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Do Bats Avoid the Urban Core in the Breeding Season? A Case Study from Temperate Latitudes

Anton Vlaschenko [1,2,3,*], Olena Rodenko [4], Vitalii Hukov [5], Viktor Kovalov [6], Alona Prylutska [1,7] and Kseniia Kravchenko [1,8]

1 Ukrainian Bat Rehabilitation Center of NGO "Ukrainian Independent Ecology Institute", Plekhanov St., 40, 61001 Kharkiv, Ukraine
2 Bat Biology Laboratory, H.S. Skovoroda Kharkiv National Pedagogical University, Valentynivska St., 2, 61168 Kharkiv, Ukraine
3 National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Pushkinska St., 83, 61023 Kharkiv, Ukraine
4 Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, ul. Golebia 24, 31-007 Krakow, Poland
5 Biological Department, V.N. Karazin Kharkiv National University, Svobody Sq. 4, 61077 Kharkiv, Ukraine
6 Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
7 Max-Planck Institute of Animal Behaviour, Am Obstberg 1, 78315 Radolfzell, Germany
8 Department of Ecology and Evolution, University of Lausanne, Biophore, 1015 Lausanne, Switzerland
* Correspondence: anton.vlaschenko@gmail.com

Abstract: Seasonal utilization of urban areas by bats remains inadequately explored. This study aimed to comprehensively survey the species composition and population dynamics of bats during both the breeding season (May–July) and autumn migration season (August–September) within a large urban area, specifically Kharkiv city, Ukraine. We conducted multiyear data collection from 2014 to 2016, employing a combination of acoustic recordings, mist-netting, and registration of opportunistically found bats. The results revealed the identification of seven bat species using all methods (*Eptesicus serotinus*, *Nyctalus noctula*, *N. leisleri*, *Pipistrellus pygmaeus*, *P. kuhlii*, *P. nathusii*, and *Vespertilio murinus*), with notable differences in species composition between the studied periods. During the migration season, *N. noctula* was a numerically predominant species, while *P. kuhlii* and *E. serotinus* were the most abundant during the breeding season. The urban core bat population during the breeding season primarily consisted of males and solitary reproductive females, mainly represented by *P. kuhlii* and *E. serotinus*. Acoustic recording data indicated that *N. noctula* actively avoided the urban core during the breeding season, but was more common on the city periphery. In contrast, during the migration season, the city experienced a significant surge in bat abundance, both in general and specifically among noctule bats, with their numbers increasing tenfold compared to the breeding season. Moreover, a considerable number of young individuals were observed during the migration season. These findings provide evidence that bats tend to avoid the urban core in large cities at temperate latitudes during the breeding season but actively utilize urban areas during autumn migration. Understanding the seasonal preferences and movements of bats in urban environments is crucial for effective conservation and management strategies.

Keywords: Chiroptera; breeding; migration; urban ecology; Kharkiv city; Ukraine

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1. Introduction

Exponential urbanization is a defining characteristic of the Anthropocene era. It is marked by the increasing concentration of people in urban areas [1,2] which resulted in the emergence of unique and distinct landscapes known as urban or city areas [3,4]. Urban areas are distinct and represent a small proportion (less than 3%) of the Earth's total surface [5]. However, despite their limited size, these landscapes exhibit unique and characteristic features, including densely populated and built-up areas, minimal vegetation coverage, specific microclimates, and a variety of pollution sources [4]. Moreover, urban landscapes are notable for their significant energy consumption, which contributes to the formation of a warmer local microclimate [6]. In addition, the physical characteristics of urban areas mimic those of mountainous and rocky regions, creating a unique and complex environment.

The conversion of natural landscapes into urban environments has resulted in a significant loss of biodiversity [7,8] due to drastic changes in land use and the loss of natural vegetation. However, various factors within urban ecosystems enable animal species to thrive in urban areas. Firstly, urban landscapes are characterized by significant energy consumption, contributing to warm microclimates and creating heat islands [3], and providing a favorable environment for animals during cold seasons. Secondly, there is an abundance of man-made structures that serve as shelters and roosts, increasing the chances of survival for animals [9,10]. Lastly, generalist and granivorous animals (for example, pigeons and sparrows) benefit from ample food sources in urban areas, leading to shifts in timing of breeding and an increase in the number of breeding cycles [11,12]. These factors have facilitated the repopulation of urban landscapes by animal species, allowing them to adapt to the challenges of the Anthropocene era [13].

Bats belong to a group of species that exhibit a certain degree of tolerance or even potential benefit from living in urban environments [14,15]. Indeed, when it comes to diversity in urban areas, bats often exhibit greater diversity than other mammals, especially in the Northern Hemisphere [10,16]. However, bats are highly sensitive to the environmental changes resulting from human activities, and they are widely regarded as excellent bio-indicators [17]. They are typically more diverse and abundant in natural, pristine habitats than in urban areas [10,17,18], especially when it comes to insectivorous bats. Therefore, one of the questions necessitating future research is whether the species diversity and abundance of bats in urban areas truly signify a successful adaptation to the conditions of modern urban landscapes, or if the utilization of urban areas by bats is a more intricate and selective phenomenon.

The loss of natural vegetation in urban landscapes leads to a significant decline in insect abundance [19,20]. Insectivorous bats of the Northern Hemisphere rely heavily on insects as a food source during the breeding and pre-hibernation seasons [21–23]. It is reasonable to assume that food resources in urban areas may be insufficient to support breeding colonies of local bat species. Previous observations in the Kharkiv city area (Ukraine) (e.g., [24]) have led us to hypothesize that a majority of bats, particularly reproductive females, avoid the urban core area during the breeding season (May–July), and moved back from the city periphery towards central districts in the autumn migration period (August–September) [24–26]. However, this hypothesis has not been adequately tested. Therefore, the present study aimed to investigate the species composition and seasonal population dynamics of bats within the urban core vs. city periphery at temperate latitudes, in order to rigorously examine this hypothesis.

2. Materials and Methods

2.1. Study Area and Local Bat Fauna

The study was conducted in the city of Kharkiv (Figure 1) from 2014 to 2016. The city is located in the northeastern part of Ukraine (50°0′ N, 36°15′ E, 135 m.a.s.l.). It covers an area of 350 sq. km and its population before the full-scale war started in 2022 was ca. 1.5 million. The mean annual temperature in Kharkiv is 7.5 °C, with an isotherm in January of −7 °C, and the absolute minimum temperature is −35.6 °C. The isotherm in July is +21 °C, and the absolute maximum temperature is 38.9 °C. Annual precipitation does not exceed 540 mm [24]. A city scheme depicting green areas and water bodies is presented in Figure 1, while a more detailed description of the city area can be found in Kravchenko et al., 2017 [24]. The exact location of Kharkiv city center, or the urban core center [4], was selected in Google Earth as the geographical center of the city.

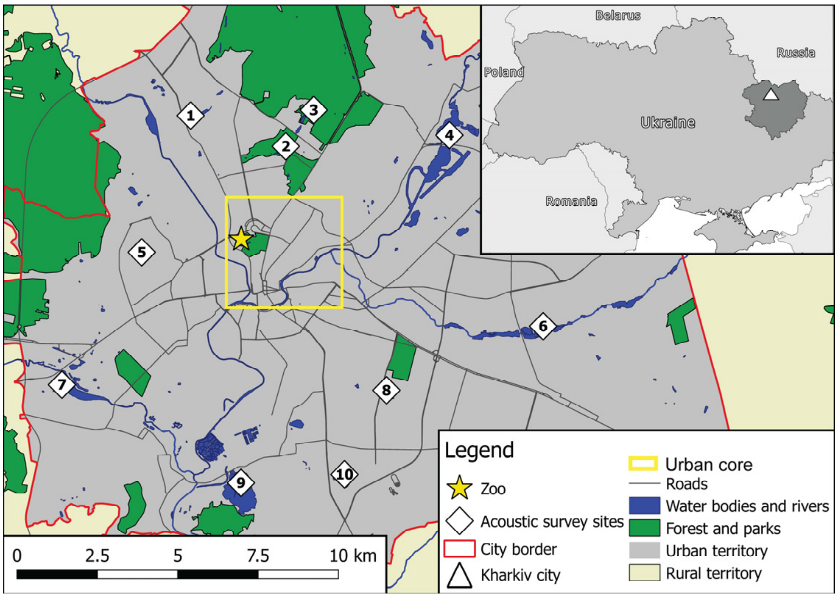

Figure 1. Location of acoustic survey and mist-netting sites on the Kharkiv city map, including Kharkiv Zoo (yellow star), where acoustic surveys and mist-netting were carried out, and water bodies (WB), where acoustic surveys only were performed (numbered rhombs: 1—Oleksiivka, 2—Vivary, 3—Lesopark Lake, 4—Zhuravlivka Hidropark, 5—Savkin Yar, 6—Niemyshlia, 7—Zhovtnevyi Hidropark, 8—Morozov Street Lake, 9—Osnova Lake, 10—Metro Lake).

The bat fauna of the Kharkiv city area has been widely studied for over a century, with special research efforts since 1999 [24–27]. Eleven bat species of the Vespertilionidae family have been recorded within the municipal administrative boundaries of Kharkiv: *Myotis daubentonii* (Kuhl, 1817); *M. dasycneme* (Boie, 1825); *Nyctalus leisleri* (Kuhl, 1817); *N. noctula* (Schreber, 1774); *Eptesicus serotinus* (Schreber, 1774); *Pipistrellus pygmaeus* (Leach, 1825); *P. nathusii* (Keyserling et Blasius, 1839); *P. kuhlii* (Kuhl, 1817); *Vespertilio murinus* Linnaeus 1758; *Plecotus austriacus* (Fischer, 1829), and *Pl. auritus* (Linnaeus, 1758) and one individual (accidental finding) from the family Molossidae *Tadarida teniotis* (Rafinesque, 1814) [28].

2.2. Study Design and Periods of the Survey

To test the hypothesis, three methodological approaches were applied: mist-netting, acoustic recordings and rescuing bats that were opportunistically (accidentally) found by the city residents (hereafter referred to as opportunistic bat records). We applied the combination of all three methods for several years (2014–2016). The sites for acoustic recording were carefully selected to focus on areas with increased bat activity, irrespective of their location within zones of the urban landscape that usually have low bat activity. Since bats have a high level of fidelity to aquatic habitats for feeding and drinking, acoustic surveys were concentrated mainly near waterbodies located within the urban core as well as the city periphery (Figure 1). The water bodies were chosen instead of other urban habitat types, e.g., streets, industrial and residential areas, etc. (e.g., [29]). The mist-netting sessions were conducted within the urban core in the territory of Kharkiv Zoo. The territory of the zoo (22 ha) was chosen as the only green area within the urban core surrounded by woodlands and providing access to open water (artificial ponds). Moreover, two multistorey buildings, V.N. Karazin Kharkiv National University building (KhNU) and the Derzhprom building, located 300 m and 350 m away from the zoo, respectively, known as hibernation places and late migration stopovers for numerous *N. noctula* individuals and single individuals of *E. serotinus* [24,30]. The exact location for mist-netting in the zoo was selected based on a preliminary survey performed with a Pettersson D200 ultrasound detector in the territory of the zoo in June/July 2013 and April 2014. The highest bat activity was observed in the southern part of the zoo, above the surface of three artificial ponds used by pelicans, swans, and for boating activity. These ponds were selected for further mist-netting and acoustic survey. The third methodological approach involved rescuing bats found in buildings, apartments, and other locations throughout the city [24,26,31], and covered multiple sites across the whole city.

2.3. Period of Survey and Bat Life Cycle Phenology

Our surveys were conducted during two periods of the bat annual life cycle: the breeding period from 1 May to 31 July, and the period of autumn migration (previously referred to as invasion [24]) from 1 August to 15 September. This division was based on previously obtained data [24,32]. The time frames for the application of different methodological approaches are presented in Table 1.

Table 1. Periods of acoustic surveys, mist-netting and recording of opportunistic bat findings in Kharkiv city during the two years of data collection.

Method	**The Period of Bat Life Cycle**	
	Breeding	**Autumn Migration**
Acoustic survey—zoo	05.06.2015–23.07.2015	12.08.2015–10.09.2015
Acoustic survey—water bodies	04.06.2015–12.07.2015	16–25.08.2016
Mist-netting—zoo	19.05.2014–23.07.2014 07.05.2015–25.07.2015	10.08.2014–08.09.2014 13.08.2015–13.09.2015
Bat findings	01.05.2014–31.07.2014 01.05.2015–31.07.2015	01.08.2014–15.09.2014 01.08.2014–15.09.2015

2.4. Data Collection

2.4.1. Acoustic Surveys

Acoustic recordings were conducted both in the zoo and near water bodies using a time-expansion ultrasound detector Pettersson D240X (Pettersson Elektronik AB; Uppsala, Sweden) and a Tranquility Transect Bat detector (Courtpan Design Ltd., Cheltenham, UK) with a Zoom H2 digital recorder (Samson technologies, Hicksville, NY, USA). In all locations, recordings started 15 min after sunset: (i) in the zoo, 12 records of 5 min each were performed; (ii) near water bodies, the total duration of the recording was 1 h, performed for each water body as four separate records of 15 min each with 5–10 min intervals between the recordings. During the recording, the operator moved around the water body and performed each of the four records at a new location on the bank. In total, there were 30 recording nights (17 during the breeding season and 13 during the migration period) (Table 1).

2.4.2. Mist-Netting

Mist-netting was conducted at a site located on the dam between two ponds in the zoo area (see above), with areas of 51 m^2 and 23 m^2, respectively. We used two ultrathin nylon monofilament Chinese mist-nets with a length of 12 m and heights of 2.5 and 3.5 m, respectively, both with a mesh size of 15 mm. The two mist-nets were positioned 30 m apart, in a straight line, and were set up in the same location for each mist-netting session. Mist-netting was conducted from sunset to sunrise. The duration of the mist-netting session was measured from the moment the first bat appeared until sunrise, when the nets were taken down (with an accuracy of 0.25 h = 15 min) separately. In total, there were 23 mist-netting night sessions of 5 to 9 h, with 2 to 6 sessions per month, constituting 367 h of mist-netting in total. Captured bats were placed in textile bags and kept near the net throughout the night until being released back at the same location the next evening. Lactating females were released one hour after capture. All bat handling methods were ethical and respectful to animal welfare and met the conservation requirements of protected species, according to international standards [33] and Ukrainian National Legislation [25].

2.4.3. City Bat Records

Opportunistic (accidental) bat findings were reported by citizens to the Bat Rehabilitation Center of Feldman Ecopark through a call-center. A Rehabilitation Center team member then transported the bat (or bats) from the location to the center for further care, such as watering and/or feeding, if necessary, as well as for measuring and banding. Bats without injuries were released the same evening (during the warm season). The Bat Rehabilitation Center also collected and recorded information about bat carcasses. Whenever possible, the same parameters were recorded for dead bats as for live ones. For more information about bat findings, and the full dataset of records, please see [24,31,34].

2.4.4. Bat Measurements

All bats caught using mist-nets or found accidentally were carefully examined with regard to their species, sex, age, and reproductive status. The forearm length (calliper with a precision of 0.1 mm) and weight (digital scale Tanita 1479Z (Tanita, Tokyo, Japan) with a precision of 0.1 g) of each bat were also measured. The recorded bats were classified into three age groups: *ad* for adult bats (older than 1 year), *sad* for 1st year individuals (younger than 1 year), and *juv* for juvenile bats (this-year-born not flying bats). Bats with an unclear age were classified as uncertain (*un*). For further details on age classification and features of identification, refer to [24,31]. A full list of all recorded bats is presented in the Supplementary Materials. All live bats were banded using special bat rings marked "Kiev, Ukraine" in three different sizes, manufactured by Aranea, Poland [35].

2.5. Data Analysis

2.5.1. Analysis of Acoustic Data

The acoustic recordings were analyzed using Bat explorer (version 1.11.04) and Bat sound (Version 4.1) software. Calls were identified at the species level by examining the following parameters of the echolocation signal: peak frequency (kHz), minimum and maximum frequencies (kHz), pulse width (ms), duration of the interval between pulses (ms), as well as the waveform. Due to the difficulty of distinguishing between the calls of *Pipistrellus kuhlii* and *Pipistrellus nathusii* [36], these species were pooled together in one group labelled as *P. kuhlii/nathusii*. Frequency-modulated pulse sequences not identifiable at the species level were classified as Unidentified (Vespertilionidae gen. sp.), and they were not used in the analysis. Bat activity was calculated as the number of recorded sequences per hour, where a sequence is a series of call pulses belonging to an individual bat.

2.5.2. Statistical Analysis

We conducted all statistical analyses using the R software (www.r-project.org, R version 3.5.0, accessed on 31 August 2018). We compared the species composition between survey years and periods of bat life cycle using the chi-square test. To account for the multiple test repetitions, we applied Holm–Bonferroni post hoc correction to all *p*-values. We used the acoustical survey data (number of sequences), results of mist-netting (number of individuals), and city bat records independently (number of individuals) for this analysis.

We utilized generalized linear model analysis (GLM) to determine if individuals of *E. serotinus* had a different pattern of occurrence in the city over the entire study period. In our GLM analysis, the occurrence of *E. serotinus* within the city served as the dependent variable, while variables such as the periods of the bat life cycle and year of record were predictors. These analyses were performed using the spaMM package. For examining the influence of the periods of the bat life cycle on sex and age categories within the city, we conducted GLM analysis with a binomial distribution (stats package), followed by ANOVA. The dependent variable in this model was the age or sex category, while the bat life period was the predictor. This analysis was restricted to *E. serotinus* findings due to the large sample size of this species across both periods of the bat life cycle.

Correlation analysis (in R) was performed to determine the relationship between the number of sequences corresponding to each species and species group and the distance from the acoustic survey site to the city center for each period of the bat annual cycle. To test this, we calculated the distance from each acoustic survey site to the city center. Using these data, we performed a correlation analysis to assess whether there was a significant relationship between the number of sequences and the distance to the city center. We performed this analysis separately for each period of the bat annual cycle.

We employed two indexes to measure bat abundance levels. The first one was the bats per hour index, which was used for the mist-netting results (e.g., [37]). The total number of captured bats in one night (Nm) was divided by the time (hours) of mist-netting (H) (b/h = Nm/H). We added the mist-netting time of each net. The second one was the bat per day index, which was used to estimate the abundance of bat records in the city. The total number of gathered bats in each period of the bat life cycle (Nd) was divided by the number of days (D) in the period (b/d = Nd/D), breeding—92 and migration 42 days.

All maps were created using QGIS software (version 2.18.11).

3. Results

3.1. General Findings

Using all available methodological approaches over 3 years, seven bat species and one species group were identified. Acoustic recordings of bat echolocation calls identified *E. serotinus*, *N. noctula*, *P. pygmaeus*, and the *P. kuhlii/nathusii* from 3456 recorded and identified sequences. Echolocation calls of most species were detected in both periods of the bat life cycle. Additionally, *E. serotinus*, *N. leisleri*, *N. noctula*, *P. kuhlii*, *P. nathusii*, and *P. pygmaeus* were captured via mist-netting in the zoo area, with a total of 364 individuals caught. However, only 19.2% of individuals were captured in the breeding period. City bat records counted a total of 871 individuals from four bat species: *E. serotinus*, *N. noctula*, *P. kuhlii*, and *V. murinus*, with most bats found during the migration period (96%).

The numerous individuals of *E. serotinus* and *P. kuhlii* were mist-netted and recorded in both the breeding and migration periods. In contrast, only one individual of *N. noctula* was mist-netted during the breeding period, while 1039 were found in the city and mist-netted during the autumn migration period.

Among the adult individuals of *E. serotinus* and *P. kuhlii* mist-netted and occasionally found in the city during the breeding period, there was a significant predominance of males (81.4%) and non-reproductive females.

3.2. Acoustic Data

Acoustic surveys showed that *N. noctula*, *E. serotinus* and *P. kuhlii/nathusii* were present at all sites, at least during one of the bat life cycle periods. *Pipistellus pygmaeus* was only recorded at two sites, WB-2 (breeding) and WB-3 (breeding and migration) (Table 2).

There were significant differences in the species composition between periods of the bat annual cycle (see the *p*-value for Chi-square test in Table 2). During the breeding period, *N. noctula* was not detected at two sites (WB-5 and WB-8). However, during the migration period, *N. noctula* was detected at all recording sites and made up, on average, 56.1% of the total number of sequences, with a minimum of 38.3% (at WB-4) and a maximum of 77.4% (at WB-8). *Eptesicus serotinus* was identified at all sites during the entire period of the acoustic survey, being absent only at one site (WB-8) during the breeding period.

Pipistrellus kuhlii/nathusii was present at all sites during the migration period and virtually at all sites except for two (WB-6 and WB-7) during the breeding season, being the only species detected at WB-8. Comparing the species composition based on acoustic data, we found differences at each site of the acoustic survey between the breeding and autumn migration periods (Table 2).

Correlation analysis did not show any significant correlations between the number of sequences related to species during each period of the bat annual cycle and the distance from the acoustic survey site to the city center.

Acoustic surveys at the Zoo revealed the presence of *N. noctula* and *E. serotinus* species as well as *P. kuhlii/nathusii* during both periods of the bat annual cycle (Table 2). However, there is a significant difference in species composition between the breeding period and the autumn migration period (Chi-sqr = 379.55, df = 2, $p < 0.001$). During the breeding period, *P. kuhlii/nathusii* was the most commonly recorded in acoustic surveys (60.03%), followed by *E. serotinus,* with 39.47%. *N. noctula* was only present in 0.5% of the recordings. During the autumn migration period, the number of *N. noctula* increased dramatically and accounted for 45.23% of all sequences. The number of sequences for the *P. kuhlii/nathusii* species group also increased, but the percentage of calls to all recorded sequences decreased to 42.73%. Although the number of *E. serotinus* sequences remained almost unchanged, there was a decline in percentage to 12.04%.

Table 2. Results of bat echolocation call identification for water bodies and the zoo area at different acoustic survey sites with the result of chi-square analysis.

	Sites	WB-1	WB-2	WB-3	WB-4	WB-5	WB-6	WB-7	WB-8	WB-9	WB-10	Zoo
Breeding	*E. serotinus*	16.8	62.1	31.7	50.3	50.0	73.1	27.7	0	27.6	55.3	39.5
	N. noctula	77.6	32.4	59.0	46.9	0	26.9	72.3	0	56.3	31.6	0.5
	P. pygmaeus	0	1.4	0.4	0	0	0	0	0	0	0	0
	P. kuhlii/nathusii	5.6	4.1	6.5	5.6	50.0	0	0	100	16.1	13.2	60.0
	Number of sequences	107	145	278	324	4	26	47	31	87	38	603
	Species number	3	4	4	3	2	2	2	1	3	3	3
Migration	*E. serotinus*	7.4	18.4	32.7	46.9	19.2	33.8	41.6	17.2	58.5	16.1	12.1
	N. noctula	72.3	49.1	41.8	38.3	76.0	58.4	46.8	77.4	41.5	70.1	45.2
	P. pygmaeus	0	0	2.0	0	0	0	0	0	0	0	0
	P. kuhlii/nathusii	20.2	32.5	23.5	14.8	4.8	7.8	11.6	5.4	0	13.8	42.7
	Number of sequences	94	114	98	162	146	77	173	93	41	87	681
	Species number	3	3	4	3	3	3	3	3	2	3	3
Total	Number of sequences	201	259	376	486	150	103	220	124	128	125	1284
	Species number	3	4	4	3	3	3	3	3	3	3	3
	Chi-square	12.3	65.3	25.3	12.8	18.2	12.7	11.9	101.4	14.9	21.2	379.6
	Df	2	3	3	2	2	2	2	2	2	2	2
	p-value	<0.01	<0.001	<0.001	<0.01	<0.001	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001

3.3. Mist-Netting

Table 3 (with footnotes) shows the number of bats of different species and sex/age groups captured during the breeding and migration periods within the urban core area. During the breeding period, five bat species were captured, with a numerical predominance of *P. kuhlii* (51.5%) and *E. serotinus* (42.8%), and only one *N. noctula*. Also, in the autumn migration period, five species were captured, and *N. noctula* became the numerically predominant species, with a ratio of 86.7%. The other three species (*N. leisleri*, *P. pygmaeus* and *P. nathusii*) were represented by single individuals. During the breeding period, among adult individuals of *E. serotinus* and *P. kuhlii*, males significantly predominated, and among females, only a few were lactating (Table 3). Thus, the predominant proportion of individuals captured during the breeding period belonged to the non-breeding portion of the population.

Table 3. Bat species and sex/age group composition during the breeding and migration periods according to the results of mist-netting in the Kharkiv Zoo area, 2014–2015 *.

Period	***N. noctula***					***E. serotinus***					***P. kuhlii***					***P. nathusii***			
	N	**Females**		**Males**		**N**	**Females**		**Males**		**N**	**Females**		**Males**		**N**	**Females**		**Males**
		ad	**sad**	**ad**	**sad**		**ad**	**sad**	**ad**	**sad**		**ad**	**sad**	**ad**	**sad**		**ad**	**sad**	**sad**
Breeding	**1**	-	-	1	-	**30**	2 [1]	-	28	-	**36**	8 [2]	-	28	-	**1**	1 [3]	-	
Migration	**255**	34	155	7	59	**7**	1	2	3	1	**29**	1	6	10	12	**2**	-	1	1
Total	**256**					**37**					**65**					**3**			

*—*N. leisleri* Fsad in migration period and *P. pygmaeus* 2Fad non-reproductive in breeding period not included in the table; [1]—1 non-reproductive and 1 reproductive; [2]—5 non-reproductive and 3 reproductive; [3]—non-reproductive.

The b/h-index for the breeding seasons of 2014 and 2015 years were 0.18 and 0.36, respectively (the two-year average was 0.28). The b/h-indexes for the migration period were 5.44 (2014), 0.97 (2015), and 3.48 (two-year average).

The species compositions differed significantly between the breeding and migration periods in 2014 (chi-squared = 185.49, df = 4, p-value < 2.2×10^{-16}) and 2015 (chi-squared = 19.771, df = 4, p-value = 0.0005541). In addition, a significant difference was detected between periods of the bat annual cycle in total for the summed two years as a whole (chi-squared = 213.35, df = 5, p-value < 2.2×10^{-16}).

Although the numbers of bats within each species were different between the breeding periods of 2014 and 2015 (Table 3), there were no differences in species composition between these two periods (Chi-squared = 3.7411, df = 4, p-value > 0.05), in contrast to the autumn migration periods, where a difference between years was detected (Chi-squared = 160.41, df = 4, p-value < 0.001).

3.4. City Bat Records

Table 4 (with footnotes) shows the number of bats of different species and sex/age groups found in the Kharkiv city area during the breeding and migration periods. During the breeding period, the findings were solely represented by *E. serotinus* (as shown in Table 4). During the migration period (as shown in Figure 2), *N. noctula* was the most commonly found species in the city. It was not uncommon to find large groups of *N. noctula* during the migration period, with the number of individuals per group reaching up to 229 bats. A comparison of the species composition between the two periods revealed significant differences (chi-squared = 299.86, df = 3, p-value < 2.2×10^{-16}).

Table 4. Bat species and sex/age group composition during breeding and migration periods according to the results of city bat records in Kharkiv, 2014–2015 *.

Period	*N. noctula*						*E. serotinus*							*V. murinus*		
	N	Females			Males		N	Females			Males			N	Males	
		ad	sad	un	sad	un		ad	sad/juv	un	ad	sad/juv	un		sad	un
Breeding	**0**	-	-	-	-	-	**35**	2 **	1	-	28	3	1	-	-	-
Migration	**784**	8	282	44	317	39	**49**	2	8	2	5	26	2	**2**	1	1
total	**784**						**84**							**2**		

*—*P. kuhlii* Msad in migration period not included in the table; **—1 non-reproductive, and 1 unidentified.

The b/d-indexes for the breeding seasons were 0.17 and 0.2 for 2014 and 2015, respectively, and increased up to 3.1 and 15.1 in the migration season, respectively.

3.5. Seasonal Changes in Bat Species Composition and Spatial Distribution of Records

The results of mist-netting and opportunistic findings of bats in Kharkiv showed similar species composition, abundance, sex/age structure and seasonal dynamic. These results (except for sex/age structure) align with the findings from acoustic recordings within the urban core. However, the results of the bat acoustic surveys near water bodies outside of the urban core demonstrate slightly different outcomes. Thus, echolocation pulses of *N. noctula* were recorded above the surface of most of the water bodies both in the breeding and migration period (Table 2).

It was observed that the presence of *E. serotinus* within the city remained consistent throughout the entire study period (both the breeding and the migration).

The distribution of city bat records is presented in Figure 2a–c. Generally, the records were concentrated in the city center area, and a few were on the periphery (Figure 2a). The records of *N. noctula* (that were found in the migration period only) were concentrated within the urban core of Kharkiv (Figure 2b) and were obtained in two buildings that aggregated large winter colonies (mentioned above Derzhprom and KhNU buildings).

Similarly, the records of *E. serotinus* were obtained in the central part of Kharkiv, both in the breeding (bachelor male groups) and migratory period (groups with subadult bats).

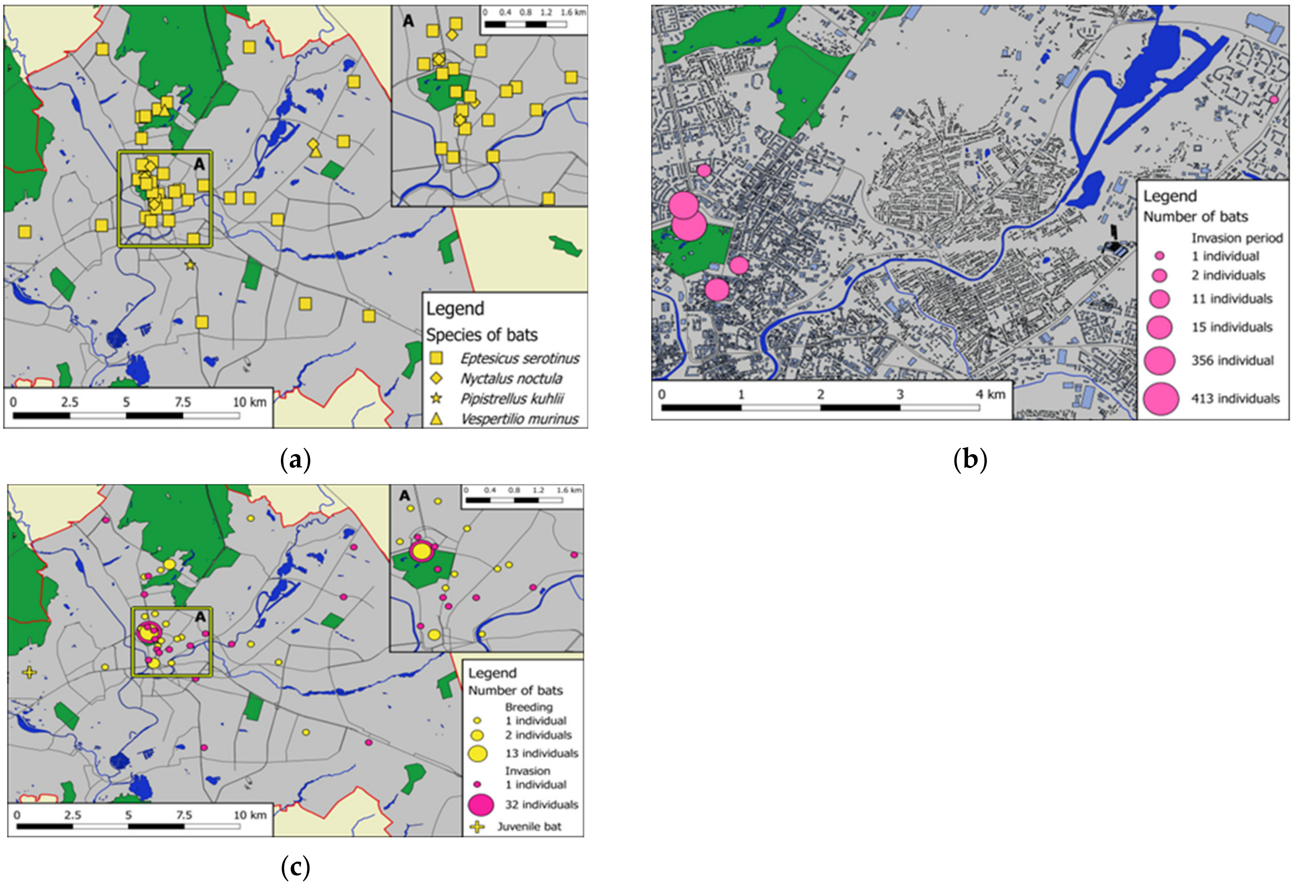

Figure 2. Spatial distributions of city bat records in Kharkiv city in 2014–2015 (with north at the top of the map). (**a**) Points of records by species, (**b**) distribution of records and numbers of *N. noctula* during the migration (call invasion in the map legend) period, and (**c**) distribution of records and numbers of *E. serotinus* in both periods. Zone A on maps **a** and **c** is a zoom of the city center area.

3.6. Sex Ratio and Reproductive Status

During the breeding period, only adult males and non-breeding females of *E. serotinus* were occasionally found throughout the city (Table 2). The same sex/age groups of *E. serotinus* were caught within the urban core. During the breeding season, individuals of *P. kuhlii* were not recorded in the city, but adult individuals of both sexes of *P. kuhlii* were mist-netted, including a few lactating females. All sex/age groups of *E. serotinus* were present in the city in the migration period, and young individuals of *P. kuhlii* were recorded using both handling methods as well.

During the migration period, all sex/age groups of *N. noctula* were mist-netted. Females predominated among both subadult (71% of all young individuals) and adult (94% of all adults) individuals captured within the urban core. Of the 784 *N. noctula* found in the city, sex and age were identified for 610 individuals, and all but 8 adult females were subadults. The sex ratio of subadult *N. noctula* found in the city was significantly different from 1:1 (chi-squared = 18.4181, df = 1, p-value = 1.774×10^{-5}). The sex ratio of subadult *N. noctula* captured at the zoo was not significantly different from 1:1 (chi-squared = 2.1969, df = 1, p-value = 0.1383). However, the sex ratio of subadult *N. noctula* between these two datasets was not significantly different from each other (Chi-squared = 0.0904, df = 1, p-value = 0.7637).

An analysis of the sex composition of *E. serotinus* showed that the period of the bat life cycle did not significantly influence the sex ratio, both among adult (ANOVA, chi-squared = 2.27, df = 1, $p > 0.05$) and subadult individuals (ANOVA, chi-squared = 1.53, df = 1, $p > 0.05$), with male predominance observed in both age categories and periods (Supplementary Materials). There is a significant difference in the relation of age categories between bat life periods for *E. serotinus* and *N. noctula* together (for males: ANOVA, chi-squared = 38.59, df = 1, $p < 0.001$; for females: ANOVA, chi-squared = 5.27, df = 1, $p < 0.05$). Thus, during the breeding period, adult males and females predominate. However, during the migration period, subadult bats within each sex group begin to prevail.

There was one finding of a juvenile female bat of *E. serotinus* (Figure 2c). This happened in July 2014, during the breeding period. This is the only finding of juvenile bats within the city in 2014–2015.

4. Discussion

In this study, we tested the hypothesis that bats avoid the urban core at temperate latitudes (using Kharkiv city as a case study) during the breeding season, and that species richness is low in urban habitats during this season. We assumed that urbanized landscapes could not provide sufficient food sources (insects) for female bats during the peak of their energetic requirements (pregnancy and lactation). By comparing the results of bat mist-netting, bat records in the city, and ultrasound recordings made over three years, we demonstrated that only a few species (*E. serotinus* and *P. kuhlii*) inhabit the urban core area of Kharkiv city during the breeding season. The local bat population during this season predominantly consists of males and solitary reproductive females. However, the results of acoustic recordings show that species (*N. noctula*) that avoid the urban core are common on the city's periphery. During the autumn migration period, the city becomes populated with bats, with *N. noctula* becoming numerically predominant in mist-netting within the urban core and opportunistic bat findings across the city. However, acoustic recordings on the city periphery did not show a significant increase in *N. noctula*'s sequences. This period also sees the appearance of a large number of newly born bats from three common bat species (*E. serotinus*, *N. noctula* and *P. kuhlii*). In total, seven bat species were identified in the city (*E. serotinus*, *N. noctula*, *N. leisleri*, *P. pygmaeus*, *P. kuhlii*, *P. nathusii* and *V. murinus*) during our survey. To the best of our knowledge, this is the first study to clearly evidence that bats, especially reproductive females, avoid the city's urban core in the territory of Eastern Europe. However, the results of acoustic recording (presence of bats on the city periphery) need to be verified through mist-netting in order to identify which sex/age groups of bats inhabit the suburbs of Kharkiv city.

4.1. Methodology

In this study, we applied three methodological approaches with different biases regarding sample quantity and quality. Thus, the sample of the opportunistic bat findings has a potential behaviorally based bias. In most cases, individuals that explore the urban space and, for unknown reasons, fly into building interiors during the breeding season have a higher probability of being found by citizens, as do bats that fly in during the migration season, probably driven by instinct to find roosts. The bat mist-netting approach should provide the most accurate results of the local bat species list and sex and age structure, as most bat species were successfully captured using this method in previous research. There is an opinion that air-hawking bats may avoid mist-nets (e.g., [38]). This might be true in the case of an uncovered mist-net set in an open space, and if a mist-netting session only lasts for the first few hours of the night. However, we always followed the protocol of keeping the captured individuals in textile bags near the mist-nets (inter-species and species-specific distress calls lure more individuals from around) and conducted the mist-netting all night long (after midnight, air-hawking bats tend to fly lower) [37]. This approach increases the proportion of air-hawking bats in a sample and provides a better picture of species diversity and relative abundance for each location and habitat type [37]. The third methodological approach involved the recording of bat calls of all species flying around. However, acoustic sampling is biased by signals from air-hawking bats that produce stronger signals at a lower frequency and therefore spread further in space. Despite the disadvantages and advantages of each methodological approach, the combination of all of them gave us the most comprehensive picture of the utilization of Kharkiv's urban landscape by bats. However, a significant advantage of mist-netting and opportunistic bat findings is the ability to identify the sex, age, and reproductive status of individuals.

The results of opportunistic bat findings during the breeding season suggest that this method is least efficient in terms of revealing species diversity, as only one species, *E. serotinus,* was found. Nevertheless, this approach allows us to estimate the sex and age structure in urban populations of bats. Moreover, the sex and age structure among opportunistically found individuals was similar to that revealed via mist-netting efforts.

At the same time, according to both mist-netting (Table 3) and acoustic recording (Table 2), *E. serotinus* is only the second most abundant species after *P. kuhlii* within the urban core. These results might also suggest that *P. kuhlii* is more behaviorally adapted to the urban environment and less likely to be trapped in anthropogenic traps, and therefore less likely to be found by citizens. Interestingly, only a few sequences of *N. noctula* recorded in the urban core in the breeding season were followed by the capture of an adult male—in June 2015. During the migratory period, the number of species increased in both mist-netting and city records samples due to the high mobility of bats, and *N. noctula* became the predominant species. Acoustic records within the urban core confirmed this trend. The city became populated with young of-the-year individuals, similarly trapped and mist-netted. Thus, during the migration period, all methodological approaches revealed similar patterns in terms of bat diversity and abundance.

Acoustic recordings near water bodies provided us with contrasting results in comparison to the urban core. First of all, echolocating calls of *N. noctula* were recorded near most of the water bodies in Kharkiv during the breeding season, and second, there was no drastic increase in the number of call records of *N. noctula* during the migration season. This method complements the results of city records and mist-netting efforts, showing that at the city periphery, air-hawking bats might be present even in the breeding season. However, the sex and reproductive status of these bats remain unknown, and further mist-netting surveys are needed to reveal the reproductive status of bats at the city periphery.

4.2. Particularities of Species-Specific Urban Core Utilisation by Bat Species

The bat species list of Kharkiv, excluding *T. teniotis*, comprises eleven species. The richest area in bat species is located at the northern city frontier, where a woodland area is deeply entrenched within the built-up space (Figure 1) [25]. This woodland area harbors the breeding colonies of several forest-dwelling species, including *N. noctula* [25] (point 3 in Figure 1). The echolocating calls of *N. noctula* were recorded at this point, and the nearby point 2 (Figure 1) is located approximately 5 km away from Kharkiv city center. Interestingly, at two other water bodies (points 5 and 8, Figure 1), which are located at the same distance from the city center, but surrounded by dense built-up area, there were no sounds of *N. noctula* in the breeding season (Table 1). Therefore, we can suggest that the utilization of the urban core by tree-dwelling species, such as *N. noctula*, is highly selective and lies in close proximity to tree-roosting sites.

It is important to highlight that the case of Kharkiv's urban core may not be universally applicable to other cities in Ukraine and Europe. First of all, before the full-scale war in 2022, Kharkiv was the second-largest city in Ukraine in terms of population size and the third-largest by area. Second, Kharkiv is structured differently from cities located on large rivers, such as Kyiv, Dnipro, and Zaporizhzhia in Ukraine, and Vienna and Budapest elsewhere in Europe. The presence of large rivers and their associated floodplains, lakes, and forests in these cities could significantly enhance the habitability of urban landscapes for bat breeding. Finally, only eight cities in Ukraine have a diameter of more than 10 km. We preliminarily estimated that the urban core of Kharkiv, which is the least suitable for bats, might be roughly evaluated as having a 8–10 km diameter around the city center. Few cities in Europe can match these parameters. The findings obtained for Kharkiv need verification across different cities; however, most urban bat research relies on acoustic methods [16], which does not facilitate an estimation of the sex and age structure of urban bat populations. Therefore, we suggest, in addition to acoustic surveys, conducting mist-netting sessions within the urban areas in order to verify whether urban landscapes are suitable for the breeding of urban bat species.

This study provides robust evidence that two bat species (*E. serotinus* and *P. kuhlii*) predominantly inhabit the urban core of Kharkiv city during the breeding season, with only a few individuals of other species. Despite being highly mobile volant animals (e.g., [38–41]), most bat species are absent from the urban core area during the breeding season, likely due to the unsuitability of the habitat. Several global reviews emphasize species traits (morphological, acoustical, taxonomical, etc.), enabling different bat species to adapt better to urban landscapes [42,43]. However, trait characteristics do not account for distinct species' seasonal needs, especially at temperate latitudes. If we associate adaptation to urban landscapes with species' relative abundance in Kharkiv, then the most adaptive species appears to be *N. noctula*. However, during the breeding season, this species avoids build-up areas and tends to occupy woodlands such as forests and urban parks. Therefore, we suggest that the presence or absence of bat species in urban areas is not a reliable indicator of the adaptability and suitability of urban habitats. Rather, resident groups of reproductive females should be considered as an indicator of habitat quality to support viable populations of bats.

4.3. Spatial and Seasonal Difference of Bat Sex/Age Groups of Urban Landscape Utilisation

The pattern of seasonal sex and age segregation in temperate-latitude bats is well-established (e.g., [44–46]). Different sex and age groups utilize diverse habitats or even landscapes during varying seasons throughout the annual cycle. These preferences stem from the need for roosting sites with differing microclimatic conditions: cooler for males and warmer for females (e.g., [21,47,48]), and the substantially higher amount of food required by breeding females compared to males (e.g., [23]). In spring, female bats frequently migrate from high-altitude winter habitats to lowland regions with an abundance of wetlands and woodlands (e.g., [49,50]). Conversely, male bats usually remain close to their wintering areas during the breeding period [47,51,52]. While these bat seasonal movements have been extensively documented in natural or rural areas [44–53], the role of urban environments, and the urban core in particular, in seasonal movements remains largely unexplored. Nonetheless, a few reports have indicated a prevalence of males in urban landscapes [54] and poorer body conditions among females [55,56]. Our findings offer valuable insights into how bats adapt to their natural annual cycles within urban landscapes in temperate latitudes. We propose that contemporary large cities, particularly their urban cores, located in lowland terrains, serve a similar ecological role for bats as high-altitude wintering areas. Despite being poor in vegetation and water bodies, these urban cores provide ample urban roosts that attract bats during autumn swarming and hibernation seasons. This seasonal pattern demonstrates an adaptation of bats to modern urban ecosystems, where they take advantage of the abundance of potential roosting sites and the generally warmer winter climates compared to rural surroundings. However, during the summer, the scarcity of natural vegetation and insects in cities could deter urban bat populations.

Understanding how bat sex/age groups utilize urban landscapes spatially and seasonally offers crucial insights into how urbanization impacts the life histories and survival strategies of bat species. For example, our study in Kharkiv city highlights the significance of investigating the dynamics of sex and age within the urban core to explain the low bat population during the breeding period and the contrasting surge during the migration period. This knowledge is essential for developing effective conservation strategies to support urban bat populations.

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