees	Building		
REPSA	55.96	15.58	17.27	2.2	2.56	6.44	0.29	1.57
Ecoguardas	74.54	16.74	1.58	0.42	1.16	5.57	0.51	1.44
PECM	47.14	14.37	26.54	1.57	2	8.38	0.35	6.37
Total	52.82	14.94	21.16	1.50	1.96	7.62		9.38



coverage of each land cover class. However, Ecoguardas showed differences compared to the other two sites, since more than 90% of its area was occupied by forest vegetation (75% mixed shrub forest and 17% forest), and a low percentage by shrub (2%). Moreover, REPSA and PECM, also had a high percentage of forest vegetation (72% and 61%), but presented 17% and 27% of shrub, respectively (Table 4; Fig. 4). Additionally, Ecoguardas showed the lowest urban components with 7.2%, versus 11.2% in PECM and 12% recorded in REPSA.

DISCUSSION

Our study integrates data from field surveys, sound recording systems, and satellite imagery (by a geographic information system), to assess the richness, encountered rates, and changes in vocal behavior of amphibians in the fragmented landscape of three urban protected areas in Mexico City. Integrating these passive and active methods, we found differences in abundance and diversity between protected areas in the Pedregal ecosystem from south of Mexico City. We hypothesize that urbanization and the management regime of protected areas influence the components of land cover and microhabitat within them, and that all these factors are behind the abundance and richness of amphibians. Additionally, we found differences in the vocal activity of Pedregal frogs among protected areas, these differences are associated with the proximity of exposure to urban noise bordering each protected area presented. These findings are useful for future amphibian monitoring in Pedregal del Xitle, a xeric ecosystem highly impacted by urbanization.

Interestingly, *C. orculus* was mostly found in the two highland urban areas (Ecoguardas and PECM), whereas *E. grandis* was mainly found in the basin area covered by REPSA. These findings are indicative of the importance of all these urban protected areas, because *C. orculus* and *E. grandis* have a distribution restricted to the metropolitan area of Mexico City, and both amphibians are cataloged as threatened in the IUCN Red List, specifically as vulnerable and endangered categories, respectively (IUCN SSC Amphibian Specialist Group, 2020a; 2020b).

The amphibian counts in Pedregal del Xitle did not reflect the size of the protected areas where they were found, and ER values varied between protected areas and species. Thus, Ecoguardas had twice the amphibian ER as PECM and REPSA, although PECM has an area that approximately doubles that of Ecoguardas and REPSA combined. The values of ER contrasts between species with the effort of 3.1 person-hours required to find two plethodontid salamanders (founding 214 individuals *Parvimolge townsendi* and *Thorius pennatulius* in 672 person-hours)

in fragments of cloud forest located in the central mountains of eastern Mexico (Sandoval-Comte et al., 2012). Instead, in the same area of cloud forest, the effort to find a single microendemic salamander *Aquiloerycea cafetalera* is 10.8 person-hours (with 109 individuals found in 1,174 person-hours) (Aguilar-López et al., 2017). Reporting such standardized measurements of ER and sampling efforts contributes to compare trends of threatened amphibian populations (Rovito et al., 2009). Unfortunately, there are no previous efforts with a comparable search effort to estimate the current situation of the amphibians in the Pedregal area of Mexico City. So, our data represents the first information to evaluate populations of these terrestrial amphibians in this urbanized habitat.

Variations in amphibian composition between Pedregal del Xitle protected areas were also related to microhabitat features that are unique to each protected area. In Ecoguardas and PECM, leaf litter is vastly available, mainly due to the presence of *Quercus* trees (Mendoza-Hernández et al., 2013). Whereas REPSA, outstands by the arrangement of rocks bare of vegetation, a feature that probably helps *E. grandis* to broadcast its vocalization (Serrano, 2016). Altogether, rocky soils and vegetation are important elements in the microhabitat of amphibians in the Pedregal del Xitle ecosystem. However, the amount of leaf litter is relevant for amphibians, due to the provision of refuge, food, and humidity (e.g., Van Sluys, 2007), particularly for plethodontid salamanders. The attributes of Ecoguardas having greater coverage of mixed scrub forest, and a medium level of evenness of amphibians, is probably due to its greater restriction on the transit of people, compared to that registered in the other two urban protected areas studied, where relatively frequent induced fires occur (Bonfil, 2009; Martínez-Orea et al., 2019).

Prior to this study, the information about amphibians from Pedregal del Xitle were scarce. We found four (*C. orculus*, *P. leprosa*, *A. cephalica*, and *E. grandis*) of the nine amphibian species reported historically. Amphibians not found comprise anurans that require permanent bodies of water, such as *Dryophytes arenicolor* (Sánchez-Herrera, 1980), *Spea multiplicata* and *D. eximius* (Ramírez-Bautista et al., 2009). These historical reports are possible records of animals in transit to rain streams or to artificial bodies of water between neighboring areas, since the stony lava soil in Pedregal del Xitle area is highly permeable and does not allow a prolonged retention of water (Sánchez-Herrera, 1980). Introduced species of amphibians into artificial bodies of water at REPSA, such as *Lithobates montezumae* (Díaz de la Vega-Pérez et al., 2016) and *Ambystoma mexicanum* (from which an *ex-situ* conservation site was established in the area known as



Cantera Oriente; Aguilar-Moreno & Aguilar-Aguilar, 2019), does not appear to influence the amphibian community.

It is important to consider that shrubby vegetation, such as that present in Pedregal del Xitle ecosystem (Castillo-Agüero et al., 2004; Mendoza-Hernández et al., 2013), can influence the detectability of terrestrial anurans in microhabitats where they are more abundant, for example, when the soil of forests has dense understory vegetation (Valenzuela-Sánchez et al., 2019). In the same case, the differences in abundance between sites and species could be caused by the temporal scope of our study since it was limited from June to August. It is possible that the reduced number of *A. cephalica* and *P. leprosa* could be due to the study period, considering that the reproductive peak of these plethodontid salamanders occurs at the beginning of autumn (Uribe-Peña et al., 1999).

The analysis of vocal activity of *E. grandis*, showed that it was notably influenced by the decrease in temperatures and the increase in humidity, probably linked to precipitation. The effects of urban noise exposure on the vocal activity of *E. grandis* can be observed on the repetition rate. Within the protected areas, the call rate of *E. grandis* was two to three times greater in the bordering sites to traffic noise sites, in contrast to the lowest call rate, observed in non-bordering sites. Call rate is the acoustic parameter that is most affected by urban noise in most studies of anurans. The most common effect of anthropogenic noise is to decrease the call rate, followed by no changes, and in a few cases, an increase of call rate (reviewed by Simmons & Narins, 2018). Different abiotic noises can increase the call rate when added to specific chorus sounds, such as wind and rain stimuli (Penna & Zuñiga, 2014). It is possible that the vocal activity of *E. grandis*, is being affected by different stimuli depending on the location of the monitored site, being stimulated to increase its call rate by specific abiotic noises in some sites and decrease it in others.

Similarly, a recent study showed how bird communities avoided areas with higher levels of urban noise within the urban area of REPSA (Manzanares-Mena & Macías-García 2018). Several other studies have demonstrated how urban noise substantially modifies singing exhibitions, sound properties, and physiological functions of birds and anurans (Brumm, 2010; Macías-García et al., 2017; Halfwerk et al., 2019). However, the evolutionary mechanisms behind changes related to the vocal behavior of organisms in urban environments remain relatively unexplored. Whether the features that allow species to settle in cities corresponds to a process of adaptation or phenotypic plasticity (Alberti et al., 2017; Ouyang et al., 2018), or whether this plasticity arose before or after the link with urban environments

(Macías-García et al., 2017), it is unknown. Discovering which mechanisms are behind vocal behavior in relationship to the urban environment where *E. grandis* lives, should be a priority issue for the conservation of this microendemic species.

Our sampling effort was achieved thanks to the participation of volunteers. Throughout the project, we had a total of 24 volunteers involved in fieldworks, some of them participated more than once. The support of volunteers in biodiversity assessments is increasing around the world, mainly in projects focused on conservation (Pocock et al., 2018). The participation of volunteers represents a potential huge workforce, particularly when low financial resources are available (Foster-Smith & Evans 2003), but at the same time, their involvement provokes a positive impact on them, by involving a potentially interested public in conservation efforts (Albergoni et al., 2016; Hobbs & White 2016). Although field experience and knowledge of the amphibians may influence their encounter in the Pedregal area (especially by their hiding habits), we tried to balance search teams by keeping at least one or two experienced personals within sampling groups.

CONCLUSION

We found that the most abundant amphibians in Pedregal are the frog *E. grandis* and the salamander *C. orculus*, the first in REPSA and the last in Ecoguardas and PECM. In addition, we found that the protected area with the greatest diversity and abundance of amphibians is Ecoguardas, this is the only one among the three protected areas that has restrictions for public access and has a higher proportion of moss and lichens and forested vegetation. The approach of our study takes advantage of the integrated use of remote sensing systems, the collection of data in the field, and the participation of volunteers in fauna monitoring. All these tools allowed us to achieve the objective of evaluating the status of taxa that are difficult to observe, scarcely studied and inhabiting urban landscapes difficult to explore. An effort was made to involve part of the community in the monitoring and conservation importance of amphibians, through the participation of volunteers in fieldwork.

In addition, our study contributes to highlighting the relevance of the endemic amphibians that inhabit this xeric ecosystem, inhabiting over a water-collecting surface in the city (Cordova et al., 1994). Our study is the first evaluation assessing the conditions affecting terrestrial amphibians in a xeric urban ecosystem and provides relevant information for future management of the urban protected areas from Pedregal del Xitle. To monitor population trends of the Pedregal amphibian



community, periodic visual and acoustic monitoring of frogs and salamanders should be implemented in these urban protected areas, and amphibians should be included in the management plans for these protected areas and its surrounding unprotected land.

Acknowledgements .— We thank Diego Gutiérrez and all the volunteers involved for their invaluable assistance in fieldwork. Juan Francisco Torres helped with geographic methods. We are very grateful to Centro de Educación Ambiental Ecoguardas (Secretaría de Medio Ambiente de la Ciudad de México), Centro de Educación Ambiental del Ajusco Medio (PRONATURA México A.C.), and Reserva Ecológica del Pedregal de San Ángel (UNAM) for the facilities and authorization to carry out this study. The Conservation Leadership Programme supported this work through the Future conservation award (Project 02244015). Permit number SGPA/DGVS/06516/15 was provided by Secretaría de Medio Ambiente y Recursos Naturales to conduct studies on wild animals under ethical and humane conditions. Commentaries from two anonymous reviewers contributed to the substantial improvement of this manuscript.

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