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Stratifying the urban matrix using zoning laws: a protocol for bats and their pathogens

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Abstract
Urbanization implies important ecological changes in bat communities and in their intra and interspecific pathogen transmission. However, little is known about the influences of the urban components on the ecology and structuring of these communities. The urban environment is a complex and heterogeneous matrix, in which different physical, structural, and socioeconomic factors influence the ecological dynamics of bats. A scale that better contemplates such spatial heterogeneity and complexity can contribute to improving the understanding of urbanization influences on bats. Urban zoning legislation determines a city's land subdivision, use, and occupation, acting as a detailed division of the urban matrix itself. We used urban zoning to develop a sample design for an eco-epidemiological study of bats in São Paulo, the biggest Metropolitan Area of Brazil. The zones defined by municipal laws were validated through visual analysis using remote sensing images to distinguish the urban matrix into strata as distinct as possible from each other and to meet the logistical assumptions of fieldwork. Six different strata were obtained: forested, peri-urban rural, slum, residential with houses, condominiums, and industrial areas. Although complementary to other landscape analysis techniques, municipal zoning can be useful to access the urban heterogeneity that general divisions of land use (e.g., urban patch) do not supply. Thus, it can be useful to compare animal communities between urban strata and to aid formulation of hypotheses about urbanization's influences on these communities. In addition, this tool steps up the connection between ecology and urban planning, subsidizing One Health actions in interdisciplinary approaches.

Keywords Bats · Urban · Zoning · Planning

Introduction

Urbanization is an extreme anthropization process. Beyond the gigantic modification of the environment, the functioning of a city demands several other anthropic activities, such as deforestation, agricultural monocultures, and reforestation with non-native species (Pacheco et al. 2010). Therefore, cities act as hotspots of environmental effects (Moll et al. 2019), and implies in ecological changes in communities (Fisher, Streicker and Schnell, 2018; Russo and Ancilotto 2015). Like for other animal groups, some species of bats (order Chiroptera) are negatively impacted by urbanization, which may even lead to local population decline processes (Rosa et al. 2011; Czech et al. 2000), while others are benefited by the good opportunities that cities offer, such as shelter, food, and few predators (Rosa et al. 2011).

Insectivore bats is the guild that best adapted to the urban environment and to the use of artificial roosts, such as ceilings and roofs of houses, basements, hollow concrete blocks, spaces between buildings, garages, and others (Rosa et al. 2011). Some of them may benefit from aerial insects attracted by artificial illumination (Lima 2008). Phytophagous species may be also benefited, from using urban vegetation, such as forest fragments, afforestation patches, and landscaping vegetation (composed mostly of invasive plant species), to feed and shelter (Almeida et al. 2015; Lima 2008).

Besides being the second richest mammal order (Almeida et al. 2015), with 1,386 species arranged in 227 genera and 18 families (Burgin et al. 2018), bats are animals with significant ecological, human, and animal health relevance.

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They are seed dispersers, pollinators, bug controllers, and predators, providing several ecosystem services (Kunz et al. 2011). However, they also act as carriers, reservoirs, or transmitters of pathogens to humans and animals and may be involved in the cycle of emerging diseases, such as the pandemic COVID-19 (Temmam et al. 2022).

Considering the possibility of changes in intra or interspecific dynamics of pathogen transmission because of urbanization (Fisher et al. 2018; Russo and Ancilotto 2015; Lima 2008), the investigation of these dynamics is of paramount importance. This fits as a One Health approach since, currently, most of humanity lives in cities (UN, 2014), where there is also a massive population of domestic animals (mainly dogs and cats), and the proximity of bats to this scenario raises concern about the transmission of zoonotic pathogens (Reynolds et al. 2021). Active surveillance is an approach to monitor not only the bat communities themselves but their pathogens. It has been used in some places in Europe to investigate Lyssavirus (Leopardi et al. 2018; Šimić et al. 2018; Picard-Meyer et al., 2011), and in Brazil, for the rabies virus (Horta et al. 2018). However, it is still little explored, especially in urban areas. Even ecological studies, frequently used in the monitoring of bats, are usually performed in forest patches and are scarce for urban bats (De Lucca et al. 2013).

Some well-spread approaches to fauna studies in urban environments are based on binary comparisons between urban versus rural environments, through gradient sampling on a rectilinear axis from an urban center to rural areas (Moll et al. 2019; McKinney 2002; Alberti et al. 2001), or studies in urban green areas such as parks and squares (Nunes et al. 2017). However, despite pointing out significant differences in biodiversity between urban and rural or preserved environments, these studies rarely identify and quantify the variables or components of the landscape that generate such changes (McKinney 2008). In addition, the urban spatial configuration, especially in metropolitan areas, is extremely heterogeneous. Instead of well-defined transition gradients between urban, rural, and forested ecosystems, metropolitan regions form mosaics of built and natural structures, which might influence biodiversity in several ways (Forman 2014). Furthermore, aspects other than these, like the sociopolitical configuration of a city, may also potentially influence species (Bhakti et al. 2021).

Therefore, it is important to access the complexity and heterogeneity of the landscape within the matrices (i.e. forested, rural and urban matrices) to advance the understanding of the influence of urbanization on ecological processes. However, simple transect approach do not contemplate that. On the other hand, covering all the heterogeneity of a city is not compatible with fieldwork. Thus, it would be useful to have a tool to assist the definition of sampling sites by stratifying the landscape, to better represent the different aspects of a city that might be important for the ecological process being accessed.

The municipal zoning legislations, or the master plans, determines the subdivision, use, and occupation of the land, and can be a tool in understanding the spatial organization of a municipality and its physical and socioeconomic structures. It is an easily accessible document, being advantageous compared to several other proposals to represent a more detailed urban landscape for ecological purposes (Moll et al. 2019; Cadenasso et al. 2007), which require mastery of specific and complex techniques or access to non-free tools. In Bhakti et al. (2021), municipal zoning influenced the modeling of permeability of birds to the urban environment. In case of bats, for example, which use human constructions for shelters, it is interesting to access and compare them in areas of the city that have a predominance of different components of the landscape, such as different types of roof, different levels of afforestation, verticalization, or presence of open areas or backyards in the residences. Urban zoning legislation allied with visual interpretation of satellite imagery, allows to access part of the urban heterogeneity, and to identify such components, helping in stratifying the sample design and planning bats' collection in the field.

The present study explored the municipal zoning of some cities in the Metropolitan Area of São Paulo (MASP), in Brazil, to develop a design for an eco-epidemiological study of bats in a gradient of urbanization. Brazil has 181 species (13.1%) of bats distributed in 68 genera (30%) and 9 families (50%) (Garbino et al. 2022), of which 84 (47.2%) occur in urban areas (Nunes et al. 2017), mainly from the Phyyllostomidae, Molossidae and Vespertilionidae families (Pacheco et al. 2010; Dias et al. 2019). As the main urban agglomeration of the South America and the sixth biggest in the world (UN, 2014), the MASP shows a great physical-structural and socioeconomic heterogeneity of a metropolitan mosaic. Thus, the framework presented here, although being developed for a particular case study, can be used for other animal groups (with proper adaptations), and aims to help in designing studies of urban fauna that deepen the knowledge of the impacts of urbanization on them, for beyond the urban *versus* non-urban comparisons.

Materials and methods

Contextualizing the metropolitan area of São Paulo

The Metropolitan Area of São Paulo (MASP) is in the southeast of the state of São Paulo, Southeastern Brazil, and occupies an area of 7,946.96 km^2, about 3.2% of the total area of the state (EMPLASA, 2019). It comprises 39 municipalities, with a population of 21,571,281 inhabitants, about 47.4% of the total population of the state (EMPLASA, 2019). The region represents the largest center of national wealth, or 18% of Brazilian gross domestic product (GDP) GDP and 54% of State GDP (EMPLASA, 2019), encompasses important industrial, commercial, and financial centers and is the center of political decisions of the state (EMPLASA, 2019). However, it also has an extensive and important rural matrix, including within the municipality of São Paulo itself, the State capital, and main national metropolis (EMPLASA, 2019). The main landscape matrices of the region (urban, rural and forest fragments) are represented in Fig. 1.

The MASP is within the Atlantic Forest biome and has an expressive urban growth and fragmented vegetation. The region has several conservation units and forest fragments, including within the urban matrix. Several urban parks and squares also contribute with the internal vegetation to the urban matrix of the MASP, in addition to the afforestation of various built spaces (houses, condominiums, clubs, etc.), and the roads themselves. In São Paulo, for example, approximately 75% of urban households are located on public roads with trees (IBGE, 2010). However, the most expressive forest fragments are found on the edges of the MASP, mainly in the North and South zones. Such regions concentrate a continuous set of conservation units that cover several municipalities. The forest fragments to the east are less expressive, and to the west, one of the most significative forest patches is the Morro Grande Forest Reserve, located in the municipality of Cotia. The reserve encompasses the local water and sewer company (Sabesp) and two dams (Ipatrimônio, 2022).

Selection of the study area

The study area was selected according to the following criteria: (1) be within the limits of the MASP; (2) consist of a distinguishable gradient between forest fragments, rural areas, and urban areas; (3) have dimensions and characteristics that are necessary, viable and compatible with field data collection. The expanded center of the city of São Paulo (Prefeitura de São Paulo 2022a) was discarded as a possible

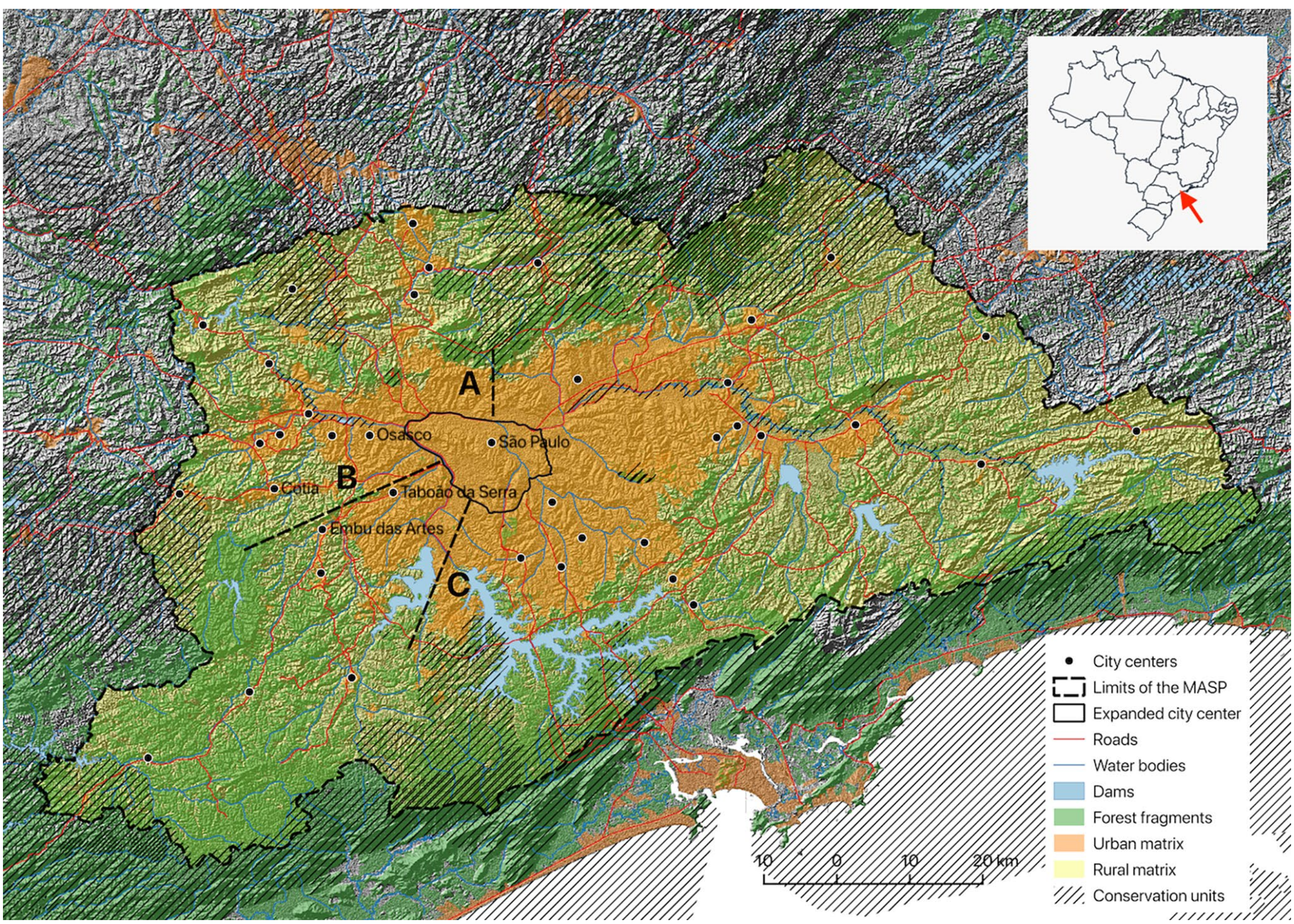

Fig. 1 Main matrices of the Metropolitan Area of São Paulo (MASP) and axes eligible as study areas, traced from forest fragments to the limit of the expanded center of the city of São Paulo (**A**, **B** and **C**)

study area due to the difficult to carry out fieldwork in this region. Thus, starting from expressive forest fragments of the MASP towards the limits of the expanded center of São Paulo, three possible areas of study were traced, defined by the axes represented in Fig. 1:

Axis A – North: From the Cantareira State Park to Campo de Marte in São Paulo, with an approximate length of 9 km;

Axis B – West: From the Morro Grande Forest Reserve (or Private Natural Heritage Reserve of Sabesp) in Cotia to the University of São Paulo campus in São Paulo, with an approximate length of 27 km;

Axis C – South: Municipal Environmental Protection Area from Capivari-Monos to Congonhas Airport in São Paulo, with an approximate length of 22 km.

Axis A presents an abrupt change from the forest (Parque Estadual da Cantareira) to an urban area, not comprising an explicit rural area along the axis and, therefore, not meeting the established transition criterion. Axis C, despite showing a satisfactory transition between the desired matrices, the forest fragments are located on the slope of Serra do Mar, whose characteristics (altitude, relief, temperature, humidity, etc.) could act as confounding factors for the research. Axis B, in turn, does not present major changes in relief between the matrices, and has a marked transition gradient, in addition to the matrices occupying similar dimensions along the axis. No axis was traced in the East Zone due to the excessive distance from the limit of the expanded city center to any forest fragment, which would not be compatible with fieldwork since it would over-represent the urban matrix. Axis B was chosen as the study area, which was delimited from 3 km in width bilaterally to the axis, totaling an area of approximately 81 km^2, including the municipalities of Cotia, Embu das Artes, Osasco, São Paulo, and Taboão da Serra (Fig. 2).

Municipal zoning

To understand the use and occupation of land in the study area, a search was carried out on the Internet pages of the municipalities of the study area to access their urban zoning laws and their respective spatial data, when available. For the municipality of São Paulo, Law 16,402/16 (São Paulo 2016), governs the subdivision, use, and occupation of land, according to Law 16,050/14, the city's Urban Plan (São Paulo 2014). The Urban Plan of São Paulo (Law 16,050/14) was used for the purpose of consulting and understanding the general territorial ordering of the municipality, however, the analysis and interpretation of the zones were carried out based on Law 16,402/16, which is specific on zoning. To this end, georeferenced databases of the areas of São Paulo were used (Prefeitura de São Paulo 2022b).

Complementary Law 95/08 was used to describe the zones in the municipality of Cotia, which establishes the zoning plan and rules for the uses, subdivision, and occupation of land in the municipality of Cotia (Cotia 2008). However, the zoning map used comes from Complementary Law 204/14, which amends Complementary Law 95/08" (Cotia 2014). For Embu das Artes, Complementary Law 186/12 (Urban Plan) (Embu das Artes 2012) was used, along with an attached map of the zones (Prefeitura de Embu das Artes

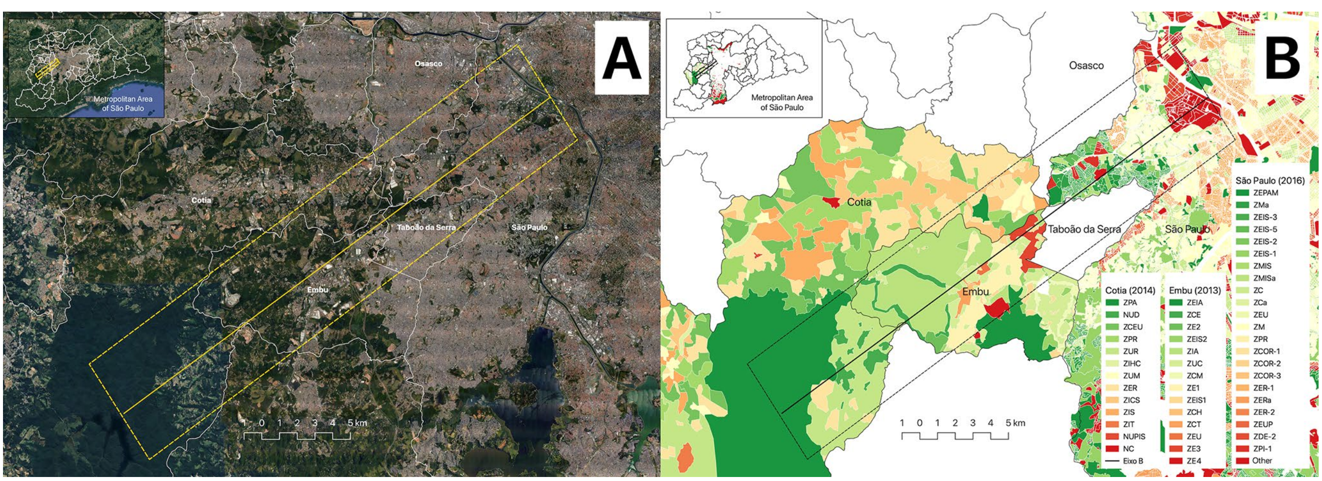

Fig. 2 (**A**) Satellite image of the study area along with the Axis B and (**B**) Municipal zoning of the municipalities of São Paulo (Law 16,402/16), Cotia (Complementary Law 204/14) and Embu das Artes (Complementary Law 186/12). ZEIA, ZEPAN and ZPA = environmental protection zones; ZIA = environmental interest zone; ZCEU = urban expansion containment zone; ZUR = rural zone; ZCE, ZCOR-1, ZCOR-2 and ZCOR-3 = business corridor zone; ZCM = mixed corridor zone; ZER-1, ZER-2, ZER and ZERa = exclusively residential zones; ZPR = predominantly residential zones; ZEU = urban expansion zone; ZM, ZMa and ZUM = mixed zones; ZUC = consolidated urban zone; ZEIS-1, ZEIS-2, ZEIS-3, ZEIS-5, ZIS, ZMIS and ZMISa = special zones of social interest; NUD = disaggregated urban nuclei; ZEU and ZEUP = urban transformation structuring axis zones; ZC and ZCa = centrality zones; ZDE-2 = economic development zone; ZPI-1 = predominantly industrial zone; ZE-1, ZE-2, ZE-3 = business zones; ZICS = trade and service industry zone; ZCH, ZCT and ZIHC = historical and cultural interest zones; ZOE = special occupation zone

2022). The maps of Cotia and Embu das Artes, originally available in the format of PDF or TIFF image files, were georeferenced using QGIS (QGIS Development Team 2022), through the "Georeferencer" function. Zoning laws or maps were not found for the municipalities of Osasco and Taboão da Serra.

A total of 71 zones were identified, 46 in São Paulo, 13 in Cotia and 13 in Embu das Artes. The spatial data of the original zones were superimposed on the delimitation map of the study area through the QGIS software, and the zones that were absent in the study area were excluded from the study. A total of 46 zones composed the study area, 23 in São Paulo, 13 in Embu das Artes and 10 in Cotia (Fig. 2 and **Supplementary Information**).

In an Excel® spreadsheet, information was compiled on the uses that each zone exerts or is intended to exert, constructive or demographic density and other additional characteristics relevant to the understanding of its spatial constitution. Only the descriptions of the zones of the municipalities were used, but no other sections or chapters of the laws that deal with the use or subdivision of land.

Landscape stratification

Step 1 - Urban zoning analysis

The "zones" of the municipalities of São Paulo, Cotia and Embu das Artes were classified and pre-grouped according to their official descriptions documented in the master plans or zoning laws. Firstly, they were grouped based on the main use intended for each one, and later, to deepen the understanding of the constitution of the zones and verify if they are similar or different from each other, based on additional information such as demographic or construction density and other relevant features.

Step 2 - Descriptive visual analysis of the landscape

Satellite image provided by the Google Earth® and implemented in QGIS (QGIS Development Team 2022) was used to check the spatial composition of the zones described in the previous step. Two sampling areas were randomly selected for each zone. Some of them presented only an area within the limits of the study area. A total of 80 areas were chosen, as illustrated in Fig. 3. For each sampled area, the predominant characteristics of the landscape were recorded in an Excel® spreadsheet. This step was performed by two researchers from the team, independently. Their notes were compared and discussed until the consensus was reached. The recorded characteristics were: building density, presence and type of vegetation (dense trees, pasture, open green area), water bodies, exposed or paved soil, number of floors and spacing between buildings, type of roof (clay or asbestos tiles) and size of houses, presence of swimming pools, agricultural plantations, warehouses, and condominiums (verticalization).

Step 3 - Comparison between urban zoning and observed landscape

Through the comparison between the legal description of the zones and their observation in satellite images, the original zones were reviewed for their coherence, and modified when necessary. The spatial constitution observed by satellite image was prioritized, that is, areas that presented a similar constitution of the landscape observed in the satellite image even with different land uses in their official descriptions of the law were merged, while zones that differed from the others of the original zone to which they belonged, were splitted. The result of this step was called "intermediate stratification".

Step 4 - Regrouping

The intermediate stratification was reviewed and evaluated for their biological relevance to bat biology and feasibility of field work, since the number of sampling regions in a bat data collection campaign must be be as low as possible to avoid significant climatic and lunar variations. The time criterion established for future fieldwork was to sample all strata in at least one week. The result of this stage was the "definitive strata".

After defining the "intermediate strata" (Step 3) for the municipalities of Cotia, Embu das Artes and São Paulo, the construction of the strata for Taboão da Serra and Osasco was carried out. A general visual analysis of the landscape of the parts of Osasco and Taboão da Serra contained in the study area was carried out, and the "intermediate strata" of the other municipalities were extrapolated to these, according to the constitution of the landscape consistent with the characteristics and compositions of each stratum. The "definitive strata" were therefore adjusted for Taboão da Serra and Osasco according to the other municipalities.

Sampling areas

Based on the definitive strata, sampling areas could be selected. The criteria for choosing the sampling areas included: (a) being located (or as close as possible) in the strata, (b) providing a safe condition for carrying out the activities and (c) authorization to carry out field samplings. At least one sampling area was selected in each stratum, which were later used in a sanitary, ecological, and bioacoustics monitoring of bats in MASP.

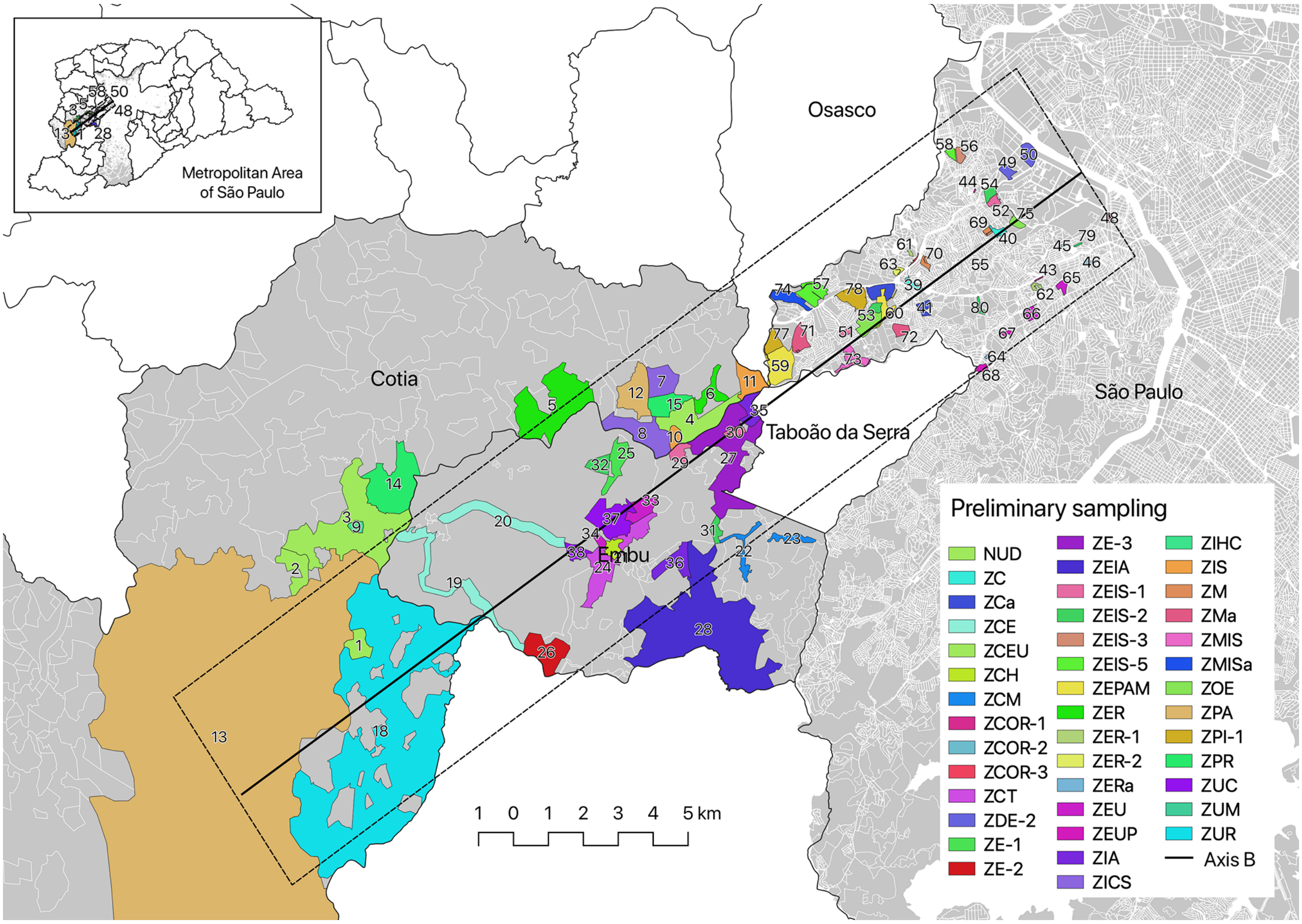

Fig. 3 Sampling for the descriptive visual analysis of the landscape of the urban zoning of São Paulo (Law 16,402/16), Cotia (Complementary Law 204/14) and Embu das Artes (Complementary Law 186/12) and delimitation of the study area along with Axis B. ZEIA, ZEPAN and ZPA = environmental protection zones; ZIA = environmental interest zone; ZCEU = urban expansion containment zone; ZUR = rural zone; ZCE, ZCOR-1, ZCOR-2 and ZCOR-3 = business corridor zone; ZCM = mixed corridor zone; ZER-1, ZER-2, ZER and ZERa = exclusively residential zones; ZPR = predominantly residential zones; ZEU = urban expansion zone; ZM, ZMa and ZUM = mixed zones; ZUC = consolidated urban zone; ZEIS-1, ZEIS-2, ZEIS-3, ZEIS-5, ZIS, ZMIS and ZMISa = special zones of social interest; NUD = disaggregated urban nuclei; ZEU and ZEUP = urban transformation structuring axis zones; ZC and ZCa = centrality zones; ZDE-2 = economic development zone; ZPI-1 = predominantly industrial zone; ZE-1, ZE-2, ZE-3 = business zones; ZICS = trade and service industry zone; ZCH, ZCT and ZIHC = historical and cultural interest zones; ZOE = special occupation zone

Results

Landscape stratification

Step 1 - Urban zoning analysis

The analysis and regrouping of the original zones, and the revision of this initial grouping was based on other characteristics of the zones described by the laws. Eleven different descriptions of the use of the zones were identified in the laws (described as "main use"), which designated the division of the zones of the municipalities of São Paulo, Taboão da Serra and Embu das Artes into 11 groups: (I) "Environmental preservation"; (II) "Rural"; (III) "Exclusively residential"; (IV) "Mainly residential"; (V) "Social interest"; (VI) "Residential and non-residential"; (VII) "Mainly non-residential"; (VIII) "Industrial, residential and commercial"; (IX) "Industrial and commercial"; (X) "Historical and cultural preservation" and XI) "Other". Other relevant information about the zones, in addition to the "primary use", contained in the official descriptions of the zoning laws of each municipality were also consulted.

Groups I ("Environmental preservation"), II ("Rural"), VI ("Residential and non-residential") and XI ("Other") remained intact. The zones of groups III ("Exclusively residential"), IV ("Mainly residential"), VIII ("Industrial with residential and commercial") and IX ("Industrial and commercial") resulted in this stage of the stratification as "inconclusive", as they differed in their additional description or in the demographic/constructive densities presented or did not present sufficient data in their descriptions. Therefore, it was not possible to conclude whether the zones of these strata could be grouped in such a way. Groups VII ("Mainly non-residential") and X ("Historical and cultural preservation") were partially maintained: Group VII presented two inconclusive zones (ZC and ZCa of São Paulo) because they have different densities from the others and lack of data in the description, and in group X, there was an inconclusive zone (ZIHC of Cotia), again due to insufficient data in the description. Group V ("Social Interest") was the largest group (10 zones) and the most heterogeneous, so it was subdivided into two strata and still had five inconclusive zones due to divergences in descriptions. The ZMIS and ZMISa zones of São Paulo, however, present the same description, but remained inconclusive as they belong to different macrozones. Two strata that emerged from the V group: V-a ("Slums"), with zones ZEIS-1 of Embu das Artes and ZEIS-1 of São Paulo; and V-b ("Empty or underused lots"), with zones ZEIS-2 of Embu das Artes and ZEIS-2 and ZEIS-5 of São Paulo.

Step 2 - Visual analysis of the landscape

Both researchers looked at the satellite images of the two samples from each zone, noted their observations and compared them, reaching a consensus of the description, compiled in the **Supplementary Information**.

Step 3 - Comparison between urban zoning and observed landscape

Nine "intermediate strata" were formed, as described in the **Supplementary Information** and Fig. 4. All zones of Group I "Environmental Preservation" (ZPA of Cotia, ZEIA of Embu das Artes and ZEPAM of São Paulo) were considered to belong to the same group and remained as "Environmental Preservation" as an intermediate stratum, as they are predominantly formed by forested areas. The Group II "Rural" (ZCEU and ZUR of Cotia, and ZCE and ZIA of Embu das Artes) remained as the intermediate stratum "Rural". The NUD zones of Cotia and ZE-2 of Embu das Artes, previously belonging to the initial Groups V – "Social

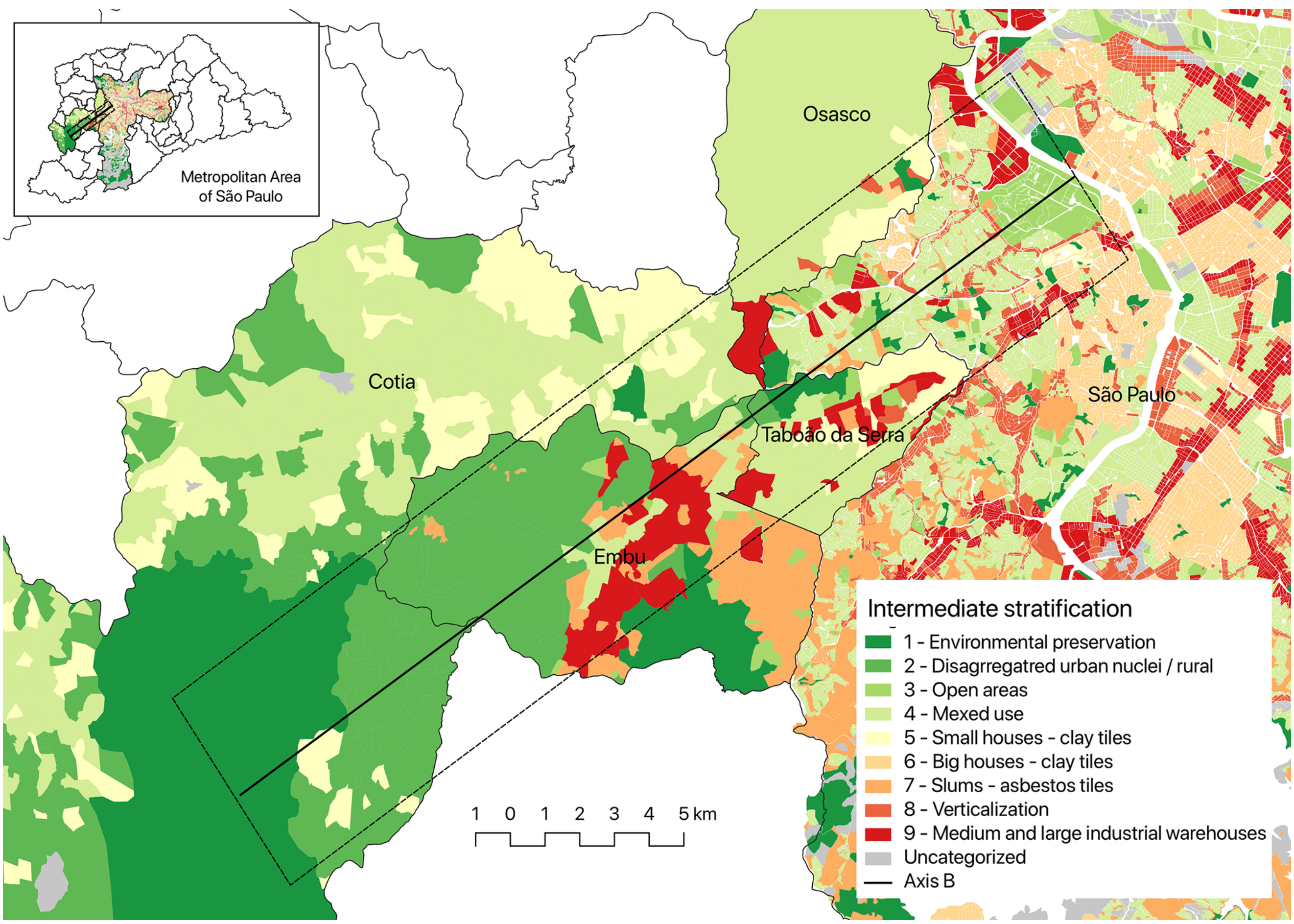

Fig. 4 Intermediate stratification of zones described in urban zoning of São Paulo (Law 16,402/16), Cotia (Complementary Law 204/14) and Embu das Artes (Complementary Law 186/12) and delimitation of the study area along with Axis B

interest" and IX – "Industrial and commercial", respectively, but classified as "inconclusive", that is, without forming a precursor stratum, were incorporated into the intermediate stratum "Rural" because they have landscape characteristics similar to those of the other areas of this group.

Of the zones of Group III, which had been classified as "inconclusive" by the description of the law at the end of Stage 1, for ZER-1 and ZERa (both in São Paulo), after the visual analysis, were considered to belong to the same stratum, while ZER-2 (São Paulo) should be splitted. The first two gave rise to the intermediate stratum "Big houses - clay tiles", as they mostly constitute this type of building, while the third provided buildings, giving rise to the intermediate stratum "Verticalization". The ZEUP (São Paulo, Group VI) and ZC (São Paulo, Group VII) zones also made up the "Verticalization" stratum. As for the intermediate stratum "Big houses - clay tiles", ZCOR zones 1, 2 and 3 of São Paulo were also included in this category, even though they were previously grouped as "mainly non-residential" use by the official description of the law.

The zones of Group IV, all classified as "inconclusive" at the end of Stage 1, proved to be quite heterogeneous in the analysis of the landscape and were divided into four intermediate strata: The ZER (Cotia) and ZPR zones (São Paulo) remained as a mainly residential group, forming the intermediate stratum "Small Houses - clay tiles", as they mostly constitute this type of building; The ZPR (Cotia), ZM (São Paulo) and ZMa (São Paulo) zones had a very heterogeneous constitution, and that is why they gave rise to the intermediate stratum "Mixed Use"; The ZEU zone (Embu das Artes) had warehouses in its constitution, giving rise to the intermediate stratum "Medium and large warehouses"; and the ZUC zone (Embu das Artes) joined with the zones of the precursor stratum V-a (ZEIS-1 of Embu das Artes and ZEIS-1 of São Paulo) to form the intermediate stratum "Popular Housing". In addition to these three, the ZMIS (São Paulo) and ZMISa (São Paulo) zones also form the initial group V ("Social interests"), but classified as "inconclusive", and the ZCM (Embu das Artes), previously classified as inconclusive in Group VIII.

The zones of the Group V-b "Empty or underused lots" (ZEIS-2 of Embu das Artes and ZEIS-2 and ZEIS-5 of São Paulo) remained as a group, giving rise to the intermediate stratum "Open Areas". The two remaining zones of Group V (ZEIS-3 of São Paulo and ZIS of Cotia) previously classified as "inconclusive", made up the intermediate stratum "Mixed Use", along with the ZPR of Cotia and ZM and ZMA of São Paulo (previously mentioned), and with the following zones: ZCa of São Paulo (previously inconclusive of the initial Group VII), ZICS of Cotia, and ZE-3 of Embu das Artes (both previously inconclusive of the initial Group IX), ZUM of Cotia (previously inconclusive of the Group VIII), ZOE of São Paulo (Group XI "Others"), and the three of Group X "Historical and cultural preservation (ZIHC of Cotia and ZCH and ZCT of Embu das Artes). Finally, the intermediate stratum "Medium and large warehouses", in addition to the aforementioned ZEU of Embu das Artes, was also formed by the ZEU of São Paulo (precursor extract VI), ZDE-2 and ZPI-1 (both from São Paulo and previously inconclusive from Group VIII), and ZE-1 from Embu das Artes (previously inconclusive from group IX). The intermediate strata were extrapolated to the municipalities of Taboão da Serra and Osasco according to the characteristics observed in the landscape of these two municipalities, in a manner consistent with the spatial constitution of the stratum (Fig. 4).

Step 4 - Regrouping

The intermediate stratum "Mixed Use" was excluded from the study, as there was no common feature between the areas of this stratum that stands out, and such spatial heterogeneity is not reflected in a biological sense for the study of bats. The intermediate stratum "Open areas" was excluded from the work due to the difficulty in assembling equipment and safety of the team for field work. The intermediate strata of "Large houses - clay tiles" and "Small houses - clay tiles" were united because both deal with residential areas, and such a level of detail is not justified, especially for bat study. Thus, together with the intermediate strata, their respective zones were excluded, totaling 16 excluded zones. The design resulted in six "definitive strata" (**Supplementary Material**; Fig. 5): Definitive Stratum 1 – Environmental Preservation: ZPA (Cotia), ZEIA (Embu das Artes) and ZEPAM (São Paulo) zones; Definitive Stratum 2 - Rural: Zones NUD, ZCEU, and ZUR (Cotia), and ZIA, ZCE, and ZE-2 (Embu das Artes); Definitive Stratum 3 - Houses (with clay tiles): ZER Zones (Cotia), and ZPR, ZER-1, ZERa, ZCOR-1, ZCOR-2, and ZCOR-3 (São Paulo); Definitive Stratum 4 - Slums (houses with asbestos tiles): ZUC, ZEIS-1, and ZCM (Embu das Artes); and ZEIS-1, ZMIS, ZMISa (São Paulo); Definitive Stratum 5 - Verticalization: ZER-2, ZEUP, and ZC (São Paulo); Definitive Stratum 6 - Industrial warehouses: ZE-1 and ZEU (Embu das Artes), and ZEU, ZDE-2, ZPI-1 (São Paulo).

Sampling areas

The sampling points are illustrated in Fig. 5: Morro Grande Reserve, Pedro Beicht - SABESP (Definitive Stratum 1): -23.7149 S; -46.9602 W; Nagmo Forest, Odsal Ling Buddhist Temple) (Definite Stratum 2): -23.6241 S; -46.8751 W; Butantã Sports Educational Center (Definitive Stratum 3): -23.5752 S; -46.7237 W; Faculty of Veterinary

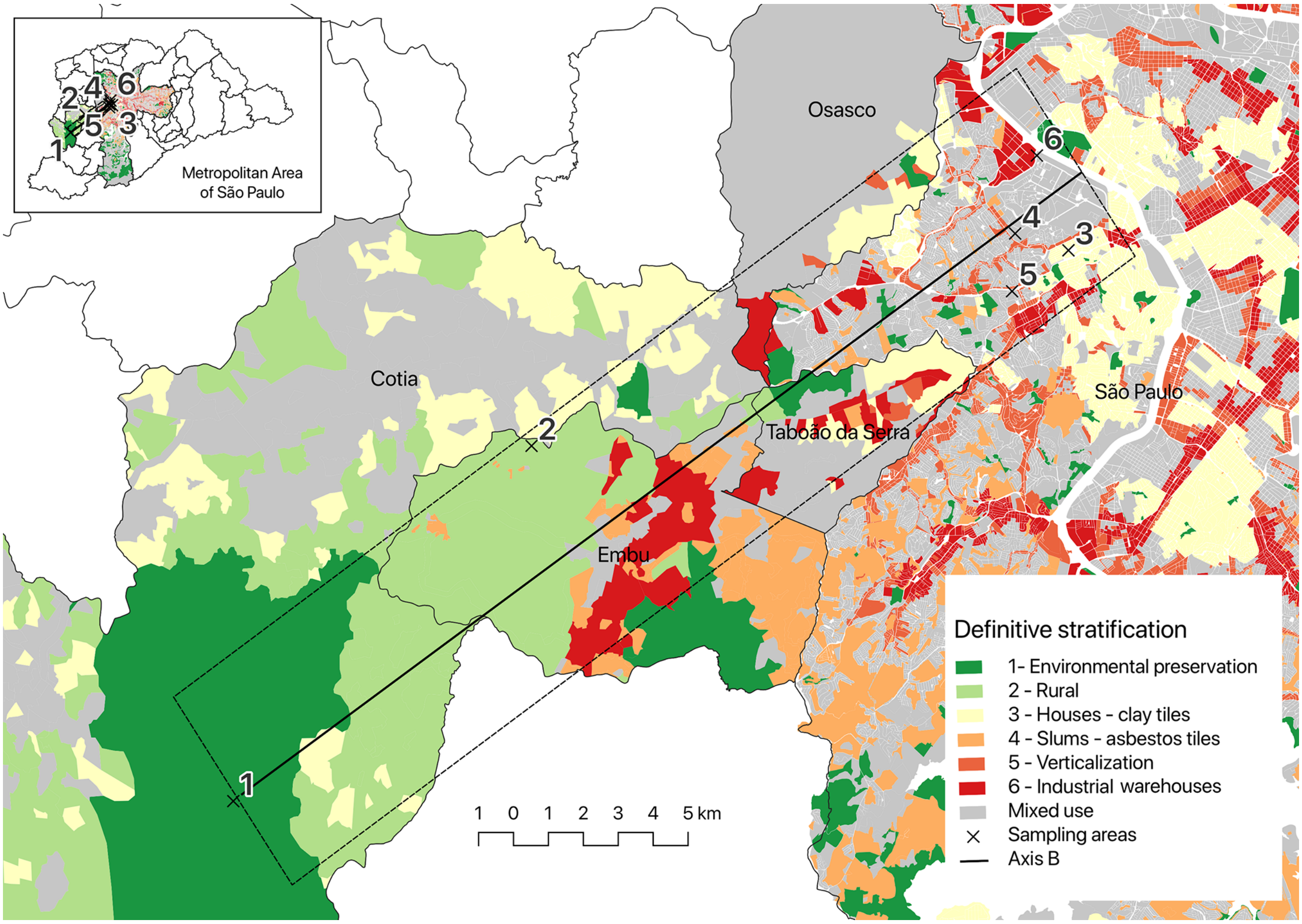

Fig. 5 Definitive stratification of a sample design for an ecoepidemiological study of bats, which involved the recategorization of the zones established in the urban zoning of the municipalities of São Paulo (Law 16,402/16), Cotia (Complementary Law 204/14) and Embu das Artes (Complementary Law 186/12) and subsequent extrapolation of the strata to the municipalities of Taboão da Serra and Osasco, along with field sampling areas numbered from 1 to 6

Medicine of the University of São Paulo (Definitive Stratum 4): -23.5704 S; -46.7392 W; EcoLife Butantã Condominium (Definitive Stratum 5): -23.5856 S; −46.7397 W; USP Olympic Rowing Lane (Definite Stratum 6): -23.5505 S; -46.7323 W.

Discussion and conclusions

Urban zoning and landscape stratification

Municipal zoning deals with the heterogeneity of the urban area, but under administrative aspects. It refers to the destination of use and occupation of the land in different areas of the municipality, addressing both socioeconomic and physical-structural characteristics of the environment. It is, therefore, a document that brings qualitative information that can be useful in the development of an ecological study that intends to contemplate the urban nuances, and not only to compare landscape matrices. However, as the zoning is not developed for scientific purposes, the categorical divisions of land use and occupation as they were originally found need to be interpreted with the lens of ecology or epidemiology to create strata that have biological relevance for the taxa studied, and that meet the objectives of each study.

Stratification aimed to group similar areas according to their predominant components, together with land use and occupation, that is, predominantly forested areas would compose the preserved stratum, followed by predominantly rural, residential, verticalized, industrialized areas, and so on. Note that the level of detail that defines each stratum depends on the scale at which the design is drawn, and that, to contemplate the different compositions of the urban landscape, it is necessary to assume an intrinsic heterogeneity of each stratum. Thus, no stratum is homogeneous. The rural stratum, for example, ranges from agricultural crops and pasture to the presence of few buildings, sheds, farms or even disaggregated urban centers.

The municipalities have distinct zoning, but the strata must be categorized for the entire extension of the study area. Therefore, efforts to group zones according to their spatial similarities, or land use and occupation, both within the same municipality and between municipalities, are also aimed at standardizing the categorization of strata. It should also be noted that the zoning does not necessarily reflect the real occupation of the land at the exact moment of the study, since it is a long-term planning of the municipality. Thus, the importance of using, together with this tool (zoning), a landscape analysis for the construction and checking of the strata, is also highlighted.

Urbanization

Quantifying effects of landscape components on biodiversity and determining how generalizable they can be across urban ecosystems depends on how urbanization is defined and measured (Moll et al. 2019). The definition of "urban", in turn, varies in academia (McIntyre et al. 2000). The scope of the present work was not to delve into the discussion on what is considered urban, or on how to measure or quantify urbanization. However, it should be noted that numerous covariates can be modeled to access urban effects in each community, and the resulting inference from each study depends on how urbanization itself is modeled (Moll et al. 2019). This directly influences the objectives and design of a study in each community.

The present study consisted of an initial exploration of the use of municipal zoning as a tool for the design of ecological studies. Therefore, it is not a study that aims to quantify landscape components. The current reading of the landscape has a qualitative descriptive character, which aims to plan data collection in the field to contemplate different strata of the metropolitan landscape (characterized by land use and predominant components) in an urbanization gradient. Random sampling, for example, might not represent all the strata of interest to the study. However, the present work can help in the development of hypotheses about the influence of urbanization on bat communities since it compares the chiropterofauna in strata within the urban matrix.

In addition, the stratification used in the present study aims to analyze the territory prior to choosing the sampling points to optimize the collection of bats in the field, especially in metropolitan and extensive regions. Cities present challenges for this type of field work, such as the need to obtain permission to carry out research on private properties, find adequate physical spaces for setting up a fog net to capture bats, concerns about security for the integrity of equipment or team members (McIntyre et al. 2000), or physical challenges such as light or noise pollution (Reynolds et al. 2021).

Landscape stratification

Step 1 - Urban zoning analysis

There was no standard in the information on the zones established in the Urban Planning Plans of the evaluated municipalities, making it impossible to group some zones only by this criterion. The main use of each zone could be identified, which allowed the initial grouping of zones. However, additional information to deepen the understanding of each zone was not homogeneous. Data on population or building density, for example, were absent for 30 zones, and when available, they could be classified as "low, medium or high" or as inhabitant/hectare. Thus, the zones were pre-grouped according to the main use, but if they differed in the other information described, or if there was not enough data to conclude the similarity or difference between the zones of the same group, the zone was classified as " inconclusive" at the end of this step. The zones that showed similarities in the description of the law were classified in some precursor stratum, according to the initial group given by the main use.

The municipality of São Paulo has some zones of the same category but located in different macrozones (Urban Structuring and Qualification Macrozone and Environmental Protection and Recovery Macrozone), such as for example, ZM and ZMa, ZC and ZCa, and ZMIS and ZMISa. These zones, despite having the same description, may have relevant differences in the spatial constitution, as these two macrozones have different objectives and patterns of land use and occupation, but not clearly specified in their descriptions in the law. This also makes it impossible to complete the grouping of these zones and reinforces the need for a visual analysis of the landscape to check and complement the construction of the strata.

Step 2 - Visual landscape analysis

The importance of carrying out this step in duplicate is emphasized, since the descriptive visual analysis of the landscape is subjective and subject to the interpretation of each researcher. Therefore, there must be a consensus among researchers on the observed components. The result of this step also depends on the scale at which the landscape is being analyzed, which must be previously agreed between the researchers. Landscape analyses, especially those from the field of landscape ecology (Melo et al. 2020), which require mastery of the technique, would add a lot of quality to compose the proposed methodology.

Step 3 - Comparison between urban zoning and observed landscape

Few initial groups remained as intermediate strata, only "Environmental preservation" and "Rural", which are precisely the ones with less spatial heterogeneity. It is possible to notice that the heterogeneity of the landscape increases with the increase of urbanization, and therefore the more central areas of the city have more strata and smaller strata. A single block or neighborhood, for example, can present more than one stratum or zone. As many zones ended up as "inconclusive" for the precursor strata, and many zones were reclassified at this stage of stratification, giving rise to new strata that had not yet been identified from the legislation alone, it is concluded that the analysis of the landscape had weight greater in the stratification of the landscape.

Step 4 - Regrouping

This step reveals the importance of a team review of the identified strata. Checking the logistics of field collections in each stratum, as well as their biological relevance are of paramount importance. It is necessary to assume an intrinsic heterogeneity in each extract, but areas that are too heterogeneous do not add any meaning to the research and should be excluded, as was the case with the "mixed use" extract of the present work.

Sampling areas

This method was used in the field in a sanitary, ecological and bioacoustics monitoring project in MASP between June 2021 and April 2022. Even though bats not necessarily live in the areas that they were captured but use them as one (of maybe many) foraging area and a single sampling point per strata has been chosen, each location was monitored four times during this period, increasing the variability of results for each strata represented by the sampling point. These results will be discussed elsewhere but could be promptly used in other places and to the study of other taxa, despite primarily dealing with a specific problem of bats.

There was a concentration of sampling areas in the city of São Paulo, precisely the areas that represent the most urbanized strata, such as the definitive stratum 3 "Houses", 4 "Slums", 5 "Verticalization" and 6 "Industrial warehouses". The sampling area should be located as centrally as possible in the stratum, to avoid edge effect, but for Strata 4 and 6 it was not possible to acquire a collection area within the stratum, and therefore the points were placed close to these strata. The choice of collection areas prioritized the safety of the team due to field trips being carried out at night, and therefore, they were carried out on properties with restricted access. Furthermore, access authorizations for the research had to be obtained during the COVID-19 pandemic period, which made communication with some locations difficult.

Sampling areas that best represent the stratum in question should be sought. However, as the stratum is not homogeneous, the collection area itself is not fully composed of the predominant components of the stratum. The collection area of the "Houses" stratum, for example, was not necessarily a house, but a sports center inserted in a residential neighborhood. It is noteworthy that, depending on the objective of the study and the rate studied, the inferences to be made in possession of the collected database may be more related to the collection area than to the stratum itself. Stratification, however, is quite useful to select sampling sites that best represent the heterogeneity of the landscape. Therefore, it is recommended that an analysis of the landscape of the study areas is also carried out with other GIS techniques, to assist in an even more detailed and precise description of each stratum, preferably measuring the landscape components (Moll et al. 2019; Cadenasso et al. 2007). More than one collection area per stratum would better represent the strata and are recommended for future approaches, but it is important to highlight that the number of sample areas per stratum should be compatible with the size of the team and the logistics of fieldwork.

Interdisciplinarity and one health

Reflections and discussions about the connection between ecology and urban planning have been growing, especially about sustainability, both popularly and in the scientific community (Melo et al. 2020; Formam, 2014). However, even with this advance in the discussion about the city planning, the fauna is usually neglected. Several influences that animals may exert and suffer from the city are rarely considered outside the academy. Although zones of a municipality were not developed with the scientific purpose of ecological studies of fauna, using them for this purpose reinforces the importance of connecting ecology with urban planning (Bhakti et al. 2021; Melo et al. 2020), and can help eventual actions of conservation of biodiversity, surveillance of zoonoses or control of synanthropic fauna, since it uses a tool of the public power itself, which already has the purpose of managing the municipality.

However, interdisciplinarity is extremely relevant to this topic. Joint action of professionals from different areas such as Landscape Ecology, Urban Ecology, Veterinary Medicine, Urban Planning, Public Management, Epidemiology, Biology, Environmental Management, among others, would make this approach more robust. It would characterize a One Health approach, seeking a connection between animal, human and environmental health.

However, it is necessary to make the population aware of Ecology and One Health as a whole, especially when it involves fauna. In this sense, the study and monitoring of bats in urban areas, in addition to being important for basic and applied science, shows great didactic utility, as it is an animal covered in myths and beliefs (Pacheco 2004), living relatively close to humans, eventually causing encounters, and as previously stated, with great ecological and public health importance. With this, it is emphasized the importance of developing specific study designs for urban bats, which consider the physical-structural and socioeconomic heterogeneity of this ecosystem. In this study, municipal zoning proved to be a complementary tool to landscape analysis, which allows accessing part of this heterogeneity.

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Declarations

Competing interests The authors declare no competing interests.

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